

The evolution of
cooperation and diversity
in
public good producing organisms

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Declaration

I, William Lee, hereby declare that this thesis and the work presented in it is entirely my own. The contribution of the other authors consisted of suggestions and corrections within the normal scope of supervision.

Signed:

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Abstract

In a population of altruists, all individuals thrive. But altruists are exploited by cheating individuals which do not perform altruistic acts but still benefit from those. In these conditions cooperation cannot easily evolve. This issue is resolved by kin recognition: altruists recognise each other through the use of a conspicuous tag. These altruists do well until cheaters acquire the signalling tag and disrupt the cooperation. But altruists using a different tag can then invade the population, followed by new cheaters. This mechanism can lead to a diversity of tags coexisting in the population. However it has not yet been applied in realistic biological systems.

In this thesis, I formulated mathematical and simulation models to investigate the effect of diversity on the evolutionary dynamics in systems where different altruists compete with cheaters. In particular, I focused on organisms producing public goods, i.e. goods that can profit to the whole population. I considered two biological systems models: gynodioecious populations of plants, where hermaphrodites produce pollen that can be used by female-only individuals, and bacteria producing an iron-chelating molecule, called siderophore, that can be exploited by both producers and non-producers.

I found that diversity in gynodioecious plants is dependent on population structure. In particular, I found that the maximal level of diversity occurs when the population structure does not favour altruists or cheaters. Next, I found a number of important results in siderophore-producing bacteria. By considering a detailed ecological model, I derived Hamilton's rule in a metapopulation and found that the level of cooperation in a population depends on the length of interaction between strains. Finally, I discovered a novel evolutionary mechanism generating and maintaining diversity and showed that it results from non-equilibrium mechanisms. These findings explain why cheaters appear readily in experiments but are rare in natural populations.

My results demonstrate the importance of integrating ecological details in order to understand the mechanisms leading to cooperation and diversity, and will provide a basis and framework for future studies on the emergence and maintenance of diversity.

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Thesis overview

Understanding the mechanisms underlying the evolution of biodiversity is one of the major challenges in evolutionary biology. It has been shown that social interactions can drive the evolution of diversity. For instance, the idea of chromodynamics (or tag-based cooperation) is that individuals can recognise each other through the use of a conspicuous tag. Altruists using a secret signal thrive, until a cheater acquires the signal through mutations, and destroy the cooperation based on this signal. However new altruists using another signal, unusable by the resident cheaters, can re-establish cooperation in the population, before another cheater acquires the new tag.

In this thesis, I used mathematical and simulation models in order to explore the potential for the emergence and maintenance of diversity in systems of cooperators/cheaters. In particular, I focused on organisms producing public goods, i.e. goods from which all individuals in the population benefit. I considered two biological systems models: gynodioecious populations of plants, where hermaphrodites produce pollen that can be used by female-only individuals, and bacteria producing an iron chelating molecule, named siderophore, which can be exploited by producers and non-producers.

I found a number of important results. In Chapter 2, by developing a model simulating gynodioecious plants dynamics I found that diversity is dependent on population structure. In particular, I determined that a maximal level of diversity can occur for intermediate values of viscosity. The dynamics produced by the model are concurring with field observations, showing a high polymorphism, associated with spatial

structure. Next, I developed in Chapter 3 a model of siderophore-producing bacteria competing in a metapopulation, and where the siderophore production level evolves. I found that in this setting, the level of siderophore production reach an evolutionary single strategy, which is determined by the interplay between altruists and cheaters. Importantly, I also derived Hamilton's rule from ecological principles.

How diversity has arisen in siderophore-producing bacteria is not well known. In Chapter 4 I explored the hypothesis that siderophore diversity arises as a defence mechanism preventing the exploitation of producers by non-producing strains. By formulating and analysing a mathematical model of siderophore-producing bacteria where different siderophore types can appear, I showed that in fact siderophore cheaters work as an evolutionary force promoting diversity by counteracting genetic drift. The resulting dynamics is unstable because of the stochastic processes that take place. In Chapter 5, I derived conditions for the local stability of the equilibrium in this model. I found that the stability depends on the level of diversity and on the mutation rates.

My results demonstrate how diversity can arise and be maintained in two biologically realistic examples. Moreover, I showed that diversity results from non-equilibrium mechanisms. I argue that this has relevance in many other biological systems, such as budding yeasts or side-blotched lizards, and can help developing new theoretical frameworks and experimental protocols for the evolution of cooperation and diversity.

Chapter 1

Introduction

1.1 Evolution of cooperation

How does evolution work? In 1859 in the “Origin of species”, Darwin proposed a theory that we now know as natural selection. He noted that evolution selects on biological traits found within a population arguing that, if a specific trait will allow its bearer to have more offspring, this trait will spread in the population. On the contrary, if the trait is detrimental, it will disappear. This theory led to the term “survival of the fittest” (Spencer, 1864).

According to this theory, an individual should always seek to maximise its number of offspring: selfish behaviour should dominate. The evolution of altruism thus poses a Darwinian paradox. In a population of cooperators, all individuals would thrive. However, a selfish individual could exploit the act of cooperation of others without putting any effort in itself. We can assume that it would produce more offspring than the cooperators, and consequently the frequency of the selfish (cheating) individuals will increase in future generations. On the face of it, natural selection would eventually lead to the disappearance of the cooperators from the population.

Given this paradox, one would expect cooperation to be rare or not exist at all. But examples of cooperating behaviour are numerous in Nature, ranging from production

of chemical compounds beneficial to all individuals in bacteria, to colony defence in mammals (Wilson, 1975, Fehr and Fischbacher, 2003). There should thus exist mechanisms that will generate such cooperation which are consistent with the tenets of Darwin's theory.

In this thesis, I will focus on those scenarios where cooperative behaviour involves the production of public goods. Here I will use the definition of "public good" commonly used in the field of evolutionary biology: that is something which can be exploited by all individuals in the population (Rankin et al., 2007). Examples of public goods are the production of iron scavenging molecules (siderophores) or toxins, by bacteria.

A famous example of cooperation on a public good is Hardin's tragedy of the commons (Hardin, 1968), which provides an insightful illustration on the issue of cooperation versus cheating, when exploiting a common resource. The original tragedy of the commons applies to a group of cattle farmers exploiting a common pasture. This pasture constitutes a public good because it is shared by all the farmers. Here we suppose that each farmer intends to maximize their profit derived from using the pasture. Given this, any particular farmer would put as many cows on the pasture as possible. Unfortunately, this will eventually lead to the overexploitation of the pasture and its tragic destruction.

Analogous to the tragedy of the commons there are also situations where the members of the population generate the shared resource themselves. In these cases, there is an incentive to contribute less, and this is often referred to as the "temptation to cheat" (Axelrod and Hamilton, 1981, Axelrod, 1984, Bronstein, 2001, van Baalen and Jansen, 2003). As with the tragedy of the commons, the cost of these lower contributions is shared by the whole population.

1.1.1 Modelling evolution

There are two approaches when doing scientific research. Either we try to understand Nature by observation and experimentation, or we use simplified representations (modelling). This thesis falls in the second category. Rather than presenting all the concepts classically used to study evolution, for example (Fisher, 1930, Haldane, 1932, Maynard Smith, 1982, Hamilton, 1996), I focus here on the concepts and techniques used in the present thesis.

Evolutionary game theory

As Darwin pointed out in his “Origin of species”, the aim for any individual is to maximise its fitness. In order to understand how a trait evolves in a population, Maynard Smith and Price (1973) applied the concepts of game theory to evolutionary biology to form what we now know as evolutionary game theory (EGT). In EGT, individuals in a population are characterised by a heritable strategy, and the aim is to study how good this strategy is depending on the frequencies of other strategies in the population. A better strategy leads to a higher fitness, which translate to a more frequent representation of this strategy in the population in future generations.

A key concept is that fitness is not an absolute measure, but is relative to other individuals’ fitnesses: the success of a strategy is not solely determined by itself, but is context-specific, meaning that it depends on the other strategies present in the population. I will here present the classical example of EGT, called the “Prisoner’s dilemma”.

The prisoner’s dilemma is the most famous evolutionary game and the most studied. In this scenario, two thieves have been caught by the police and are held in two separate cells. The head of the police proposes to each of them the following deal: If they both stay silent (cooperation), they will both serve only one month for minor charges. If one stays silent and the other denounces him (defection or cheating), then

the first will serve one year and the second will walk out free. Finally, if they both denounce each other, they will both serve six months (Idea formulated by Tucker in 1950).

What should they do? The dilemma here is that the best strategy all round would be for both to cooperate, but each prisoner will be better off denouncing the other, irrespective of the other's decision. Thus, both prisoners defecting is the expected outcome of the game. The tragedy of the commons game is similar to the prisoner's dilemma, except that the game is played with many individuals instead of two. In both scenarios, the best strategy for an individual is the selfish one.

Adaptive dynamics

Evolutionary game theory is interesting but it lacks biological realism, and it does not take population dynamics into account. To overcome this issue, other techniques have been developed, in particular adaptive dynamics, which combine evolutionary game theory with population dynamics. I will here present a brief summary of adaptive dynamics (for a detailed review, see Geritz et al., 1998). The main idea of adaptive dynamics is to study the evolution of a phenotypic trait in a population of haploid individuals, through the analysis of differential equations. To do so, it makes a crucial assumption, which is a time scale separation between ecology and evolution. Indeed, it is considered that evolution is a mutation-limited process, so that a population is at an ecological attractor (equilibrium) before a mutation event. If the new trait arising by mutation is beneficial for its bearer, then it will grow in the population and replace the former trait eventually becoming the new resident trait.

Consider a monomorphic population of resident A , with a quantitative trait a (such as size). Its dynamics is represented by the following differential equation

$$\frac{dA}{dt} = r(a, E_A)A$$

where $r(a, E_A)$ is the per capita growth rate of A , determined by ecological principles and E_A is the environment of A . At the ecological equilibrium, this per capita growth rate is zero. Consider now a mutant A' with a slightly different trait a' . Similarly, its dynamics is given by

$$\frac{dA'}{dt} = r(a', E_A)A'$$

where $r(a', E_A)$ is the per capita growth rate of A' in the environment of A (because A' is very rare). This growth rate is equivalent to the invasion fitness, dubbed $s_a(a')$. If the invasion fitness is positive, the mutant invades, and if it is negative the mutant disappears.

In adaptive dynamics, mutations are assumed to be small, so that the mutated trait drifts only a little from the resident trait. To determine the direction of evolution, we consider that the mutated trait is almost identical. Thus it suffices to calculate the sign of the gradient of selection which is the derivative of the fitness function

$$\left. \frac{\partial s}{\partial a'} \right|_{a'=a}$$

However, for some values of a , that we can call a^* , this selection gradient can become 0, i.e. when

$$\left. \frac{\partial s}{\partial a'} \right|_{a'=a=a^*} = 0$$

These points are known as evolutionary singularities, and represent either a maximum or a minimum of the fitness function s . On the one hand if it is a maximum,

this means that the a^* trait cannot be invaded by nearby mutants: it is an evolutionary stable strategy (ESS). On the other hand if it is a minimum, this means that the a^* trait is invadable by any nearby mutant: the population will be subject to disruptive selection, eventually leading to an evolutionary branching. To determine whether the evolutionary singularity is an ESS or a branching point, it suffices to determine the sign of the second derivative of the fitness function at the singularity. The criterion for an ESS is

$$\left. \frac{\partial^2 s}{\partial a'^2} \right|_{a'=a=a^*} < 0$$

The reverse condition logically leads to a branching point. The evolutionary dynamics can be represented graphically using a Pairwise Invasibility Plot (PIP). A PIP is a diagram showing the sign of the fitness on the (a, a') domain (Figure 1.1)

1.1.2 How can cooperation arise ?

I have earlier stated that cooperation needs enabling mechanisms to arise. I will here focus on the effect of spatial structuring. In the area of ecological and evolutionary dynamics, two families of models exist: non-spatial, and viscous models. In non-spatial models (or well-mixed population models), it is assumed that all individuals in the population interact with each other with equal probability. As a consequence, the benefits of cooperating are shared between all individuals, cooperators or cheaters, and thus cooperation cannot persist.

The second family includes viscous models. The term “viscous” was first used by Hamilton (1964). In these models, the interactions between individuals are not random. For instance, individuals either interact only with their nearby neighbours or the probability of interacting with another individual decreases with the distance

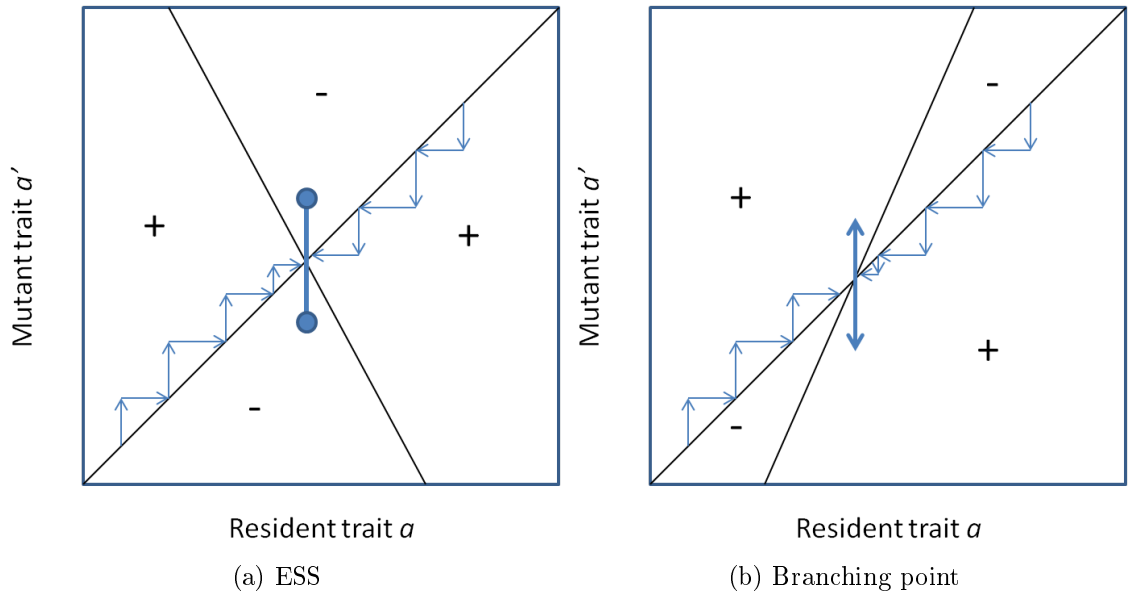


Figure 1.1: Illustration of the adaptive dynamics. The “+” regions are areas where a mutant trait can invade the resident trait, and the “-” regions areas where a mutant trait cannot. (a) Evolutionary stable strategy. (b) Branching point. The PIPs can be read as follows: starting at any point on the diagonal, giving the value of the resident trait, a mutation can occur. If the mutant trait is in the “+” regions, the mutant invades and replaces the resident, and becomes the new resident. This eventually leads to the evolutionary singularity (point where the fitness function crosses the diagonal), corresponding to a maximum (ESS) or a minimum (Branching point). If it is a ESS, it cannot be invaded by any nearby mutant, and the ESS is the endpoint of evolution. If it is a branching point, it can be invaded by any nearby mutant and the population experiences disruptive selection.

between them. If we consider that a given individual carries an altruistic gene, the limited dispersal of its offspring will mean the formation of a cluster of individuals with the same gene. Thus, an altruist individual is more likely to interact with another individual carrying the same altruistic gene than it would when it interacts with a random individual in the population.

Thus, even if the act of cooperation does not directly benefit the actor, there is an indirect benefit through the increase of fitness for the recipient, which is likely to be a relative (kin). This is the basis of “kin selection” or “inclusive fitness” theory (Hamilton, 1964, Maynard Smith, 1964). Hamilton’s verbal argument has also been accompanied by an inequality, which we now know as Hamilton’s rule:

$$RB > C$$

where B is the benefit and C the cost of performing the altruistic act. Hamilton's rule explains how altruism can evolve if the benefits gained by receiving an altruistic act, modulated by the relatedness between actor and recipient, outweigh the cost of performing the altruistic act. In simple terms, altruism can evolve if the benefits go towards related individuals, which will indirectly transmit the gene of the cooperating actor and spatial structure is a way of ensuring that the benefits are so directed. The R parameter is called relatedness and has received many interpretations. A commonly accepted definition is that it is the probability for an individual of encountering another individual which carries the same gene in its local group, relative to the probability of encountering one in the global population (Queller and Goodnight, 1989). Thus relatedness should be higher in populations exhibiting limited dispersal, and a high relatedness should favour the emergence/maintenance of cooperative behaviour (Hamilton, 1964).

1.1.3 Examples of spatial structure

In this thesis, I will present a model of the dynamics of gynodioecious plants on a square lattice in Chapter 2. The motivation behind choosing a squared lattice (instead of a random) is for biological realism. Typically, I consider that the spatial structure resembles an agricultural field, where every plants are fixed on a particular plot, and are evenly spaced with the other plants. The model is a cellular automaton consisting of a grid, where all cells exhibit a given state (determined by the ecology), such as "alive" or "dead" (Figure 1.2). Each cell experiences a neighbourhood, which defines the cells it can interact with. The state of each cell is then updated through updating rules determined by the ecological dynamics. Note that on figure 1.2a, the cellular automaton resembles an agricultural field, but in fact the boundaries are interconnected: the east end joins the west end, and the north end joins the south, so that the actual shape is a torus.

In chapter 3,4 and 5, I will model siderophore evolution in a metapopulation. Indeed, in experimental studies of siderophore evolution, the set-up is made such that bacteria interacts both within wells (local competition) and between wells (global competition). I chose to follow theoretically these set-ups, hence the use of metapopulation models. A metapopulation consists of a set of spatially separated populations (or patches), which interact with each other (Figure 1.2b). Each population behaves independently of the others apart from migration between the populations. In the model, these migration events perturb the equilibrium of the populations.

A metapopulation is an example of extreme spatial structuring: individuals in a patch are assumed to interact almost exclusively with individuals of the same patch. This interaction within a patch constitutes the local dynamics. The global dynamics are often at a different time scale and are defined by migration and mixing between patches. Depending on the model's assumptions, patches can have internal structure as well (or not). I will present a modelling of the dynamics of siderophore producing bacteria in a metapopulation in Chapters 3, 4 and 5. I consider that each patch is well mixed, so that the spatial structure is given only through the metapopulation set-up.

1.2 How diversity can arise from social interactions

The classical models of cooperation are constrained on the interaction between altruists and cheaters. Recent developments have shown a richer image of social interactions, which are not limited to one altruist versus one cheater. A famous example is the “rock-paper-scissors” game. This children’s game is simple: rock crushes scissors, scissors cut paper, and paper covers rock. When the game is played with pure strategies (which is the case in biological organisms), the resulting dynamics are unstable (May and Leonard, 1975), because no strategies dominate the other two. Thus, we observe cycles of invasion of the different strategies, which are always

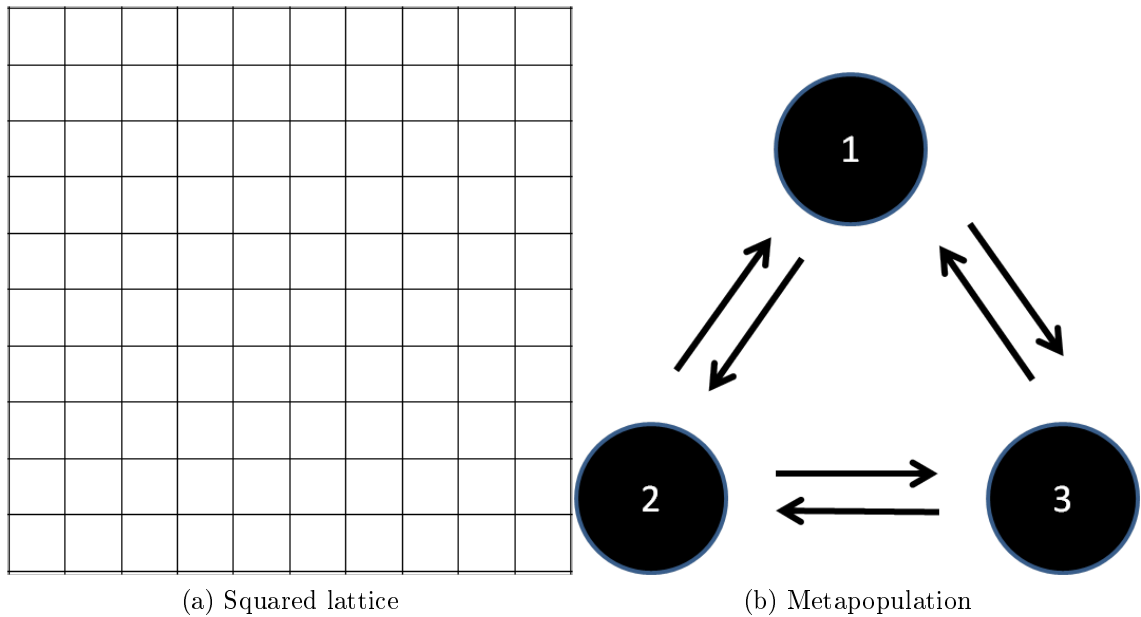


Figure 1.2: Examples of spatial structures.

bested by another one. Kerr et al. (2002) found that diversity of strategies is maintained when the competition is local but that it is lost when competition is global. I will also discuss the effect of the scale of competition on diversity in Chapter 2. The “rock-paper-scissors” game has recently been discovered to be played in a population of lizards (side-blotched lizards), which indeed exhibit alternate cycles of dominance (see Sinervo et al., 2006 for more details).

In this thesis, I focus on another type of diversity, known as chromodynamics (Jansen and van Baalen, 2006). This follows from the “green beard effect” (Dawkins, 1976, Gardner and West, 2010) which assumes that organisms interact via the recognition of a conspicuous tag (coded by a single gene, the so-called green beard gene). Such a tag, used as a marker to discriminate carriers and non carriers of the correct allele, would serve to favour any individuals exhibiting the same tag. By interacting preferentially with each other, the green beard gene could thus increase in frequency in the population and cooperation can arise. However, if a non-cooperating individual (cheater) acquires the tag (by mutation for instance), it would succeed in reaping the benefits of cooperation without paying any cost and cooperation would break down. With chromodynamics, new tags can also be generated through mutations, resulting in a diversity of distinct sub populations of altruists and cheaters coexisting in the

population.

The principle is simple: consider a subpopulation of altruists called A_t exhibiting a tag t . Initially, this subpopulation does well, until cheaters also acquire the tag t (we will call the cheaters C_t). These cheaters oust the altruists, but a mutant altruist A_v , using a new tag v , unrecognisable by cheaters C_t , could arise and in turn invade the population, before being itself invaded by a cheater C_v . These cycles of invasions can continue indefinitely (Figure 1.3). Depending on the model's assumptions, dynamics can either exhibit successive invasions of one population, so that at any time, only one type of individual is present in the population (see Traulsen and Nowak, 2007), or a diversity of different altruists and cheaters coexisting simultaneously (see Jansen and van Baalen, 2006).

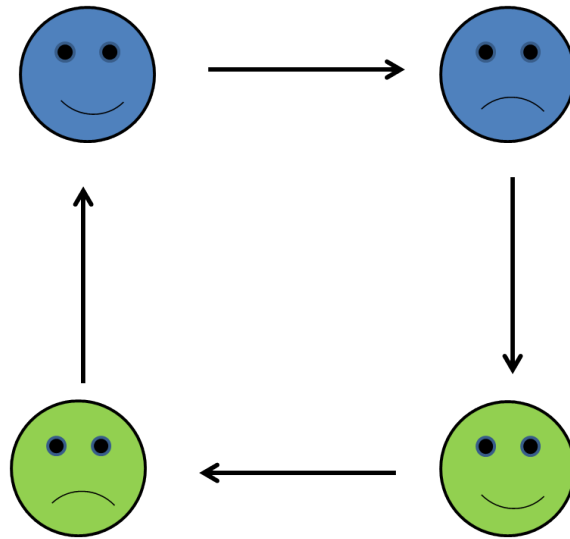


Figure 1.3: The green beard effect. A cooperator type “blue” (happy blue face) is invaded by a cheater type “blue” (sad blue face), which is in turn invaded by a cooperator type “green”, which is in turn invaded by a cheater type “green”. In this example only two colours are present, so that a cooperator type “blue” can invade the cheater type “green”, but the same mechanisms apply for greater number of “beards”.

Greenbeards have long remained a theoretical toy. Because of their supposed complexity, it has been speculated that greenbeards would be very rare in Nature (Hamilton, 1964, Dawkins, 1976). Indeed, the original idea was that a green beard

gene should confer both the conspicuous signal, and the altruistic behaviour, which seems rather unlikely (but such green beard genes have been discovered, see, e.g., Smukalla et al., 2008). However, if two genes, one coding for the conspicuous signal, and the other coding for the altruistic behaviour, are closely linked, they can act similarly as a green beard gene (this example is studied in Jansen and van Baalen, 2006).

In this thesis I investigate the dynamics of cooperation and diversity in populations where individuals cooperate via the production of a public good. If the public good is not utilizable by the whole population but only accessible to some individuals which can recognise it, I consider that it is in fact a *restricted* public good. Examples of these restricted public goods, similarly to the GB effect, are few. In the next section I present two biological systems using these. Other examples are the *FLO1* gene causing flocculation in *saccharomyces cerevisiae* (Smukalla et al., 2008) and the *csA* gene causing adhesion in *Dictyostelium discoideum* (Queller et al., 2003), but studies on their diversity are lacking.

1.3 Restricted public good systems

1.3.1 Gynodioecious plants

In gynodioecious plants, two types of individuals coexist in the population: hermaphrodites, which produce both pollen and seeds, and female-only individuals, which do not produce pollen (Delannay, 1979). The female's reproduction mode is pseudogamy (or gynogenesis): it is an *incomplete* asexual form of reproduction. Pseudogamy resembles parthenogenesis because the egg develops with only maternal genetic material, but it is *incomplete* because the development must be triggered by pollen (or sperm)(Beukeboom and Vrijenhoek, 1998). Pseudogamy occurs in a wide range of organisms, from animals to plants (Richards, 2003, Schlupp, 2005).

Pseudogamy has often been referred to as “sperm parasitism” or “sperm-dependent parthenogenesis” (Hubbs, 1964, Vrijenhoek, 1994), because females survive by exploiting the hermaphrodites’ pollen, similarly to a parasite-host situation. However, assuming that the production of pollen is costly for the hermaphrodites, this biological system can also be interpreted as a public good-producing system, the public good being the pollen, which altruists (hermaphrodites) produce and which is hijacked by the cheaters (female-only plants) that do not produce any.

Moreover, there is evidence that pollen incompatibility mechanisms have evolved: not every type of pollen is utilizable by all plants in the population (Goswami and Matfield, 1974). This indicates that the pollen might be tagged.

This biological system is thus an example of the struggle between altruists and cheaters. Females do not produce pollen, so they can produce more seeds than the hermaphrodites and should thus dominate the population. Moreover in this example the tragedy of the commons is emphasized because the females need pollen for reproduction. Without pollen they cannot produce any seeds. In simple, non-spatial models, coexistence is not possible because females oust the hermaphrodites of the population and die out due to the scarcity of pollen. By introducing spatial structure (viscosity) in a cellular automaton, more recent models have showed that the coexistence is possible (Wilson, 2000, Stewart-Cox et al., 2005, Preece and Mao, 2010). The argument is that pollen and seed do not disperse anywhere; instead they should disperse close to the plants which produced them.

These theoretical studies investigated the possibility of coexistence between hermaphrodites and females, but did not take into account the incompatibility between different pollen types. In Chapter 2 I explore the consequences of adding a pollen incompatibility system in a population of gynodioecious plants. I use both kin selection and tag-based cooperation theory to investigate the consequences of limited dispersal on cooperation and diversity.

1.3.2 Siderophore-producing bacteria

Like many organisms, bacteria need iron for survival and growth. However, iron is a scarce resource for host-dwelling bacteria because it is encapsulated in larger molecules, such as blood proteins in mammals. To scavenge for iron in these hostile environments, bacteria have developed sophisticated mechanisms. In particular, some bacteria can produce molecules that have a very high affinity for iron, called siderophores (Guerinot, 1994). These siderophores are very diverse (Ratledge and Dover, 2000, Wandersman and Delepelaire, 2004) but have the same structure: a functional unit that ligates with iron molecules (transferrins, lactoferrins) and a peptide backbone interacting with a receptor on the surface membrane of the bacteria. This peptide differs from strain to strain, and the receptor is specific, only recognising the corresponding peptide backbone (Hohnadel and Meyer, 1988, Cornelis et al., 1989, Spencer et al., 2003, de Chial et al., 2003). The siderophores are secreted and sent into the extracellular environment, where they can bind with iron. Once bound, they can be taken up by any bacteria possessing a corresponding receptor. Because the production of these siderophores is costly, they constitute a public good, that can be hijacked by non-producing bacteria. Moreover, their specificity means that they are restricted to a certain portion of the population. Thus the siderophore-producing bacteria are also an adequate example of tag-based cooperation.

The most famous and studied example of cooperation via the use of siderophores occurs in the bacterium *Pseudomonas aeruginosa*. *P. aeruginosa* produces several types of siderophores, the main class of which are called pyoverdines (Cornelis and Matthijs, 2002). Of these there exist three distinct structural types: types 1, 2 and 3. Each strain of *P. aeruginosa* can produce only one of these types, and possesses the corresponding type-specific pyoverdine receptor (Cornelis et al., 1989). Moreover, considerable variation has been reported within each structural type (Smith et al., 2005, Bodilis et al., 2009).

The question of emergence and maintenance of diversity in this biological system has

been investigated recently by Smith et al. (2005). Their verbal explanation has much in common with the chromodynamics mechanism. They argue that siderophore diversity could be a defence against exploitation by non-siderophore producing bacteria. In an environment of cheaters, it would be beneficial for a mutant to produce a siderophore that is distinct in structure, unusable by the dominant cheaters. The new altruists would invade, before the appearance of a compatible cheater render them vulnerable again. This can result in a type of Red Queen evolutionary race, in which cheaters chase the altruists when they acquire the ability to use the siderophores produced by a strain of altruists, while the altruists constantly evolves new structures of siderophores to escape the exploitation by cheaters.

Siderophore-producing bacteria are a very well studied biological system. So far, most studies focused on the evolution of cooperation and virulence (West and Buckling, 2003, Griffin et al., 2004, Brown et al., 2009, Alizon and Lion, 2011). None of these studies take into account the diversity and specificity of the siderophores. Chapter 4 and 5 of this thesis are thus completely novel and innovative to study the dynamics of cooperation in this system. Also, these studies have a strong focus on local competition and do not link the global competition to the local production. In Chapter 3 I present a framework to model the evolution of siderophore production that take into account both local and global competition.

Chapter 2

Intermediate dispersal ability can promote diversity in gynodioecious plants

2.1 Introduction

Pseudogamy (also known as gynogenesis) is a peculiar form of reproduction, halfway between parthenogenesis and sexual reproduction (Figure 2.1). It occurs in a wide range of organisms, from animals to plants (Richards, 2003, Schlupp, 2005). In pseudogamous organisms, an offspring only receives the maternal genetic information, but the embryogenesis must still be triggered by sperm (Beukeboom and Vrijenhoek, 1998). For instance in angiosperms, where reproduction involves the double fertilisation of zygote and endosperm, pseudogamous individuals develop their zygote parthenogenetically, but the endosperm is fertilised by pollen, resulting in a triploid endosperm. A classic example of plant pseudogamy occurs in gynodioecious plants. In this breeding system, female individuals only producing ovules, coexist in the population alongside hermaphrodites that produce both ovules and pollen (Delanney, 1979).

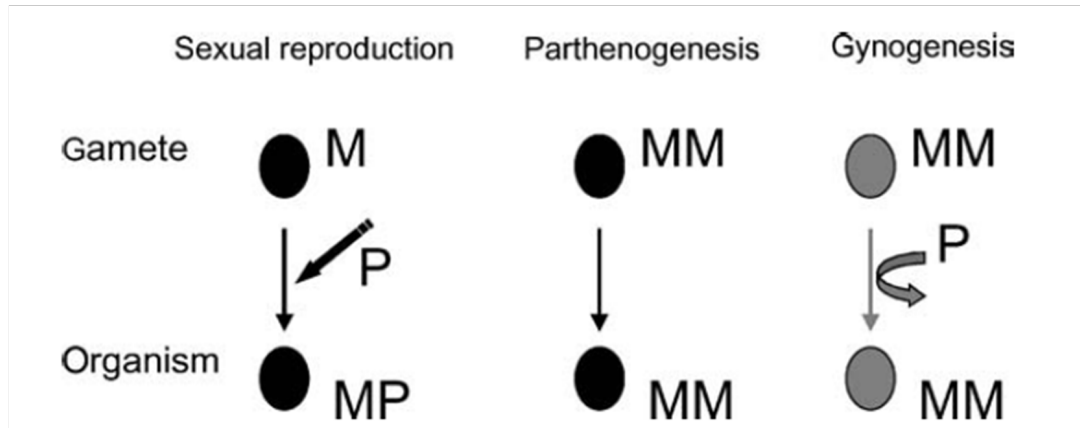


Figure 2.1: Schematic representation of sexual reproduction, parthenogenesis and gynogenesis (pseudogamy). M refers to maternal contributions and P to paternal contributions (Illustration extracted from Schlupp 2005)

It has been argued that males are a burden for sexual populations because they do not make any progeny (Maynard Smith, 1982): they parasitise females' reproductive effort. In pseudogamy, this so-called host-parasite relationship is reversed: the male is needed for fertilisation, but its genetic information is not transmitted by the females. Thus, pseudogamy has been referred to as sperm parasitism (Hubbs, 1964), or sperm-dependent parthenogenesis (Vrijenhoek, 1994). Alternatively, one could consider this system as a form of cooperation via the production of a costly public good, the pollen. In this light, the hermaphrodites are the cooperators, while the females are the cheaters, because they can exploit the pollen without paying the cost of production. Hence pseudogamy is not necessarily a cooperative system. In this study I will consider that it is an act of altruism.

The coexistence of hermaphrodites and females is a paradox: because females do not produce pollen, they should have a higher fitness and hence invade population of hermaphrodites. Simple models suggest that coexistence is not possible, and that the system will always collapse due to the scarcity of pollen: females drive the population of hermaphrodites to extinction, and as they need pollen to reproduce, also go extinct themselves. However, if spatial structure is taken into account, i.e. if pollen/seed dispersal is limited in space, coexistence is possible (Wilson, 2000,

Stewart-Cox et al., 2005, Preece and Mao, 2010).

From this example, we can infer that assortment is very important for the coexistence between cooperators and cheaters: if pollen can be shared preferentially with other pollen-producers, the hermaphrodite population can survive despite being exploited by the females. This suggests that the limited dispersal of pollen leads to an inclusive fitness argument (Hamilton, 1964, Dawkins, 1976). Another mechanism by which cooperators could avoid exploitation by cheaters exists: kin discrimination. If the hermaphrodites could evolve a strategy to discriminate against “helping” the females, they would also do better.

In plants, discrimination can occur with the installation of reproductive barriers. These include pre and post pollination barriers (See Rieseberg and Willis, 2007 for a review). In gynodioecious plants, there is substantial phenotypic and genetic diversity, and pollen from different sub-species cannot be used indifferently (Goswami and Matfield, 1974), suggesting that discrimination occurs. In this chapter we will explore the consequences of adding a pollen incompatibility system in a population of gynodioecious plants. In particular, we will investigate if and under what conditions a single type population can be invaded by a new sub-species, and if different sub-species competing for the same space can coexist in the population.

Our approach combines both the mechanisms of inclusive fitness theory and tag-based cooperation to investigate the consequences of limited dispersal on cooperation and diversity.

2.2 The model

The model we use for our simulations is based on Wilson’s model (2000) for the maintenance of gynodioecy. It is a probabilistic cellular automaton, representing a 2-dimension lattice (Figure 2.2). This is a model of perennial flowering plants, constituted of two types of individuals: hermaphrodites who produce both ovules

and pollen, and pseudogamous apomict females, who produce only ovules, but still need pollination to trigger embryogenesis. We also assume that hermaphrodite seeds are all hermaphrodites, and female seeds are all females.

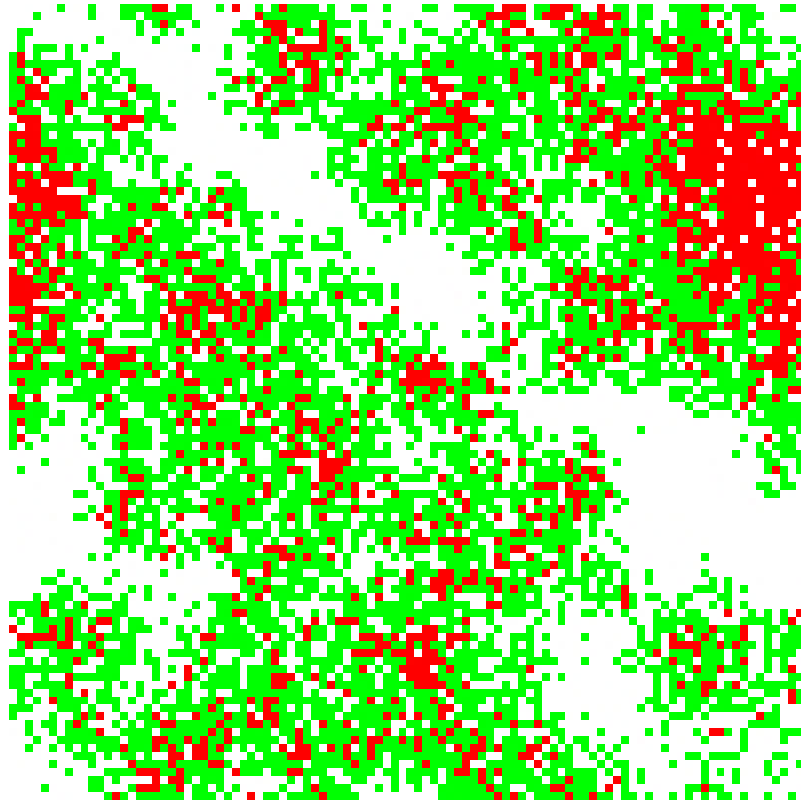


Figure 2.2: The probabilistic cellular automaton. Each pixel represents a cell. Red: hermaphrodites, green: females, White: empty cells.

We assume that females are hermaphrodites that have lost the male function, which is coherent with observations of rudimentary males organs (Couvet et al., 1990). We also assume that females have an enhanced capacity of producing ovules, as they do not pay the cost of producing male gametes (Lloyd, 1976, van Damme, 1984). In all the simulations, we set the number of seeds produced at 4/hermaphrodite individual and 5/female individual. Although changing these values modifies quantitatively the results, qualitatively conclusions are similar.

We assume logistic growth, limited by the amount of space to put down roots. When one individual germinates, it occupies a site (a cell), and stays there until its death. Thus cells are either empty, occupied by a hermaphrodite, or occupied by a female.

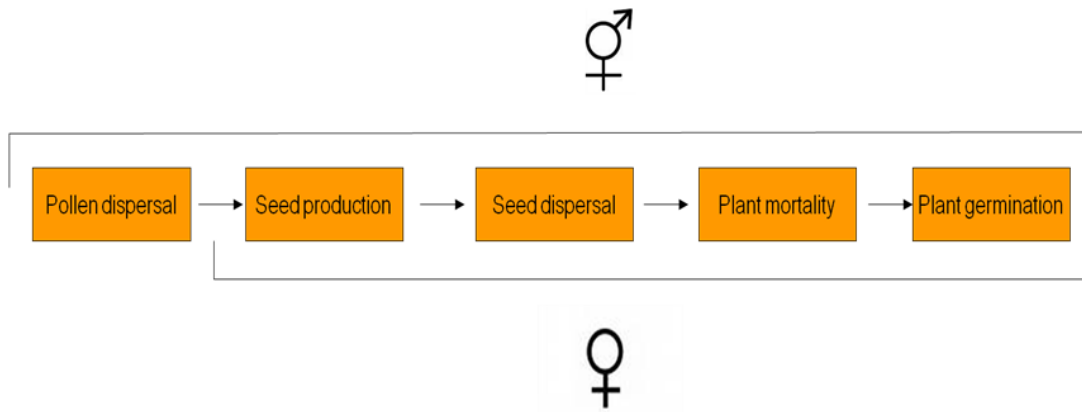


Figure 2.3: Schematics of the different biological processes executed by the model. Hermaphrodites and females differ only by the process of pollen dispersal: females do not spread pollen.

The biological processes included in the model are: dispersal of pollen, production and dispersal of seeds, mortality of plants, and germination of seeds. Mortality is assumed to be the same for both hermaphrodites and females. The model operates in discrete time and uses synchronous updating rules, summarised in Figure 2.3. The only difference in the processes of hermaphrodite and female reproduction is that there is no pollen dispersal for the females.

Pollen and seed are assumed to be dispersed by wind. The dispersal is limited in space, and each individual can spread pollen and seed in a given radius around it (Figure 2.4). For simplicity, we assume that pollen and seed have the same radius of dispersal. The number of cells a plant is interacting with depends on this radius. For example, if the radius is 1 (arbitrary distance), each individual interacts with 4 others. If the radius is 2, it will have interactions with 12, etc. A seed can germinate only in an empty site, and is randomly picked from the total seed pool arrived on the site.

First, pollen is produced and dispersed by the hermaphrodites on the lattice. Then, any individual that has received pollen produces seeds. Seeds are then dispersed over the dispersal neighbourhood. If the cell is already occupied by a plant, the seeds die. If there is no adult plant, one of the pool of seeds germinates to form

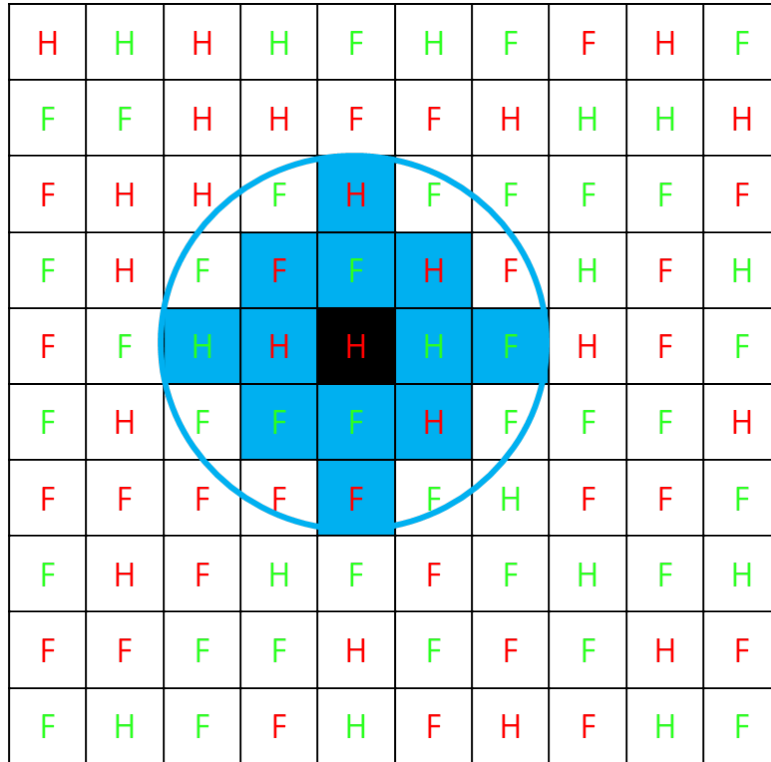


Figure 2.4: Representation of the pollen dispersal. The hermaphrodite in the black cell spreads pollen/seed in its vicinity. Each cell within the range of pollen dispersal (the entire cell must be included in the range of dispersal) are represented by blue cells and has an equivalent chance of being sent pollen to. We assume that pollen can be spread in every direction. For example, here the radius of dispersion is 2, the number of cells interacting with the black cell is 12.

an individual. Note that we included a process of plant mortality between seeds dispersal and plant germination: if a plant dies it is immediately removed and the cell becomes empty and available for germination.

2.3 Results

We will start by investigating the effect of the dispersal distance of pollen and seed on the level of cooperation in a single-type population (i.e. only one type of hermaphrodite and female). Then in the second part, we assume that a pollen incompatibility system can arise in the population through mutation: hermaphrodites with a new type of pollen unusable by the resident can appear and compete for space. We will study the effect of the dispersal distance on the level of pollen-type diversity.

2.3.1 The effect of viscosity on cooperation

Figure 2.5 shows the phase diagrams for different values of the dispersal distance and for different initial conditions. For each value of d , the system converges towards a stable equilibrium of hermaphrodite and female densities, determined by the carrying capacity (the entire grid) and dependent of d . The proportion of hermaphrodites can be interpreted as the level of cooperation in the population.

Figure 2.6 shows a negative relationship between the level of cooperation and the dispersal distance: cooperation is favoured if hermaphrodites disperse pollen and seed close to themselves (i.e. when the population is spatially structured). Moreover, when d is very high (i.e. when individuals disperse far from their location), the population collapses (Figure 2.5f). This is because the females get an overwhelming advantage over the hermaphrodites and wipe them out. However, with the resulting scarcity of pollen, they also go to extinction and the system collapses to the zero equilibrium.

The dispersal distance sets the number of neighbours an individual can interact with, for dispersal of pollen and seeds. Hence, it reflects the number of individuals who can receive the pollen of a hermaphrodite. In order to spread, females must be within the range of dispersal of hermaphrodites' pollen. When a female receives pollen, she produces more seeds than a hermaphrodite, which allow them to colonise more efficiently the field. Females are hence advantaged when the dispersal distance is important.

We have seen that hermaphrodites cannot persist if there is no spatial structure in the population. On the global scale, hermaphrodites have a reproductive disadvantage against the females, eventually leading to their extinction. If we focus on a hermaphrodite, it can set seeds if it is pollinated by another hermaphrodite.

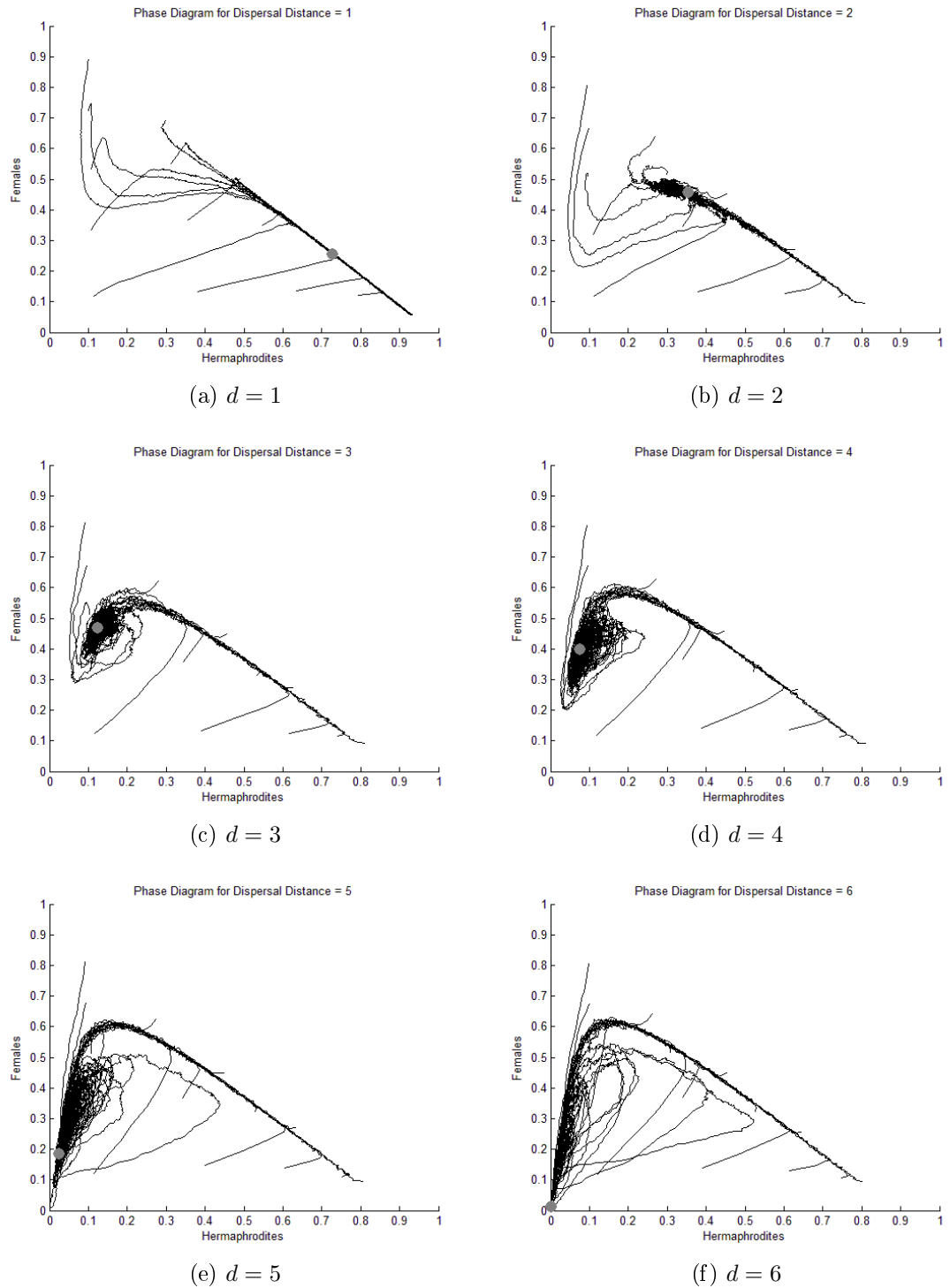


Figure 2.5: Phase diagrams of females versus hermaphrodites density, for different values of the dispersal distance d . The final density is shown by the grey dot. Parameters : grid size : 100*100, deathrate=0.01.

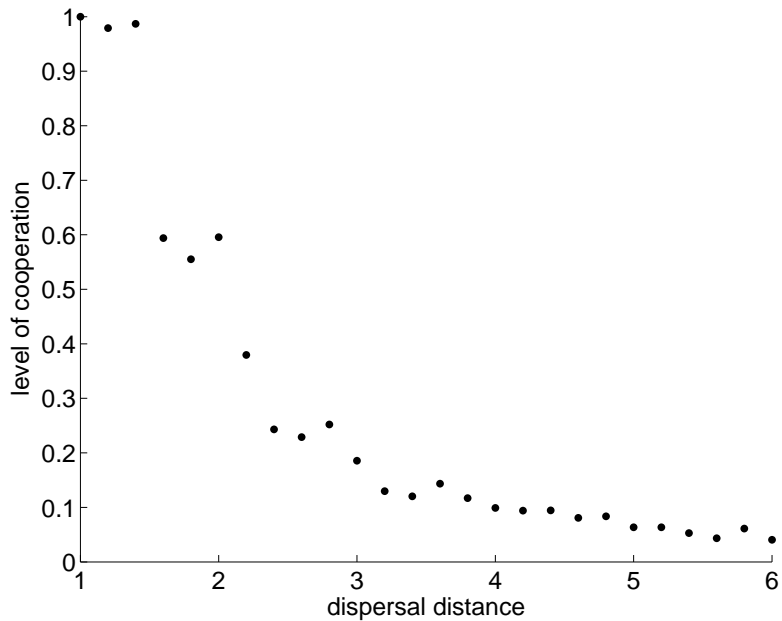


Figure 2.6: The level of cooperation versus dispersal distance. The level of cooperation is shown by the proportions of hermaphrodites in the population. It is high for small values of the dispersal distance and decreases very rapidly for higher dispersals. Parameters: grid size=100*100, deathrate=0.01.

Thus hermaphrodites would do better if they remain close geographically. This is analogous to a kin selection argument: altruism can evolve if the benefits of altruism are disproportionately received by related individuals, which are likely to share the gene causing the altruistic behaviour. In viscous populations, related individuals are close geographically, because individual dispersal is limited.

We now add a kin discrimination mechanism: a pollen incompatibility system. Hermaphrodites are now divided into subgroups (denoted $H_1, H_2, \dots, H_i,$), producing a specific type of pollen i . Females F_i can use the pollen produced by H_i , but not the pollen produced by the other subgroups. We will now investigate the effect of dispersal distance on the diversity of pollen types in the population.

2.3.2 The effect of viscosity on pollen type diversity

At equilibrium, both populations persist globally. But the coexistence is spatially and temporally dynamic: hermaphrodites colonise by spreading into the empty spaces in clusters, while females “chase” the hermaphrodites. In this system, kin

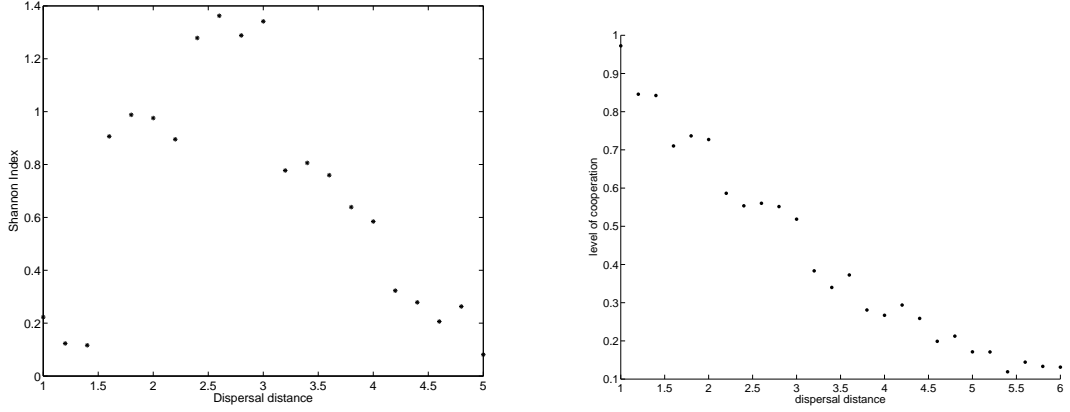
selection is blind: hermaphrodites have more interactions with each other only because they do not disperse far. But another mechanism could be of importance for the evolution of cooperation: kin recognition (Hamilton, 1964). If a small number of hermaphrodite mutants arises in the population, having a different pollen recognition system that is incompatible with the dominant females, it will enter in competition with the former population. Assuming all else is equal, on the one hand they are in direct competition with the resident population of hermaphrodites, with no intrinsic advantages. But on the other hand, they are free of female competitors (at least in the beginning). This means that they could outcompete locally the resident subpopulations, before being invaded themselves by a compatible female subgroup (appearing through mutation).

The diversity in such a system will depend on a balance between generation of new pollen types through mutations, and loss of such types through competition between them. We first study the effect of the dispersal distance d on the final level of diversity in the population, represented by the Shannon Index. Here we calculate the Shannon Index with the formula

$$H' = \sum_{i=1}^R -p_i \log(p_i)$$

where p_i is the frequency of altruists of type i , and R is the total number of types. Surprisingly, we observe a maximum level of diversity for intermediate values of the dispersal distance, and low levels of diversity for both low and high values of d (Figure 2.7a). This is in contrast with our results on cooperation, and shows that the criteria for cooperation and diversity to evolve are different. In order to explain why diversity is not favoured at low and high values of the dispersal distance, we study the dynamics of a 2-type population (Figure 2.8).

If the dispersal distance is short, the population goes rapidly to a high cooperation



(a) Shannon Index versus dispersal distance (b) Corresponding levels of cooperation

Figure 2.7: Diversity and corresponding levels of cooperation versus dispersal distance. (a) The diversity is low for high and low d , whereas it is maximum for $d = 3$. (b) Cooperation levels decrease but are higher than in the non-diverse model. Parameters : grid size=100*100, deathrate=0.05, mutation rate=0.001

level (high density of hermaphrodites). Here we observe that mutant hermaphrodites cannot invade the population easily: they have no advantage over the resident hermaphrodites, and though they have an advantage over the resident females (because they are incompatible), these are at a low density. It is thus difficult for a hermaphrodite mutant to invade a very viscous population (Figure 2.8a).

If the dispersal distance is long, the system goes rapidly to an equilibrium, with a high frequency of females. Here on the contrary, we observe that mutant hermaphrodites can easily invade the population: they have an important advantage over the resident females and can rapidly outcompete them. The invasion is so intense that the resident subgroups are completely ousted of the population, and the system goes towards an equilibrium with the new pollen type. However, if an incompatible hermaphrodite mutant reappears, it can re-spread and create the same process of invasion. Thus, the system is almost always monomorphic, which explains the low level of diversity at high dispersal distance values (Figure 2.8b).

Finally, if the dispersal distance is at an intermediate value, the system favours diversity: when a hermaphrodite mutant appears, it can spread and outcompete locally the resident subgroups, but this time the invasion is limited and the res-

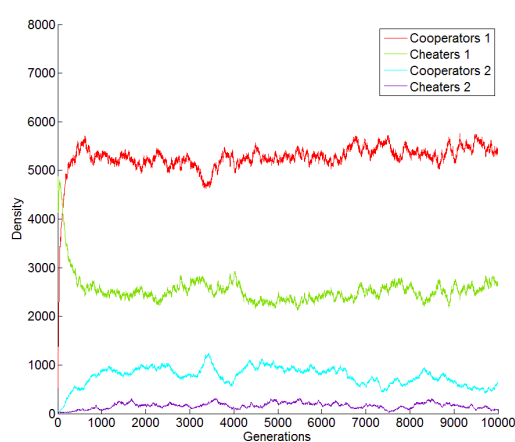
idents are not ousted. This create a cycling dynamics, with alternate periods of one hermaphrodite invasion, followed by its compatible female invasion, followed by the new hermaphrodite invasion, followed by its compatible female invasion (Figure 2.8c).

In this model, the level of cooperation still decreases with the dispersal distance, but if we compare Figures 2.6 and 2.7b we observe that cooperation decreases less quickly with a diverse population (for instance for $d = 3$, cooperation is at 0.2 in the non-diverse model whereas it is at 0.5 in the diverse model. Thus we can conclude here that diversity is aiding cooperation.

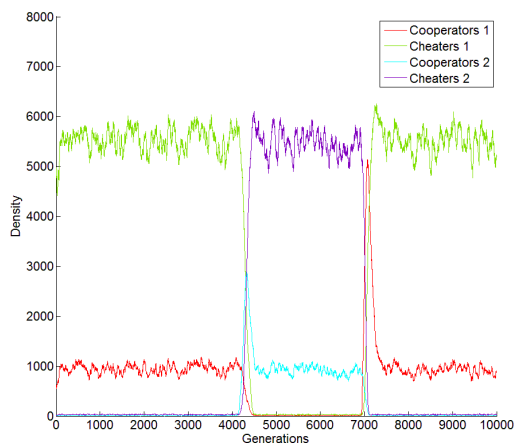
2.4 Discussion

We used a simulation model to investigate how hermaphrodites and females can coexist and how incompatibility can arise and be maintained in populations of gynodioecious plants. We confirmed the results that coexistence is favoured when the population is spatially structured: dispersal has a strong influence on the densities of hermaphrodites and females. High dispersal rates are advantageous for females, which profit more from hermaphrodite's pollen. Hermaphrodites have an advantage with short dispersal distances, which allow them to remain close to each other, thus preventing females from exploiting them. Furthermore, we found that pollen type diversity is maximised for intermediate values of dispersal distance: low values of dispersal distance do not allow diversity to evolve, because new hermaphrodites do not have enough available space, and high dispersal distances give an overwhelming advantage to the mutant hermaphrodites, which can invade and erase all diversity.

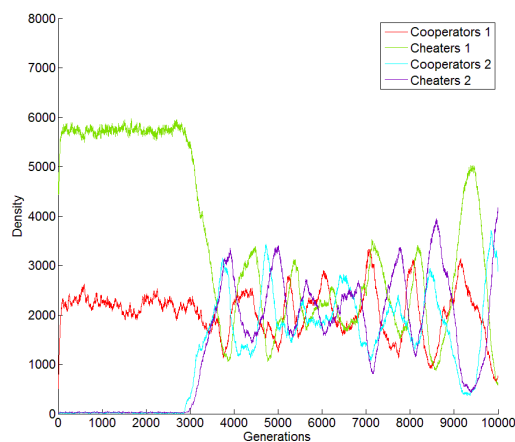
Our results on coexistence confirm those of Wilson (2000) and Stewart-Cox et al. (2005), who studied the maintenance of gynodioecy in regular and random lattices. Moreover, here we also highlighted that this biological system is an example of



(a) $d = 2$



(b) $d = 5$



(c) $d = 3$

Figure 2.8: Dynamics of a unique pollen-type population invaded by a new incompatible pollen-type. Hermaphrodites 1 and 2 are represented by red and blue lines respectively, and the corresponding females 1 and 2 in green and purple respectively. (a) For a short dispersal distance, ($d = 2$) the new pollen type remains at a low density. (b) For a long dispersal distance ($d = 5$) the new pollen type invades very easily and oust the resident. However, the former resident can re-invade, and the cycle can go on. The diversity is thus low if we take a screenshot of the population at any given time. (c) For an intermediate value of the dispersal distance ($d = 3$) Both types coexist in the population, in a cycling manner: a hermaphrodite is invaded by its corresponding female, which creates a pollen shortage. The other hermaphrodite can thus invade in the population, before being itself invaded by its corresponding female. Parameters : grid size : 100×100 , deathrate=0.01, mutation rate=0.001.

cooperation via the production of a public good, the pollen. In this light, we can consider hermaphrodites as cooperators because they produce pollen, and females as cheaters. Cooperation via a public good has been shown to occur in animals (Packer and Rutan, 1988) and microbes (Griffin et al., 2004), and studies on limited dispersal concur with ours.

The existence of a pollen incompatibility system, however, makes that all individuals cannot use all kinds of pollen. By continuing the analogy with public good cooperation, the production of different kinds of pollens, only usable by specific subgroups, is an example of a tagged public good system (Lee et al., 2012). We can thus relate our results on diversity with those on chromodynamics of cooperation (Riolo et al., 2001, Axelrod et al., 2004, Jansen and van Baalen, 2006, Rousset and Roze, 2007, Traulsen and Nowak, 2007).

In particular, we found that pollen type diversity is maximum for intermediate values of the dispersal distance (i.e medium spatial structure). This result is different from Jansen and van Baalen's (2006), who found that diversity increases with weak spatial structure. However, in their model, there is no empty space. This means that even if an individual is capable to disperse far, it has no real advantage because the seeds cannot easily germ. Thus, the invasion periods we observe in our model for high dispersal, where a new type erases most of the diversity, does not occur in their model, and the diversity hence increases with weak spatial structure.

In the absence of a pollen incompatibility, the dynamics of our system is very stable: hermaphrodites and females go to a stable equilibrium of density. However, by introducing mutations, we obtain unstable dynamics, with local spreading and disappearance of phenotypes. Frank (2000) and Couvet et al. (1985) observed similar dynamics, driven by the mutations, generating constant novelty for label/strategy combination.

The mechanism governing diversity is very similar to chromodynamics: in a system of cooperators and cheaters, with incompatibility between subgroups, we observe a

kind of Red Queen process, where no strategy has a complete advantages over all the others. A hermaphrodite H_α can easily be invaded by its compatible female F_α . This female is then easily outcompeted by an incompatible hermaphrodite H_β , itself vulnerable to F_β , and the cycle goes on.

Thus, we expect that a recognition system will appear in a population when the level of cooperation created by dispersal distance is low: in a population with high hermaphrodite density and few females, the global level of cooperation is high, so that a new mutant has a low probability of invasion, whereas in a population with low hermaphrodites and high female densities, the general level of altruism is low, facilitating the invasion of a new mutant.

For simplicity, we linked the mutation rate of both strategy and label. However, it is likely that these are different. Moreover, in nature, male sterility is often caused by cytoplasmic male sterility, which is determined by the mitochondrial genome causing cytoplasmic dysfunction, whereas male-function restorer genes tend to be determined by nuclear genes repairing the cytoplasm (Charlesworth, 2002, Eckardt, 2006). It would then be interesting in future studies to disassociate the different mutation rates in order to determine the role of each.

Our results can also provide a scope for sympatric speciation. We assumed here a gamete recognition system, providing incompatibility between different groups of plants. We argue that barriers between groups can thus arise rapidly, hence leading to sympatric speciation. Van Doorn et al. (2001) obtained similar results: in their theoretical model, they found that sexual selection and sympatric speciation was responsible for a rapid divergence of gamete recognition proteins.

Diversity in gynodioecious plants has been reported in previous studies. In particular, Laporte et al. (2000) found a high polymorphism of nuclear and mitochondrial markers in populations of *Beta vulgaris ssp. Maritima*. In their study, they observe highly diverse and structured populations, which they explained by the difference in cytoplasmic male sterility and male fertility restorer genes. This provides a strong

argument for the importance of these mechanisms on the emergence of polymorphism in our model.

Examples of pseudogamy also exist in animals (Schlupp, 2005), but to our knowledge, diversity (as incompatible subgroups) has not been reported. Whether this is because of a high spatial structure, or simply because studies are currently not oriented towards the potential for diversity, remains to be studied.

Chapter 3

The evolution of siderophore production: a model for cooperation through a public good

3.1 Introduction

Explaining the evolution of altruism and cooperation is one of the main challenges in evolutionary biology. Populations of altruists are vulnerable to cheaters, who can benefit from the act of cooperation without paying the cost. For cooperation to evolve, populations need to have structure, so that the benefits of cooperation fall towards individuals carrying copies of the altruistic gene, a mechanism called kin selection (Hamilton, 1964). In viscous populations, in which individuals interact mainly with other spatially near individuals, clusters are formed of more or less closely related individuals. The presence of this structure facilitates the evolution of cooperation.

Cooperation can take several forms. It can be direct, but it can also be indirect, through the production and sharing of goods. If the good is accessible by all members of a population, it is referred to in the biological literature as a public good (Rankin

et al., 2007). A common, for example, is a land that can be jointly exploited, for instance, by grazing cattle, by all with rights to do so. Exploitation of a common benefits the individual but depletes its quality. Because the costs of depletion are carried by the community this easily leads to overexploitation and the tragedy of the commons (Hardin, 1968).

Here we will explore how cooperation can evolve if individuals interact through the use of a public good. The production of iron-scavenging siderophores by bacteria is a well-documented example of cooperation via the production of a public good (Strassmann et al., 2011). Bacteria need iron for their growth but soluble iron is scarcely found in living organisms. In order to take up iron many bacteria excrete siderophores, which are molecules that bind to iron. Once bound, they can then be taken up by any cell with appropriate siderophore receptors (Ratledge and Dover, 2000, Wandersman and Delepelaire, 2004). This has been particularly well studied for the bacterium *Pseudomonas aeruginosa*, which produces the siderophore pyoverdine. Experimental work has shown that when iron is limiting, pyoverdine producing strains reach higher densities than strains which do not produce pyoverdine, yet when put in competition the pyoverdine producers are outcompeted, which shows that there is a cost associated with siderophore production (Griffin et al., 2004). Thus, because the production of these siderophores is costly, strains that produce less or altogether no siderophores should be advantaged when competing against high siderophore producers, and thus cooperation should not evolve if selection is dominated by local competition. However, although non siderophore producers evolve rapidly under laboratory conditions, they are rarely found in natural populations (West and Buckling, 2003, Kummerli et al., 2009, 2010).

The evolution of public good use depends both on local and global competition. Bacterial populations which produce more siderophores reach higher densities and should therefore contribute more to the next generation. Studies have found a strong effect of the scale of competition on the level of cooperation in siderophore-producing bacteria (Griffin et al., 2004, Kummerli et al., 2009, 2010). In these experiments

colonies are seeded from very few individuals, so local populations contain a high proportions of related individuals. If competition is strictly local, there is little advantage to a higher productivity because the competition will mainly be with relatives. On the other hand, if competition is global, there is a strong advantage to have a higher productivity because competition will mainly occur with non-relatives.

Previous theoretical studies of the evolution of siderophore production tended to focus on local competition and did not link the global competition to the local production in detail (West and Buckling, 2003, Brown et al., 2009). Studying the evolution of siderophore production only at a within host level, although simple, has a number of drawbacks. Firstly, it can lead to wrong predictions. For instance, in Brown's model (2009), cheaters can always invade in well-mixed populations of wild-types, because they always benefit locally from the use of siderophores without paying the cost of production. In this model, cooperation can thus not be maintained. However, in subdivided populations, or in environments such as studied in Griffin et al. (2004), the level of cooperation will depend on the interplay between local competition (the dynamics within a host or patch) and global competition (dispersal and colonisation of new hosts or patches). This creates a trade-off: cheaters are better local competitors, so they outcompete cooperators at the host level, but have a decreased productivity, so that their dispersal potential is inferior to that of the cooperators.

A further issue is that these theoretical predictions are often interpreted in terms of individual fitness, based on the idea that the interaction between two individuals conveys a certain amount of fitness to the recipient, at a cost to the donor. In terms of interactions based on public goods this leads to an interpretation of fitness costs only in terms of the physiological cost of producing the public good, and fitness benefits as the reproductive success that be gained by consuming the public good. How these individual cost and benefit translate into the costs and benefits at the metapopulation level is not immediately obvious if the local and global dynamics are not linked. Furthermore, the degree of assortment in a metapopulation, as measured by

the relatedness, also depends on the local dynamics. This feedback is important for the end point of the evolutionary process. Both the value of the unbeatable strategy (ESS), as well as its evolutionary stability, depend on the parameters affecting the local dynamics.

In previous work we have shown that social interactions can lead to the emergence of a diversity of competing types of cooperators and cheaters (Lee et al., 2012, this work is presented in Chapter 4). For this model we assumed that all cooperators (and all cheaters) have the same level of siderophore production, and hence, a strain can be characterised by the type of siderophore it produces, and its strategy (cooperate or cheat). However, there exists a high diversity of siderophore production rate between different strains (Jiricny et al., 2010). Here, we focus on the evolution of the production rate of a single siderophore type. We assume that all strains produce the same type of siderophore, but each strain is now characterised by its rate of production. Similarly to Lee et al (2012), we determine if social interactions can lead to the emergence of a diversity of different coexisting strains. We formulate and analyse a model for the evolution of siderophore production that takes into account both the interactions at an individual and at a metapopulation level. The model is based on similar assumptions as in Brown et al. (2009) for the local dynamics.

3.2 The model: description and results

The model is inspired by the Haystack model (Maynard Smith, 1964) and its mathematical analysis follows Jansen and Mulder (1999) and Jansen (2011). We consider a collection of subpopulations inhabiting identical environments, which are referred to as patches. At regular intervals (corresponding to a season or a fixed time interaction in a tube or chemostat) there is a round of dispersal and recolonisation (Figure 3.1). Thus we have two levels of dynamics: the local dynamics (what happens inside a patch) and the global dynamics (what happens at the metapopulation level).

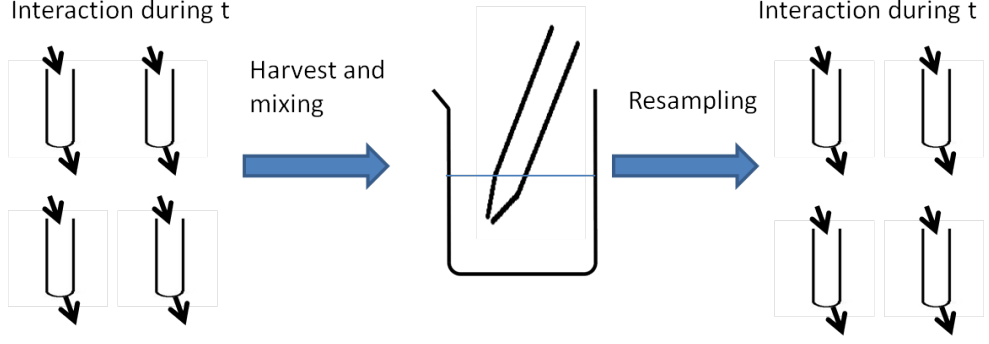


Figure 3.1: Illustration of the model. Patches are represented by individual tubes set-up as chemostats. First, the bacteria compete in each tube for a fixed time τ (local competition). Then, the solutions are harvested and put into a beaker. The resulting solution is mixed and a fixed volume is taken and put into new tubes (global competition). The process can then be repeated after a time τ .

3.2.1 Within-patch dynamics

Within a patch several strains of bacteria may coexist. A given strain i has a density of Q_i cells, which produce siderophores with per capita rate b_i . The production of siderophores is costly and reduces the per capita growth rate of the strain by ab_i . We assume that iron concentration, denoted F , is low and constant. Siderophores can be either free in the environment, or bound with iron. The dynamics of the free siderophores is described by the differential equation

$$\frac{dS}{dt} = \sum_j b_j Q_j - uFS - \lambda_S S \quad (3.1)$$

where $\sum_j b_j Q_j$ is the total amount of siderophores produced, uFS the rate with which siderophores bind with iron, and $\lambda_S S$ the washing out of siderophores.

The dynamics of bound siderophores is:

$$\frac{dS_F}{dt} = uFS - \theta S_F \sum_j Q_j - \lambda_S S_F \quad (3.2)$$

where $\theta S_F \sum_j Q_j$ is the assimilation of bound siderophores by bacteria.

F	Concentration of iron (constant)	10^{-3}
S	Density of free siderophores	
S_F	Density of bound siderophores	
Q_i	Density of strain i	
Q	Density of the population	
K	Carrying Capacity	1
b_i	Rate of siderophore production of the strain i	0 to 20
u	Affinity of siderophore to iron	1
λ	Loss of bacteria (mortality and dilution)	0.01
λ_S	Dilution of siderophores	0.01
θ	rate of assimilation of siderophores	1
ϵ	Efficiency of assimilation of siderophores	1
α	Cost of siderophore production	0.1
r	Growth rate	2
μ	Probability of transfer	0.1
τ	Duration of local interaction	10

Table 3.1: Table of all the variables used , with their typical used value.

Next we describe the dynamics of the different populations of bacteria. The dynamics of a strain Q_i is given by

$$\frac{dQ_i}{dt} = Q_i(r(K - Q) - \alpha b_i + \epsilon \theta S_F - \lambda) \quad (3.3)$$

where $r(K - Q)$ is the basic density dependent reproduction and where Q is the total population of bacteria $Q = \sum_i Q_i$, αb_i the cost of siderophore production, $\epsilon \theta S_F$ the successful assimilation of bound siderophores, and λ the washing out of microbes (as would happen if the subpopulations are grown in a chemostat). Together with the initial conditions for these equations this completely describes the within-patch dynamics.

In order to describe the global dynamics, we need a solution for the within-patch dynamics. An explicit solution does not exist but one can derive a sufficiently close approximation, based on Jansen and Mulder (1999). To do so we will describe the dynamics in terms of the total local bacterial population Q , and the fraction f_i of

the population of strain i , where $f_i = Q_i/Q$. We can now rewrite the dynamics as:

$$\begin{cases} \frac{dS}{dt} &= Q\bar{b} - uFS - \lambda_S S \\ \frac{dS_F}{dt} &= uFS - \theta S_F Q - \lambda_S S_F \\ \frac{dQ}{dt} &= Q(r(K - Q) - \alpha\bar{b} + \epsilon\theta S_F - \lambda) \\ \frac{df_i}{dt} &= \alpha f_i (\bar{b} - b_i) \end{cases} \quad (3.4)$$

where $\bar{b} = \sum_i b_i f_i$.

The equations for f_i can now be solved (See Appendix 3.4.1 for the derivation)

$$f_i(t) = \frac{f_{i0} e^{-\alpha t b_i}}{\sum_j f_{j0} e^{-\alpha t b_j}}. \quad (3.5)$$

With this we find that the average siderophore production changes as

$$\bar{b}(t) = \frac{\sum_i b_i f_{i0} e^{-\alpha t b_i}}{\sum_j f_{j0} e^{-\alpha t b_j}}. \quad (3.6)$$

To proceed we assume that the cost of competition is relatively small compared to the rate of basic bacterial growth ($rK - \lambda \gg \alpha$). Then, the growth of the total bacterial population and the siderophore pool is much faster than the competition between strains of bacteria and we can apply a time scale separation. To do so we assume that the total bacterial and siderophore densities go to quasi steady states (denoted with tildes) given by

$$\begin{cases} \tilde{S}(\bar{b}) &= \frac{\bar{b}\tilde{Q}(\bar{b})}{uF + \lambda_S} \\ \tilde{S}_F(\bar{b}) &= \frac{C\bar{b}\tilde{Q}(\bar{b})}{\theta\tilde{Q}(\bar{b}) + \lambda_S} \end{cases} \quad (3.7)$$

and the quasi steady state of the bacteria \tilde{Q} can be solved from

$$r\tilde{Q}(\bar{b}) = rK - \alpha\bar{b} + \epsilon \frac{\theta C \bar{b} \tilde{Q}(\bar{b})}{\theta \tilde{Q}(\bar{b}) + \lambda_S} - \lambda \quad (3.8)$$

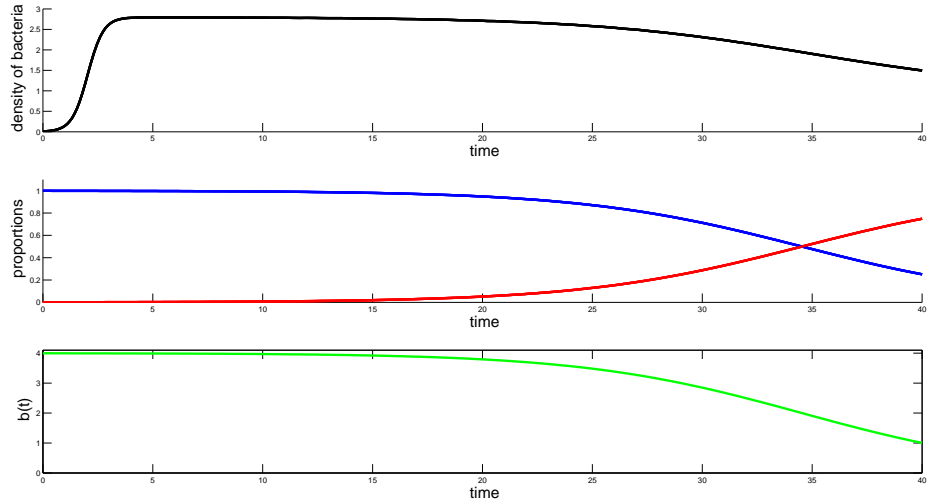


Figure 3.2: The dynamics of a resident being invaded by a competitor. The top graph shows the density of bacteria in the patch. The middle graph shows the frequency of the high siderophore producing resident (in blue) and the low siderophore producing competitor (in red). The lower graph shows the mean production of siderophores. As the competitor increases in frequency, the density of bacteria decreases because there are less siderophores produced. Parameters: $b_{res} = 4$, $b_{comp} = 3$.

where $C = \frac{uF}{uF + \lambda_s}$. Note how all these densities change slowly over time with \bar{b} , which depends on time through equation (3.6) (Figure 3.2). If there is only one strain present, then $\tilde{Q}(\bar{b})$ is simply the equilibrium density.

3.2.2 Global population dynamics and fitness

In the previous section, we considered the dynamics of a collection of strains with different siderophore production rates within a patch. In this section we will focus on the evolution of this production rate at the metapopulation level, and ask whether there is an unbeatable siderophore production rate, such that populations which produce siderophores at this rate cannot be invaded by strains which produce either more or less siderophores. To find this unbeatable rate we will establish which strains are evolutionarily stable by applying the approach used in adaptive dynamics, based

on whether a strain with a given siderophore production rate can be invaded by a rare mutant with a different siderophore production rate.

As outlined above, we consider a discrete-time metapopulation of patches, where at every time step the patches are inoculated with a variable number of bacteria. We assume that the distribution of these inocula follows a Poisson distribution $\mathcal{P}(i, N)$ with mean N , which indicates a random number in a fixed volume as it would if the inocula are pipetted from a larger volume.

We will start with the description of a global population consisting only of a resident strain with siderophore production rate b . In all inoculated patches, the production rate is therefore $\bar{b} = b$ (we assume that the length of the interaction, τ is sufficiently long for the local populations to converge to their equilibrium densities). These patches will thus produce $\tilde{Q}(b)$ bacteria. There is obviously no output if a patch receives no inoculum, which happens with a probability $\mathcal{P}(0, N_T) = e^{-N_T}$. The average production of bacteria per patch is $Q(1 - \mathcal{P}(0, N_T))$. If we further assume that a fraction μ of these bacteria is transferred to the next generation, the global dynamics of the resident is given by

$$N_{T+1} = \mu \tilde{Q}(b) (1 - \mathcal{P}(0, N_T)) \quad (3.9)$$

The dynamics will proceed to an equilibrium, which is the solution of

$$N_{eq} = \mu \tilde{Q}(b) (1 - \mathcal{P}(0, N_{eq})) \quad (3.10)$$

Next, we consider a rare mutant appearing in the population, which has a siderophore production rate of b^* . The output of this mutant at the next generation depends on how many of its propagules arrive in every patch. The distribution of mutant inocula, is also random and therefore given by the Poisson distribution $\mathcal{P}(j, N_T^*)$, where N_T^* is the average number of mutants in the inocula in generation T . As illustrated in Figure 3.2, the introduction of a new competitor in a patch will set

off a process of competition and partial replacement, which will affect the overall bacterial density. The global dynamics are given by

$$\begin{cases} N_{T+1} &= \mu \sum_{i=1}^{\infty} \sum_{j=0}^{\infty} \mathcal{P}(i, N_T) \mathcal{P}(j, N_T^*) \tilde{Q}(\bar{b}) f_{res}(\tau) \\ N_{T+1}^* &= \mu \sum_{i=0}^{\infty} \sum_{j=1}^{\infty} \mathcal{P}(i, N_T) \mathcal{P}(j, N_T^*) \tilde{Q}(\bar{b}(\tau)) f_{mut}(\tau) \end{cases} \quad (3.11)$$

where $f_{mut}(\tau)$ and $f_{res}(\tau)$ denote, respectively, the fraction of mutants and residents at the end of the interaction, when $t = \tau$. The average siderophore production at the end of the interaction is given by

$$\bar{b}(\tau) = f_{mut}(\tau) b^* + f_{res}(\tau) b, \quad (3.12)$$

and the total amount of bacteria in the patch is $\tilde{Q}(\bar{b})$. The fractions relate to the number of inocula, through (3.5), with the initial fractions given by $f_{mut,0} = \frac{i}{i+j}$ and $f_{res,0} = \frac{j}{i+j}$.

Next, we make the standard assumption that the mutant is globally very rare, so that the resident's dynamics are not affected by the mutant (Metz et al., 1992). The resident's dynamics will thus settle at the equilibrium value N_{eq} . As the mutant is rare it is very unlikely to have a patch inoculated with more than one mutant. This follows from the Poisson distribution for which for small N : $\mathcal{P}(1, N) \approx N$ and $\mathcal{P}(i, N) \approx 0$ for $i > 1$. Using this the dynamics simplify to

$$N_{T+1}^* = \mu N_T^* \sum_{i=0}^{\infty} \mathcal{P}(i, N_{eq}) \tilde{Q}(\bar{b}(\tau)) f_{mut}(\tau) \quad (3.13)$$

The fitness of a strain with a siderophore production rate b^* in a population which produces siderophores at rate b is thus

$$\begin{aligned} W(b^*, b) &= \mu \sum_{i=0}^{\infty} \mathcal{P}(i, N_{eq}) \tilde{Q}(\bar{b}(\tau)) f_{mut}(\tau) \\ &= \frac{\sum_{i=0}^{\infty} \mathcal{P}(i+1, N_{eq}) \tilde{Q}(\bar{b}(\tau)) f_{mut}(\tau) (i+1)}{(1 - \mathcal{P}(0, N_{eq})) \tilde{Q}(b)} \end{aligned} \quad (3.14)$$

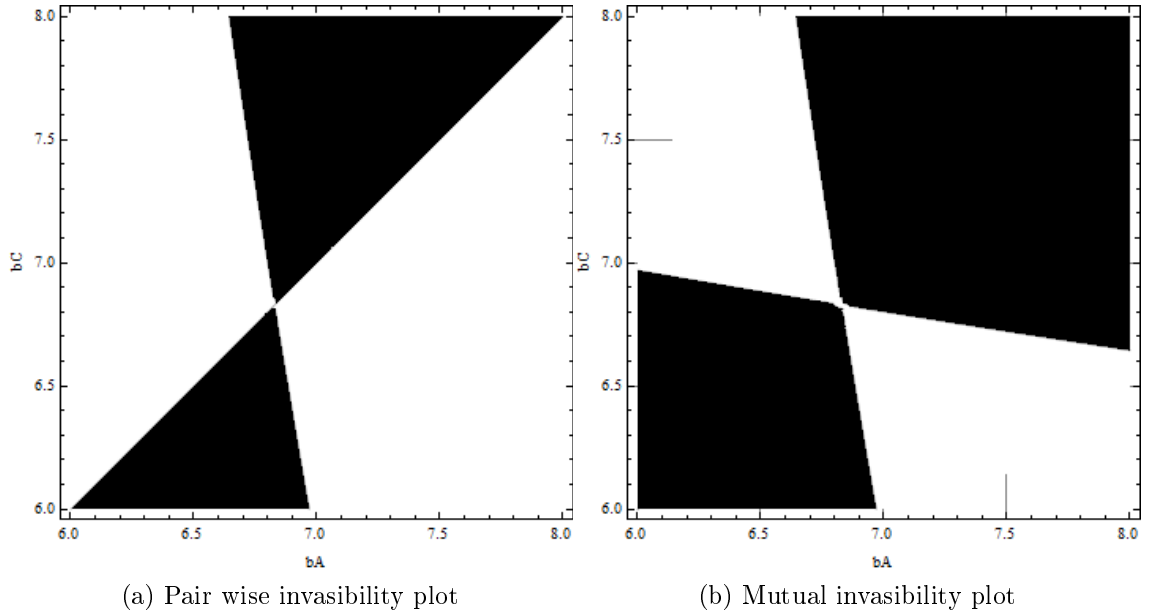


Figure 3.3: Diagrams of invasion. (a) Pairwise invasibility plot. Areas where the fitness of the mutant is positive are white and areas where the fitness of the mutant is negative are black. (b) Mutual invasibility plot. Black areas designate combination of trait values that are mutually invisable. Parameters: $\alpha = 0.01$, $F = 10^{-3}$, $\lambda = 0.01$.

where the last step uses the equilibrium condition (3.10) and the relation

$$\mathcal{P}(i, N_{eq}) = \frac{\mathcal{P}(i+1, N_{eq})(i+1)}{N_{eq}}$$

With this expression for mutant fitness, we can construct a pairwise invasibility plot (Figure 3.3a). This shows the region where a mutant with trait b^* can invade a resident population, with trait b . We can also construct the mutual invasibility plot, showing the areas where both resident and mutant have a positive fitness and where strain with these traits can coexist (Figure 3.3b).

3.2.3 Marginal fitness and Hamilton's rule

The pairwise invasibility plot shows the existence of an evolutionarily stable point. To find and interpret the trait value for this point we will derive the marginal fitness, which is the change of fitness with a small change of siderophore production rate $\frac{\partial W}{\partial b^*}$. To do so we first derive from (3.5) that if the mutant differs marginally from the resident

$$\left. \frac{df_{mut}(\tau)}{db^*} \right|_{b^*=b} \approx -\alpha\tau f_{mut,0}(1 - f_{mut,0}), \quad (3.15)$$

and from (3.6) that

$$\left. \frac{d\bar{b}(\tau)}{db^*} \right|_{b^*=b} \approx f_{mut,0}. \quad (3.16)$$

Because we only need to consider patches with one mutant inoculum we have $f_{mut,0} = \frac{1}{1+i}$. Putting this all together gives for the marginal fitness

$$\begin{aligned} \left. \frac{\partial W(b^*, b)}{\partial b^*} \right|_{b^*=b} &= \frac{\sum_{i=0}^{\infty} \mathcal{P}(i+1, N_{eq}) \left[\tilde{Q}'(b) f_{mut,0} - \alpha\tau \tilde{Q}(b)(1 - f_{mut,0}) \right]}{(1 - \mathcal{P}(0, N_{eq})) \tilde{Q}(b)} \\ &= \frac{\sum_{i=1}^{\infty} \mathcal{P}(i, N_{eq}) \left[\tilde{Q}'(b) \frac{1}{i} - \alpha\tau \tilde{Q}(b)(1 - \frac{1}{i}) \right]}{(1 - \mathcal{P}(0, N_{eq})) \tilde{Q}(b)} \end{aligned} \quad (3.17)$$

We now introduce the relatedness measure (See Appendix 3.4.2 for the derivation)

$$R = \frac{\sum_{i=1}^{\infty} \mathcal{P}(i, N_{eq}) \frac{1}{i}}{1 - \mathcal{P}(0, N_{eq})}, \quad (3.18)$$

which is the probability to pick two individuals of the same type from the same patch over and above the probability of picking two of the same type from the global population, relative to the probability of picking the same individuals in the population at large (Queller and Goodnight, 1989, Jansen, 2011). With this we can

rewrite the marginal fitness as

$$\left. \frac{\partial W(b^*, b)}{\partial b^*} \right|_{b^*=b} = \frac{\tilde{Q}'(b)R}{\tilde{Q}} - \alpha\tau(1 - R) \quad (3.19)$$

In the first term ($\tilde{Q}'(b)R$) we recognise that a mutant which produces more siderophores gains a benefit from the change of output from the patch: the share of the benefit is proportional to the relatedness. The second term $\alpha\tau\tilde{Q}(b)(1 - R)$ is the cost this mutant will pay through the decreased competitive ability, from its reduction in growth rate that results from siderophore production. As competition amongst the mutants is neutral, this cost is proportional to the number of unrelated individuals $\tilde{Q}(b)(1 - R)$.

An alternative way of writing marginal fitness is

$$\left. \frac{\partial W(b^*, b)}{\partial b^*} \right|_{b^*=b} = R \underbrace{\left[\frac{\tilde{Q}'(b)}{\tilde{Q}} + \alpha\tau \right]}_B - \underbrace{[\alpha\tau]}_C \quad (3.20)$$

in which we recognise Hamilton's rule. Remarkably, we see here that the fitness cost and benefit are different from the physiological costs and benefits: the benefit consists of the increase of the output of a patch plus the benefit of not suffering from competition with related individuals. Also note that the cost and benefits are not constant but depend on the length of the interaction.

We can now easily calculate the candidate evolutionary stable siderophore production rates from the equation $RB = C$. To do so we need to demonstrate that for any value of b for which the marginal fitness is zero, the solution is evolutionary and convergence stable. This involves the evaluation of the second derivative of the fitness. We do this in the appendix and show that all candidate ESS production rates are indeed evolutionary stable. Figure 4 shows the values of b at the ESS as a function of the length of the interaction. The ESS value decreases rapidly when the length of interaction increases.

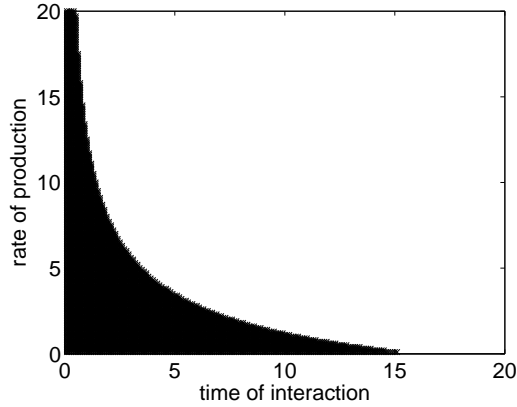


Figure 3.4: ESS value of siderophores production as a function of the duration of the local interaction. The area in black shows all values where the marginal fitness is positive. Parameters: $K = 1$, $r = 1$, $\theta = 2$, $\alpha = 0.1$.

3.3 Discussion

We used a mathematical model to investigate how siderophore production evolves when bacteria compete both at local and at metapopulation levels. We found that different strains can coexist stably at an ecological level. However, evolution leads to a single unbeatable (ESS) level of siderophore production that does not allow for the coexistence of strains with different production levels. We found that the ESS depends on the length of interaction between strains: invasion by cheaters, which are theoretically better competitor, is dynamical; the longer the interaction, the easier it is for them to invade. Also, we derived Hamilton’s rule from first principles in a metapopulation. We found that the fitness costs and benefits are different from the physiological costs and benefits defined in our model. This provides an insight in the processes taking place: a mutant obtains a benefit, which is a change in output shared by related individuals, and pays a cost only through competition with non-related individuals. Finally, we found that all ESS are evolutionary stable. This means that no branching is possible: cheaters cannot stably coexist with cooperators.

Most studies for public good production often implicitly assume total populations being of a fixed size. In such models the benefits of increased cooperation are

cancelled by a simultaneous increase in competition for resources. In such inelastic populations cooperation does not easily evolve. In our model however, the size of a population depends on its strategy. Therefore, a cooperator will benefit from the production of public goods, and cooperation can thus be favoured, even in the face of local competition. Moreover, the effect of competition is modulated through the length of the local interaction. If the interaction is long lasting, then competition dominates and the ESS level for public good production is low. If the interaction is for a short period of time, cooperation is promoted and high levels of public good production arise. This suggests novel ways to evaluate the role of competition in evolutionary studies in elastic populations, through a manipulation of the duration of the interaction. Current methods to assess the role of competition are mostly based on inelastic populations (West et al., 2002).

In our model, evolution leads to siderophore production that is dependent on ecological factors, in particular on the interactions between different strains. Because there is a single ESS attractor, there is no lasting diversity and hence a cheating strain cannot invade or establish itself if the population has attained the ESS. This result is in agreement with the observation that cheating strains are rarely found in natural populations, (West and Buckling, 2003), yet appear readily in laboratory experiments (Harrison and Buckling, 2009), suggesting that such strains emerge, but cannot invade the population. Moreover, our results also show why “ultra cooperators” (i.e. strains that invest highly in the production of siderophores (Brown et al., 2009) cannot invade: although such strains have a high reproductive success when they are on their own, they will lose out in the local competition against cheaters, so that they cannot invade a global population. This follows from the ecology of the bacterium and there is no need to invoke a physiological limit to explain this (as in Brown et al., 2009).

Even though there is no long lasting coexistence of cheaters and siderophore producers in our model, this does not mean that cheaters play no role in the evolutionary dynamics. We have previously explained the important role that cheaters can have

in the regulation of the diversity of siderophore types. Even if they rarely appear in the population, they can stabilise the balance of the different types of siderophore by episodically counteracting genetic drift, and promote diversity (Lee et al., 2012).

In our model we tried to capture the essence of the biology and the ecological interactions between social microbes, by linking their local dynamics to their global distribution. We analyse the adaptive dynamics that follow from these interactions and this allows us to derive an expression for fitness from first principles. Taking the derivative to assess selection pressure on siderophore production results in a form of Hamilton's rule. This leads to a number of important insights and advances. First, most studies on the evolution of social traits start with postulating fitness functions. Central in the formulation of these functions is the idea that actions have costs and benefits, and these are therefore postulated. Because of the absence of a mechanistic basis, it is often difficult to explain these costs and benefits in a biologically meaningful and observable manner. By using an explicit and detailed ecological model for the local demography, we can interpret the evolutionary costs and benefits that we uncover in terms of a change in local population density, and the effects of competitive replacement. As we have based our model on an ecologically meaningful basis, both of these fitness components can be interpreted in terms of ecological interactions, which could lead to experimental quantification. This interpretation also shows that, for instance, the increase in the reproductive success of a siderophore user is not matched to the decrease in reproductive success of others (Taylor, 1992, West et al., 2002).

We derived a relatedness measure, which is equivalent to Queller's relatedness measure (Queller and Goodnight, 1989), and we show that it depends on the population dynamics. Finally, because we have a full description of the dynamics, we not only can derive the marginal fitness, but we can also go beyond and consider the evolutionary stability of the solutions we found. This involves properties of the population that go beyond costs, benefits, and the relatedness measure that we have derived.

This leads to the important conclusion that selection in our model does not favour lasting differences in the siderophore production rates.

Our results are concurring with empirical studies on siderophore producing bacteria, at both local and global levels. For instance, Harrison et al. (2006), studying infections in isolated hosts (i.e. local competition level), have shown that mixed clone infections can favour the evolution of cheats, which result in a decreased mortality of hosts. Other studies showed the same pattern: in local competition cheats always have a higher fitness than cooperators, and the less public good they produce, the higher their local fitness (Jiricny et al., 2010).

At the metapopulation level, Griffin et al. (2004) have shown that the level of cooperation is highly dependent on the scale of competition and on relatedness. In particular, they showed that when in competition (i.e. low relatedness), the cheating strains were always favoured. However, their experimental set up did not take into account the effect of the time of interaction between strains. For longer or shorter durations of interactions, results could well be very different. Our analysis suggests that to fully understand how cooperation evolves in a metapopulation, the length of interaction must be considered in future studies.

This prediction is experimentally testable. For instance, the experimental protocol could be similar to Griffin et al. 2004's, under global competition and low relatedness (two competitors), but with the additional twist of adding the length of interaction's dimension. For example, one could set two different experiments with different lengths: short and long, and measure the frequencies of the competitors at each time step. We predict that the frequency of cheaters will be higher for a long time of interaction and the frequency of cooperators higher for a short time of interaction. It would be interesting to elucidate whether the effects of dispersal on relatedness and competition always cancel out, as it appears to be the case in the some experiments (Kummerli et al., 2009), or whether this conclusion depends on the length of the interaction, as is predicted by our model.

An alternative experimental protocol would be to create/collect a wide range of

strains varying in their siderophore producing rate (as in Jiricny et al., 2010) and make them compete in a metapopulation. We predict here that the mean level of cooperation (which could be measured by assessing the quantity of siderophores) would be negatively correlated to the time of interaction.

3.4 Appendix

3.4.1 Derivation of equations 3.5 and 3.6

The differentiation by t of equation 3.5 gives

$$\frac{df_i}{dt} = \frac{-\alpha b_i f_{i_0} e^{-\alpha t b_i}}{\sum_j f_{j_0} e^{-\alpha t b_j}} + \frac{\alpha f_{i_0} e^{-\alpha t b_i}}{\sum_j f_{j_0} e^{-\alpha t b_j}} \frac{\sum_j b_j f_{j_0} e^{-\alpha t b_j}}{\sum_j f_{j_0} e^{-\alpha t b_j}}$$

which simplifies into

$$\frac{df_i}{dt} = -\alpha b_i f_i + \alpha f_i \sum_j b_j f_{j_j}$$

using $\bar{b} = \sum_i b_i f_i$ we obtain

$$\frac{df_i}{dt} = \alpha f_i (\bar{b} - b_i)$$

Equation 3.6 is obtained by replacing \bar{b} by $\sum_i b_i f_i$ in equation 3.5.

3.4.2 Derivation of the relatedness (Equation 3.18)

We consider 2 alleles “1” and “2”. We note ϕ the probability of picking an individual which carries allele “1” in the global population. We note p_x the probability of picking 2 individuals which carries the same allele x in the local population. In a population initialised by j individuals carrying allele “1” and i founders, the probability of picking an allele “1” individual follows a binomial law given by

$$\mathcal{B}(j, \phi) = \binom{i}{j} \phi^j (1 - \phi)^{i-j}$$

Thus we have we can determine p_1 and p_2

$$p_1 = \sum_{i=1}^{\infty} \sum_{j=0}^i \frac{\mathcal{P}(i, N_{eq})}{1 - \mathcal{P}(0, N_{eq})} \mathcal{B}(j, \phi) \left(\frac{j}{i}\right)^2$$

$$p_2 = \sum_{i=1}^{\infty} \sum_{j=0}^i \frac{\mathcal{P}(i, N_{eq})}{1 - \mathcal{P}(0, N_{eq})} \mathcal{B}(j, \phi) \left(\frac{i-j}{i}\right)^2$$

which simplifies into

$$p_1 = \sum_{i=1}^{\infty} \frac{\mathcal{P}(i, N_{eq})}{1 - \mathcal{P}(0, N_{eq})} \sum_{j=0}^i \mathcal{B}(j, \phi) \left(\frac{j}{i}\right)^2$$

$$p_2 = \sum_{i=1}^{\infty} \frac{\mathcal{P}(i, N_{eq})}{1 - \mathcal{P}(0, N_{eq})} \sum_{j=0}^i \mathcal{B}(j, \phi) \left(\frac{i-j}{i}\right)^2$$

Now $P_{local} = p_1 + p_2$ is probability of picking two individuals of the same type in the local population

$$P_{local} = \sum_{i=1}^{\infty} \frac{\mathcal{P}(i, N_{eq})}{1 - \mathcal{P}(0, N_{eq})} \sum_{j=0}^i \mathcal{B}(j, \phi) \left(1 + 2 \frac{(j^2 - j)}{i^2} \right)$$

which simplifies into

$$P_{local} = 1 - 2 \sum_{i=1}^{\infty} \frac{\mathcal{P}(i, N_{eq})}{1 - \mathcal{P}(0, N_{eq})} \sum_{j=0}^i \mathcal{B}(j, \phi) \frac{j^i - j^2}{i^2}$$

We can simplify $\sum_{j=0}^i \mathcal{B}(j, \phi) \frac{j^i - j^2}{i^2}$ into

$$\sum_{j=0}^i \mathcal{B}(j, \phi) \frac{j^i - j^2}{i^2} = (1 - \phi)(\phi) \left(1 - \frac{1}{i} \right)$$

P_{local} becomes

$$P_{local} = 1 - 2 \sum_{i=1}^{\infty} \frac{\mathcal{P}(i, N_{eq})}{1 - \mathcal{P}(0, N_{eq})} (1 - \phi)(\phi) \left(1 - \frac{1}{i} \right)$$

which simplifies into

$$P_{local} = 1 - 2(1 - \phi)\phi + 2 \sum_{i=1}^{\infty} \frac{\mathcal{P}(i, N_{eq})}{1 - \mathcal{P}(0, N_{eq})} \frac{(1 - \phi)\phi}{i}$$

The probability of picking two individuals carrying the same allele in the global population is given by

$$P_{global} = \phi^2 + (1 - \phi)^2 = -2\phi(1 - \phi) + 1$$

We use the definition of relatedness given by Queller and Goodnight [1989], which is the probability to pick two individuals of the same type from the same patch over and above the probability of picking two of the same type from the global population, normalised so that the relatedness is 1 if there is only one allele in the population. Thus relatedness R is

$$R = \frac{P_{local} - P_{global}}{1 - P_{global}}$$

which gives

$$R = \frac{\sum_{i=1}^{\infty} \mathcal{P}(i, N_{eq}) \frac{1}{i}}{1 - \mathcal{P}(0, N_{eq})}$$

Chapter 4

An evolutionary mechanism for diversity in siderophore-producing bacteria

4.1 Introduction

In Chapter 3 we found that evolution leads the level of siderophore production to an ESS: there is no diversity for the level of siderophore produced. However, siderophores are diverse in structure, (more than 500 different siderophores are known; Ratledge and Dover, 2000, Wandersman and Delepelaire, 2004), and are specific: the siderophore receptor of a bacterium can generally only recognise the peptidic chain of a single siderophore (Hohnadel and Meyer, 1988, Cornelis et al., 1989, Spencer et al., 2003, de Chial et al., 2003), although some strains can recognise multiple siderophore types by expressing different receptors (Barelmann et al., 2002, Ghysels et al., 2004).

How siderophore diversity has emerged and why it persists is not completely understood. Here, we will investigate this question focusing on *Pseudomonas aeruginosa*. *P. aeruginosa* produces several types of siderophores, the main class of which are

called pyoverdines (Cornelis and Matthijs, 2002). Of these there exist three distinct structural types: types 1, 2 and 3. Each strain of *P. aeruginosa* can produce only one of these types, and possesses the corresponding type-specific pyoverdine receptor (Cornelis et al., 1989). Moreover, considerable variation has been reported within each structural type (Smith et al., 2005, Bodilis et al., 2009).

Smith et al. (2005) proposed an explanation of how selection could cause diversity at the pyoverdine receptor locus: they argue that siderophore diversity could be a defence against exploitation by non-siderophore producing bacteria. If there are many non-siderophore producers, it would be beneficial for a mutant to produce a siderophore that is distinct in structure and then incompatible with the dominant population, which would create diversifying selection on the pyoverdine gene (Smith et al., 2005, Tummler and Cornelis, 2005). However, this explanation assumes that non-siderophore producing strains are sufficiently abundant to drive this selection.

The ecology and epidemiology of *P. aeruginosa* are not known in great detail, but although there are reports of pyoverdine-negative mutants, these are normally considered to be exceptions. Indeed, such negative strains readily appear in vitro, when cultures are kept for sufficiently long time (Harrison and Buckling, 2009). Pyoverdine-negative strains have been reported in cystic fibrosis sufferers, but they appear normally after a patient has been infected with a pyoverdine positive clone (De Vos et al., 2001). Although coinfection of *P. aeruginosa* has been reported (McCallum et al., 2001) this suggests that infection with pyoverdine-negative strains would normally arise through mutation from pyoverdine positive strains in the same host. All these observations suggest that the natural abundance of pyoverdine-negative strains is low, which raises the question of whether the defence against cheating can actually explain the observed pyoverdine diversity.

Siderophores are secreted into the extracellular environment to bind iron and where they can be taken up by any organism that has a suitable receptor. Clones of the same strain share siderophores between them, but not with other strains. Hence, siderophores can be considered as a receptor-specific public good. Assuming that

the production of siderophores is costly, this public good system favours pyoverdine-negative mutants. In the context of this public good game, not producing pyoverdine is a cheating strategy (De Vos et al., 2001, West and Buckling, 2003, Harrison and Buckling, 2009).

If a public good can be exploited by cheaters, a feedback may arise that leads to significant diversity. The reason is that strategies may evolve recognition schemes to exclude the cheaters, as already hypothesised by Hamilton [1964]. Hamilton also realised that such discrimination schemes themselves are vulnerable to new cheaters. A number of studies have shown that this can result in a type of Red Queen evolutionary race, in which cheaters continually attempt to “crack” the recognition scheme while the cooperators continually devise new schemes to outrun the cheaters. A siderophore’s variable part acts as a tag used in recognition (West et al., 2007). The dynamics that govern siderophore dynamics could thus be very similar to the Red Queen’s races reported in the studies of green beard genes and other tag-based cooperation models (Riolo et al., 2001, Axelrod et al., 2004, Jansen and van Baalen, 2006, Traulsen and Nowak, 2007, Rousset and Roze, 2007).

However, as we will see, some aspects of the bacteria metapopulation make that this system has a rather different dynamics, leading to alternating periods of random drift and episodes of intense selection. The interplay between complex population dynamics and selection that favours diversity is even richer than previously thought. The episodic nature of selection by cheaters has important consequences for the design of experimental tests of the hypothesis that diversity is generated by social dynamics, as we will discuss in some detail.

In the methods and results sections we will study the maintenance of siderophore diversity using a mathematical model. For those not interested in the mathematical details we will attempt to explain our results verbally in the discussion section.

4.2 Methods: Model description

The model describes a set of hosts which can be colonised by bacteria. Some strains of bacteria can produce a common good in the form of a siderophore, and the production of siderophores increases the output of the colony. We assume that there are different strains of bacteria, each producing a different type of siderophore and having only the corresponding receptor for the siderophores produced: a strain cannot use siderophores produced by other strains. The production of siderophores and the expression of the receptor are controlled by different but adjacent genes (Merriman et al., 1995, Ghysels et al., 2004, Smith et al., 2005) (*fpvA* and *pvdS* respectively in *P. aeruginosa*). We will consider them as a single locus, since a single mutation would obviously be detrimental because of a mismatch between the siderophore and its receptor. We also assume that there are siderophore negative strains, which have lost the ability to produce siderophores, but are still capable of using the siderophore for which they possess the corresponding receptor. Therefore we characterise a strain by two traits that evolve independently: the strategy (cooperation or cheating) and the type of siderophore it produces and/or can utilise. This conveniently captures the functionality of the biology of the system whilst allowing mathematical analysis.

4.2.1 Within a patch: local dynamics

Patches colonised by a strain of cooperators are vulnerable to compatible cheaters, because cheaters benefit from the siderophores while not paying any production cost. The appearance of compatible cheaters leads to the breakdown of cooperation, as they invade and oust the cooperators. Patches colonised by the cheaters are vulnerable to incompatible cooperators however, because these cooperators can keep the benefits of siderophores to themselves. Finally, because of positive frequency dependence, we assume that a patch dominated by one strain of cooperators cannot be invaded by a rare cooperator with a different siderophore type. Similarly, cheaters cannot invade patches of different cheaters. Assuming local dynamics are fast, the

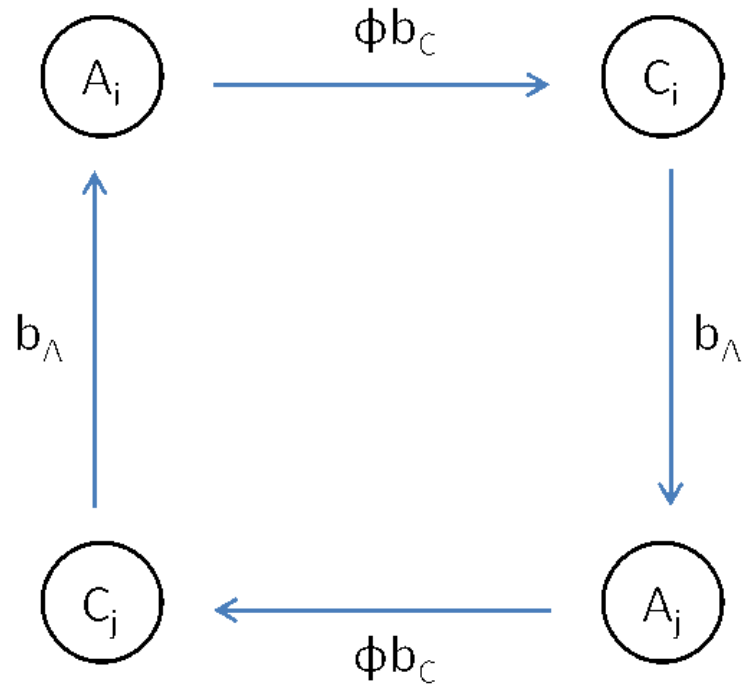


Figure 4.1: Outcomes of the common good game. If a patch is occupied by a cooperator, it is vulnerable to invasion by a compatible cheater (with rate ϕb_C). If a patch is occupied by a cheater, it is vulnerable to invasion by an incompatible cooperator (with rate b_A). In principle, the diagonal transitions ($C_i \leftrightarrow C_j$ and $A_i \leftrightarrow A_j$) are possible too through random drift, but this will be such a slow process that it can be ignored.

transitions are instantaneous, and patches only contain a single strain of bacteria. We will therefore characterise the state of a patch by the type of strain that is currently inhabiting it: we denote A_i and C_i the fraction of patches that contain cooperators of type i and cheaters of type i , respectively. Figure 4.1 summarises the transitions in the states that the model allows.

4.2.2 Between patches : The metapopulation model

Patches are colonised by dispersing bacteria propagules, which are produced with rates b_A and b_C by altruists and cheaters respectively. We assume that cooperators are more efficient in exploiting their hosts than cheaters (on their own), therefore cooperator patches will produce more dispersers, and we have $b_A > b_C$. We differ-

entiate the invasion by cheaters in an empty patch and in a patch already occupied by a compatible cooperator: as cheaters benefit from siderophores already produced by compatible cooperators in a cooperator patch, we could assume that colonisation of a cooperator patch by compatible cheaters is facilitated by a factor $\phi > 1$. For simplicity however, we will assume that cheaters will invade cooperator patches as easily as empty patches, so that $\phi = 1$ and will thus not appear in the model (See Appendix 4.5.1 and Chapter 5 for results taking $\phi > 1$ into account).

New strains appear in the metapopulation through mutation. We describe two types of mutation affecting the local dynamics: with rate ϵ , the mutation from siderophore-producing to non-producing strategy leads to the replacement of cooperators by compatible cheaters inside a patch, and with rate δ , the mutation of siderophore type leads to the replacement of cheaters by incompatible cooperators inside a patch. Since the mutation of siderophore type needs a double mutation (on the siderophore and the receptor genes), we impose $\epsilon > \delta$. We ignore all the other mutation possibilities, as they are irrelevant for the local dynamics: for instance, the mutation from non-siderophore producing strategy to producing would render the mutant (now cooperator) vulnerable to the all-cheater resident population, and thus it could not invade. Thus, an empty patch can change status when it is colonised, a colonised patch can change status either by local extinction, by competition from other strains, or as a consequence of the fixation of a mutant. The dynamics of a metapopulation with n types of siderophore producers are governed by the following differential equations

$$\frac{dA_i}{dt} = (1 - \Sigma A - \Sigma C)b_A A_i - mA_i - b_C A_i C_i + b_A A_i (\Sigma C - C_i) - \epsilon A_i + \frac{\delta}{n-1} (\Sigma C - C_i)$$

$$\frac{dC_i}{dt} = (1 - \Sigma A - \Sigma C)b_C C_i - mC_i + b_C A_i C_i - b_A C_i (\Sigma A - A_i) + \epsilon A_i - \delta C_i$$

with $\Sigma A = \sum_{j=1}^n A_j$ and $\Sigma C = \sum_{j=1}^n C_j$.

Here, $(1 - \Sigma A - \Sigma C)b_A A_i$ represents the colonisation of empty patches by A_i , $m A_i$ the extinction of A_i patches, $b_C A_i C_i$ the colonisation of A_i patches by C_i , $b_A A_i (\Sigma C - C_i)$ the colonisation of $C_j (j \neq i)$ patches by A_i , ϵA_i the conversion from A_i to C_i , $\frac{\delta}{n-1} (\Sigma C - C_i)$ the conversion from patches $C_j (j \neq i)$ to A_i . Similarly, $(1 - \Sigma A - \Sigma C)b_C C_i$ represents the colonisation of empty patches by C_i , $m C_i$ the extinction of C_i patches, $b_A C_i (\Sigma A - A_i)$ the colonisation of C_i patches by $A_j (j \neq i)$ patches, δC_i the conversion from patches C_i to A_j .

4.3 Results

We will start by assessing under what conditions new types can invade in the population and first analyse the existence and invasibility of equilibria without explicit mutation, so that $\epsilon = \delta = 0$. We first determine the equilibria of the system. Then, we explore under which conditions a metapopulation using a single siderophore type can be invaded by a strain utilising a new type of siderophore.

4.3.1 Equilibria

We denote \bar{A} and \bar{C} the equilibria for the total populations of altruists and cheaters, respectively. We will only consider the simultaneous non-zero equilibrium for both strategies ($\bar{A} > 0$ and $\bar{C} > 0$). Solving the equilibrium for arbitrary n yields

$$\bar{A} = n \left(\frac{m}{b_A + b_C - b_A n} - \frac{b_C}{b_A + b_C - b_C n} \right)$$

$$\bar{C} = n \left(-\frac{m}{b_A + b_C - b_A n} + \frac{b_A}{b_A + b_C - b_C n} \right)$$

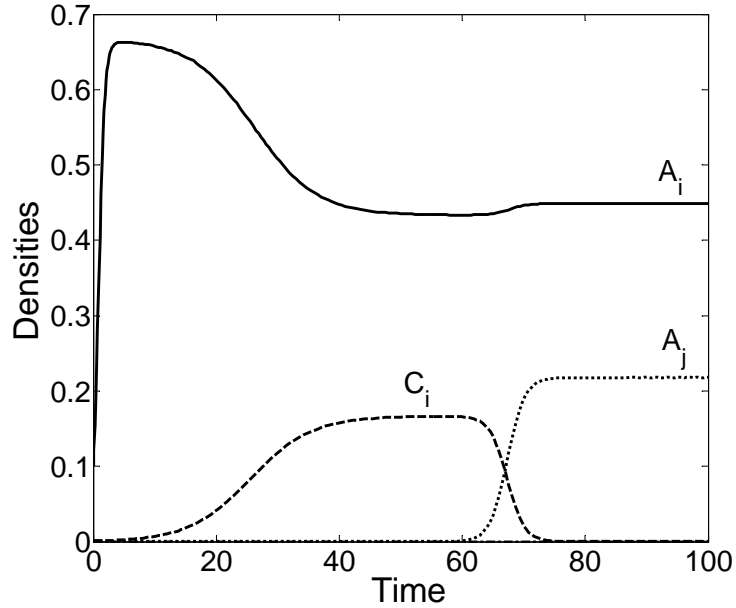


Figure 4.2: How cheaters disappear from patches when there is more than one type of siderophore. First both A_i and C_i are going to equilibria \bar{A} and \bar{C} . The appearance of a competitor (A_j) drives the population of C_i to extinction and A_j settles in an all-cooperator system (neutrally stable). Parameters : $b_A = 3$, $b_C = 1.2$, $m = 1$.

As \bar{A} and \bar{C} are proportions, feasible equilibria constrain between 0 and 1. For a metapopulation with only one type of siderophore produced ($n = 1$), a feasible equilibrium can exist, with cooperators and cheaters present. If we increase the number of siderophore types ($n \geq 2$) however, there is no solution with $0 < \bar{C} < 1$ anymore: the cheaters cannot stably persist in the metapopulation if there is more than one siderophore type (Figure 4.2).

The fact that no diverse equilibrium is possible raises the question what will happen in the long run when siderophore diversity increases.

4.3.2 Invasibility

Even if cheaters cannot persist in a diverse metapopulation this does not mean they do not play a role in determining siderophore diversity. In order to gain an intuitive understanding of their role, we now investigate the invasion by mutant types utilising

a novel siderophore into a system which has a resident cooperator and cheater who share a single siderophore ($n = 1$). We consider first what happens if a mutant cheater appears in the metapopulation. This mutant cheater has no advantage because it cannot use the siderophores produced by the resident cooperator, and therefore, it cannot invade (see Appendix 4.5.2 for a mathematical description).

Then, we consider what happens if a mutant cooperator that uses a new type of siderophore arises in a population. This mutant is initially not encumbered by any cheaters, so it has an advantage over the resident cooperator which is being exploited by its cheater, and will invade the system. When this mutant invades, the resident cooperator will decrease in density. Eventually, the resident density will drop below a threshold density, denoted A_T , which is the density value above which a cooperator can support the presence of a corresponding cheater (see Appendix 4.5.3 for a mathematical derivation). As a result, the resident cheater decreases and once it has gone extinct, the two strains of cooperators will coexist in a neutral equilibrium (Figure 4.3). However, a cheater can reappear whenever one particular corresponding strain of cooperators exceeds the A_T threshold, triggering the same mechanism to regulate the cooperators, protecting the emerged diversity. Thus, although they cannot persist in the metapopulation, cheaters have an ephemeral but important role in regulating siderophore diversity. The overall effect will depend on the level of the threshold density A_T , the processes (such as drift) that allow some cooperator strains to exceed this threshold, and the processes (such as mutations) that reintroduce cheaters into the population. We will explore these effects in the next section using stochastic simulations.

4.3.3 Stochastic simulations

Mutations have a number of important effects. The first is that it may lead to genetic drift, which has the potential to favour (at random) one of the cooperator strains.

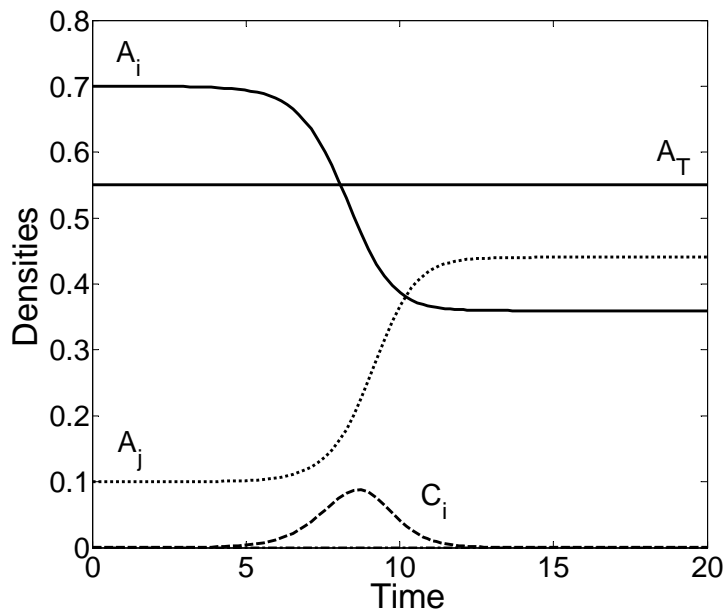


Figure 4.3: The role of the cheaters. Cheaters can appear in the population if one of the cooperating strains exceeds a threshold density A_T . The cheater of the abundant cooperator increases and regulates its population, then disappears again. The amplitude between high and low cooperators is reduced, and cheaters cannot re-invade until the same process perturbs the relative frequency of the cooperators. Parameters : $b_A = 5$, $b_C = 3$, $m = 1$.

The second is the occasional appearance of cheater mutants which, as we have seen, protects diversity. The diversity in such a system thus depends on a balance between generation of new types through mutation, loss through drift but also regulation by cheaters. To study the dynamics of this diversity, we used a simulation model. This simulation model is a stochastic version of the deterministic metapopulation model, in which the number of patches is finite. Otherwise, the patch state transitions follow the same rules as in the deterministic model (colonisation, extinction, competition between strains, mutations), in discrete time.

We first study the effect of stochasticity in a polymorphic metapopulation of cooperators without cheaters. Because all strains of cooperators are competitively neutral with respect to each other, stochastic variation in the number of offspring makes that types eventually disappear. Unsurprisingly, in the absence of cheaters, the metapopulation eventually loses all its initial diversity and becomes monomorphic (Figure 4.4a) due to genetic drift.

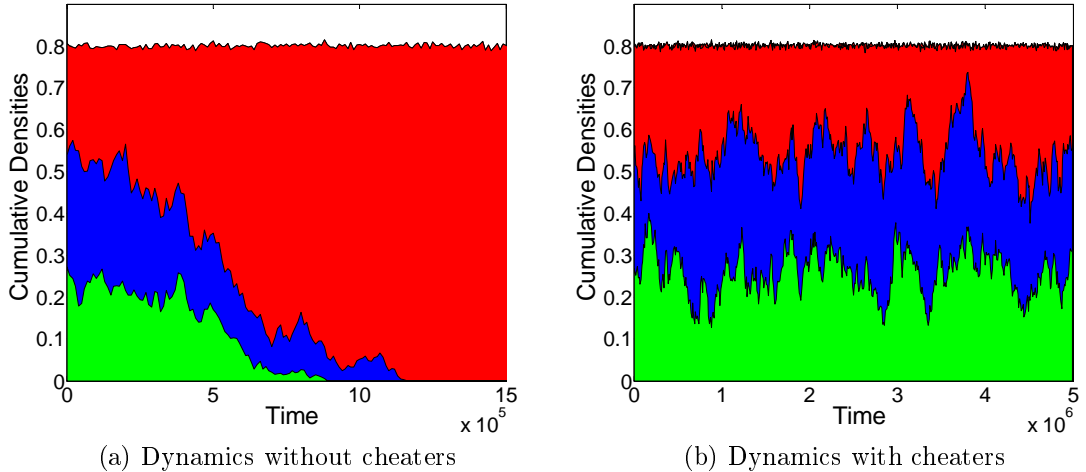


Figure 4.4: Stochastic simulations for an initially diverse system of 3 different cooperators. Graphs show the cumulative densities of cooperators: A_1 in green, A_2 in blue, A_3 in red. 4.4a: Dynamics in absence of cheating strains. Over time, some strains disappear (A_1 and A_2) and eventually the system reverse to a monomorphic population. 4.4b: Dynamics in presence of cheating strains. Strains vary in densities, but do not disappear even for a longer period of time. Parameters: $b_A = 5$, $b_C = 3$, $m = 1$.

The presence of cheaters in the metapopulation has a protective effect on the population diversity: diversity is invariably maintained in all the simulations we carried out (Figure 4.4b, 4.6a, 4.6b). This is in stark contrast with the results of our simulations without cheaters and shows how cheaters can act as a force against the loss of diversity in siderophores types through genetic drift. Moreover, in agreement with our theoretical analysis, while being critical to diversity, cheaters remain at very low frequencies in the population, and increase only episodically. Figure 4.5 shows clearly the mechanism maintaining diversity: through genetic drift, one type is starting to increase in frequency, reducing the frequencies of the other types present. At some point, when the frequency of this cooperator crosses the threshold A_T , we observe some time later a short appearance of the corresponding cheater, reducing the frequency of the cooperator below A_T , before disappearing again. The genetic drift is thus counteracted by the cheaters, which act as a regulating force which is absent most of the time but appears when one of the cooperating strains becomes dominant, thus protecting diversity.

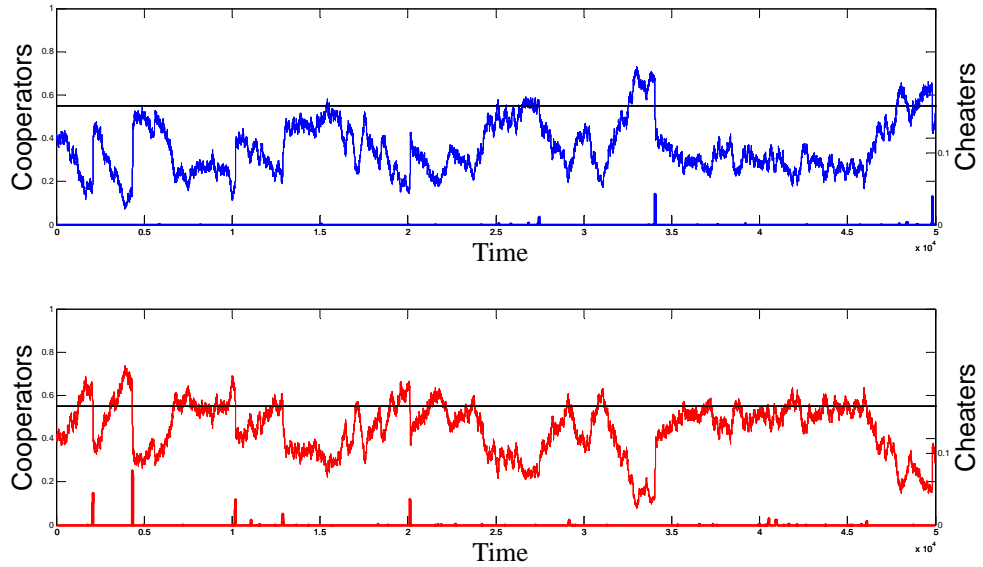


Figure 4.5: Dynamics for a 2-type system. In blue, cooperator and cheater 1, and in red, cooperator and cheater 2. Fluctuating lines correspond to the densities of cooperators, and peaks to the densities of cheaters. When one of the cooperating strains increases in frequency, the other decreases. If one of the cooperators goes above the threshold A_T (black horizontal line), the corresponding cheating strain invades, which regulates the cooperators, and then disappears. Parameters: $b_A = 5$, $b_C = 3$, $m = 1$, $N = 5000$.

Figure 4.5 demonstrates how diversity is promoted and maintained by cheaters in the metapopulation. But it leaves unanswered the question as to whether this diversity is bounded, and if so, to what level it is regulated. Figure 4.6a and 4.6b show the effect of mutation rates on the resulting level of diversity. High mutation rates of the cooperating to cheating strategy (ϵ) lead to high diversity in the metapopulation (Figure 4.6a). This is because the cheating strains appear quicker in the population, preventing the disappearance of types through the genetic drift: if a strain of cooperator increases in frequency, it is regulated when the corresponding cheaters appear (Figure 4.5), but the delay between the increase of cooperators and the appearance of the corresponding cheaters can lead to the extinction of other less abundant types. The level of diversity also depends on the rate of appearance of new siderophore types δ . If the new strains appear quickly, diversity will be high, and on the contrary if new strains appears rarely, the diversity is lower (Figure 4.6b).

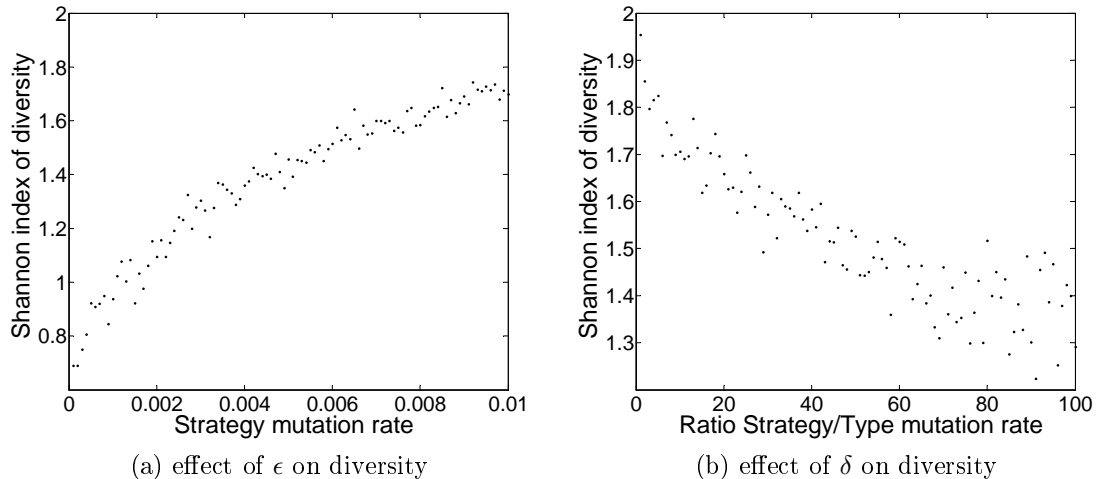


Figure 4.6: The effect of mutations on diversity. Simulations start with an initial monomorphic metapopulation. 4.6a: The effect of the cooperation to cheat strategy mutation rate (ϵ) on the diversity. Diversity is higher if the rate ϵ is high. Parameters : $N=10000$, $\delta=0.1\epsilon$. 4.6b: The effect of the type-mutation (δ) rate on diversity. Diversity decreases and is low when the ratio ϵ/δ is high. Parameters: $N=10000$, $\epsilon=0.01$.

4.4 Discussion

We used a mathematical model to investigate how siderophore diversity can be maintained in populations of siderophore-producing bacteria. We found that cheaters can play a crucial role in the maintenance and regulation of siderophore diversity. Because siderophore-negative cheaters can act as a regulating agent, the cheating strains increase in density if the specific siderophore they can use is produced in sufficient abundance, which results in a decrease of the cooperators that produce this siderophore. The cheaters then disappear. This frequency-dependent mechanism selects against all siderophore-producing strains that becomes sufficiently dominant. Our analysis suggests that this mechanism favours diversity even if the cheaters occur infrequently in the metapopulation and at a very low density.

Our results thus support the hypothesis that siderophore diversity results from cheating, as suggested by Smith et al. [2005]. However, this hypothesis appears to rely on

the selection pressure caused by cheaters, which often seem to be absent. Although cheating strains arise readily in laboratory experiments (Harrison and Buckling, 2009), they seem rare in natural populations (West and Buckling, 2003). Is it then possible that siderophore diversity has nevertheless arisen as an evolutionary response to non-siderophore producing strains? Our model shows that this can indeed be the case: fluctuations from drift in the relative densities of siderophore can make certain strains temporarily abundant. If this happens, non-siderophore producing strains will be selected for and rapidly increase, and will reduce the density of these abundant strains, increasing the densities of other producers of siderophores in the process. This mechanism prevents producing strains from reaching very high or very low densities and thus maintains diversity through episodic outbreaks of non-siderophore producing strains.

Siderophore diversity is not regulated as a dynamic equilibrium, in which the forces that increase and decrease the diversity counteract each other and balance out, but as a dynamic process in which the drift in the population keeps changing the densities of the different siderophore-producing strains in the population. Only once a strain becomes too numerous do the cheating strains emerge and does the regulation kick in. With the hypothesis of all b_i being identical, this implies that stochastic processes play a key role: they govern both the drift in a strain's frequency and the appearance of new mutants. The dynamics are thus characterised by an alternation of periods of steady genetic drift with episodes of rapid and intense evolution. However, if the b_i are different, the same mechanisms regulating diversity will remain, but the dynamics will not be as affected by the drift: the type with the higher b_i will always be the better competitor, going to the threshold and being regulated.

This is also found in other tag-based cooperation models (Axelrod, 1984, Riolo et al., 2001, Jansen and van Baalen, 2006, Rousset and Roze, 2007) in which diversity results from non-equilibrium dynamics. If there were no incentives to cheat it would pay all cooperators to produce the same type of siderophore, that is to equally share the benefits of their altruistic acts. Only when there are cheaters around it pays

for cooperators to deviate from the dominant type and share benefits with members using the same receptor. This results in (locally) unstable dynamics governed by rarity advantage and commonness penalty. In Jansen and van Baalen's (2006) beard chromodynamics model different types occur in amorphous clusters, leading to a dominance of cheaters. In the model presented here types appear in homogeneous clusters, leading to dynamics in which altruists prevail. Whether a similar relationship between siderophore production costs and tag diversity exists as in the Jansen and van Baalen's (2006) model remains to be tested.

In our model, the final levels of siderophore diversity depend on the mutation rates. Moderate diversity can evolve when mutation rates are low, but high mutation rates lead to a high diversity. This result is consistent with the discovery of hypermutant strains of *P. aeruginosa*, which have unusually high mutation rates (Oliver et al., 2000, Buckling et al., 2007), particularly for the *mutS* gene, involved in the DNA-mismatch repair mechanism (Oliver et al., 2002). As in the classical Red Queen, the mutation rate has the general effect of speeding up the dynamics: cooperative strains can escape their cheating corresponding parasite quicker, by producing new types of siderophores, and vice versa, the cheating strains can acquire the compatible siderophore receptor quicker.

Our results show that the diversity in the siderophore receptor locus can result from the selection imposed by the episodic presence of cheating strains. This finding has relevance beyond explaining siderophore diversity: a number of other organisms have been suggested as having tag based recognition, for instance *Saccharomyces cerevisiae* has genes (*FLO*) that cause flocculation which is associated with social behaviour (Smukalla et al., 2008). It has also been suggested that there is considerable diversity in these genes, with closely related strains of *S. cerevisiae* often displaying very distinct phenotypes, due to an unstable tandem repeat sequence in the *FLO1* gene (Verstrepen et al., 2004, 2005, Smukalla et al., 2008). Moreover, mutation rates of the *FLO1* gene have been found to be at least 100-fold greater than the average mutation rates in the genome, suggesting that the same mechanism, as

the one we highlight here, is operating in this system. We thus suggest that diversity in the *FLO* genes could also have emerged as an evolutionary response to the presence of cheaters. A further example are side-blotched lizards where the males form cooperative mate-guarding dyads based on throat coloration (Sinervo and Clobert, 2003, Sinervo et al., 2006). Different throat colours have been observed together with the unstable dynamics in which altruistic and selfish traits alternate in dominance.

Our results suggest that to better understand diversity in siderophores, or tag-based cooperation in general, one needs to go beyond studying strains in isolation or even snapshots from a population. One needs to investigate the change in tag composition over sufficient time within such populations, and in sufficient detail. Our analysis suggests that siderophore producers, such as *P. aeruginosa*, can serve as an experimental model system to demonstrate how altruistic behaviour can go together with the dynamically unstable behaviour known as chromodynamics.

The experiment is simple in essence: create a metapopulation of bacteria and allow some movement between the patches. What we predict is that the diversity in siderophores is more persistent if cheating strains are occasionally introduced in this population. A further prediction is that the densities of cheaters will be negatively correlated to the siderophore diversity.

4.5 Appendix

4.5.1 Inclusion of ϕ

If we take into account the facilitation of colonisation by cheaters in compatible cooperator patches, the between patches differential equations become

$$\frac{dA_i}{dt} = (1 - \Sigma A - \Sigma C)b_A A_i - mA_i - \phi b_C A_i C_i + b_A A_i (\Sigma C - C_i) - \epsilon A_i + \frac{\delta}{n-1} (\Sigma C - C_i)$$

$$\frac{dC_i}{dt} = (1 - \Sigma A - \Sigma C)b_C C_i - mC_i + \phi b_C A_i C_i - b_A C_i (\Sigma A - A_i) + \epsilon A_i - \delta C_i$$

The equilibria are then given by

$$\bar{A} = n \left(\frac{m}{b_A + \phi b_C - b_A n} - \frac{b_C}{b_A + \phi b_C - b_C n} \right)$$

$$\bar{C} = n \left(-\frac{m}{b_A + \phi b_C - b_A n} + \frac{b_A}{b_A + \phi b_C - b_C n} \right)$$

In the main text, the cheaters cannot stably persist in the metapopulation if there is more than one siderophore type (Figure 4.2). This result can change with $\phi > 1$: The maximum number of siderophore types n_{max} in which the cheaters can stably persist increases with a sufficiently high ϕ . However, if a new siderophore type appears in the metapopulation ($n > n_{max}$), the stable equilibrium for the cheaters disappears and again the cheaters cannot stably persist in the metapopulation (this is studied more precisely in Chapter 5).

4.5.2 Invasion of a second cooperator

In order to study the possible non-equilibrium persistence of cheaters, we investigate the potential for invasion into a single siderophore type system at equilibrium ($n = 1$, but with both cooperator and cheater) by a mutant cooperator or by a mutant cheater, utilising a new type of siderophore. We denote $G(X_{eq})$ the growth rate of a strain X at equilibrium. At equilibrium, both densities of cooperator and cheater residents are constant, so the growth rate is null for both the cooperator and the cheater:

$$G(A_{eq}) = \frac{1}{A_{eq}} \frac{dA_{eq}}{dt} = (1 - A_{eq} - C_{eq})b_A - m - b_C C_{eq} = 0$$

$$G(C_{eq}) = \frac{1}{C_{eq}} \frac{dC_{eq}}{dt} = (1 - A_{eq} - C_{eq})b_C - m + b_C A_{eq} = 0$$

The invasion fitnesses of a mutant cooperator A_m and of a mutant cheater C_m are given by:

$$G(A_m) = \frac{1}{A_m} \frac{dA_m}{dt} = (1 - A_{eq} - C_{eq} - A_m - C_m)b_A - m - b_C C_m$$

$$G(C_m) = \frac{1}{C_m} \frac{dC_m}{dt} = (1 - A_{eq} - C_{eq} - A_m - C_m)b_C - m + b_C A_m$$

Because $G(A_{eq}) = 0$, $G(A_m) = G(A_m) - G(A_{eq})$. As at invasion A_m and C_m are negligible for they are rare, the invasion fitnesses become:

$$G(A_m) = G(A_m) - G(A_{eq}) = b_C C_{eq}$$

$$G(C_m) = G(C_m) - G(C_{eq}) = -b_C A_{eq}$$

If a mutant patch A_m appears in the metapopulation, the A_m strain will invade if its growth rate is positive. These mutant cooperators are not likely to invade A

patches because of positive frequency dependence. However, because siderophores make them superior competitors they win the competition against the incompatible cheater residents, and hence they will invade C patches. The growth rate of A_m is thus dependent on the density of C , and is given by $G(A_m) = b_C C_{eq}$.

If we conduct the same reasoning for a mutant patch C_m , we obtain that mutant cheaters do not invade C patches because of positive frequency dependence, and that they lose the competition against the incompatible resident cooperators, and hence they cannot invade the metapopulation. The growth rate of C_m is also dependent on the density of A , and is given by $G(C_m) = -b_C A_{eq}$.

It follows from this result that if the population contains cooperators and cheaters which share a single siderophore type, a cooperator using a new siderophore type can invade this population, because it will initially be free from cheaters. It will therefore increase in density and (in absence of cheaters targeting the new siderophore type) this will eventually result in the ousting of the cheating strain from the population. Eventually, the metapopulation is constituted of cooperator patches only (Figure 4.2).

4.5.3 Reinvasion by cheaters

Although they cannot persist, cheaters are not always absent and have an ephemeral, yet important, role: in this all-cooperator metapopulation, a cheater C_i can re-invade if its invasion fitness becomes positive:

$$G(C_i) > 0 \iff (1 - \Sigma A)b_C - m + b_C A_i - b_A(\Sigma A - A_i) > 0 \iff A_i > \bar{A} - \frac{b_C - m}{b_A + b_C}$$

This means that cheaters can appear and invade when their corresponding cooper-

ators exceed a threshold level:

$$A_T = \bar{A} - \frac{b_C - m}{b_A + b_C}$$

The invasion of these cheaters has the immediate consequence of lowering the density of the corresponding cooperators, and when this density goes back below the threshold A_T , The fitness of the cheater re-becomes negative, leading to their disappearance. Thus, A_T is the density value above which a cooperator can bear the presence of a corresponding cheater.

Chapter 5

The effect of diversity on the dynamics of siderophore producing bacteria

5.1 Introduction

In Chapter 4, we investigated the hypothesis proposed by Smith et al. (2005) and found that diversity can arise from the interplay between different altruists and cheaters. The mechanism we described explains the rarity of the cheaters: we found that they cannot persist in a diverse metapopulation, because their invasion rates is negative most of the time. However, they can invade in non equilibrium situations, when the densities of the different altruists are very different and one strain starts to dominate the others due to genetic drift. The invasion of these cheaters lowers the density of the numerous altruists, preventing the least numerous types from disappearing from the metapopulation, hence protecting the diversity. The cheaters then disappear, until the altruist densities become unbalanced again. The dynamics are thus characterised by an alternation of genetic drift periods (without cheaters), with episodes of rapid and intense selection (with cheaters). However,

this analysis was based on a model for which we made the simplifying assumption that cheaters invade unoccupied habitats as efficiently as habitats occupied by siderophore-producing populations. But it is very well possible that invasion of habitats of altruists is easier because these already contain siderophores produced by the altruists. Indeed, in in-vivo infections of insect hosts by the bacterium *Pseudomonas aeruginosa*, cheaters have been showed to be more virulent when coinfecting the host with an altruist (Harrison et al., 2006). This shows that cheaters are capable of using the siderophores to enhance their growth and dispersal.

Here, we investigate a variant of our previous model that takes the differential invasion capacities by the cheaters into account: cheaters invade better in an altruist patch than in an empty patch. In order to do so, we explicitly introduced a new parameter ϕ which describe the relative increase of the success of invasion of cheaters in an altruist patch, and perform a stability analysis for both cases, when $\phi = 1$ and when $\phi > 1$. This variant has different dynamics than the previous model, because it allows the coexistence of altruists and cheaters of different type in the metapopulation, under certain conditions. The stability analysis shows that there is a neutrally stable diverse equilibrium without mutations. We then show that this equilibrium becomes stable when mutations arise in the system. Finally, we investigate how the different dynamics are linked by analysing simulations, and find that the dynamics revert to a metapopulation of altruists regulated by cheaters outbreaks when the level of diversity becomes sufficiently high.

5.2 Methods: Model description

n	number of siderophores types	2
b_A	rate of propagule production by the altruists	3
b_C	rate of propagule production by the cheaters	1.2
m	rate of patch extinction	1
ϵ	rate of patch conversion from altruist to cheater	0.01
δ	rate of patch conversion from cheater to incompatible altruist	0.001
ϕ	relative increase of the success of invasion of cheaters in an altruist patch	
A	total density of altruist patches	
C	total density of cheater patches	
A_i	density of patches of altruist strain i	
C_i	density of patches of cheater strain i	
\bar{A}	density of patches of one strain of altruists at equilibrium	
\bar{C}	density of patches of one strain of cheaters at equilibrium	

Table 5.1: Table of all the variables used, with their typical value.

We use the model for siderophore diversity described in Lee et al. [2012] (See Chapter 4). The model corresponds to a set of patches which can be colonised by bacteria. The bacteria can produce a public good in the form of a siderophore, and the production of siderophores increases the output of the patch. Bacterial strains have different strategies and produce different types of siderophores. There are two different strategies in the population: Either a strain produces the public good, and is considered an altruistic strain, or it does not produce the public good and is considered a cheating strain. Additionally, a strain produces a specific type of siderophore and has only the corresponding receptor for the siderophores produced: a strain cannot use siderophores produced by other strains. Cheating strains have lost the ability to produce siderophores, but are still capable of using the siderophore for which they possess the corresponding receptor. Therefore we characterise a strain by two traits that evolve independently: the strategy (cooperation or cheating) and the type of siderophore it produces and/or can use. Bacteria compete on two levels: they interact locally, inside a patch, and they interact globally, by dispersal in the metapopulation.

The local dynamics depend on which strains compete inside a patch. Altruists can be invaded by cheaters in a patch if the cheaters can exploit the siderophores produced by the altruists. However, altruists are not vulnerable to either incompatible cheaters

or different altruists. Similarly, subpopulations of cheaters can be invaded and are replaced by incompatible altruists because altruists are more productive and can keep the benefits of the siderophores for themselves. However, cheaters are not vulnerable to either compatible altruists or different cheaters. We assume strong selection when bacteria compete inside a patch: There is immediate replacement and therefore the state of a patch is characterised by the type of strain that is inhabiting it at the end of the interaction: A_i and C_i denote the fraction patches that contain altruists of type i and cheaters of type i , respectively.

Bacteria disperse in the metapopulation by producing propagules, produced with rates b_A and b_C by altruists and cheaters respectively. Because altruists are more efficient in exploiting their hosts than cheaters (on their own), we assume that altruists produce more dispersers than cheaters, so that $b_A > b_C$. Also, the two bacterial traits (strategy and siderophore type) can be modified through mutation, which creates new strains. Two types of mutation affect the local dynamics: with rate ε , the production gene is broken, so that a mutant cheater appears in an altruist strain. Because the cheater profits on the siderophores, this leads to the replacement of altruists by compatible cheaters inside a patch. With rate δ , a mutated siderophore type appears, and the new altruists outcompete resident cheaters inside a patch. Since the mutation of siderophore type needs a double mutation (on the siderophore and the receptor genes), we impose $\varepsilon > \delta$. All the other mutation possibilities are ignored, as they are irrelevant for the local dynamics.

We will present the analysis of this model, with increasing levels of complexity. First, we study a simplified model without mutation and with only a single type of siderophore produced (no diversity) for which we show that altruists and cheaters can coexist in a stable equilibrium. Then, we introduce diversity by adding a new subpopulation of altruists and a new subpopulation of cheaters that use an incompatible type of siderophore, and determine if these subpopulations can invade.

After invasion, the metapopulation is now diverse and has different dynamics compared to the non-diverse system. Two different behaviours emerge depending on

the value of ϕ . If we consider that cheaters invade unoccupied and altruist patches with the same rate ($\phi = 1$), cheaters cannot persist in the metapopulation, which goes to a neutral equilibrium constituted of altruists only. But if we consider that cheaters invade altruist patches more efficiently ($\phi \geq 1$), cheaters can persist and the dynamics will follow a cycling pattern. We will determine that neither cases give a stable equilibrium. Finally, we will introduce mutations, and determine that they stabilise the equilibria in both cases.

5.3 Results

5.3.1 System without mutation

We first study the system with no mutation occurring ($\epsilon = \delta = 0$).

In a single-type population ($n = 1$), the system is simplified so there is only one type of altruists (A) and one type of cheaters (C). The differential equations are

$$\begin{cases} \frac{dA}{dt} = b_A A(1 - A - C) - mA - \phi b_C AC \\ \frac{dC}{dt} = b_C C(1 - A - C) - mC + \phi b_C AC \end{cases} \quad (5.1)$$

Where $b_A A(1 - A - C)$ and $b_C C(1 - A - C)$ represents the colonisation of empty patches by A and C respectively, mA and mC the extinction of A and C patches respectively, and $\phi b_C AC$ the colonisation of A patches by C .

If we denote \bar{A} the density of altruists at equilibrium and \bar{C} the density of cheaters at equilibrium, the equilibria are the couples (\bar{A}, \bar{C})

$$(0, 0) \quad (5.2)$$

$$\left(\frac{b_A - m}{b_A}, 0\right) \quad (5.3)$$

$$\left(0, \frac{b_C - m}{b_C}\right) \quad (5.4)$$

$$\left(\frac{m}{\phi b_C} - \frac{b_C}{b_A + b_C(\phi - 1)}, \frac{b_A}{b_A + b_C(\phi - 1)} - \frac{m}{\phi b_C}\right) \quad (5.5)$$

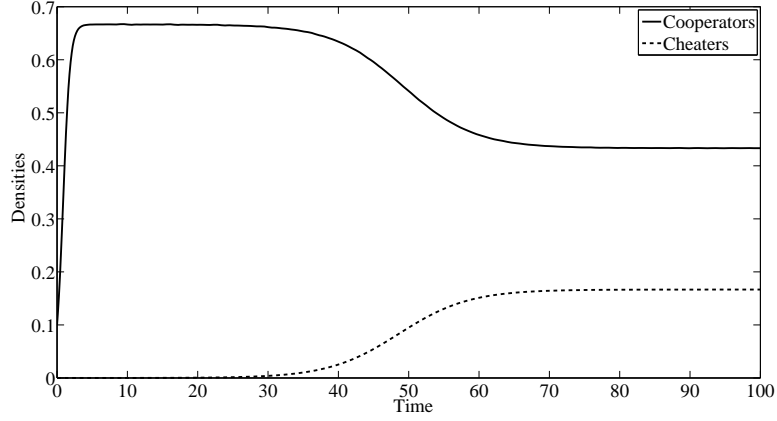
Equation (5.5) gives the only equilibrium with coexistence of both the altruist and the cheater. The condition of coexistence is given by

$$\begin{cases} 0 < \bar{A} < 1 \\ 0 < \bar{C} < 1 \end{cases} \iff b_A > b_C \left(1 + \phi \left(\frac{b_C}{m} - 1\right)\right) \quad (5.6)$$

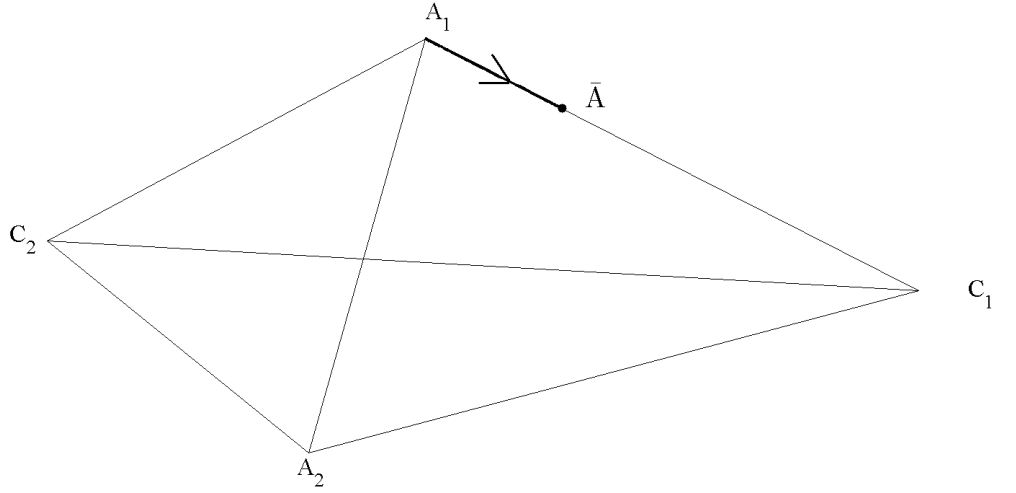
We use standard linearisation techniques and Routh-Hurwitz criteria to determine the stability of the equilibrium. The equilibrium is stable if the trace of the matrix is negative, and the determinant positive (Kot, 2001). The Jacobian matrix J_s for this single type system is

$$J_s = \begin{bmatrix} -b_A \bar{A} & -\bar{A}(b_A + \phi b_C) \\ b_C \bar{C}(\phi - 1) & -b_C \bar{C} \end{bmatrix} \quad (5.7)$$

Given that \bar{A} and \bar{C} exist, the Routh-Hurwitz criteria of stability are fulfilled, and the equilibrium is stable. Figure 5.1 shows the dynamics of a single-type system. First, altruists invade following a logistic growth. Then, cheaters invade, reducing the densities of cooperators, and both coexist at a stable equilibrium level.



(a) Densities



(b) Frequencies

Figure 5.1: Dynamics of a single-type system. The population start with altruist individuals and a small fraction of cheaters. The altruists go to the cheater-free density equilibrium (because the density of cheaters is initially negligible), before being invaded by the cheaters. The system then converges to the equilibrium (\bar{A}, \bar{C}) with the coexistence of both altruists and cheaters. Parameters: $b_A = 3$, $b_C = 1.2$, $m = 1$, $\phi = 1$.

We now investigate the potential for invasion into a single siderophore type system at the coexistence equilibrium (Equation (5.5)) by a new altruist or by a new cheater, using a different type of siderophore. We denote $G(X)$ the per capita invasion rate of a strain X . At equilibrium, both densities of altruist and cheater residents are constant, so the per capita invasion rate is zero for both the altruists and the cheaters

$$\begin{cases} G(A) = (1 - A - C)b_A - m - \phi b_C C = 0 \\ G(C) = (1 - A - C)b_C - m + \phi b_C A = 0 \end{cases} \quad (5.8)$$

The invasion fitnesses of a mutant altruist A_m and of a mutant cheater C_m are given by

$$\begin{cases} G(A_m) = (1 - A - C - A_m - C_m)b_A - m - \phi b_C C_m + b_A C \\ G(C_m) = (1 - A - C - A_m - C_m)b_C - m + \phi b_C A_m - b_A A \end{cases} \quad (5.9)$$

Because $G(A) = 0$ and $G(C) = 0$, we have $G(A_m) = G(A_m) - G(A)$ and $G(C_m) = G(C_m) - G(C) = 0$. As at invasion A_m and C_m are negligible because they are rare, the invasion rates become

$$\begin{cases} G(A_m) = (\phi b_C + b_A)\bar{C} \\ G(C_m) = -(\phi b_C + b_A)\bar{A} \end{cases} \quad (5.10)$$

If a new strain A_m appears in the metapopulation, the A_m strain will invade because its invasion rate is positive (Equation (5.10)). These new altruists win the competition against the incompatible cheater residents, and hence they invade C patches. If we conduct the same reasoning for a new patch C_m , we obtain that new cheaters do not invade (at least not before the mutant altruists have settled into the metapopulation) because their invasion rate is negative.

The invasion of a new altruist leads to very different dynamics. Two possibilities arise: either the new altruist ousts the resident cheater and the metapopulation becomes all-altruist, or the different subpopulations coexist. We will now analyse the conditions leading to either cases.

5.3.2 Multiple-type system

The metapopulation becomes diverse when the second type invades it. We now note A and C the total densities of altruists and cheaters, respectively. Because cheaters are vulnerable to incompatible altruists, the differential equations become

$$\begin{cases} \frac{dA_i}{dt} = b_A A_i (1 - A - C) - mA_i - \phi b_C A_i C_i + b_A A_i (C - C_i) \\ \frac{dC_i}{dt} = b_C C_i (1 - A - C) - mC_i + \phi b_C A_i C_i - b_A C_i (A - A_i) \end{cases} \quad (5.11)$$

where $b_A A_i (C - C_i)$ and $b_A C_i (A - A_i)$ represents the colonisation of patches of cheaters by incompatible altruists.

We can determine the equilibrium where all strains of altruists have the same density \bar{A} and all strains of cheaters have the same density \bar{C} . The equilibria are the couples (\bar{A}, \bar{C})

$$(0, 0) \quad (5.12)$$

$$\left(\frac{b_A - m}{b_A n}, 0 \right) \quad (5.13)$$

$$\left(0, \frac{b_C - m}{b_C n} \right) \quad (5.14)$$

$$\left(\frac{m}{b_A + \phi b_C - b_A n} - \frac{b_C}{b_A + \phi b_C - b_C n}, \frac{b_A}{b_A + \phi b_C - b_C n} - \frac{m}{b_A + \phi b_C - b_A n} \right) \quad (5.15)$$

Beside the trivial equilibrium, the system goes to either equilibrium (5.13), (5.14) or (5.15), depending on whether altruists and cheaters can both persist in the metapopulation or not. To determine this, we analyse if a cheater can invade in a metapopulation at the multi-altruist equilibrium (5.13), where all altruists have the same density, and also if an altruist can invade in a metapopulation at the multi-cheater equilibrium (5.14), where similarly all cheaters have the same density. The invasion rates of an altruist strain A_i in a metapopulation of cheaters, and of a cheating strain C_i in a metapopulation of altruists, are respectively

$$\begin{cases} G(A_i) = (1 - n\bar{C})b_A - m - \phi b_C \bar{C} + b_A \bar{C}(n - 1) \\ G(C_i) = (1 - n\bar{A})b_C - m + \phi b_C \bar{A} - b_A \bar{A}(n - 1) \end{cases} \quad (5.16)$$

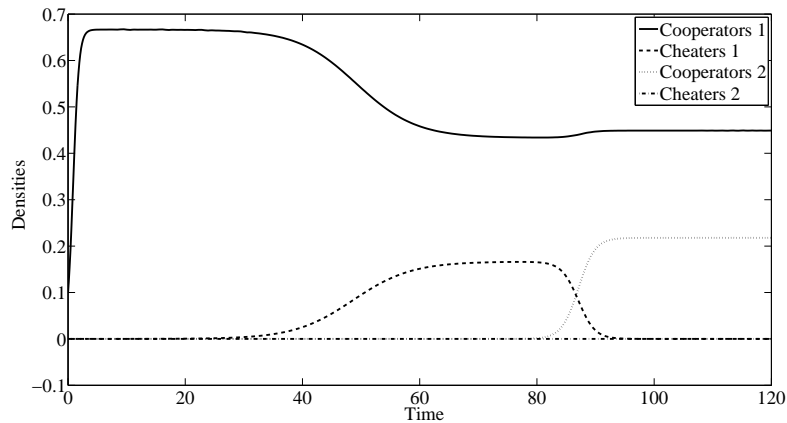
A necessary condition for the multiple-type equilibrium to exist is that both invasion rates are positive. Using Equilibria (5.13) and (5.14), the conditions are

$$\begin{cases} G(A_i) > 0 \iff b_A - m - \frac{(b_A + \phi b_C)(b_C - m)}{b_C n} > 0 \\ G(C_i) > 0 \iff \frac{(b_A + \phi b_C)(b_A - m) - b_A^2 n + n m b_C}{n b_A} > 0 \end{cases} \quad (5.17)$$

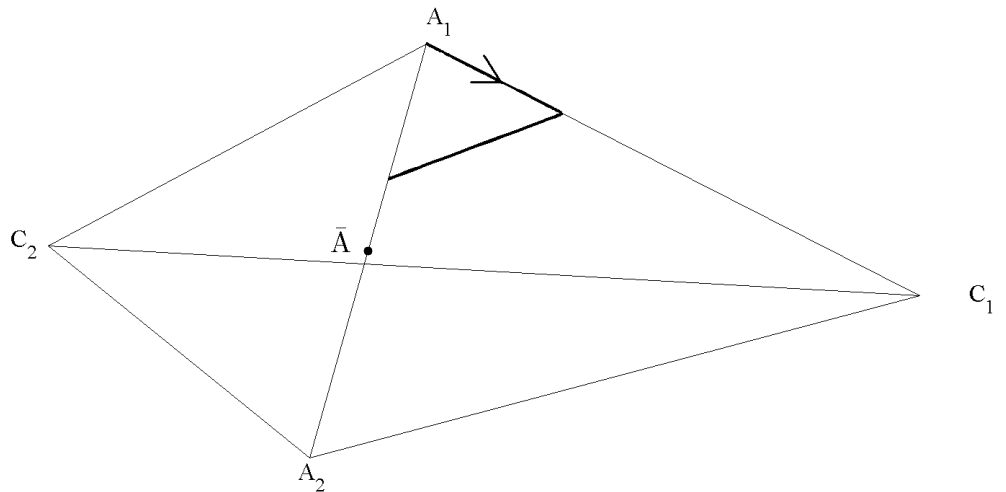
5.3.3 The effect of ϕ

If $\phi = 1$, conditions in equation (5.17) cannot be fulfilled simultaneously: the multi-type equilibrium (5.15) never exists. More precisely, as the cheaters have a negative invasion rate, they cannot stably persist in the metapopulation if there is more than one siderophore type. It follows from this result that if the population contains altruists and cheaters which share a single siderophore type, an altruist using a new siderophore type can invade this population, because it will initially be free from

cheaters. It will therefore increase in density and (in absence of cheaters targeting the new siderophore type) this will eventually result in the ousting of the cheating strain from the population. Eventually, the metapopulation is constituted of altruist patches only (Figure 5.2). The multiple altruist equilibrium (5.13) is thus a neutral equilibrium.



(a) Densities



(b) Frequencies

Figure 5.2: Dynamics of a single-type system invaded by a second type (no mutations). With a non-zero initial population of cheaters C_1 , The system first converges to the (\bar{A}, \bar{C}) equilibrium. When a new altruist appears, it ousts the cheaters from the metapopulation, and both altruists settle in an all-altruist system (neutrally stable). The system does not converge to the two-altruist equilibrium. Parameters: $b_A = 3$, $b_C = 1.2$, $m = 1$, $\phi = 1$.

The results change importantly if $\phi > 1$, as now the multiple type equilibrium (5.15) can exist. Both conditions in (5.17) are fulfilled if

$$\frac{b_A m + b_A^2(n-1) - n b_C m}{b_C(b_A - m)} < \phi < \frac{(b_A - m)n}{b_C - m} - \frac{b_A}{b_C} \quad (5.18)$$

Figure 5.3 shows an illustration of the effect of ϕ on the invasion rates of equation (5.16). If ϕ is too small, the cheaters cannot invade and the metapopulation is altruist-only. If ϕ is too large, the altruists cannot invade and the metapopulation is cheater-only. For a certain range, both invasion rates are positive: there is coexistence of several types of altruists and cheaters.

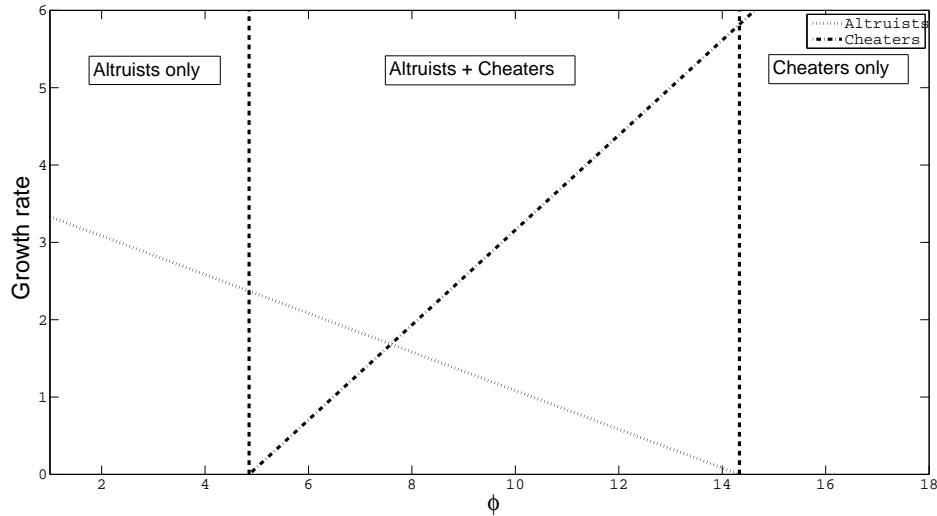


Figure 5.3: The effect of ϕ on the invasion rate of altruists and cheaters (Equation 5.16). If ϕ is too low, cheaters invasion rate is negative: they cannot invade the metapopulation, which is thus constituted of altruist-only patches. On the contrary, if ϕ is too large, altruists invasion rate is negative: they are ousted of the metapopulation, thus constituted of cheater-only patches. Inbetween, Several types of altruists and cheaters can coexist. Parameters: $b_A = 5.5$, $b_C = 1.5$, $m = 1$, $n = 2$.

5.3.4 The effect of increasing diversity

Thus, two types of dynamics are possible, depending on the value of ϕ : on the one hand if we don't take into account an improved capacity of invasion when cheaters invade an altruist patch ($\phi = 1$) then the dynamics present a neutrally stable equilibrium. On the other hand if cheaters invade better patches of altruists ($\phi > 1$), then we can observe a cycling behaviour. But the dynamics (5.15) also depend on the number of types in the metapopulation. When diversity increases (i.e. when a new type of siderophore arises in the metapopulation), the minimum value of ϕ for which the equilibrium exists also increases. This means that the cycling behaviours which could exist for a given value of n would disappear when n becomes larger (Figure 5.4). Thus the metapopulation would go back to equilibrium (5.13): a neutral equilibrium. Note that if the value of ϕ exceeds the maximum value, the metapopulation goes to the cheaters neutral equilibrium (5.14).

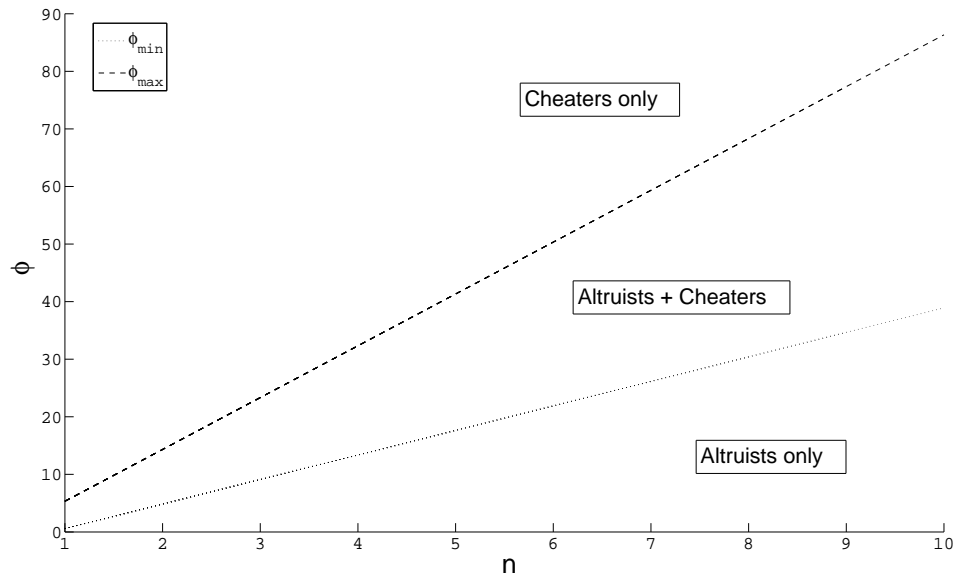


Figure 5.4: The effect diversity on the minimum (ϕ_{min}) and maximum (ϕ_{max}) value of ϕ for which a multiple-type equilibrium exists. Both ϕ_{min} and ϕ_{max} increases when n increases. Parameters: $b_A = 5.5$, $b_C = 1.5$, $m = 1$.

We can now determine the stability of the multiple type equilibrium (5.15) with a stability analysis.

5.3.5 Stability analysis

The linearisation of the complete system is presented in Appendix 5.5.1. To determine the Jacobian matrix from the linearised equations for any number of types is possible but becomes difficult for large n . To simplify the problem, we will introduce a transformation of variables, by considering the total density of altruists and cheaters, denoted A and C , and the deviation from the mean, denoted A' and C' (Note that $A' = \frac{A}{n} - A_i$ and $C' = \frac{C}{n} - C_i$). This transformation achieves a decoupling of the system (See Jansen and Lloyd, 2000).

The linearised dynamics of the total densities of altruists and cheaters are

$$\begin{cases} \frac{dA}{dt} = (-nb_A\bar{A})A - \bar{A}(b_A + \phi b_C)C \\ \frac{dC}{dt} = (\bar{C}((b_A + \phi b_C) - (b_A + b_C)n))A + -nb_C\bar{C}C \end{cases} \quad (5.19)$$

and the linearised dynamics for the deviation from the mean are

$$\begin{cases} \frac{dA'}{dt} = -\bar{A}(b_A + \phi b_C)C' \\ \frac{dC'}{dt} = \bar{C}(b_A + \phi b_C)A' \end{cases} \quad (5.20)$$

The Jacobian matrix is determined by the partial derivatives of the variables that describe the system. The Jacobian matrix can be written as a block matrix:

$$J = \begin{bmatrix} H & & & & \\ & J_1 & & & \\ & & J_2 & & \\ & & & \ddots & \\ & & & & J_n \end{bmatrix} \quad (5.21)$$

Because every types have the same demography, they have the same description as deviation from the mean: all the J_i are identical. We have

$$H = \begin{bmatrix} \frac{\partial \frac{dA}{dt}}{\partial A} & \frac{\partial \frac{dA}{dt}}{\partial C} \\ \frac{\partial \frac{dC}{dt}}{\partial A} & \frac{\partial \frac{dC}{dt}}{\partial C} \end{bmatrix} = \begin{bmatrix} -nb_A \bar{A} & -\bar{A}(b_A + \phi b_C) \\ \bar{C}((b_A + \phi b_C) - (b_A + b_C)n) & -nb_C \bar{C} \end{bmatrix} \quad (5.22)$$

$$J_i = \begin{bmatrix} \frac{\partial \frac{dA'_i}{dt}}{\partial A'_i} & \frac{\partial \frac{dA'_i}{dt}}{\partial C'_i} \\ \frac{\partial \frac{dC'_i}{dt}}{\partial A'_i} & \frac{\partial \frac{dC'_i}{dt}}{\partial C'_i} \end{bmatrix} = \begin{bmatrix} 0 & -\bar{A}(b_A + \phi b_C) \\ \bar{C}(b_A + \phi b_C) & 0 \end{bmatrix} \quad (5.23)$$

Because all blocks are independent (the off diagonal blocks are zero), the overall stability of the equilibrium can be determined by studying the stability of each block. The H block gives

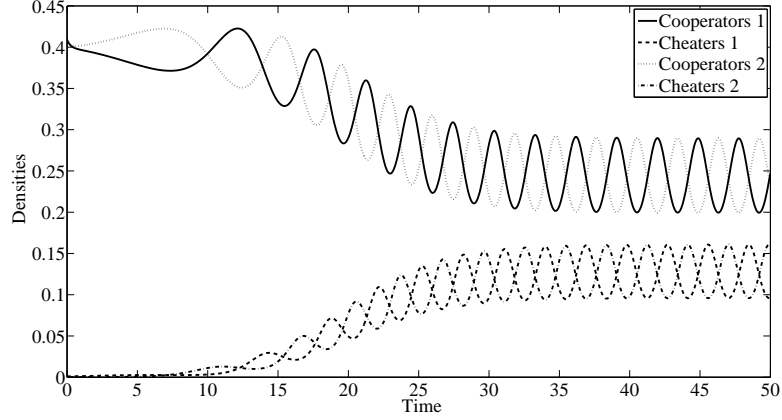
$$\begin{cases} \text{Tr}(H) = -nb_A \bar{A} - nb_C \bar{C} < 0 \\ \text{Det}(H) = \bar{A} \bar{C} (n^2 b_A b_C + (b_A + \phi b_C)(b_A + \phi b_C - (b_A + b_C)n)) \end{cases} \quad (5.24)$$

The determinant is positive in the range of the multiple-type equilibrium (5.15) (See the proof in Appendix 5.5.2).

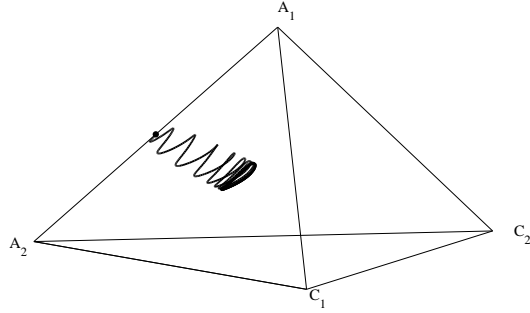
The J_i block gives

$$\begin{cases} \text{Tr}(J_i) = 0 \\ \text{Det}(J_i) = \overline{AC}(b_A + \phi b_C)^2 > 0 \end{cases} \quad (5.25)$$

The determinant of J_i is positive but the trace is zero. This means that the system has a pair of complex eigenvalues with zero real parts. Although the multiple-type equilibrium exists, the different subpopulations of altruists and cheaters need not converge towards the equilibrium. Instead, the dynamics follows a cycling pattern around the multiple-type equilibrium (Figure 5.5).



(a) Densities



(b) Frequencies

Figure 5.5: Dynamics of a two-type system at the two-altruist equilibrium invaded by cheaters. If the two-cheater equilibrium exists, cheaters invade and the dynamics follow a cycling pattern with alternate period of high and low amplitudes for both types. Both types cycle around the equilibria, but the system does not converge to the two-altruist equilibrium. Parameters: $b_A = 5.5$, $b_C = 1.5$, $m = 1$, $\phi = 5$.

5.3.6 The effect of mutations in a multiple-type system

We now investigate the effect of mutations on the dynamics previously determined.

The complete system for any number of siderophore types is

$$\begin{cases} \frac{dA_i}{dt} = b_A A_i (1 - A - C) - mA_i - \phi b_C A_i C_i + b_A A_i (C - C_i) - \epsilon A_i + \frac{\delta}{n-1} (C - C_i) \\ \frac{dC_i}{dt} = b_C C_i (1 - A - C) - mC_i + \phi b_C A_i C_i - b_A C_i (A - A_i) + \epsilon A_i - \delta C_i \end{cases} \quad (5.26)$$

where ϵA_i is the conversion from A_i to C_i , $\frac{\delta}{n-1}(\widehat{C} - C_i)$ is the conversion from patches $C_j (j \neq i)$ to A_i and δC_i the conversion from patches C_i to A_j .

The equilibrium is only needed to determine the linearised equation and is given in Appendix 5.5.1. The blocks of the Jacobian matrix becomes

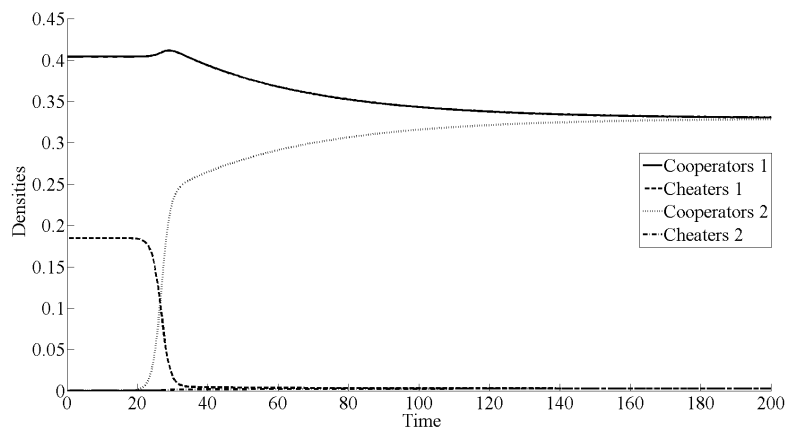
$$H' = \begin{bmatrix} -nb_A \bar{A} - \delta \frac{\bar{C}}{\bar{A}} & \delta - \bar{A}(b_A + \phi b_C) \\ \bar{C}((b_A + \phi b_C) - (b_A + b_C)n) + \epsilon & -nb_C \bar{C} - \epsilon \frac{\bar{A}}{\bar{C}} \end{bmatrix} \quad (5.27)$$

$$J'_i = \begin{bmatrix} -\delta \frac{\bar{C}}{\bar{A}} & -\bar{A}(b_A + \phi b_C) - \frac{\delta}{n-1} \\ \bar{C}(b_A + \phi b_C) + \epsilon & -\epsilon \frac{\bar{A}}{\bar{C}} \end{bmatrix} \quad (5.28)$$

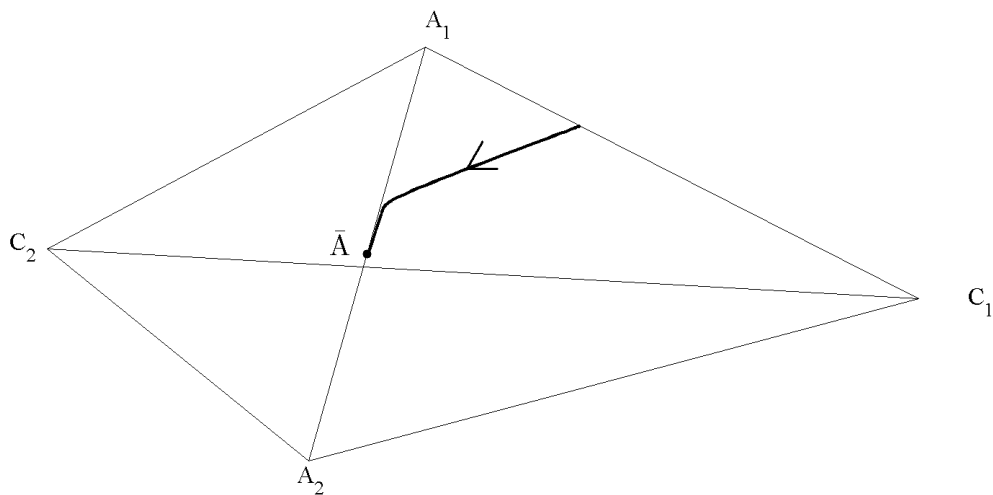
If ϵ and δ are much smaller than the other variables, the properties of H' and H are similar, in that $Tr(H') < 0$ and $Det(H') > 0$. We now look at the J'_i block:

$$\begin{cases} Tr(J'_i) = -\delta \frac{\bar{C}}{\bar{A}} - \epsilon \frac{\bar{A}}{\bar{C}} < 0 \\ Det(J'_i) = \delta \epsilon + (\bar{C}(b_A + \phi b_C) + \epsilon) \left(\bar{A}(b_A + \phi b_C) + \frac{\delta}{n-1} \right) > 0 \end{cases} \quad (5.29)$$

Mutations render the trace negative. Both subsystems now satisfy the Routh-Hurwitz criteria, and the equilibrium becomes stable. Figure 5.6 and 5.7 show the dynamics of a multiple-type system (with $n = 2$) with $\phi = 1$ and $\phi > 1$ respectively. We have seen that when $\phi = 1$, cheaters cannot persist stably in the metapopulation. However, mutations cause a small but continuous presence of cheaters. Initially, two altruists can be at different density levels, but they converge towards the stable equilibrium \bar{A} because of the continuous presence of cheaters, who converge towards the stable equilibrium \bar{C} (Figure 5.6).



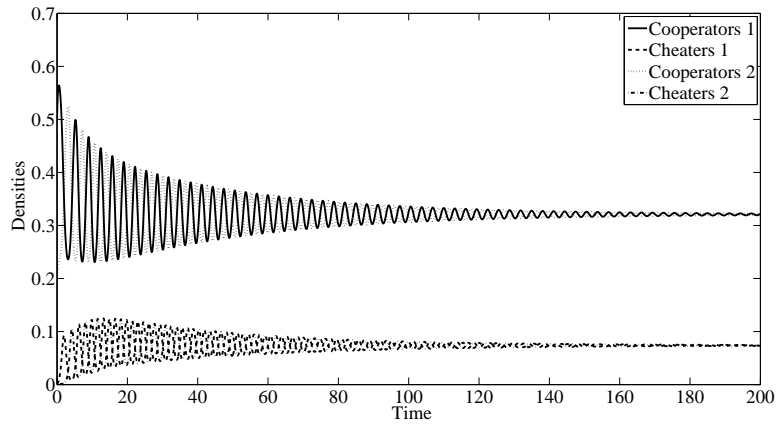
(a) Densities



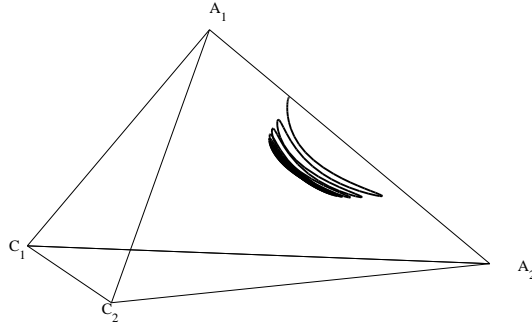
(b) Frequencies

Figure 5.6: Dynamics of a single-type system invaded by a second type, with mutations. Initially, the two cooperators have different densities in the metapopulation, but because of the presence of cheaters, they converge towards the altruist equilibrium. Cheaters also converge towards the cheaters equilibrium, which is non-zero due to the mutations. Parameters: $b_A=3$, $b_C = 1.2$, $m = 1$, $\phi = 1$.

We have seen that when $\phi > 1$ the dynamics follow a cycling pattern because of Equation (5.25). Mutations now allows a small negative trace (Equation (5.29)), which stabilises the system: the cycles disappear and the system converges to the now stable equilibria \bar{A} and \bar{C} (Figure 5.7).



(a) Densities



(b) Frequencies

Figure 5.7: Dynamics of a two-type system with mutations. Mutations stabilise the system: altruists and cheaters converge towards the multi-type equilibrium with mutations. Parameters: $b_A = 5.5$, $b_C = 1.5$, $m = 1$, $\phi = 5$.

5.3.7 Stochastic simulation

We can observe the dynamics revealed by the deterministic model by using a simulation model. This simulation model is a stochastic version of the deterministic model, in which the number of patches is finite. It takes the parameters set in figure 5.4, and we fixed the ϕ parameter at 5.5. We start with a monomorphic population of altruist. At the single type population, the altruist cannot persist and is invaded by its compatible cheater. The metapopulation thus goes to the single type cheater equilibrium. If a mutant altruist appears without its cheater, it invades and ousts the resident cheater. But if it appears with its compatible cheater, it results in the coexistence of all subpopulations, with a cycling behaviour. The increase of diversity at this level of ϕ leads to the system changing to an altruists only equilibrium. So when a third altruist appears, the cycling behaviour disappears and the cheaters disappear of the metapopulation. The different subpopulations of altruists are neutral with respect to each others, but are now subject to genetic drift. At this point we recover previous results: diversity does not decrease because of the regulatory action of the cheaters (See Lee et al., 2012).

5.4 Discussion

We used a mathematical model to investigate the stability of equilibria under different conditions in siderophore producing bacteria. We found that in a single siderophore type metapopulation, the equilibrium is stable and coexistence between altruists and cheaters occurs if the advantage gained by producing siderophores is sufficient. In a multiple type metapopulation without mutation, there is a strong effect of ϕ . If we assume that cheaters do not invade altruist patches more easily than empty patches ($\phi = 1$), we found that the altruist equilibrium is neutrally stable: different altruists can coexist, but a slight perturbation (for example the arrival of

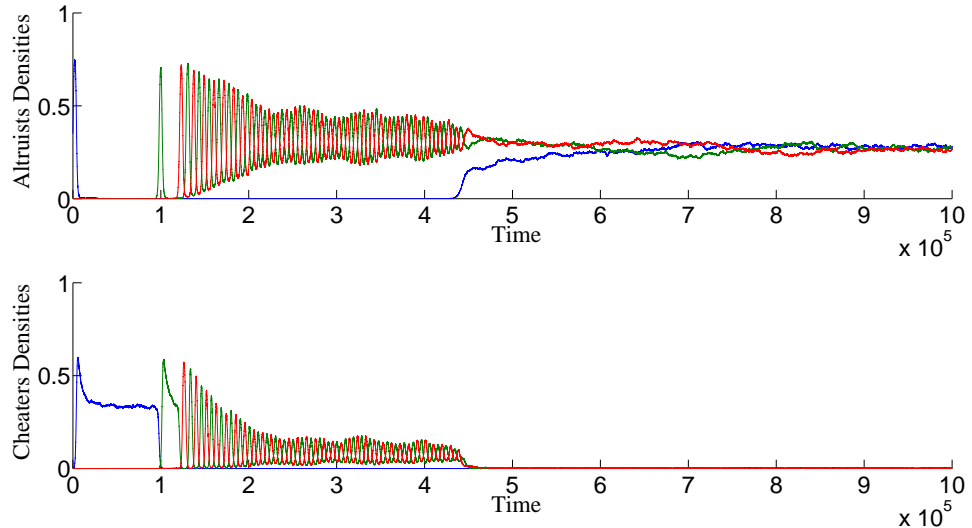


Figure 5.8: Stochastic simulation of the metapopulation model. The top graph corresponds to the densities of the different subpopulations of altruists, and the graph below to the subpopulations of cheaters. We start with a single population of altruists. The dynamics is stable with a single type, then it switches to a cycling behaviour when $n = 2$, and finally goes to an all-altruist diverse equilibrium without cheaters. Thus the behaviour of the system depends on the number of different types of subpopulations. Parameters: $b_A = 5.5$, $b_C = 1.5$, $m = 1$, $\phi = 5.5$.

cheaters of one type) changes the densities of the different altruists. The cheater equilibrium does not exist however and cheaters do not persist in the metapopulation. But by considering that cheaters benefit from the siderophores already present in a compatible altruist patch, by producing more propagules ($\phi > 1$), we found that a non zero equilibrium for altruists and cheaters can exist if ϕ is sufficiently high. However, this equilibrium is neither stable nor unstable, and the dynamics show oscillating behaviours. By introducing mutations, we found that the system becomes stable in both cases: if ($\phi = 1$), mutations stabilise the equilibrium, by allowing a continuous and very low density of cheaters patches to exist. If ($\phi > 1$), the cycling pattern attenuates and eventually disappears.

Our results concur and complete the results obtained in Lee et al. (2012). We previously found that cheaters could not persist in a diverse metapopulation if we assumed that cheaters invade unoccupied and altruist patches with the same rate. This need not be true as cheaters potentially could invade altruist patches more easily because of the siderophores present in these patches. By relaxing this as-

sumption, we uncovered a richer dynamics than in the original model. The type of dynamics depends on the diversity in the metapopulation: it is possible to have coexistence of different altruists and cheaters for a low to intermediate level of diversity, but eventually the cheaters disappear as diversity increases and the system reverts back to a metapopulation dominated by different types of altruists, subjected to genetic drift and regulated by cheaters. Our variant is thus robust with respect to the influence of ϕ .

Siderophore diversity is a form of kin recognition. It has been stated that kin recognition erodes the diversity that underlies the recognition system, a result known as Crozier's paradox (Crozier, 1986). In the model studied here we found that the diversity is protected by the presence of cheaters. The way this works is that, after diversity has increased, the dynamics settle on a state in which the altruist populations consist of a more or less evenly distribution of siderophore types. Once a single type gets too dominant, cheaters appear that will reduce the density of this siderophore type, restoring the more or less even distribution. As in Jansen and van Baalen (2006) and Lee et al. (2012) the diversity of recognition signals is maintained through chromodynamics: a dynamic process of regulation. This provides a solution to Crozier's paradox. We argue that this non-equilibrium process could regulate the diversity in many species.

Cheaters are rare in natural populations (West and Buckling, 2003). We hypothesise that this is because the level of diversity is high in these natural populations. It is thus not likely to observe cycles of cheaters invasions in these conditions. However, it would be possible to observe these cycles in non natural conditions, for example in populations of human patients suffering from cystic fibrosis. Indeed, infection control measures are often set up in order to prevent cross infections from patient to patient (Saiman and Siegel, 2004), so that contacts between patients are limited. Observing these cycles could also be possible in experimental conditions, where the diversity can be artificially maintained at intermediate levels.

Our model predicts that the level of cooperation depends on the diversity. The

hypothesis can be easily verified by setting experimental metapopulations of altruists and cheaters with different levels of diversity, and measure the frequency of the altruists. We predict that cheaters should dominate at low diversity levels, and disappear when diversity increases. This result is similar to Jansen and van Baalen, 2006's study of beard chromodynamics, who found that altruism is facilitated when many types coexist in the population.

Finally, in our study, we set arbitrarily the ϕ parameter. However, it is possible that ϕ depends on b_C , because it represents the increased capacity of dispersal. More precisely, ϕ should be small if the propagules productions of altruists and cheaters are close, and it should be higher when the cheaters rate of propagule production is very different from the altruists'. It would also be interesting to have a measurement of its value. If we suppose that the number of propagules produced in a patch depends on the density of bacteria, this would be simple: we would just need to determine the density of cheaters when invading an empty patch, and compare it to the density of cheaters when invading a patch of altruists.

5.5 Appendix

5.5.1 Linearisation of the complete system with mutations

We can determine the values of A_i and C_i at equilibrium, denoted \bar{A} and \bar{C} :

$$\begin{cases} \frac{dA_i}{dt} = 0 \iff \bar{C} = \frac{\bar{A}(b_A - \epsilon - nb_A\bar{A} - m)}{\bar{A}(b_A + \phi b_C) - \delta} \\ \frac{dC_i}{dt} = 0 \iff \bar{A} = \frac{\bar{C}(b_C - m - \delta - nb_C\bar{C})}{-\epsilon + \bar{C}(-b_A - \phi b_C + nb_A + nb_C)} \end{cases} \quad (5.30)$$

We then linearise and simplify the system. We note $A_{i_{Lin}}$ and $C_{i_{Lin}}$ the linearised approximations of A_i and C_i respectively:

$$\frac{dA_{iLin}}{dt} = -b_A A_{Lin} \bar{A} - \bar{A} C_{iLin} (b_A + \phi b_C) + \delta \left(\frac{(C_{Lin} - C_i)}{n-1} - \frac{\bar{C} A_{iLin}}{\bar{A}} \right) \quad (5.31)$$

$$\frac{dC_{iLin}}{dt} = -b_C C_{Lin} \bar{C} + (b_A + \phi b_C) (A_{iLin} - A_{Lin}) \bar{C} + \epsilon \left(A_{iLin} - \frac{\bar{A} C_{iLin}}{\bar{C}} \right) \quad (5.32)$$

We could determine the Jacobian matrix from these linearised equations for any number of types, but it becomes increasingly difficult for high n . Alternatively, it is possible to describe the system with new variables: the total density of altruists and cheaters, denoted A and C , and the deviation from the mean, denoted A' and C'

First we determine the dynamics of the total density of altruists:

$$\frac{dA}{dt} = \left(-nb_A \bar{A} - \delta \frac{\bar{C}}{\bar{A}} \right) A + (\delta - \bar{A}(b_A + \phi b_C)) C \quad (5.33)$$

Similarly we determine the dynamics of the total density of cheaters:

$$\frac{dC}{dt} = ((b_A + \phi b_C)(1-n)\bar{C} + \epsilon) A + \left(-nb_C \bar{C} - \epsilon \frac{\bar{A}}{\bar{C}} \right) C \quad (5.34)$$

The dynamics for the deviation from the mean for the altruists is

$$\frac{dA'}{dt} = \frac{d\left(\frac{A}{n} - A_i\right)}{dt} = -\delta \frac{\bar{C}}{\bar{A}} A' + \left(-\bar{A}(b_A + \phi b_C) - \frac{\delta}{n-1} \right) C' \quad (5.35)$$

The dynamics for the deviation from the mean for the cheaters is

$$\frac{dC'}{dt} = \frac{d\left(\frac{C}{n} - C_i\right)}{dt} = (\bar{C}(b_A + \phi b_C) + \epsilon) A' - \epsilon \frac{\bar{A}}{\bar{C}} C' \quad (5.36)$$

The Jacobian matrix is thus

$$J = \begin{bmatrix} -nb_A \bar{A} - \delta \frac{\bar{C}}{\bar{A}} & \delta - \bar{A}(b_A + \phi b_C) & 0 & 0 \\ (b_A + \phi b_C)(1 - n)\bar{C} + \epsilon & -nb_C \bar{C} - \epsilon \frac{\bar{A}}{\bar{C}} & 0 & 0 \\ 0 & 0 & -\delta \frac{\bar{C}}{\bar{A}} & -\bar{A}(b_A + \phi b_C) - \frac{\delta}{n-1} \\ 0 & 0 & \bar{C}(b_A + \phi b_C) + \epsilon & -\epsilon \frac{\bar{A}}{\bar{C}} \end{bmatrix} \quad (5.37)$$

5.5.2 Determination of the sign of the determinant

We look at the value of C_i at equilibrium (equation 5.15), which rewrites as

$$\bar{C} = \frac{b_A(b_A + \phi b_C - b_A n) - m(b_A + \phi b_C - b_C n)}{(b_A + \phi b_C - b_C n)(b_A + \phi b_C - b_A n)} \quad (5.38)$$

The numerator corresponds to the condition of equation (5.17) and the denominator is proportional to the determinant of equation (5.24). We next determine if \bar{C} exists when the determinant is negative. Let a new variable $x = b_A + \phi b_C$. Equation (5.38) rewrites as

$$\bar{C} = \frac{b_A}{x - b_C n} - \frac{m}{x - b_A n} \quad (5.39)$$

The derivative of \bar{C} with respect to x is

$$\frac{d\bar{C}}{dx} = \frac{m}{(x - b_A n)^2} - \frac{b_A}{(x - b_C n)^2} \quad (5.40)$$

Solving for 0 equation (5.40) gives 2 solutions, but only one with a negative denominator for equation (5.38). The solution is

$$x_0 = -\frac{n(\sqrt{b_A m}(b_A - b_C) + b_C m - b_A^2)}{b_A - m} \quad (5.41)$$

Which corresponds to a minimum for \bar{C} . Hence the minimum value of \bar{C} , called \bar{C}_0 is

$$\bar{C}_0 = \frac{b_A + m + 2\sqrt{b_A m}}{(b_A - b_C)n} \quad (5.42)$$

\bar{C}_0 is an increasing function of b_C , so the lowest value of \bar{C}_0 is obtained when $b_C = 0$.

Equation (5.42) thus rewrites as

$$\bar{C}_0 = \frac{1}{n} + \frac{m}{b_A n} + \frac{2\sqrt{b_A m}}{b_A n} > \frac{1}{n} \quad (5.43)$$

The minimum value of \bar{C} is superior to $\frac{1}{n}$, which is impossible because $\frac{1}{n}$ is the maximum frequency of one type at equilibrium. Thus \bar{C} does not exist when the determinant (Equation 5.24) is negative. Hence the determinant is always positive.

Chapter 6

Conclusion

In this thesis I investigated the effect of diversity on the evolutionary dynamics in systems where altruists compete with cheaters. In both gynodioecious plants and siderophore producing bacteria, diversity arises through an incompatibility mechanism, so that the public good is in fact a restricted good, that can be used only by individuals capable of recognising it. Rather than being characterised only by the strategy (cooperation or cheating), individuals are characterised by both a strategy and a type. Diversity is generated through mutations changing the strategy and/or the type of public good.

Rather than using classic models I have used biologically realistic examples and explicit models of interactions between altruists and cheaters via the exploitation of a public good. Previous studies (for instance Frank [1998], West and Buckling [2003], Brown et al. [2009]) were using very strong ecological assumptions. In contrast, I tried to stay as close as possible to the ecology of these organisms, which allowed me to make important findings. In particular in Chapter 3, I derived both relatedness and Hamilton's rule directly from ecological principles, instead of simply assuming them. In Chapter 3 and 4 I modelled the local dynamics which were crucial to understand the global dynamics.

I found that populations rarely stay monomorphic when mechanisms of incompatib-

ity exist: diversity arises readily in most conditions. The level of diversity depends on different factors: in Chapter 2 I found that it depends on the spatial structure, determining the “balance of power” between altruists and cheaters. If it’s biased towards altruists (with low dispersal rates) or cheaters (with high dispersal rates) the diversity is low. Diversity is highest when altruists can be invaded but not overwhelmed by cheaters, and similarly when new altruists invade without erasing all the previous established diversity. In Chapter 4, I found that the level of diversity depends on the mutation rates. In particular, high mutation rates lead to high levels of diversity. In fact, the mutation rates also determine the balance of power between altruists and cheaters: they control how fast a cheater can appear in a population of altruists and how fast a new altruist appears in a population of cheaters.

I argue in this thesis that diversity results from non-equilibrium processes. When there are no incompatibility mechanisms, the population dynamics are stable. But when we include these mechanisms, the dynamics become unstable. In Chapter 2, the instability arises from the competition for space: cheaters invade rapidly altruists, so altruists must constantly disperse away from the cheaters. In these gynodioecious plants, the females cannot survive without the public good (pollen), so they also have to “follow” the hermaphrodites in space. In Chapter 4, I found that the instability arises from the associations of genetic drift and mutations. Indeed, genetic drift leads to frequency fluctuations of altruist populations. When a particular strain of altruists become too numerous, a compatible cheater appears through mutations, and regulate the frequency of the altruist. This mechanism maintains the diversity, but the resulting dynamics are very unstable because populations are regulated by a stochastic process. My results suggest that biodiversity is regulated by non-equilibrium processes. They also suggest that to understand better how diversity works, one must also look at the composition of the populations over time and not only through simple “snapshots”.

The theoretical idea behind this thesis is the chromodynamics. As I have showed in Chapter 2 and 4, this leads to a kind of Red Queen race, where no individuals

or groups are constantly better overall and through time. The results of this race is that it might lead to the appearance of organisms with high mutation rates. Indeed in the chromodynamics the advantage occurs when novelty arises. High mutating organisms should thus be favoured, and in fact have been discovered in the biological systems I studied, but further investigations need to be led on other similar systems to determine if this is a common feature.

In this thesis I extended the pioneering studies of chromodynamics to real biological examples. I have used realistic models and related most results to the biology and ecology of the studied species. This suggests that the chromodynamics might not only be a theoretical toy but a real ecological mechanism that takes place in Nature. The main insight I obtained is that I discovered a novel evolutionary mechanism generating and maintaining diversity in Chapter 4, which could pave the way for future theoretical and experimental studies in many systems.

To conclude I argue that the emergence of diversity should be quite common in this type of system. This thesis provides a basis and framework for future studies on the emergence and maintenance of diversity. In some related systems, such as the *FLO1* gene causing flocculation in *saccharomyces cerevisiae* and the *csA* gene causing adhesion in *Dictyostelium discoideum*, diversity has not been reported. Whether this is because studies are not yet focused on diversity or because there exists a mechanism preventing its emergence remains to be investigated.

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