Competition and Cooperation in Host-Associated Microbial Communities: Insights from Computational and Mathematical Models.

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Statement of Originality

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated in the text. This work has not been submitted for any degree or professional qualification except as specified.

Jonas Schluter, Hilary 2014

For Michelle

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Publications and statement of authorship

The majority of each chapter in this thesis has been my own work. I have received help from collaborator as detailed below. Chapter 4 has been published as:

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Kevin Foster was involved in the interpretation of results and has commented on the manuscript.

The unpublished chapters 2, 3 and 5 are my own work but have benefitted from the help of others. In particular, in chapter 2 Armin Schoech, a summer student, conducted a pilot study not contained in the current chapter, Sara Mitri and Kevin Foster contributed to the study design and commented on the manuscript. Chapter 3 has led to testable predictions and the resulting experiments were performed by Carey Nadell in the laboratory of Bonnie Bassler at Princeton. I have been involved in the design, data analysis and writing up of the experimental work presented in this chapter but have not performed any of the lab work myself. Kevin Foster has commented on the manuscript. Finally, in chapter 5, Katherine Coyte has helped with the analysis and Kevin Foster with comments on the manuscript.

Further, I have contributed a quantitative model to a large collaborative project which is currently under review.

Abstract

Our bodies contain a vast number and diversity of microbes. These microbes interact, and these interactions can define how microbes affect us. Microbial ecology and evolution, therefore, are important for both microbiology and human health. However, our understanding of microbial communities remains limited. There is a need for theory that dissects the complexity and identifies the key factors and processes affecting microbial groups. Here I develop realistic computer simulations and population models of microbial communities. My first project seeks to explain microbial communication (quorum sensing) and argues that quorum sensing is a way to infer when competing genotypes are no longer a threat. The second project proposes an evolutionary explanation for another major microbial trait: adhesion. I argue that adhesion is a weapon allowing cells to compete within microbial groups and push competitors out, particularly when growing on a host epithelium. The third project moves from microbes to the host and asks whether a host can control which microbes grow and persist inside it. I develop a model of the human gut epithelium and show that the gut architecture amplifies the ability of hosts to select helpful microbes over harmful ones using nutrient secretion. In addition to selecting particular microbial strains, a host will also benefit from stable symbiotic communities that behave in a predictable manner. But what determines whether host-associated communities are ecologically stable? My final project uses ecological network theory to show that ecological stability is likely to be a problem for gut communities that are diverse and contain species that cooperate with each other. However, I argue that the host should function as an ecosystem engineer that increases ecological stability by weakening the strong dependence of cooperating species upon one another. While host-associated communities are complex ecological systems, my thesis identifies key factors that affect their form and function.

Contents

1 Introduction

Contents

1.1 Why care about microbes.

Microbial life is ubiquitous - virtually every habitable environment is colonised by microbes (Martiny et al. 2006). The world of microbes has profound effects on humans, both positive and negative, and understanding microbial evolution will allow us to better harness their useful abilities or fight them when they attack us.

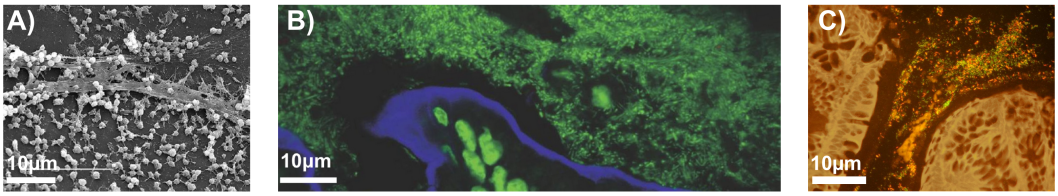

Figure 1.1: **Microbes affect human life.** A) *Staphylococcal* cells growing on a medical implant, scanning electron micrograph from Donlan 2002 and therein used with permission of Lippincott William & Wilkins. B) Confocal fluorescence image of bacteria (green), epithelial border brush (blue) and the gut epithelium (large green cells) separated by mucus (transparent). From Stecher et al. 2007. C) Bacteria of the normal human gut microbiota (different colours) on the gut epithelium (grey-orange). Image taken from a human cecal biopsy, personal communication with Alexander Swidsinski. URL: www.charite.de/arbmkl/themen/ubersicht/intestinalmicrobiota.pdf

In order to better understand microbial evolution, we need to know how they live in natural environments. It is now clear that in most environments, microbial species mix with many others (Lozupone & Knight 2007) and live highly "social" lives as opposed to being solitary, individualistic cells they were once thought to be (Cornforth & Foster 2013, Mitri & Foster 2013, West et al. 2006). It is most useful to think of sociality between single cell organisms by considering how a microbial trait affects the fitness of the focal and other cells. Then, we can categorise social traits as either *mutually beneficial* (actor/recipient: $+/+$), *selfish* ($+/-$), *altruistic* ($-/+$), or *spiteful* ($-/-$) (West et al. 2006).

Another common characteristic of microbial communities is high cell density. For example, a single gram of soil can contain up to $10^{10} - 10^{11}$ microbes (Curtis et al. 2002). Record densities of microbes, however, have been found to exist in all of us. The gastrointestinal tract of mammals is colonised by up to $10^{11} - 10^{12}$ cells per gram (Figure 1.1, Ley et al. 2006).

Microbes then do not only interact among themselves but also with multicellular host organisms (Bevins & Salzman 2011, Hooper et al. 1999, Ley, Lozupone, Hamady, Knight & Gordon 2008, Lozupone, Stombaugh, Gordon, Jansson & Knight 2012). How do such microbes affect their host? Multicellular hosts and microbial communities are tightly associated and evolve together (Ley, Lozupone, Hamady, Knight & Gordon 2008). This is dramatically reflected in most recent discoveries that show how normal and healthy development of the human body is dependent on the presence of microbial communities, collectively called the microbiota (De Vadder et al. 2014, Round & Mazmanian 2009, Sommer & Bäckhed 2013). Relationships between microbes and their hosts can be beneficial for the host, as occurs in the gut where microbes provide us with nutrients and protection (De Vadder et al. 2014, Ley et al. 2006, Sekirov et al. 2010). On the other hand, many microbes adopt a pathogenic lifestyle and harm their hosts which can fight back and try to kill or rid themselves of those invading microbes (Dethlefsen et al. 2007, Salzman 2010).

Two critical aspects of microbial life emerge that I will be focussing on in this thesis: 1) high microbial cell densities, and 2) extensive social interactions among and between microbes and hosts. Experimental microbiology has focussed on lab cultures where species grow in clonal populations. Working with such mono-cultures is easier but will inevitably miss most of a microbe's real ecology. Investigating natural microbial ecologies in the lab is further hampered by the fact that up to >99% of bacteria cannot be cultivated (Amann et al. 1995). But how can we hope to understand microbial ecology and evolution if

both are so dependent on inter–species interactions?

One way is to reduce complexity of real microbial communities by constructing mathematical and computational models. These have proven powerful tools to gain better understanding of fundamental dynamics that arise through microbial interactions (Brown 1999, Bucci et al. 2012, Cornforth et al. 2012, Mitri et al. 2011, Nadell et al. 2010, 2008, Xavier & Foster 2007). In this thesis, I develop theoretical models of microbial ecology and evolution. For the most part I will be focussing on high density, surface attached microbes - a typical form of microbial life in hosts as well as in abiotic environments. In the final results chapter I develop more abstract population models of microbes that live in the gut.

All of these models rest upon their relevance for the biology of microbes. For the remainder of the introduction, therefore, I will consider what we know about microbial communities, their ecology, and the interactions between their members. First, I will begin by discussing the interactions between microbial strains and species and how one can use social evolution to dissect and understand these interactions. Second, I will consider a key ecological context of microbes that is a focus of my thesis: microbial communities in association with a host organism and how this can affect their ecology and evolution. Finally, I discuss biofilms – a common form of microbial life within and outside of multicellular hosts – and briefly review previous models thereof.

1.2 Sociality among microbes.

Lozupone & Knight have conducted a meta-analysis from 111 different, globally

distributed environments and created a tree of microbial diversity from 21752 sequences (2007). This provided an overview of microbial diversity across environments. For example, the soil is particularly species-rich compared with other environment which indicates a highly diverse community. However, many of these species are genetically very similar (Faith 1992, Lozupone & Knight 2007) . Such co-occurrence of many highly related species is consistent with a strong metabolic overlap and similar nutrient requirements (Mitri & Foster 2013). This suggests that microbes will often compete for nutrients and space with surrounding cells, something that was also suggested by a recent empirical survey of competition between microbial species from a single environment (Cornforth & Foster 2013, Foster & Bell 2012, Mitri & Foster 2013). Strong competition is further indicated by the armoury of secretions that microbes possess, such as toxins and antibiotics, which can negatively affect other cells' growth (Cornforth & Foster 2013, Foster & Bell 2012, Mitri & Foster 2013). Secretions usually come at a cost because their gene expression diverts resources away from pure growth. Toxin secretion, therefore, can be considered spiteful because both the actor and the recipient of this interaction are negatively affected (West et al. 2006).

Beside such microbial warfare (Czárán et al. 2002, Keller & Surette 2006), microbes also have the potential to communicate and cooperate with other cells (Darch et al. 2012, Fuqua et al. 1994, Nogueira et al. 2009, Popat et al. 2012, West et al. 2007, 2006). A well-known example is quorum sensing (microbial communication via diffusible molecules) regulated secretion of proteases that break down proteins in the environment where resulting amino acids then are

available to all cells in the vicinity (Brown & Buckling 2008, Diggle, Griffin, Campbell & West 2007). This then is a cooperative phenotype through which actors and recipients can both gain a benefit. Mutually beneficial relationships between microbes do not always require secretion of public goods (Mitri & Foster 2013). An example of secretion-independent cooperation is when microbes switch their metabolic state between fast growth where each unit of consumed nutrients yields little new biomass (wasteful, but rapid growth), and prudent nutrient utilisation with high yield, albeit at a slower rate (MacLean & Gudelj 2006). The latter strategy is a cooperative phenotype because it utilises a common resource most efficiently.

Akin to the classical "tragedy of the commons", costly cooperative traits can be fragile in the face of competition (Diggle, Griffin, Campbell & West 2007, Griffin et al. 2004, Hardin 1968, MacLean & Gudelj 2006, Van Dyken et al. 2013). For example, consider a focal "cheat" cell that is surrounded by others who secrete public goods such as proteases that release nutrients from the environment. The focal cell consumes those nutrients but does not itself invest in enzyme secretion and, therefore, divides more rapidly than its neighbours. The cheater genotype then increases in frequency and cooperation collapses which can lead to the eventual eradication of the cooperative trait from the gene pool. How is it then that we see so much evidence for cooperation among microbes (Brown & Buckling 2008, Crespi 2001, Diggle, Gardner, West & Griffin 2007, West et al. 2007, 2006)?

Inclusive fitness theory is the most useful formalism to predict the evolution of social traits. It states that a cooperative trait can evolve, independent of

the cost to the actor genotype, when its inclusive fitness effect on the actor is greater than zero. This is formalised by Hamilton's rule (Hamilton 1964)

$$rb - c > 0,$$

which says that for a trait to be under positive natural selection, the fitness cost c incurred by a focal individual has to be less than the benefit b of recipients weighted by their relatedness r to the actor. Indeed, in one of the most dramatic examples of altruism, the cell-suicide in slime moulds of *Dictyostelium discoideum*, dead cells form a rigid stalk which allows genetically highly similar cells to spread to new environments, thereby outweighing the evolutionary cost of cell suicide.

It is clear then that sociality among and between microbial species is complex and under strict constraints. Mutually beneficial relationships within species are less difficult to evolve than between species because of higher relatedness. However, the latter form of cooperation can happen by chance: an "accidental" +/+ relationship may arise, for example, when microbes form cross-feeding networks[1] where one species feeds on the by-products of another species (Mitri & Foster 2013, West et al. 2006). This is generally considered

[1]Cross-feeding is usually not considered *cooperation* in social evolution theory as neither partner bares a cost and instead directly benefits from their respective behaviour implying fewer evolutionary constraints than costly cooperation (Mitri & Foster 2013, West et al. 2006). For a cross-feeding relationship to be considered cooperative, it is required that both species have evolved increased by-product secretion, beyond the amount that would selfishly be required, and in anticipation of returned benefits from their respective interaction partner. It is important to be aware of this distinction when investigating the evolution of cooperation. However, in my thesis, especially in chapter 5, I will be calling any +/+ interaction *cooperation* and clarify when necessary. The reason is that in chapter 5, I am not investigating the evolution of cooperative traits explicitly, but rather assume their existence and ask ecological questions at the ecosystem level about the effect +/+ interactions between species can have. I there use the term *cooperation* loosely but in accordance with standard nomenclature in the relevant literature (Allesina & Tang 2012, Mougi & Kondoh 2012) because the focus is not on how the +/+ interactions came about or may be maintained.

an important mechanism among gut microbes. Complex food sources from the host's diet are broken down step-by-step in cross-feeding networks of beneficial microbes. Eventually, this yields additional energy for the host from otherwise indigestible nutrients (Belenguer et al. 2006, Ze et al. 2012, 2013).

The helpful function of microbes in the gut has led to the interpretation that the microbial community in the gut "*can be pictured as a microbial organ [...] (that) endows us with functional features that we have not had to evolve ourselves*" (Bäckhed et al. 2005). A collection of independent organisms such as the microbiota will have different evolutionary constraints than an actual organ of the human body and I therefore disagree with this point of view and take such statements as indicators that the inevitable competition among microbes in the microbiota is often underestimated. It is far from being understood how dynamically changing microbial communities and in turn microbiota-host relationships evolve, and there are only few theoretical approaches to these problems yet (Bucci et al. 2012, dos Santos et al. 2010, Foster et al. 2008, Karlsson et al. 2011, Schluter & Foster 2012, Stein et al. 2013). Besides between-microbe interactions, host-microbe interactions, too, are plentiful, but it is clear that microbes in our body are not there simply to fulfil our metabolic needs. Instead, the host appears to actively keep its microbiota at bay to protect itself (Salzman 2010), and enforces a useful species composition despite enormous microbial loads and diversity (Hooper & Macpherson 2010, Ley et al. 2006, Schluter & Foster 2012).

1.3 Host-microbe interactions

Virtually every surface of the human body is home to its own complex community of microbes, collectively called the "microbiome". High-throughput sequencing data reveals mind-boggling facts: there are $\tilde{1}00$ trillion microbes inhabiting the average human body - this is ten times more than there are human cells - and the total number of microbial genes in the human microbiome exceeds the number of human genes by even larger magnitudes (Hooper & Macpherson 2010, Ley et al. 2006). Skin (Grice et al. 2008), dental and oesophageal (Pei et al. 2004), vaginal (Macklaim et al. 2011) and gut microbiota (Eckburg et al. 2005) all differ from each other (Reid et al. 2010), and have species compositions distinct from those found in non-host environments such as soil or sea water (Ley, Hamady, Lozupone, Turnbaugh, Ramey, Bircher, Schlegel, Tucker, Schrenzel, Knight & Gordon 2008).

The gut stands out as the by far most populous microbial habitat in the human body. It dawns upon us that this microbial "*world within a world*" (Ley, Lozupone, Hamady, Knight & Gordon 2008) in our bodies has profound effects on human health and development. For example, it has been shown that obesity (Bäckhed et al. 2004, Turnbaugh et al. 2009, 2006), normal development of the immune system (Mazmanian et al. 2005, Round & Mazmanian 2009), inflammatory bowel diseases such as Crohn's disease (Mazmanian et al. 2008, Wehkamp et al. 2005), colorectal cancer (Hu et al. 2013, Tlaskalová-Hogenová et al. 2011), neuronal development (Diaz Heijtz et al. 2011), and even neuronal disorders such as autism (Hsiao et al. 2013) correlate with changes in gut microbiota species composition. While often the reason for these correlations

remains unclear, those studies highlight the importance of understanding our microbial cohabitants.

What factors determine which species live in the gut? First, the type of food consumed by the host has significant effects on the microbes living off those nutrients (David et al. 2013, De Vadder et al. 2014, Faith et al. 2011, Muegge et al. 2011). For example, herbivores and carnivores have been shown to form separate clusters when analysing microbiota of various species (Ley, Lozupone, Hamady, Knight & Gordon 2008). There is currently a hunt for a "core microbiome" of humans trying to identify a minimum set of microbes that will make a functional microbiota. These studies have shown that a "core microbiome" is in fact rather thought of as a set of metabolic capabilities of a microbiota than specific species (which clearly contradicts the idea of a microbial organ). Species compositions between individuals vary, but the sets of catabolic genes in different human microbiota are very similar (Turnbaugh et al. 2009). The gut environment appears to consist of a range of ecological niches that may be occupied by slightly different species with similar niche specialisation. Such an ecological perspective fits with data showing that sudden changes in diet can alter microbiota compositions, presumably by widening or shrinking niches. Accordingly, sudden diet changes tend to shift abundances of species from before and after the change rather than altering the identities of species present (David et al. 2013). Importantly though, while the microbiota between healthy humans may differ, the majority of species within an individual stays stable even over years (Costello et al. 2009, Dethlefsen & Relman 2011, Faith et al. 2013, Vanhoutte et al. 2004).

How is stability and identity of species in the gut maintained? Besides food, the host actively influences species compositions in the gut. This is mostly achieved by the epithelium, the first point of contact between the body and the gut microbiota. Epithelial secretions comprise structural compounds in form of mucus that functions as a physical barrier to separate host tissue from microbial cells (Derrien et al. 2010, Johansson et al. 2008). Further, the host secretes toxins and antibodies that can kill microbes but also, astonishingly, releases nutrients from the epithelium (Hooper 2009, Macpherson 2000, Macpherson et al. 2005, 2008, Macpherson & Uhr 2004, Petnicki-Ocwieja et al. 2009, Salzman et al. 2010, Wehkamp et al. 2004, 2005). Additionally, the mucus itself may serve as nutrients for microbes (Derrien et al. 2010) and we have shown how this feeding of the microbiota may be a powerful tool to select helpful microbes (chapter 4, (Schluter & Foster 2012)). Understanding internal microbiota dynamics and host control that together determine composition and stability of the microbiota opens exciting research questions and the need for novel theoretical explanations has been recognised (Foster et al. 2008, Gordon & Klaenhammer 2011) and they are on the way (Bucci et al. 2012, Schluter & Foster 2012, Stein et al. 2013).

To develop biologically relevant models of microbe–microbe and microbe–host dynamics, we have to consider the spatial ecology of the gut. Genomic data provides information about species identities but cannot inform on the spatial organisation of cells. Spatial structure is pivotal when analysing social traits as it affects the critical relatedness factor, r, in Hamilton's rule (Frank 1994, Hamilton 1964, Korolev et al. 2011, Nadell et al. 2010). There are a few

attempts at microscopic imaging of microbial communities in the gut (Bollinger et al. 2007, Palestrant et al. 2004, Stecher et al. 2007). It appears though that this is difficult and that there are no clear protocols on how to best preserve the microbes in their natural state (Bollinger et al. 2007). However, those microscopic studies show that microbes near the gut epithelium can occur in biofilms. Life in biofilms imposes different evolutionary constraints on social interactions between microbes compared with free-floating cells in well-mixed environments (Nadell et al. 2009). It is clear then that understanding biofilms is important to understand host–microbiota interactions as well as evolution of sociality among microbes.

1.4 Biofilms - a common form of microbial life

Biofilms are "*a collection of microorganisms and their associated extracellular products at an interface and generally attached to a biological or abiological substratum*" and are thought to contain the majority of microbial life on earth (Palmer & White 1997). Microbes attach to all kinds of surfaces - inorganic or organic (Donlan 2002, O'Toole et al. 2000, Reid et al. 2010). Host-beneficial microbes not only form biofilms in the gut but also, for example, on plant roots in the soil (Beauregard et al. 2013, Palestrant et al. 2004, Vlamakis et al. 2013). Further, many pathogens live in biofilms on host tissues (Dapa et al. 2013, Stapper et al. 2004, Tamayo et al. 2010) or as contaminants on medical implants (Costerton et al. 2005). Cells in a biofilm are held together and adhere to surfaces with extracellular polymeric substances (EPS), which encompass polysaccharides, proteins, and nucleic acids (Berk et al. 2012, Petrova & Sauer

2012, Stoodley et al. 2002). EPS increases the rigidity of the biofilm and protects it from toxins in the environment (Romero et al. 2010). This results in much higher antibiotic resistance of cells in a biofilm compared with free-swimming cells (Costerton et al. 2005), which poses an immediate threat to human health as the resilience of biofilms makes them hard to eradicate (Mah & O'Toole 2001). Given that a large proportion of microbial life and reproduction takes place in biofilms, any trait that influences fitness here will be under strong natural selection. Understanding biofilm evolution is therefore important for our understanding of microbial life in general and to improve human health in particular.

The standard model for biofilm formation distinguishes discrete stages. At first, cells attach to a substratum in a reversible manner. This is followed by one of at least three possible aggregation mechanisms: cells may move over the surface and group up (O'Toole & Kolter 1998), they may recruit further cells from the surrounding environment (Tolker-Nielsen et al. 2000) or begin to divide and increase in number while adhering to the substratum (Stoodley et al. 2002, Tolker-Nielsen et al. 2000). Once a biofilm has been firmly established, the maturation of the biofilm follows during which cells grow and replicate. This stage then will be especially important from an evolutionary perspective, in particular when different genotypes compete within the biofilm. My work, therefore, will be focusing on biofilm growth through division rather than attachment of cells from the environment. Eventually, the life-cycle of a biofilm concludes in dispersal and/or release of cells (Stoodley et al. 2002).

Because of their impact on human lives, biofilms have been studied ex-

tensively both in the lab and with mathematical and computational models. Biofilms in the lab are typically grown in flow chambers where a nutrient containing medium flows over an inert substratum to which cells adhere. Typically, these studies include only one or at most few different genotypes while real biofilms often contain multiple species (Hall-Stoodley et al. 2004, Mitri et al. 2011, Nadell & Bassler 2011). Mathematical and computational models , on the other hand, are often only concerned with the physical mechanisms that determine biofilm growth (Alpkvist et al. 2006, Klapper & Dockery 2010, Kreft et al. 2001, Picioreanu et al. 2001, Ward et al. 2003, Xavier et al. 2005). The importance of competition and cooperation among microbes, which is often achieved through diffusible secretions, and the problem of having to consider many different species at once has opened a new avenue for computational models that explicitly resolve concentration gradients of such secretions and allow simulation of many species in one biofilm to investigate microbial ecology and evolution (Hense et al. 2007, Mitri et al. 2011, Nadell et al. 2010, 2008, Xavier & Foster 2007). I adopt this approach in my thesis and develop individual-based computational models of biofilms.

1.5 Models of biofilms

Much of the theoretical work on the growth of a biofilm originates from engineering problems due to their roles in bioremediation and in waste-water treatment (Xavier et al. 2007). First models of microbial biofilms were created using continuum models in one spatial dimension (Ward et al. 2003). These models are limited as they do not capture spatial complexity in biofilms be-

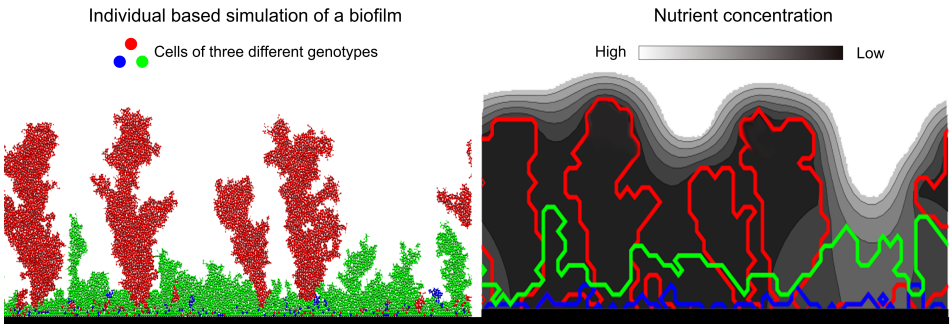

Figure 1.2: **Individual-based simulation of a biofilm.** Left: Cells of the red genotype dominate in the biofilm. Right: Nutrient gradients amplify small perturbations in the growing front and lead to bottlenecks that only allow few cells to continue to grow as other cells have no access to nutrients anymore (biomass silhouettes coloured corresponding to genotype).

yond stratification (Beun et al. 2001). Extension of similar partial differential equation models to higher dimension have subsequently provided insight into what factors create the macroscopic structures seen in biofilms. This has identified nutrient gradients above a growing biofilm as a key factor for structural development of the biofilm front (Nadell et al. 2010, Picioreanu et al. 2001).

These models demonstrate two key features of biofilm growth: First, spatial structure in biofilms is likely to be a result of small perturbations amplified through nutrient limitations which has been confirmed in experimental systems (Korolev et al. 2011). Second, those structures mean that there is strong potential for bottlenecks in growing biofilms – only a few cells will be fortunate enough to form a cluster that continues to grow towards the nutrients (Figure 1.1, Mitri et al. 2011).

This highlights a key limitation of continuum models when investigating the evolution of biofilms: To compete one strategy against another in an evolutionary game, we need to know who it is that makes it through the bottleneck and

succeeds. Due to their nature, continuum models do not easily allow tracking of an individual cell's fate. An important solution for this was the development of combinations of continuum with discrete models. Such individual-based hybrid models allow realistic simulations of microbial biofilm growth (Alpkvist et al. 2006, Kreft et al. 2001, Picioreanu et al. 1998, Xavier et al. 2007, 2005), but also can be used for the investigation of evolution in microbial communities (Mitri et al. 2011, Nadell et al. 2010, 2008, Xavier & Foster 2007). Here I adopt this approach and extend a software framework developed over the past 15 years (Kreft et al. 2001, Picioreanu et al. 1998, Xavier & Foster 2007, Xavier et al. 2005). Individual cells are defined explicitly and we can therefore now follow the "fate" of a single cell. This means that we calculate fitness of different cell types by tracing how many offspring each cell in a given simulation generates. By assigning different abilities ("genotypes") to the cells, we can compete different strategies against each other. For example, we can mimic the occurrence of a rare mutant in a group of cells and estimate its evolutionary success, or simulate multi-species biofilms which are difficult to investigate in experimental systems. In this thesis, I focus on simulations of biofilms where multiple genotypes mix. I develop a model that implements a host-microbiota system such as the gut and use it to investigate constraints for the evolution of microbial traits and host-microbiota interactions.

1.6 Thesis outline

In particular, I simulate competition and cooperation between microbes as well as how a host may exert control over its microbiota. Specifically:

- Chapter 2: Microbial communication via diffusible signalling molecules is called quorum sensing and regulates public good secretion in many microbes. It is unclear how this conveys a competitive advantage for microbes that compete with non-secretors or constitutive secretors in the setting of a biofilm. I present a possible mechanism that allows a quorum-sensing genotype to outcompete both, a constitutive secretor strain and a non-cooperating cheater strain and infer kinship from signalling molecule concentration.

- Chapter 3: A key feature of all biofilms is adhesion to surfaces and of cells to each other. While the ability to adhere is recognised as a key characteristic of cells in biofilms, the competitive effects of differences in adhesive properties between different genotypes within biofilms has not been investigated. I am simulating biofilms both on inert and on nutrient-rich host surfaces and show that on the latter, an increased ability to adhere can convey a strong competitive advantage making it a likely trait by which host-associated microbes can compete with each other. My predictions have been successfully tested in experiments conducted by researchers at Princeton and the results of those experiments are also included in chapter 3.

- Chapter 4: The host-microbiota relationship is assumed to be mutualistic. How this mutualism is maintained, however, is controversial. I show that the host needs to actively choose its partner microbes to maintain a "helpful" microbiota that competes with potentially faster growing

detrimental species. I create a model of the gut and simulate competing microbes that live in the mucus layer on the interface between the host epithelium and the gut lumen. I show how the architecture of this environment amplifies selective effects of epithelial secretions and hypothesise that nutrient secretion from the epithelium into the microbiota is particularly powerful at selecting helpful over non-helpful microbes.

- Chapter 5: The previous chapter presented a mechanism of host-selection in epithelium associated communities. How strongly this selection will translate into a host's ability to control the entire microbiota is unclear. I therefore develop an ecological network model of many microbial species and ask how the highly complex microbial community in a healthy human gut can remain so remarkably stable. It again emerges that feeding of microbes by the host epithelium may be a powerful tool to stabilise otherwise potentially fragile microbial communities.

2

The Evolution of Quorum Sensing as a Mechanism to Infer Kinship

2.1 Abstract

Bacteria regulate a large number of phenotypes via quorum sensing systems. Explanations for the function of quorum sensing focus upon cell density and concentration dependent benefits of the regulated traits. However, many evolutionary studies of quorum sensing focus on a separate issue: how does evolved quorum sensing remain stable in the face of cheater mutants? Here we combine these two perspectives using an individual-based model that captures the natural population structuring that occurs within dense bacterial communities. We first recapitulate the key argument for the origin of quorum sensing: quorum sensing allows effective use of products that are only useful at high concentrations. We then demonstrate the classical problem of competition and cheating for traits like quorum sensing. Finally, we show that combining these

two perspectives reveals a strong and evolutionarily stable benefit for quorum sensing in dense microbial communities like biofilms. In crowded environments, initially mixed groups of microbes tend to spatially segregate into patches of single genotypes. This leads to a benefit of quorum-sensing that is not directly linked to the inference of cell density. A quorum sensing cell that lands and is surrounded by competing genotypes will grow as fast as non-secretors and only activate costly secretions once safely surrounded by its own genotype. Our results suggest that quorum sensing can evolve because of the ability to infer the absence of competitors.

2.2 Introduction

Quorum sensing is used by microbes to regulate a large number of phenotypes. During growth, cells secrete autoinducers, which are small, diffusible compounds that accumulate in the environment. High autoinducer concentration around cells induces expression of many metabolically costly secretions (Bassler 2002, Bassler & Losick 2006, Cornforth & Foster 2013, Diggle, Griffin, Campbell & West 2007, Fuqua et al. 1994, Mitri & Foster 2013).The canonical explanation for the function of quorum sensing is that autoinducer concentrations can be used as a proxy for local cell density. Estimating the density of cells in a given environment allows microbes to tune the onset of the expression of secreted traits, such as digestive enzymes, so that they are only expressed when there are enough cells to make them useful (Diggle, Griffin, Campbell & West 2007, Fuqua et al. 1994). A proposed alternative explanation for quorum sensing is “diffusion sensing”, whereby the signalling molecules

are used to probe the mass transfer properties of the environment, such as diffusion rates, before investing in more costly secreted products that may quickly diffuse away (Redfield 2002). From the perspective of a cell perceiving autoinducer concentrations, these two measures are indistinguishable (Hense et al. 2007). Accordingly, a more recent proposal has been to view the mechanism of quorum sensing as "efficiency sensing", whereby a population of cells uses quorum sensing to avoid the secretion of products that will go to waste, either because too few cells are present, or because the products will diffuse away before they can be used (Hense et al. 2007).

The above discussions then focus on why cells benefit from regulating beneficial secretions in a density or concentration-dependent manner. Specifically they ask: why do quorum sensing strains perform better than strains that produce beneficial secretions all of the time? Other studies focus more upon a different question: why do quorum sensing strains perform better than cells that do not make beneficial secretions, often known as "cheater" genotypes (Brown 1999, Cornforth et al. 2012, Diggle, Griffin, Campbell & West 2007, Popat et al. 2012, Sandoz et al. 2007)? For example, in mixed liquid cultures, *Pseudomonas aeruginosa* cells with a defective *lasR* gene preventing them from responding to quorum sensing signals can outcompete cooperating wild-type cells (Popat et al. 2012). This is because the mutants have a higher growth rate since they do not produce the costly secretions, but can benefit from the secretions of wild-type cells. Quorum sensing then has the potential to be evolutionarily unstable in mixed genotype cultures and needs population structure that keeps different genotypes apart from one another (Brown 1999, Brown &

Johnstone 2001, Cornforth et al. 2012, Diggle, Griffin, Campbell & West 2007, Rumbaugh et al. 2012, Wilson 1975)

Microbes commonly live in highly diverse and dense communities where many different genotypes meet and compete (Cornforth & Foster 2013, Gans et al. 2005, Lozupone & Knight 2007, Roesch et al. 2007, Sekirov et al. 2010). What then maintains quorum sensing? One explanation is the spontaneous formation of clonal patches within microbial communities by cell division (also see (Dandekar et al. 2012)). As cells divide and grow in dense and nutrient-limited conditions, bottlenecks occur that cause genotypes to segregate into large clonal patches. Theoretical work suggested that this patch formation in microbial colonies can stabilise the use of beneficial secretions (Nadell et al. 2010), which has recently been verified experimentally (Datta et al. 2013, Van Dyken et al. 2013).

Here we investigate the costs and benefits of quorum sensing in diverse and dense microbial communities. We use a realistic individual-based model of microbes that captures key features of the natural ecology of microbial groups (Nadell et al. 2010). We first recapitulate the most cited benefit of quorum sensing in single genotype groups: it allows cells to respond to cell density and diffusive conditions. We then consider what happens when quorum sensing cells are surrounded by competing genotypes. These competitors can both be cells that constitutively produce beneficial secretions (cooperators) or non-producers that have the potential to act as cheater genotypes. This analysis shows how quorum sensing can evolve in dense and diverse microbial communities. However, this occurs via a benefit that is not associated with the inference of cell

density or diffusion. In competitive environments containing many different genotypes, quorum sensing can infer when a cell is surrounded by clonemates.

2.3 Results and Discussion

Quorum sensing increases performance in clonal groups.

According to the canonical view, quorum sensing allows cells to initiate the production of beneficial secretions only once other secretors are frequent enough for the group to benefit. We first test the prediction that this will allow quorum sensing cells (Q) to have an advantage over both constitutive secretors (C) and non-secretors (N). Starting from a single cell in a well-mixed, "liquid" environment (Materials and Methods), N cells grow and divide, such that the biomass of the population increases exponentially over the course of the simulation (Figure 2.1, green line). C cells, on the other hand, initially grow slower than N because a fraction of their growth is redirected into the secretion of a factor that helps all cells around them to grow. For example, this could be a protease that breaks down proteins into amino acids or peptide for import into the cell (Cicmanec & Holder 1979, Sandoz et al. 2007). After an initial phase of slow growth, C cells then experience a burst in growth once the concentration of the secreted factor is high enough to have an effect (Figure 2.1 A, blue line, Figure 2.1 C). The idea of this strongly density dependent benefit to secretions is consistent with experimental evidence (Darch et al. 2012, Koschwanez et al. 2011) and is in accordance with previous models (Brown & Johnstone 2001, Mitri et al. 2011, Nadell et al. 2010).

Q cells outcompete both N and C. In our model, quorum sensing cells se-

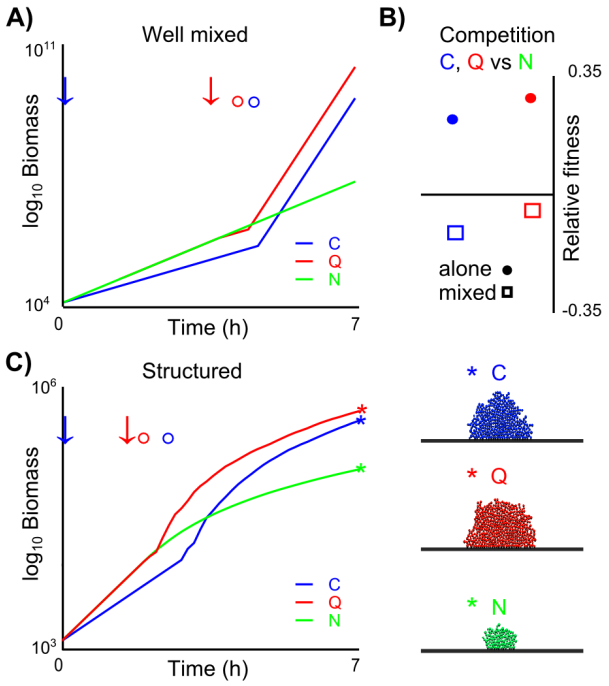

Figure 2.1: **Canonical QS.** A) Growth of biomass over time in simulations of well-mixed cultures of a constitutively public good secreting genotype (C, blue), a quorum-sensing genotype that only begins secreting public goods after a threshold concentration of the autoinducer has been reached (Q, red) and a control strain that does not produce any public goods (N, green). The arrows on the time axis indicate the onset of public good secretion by the two secretor genotypes, circles indicate the first time point at which the public good has reached above-threshold concentration in the simulation and cells begin to benefit from the public good. B) Relative fitness of C and Q (Material and Methods) compared with competitors of genotype N when growing alone (filled circles) or in direct competition in well-mixed culture (open squares). Secretion (C and Q) is only favoured over non secretion when strains grow alone. C) Comparison of biomass growth for the three genotypes in spatially structured clonal colonies. Time axis labels as in A). On the right: snapshots of the simulated colonies at t = 7 h.

crete a non-costly diffusible autoinducer, sense its concentration and only begin to produce the costly public good once a threshold concentration of the autoinducer is perceived. Quorum sensing cells grow identically to the N cells initially, and only start to produce public goods once they reach quorum, at which point the public good quickly accumulates to the threshold concentration such that all cells benefit (Figure 2.1 A, C, solid red lines). These results confirm that

our simulations capture the canonical paradigm of quorum sensing, in which Q cells can prevent wasteful secretion of public goods and maximise the efficiency of public good secretion. We assume that the cells constantly secrete the autoinducer and do not consider positive feedback in its production, which can sometimes occur (Wang & Ding 2001, Williams et al. 2007). Introducing positive feedback would allow an increased potential to tune and optimise the timing of the quorum sensing response. Our predictions on the evolution of quorum sensing, therefore, are conservative in the sense that adding in additional complexity should only improve the scope for quorum sensing to evolve.

The problem of cheating for the evolution of quorum sensing

Our first model assumes that the different genotypes live alone in clonal communities with no direct interactions between genotypes. This assumption favours strains with the maximum yield of biomass: the more cells a colony can generate, the higher the probability that its offspring will colonise new patches. While distinct clonal patches may well capture the biology of some microbial species, the extreme levels of diversity found in nature (Gans et al. 2005, Lozupone & Knight 2007, Lozupone, Faust, Raes, Faith, Frank, Zaneveld, Gordon & Knight 2012, Roesch et al. 2007) suggest that strains may often be surrounded by other genotypes. Implementing such competition in the model recapitulates previous experimental results (Dandekar et al. 2012, Diggle, Griffin, Campbell & West 2007) and confirms theoretical work (Brown & Johnstone 2001, Hense et al. 2007) showing how quorum sensing can be evolutionarily unstable in genetically diverse and well-mixed environments. Specifically, we next

initialise the system with two cells that use secretions, one cell of either the C or Q genotype together with a non-secretor cell (N), in a well-mixed environment, equivalent to growth in liquid. While both C and Q outcompete non-secretors in the clonal groups (Figure 2.1 A), direct competition results in cells from all genotypes benefiting from the public good while non-secretors do not incur the costs of its secretion. They therefore outcompete both secretor genotypes (Figure 2.1 B). As predicted by previous theoretical and experimental models (Brown & Johnstone 2001, Dandekar et al. 2012, Diggle, Griffin, Campbell & West 2007, Hense et al. 2007, Nadell et al. 2013, Rumbaugh et al. 2012), then, our simulations demonstrate how secretors can be exploited by non-secretors in well-mixed groups and our model is an example of a public goods dilemma.

Constitutive secretors lose when surrounded by competing genotypes

While direct competition in liquid favours non-secretors, theoretical and experimental work shows that secretor genotypes can outcompete non-secretor genotypes in direct competition in a spatially structured environment. We begin therefore by mapping out the effects of spatial structure on direct competitions between secretors and non-secretors (Nadell et al. 2010), before then considering how quorum sensing genotypes will fare under the same conditions (Figure 2.2). Previous work has shown that when nutrients are limiting, secretors can sometimes outcompete non-secretors in direct competition (Nadell et al. 2010). The reasons is that low nutrient levels lead to population bottlenecks and the emergence of large patches of a single genotype, which prevents N strains from using the secretions of C strains (Figure 2.2) (Datta et al. 2013,

Nadell et al. 2010, Van Dyken et al. 2013). In social evolution terminology, the population bottlenecks drive an increase in genetic relatedness – the probability that two individuals are more genetically similar than the population average – one of the major predictors of the evolution of cooperation (Hamilton 1964, Nadell et al. 2010).

However, secretors do not always succeed against non-secretors in this way. In particular, whenever strains start to grow surrounded by cells of competing genotypes (low genetic relatedness, (Mitri et al. 2011, Nadell et al. 2010)), it is more difficult for constitutive secretion to first evolve (Figure 2.2 A, 1:4 competitions). Why does a rare constitutive secretor fare badly when surrounded by non-secretors? The reason is that constitutive secretors grow slowly at first when they are at low numbers and are not yet producing enough secretions to benefit. The result is that faster growing non-secretors can overgrow them and prevent secretors from capitalising on the benefits of cooperation. Importantly, this represents a condition of nutrient limitation and high diversity that may often occur in natural microbial communities (Cornforth & Foster 2013, Hibbing & Fuqua 2009, Lozupone & Knight 2007, Mitri et al. 2011, Roesch et al. 2007). Indeed, these conditions may occur whenever a focal genotype lands in an environment that has already been populated by other genotypes.

Quorum sensing evolves under strong competition

Our initial analysis shows that when the social environment is highly competitive - nutrients are limiting and there are many genotypes - constitutive secretion can be a disadvantageous strategy. However, if secretors do manage

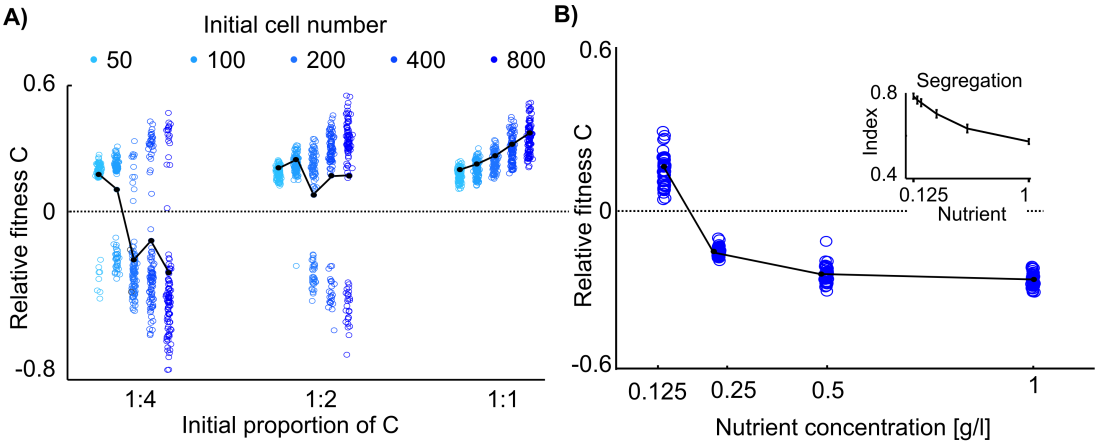

Figure 2.2: **Constitutive secretors lose when surrounded by non-secretor cells.** A) Relative fitness of a constitutive secretor genotype (C) in competition with non-secretor genotypes (N). Constitutive secretors can outcompete non-secretors when competing with few other genotypes and cells. At high evolutionary competition where many non-secretor genotypes compete with the secretor genotype (1:4), constitutive secretors will be out-competed by non-secretors. The black line connects mean relative fitness values (Materials and Methods). B) Constitutive secretion can evolve (relative fitness > 0) when genotypes segregate from each other. This occurs when nutrients are limited (strong ecological competition). Here, 400 cells of genotype C were competed with 400 cells of N and nutrient concentrations were varied. Inset: The segregation index at the end of simulations in pure colonies of N decreases with nutrient concentrations and the colony remains more mixed. Non-secretors were used for this analysis as the positive feedback of successful public good cooperation will also increase the segregation index (Materials and Methods).

to get through the initial bottlenecks that characterise competitive environments, the use of secretions can give them a strong fitness advantage. From this result, we hypothesised that quorum sensing could be a key strategy under competitive conditions to delay secretion until after clonal clusters had formed. To test this hypothesis, we competed Q strains, in addition to C, in pairwise competitions against the non-secretor N strain. Again, our scenario is where the population comprises N strains and we ask: what is the fitness of a rare mutant strains that either has the Q or C phenotype? We again consider the fitness of the focal rare mutant in biofilms comprised of 2, 3 or 5 founder strains

(1:1, 1:2, and 1:4) to study the effect of increasing evolutionary competition. We consider a range of quorum sensing strains (Q1 to Q4) that all produce autoinducers at the same, constant rate, but are induced to produce public goods at different, increasing, threshold concentrations of the autoinducer.

When secretors (C or Q1–4) were seeded at a 1:1 proportion (low evolutionary competition), all five strains outcompete the N strain in each of the 100 simulations, with C achieving the highest mean relative fitness, although secretors do not differ much in their relative fitness (Figure 2.3 A). This changes when we consider strong evolutionary competition with a low initial proportion of secretors. Importantly, Q strains succeed against competing non-secretors where constitutive secretors fail. Quorum sensing then enables cooperation in highly competitive environments where non-secretors would otherwise dominate. What is the cause of the advantage to Q strains under these conditions? As hypothesised, quorum sensing allows a newly colonising strain to compete against non-secretors and establish itself prior to activating energetically costly secretions. The critical benefit to quorum sensing then comes from the fact that expressing a costly secretion early means that a strain will be rapidly overgrown by faster growing genotypes.

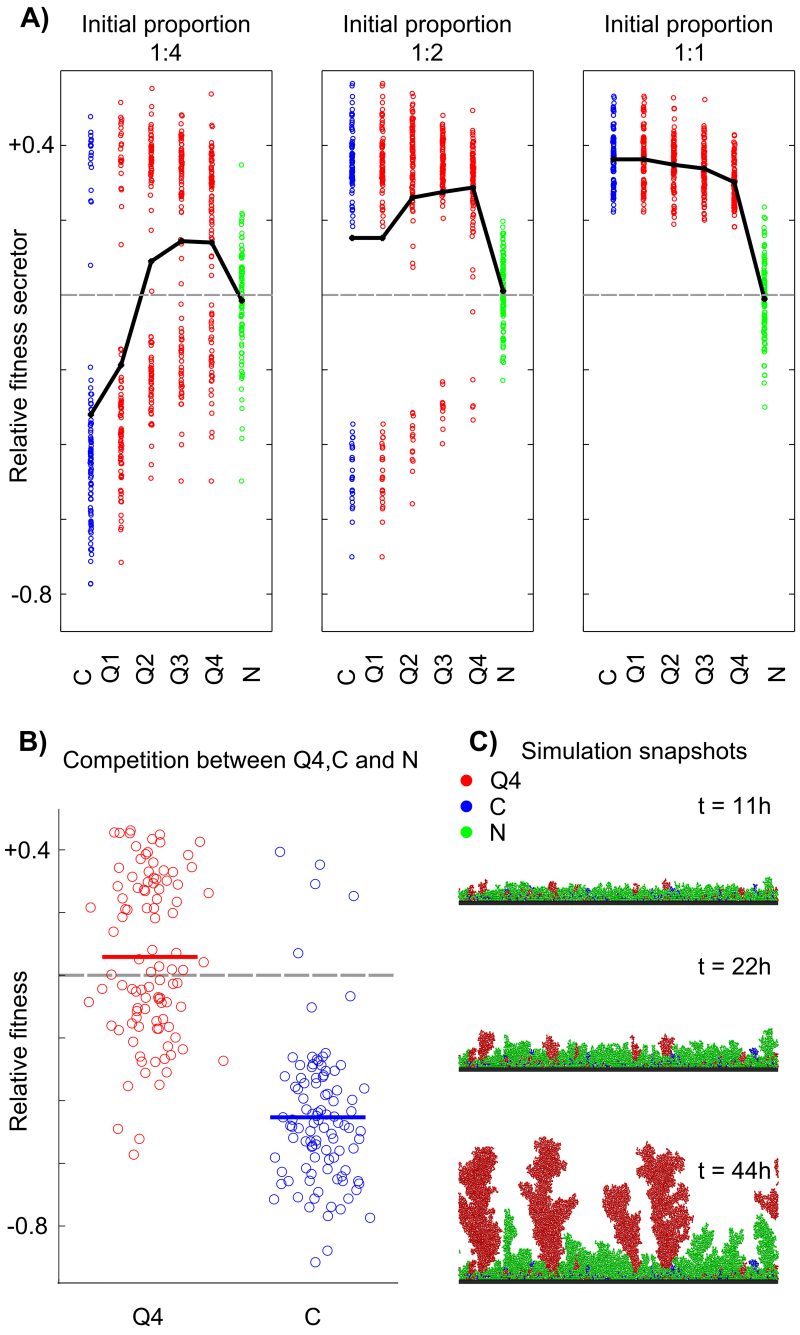

Figure 2.3: **Quorum Sensing is beneficial in competitive environments.** A) Relative fitness of different genotypes (C: constitutive, Q1-Q4: quorum sensing secretors with increasing quorum sensing thresholds, see Table S1, N: non-secretor control) in competition with non-secretor genotypes. Higher genotypic diversity is reflected in lower initial proportions of the secretor genotype, results of 100 independent simulations each. B) Direct competition between C, Q4 and N. 80 cell of C and Q4 respectively, and 640 cells of N were seeded. Relative fitness for Q4 and C was calculated by considering their fitness against the average competitor fitness (N+Q4 and N+C respectively, see Methods), 100 independent simulations. C) Snapshots of an individual simulation where Q4 succeeds.

The benefit to quorum sensing is illustrated in Figure 2.4. Here, Q1 and Q4 compete against N. Q1 turns on public good production at a 4 times lower autoinducer concentration than Q4 and Q4 therefore delays costly secretion further (see Table S1). In competition with the N genotype, Q1 gets buried while with the exact same simulation setup, Q4 can escape this and form clonal towers (Figure 2.4 D). The reason is that Q4 only initiates public good secretion when cells are surrounded by clonemates. Under these conditions then, autoinducer concentration is tracking the shift from competitive, genetically-mixed conditions to conditions where cells are surrounded by clonemates. Quantitatively, this can be seen from the fact that Q4 starts secretion at a higher "segregation index", which measures the extent to which cells are surrounded by their own genotype (Figure 2.4 C, Materials and Methods (Mitri et al. 2011, Nadell et al. 2010)). In other words, the cells are tracking a change in local genetic relatedness, from low relatedness to sufficiently high relatedness under which cooperation becomes favourable. Our model shows how a rare quorum sensing strain can succeed in a population of non-secretors, and so quorum sensing is predicted to evolve under these conditions. However, once common, how robust is quorum sensing to potential exploitation by rare non-secretors? In the supplement we ask how a rare non-secretor genotype fares in local competition with Q (Figure S2.1), which shows quorum sensing strains also outcompete non-secretors when the latter are rare.

Overall, our results show how quorum sensing can be a more robust strategy than constitutive secretion in competitive environments. Specifically, quorum sensing strains can out-compete non-secretors where constitutive secretors will

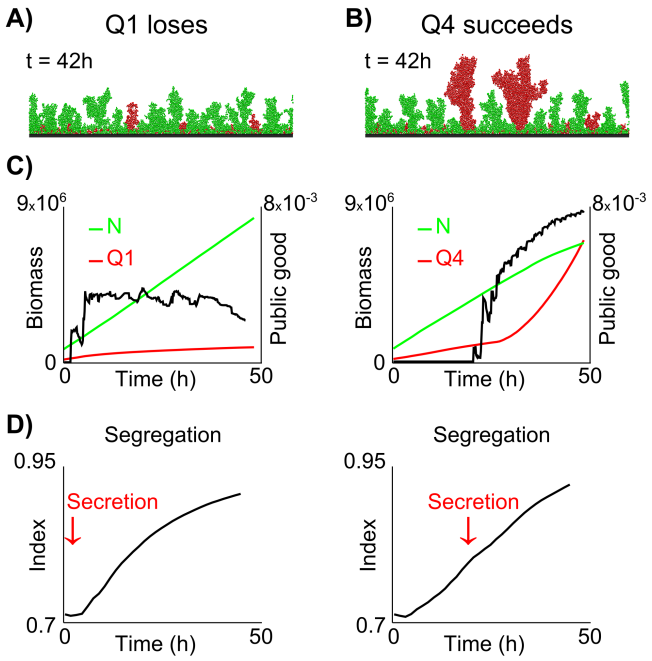

Figure 2.4: **Autoinducer concentration tracks the shift from genetically-mixed to clonal groups.** A) Snapshots of simulation show that Q1 (red) cannot outcompete non-secretors (green) in local competition, whereas in B) Q4 (red) outcompetes non-secretors (green). C) Right y-axis: Biomass of non-secretors (N) and secretor genotypes Q1 and Q4 respectively. Left y-axis: peak concentration of public good in the colony over time.Q1 initiates public good production early while Q4 delays secretion for longer due to its higher autoinducer threshold (Table S1). D) Segregation index in the two simulations over time. Q4 initiates public good production at a higher segregation index than Q1 and, therefore, when safely surrounded by clonemates with little risk of being overgrown by competitors due to investing in costly secretion.

not. But what about direct competition between a quorum sensing and a constitutive secretor strain? In general, these two genotypes perform similarly in direct competitions although quorum sensing strains do maintain a slight advantage (Figure S2.2). This is again because Q cells grow faster initially and compete well for bottlenecks and, after establishing a clonal group, Q can benefit from its public good secretion. This initial edge leads to the success of Q over C, which is robust to reciprocal competition where a rare genotype C competes with frequent cells of genotype Q. The dominance of quorum sensing

can also be seen in a competition involving all three main genotypes C, Q and D (Figure 2.3 B,C).

Invasion analyses

Across a range of competitive environments then, we find that quorum sensing strains will perform better against non-secretors than constitutive secretor strains, while the reverse is not seen. This conclusion also holds if we implement a simple meta-population model where cells in different biofilms disperse and compete globally (Figures 2.5, S3). We examine this model because our analysis so far has only asked which strain wins in direct competitions within a particular biofilm (local competition). However, if some biofilms in a population are particularly productive and make a lot of dispersing cells, this can affect evolutionary predictions by generating a global competition effect. Indeed, a classic result in social evolution is that cooperation can be disfavoured locally, but still favoured globally when groups containing cooperator genotypes are much more productive than those that do not contain cooperators (Chuang et al. 2009, Wilson 1975). We therefore examined the influence of global competition in a simple invasion analysis. This asks, for each pair of competing genotypes, whether a rare mutant genotype has a higher fitness than a common resident genotype in a metapopulation composed of many communities (Materials and Methods). Importantly, the inclusion of a global competition effect does not affect our conclusions because any differences between groups in their productivity are not great enough to overcome the effects of local competition (Figures 2.5, S3).

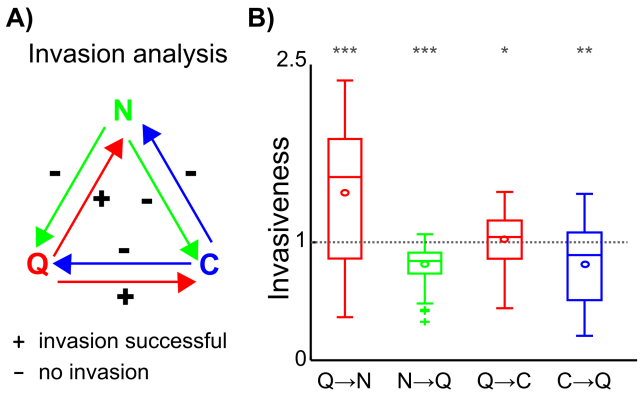

Figure 2.5: **Pairwise invasiveness analysis.** A) Summary of all pairwise invasion analyses under high evolutionary competition (initial frequency of invading strains 0.2). Here, arrows denote the direction of invasion with the rare strain at the origin of the arrow. I.e., + next to the arrow from quorum sensing genotypes (Q, blue arrows) to N denotes that Q can invade a metapopulation of non-secretors (N). On the other hand, constitutive secretors cannot invade non-secretors (C, red arrows). Both secretor genotypes, however, resist reciprocal invasion by a rare non-secretor (green arrows), see also Figure S2.3. Further, Q resists invasion by C but can itself invade C. The data from the perspective of Q is shown in B). Mean invasiveness values of 100 (200 for Q $\rightarrow$ C) independent simulations that are > 1 indicate successful invasion of a metapopulation by the rare strain (sign tests for difference of the median from 1, $^{*}: p < 0.05; ^{**}: p < 0.01; ^{***} p < 0.0001$). We verified that the mean of the invasion Q $\rightarrow$ C whose median least significantly different from 1 converges to a value > 1 and that, therefore, Q can invade C (Figure S2.3).

The evolutionary benefit of quorum sensing comes from kin inference

In our model, only quorum sensing cells respond to the autoinducers. Importantly, we also assumed that only the quorum sensing strains produce the autoinducer. This scenario will occur whenever quorum sensing strains differ from competing strains at both the loci for induction and response. And, at least when rare, this is equivalent to Q strains using an autoinducer that only they produce and respond to. The use of a genotype specific signal raises the possibility that a focal genotype can detect the number of clonemate cells in the face of high variability in genetic mixing (a form of kin discrimination (Grafen

1990)). However, such kin discrimination is not needed for the evolutionary benefits to quorum sensing that we observe. In some situations, all genotypes may produce autoinducers even if they do not all respond to them, such as when autoinducers are linked to common metabolic waste products (Hibbing & Fuqua 2009, Taga & Bassler 2003). We therefore also consider a system where all competing genotypes make the autoinducer. Under these conditions, Q cells maintain their advantage against non-secretor strains, albeit at different (higher) autoinducer thresholds (Figure S2.4).

While a genotype-specific signal is likely to be more robust to variability in strain mixing, therefore, it is not needed for the evolutionary benefits to quorum sensing that we observe. Even if the change in autoinducer concentration reflects total cell density, a Q strain can still use autoinducer concentration to infer genetic similarity. We consider the indirect inference of kinship via autoinducers key to our arguments, therefore, as opposed to strict kin detection and discrimination. This inference is possible because cell density correlates with the genetic similarity of neighbouring cells due to the changing spatial structure of the colony (Figure 2.4).

2.4 Conclusions

Two key issues have been identified as central to the evolution of quorum sensing. The first is a benefit over constitutive secretion. This is typically thought to come from the ability to infer cell density and perhaps diffusion rates. The second issue is that quorum sensing cells must resist competition from non-secreting cheater mutants. Here we show that, under conditions of

strong competition, these two issues combine to provide a benefit to quorum sensing that is not linked to the assessment of cell density – with nutrient limitation and high numbers of competing genotypes, the key benefit to quorum sensing comes from the ability to delay public good secretion and grow quickly when first in a new environment. This allows quorum sensing cells to outgrow constitutive secretors and keep up with fast growing non-secretors until clonal patches have formed in a microbial group. The relative importance of this predicted effect will, of course, depend on how often microbes find themselves in these highly competitive environments. Where species grow as isolated microcolonies, the benefits of quorum sensing are likely linked to the inference of cell density and diffusion (Brown 1999, Hense et al. 2007). However, there is a growing recognition of the importance of competition for understanding microbial phenotypes (Cornforth & Foster 2013, Hibbing & Fuqua 2009, Mitri & Foster 2013). A focal microbial genotype may often land in an environment where other genotypes are already present. Our model suggests that quorum sensing can be particularly advantageous under these conditions. When surrounded by foreign genotypes, a quorum sensing strain can grow as fast as non-secretors and only activate secretions once it is safely surrounded by its own genotype.

2.5 Materials and Methods

We are extending an individual based simulation framework developed and tested over the last 15 years (Kreft et al. 2001, Picioreanu et al. 1998, Xavier & Foster 2007, Xavier et al. 2005). Cells are modelled individually and diffusion

and reaction of chemical species are calculated by solving the reaction-diffusion equations. Briefly, cells take up and secrete chemical species, grow and divide once a certain threshold size is reached. Growth and division leads to overlapping cells. This overlap is relaxed by moving cells individually which leads to an expansion of the biofilm. There is no active movement and this relaxation mechanism is the only way cells can change their positions. Further, the substratum on which cells grow is impenetrable and cells cannot move into this area. We assume that reaction and diffusion of solutes happen on a much faster time scale than cell growth, division and biofilm expansion. The solute concentrations are, therefore, the steady state concentrations of the reaction-diffusion system for any particular biofilm conformation as in previous models (Xavier & Foster 2007, Xavier et al. 2005). The framework is written in the Java programming language and the reaction-diffusion system is solved numerically to steady state with the relaxation method. For "well-mixed" simulations, cell positions were shuffled after each iteration and secretions such as autoinducer and extracellular secreted products were allowed to accumulate in the system by imposing dynamically changing boundary condition.

Here we adopt the same basic conventions and parameters as used in previous work on the evolution of public good secretion in biofilms (Mitri et al. 2011, Nadell et al. 2010). We extended them to include quorum sensing species. The biofilm is represented in 2D. Cells grow on top of a hard, smooth surface that does not absorb or release any solutes. Above the biofilm, a diffusion layer separates cells from a region with constant nutrient concentrations (0.125 g/L, if not otherwise stated) and we impose periodic boundary conditions on the

sides of the simulation area.

Our simulations include three types of cells: non-secretors (N), constitutive secretors (C) and quorum sensors (Q). C have exactly the same properties as N except that they divert a constant fraction of the nutrient uptake to the secretion of public good (here 20%). When the local public good concentration exceeds a threshold, the yield (i.e. cell growth per nutrient invested) increases by a a factor of 3. This increase is the same for all three species, i.e. all species can benefit from the public good. Q are identical to C with the difference that they constantly secrete costless auto-inducer molecules and only spend energy on public good production if the local auto-inducer concentration exceeds a certain threshold. This threshold varies for different types of Q strains. The exact rates and stoichiometry of all these processes can be found in Table S1. The description and numeric value of all parameters are shown in Table S2.

In each simulation a chosen number of cells of each species were seeded at random positions on the bottom surface. The cells were then allowed to grow as described above. The simulation was terminated once a fixed amount of nutrients had been absorbed by the biofilm and the final biomass was recorded for each species.

Calculating fitness. We define fitness of each genotype (for example, x and y) as the mean number of rounds of cell division per unit time that cells of a focal phenotype achieve during the interval between initial seeding at t_0 and t_end when a maximum amount of nutrients were consumed. Fitness w,

therefore, is calculated as

$$w_x = \frac{1}{t_{end}} log_2 \frac{N_{x,t_{end}}}{N_{x,0}}, \tag{2.1}$$

where $N_{x,t}$ is the number of cells of phenotype x present within the cell group at time t. The relative fitness of a phenotype x in local competition with another phenotype y is defined as: $\log_{10}(\frac{w_x}{w_y})$ and, therefore, competition is successful when $w > 0$. The mean was taken over 100 such simulations and we show convergence of the results in the supplement.

Invasion Analysis. A simple meta-population analysis was conducted following the same approach as in (Nadell et al. 2010). This determines whether a rare mutant genotype would succeed in a metapopulation of cell groups with reoccurring dispersal and colonisation events. We assume a very large number of cell groups where the great majority of groups are of a single dominant genotype and only a small minority will contain the mutant. Under these conditions, a genotype x (rare mutant) can invade a meta- population of genotype y (majority resident) if the fitness of x in local competition with y is greater than the average fitness of the whole metapopulation, denoted $\langle w_y \rangle$. For each invasion analysis, w_x was computed in 100 replicates of the simulations (with varying inoculation frequencies of the two genotypes and a total of 800 cells initially, see relevant figures). Because the great majority of cell groups in the meta population consist purely of the majority genotype y, $\langle w_y \rangle$ is approximately the mean fitness of the majority strain, y, when growing on its own. To calculate $\langle w_y \rangle$, the mean of w_y over 100 simulations is computed, where the cells of strain x inoculated initially are replaced with y cells and a mono-

culture of the majority genotype is simulated. The invasion index $I_{x\to y}$ of a rare mutant x into a metapopulation with majority strain y was calculated for each of the 100 replicates as

$$I_{x\to y} = \frac{w_x}{\langle w_y \rangle}.$$

Under the assumptions of our model, we conclude that x can invade a metapopulation of y when $I_{x\to y} > 1$.

Statistical Analysis. Simulation results are from 100 independent replicates. Fitness data is non-normal and often bimodal distributed where the bimodality differs between simulations with different initial frequencies and/or initial cell densities meaning it is difficult to apply standard statistics. In some figures we show box plots and test the median fitness value with non-parametric sign tests. This is only an indicator as from an evolutionary perspective, the mean relative fitness is the determining parameter of evolutionary success. Therefore, we further conducted convergence analyses that show how the mean fitness converges after about 100 simulations (Figure S2.3).

Segregation Index. The segregation index used here is identical to that used in previous work (Mitri et al. 2011). To measure segregation in a population of M cells, we consider each cell c_i, $i = 1 \dots M$ in the population and identify all other individuals within a distance of 10μm. The N cells in this neighbourhood are indexed by c_j, with $j = 1 \dots N$. We define a phenotypic identity function, $p(c_i)$:

$$p(c_i) = \begin{cases} 0, c_j \text{ is not the same phenotype as } c_i \\ 1, c_j \text{ is the same phenotype as } c_i \end{cases} \tag{2.2}$$

Segregation with respect to a focal cell, $s(c_i)$, was calculated as the mean product of the p and m functions for every cell in its neighbourhood:

$$s(c_i) = \frac{1}{N} \sum_{j=1}^{N} p(c_j) \tag{2.3}$$

Finally, we define the segregation index σ for the entire cell group as the mean value of $s(c_i)$ across the population of cells:

$$\sigma = \frac{1}{M} \sum_{i=1}^{M} s(c_i) \tag{2.4}$$

The segregation index measures the degree to which co-localised cells are clonally related to each other.

3

Adhesion as a weapon in microbial competition

3.1 Abstract

Microbes attach to surfaces and form dense communities known as biofilms, which are central to how microbes live and influence humans. The key defining feature of biofilms is adhesion, whereby cells attach to one another and to surfaces, via attachment factors and extracellular polymers. While adhesion is known to be important for the initial stages of biofilm formation, its function within biofilm communities has not been studied. Here we utilise an individual-based model of microbial groups to study the evolution of adhesion. While adhering to a surface can enable cells to remain in a biofilm, consideration of within-biofilm competition reveals a potential cost to adhesion: immobility. Highly adhesive cells that are resistant to movement risk being buried and starved at the base of the biofilm. However, we find that when growth occurs

at the base of a biofilm, adhesion allows cells to capture substratum territory and force less adhesive, competing cells out of the system. This process may be particularly important when cells grow on a host epithelial surface. We test the predictions of our model using the enteric pathogen *Vibrio cholerae*, which produces an extracellular matrix important for biofilm formation. Flow cell experiments indicate that matrix-secreting cells are highly adhesive and form expanding clusters that remove non-secreting cells from the population, as predicted by our simulations. Our study shows how simple physical properties, such as adhesion, can be critical to understanding evolution and competition within microbial communities.

3.2 Introduction

Microbes secrete a range of products that aid in adhesion to surfaces and to other cells (Hall-Stoodley et al. 2004, Petrova & Sauer 2012, Sauer et al. 2002), but even within a single species, microbes vary widely in their tendency to adhere (Carter et al. 2011, Crociani et al. 1995, Dranginis et al. 2007, Halme et al. 2004, Macklaim et al. 2011, Smukalla et al. 2008, Sutherland 2001, Tojo et al. 1988, Vidal et al. 1998). Adhesion is critical for the formation of surface-attached communities known as biofilms, in which many species of microbes, particularly bacteria, reside (Hall-Stoodley et al. 2004). In the standard model for biofilm formation, swimming or settling cells encounter surfaces and first attach reversibly, such that they can rapidly disperse if conditions are not favourable (O'Toole et al. 2000). When conditions are suitable, cells strengthen their attachment and divide to form a growing pop-

ulation on the surface (O'Toole et al. 2000). Attachment is facilitated by a range of secreted products, including polysaccharides, proteins, nucleic acids, and amyloids (Absalon et al. 2011, Garcia et al. 2011, Linke et al. 2006, Ma et al. 2006, Romero et al. 2010, Shahid, Bardiaux, Franks, Krabben, Habeck, van Rossum & Linke 2012, Shahid, Markovic, Linke & van Rossum 2012, Timmerman et al. 1991, Veenstra et al. 1996). Microbes in biofilms adhere to each other in addition to surfaces (Conrady et al. 2012, Heilmann et al. 1996), suggesting a second possible function of adhesion: preferential attachment to cells of the same genotype, which can facilitate green-beard type cooperative interactions (Queller et al. 2003, Smukalla et al. 2008). Specifically, attaching to cells of the identical genotype allows groups to better exploit secondary phenotypes that improve final growth yield at an immediate cost to individual growth rate. An example is the secretion of enzymes that digest large nutrient sources that cannot be directly imported into cells (Drescher et al. 2013, West et al. 2007). If such secreting cells do not attach to their own kind, they can be exposed to genotypes that make use of the secreted products but do not themselves contribute to their production. These "cheater" genotypes can outcompete secreting cells and undermine the use of public goods over evolutionary time. Adhesion is therefore considered a factor that promotes the evolution of cooperation (Sachs 2008). Adhesion has almost exclusively been studied in the context of biofilm initiation and dispersal. Consequently, the evolutionary dynamics of adhesiveness over the course of biofilm growth are not well understood, even though adhesion may have a strong impact on competition between different strains and species. Here we extend an established

individual-based computer model of bacterial biofilms to investigate the costs and benefits of adhesion within communities. We first consider the familiar idea that adhesion enables cells to form biofilms. We then extend the model to examine how cells can use adhesion to resist displacement, which reveals roles for adhesion in competition within microbial communities. Finally, we perform experiments with the enteric pathogen *Vibrio cholerae* to test the predictions of our model.

3.3 Results and Discussion

We simulate a microbial community residing on a solid surface. Our model builds on an empirically tested individual-based framework designed to predict biofilm structure and composition (Kreft et al. 2001, Mitri et al. 2011, Nadell et al. 2010, 2008, Picioreanu et al. 1997, Schluter & Foster 2012, Xavier & Foster 2007, Xavier et al. 2005). Our simulations implement cells that consume nutrients, grow, and divide, which can lead to the formation of spatial nutrient concentration gradients. In some regions, nutrients may become depleted below the point at which cells can grow (Xavier & Foster 2007). We focus primarily on conditions under which biofilms are nutrient limited, which is likely to be common in many environments (Stewart & Franklin 2008). In this scenario, competition for nutrients is high, and we measure fitness after a finite amount of nutrients has been consumed. We later relax these constraints and consider conditions of high or unlimited nutrient availability. In all cases, those genotypes that achieve the highest overall growth rate and retention within biofilms increase in frequency and outcompete other genotypes. To measure the effects

of selection, we calculate a commonly used metric for relative fitness, w, which corresponds to the relative number of cell divisions (see Methods) and consider a species competitively successful if $w > 0$.

Adhesion as a mechanism to form biofilms

In this first model, nutrients diffuse into the biofilm from a bulk fluid above, and we assume that cell biomass is removed at a rate proportional to the square of the local biofilm height, a simple form of sloughing due to flow over the upper surface. Regions with more adherent cells accumulate biofilm more rapidly than other regions, because adhesiveness in this model reduces the rate at which cells are washed from the biofilm surface (Materials and Methods). This analysis reveals the first and obvious potential advantage of being adhesive: thicker biofilm formation (Figure 3.1 A). In biofilms containing more than one cell type, the more adhesive genotype dominates, as continual sloughing of cells preferentially removes the less adhesive genotype from the population (Figure 3.1 B).

Resisting displacement can be costly within biofilms

We next explored the competitive dynamics of strains with different abilities to resist displacement when they divide and push against each other. Specifically, when cells grow and divide, passive shoving occurs among neighbours to reveal space for new biomass. A more adhesive cell moves less when pushed by a less adhesive cell and vice versa, because the former attaches more securely to the surface and to adjacent cells or polymers. We assign a weighting, the adhesion parameter σ, to the distance a cell moves aside when pushed (see

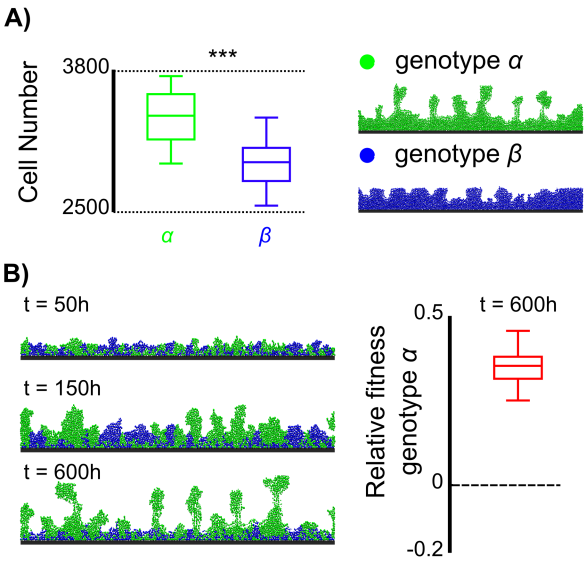

Figure 3.1: **Adhesion is beneficial when cells are sloughed from biofilms.** A) Monoculture biofilms in which equilibrium biofilm thickness is limited by cell detachment, which increases with the square of local biofilm height (bulk nutrient concentration: $2 * 10^{-3}$[g/L], detachment rate: $r = 0.1$, see Methods). Greater adhesion reduces this effect and therefore leads to thicker biofilms consisting of the more adhesive genotype α than those of the less adhesive genotype β (boxplots, *** Wilcoxon signed-rank test $p < 10^{-8}$). B) Greater adhesion allows cells of genotype α to outcompete genotype β when the two strains are co-inoculated. Snapshots show a representative simulation, with relative fitness calculated from 30 independent simulations.

Methods). We simulate competition between two genotypes, with genotype A more adhesive than genotype B ($\sigma_A = 2$, $\sigma_B = 1$), and compare results to control simulations in which the two genotypes possess the same adhesion parameter value ($\sigma_A = \sigma_B = 1$, see Methods). This comparison reveals that the more adhesive genotype has lower relative fitness when in competition with the less adhesive genotype. The more adhesive genotype performs poorly when two cells of differential adhesiveness collide because more adhesive cells tend to localise underneath less adhesive cells, where nutrient concentrations are usually lower if the nutrient source is above the biofilm. Thus, the less

adhesive genotype more frequently passes through population bottlenecks that often occur in biofilms for which nutrients are supplied from above (Hallatschek et al. 2007, Mitri & Foster 2013, Figure 3.2 B).

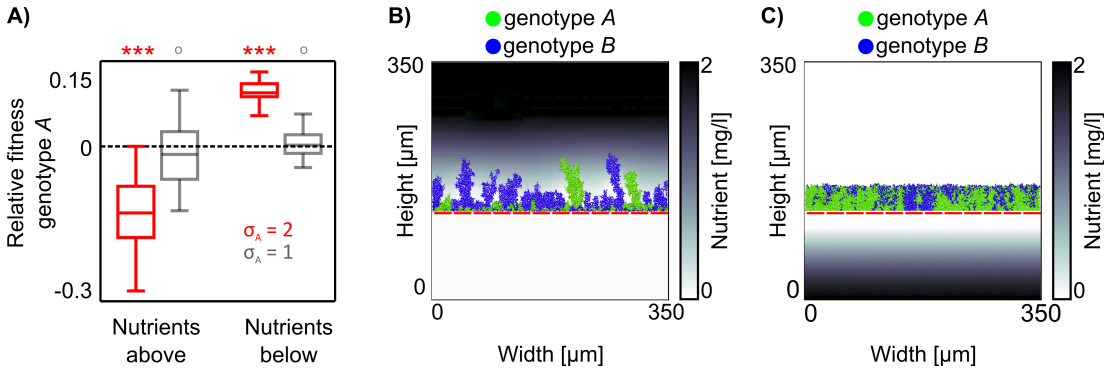

Figure 3.2: **The fitness effects of displacement by other cells.** A) Relative fitness of the more adhesive genotype A when nutrients diffuse from above and below the biofilm. The grey boxplots show when cells of competing genotypes are equally adhesive, and the red boxplots show when A is more adhesive than B the dashed line corresponds to no fitness difference, sign test for different from zero $^{***} : p < 10^{-6}$ and $\circ : p > 0.4$. Snapshots of simulations when nutrients diffuse from above (B) or below (C) showing the nutrient gradients arising from consumption in the biofilm growing on an impenetrable surface (dashed red line).

When nutrients are limited and diffuse from above, our model predicts that natural selection for adhesion will result in the following outcomes. First, provided that cells are sufficiently adherent to remain attached to a surface, we expect a general upper bound on the expression of genes promoting adhesion during biofilm growth, as high expression leads to substratum localisation away from areas of high nutrient concentration. Second, cells might be selected to regulate adhesion such that they are adherent during the initial stages of substratum attachment and then repress expression of genes encoding adhesins later during biofilm growth. Third, if there is genotypic variation in adhesin expression within a biofilm community, less adhesive genotypes might be in-

creasingly favoured as biofilms mature because they deprive nutrients to highly adhesive cells in the basal layers. Boles et al. describe a potential example of selection for less adhesive genotypes over time in growing laboratory biofilms of the opportunistic pathogen *Pseudomonas aeruginosa*. A recurring variant that arose in their experimental communities was weakly adhesive to the biofilm (Boles et al. 2004). The authors interpreted this reduction in adhesive capability to be part of an insurance policy for the biofilm as a whole: less adhesive cells can more easily detach and disperse to new potential habitats than could strongly adhesive cells. However, for less adhesive genetic variants to arise and increase in frequency by natural selection, their phenotype must also provide a direct short-term evolutionary advantage within the biofilm itself. Our model offers a putative advantage: compared to highly adhesive cells, weakly adhesive cells can more easily spread to the upper biofilm surface, where they will gain preferential access to nutrients diffusing from above.

An evolutionary advantage to resisting displacement

In the above model, due to limiting nutrients diffusing from above, only cells along the biofilm's advancing front can grow and divide, but this scenario captures only a subset of habitats that microbes occupy. First, if nutrients are abundant, growth occurs throughout a biofilm, and thus there is no cost to localising to the substratum. Second, nutrients can diffuse from the substratum itself, for example in detrital particles in the ocean (Cordero et al. 2012, Shapiro et al. 2012) or the soil (Böckelmann et al. 2003). Similarly, many host-associated bacteria catabolise host-made products on the tissues on which they grow (Bevins & Salzman 2011, Cash et al. 2006, Church et al. 2006, Derrien

et al. 2010, Meibom et al. 2004, Schluter & Foster 2012). Our model shows that high adhesiveness localises cells to the attachment surface. On this basis, we reasoned that the cost of adhesion observed in the preceding model could be converted to an advantage when nutrients are obtained from the substratum. To assess this idea, we modelled an environment in which nutrients diffuse from below the biofilm. Indeed, the adhesiveness that is detrimental in the above model becomes beneficial when access to limiting nutrients is highest at the substratum, because the more adhesive strain has preferential access to nutrients (Figure 3.2 C) and rapidly dominates the attachment surface.

Resisting displacement when abundant nutrients are present

We have shown that adhesiveness can be strongly beneficial when it fosters access to a nutrient source on which cells are growing. In some circumstances, however, nutrients enter biofilms from above and below the basal substratum. The gut epithelium is an example of such an environment. We therefore evaluated the costs and benefits of resisting displacement when nutrients simultaneously diffuse from below and above, and when cells are sloughed from the biofilm's outermost surface (Schluter & Foster 2012). In this model, relative adhesiveness has no initial effect on fitness because the entire community is nutrient saturated, which allows all cells to grow at high rates (Figure 3.3). However, the more adhesive cells dominate on longer time scales. By localising to the base of the biofilm, the most adhesive cells gradually but reliably displace less adhesive cells away from the surface and into the sloughing region. This result is also obtained when nutrients diffuse only from above the biofilm

but are not limiting, such that adhesive cells at the base of the colony are never starved (Figure S 3.1).

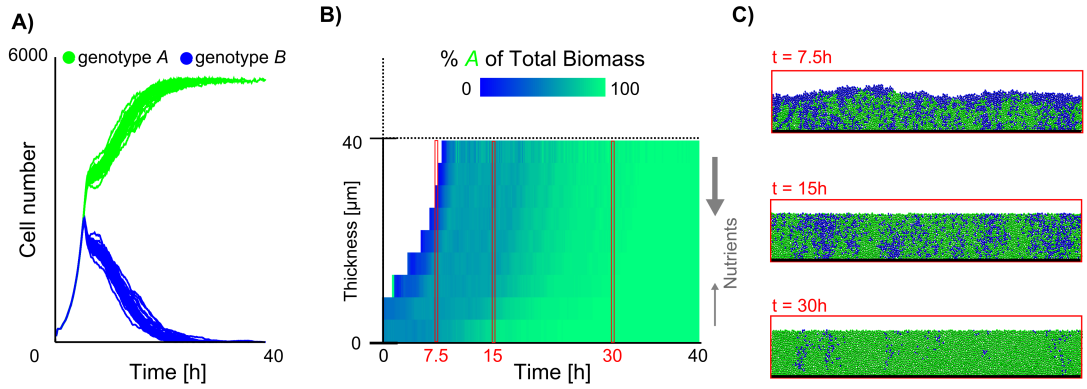

Figure 3.3: **Competition in a model of a nutrient saturated biofilm with nutrients diffusing into the colony from above (N = 4) and below (N= 0.8).** A) Cell number over time; initiated with equal cell numbers; genotype A outcompetes B over time. B) The heat map shows the biomass distribution of the two genotypes averaged over the width of a single simulated biofilm community. C) Snapshots expand these average values. Cells grow protected from sloughing in a 40μm thick layer beyond which cells are lost from the biofilm. Because genotype A is more adhesive, it displaces genotype B into the biofilm's sloughing region, leading to dominance of A. Identical results are obtained when nutrients exclusively diffuse into the biofilm from above, but remain at high enough concentration for growth to occur throughout the biofilm.

Two potential effects of secreted polymers: adhesion and expansion

A common mechanism by which bacterial cells achieve adhesion is the secretion of extracellular polymeric substances (EPS), including polysaccharides, protein, and DNA (Friedman & Kolter 2003, 2004, Nadell & Bassler 2011). In addition to the advantages of adhesion described above, theoretical work has identified another possible advantage of polymer secretion, namely an inexpensive expansion in cell cluster volume relative to biomass production (Xavier & Foster 2007). Such cluster expansion positions EPS-secreting strains into

nutrient-rich areas of the biofilm at the expense of strains that do not produce EPS. This beneficial effect could counteract the costs of adhesiveness. Previous work on EPS has only considered the case of nutrient diffusion into the biofilm from above (Nadell et al. 2008, Xavier & Foster 2007), which raises the question of whether volume expansion via EPS is advantageous when nutrients are obtained from the substratum.

We investigated whether EPS-based volume expansion can confer a competitive advantage when cells acquire nutrients from the surface on which they reside, independently of the potential adhesive properties of EPS. In contrast to adhesion, Figure 3.4 shows that volume expansion via EPS enables cells to gain preferential nutrient access by rapidly colonising the surface when nutrients diffuse from below. Likewise, when nutrients diffuse from above, EPS-producing cell lineages can expand upwards and deny competitors access to nutrients, as shown by Xavier and Foster (2007). In the supplement, we explore the interactions between the two properties of EPS, volume expansion, and adhesion, and find that volume expansion can compensate for the costs of adhesion when limiting nutrients diffuse into biofilms from above (Figure S3.2).

The benefits of an adhesive polymer in *Vibrio cholerae*

We performed an empirical test of our model predictions using biofilms produced by the enteric pathogen *Vibrio cholerae* (Alam et al. 2006, Huq et al. 1983, Meibom et al. 2004, Nalin et al. 1979). Previous research has demonstrated that an EPS-producing strain has a strong competitive advantage over an isogenic non-producing strain in mixed genotype biofilms (Nadell & Bassler 2011). These experimental data provide empirical support for the first benefit of

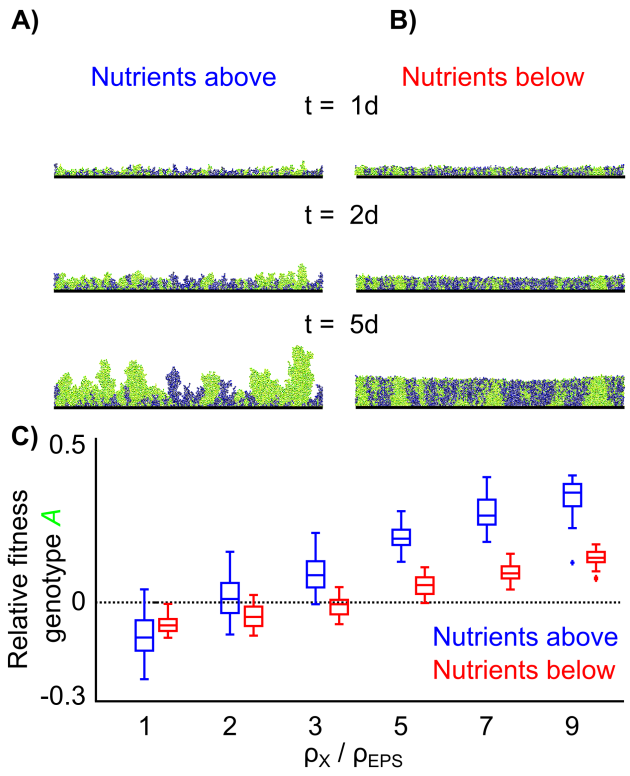

Figure 3.4: **The fitness effect of volume expansion.** EPS producing genotype A (investment in EPS 25% of total growth) is competed against a non-producing genotype, in which EPS is not adhesive and only generates volume. A) and B) Snapshots at different time-points from representative simulations with EPS modelled to be five times less costly to produce than cell biomass. The simulation is shown when nutrients diffuse from above (A) or below (B). C) Fitness of EPS secretor genotype A for different EPS costs (ρ_{EPS}) relative to the cost of biomass production (ρ_X). Decreasing the cost of EPS relative to the cost of producing new cells (increasing $\frac{\rho_X}{\rho_{EPS}}$) leads to increased fitness of the EPS-producing genotype A, irrespective of whether nutrients come from above (blue) or below (red); $N = 2 * 10^{-3}$g/L.

EPS secretion highlighted by our simulations: adhesion leads to thick biofilms (Figure 3.1,Fong et al. 2010, Nadell & Bassler 2011, Tamayo et al. 2010). Here we explored whether the two other benefits identified by our model – active displacement of competing strains and cell cluster volume expansion – also contribute to the success of EPS-secreting cells.

As observed previously, the EPS$^+$ strain forms more robust biofilms (10-20

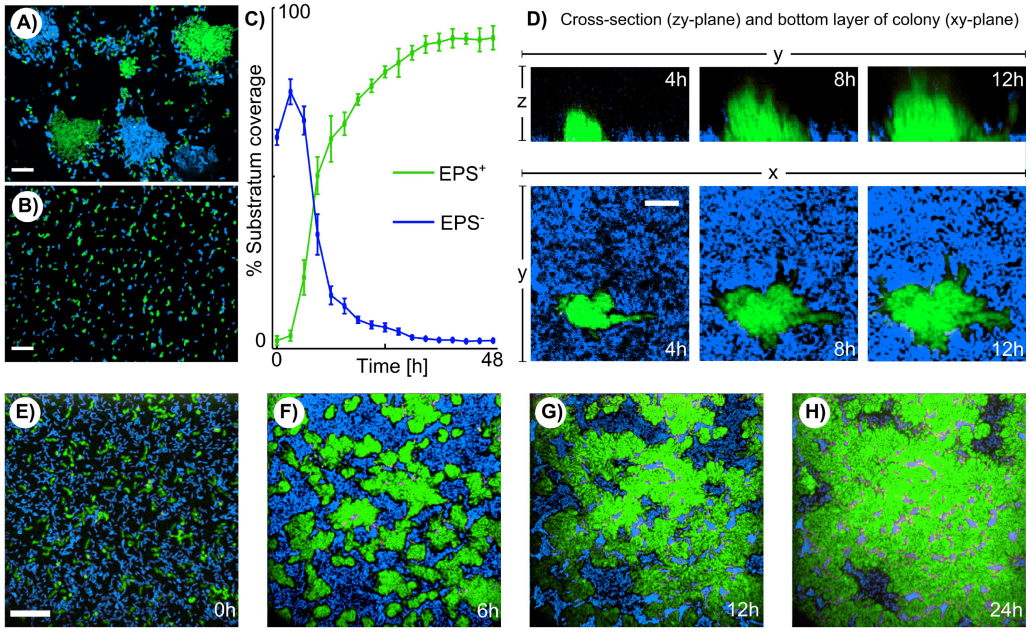

Figure 3.5: **EPS-producing cells displace non-producing cells from the substratum.** A) A 1:1 mixture of green and blue EPS^+ cells form clonal clusters in liquid culture. B) A 1:1 mixture of blue and green EPS^- cells remain dispersed. C-H) EPS^+ (green) in competition with EPS^- cells (blue) at different time points. C) Quantification of surface area coverage by the two strains over time. Bars denote SEM, n = 3 replicates. D) *V. cholerae* cells EPS^+ (green) displace *V. cholerae* EPS^- cells (blue) by burrowing under the EPS^- strain along the attachment surface in co-culture, even though EPS^- cells can attach when grown alone (Figure S3.4). E-H) A time series for the bottom layer of EPS^+ (green) competing against EPS^- cells (blue). The scale bars in panels A, B, and E-F denote 20μm. The scale bar in panel D denotes 8μm.

cell lengths thick) relative to the EPS^- strain (1-2 lengths thick), suggesting increased adhesion among EPS^+ cells. To further assess this inference, we grew gently shaken cultures of 1:1 blue EPS^+ : green EPS^+ cells and observed that they form dense mono-colour groups. This result indicates strong mother-daughter cell adherence (Figure 3.5 A). In contrast, EPS^- cells do not adhere to one another (Figure 3.5 B). To examine the competitive interaction of EPS^+ and EPS^- cells in biofilms, we inoculated the two strains together on the glass substratum of straight-chamber microfluidic devices composed of

PDMS bonded to microscope slides and imaged the resulting biofilms at regular intervals for 48h. Visual inspection of the bottom biofilm layer shows clearance of EPS^- cells along the expanding front of EPS^+ clusters; ultimately, EPS^+ cells occupy nearly 100% of the substratum (Figure 3.5 C, E-H). The mother-daughter cell adhesion of EPS^+ cells and rapid reduction in surface occupation by EPS^- cells during biofilm competition is consistent with our second proposed advantage of adhesion, namely active displacement of competitors by EPS^+ cells. However, if EPS^- cells are intrinsically more likely to detach from the substratum than EPS^+ cells, it could be that EPS^- cells are simply revealing space that is subsequently occupied by EPS^+ cells without any direct interaction between the two genotypes. Two pieces of evidence contradict this interpretation. First, EPS^- cells are equally capable as EPS^+ cells of forming and maintaining confluent monolayers on the glass surface, even when subjected to 20-fold higher flow velocities than those used in the competition experiment (Figure S3.4). Second, 3-dimensional imaging of co-cultured biofilms illustrates a sloughing effect by which expanding EPS^+ clusters displace EPS^- cells from the glass along the boundary at which the two strains meet (Figure 3.5 D).

The third predicted benefit of EPS-mediated adhesion is that matrix-secreting cells expand volumetrically more rapidly than non-secreting competitors, allowing the secreting strain to occupy more space from which to draw growth substrate and thereby deny neighbouring cells access to nutrients. Though nutrients were supplied from above the biofilm in our system, cells near cell free spaces on the substratum grew throughout the experiment, showing that they were not being denied nutrients by EPS^+ clusters. In addition, microscopy

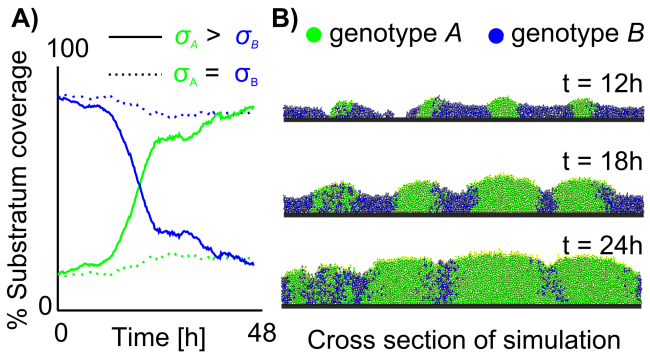

Figure 3.6: **Model of EPS$^+$ versus EPS$^-$ competition in *V. cholerae*.** Simulation in which genotype A produces adhesive EPS (investment 5% of growth rate), whereas genotype B does not. Here, nutrients diffuse from above and are saturating (N = 0.8 g/L) to enable growth throughout the biofilm, which is limited in thickness through sloughing of biomass proportional to the square of local biofilm height. Rate of sloughing (r = 20, see Methods) and investment into EPS are parameters chosen based upon the experimental system (see text), but all other parameters are the same as those used in previous simulations, Table S1). Areas containing EPS exhibit reduced sloughing of cells (see Methods) and EPS-producing cells are more resistant to displacement by non-producing cells (initial frequency of A = 0.1, 35 cells seeded in total). The graph shows coverage of the substratum by the two genotypes and shows displacement of B by A when A is more adhesive than genotype B (solid line), and no displacement in an otherwise identical control simulation with equal adhesiveness (dashed line).

did not indicate significantly increased spacing between EPS$^+$ cells compared to EPS$^-$ cells (Figure S3.3). Thus, volume expansion does not appear to be a dominant mechanism underpinning the competitive advantage of the EPS$^+$ strain over the EPS$^-$ strain in this system.

In summary, our experimental data suggest that the cell cluster formation that occurs due to the adhesiveness of *V. cholerae* EPS-producers provides a competitive advantage to secreting cells primarily via the accumulation of thick biofilm and active displacement of non-secreting cells from the glass substratum. These dynamics can be recapitulated in simulations that incorporate increased resistance to sloughing and displacement by EPS-producing cells

(Figure 3.6). Importantly, the parameters used for these simulations were the same as those in the above sections and were chosen prior to experimentation (Figures 3.1, 3.2 and 3.4, Table S1) with two exceptions. First, the rate of sloughing from biofilms was chosen to obtain a biofilm thickness comparable to that of the *V. cholerae* EPS^+ strain. Second, the fraction of biomass devoted to EPS production by EPS^+ cells was reduced from 25% to 5% to reflect that little EPS is observed between cells in micrographs obtained from the experiments (Figure S3.4 B).

3.4 Conclusions

Our work highlights the potential importance of adhesion for the evolutionary fate of microbes in biofilms, beyond simple attachment to surfaces. Resistance to displacement can be costly when nutrients do not reach the base of a biofilm. Under these conditions, highly adhesive cells can be overgrown by less adhesive genotypes, which results in starvation of the more adhesive genotype. However, when cells are able to grow at the base of a biofilm, adhesion is beneficial, as it enables cell lineages to persist during biofilm growth and to expand across the substratum by displacing other cells in their paths. Therefore, in biofilms residing on host tissues, adhesiveness is likely to be important for the outcome of competition for space and nutrients.

In vivo studies have demonstrated the importance of adhesiveness for initial surface colonisation (Liu et al. 2008), however we do not know of studies that examine whether adhesion provides a competitive advantage within host-associated communities. We do know that both pathogenic and commensal bac-

teria produce molecules that anchor them to epithelial cells or to the associated mucus layer (Chattopadhyay et al. 2012, MacKenzie et al. 2009). Moreover, bacteria have apparently evolved environment-specific adhesion molecules. For example, *Lactobacilli* living in the gut possess a suite of adhesins that differs from those of *Lactobacilli* strains residing in the vagina (Macklaim et al. 2011), and vaginal *Lactobacilli* display increased adhesiveness relative to their counterparts that exist in other environments (Boris et al. 1998, Malik et al. 2013). The hypothesis that adhesion is a key competitive strategy raises the interesting possibility that arms races occur in which strains evolve strategies to decrease adhesion of their competitors. While speculative, some evidence exists for this notion. For example, the soil bacterium *Lysinibacillus fusiformis* secretes non-bactericidal biosurfactants that can act as wetting agents that reduce attachment and biofilm formation by competitors (Pradhan et al. 2013).

Evolutionary competition in biofilms is often intense, and factors that tip the competitive balance in favour of a particular genotype can be rapidly selected. The literature emphasises roles for secreted toxins and rapid growth in competition. Our work demonstrates the importance of strategies that modify endogenous physical properties of microbial communities. A genotype can rapidly dominate a community simply being more adhesive than its competitors.

3.5 Methods

Our model considers the growth of cells that passively push against each other as they grow and divide. Nutrient concentration gradients are calculated from

consumption by cells and solute diffusion. Our simulations use periodic left-right boundary conditions and implement diffusion of nutrients into microbial colonies from the liquid above, the substratum below, or both. The model extends an individual-based framework for the simulation of growth and division of unicellular organisms that has been empirically tested. The assumptions, justifications, and implementations of these simulations are discussed at length elsewhere (Kreft et al. 2001, Picioreanu et al. 1998, Xavier & Foster 2007, Xavier et al. 2005). In brief, the model implements a multigrid solver for two or three dimensional reaction diffusion partial differential equations. Using the standard assumption that diffusion occurs on a shorter time scale than bacterial growth, these equations are solved at each iteration to steady-state based on the flux at the boundaries and the production or consumption of diffusing solutes by biomass particles (cells). The biomass domain is separated from the boundaries by a layer in which only diffusion governs the concentration of solutes. The biofilm domain is derived from the second portion of our model, a simulation of cells in continuous space. Here, cells are represented as solid spheres. Cells grow according to the local steady-state concentration of nutrients (N) following the Monod equation

$$\mu = \mu_{max} \frac{N}{N + K_s},$$

where the maximum growth rate μ_{max} and the Monod constant K_s are modelling parameters (see Table S1).

Implementation of adhesion

In the work presented here, we extend the existing modelling framework to consider an additional modelling parameter σ, which implements adhesion. We model two effects of adhesion. The first is the improved ability of more adhesive cells to persist in biofilms. This property is captured by reducing biomass at the upper surface of biofilms to simulate cell sloughing by shear forces. In regions that are exposed to the liquid above the colony, biomass is reduced in proportion to the squared local thickness of the biofilm. Biomass is locally reduced according to the following equation:

$$dR = r\frac{h^2}{2B\sigma},$$

where r is the rate constant of detachment, h is the local thickness of the biofilm, and B the amount of biomass in a computational voxel.

The second effect of adhesion is the ability to resist displacement. As a cell increases its diameter, or upon exceeding a maximum diameter (after which it divides into two cells), overlap between neighbouring cells occurs. Our algorithm probes all cells in the simulation in a random order and tests for such overlap. If overlap is found for a focal cell, a vector for cell displacement is calculated, and the cell is moved accordingly. This step is repeated until no overlap remains in the system. The consequence is that during growth, an expansion of the biofilm domain occurs, and the top of the biofilm advances away from the substratum. Locally, if a focal cell overlaps with a cell of the same adhesiveness, the resulting movement vector is simply a function of the magnitude of their overlap. If the adhesion parameters of two cells differ, a

weight to the resulting vector is assigned,

$$dV = 2\frac{\sigma_f^{-1}}{\sigma_f^{-1} + \sigma_n^{-1}})$$
$$\mathbf{v}_{new} = \mathbf{v} * dV,$$

where σ_f is the adhesion parameter of the focal cell, σ_n is the adhesion parameter of the neighbouring cell, and $\mathbf{v}$ is the original movement vector prior to including the differential adhesion effect. Hence, a more adhesive cell ($\sigma_f > \sigma_n$) will move less upon meeting a cell of lower adhesiveness, and vice versa (Figure 3.7). This implementation of adhesiveness is an approximation that substitutes for the true forces that cause more adhesive cells to remain in place more than less adhesive cells. Finally, in some simulations, cells may secrete and accumulate extracellular polymers that make their colonies increase in volume more rapidly than colonies in which cells do not secrete such polymers, as detailed in Xavier & Foster 2007.

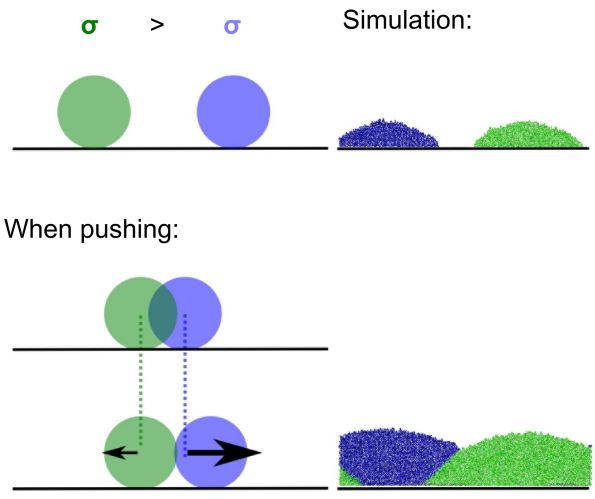

Figure 3.7: **Effect of adhesiveness on cells cell-cell shoving.** When two cells that differ in their adhesiveness contact each other, the more adhesive cell moves less than a less adhesive cell, and vice versa.

Fitness calculation

To calculate evolutionary success of different genotypes, we calculate their relative fitness. Fitness, w, of a species X is calculated as the average number of divisions in a simulation (from t_0 to t_{end})

$$w_x = \frac{1}{t_{end}} log_2 \frac{X_{t_{end}}}{X_{t_0}},$$

where X_t is the number of cells of species X at time t. The relative fitness, w, of species X in competition with Y is defined as $log_{10} \frac{w_X}{w_Y}$

3.6 *V. cholerae* experiments

All experiments were conducted using strains derived from *V. cholerae* str. C6706 using standard molecular biology protocols. Briefly, a constitutive EPS producer (EPS^+) was constructed by deleting genes encoding the quorum-sensing regulator (*hapR*) and the flagellin core protein (*flaA*). The EPS non-producer (EPS^-) was built by further deletion of *vpsL*, which encodes a protein required for EPS biosynthesis. Further detail on strain construction and characterisation can be found in *Nadell2011*. Constitutive fluorescent protein expression constructs were inserted onto the chromosomes of each strain for visual distinction and quantification of our experiments. The two fluorescent proteins used were mKate (Shcherbo et al. 2007) and mTFP1 (Ai et al. 2006), which were artificially coloured green and blue for comparison with our simulations. These expression constructs were previously shown to have negligible effects on bacterial growth rate (Nadell & Bassler 2011). Biofilms were grown in simple straight-chamber microfluidic devices as previously described (Nadell

& Bassler 2011). To determine if EPS^+ cells displace EPS^- cells by pushing against them, biofilms were initiated by inoculating the two strains in a 1:100 ratio (EPS^+:EPS^-). Biofilms were imaged using z-steps of 1μm at regular time intervals for 48 h. All microscopy was performed with a Nikon Eclipse fluorescence microscope fitted with a Yokogawa CSU-X1 confocal scanning unit. Data were analysed using ImageJ and custom software written for MatLab.

4

The Evolution of Mutualism in Gut Microbiota Via Host Epithelial Selection

4.1 Abstract

[1] The human gut harbours a large and genetically diverse population of symbiotic microbes that both feed and protect the host. Evolutionary theory, however, predicts that such genetic diversity can destabilise mutualistic partnerships. How then can the mutualism of the human microbiota be explained? Here we develop an individual-based model of host-associated microbial communities. We first demonstrate the fundamental problem faced by a host: The presence of a genetically diverse microbiota leads to the dominance of the fastest growing microbes instead of the microbes that are most beneficial to the host. We next investigate the potential for host secretions to influence

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the microbiota. This reveals that the epithelium-microbiota interface acts as a selectivity amplifier: Modest amounts of moderately selective epithelial secretions cause a complete shift in the strains growing at the epithelial surface. This occurs because of the physical structure of the epithelium–microbiota interface: Epithelial secretions have effects that permeate upwards through the whole microbial community, while lumen compounds preferentially affect cells that are soon to slough off. Finally, our model predicts that while antimicrobial secretion can promote host epithelial selection, epithelial nutrient secretion will often be key to host selection. Our findings are consistent with a growing number of empirical papers that indicate an influence of host factors upon microbiota, including growth-promoting glycoconjugates. We argue that host selection is likely to be a key mechanism in the stabilisation of the mutualism between a host and its microbiota.

4.2 Author Summary

The cells of our bodies are greatly outnumbered by the bacteria that live on us and, in particular, in our gut. It is now clear that many gut bacteria are highly beneficial, protecting us from pathogens and helping us with digestion. But what prevents beneficial bacteria from going bad? Why don't bacteria evolve to shirk on the help that they provide and simply use us as a food source? Here we explore this problem using a computer model that reduces the problem to its key elements. We first illustrate the basic problem faced by a host: Whenever beneficial bacteria grow slowly, the host will lose them to fast-growing species that provide no benefit. We then propose a solution

to the host's problem: The host can use secretions – nutrients and toxins – to control the bacteria that grow on the epithelial cell layer of the gut. In particular, our model predicts that the epithelial surface acts as a "selectivity amplifier". The host can thereby maintain beneficial bacteria with only small amounts of weakly selective secretions. Our model fits with a growing body of experimental data showing that hosts have diverse and important influences on their gut bacteria.

4.3 Introduction

Many microbial species live on or are associated with epithelia of multicellular organisms. Examples range from plants and soil bacteria interactions in the rhizosphere where plant secretions affect the composition of bacterial communities (Callaway et al. 2004, Sanon et al. 2009), through the light organs of marine animals in which specialised symbiotic bacteria are cultivated by the host (Graf 1998, Ruby & McFall-Ngai 1999, Visick & McFall-Ngai 2000) to many surfaces of the mammalian body (Dethlefsen et al. 2007).

Every human is home to roughly 100 trillion bacterial cells, collectively called the microbiota. The majority of these cells reside in the human gastrointestinal tract and, in particular, in the large intestine (Ley et al. 2006). Here, bacteria can have beneficial effects such as the digestion of complex carbohydrates, colonisation resistance against invading pathogens, maturation of the adaptive mucosal immune system and immune cells, and the production of secondary metabolites, including vitamin (Holzapfel et al. 1998, Hooper & Macpherson 2010, Sekirov et al. 2010). However, these activities are not per-

formed by all species, and the species composition of the microbiota in a healthy human is clearly distinct from bacterial communities in other environments (Ley, Lozupone, Hamady, Knight & Gordon 2008). Moreover, various diseases correlate with disturbances in the species composition of the microbiota (Dethlefsen et al. 2007, Sekirov et al. 2010). It is clear then that the gut community has the ability to both help and harm the host. Despite the potential for harmful effects of the gut microbiota, the major class of interaction with the host appears to be one of mutualism, whereby both sides benefit from the interaction. The evidence for host benefits comes both from our understanding of the metabolic services that the gut microbiota provides and studies of germ-free animal models (Bäckhed et al. 2004, 2005, Cash et al. 2006, Dethlefsen et al. 2007, Kelly et al. 2004, Martin et al. 2007, Mazmanian et al. 2005, Rakoff-Nahoum et al. 2004).

There is a growing literature on the evolution of mutualisms among species, both theoretical and empirical, which emphasizes a number of key factors required for the evolutionary stability of mutualisms (Foster & Wenseleers 2006, Frank 1994, Sachs et al. 2004, West et al. 2002). Most relevant for the gut microbiota is the issue of having multiple genetically different individuals on one side of the mutualism (microbes) involved in a single interaction with the other (host) (Foster & Wenseleers 2006, Frank 1994, West et al. 2002). On the side with multiple genotypes, this can lead to the loss of helpful mutualistic genotypes, whenever non-helpful genotypes are more competitive. How is such potential conflict among partner species resolved in other systems? Theory predicts a central role for partner choice: the selection of the best mutualis-

tic partners by a focal species (Foster & Wenseleers 2006). Moreover, partner choice is widespread in nature with evidence from many different systems (Foster & Wenseleers 2006, Sachs et al. 2004) including leaf cutter ants and their fungus (Poulsen & Boomsma 2005), legumes and rhizobia (Kiers et al. 2003), and the mutualism between the bobtail squid and the luminescent bacterium *Vibrio fischeri* (Nyholm & McFall-Ngai 2004). The predominance of partner choice mechanisms in other systems begs the question: What is the role of partner choice in the mammalian gut?

The sheer diversity of microbial species in the mammalian gut shows that hosts do not select for one or two partner species, as occurs in some mutualisms. In addition, there is a clear environmental effect on microbial species composition in the form of host nutrient intake (Faith et al. 2011, Muegge et al. 2011). Nevertheless, there are also a range of mechanisms by which vertebrate hosts affect their microbes more directly. In particular, the intestinal epithelium produces a wide range of secretions that help to maintain the barrier between the gut lumen and host tissues (Hooper & Macpherson 2010, Peterson et al. 2007, Salzman 2010, Salzman et al. 2010, Vaishnava et al. 2011). Central to this barrier is mucus secretion (Atuma et al. 2001, Brugman & Nieuwenhuis 2010, Cone 2009, Olmsted et al. 2001) that limits the direct access of bacteria to the epithelium (Johansson et al. 2010). The mucus becomes less dense, however, as it moves upwards away from the epithelium and bacteria grow in the upper layers that can feed on carbohydrates such as fucose, which the host adds to the mucus proteins (Fabich et al. 2008, Hooper 2001, Hooper et al. 1999, Sonnenburg et al. 2005).

The host also secretes a range of antimicrobials into the mucus, including defensins. Mucosal community composition has been studied in mice that lack an enzyme required for murine alpha-defensins but secrete human alpha-defensin (Salzman et al. 2010). The observed changes in community composition, in combination with other studies, led to the conclusion that defensins are essential regulators of intestinal microbial ecology (for a review, see Bevins & Salzman 2011). More work is now required to understand the exact role of defensins as a selective agent of the microbiota. In particular, the defensins of the small intestine have been the primary focus of research, and the effect of defensins in the large intestine is less well understood. Moreover, studies have shown that production and activation of defensins can themselves be dependent on the resident microbiota (Girardin et al. 2003, Petnicki-Ocwieja et al. 2009), which opens the way for feedback loops between the host and its microbiota. In addition to defensins, the adaptive immune system also has the potential for selective effects. B-cell-derived immunoglobulin A (IgA) is considered the most likely host secretion to affect the localisation, growth, and composition of the microbiota (Kawamoto et al. 2012, Macpherson & Uhr 2004, Peterson et al. 2007).

While it is clear that epithelial secretions can affect the microbiota, the primary role is often assumed to be as a simple barrier between the lumen and host tissues (Wehkamp et al. 2004, 2005). However, there is evidence that epithelial secretions differentially affect different strains and species. Sugars like fucose are more easily utilised by some microbial species than others (Fabich et al. 2008, Hooper et al. 1999, Sonnenburg et al. 2005), and defensins and

IgA have biased effects on the microbiota (Cash et al. 2006, Kawamoto et al. 2012, Peterson et al. 2007, Salzman et al. 2010). Such findings suggest that host secretions might help to control the composition of the resident microbiota (Bevins & Salzman 2011, Van den Abbeele et al. 2011). Indeed, it has even been suggested that control over a wide array of non-pathogenic microbes is the primary reason why adaptive immunity first evolved (McFall-Ngai 2007). Despite this, we understand very little about how the host might in practice select for particular microbial strains or species.

Here we build a model to evaluate the potential of a host to select their microbiota. Ecologies like the mammalian gut are extremely complex dynamical systems and will require a central role for theoretical approaches if we are to dissect their complexity (dos Santos et al. 2010, Gudelj et al. 2010). We have, therefore, developed a new model of host-associated microbial communities with the goal of bringing an evolutionary perspective to the study of host-microbiota interaction. Our model is relatively complex in that it includes realistic features such as mechanistic interactions among cells, spatial structure, and chemical gradients. However, it greatly simplifies the full complexity of the gut and is not intended as a complete description. We hope to show, nevertheless, that one can gain new understanding by the application of such simplifications to the problem of the host-microbiota interaction. In particular, our study reveals three key findings. First, we demonstrate the problem of multiple genotypes on one side of a mutualistic partnership, which renders the host-microbiota mutualism intrinsically fragile. Second, we show that a solution to this fragility is host selection: The epithelium-microbiota in-

terface acts as a selectivity amplifier that can quickly shift the composition of the microbiota at the interface. Finally, we show that central to the selectivity is the provision of nutrients, and not just antimicrobial factors, by the host. Our results suggest a host's epithelium is a remarkable environment for partner choice, which is well suited to control bacterial community composition.

4.4 Results

We model a bacterial community containing two strains, which is growing on the host epithelium (Figure 4.1) where cells are represented by spheres that consume nutrients, grow, and divide (Materials and Methods, Table S1, Figure S1).

Host-Microbiota Mutualism Is Fragile

Our first goal is to evaluate the potential effects of differences in growth rates between strains under the simplest of conditions, and then build in increasing complexity in order to understand the key factors at play. We denote two bacterial competitors A and B, where B divides more rapidly than A (Figure 4.1 B). These two strains can either represent two members of one species that differ only in their interaction with the host or two different species that differ in other ways. As such, the model can be viewed from either an evolutionary (genotypes within a species) or ecological (species within a community) perspective. We return to the differences between these two scenarios in the Discussion. While we only model two strains, the model also approximates more diverse communities in which there is selection for a set of beneficial ecotypes where each "strain" would then represent multiple strains with similar

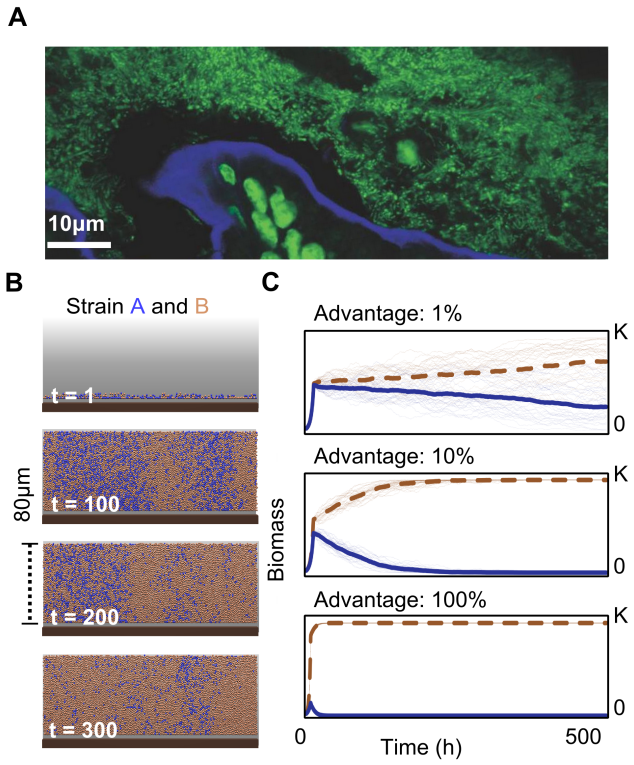


Figure 4.1: **Microscopic image and simulations of microbial growth near a host epithelium.** (A) Confocal fluorescence image of bacteria growing in the lumen on top of host epithelial cells. Sample taken from the cecum of a laboratory mouse, where there has been no intentional manipulation of the animal's microbiota. Epithelial and bacterial cells in green (DNA stained with Sytox green), and the epithelial border brush in blue (actin stained with Alexa-647-phalloidin) from (Stecher et al. 2007). (B) Simulation of bacterial growth on host epithelium; brown bacterial cells (strain B) have a 1% growth rate advantage over blue bacterial cells (strain A). Even with a modest growth rate advantage, strain B succeeds as strain A is slowly washed out. (C) Thirty independent simulations of bacterial competition. Development of biomass of strain B (brown dashed) and A (blue) with growth rate advantages for strain B of 1%, 10%, and 100% and environmental capacity K. The thick lines are mean values. doi:10.1371/journal.pbio.1001424.g001

phenotypes.

These simple models show the potential power of competition in a host-associated microbial community. Figure 4.1 C shows the increase in frequency of the fittest species over time in the epithelial community. Here and in the majority of subsequent figures, we show time as an axis. One reason we do this

is because it is impractical to run all simulations until the final frequencies of the two strains have been established, especially for very small differences in fitness. Nevertheless, we expect in the majority of cases that one strain will ultimately dominate the system (see following section and Text S1 for exceptions). Indeed, even for a modest difference in the growth rate among strains (e.g., 10%), a faster growing strain rapidly reduces the slower growing strain to negligible frequency in tens of generations (Figure 4.1 C). This corresponds to a few days for species like *Escherichia coli* in a mammalian gut (Rang et al. 1999). The constant removal of cells leads to thinning out and eventual eradication of the slower growing strain A near the epithelium (Figure 4.1 B, C). For larger difference in growth rate, such as B doubling at twice the rate of A, the eradication of A occurs in a few generations.

This demonstrates the fundamental problem faced by a host when having multiple possible genotypes competing for a niche where a mutualistic species could exist. Whenever the most beneficial bacteria do not grow the fastest, competition between bacterial genotypes will lead to the loss of mutualistic strains within the host and thus a suboptimal microbiota composition (Figure 4.2). But is it possible that mutualistic species are, without exception, intrinsically faster growing than non-mutualist species? If anything, the reverse is expected. Recent phylogenetic work shows that species from healthy guts tend to cluster with species from complex and relatively slow–growing communities (Lozupone, Faust, Raes, Faith, Frank, Zaneveld, Gordon & Knight 2012). By contrast, bacteria of infants and unhealthy guts tend to cluster with bacteria from fast-growing pioneer communities. In an entirely neutral host that

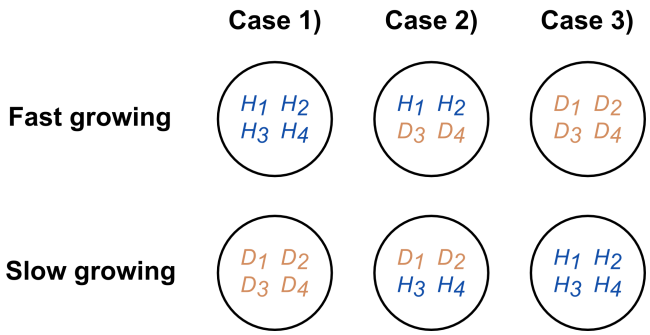


Figure 4.2: **Cartoon to illustrate the potential problem faced by a host.** Three scenarios are shown for four helpful strains (H) and four detrimental strains (D) that occupy four different niches, 1 to 4. Two extreme cases exist: beneficial strains grow faster in all niches (case 1) or all detrimental stains grow faster in all niches (case 3). In the first case, no partner choice is required, as natural selection favours the beneficial strain throughout all niches. However, any deviation (case 2 or 3) from this means that the host will experience a sub-optimal microbiota. doi:10.1371/journal.pbio.1001424.g002

does not exert any control over the bacterial composition, therefore, our model predicts that the mutualism between bacteria and a host is intrinsically fragile.

Epithelial Selection Dominates Lumen Selection

So far, there is little spatial structure in our model, and we confirmed that our first results correspond to a well-mixed (no spatial resolution) ordinary differential equation model of evolutionary competition (Text S4.1, Figure S4.2). We next extend the simulations to introduce more realism and calculate nutrient levels as a function of space and time. As cells divide, they use up nutrients such that nutrient concentration is depressed as one moves away from the nutrient source and into a group of dividing cells. These solute gradients are known to be important in natural bacterial groups and can have strong influences on community structure and composition (Kreft 2004, Kreft & Bonhoeffer 2005, Nadell et al. 2010). In our case, there is the potential for two solute gradients,

one from the lumen direction and one from the host epithelium direction. Our question is then: How do selective compounds from the epithelium and from the lumen influence the composition of this bacterial community?

Compared to the well-mixed case, the ability of nutrients to select for one strain over the other is reduced in the presence of solute gradients because not all cells have access to nutrients. With less reproduction, natural selection is less powerful. However, more striking is that lumen nutrients exert a much weaker selective effect than epithelial nutrients. This suggests a bias that may empower the host to affect the microbial communities growing on the epithelial surface (Figure 4.3). What causes this difference? When the epithelium secretes nutrients, growth occurs at the base of the bacterial colony, which can affect the whole bacterial community. By contrast, lumen selection from the opposite direction preferentially affects cells that are about to be sloughed off, which limits the effect of lumen nutrients on cells at the base of the bacterial community.

The inhibition of lumen selection only occurs beyond a certain thickness of the bacterial community (Text S4.1, Figure S4.2). While it is difficult to measure the thickness of these bacterial communities in vivo, the range of thicknesses used in our model are consistent with the outer mucus layer of mice and rats (Atuma et al. 2001). A corollary of these results is that selection from the lumen should be weakened by growth near the epithelium. Hence, we further show that the addition of non-selective nutrients at the epithelium strongly inhibits lumen selection (Figure S4.3 B). By contrast, additional non-selective lumen nutrients do not affect the ability of epithelial nutrients to select

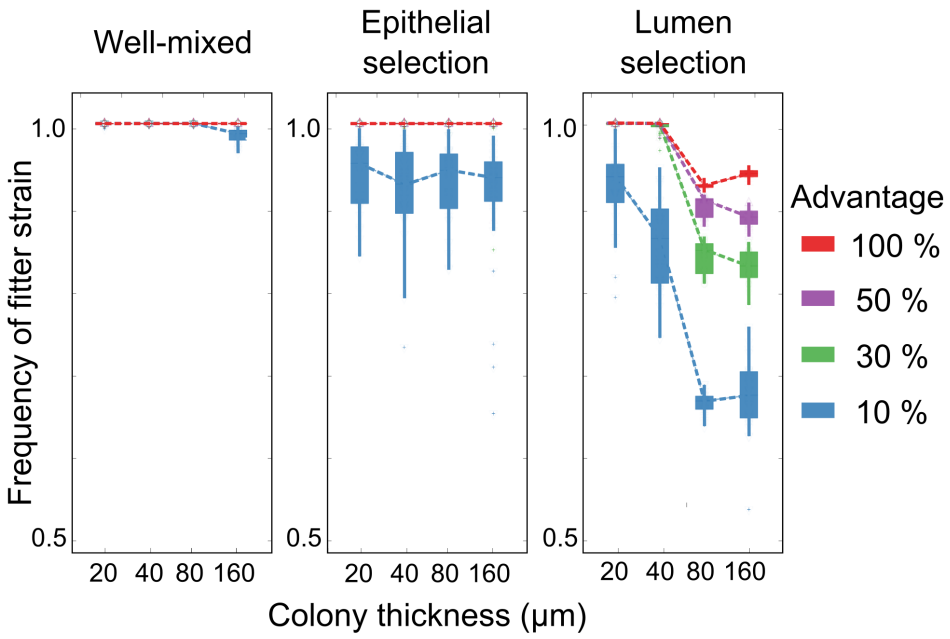


Figure 4.3: **Epithelial nutrients have more effect on a bacterial community than lumen nutrients.** Box plots show the final frequency of a faster growing strain after 12 d as a function of microbial community thickness, where the growth rate advantages of the fitter strain range from 10% to 100%. Well-mixed: No gradients of nutrients exist (Figure 4.1 B,C). Epithelial selection: Nutrients exclusively diffuse into the colony from the host epithelium. Lumen selection: Nutrients exclusively diffuse into the colony from the lumen. Dashed lines connect mean values of 30 independent simulations. The total nutrient influx into the system from the host or the lumen is kept identical. Results agree with a steady-state solution of a simplified ODE model (Figure S4.2). doi:10.1371/journal.pbio.1001424.g003.

for one strain over the other.

Our model predicts that the physical layout of the gut epithelium environment allows host secretions to have disproportionately strong effects. We next test this by pitting the two sources of nutrients against one another. We assume that epithelial nutrients select for strain A, whereas lumen nutrients select for strain B, simulating a scenario in which the slow growing strain A would be lost without host selection. We present a conservative case in which epithelial nutrients are both less abundant and less selective. Specifically, lumen nutrient

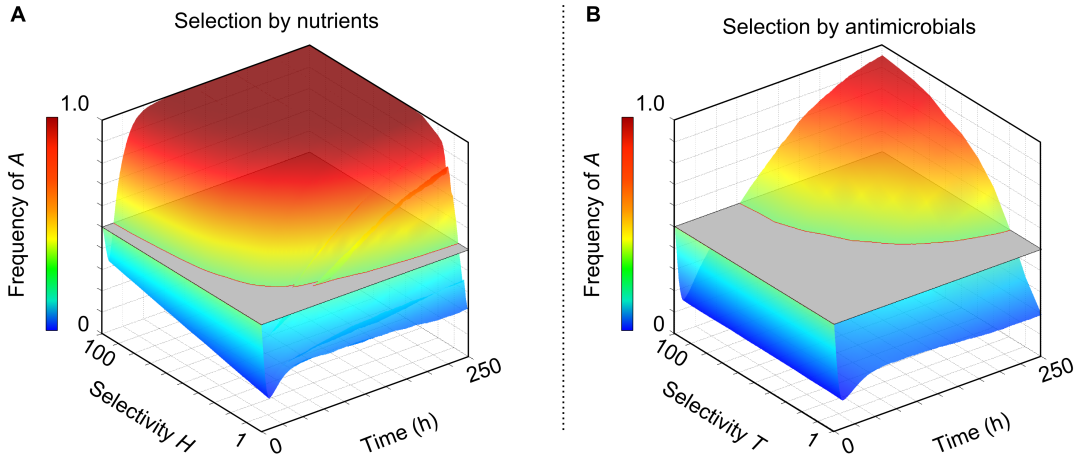


Figure 4.4: **Selectivity amplification by the host epithelium.** Weak epithelial selection dominates strong lumen selection. Strain B has a 100% growth rate advantage on nutrients from the lumen, and lumen nutrients are five times the concentration as epithelial nutrients. Grey planes mark the starting frequency of the two strains (0.5). (A) Host nutrients provide growth rate advantages to strain A ranging from 1% to 100%. (B) The host secretes antimicrobials that preferentially kill strain B; susceptibility advantages for strain A range from 1% to 100%. Host-secreted nutrients are also provided that neutral. In (A) and (B) strain A outcompetes strain B for all but the smallest selective advantages. doi:10.1371/journal.pbio.1001424.g004

concentrations are five times higher than epithelial nutrients and the growth rate advantage of strain B on lumen nutrients (100%) is always higher than or equal to the (varied) growth rate advantage of strain A on epithelial nutrients (Figure 4.4 A).

Initially, strain B outgrows strain A as the former's overall growth rate advantage from the nutrients is much greater than that of strain A. However, the advantage of strain B diminishes as the microbial community grows and the effects of lumen nutrients and epithelial nutrients separate into distinct regions. This allows strain A to establish itself at the epithelial surface, and for all but the weakest selection by the host, strain B is eliminated eventually. In

fact, in this example, the host need only provide a 5% growth rate advantage to strain A to counter the 100% growth rate advantage and five times higher concentrations that lumen nutrients provide to strain B. In summary, we find that a fast growing strain, which would rapidly replace slow growing strains in a well-mixed environment, can be eliminated by moderate counter-selection at the gut epithelium. This process is also effective when strain A is initially rare (Figure S4.4). Host selection at the epithelium, therefore, can effectively operate on an initially rare strain or species that is a minor member of a diverse community.

Nutrients Are Often Critical to Host Epithelial Selection

We next tested the effects of epithelial selection using antimicrobials that tend to harm strain B more than strain A. In our model, selection with antimicrobials is slower than with nutrients, because the antimicrobials kill both strains, which reduces the rate at which one strain outgrows the other. Antimicrobials could, in principle, select more quickly than nutrients if they could instantly kill only one of the two strains. In the absence of such extreme selectivity, however, nutrient selection is more powerful. Indeed, for a wide range of conditions, we find that it is critical that the host also supplies nutrients (Figure 4.4 B). These do not need to be selective if selective antimicrobials are secreted. However, nutrients are required because the selective effects of antimicrobials will not permeate up through the community unless there is net positive growth at the epithelial surface. With antimicrobials alone, cell death can easily outweigh the birth of new cells at the epithelial surface because lumen nutrients are at their lowest concentrations. This means that although the host kills more cells

of strain B than of strain A (depending on the specificity of the antimicrobial), if growth is limited by nutrients at the epithelium, no net positive growth of strain A will occur either. For this reason, providing nutrients at the epithelial surface greatly widens the range of conditions under which antimicrobials can be used as a selectivity mechanism by allowing sufficient growth in this critical region.

One challenging case for the host is when lumen nutrient levels are so great as to remove all nutrient gradients in the bacterial community and hence nutrients are available at high concentrations throughout the colony. However, even here, the host can use the epithelium as a selectivity amplifier (Figures 4.5, S4.5). Selectivity amplification occurs whenever the host can maintain a thin region next to the epithelium that favours strain A over strain B and allows for net positive growth. With this, strain A will eventually take over the community even though it is counter-selected in the vast majority of the community (Figure 4.5). As a control, we show in Figure 4.5 how the same amount of solutes evenly distributed throughout the system would strongly select against A, which contrasts with the selectivity amplification seen when solute gradients are present. Finally, our results are robust to fluctuations in lumen nutrient concentrations, which are inevitable in organisms that have discontinuous food intake. As our model predicts, the effects of epithelial secretions are strongest during starvation periods, because lumen nutrient concentrations are highest after feeding (Hooper et al. 1999). However, implementing a feast-famine cycle that increases the variance in lumen nutrient concentration (but does not affect the mean) suggests that the net effect of these cycles is modest (Figure S4.6).

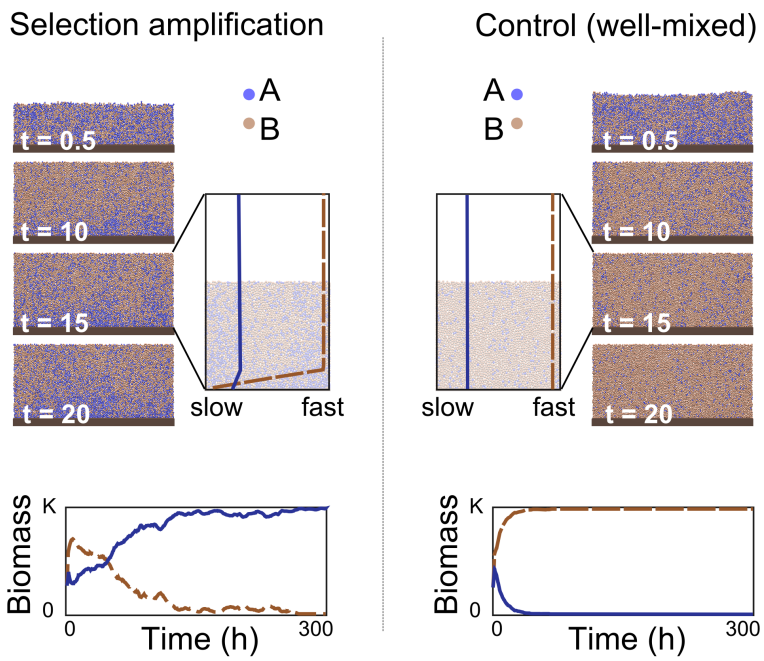


Figure 4.5: **The host need only influence a thin layer of a microbial community to exert control.** Selection amplification: To illustrate, we apply constant distributions to all solutes in the simulation (no gradient for lumen nutrients, steep gradient for host secretions) to create a thin layer in which the strain A (blue) outgrows strain B (brown). The snapshots show the progression of a representative simulation with the expanded snapshot showing the growth rates of the two strains throughout the community. Strain A only grows better very close to the surface of the epithelium. Well-mixed: Control simulation with identical total amounts of solutes but without spatial differences in solute concentrations and growth rates of the two strains. In such an environment, strain A is out-competed by strain B; environmental capacity K. 10.1371/journal.pbio.1001424.g005

4.5 Discussion

The gut is a competitive environment where the potential for high growth rates and population turnover means that slower-growing bacterial strains can be rapidly lost. This presents a problem for hosts. Natural selection of microbial phenotypes based upon intrinsic growth rate will disadvantage any microbes that grow more slowly (Figures 4.1, 4.2). Our model predicts that a host can

compensate for this effect using epithelial secretions that promote relatively slow-growing strains. Importantly, these effects do not require a highly specific selection mechanism akin to the full force of adaptive immunity. In our model, moderate selectivities that allow poorly growing strains to grow 5% to 10% faster at the epithelial surface are sufficient to reverse their fate.

Epithelial selection may occur either through growth-promoting secretions or toxic growth inhibitors, but we find that growth promotion is often critical because selectivity amplification requires net growth of the microbial community near the epithelial surface. In this context, it is interesting that host epithelial secretions include growth promoters, particularly mucosal glycans (Matsuo et al. 1997, Varum et al. 2012), in addition to the growth inhibitors of the immune system.

Positive growth at the epithelium surface is important because it causes a flow of microbial cells towards the lumen that limits the effects of lumen nutrients on the community. Cells nearest the lumen are least likely to persist due to detachment and sloughing deeper in the lumen. In our model, this motion is driven by pushing and shoving of dividing bacterial cells. In the mammalian gut, the flow towards the surface is likely to be further promoted by the constant release of mucin polymers from the epithelial surface (McGuckin et al. 2011, Varum et al. 2012). Furthermore, the diffusion of IgA – a key secretion known to influence the microbiota – is inhibited by mucins (Olmsted et al. 2001). Our work suggests that this diffusion limitation will maximise not only the residence time of IgA in the gut but particularly the residence time close to the epithelium, where IgA will have an amplified effect.

Our model requires that a host has mechanisms to differentially affect the net growth rate of different bacterial strains or species. Are such effects always possible, particularly in the face of bacterial coevolution to evade the negative effects of host selection? The greatest challenge for host selection will occur when the strains involved are variants of a single species that differ only in their cooperativity towards the host (as opposed to different species that differ in many ways). However, even here, host selection is possible if the host can select directly on the beneficial phenotype in the bacteria (Foster 2004, 2011). This appears to occur in the mutualism between bioluminescent *Vibrio fischeri* bacteria and the bobtail squid. It is thought that the squid creates an oxidising environment in the light organ that selects for cells using the luminescence reaction because this reaction uses up oxygen (Visick & McFall-Ngai 2000).

We believe comparable mechanisms to those seen in the bobtail squid may exist in the gut. Mammalian cells produce glycoconjugates of a remarkable structural complexity and diversity, which are known to favour, or disfavour, the attachment and growth of different microbial species (Hooper & Gordon 2001). These compounds may represent an evolutionarily stable way to select for bacteria, like *Bacteroides thetaiotaomicron*, which are carbohydrate specialists that convert complex carbohydrates for the host: *B. thetaiotaomicron* has over five times the number of glycoside hydrolases as species like *Salmonella enterica* or *Shigella flexneri* (Hooper 2009). Indeed, human milk contains polysaccharides that cannot be digested by the infant, suggesting that mothers may also be exercising this simple but effective form of selection (Lo-Cascio et al. 2007). But is host secretion of complex carbohydrates vulnerable

to exploitation by a variant that receives benefits but does not provide any help to the host? The use of complex carbohydrates as a selective mechanism is likely to greatly constrain the evolutionary options for bacterial species by demanding that bacteria use the glycoside hydrolases that also help the host with digestion. Of course, these species might still attempt to invade the epithelial layer. Our model is not intended to capture direct attacks by pathogens, but the detection of tissue damage is a relatively simple problem for a host as compared to selecting among more or less metabolically useful symbionts. And we know that hosts possess mechanisms to counter direct attacks, such as the inflammation response.

However effective, host selection will not preclude bacterial coevolution in the gut. Indeed, long-term bacterial evolution in the gut may allow mutualists to achieve gains in competitiveness both in the presence and absence of host selection. Consistent with such adaptation, *B. thetaiotaomicron* can induce carbohydrate secretion by the host (Hooper et al. 1999). Coevolution also brings the potential for arms-races with pathogens that adapt and use host-provided nutrients or evade host-secreted antimicrobials. For example, l-Fucose utilization provides *Campylobacter jejuni* with a competitive advantage (Stahl et al. 2011). More generally, the possibility of bacterial counter-adaptation to host selection mechanisms leaves interesting questions to be answered. These include the issue of how antimicrobial secretions can remain selective when bacteria are known to rapidly develop resistances to many antimicrobials. In addition, the fitness of a bacterial cell will be influenced by cells that possess different secretion, motility, or adhesion phenotypes (Nadell et al. 2009). We

do not yet understand how the potential for complex social interactions among cells will influence host selection. In sum, hosts may be forced to modify or increase their exact selection criteria, either during the life of the host via adaptive immunity or over evolutionary time. Interestingly, recent work has shown how the use of multiple selective mechanisms can allow a host to stay ahead in evolutionary arms races with parasites (Gilman et al. 2012).

Can multiple strains coexist within the epithelial community? We do not find evidence that coexistence is a stable state in our model in the sense that multiple strains will persist indefinitely. This can be seen in Figure 4.5, where despite lumen selection being much stronger than epithelial selection, the lumen-favoured strain does not persist. The reason for this is that epithelial selection generates a ratchet-like effect whereby the epithelial-favoured population expands and gradually pushes any other strains up and out of the community. If host selection is weak and/or growth in the community is slow, however, favoured and disfavoured strains may both persist for long periods. Moreover, a number of other processes in the gut will counter any winnowing by host selection and help to maintain bacterial diversity. This includes the existence of multiple niches, both at different positions along the epithelial surface but also within the lumen proper. Community diversity will be further influenced by the influx rates of different species (Bucci et al. 2012) and diet (Faith et al. 2011, Muegge et al. 2011).

Host epithelial selection is not the only process that influences the microbial species composition of the gut. Nevertheless, our model predicts that the control of epithelium-associated microbial communities is much easier for

a host than expected from unstructured environments. Selection of particular microbial species and strains at this position is likely to pay dividends both metabolically but also in terms of the competitive exclusion of undesirable species. Furthermore, epithelium-associated communities are relatively unlikely to be washed out and may represent a stable source community for the rest of the gut. We conclude that host influence on the composition of microbiota is both likely and likely to be powerful.

4.6 Materials and Methods

The study centres upon an individual-based simulation framework that captures bacterial growth and the concentration gradients of solutes, such as nutrients, that originate from bacterial activity while they are growing near to an epithelial host layer. While the model can capture a wide range of conditions, our analysis focuses upon a relatively nutrient-rich environment where cells grow rapidly (Table S1) and slough off at a fixed height above the epithelial surface, which is intended to reflect microbial growth in an animal intestine (Atuma et al. 2001, Rang et al. 1999). In the mammalian gut, these cells will typically grow in the loose upper mucus layer of the epithelial surface, which continually detaches and sloughs off into the lumen (Atuma et al. 2001, Johansson et al. 2010, Varum et al. 2012). We do not explicitly model the effects of these mucin polymers but implicitly include the protection from sloughing they provide for adherent bacteria in the loose layer. Note that we are only explicitly modelling the bacteria at the surface of the epithelium and not those in the lumen. Of course, selection at the epithelial surface will influence the

lumen to some degree (discussion), but we do not explicitly model this process.

The model is an extension of an established framework that has been developed and tested over the last 15 years to understand and predict the behaviour of bacterial communities growing on inert surfaces (Kreft 2004, Kreft et al. 2001, Nadell et al. 2008, Picioreanu et al. 1998, Xavier et al. 2007, 2005). While originally developed for problems in bioengineering, it has most recently been applied to understand the evolution and ecology of microbial groups (Mitri et al. 2011, Nadell et al. 2010, 2009, 2008, Xavier & Foster 2007). Subsequent empirical validation of these models has demonstrated the ability of the framework to both describe bacterial communities and identify new biological mechanisms (Korolev et al. 2011, Nadell & Bassler 2011). The model assumptions, justifications, and implementation are extensively discussed elsewhere (Kreft et al. 2001, Lardon et al. 2011, Xavier et al. 2007, 2005). In brief, bacterial cells are modelled as solid spheres that metabolise nutrients in a continuous concentration field. At each iteration, the concentration field is updated solving the two- or three-dimensional reaction-diffusion equations using multigrid solvers. This takes into account local sinks, such as a bacterium utilising the solutes around it as a nutrient source or local sources, such as secretions from a cell. Cells increase in diameter and eventually divide pushing aside neighbouring cells.

The model focuses upon the resident bacterial communities that grow in the loose upper mucus layer at the interface of the lumen and epithelium, which are most likely to be affected by host selection (Spor et al. 2011). We inoculate our simulations with a total of 250 cells in varying frequencies. This is a simplification as initial assembly of the microbiota has been shown to be

more complex and may depend on interbacterial cross-talk as well as other yet unknown factors (Rezzonico et al. 2011). Bacteria reside above a layer of host cells that secrete solutes at varying rates. We assume that this epithelial layer and the dense mucus layer immediately above it is impenetrable to the bacterial cells (Atuma et al. 2001, Johansson et al. 2008). This is supported by data on the healthy gut with a few notable exceptions, such as segmented filamentous bacteria in mice that live in the dense mucus layer (Bevins & Salzman 2011). Accordingly, we do not consider host responses to invasion of a pathogen or breach of the mucus layer, such as inflammation (but see Discussion). The bacteria grow and divide utilising nutrients diffusing in from the lumen or the epithelium. At a certain height above the epithelium, cells are sloughed and excluded from the simulation. Bacteria utilise nutrients (N) and convert them into biomass at the rate μ following Monod-kinetics

$$\mu = \mu_{max} \frac{N}{N + K_s},$$

where K_s is the Monod constant. Competing strains in our simulations differ in their maximum growth rates, μ_{max}. Bacteria may switch between different substrates, ensuring that the maximum growth rate cannot be exceeded, where switching is based upon a recent analysis of optimal foraging in microbes (Krivan 2006). Death of cells through antimicrobials is modelled using a similar equation as for growth

$$p = \frac{T}{T + S},$$

where p is the probability of death for a cell, T is the local concentration of the antimicrobial, and S the concentration at which cell death within 1h occurs

with a probability of 50%. Different strains may have different susceptibilities to the antimicrobial and hence different probabilities for cell death at a given concentration. Most of our understanding of host-secreted antimicrobials stems from secretions of the epithelium in the small intestine, whereas secretions in the larger intestine are less well understood (Bevins & Salzman 2011, Van den Abbeele et al. 2011).

5

A network model of the gut microbiota – stability and host control.

5.1 Abstract

The human gut harbours a diverse community of symbiotic microbes that cooperatively protect and provide nutrients for the host. The species composition of gut communities is extremely stable meaning that species compositions within an individual human tend to not change much over long periods of time, which is considered to be critical for good health. However, we lack a framework to understand and predict the stability of the gut microbiota. Here we present a network model of gut communities in order to identify how the biology of gut communities influences their ecological stability. We study the effects of three often discussed features of gut communities: high cooperativity between species, high species diversity and high nutrient abundance. Our analysis predicts that cooperation among microbes, which is often considered

to be key for the host, will tend to destabilise gut communities. In addition, high species diversity is also detrimental for ecological stability. How then is stability maintained? We consider a third element of the biology of gut communities: high nutrient abundance, including feeding of microbes from the host epithelium. Our analysis suggests that abundant nutrients can be key because they will tend to weaken the strength of interactions between species; something that will promote ecological stability. This prediction is supported by recently published data showing that host feeding of gut microbes is associated with increased ecological diversity. Our work suggests that hosts should act as ecosystem engineers to maintain a diverse but weakly interacting gut community.

5.2 Introduction

The gut microbiota in an individual healthy human forms a complex community (Lozupone, Faust, Raes, Faith, Frank, Zaneveld, Gordon & Knight 2012). Up to 10^3 different microbial species colonise the gut, and together outnumber all human cells in the body $10:1$ (Gill et al. 2006). The microbiota-host relationship is commonly described as mutualistic - microbes benefit from living in a nutrient rich and relatively stable environment, whilst the host receives protection from invading pathogens, and nutrients through the metabolic activity of the microbes (Bäckhed et al. 2005, Mazmanian et al. 2008).

Microbiota species composition between individual humans differ, but catabolic sets of genes in microbiota of all individuals tend to be highly similar (Turnbaugh et al. 2009). This indicates that many of the niches within the gut

environment can be occupied by a range of different species, each demonstrating a similar niche specialisation, and thus playing a similar role in the microbiota species network. Further, whilst species compositions between individual hosts is variable, the microbiota within an individual is remarkably stable, even over years (Dethlefsen & Relman 2011, Faith et al. 2013, Vanhoutte et al. 2004).

A stable microbiota is beneficial for the host by protecting against pathogen invasion (Dethlefsen et al. 2007). Inflammatory bowel diseases (Mazmanian et al. 2008, Wehkamp et al. 2005), and a range of other health problems ranging from cancer to neuronal disorders correlate with abnormal species compositions in the gut (Diaz Heijtz et al. 2011, Hsiao et al. 2013, Hu et al. 2013, Tlaskalová-Hogenová et al. 2011). While causal relationships between microbiota composition and disease are difficult to evidence, the stability of the gut microbiota composition is considered important for health (De Cruz et al. 2012). Despite the importance of the stability of the gut microbial community, our understanding of what drives stability is limited.

Theoretical ecology has developed a set of tools to understand what affects the ability of ecological communities to resist perturbations and return to their previous state. This theory has identified a number of key factors that decrease such *stability*, including increasing numbers of species and increasing strength of interactions between species (Allesina & Tang 2012, May 1972). However, there are many species in the gut, and microbial species have the potential to strongly affect one another. Outside of a host, competition between microbial strains and species may be the norm as they compete for nutrients and use antibiotics against one another (Cornforth & Foster 2013, Foster & Bell 2012).

However, microbes also have the potential to cooperate with one another (Mitri & Foster 2013, West et al. 2006, Ze et al. 2012) and, while we lack clear data, host associated communities are often assumed to contain a large number of species that cooperate with one another (Eberl 2010, Sleator 2010). Specifically, it is assumed that microbes form cross-feeding networks when metabolising the complex food sources presented to them in the human diet (Belenguer et al. 2006, Ze et al. 2012, 2013). These cooperative networks are particularly important for the host, as the breakdown of complex carbohydrates in food by the combined effect of different microbial species will eventually yield energy for the host from otherwise indigestible material (Van den Abbeele et al. 2011, Ze et al. 2013). The importance of the microbiome for the host has also led to the suggestion that the host plays a role in shaping its microbiota. In particular, it is assumed that epithelial secretions such as toxins or nutrients are key tools for a host to exert control over its microbes (Hooper et al. 1999, Salzman et al. 2010).

Our goal here is to apply and develop the theories of community stability for the biology of the gut microbiome. The need to develop better theoretical understanding of our relationship with the microbiota has been recognised (Foster et al. 2008, Gordon & Klaenhammer 2011). However, there are only few population models of microbiota (Bucci et al. 2012, Stein et al. 2013). We here present a novel network model of microbiota that is amenable to standard analysis of community stability. We study the influence of three characteristic features of the biology of mammalian gut communities: cooperativity of symbionts, high species diversity and, finally, high nutrient abundance. From

a host's perspective, a healthy microbiota is often assumed to be associated not only with stability, but also with cooperation among the species that provide nutrients for the host. However, our analysis indicates that these two objectives can be incompatible – larger proportions of cooperative interactions between microbial species will tend to destabilise a community. Mammalian gut communities are also diverse, particularly relative to many of the well-studied symbiotic associations (McFall-Ngai & Ruby 1991, West et al. 2002) and we show that, like cooperativity, diversity is a threat of community stability. Finally, we consider the effects of high nutrients and show that this may be central to the stability of gut communities. We discuss and analyse the observation that the host feeds key members of the community, and show how this can promote ecological stability.

5.3 Results and Discussion

The effect of cooperation on ecological stability

We begin by considering the effect of species interaction type on stability. As discussed above, the microbiome is often considered to contain a large proportion of positive interactions. However, the existing models of ecological networks usually consider food webs where all interactions between species are $+/-$, meaning that one species benefits to the detriment of the other (Foster et al. 2008). Other interaction types such as cooperation $(+/+)$ and competition $(-/-)$ will affect stability in different ways to $(+/-)$ interactions (Allesina & Tang 2012). Some studies have suggested that purely cooperative communities are relatively unstable (Allesina & Tang 2012), whilst others

have suggested intermediate amounts of cooperation can be stabilising (Mougi & Kondoh 2012). We, therefore, develop a new analysis here to identify the link between species cooperativity and ecological stability.

We here consider communities with a mixture of interaction types, and investigate the effect on stability of altering the proportions of each type (cooperation: $+/+$, exploitation: $+/-$ and competition: $-/-$, Figure 5.1 A). In the gut, cooperative cross-feeding networks are thought to be important for the host as they release otherwise inaccessible nutrients from the host's diet (Ze et al. 2012). Thus we first seek to develop analytical solutions for the effect of different levels of cooperation on community stability in our model. We derive a novel analytic stability criterion applicable to networks with any proportion of cooperation, competition or exploitative interactions between species, so as to account for the range of potential interactions among microbes.

Our model considers networks of interacting species in which each species' growth is determined by its own intrinsic growth rate, interactions with other members of the same species, and interactions with members of other species (see Methods for a detailed derivation). This is in accordance with previous models (Allesina & Tang 2012, May 1972, Mougi & Kondoh 2012) and a generalisation of classic Lotka-Volterra equations that allows us to incorporate multiple species and interaction types. Importantly, here species are represented as densities averaging over many individuals – an approach that is particularly well suited when considering large microbial populations.

Our analysis focuses on asymptotic stability following the classic definition used by May, whereby a steady-state of a dynamical system is defined to be

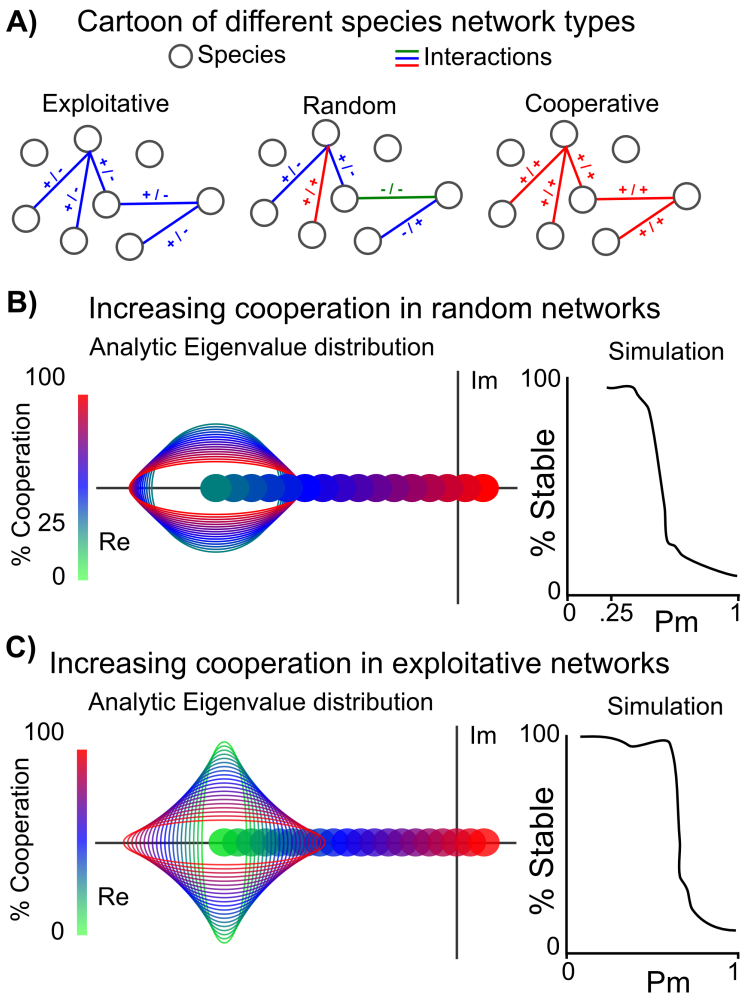


Figure 5.1: **Increased levels of cooperation decrease community stability**. A) Cartoon showing three different network types. In a purely exploitative network, interacting species (circles) form $+/-$ relationships only. In random networks, distributions of interaction types will be 25% cooperative $+/+$, 25% competitive $-/-$, and 50% $+/-$ exploitative. A purely cooperative network will only have $+/+$ interactions between species. B, C) Analytical solutions for the location of eigenvalues in the complex plane of community matrices with different proportions of cooperation ($S = 80$, $C = 0.35$) show that gradual increase of cooperation in random community matrices (A) or purely exploitative communities (B) leads to more positive eigenvalues and, therefore, decreased stability. We confirm our analytic results by simulating explicit communities and plotting the average number of stable systems as a function of the proportion of cooperative interactions, P_m (100 communities per P_m-value). A community is considered stable if the real (Re) parts of all eigenvalues are < 0. For our simulations we use the same approach as in Mougi & Kondoh 2012 and show that increased proportions of cooperation destabilise communities.

stable if the system will return to this point following a small perturbation (1972). This definition of stability has been applied widely to analyse fundamental properties of dynamical systems and ecologies (Allesina & Pascual 2007, Allesina & Tang 2012, May 1972), and tends to generate similar predictions to other measures of ecological stability, which ask whether any species will be lost from a system following perturbation (Chen & Cohen 2001, Townsend et al. 2010). It is, therefore, a suitable minimal model to capture microbiota dynamics that in reality may be more complicated. We calculate stability from the eigenvalues of the Jacobian matrix (in the following, the "community matrix") resulting from the dynamical system considered. Provided the real part of each of these associated eigenvalues lies below zero, the system will return to its equilibrium following a small perturbation, and can thus be considered "stable". Stability, therefore, can be determined from the region in the complex plane to which all eigenvalues will be restricted, and we continue Allesina and Tang's approach to derive these regions (see Methods and (Allesina & Tang 2012, Sommers et al. 1988)). The further this region lies along the positive part of the real axis, the less likely the system is to be asymptotically stable. The largest real part of the eigenvalues thus captures the *instability* of a given matrix. In order to capture *stability*, therefore, throughout the figures we plot the negative of this value. (Box: A stability criterion for mixed interaction types in ecological networks.) Our analysis shows that when we increase the proportion of cooperation between species, stability will decrease (Figure 5.1).

Gradually changing interaction types within communities towards more cooperation then leads to proportional gradual changes in stability likelihood be-

tween the extreme cases of random and purely cooperative networks presented by Allesina & Tang 2012. This result contradicts recent computer simulation based models of food webs, which predict that intermediate levels of cooperation leads to an increase in community stability likelihood (Mougi & Kondoh 2012). Our analysis proves that simply increasing cooperation will never lead to such increased stability, and we confirm our analytic results by applying identical computer simulations (Figure 5.1 B and C, Methods and Mougi & Kondoh 2012).

The effect of species diversity on ecological stability

In addition to cooperativity, another key feature of the biology of gut communities is species diversity. We, therefore, analyse here the effects of species diversity on ecological stability. Variable diversity was a central component of May's initial investigation into community stability 1972, which suggested that, for random interaction networks, increased diversity will tend to destabilise ecological networks. Here we extend this analysis for our networks where we vary the types of interactions from purely cooperative networks, through mixed interaction networks to purely competitive networks. This reveals that May's prediction holds irrespective of the nature of the interactions in an ecological network (Figure 5.2 A), confirming that species diversity is a destabilising process.

Since May, some authors (Mougi & Kondoh 2012, P. Vázquez et al. 2007) have argued that increasing species numbers will have an additional effect that is not captured by changing diversity as in May's original model. In particular, it has been argued that adding additional species will also tend to decrease the

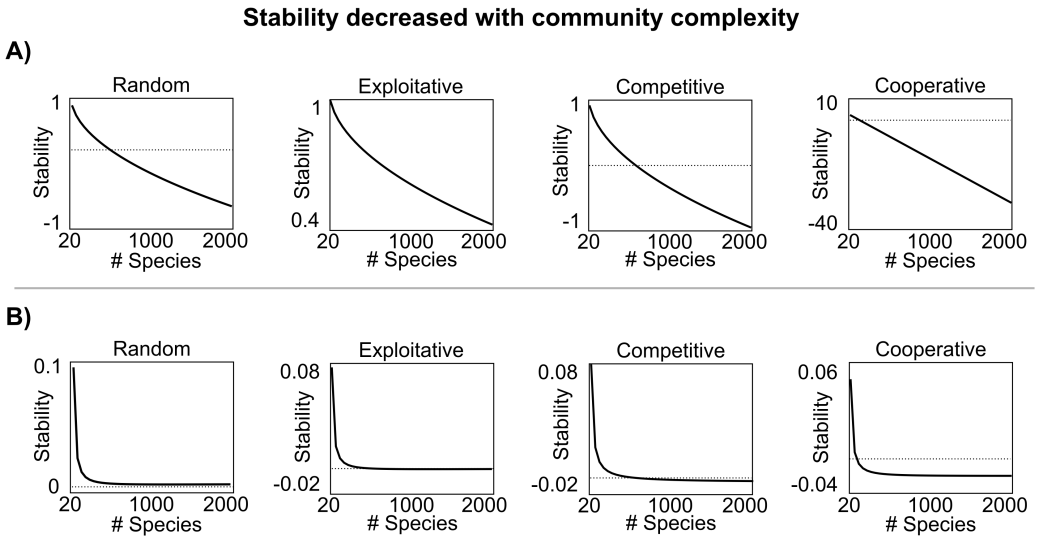


Figure 5.2: **Diversity can destabilise communities independent of interaction type.** Increasing the number of species in random, exploitative, competitive, or cooperative networks leads to less stable communities (A). These results hold even then when by increasing the number of species in the community, each individual interaction pair becomes less likely and, therefore, less strong. To demonstrate this, we scale each individual interaction strength by the average number of interaction partners (B, see Methods).

strengths of interactions between species (Mougi & Kondoh 2012), something that May suggests will *increase* stability. The logic behind decreasing interaction strength with increasing diversity is as follows. If a species only has a certain amount of time or space in which to interact, the addition of a new interaction partner should decrease the time (or space) available to interact with its original partners. One might, therefore, assume that there is a constant total amount of interaction that is possible and divide this among all of the interaction partners of a focal species. However, applying this as described does not affect our conclusions: increased diversity is still a destabilising effect (Figure 5.2 B).

Is there a way in which diversity can promote stability? Our analysis sug-

gests one route that links diversity and ecological stability. For this, the starting point is the relatively small set of highly cooperative species that are considered central to supplying nutrients to the host (Tremaroli & Bäckhed 2012). Specifically, we begin by considering an initially unstable community of 100 cooperating species (5-10% of the normal number of species found in a healthy gut, Tremaroli & Bäckhed 2012). To this, we can then add additional species that do not add to the cross feeding network but simply act as competitors that harm the focal cross feeders (Figure 5.3 A). Under these conditions, increased species numbers will tend to promote stability (Figure 5.3 B and C).

At first, this appears to contradict the result of May that predicts decreased stability with increased numbers of species (1972). However, the key difference between our scenario and May's investigation is that we are now changing *both* diversity and the nature of between species interactions. In May's random community matrices, increased diversity is not changing the types of interactions and only serves to destabilise the community because the addition of species will increase feedback loops with the potential to destabilise a community. In particular, positive feedback loops from cooperative interactions between species have a strong destabilising effect. Introducing competitors to a cooperative network then has two effects. There is a destabilising component from adding more species as in May's model. However, at least initially, this effect is dominated by the dampening of positive feedbacks in the network. The same effect can be seen when, instead of competitor species, species that interact in purely exploitative $(+/-)$ ways are added to a purely cooperative network (Figure S5.1 A). Adding randomly interacting species, however, does not have

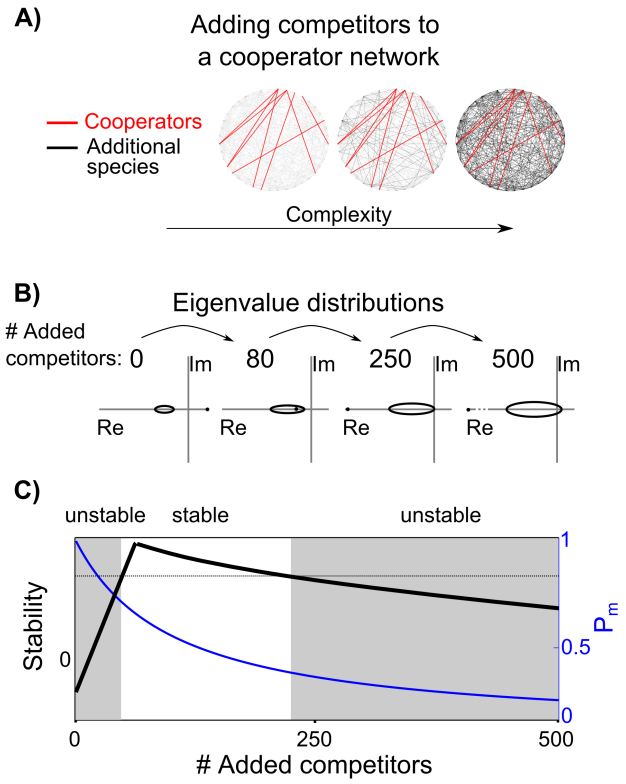


Figure 5.3: **Introducing more species can stabilise a network of cooperators**. A) Cartoon demonstrating how a network of cooperating species (red) is embedded in larger networks by adding more species that compete with the focal cooperator community (black). B, C) We add competitor species to a focal community of 100 cooperating species. Initially, additional species increase stability (black line in C) because the average interaction between species shifts from cooperation to competition (decrease in P_m, blue line in C) and the average row sums are reduced (the dot in B). Eventually, addition of even more species decreases stability because of increased between species interactions.

a stabilising effect because the dampening effect on existing positive feed back loops is cancelled out by the introduction of new feedback loops (Figure S5.1 B)

Our model then suggests that a host can maintain a cooperatively cross-feeding network by embedding it in a larger network that also contains competitive species. These additional species act to dampen the cooperative network and limit positive feedbacks that will destabilise the community. Allowing

competitors into the community that do not play a direct role in host feeding can therefore help to prevent instability. Thus, in this specific sense, species diversity may be helpful for a host.

The effects of nutrients and host feeding on ecological stability

The gut is often considered to be a high nutrient environment because of the abundance of food in the lumen (Bäckhed et al. 2005). In addition, the host secretes nutrients from the epithelial surface, especially during starvation periods when lumen nutrient concentrations are less abundant (Hooper et al. 1999). In this section, we consider how resource abundance, and particularly host feeding, may influence ecological stability.

The host epithelium secretes a range of nutrients into the microbiota, and can even be triggered to do so by a common member of a healthy microbiota community, *Bacteroides thetaiotaomicron* that can "request" additional nutrients from the host (Hooper et al. 1999, Sonnenburg et al. 2005, Tremaroli & Bäckhed 2012). The host then appears to ensure high ambient nutrient concentrations in the gut, even in addition to the nutrients from food already present in the gut. What is the effect of this additional food on ecological stability?

Intuitively, the effect of host feeding will be to decrease the dependence of a focal species on the nutrients from other species. We can then capture the consumption of host-secreted nutrients as an additional interaction for microbial species in the community. Now, host feeding will decrease the strength of interactions of a given microbial species with other species. In Figure 5.4 A, we show that consuming host-secreted nutrients leads to cooperative microbes

interacting less strongly with their cross-feeding partners, and thus the stability of the community is increased. The reason for this is that species divert some of their interaction effort into consuming the host secreted nutrients instead of engaging with other species in cross-feeding. Additional nutrients secreted by the host for cooperative species then can dampen otherwise destabilising interactions, and data suggest that host supplied nutrients are indeed specifically targeted at the cross feeders in gut communities (Hooper & Gordon 2001, Hooper et al. 1999). What happens though if host feeding affects all species in the gut? The effects are similar, and the community is again more stable (Figure 5.4 B). In sum, a high concentration of nutrients in the gut community may allow the host to control and limit the extent to which species of the microbiota interact with one another, which will have a positive effect of ecological stability.

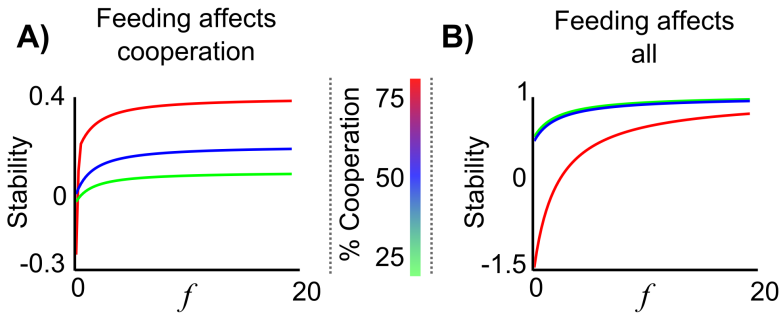


Figure 5.4: **Stabilisation of the microbiota through host feeding.** A) Stability in networks of 500 species with 25% (green), 50% (blue) and 75% (red) cooperative interactions and the remainder of the competitive type can be increased by the host through the secretion of nutrients (f, see Methods). Host feeding stabilises communities when cooperative-cross feeding interaction are reduced due to a diversion of interaction efforts away from between-species interactions and towards consuming host-secreted nutrients (A). This also works when interactions between species in general are diverted (B).

5.4 Conclusions

We present a minimal model of the microbial community in the human gut based on classic ecological network models (Allesina & Tang 2012, May 1972). The microbiota in the gut forms a diverse and stable community that can resist change in an individual human even over a period of years. How this remarkable stability is maintained is still unclear. Here we have sought to understand the factors that promote or inhibit stability in gut communities. We applied a simple network model to study how ecological stability is influenced by three of the key characteristics of gut biology. First, we studied the effects of cooperation between microbial species. Cooperative interactions among microbes are considered important for the benefits a healthy microbiota conveys to the host, namely the break-down of complex carbohydrates and release of short-chain fatty acids. However, our model predicts that this cooperativity can be problematic for ecological stability, because cooperating species create positive feedback loops in ecological networks. Secondly, in line with the classic ecological model of May, we found that the high species diversity of the gut can also be a problem for community stability. How then is ecological stability maintained in highly diverse gut communities? Our analysis suggested a way in which diversity can be good for ecological stability. In particular, when increased diversity causes an increased number of competing species to be added to an otherwise cooperating network, the effect is an increase in stability. The addition of competitors dampens the effect of positive feed-back loops between cooperators and can thereby increase stability to some extent. Last, we discuss a third key feature of gut biology: a high abundance of nutrients which in

part come from the secretion of nutrients by the host epithelium (Hooper et al. 1999, Tremaroli & Bäckhed 2012, Xu et al. 2007). When these nutrients divert interaction effort from between species interactions towards consumption of the additional host nutrients, we find that they can have a strongly stabilising effect on community stability.

It is well known that communities in which weak interactions dominate tend to have high ecological stability (McCann 2000) and we propose that nutrient secretion can achieve this effect. Consistent with this, nutrient secretion by the host epithelium is known to be common in the mammalian gut, particularly at times when there are low levels of alternative nutrients (Hooper et al. 1999). Further data comes from knockout mice lacking the ability to feed the microbiota with fucose, which is one of the key carbohydrates that is produced by the host and used by microbial species in the gut. Mice lacking the ability to make fucose show a significant decrease in alpha-diversity, meaning that in the knockout-mice, few genotypes dominate the microbiota, which is consistent with host feeding contributing to the diversity, and potentially ecological stability, of the microbial community (Kashyap et al. 2013). The mechanism by which this host feeding promoted diversity is not yet understood, and may be different to the mechanism in our model. However, these experiments support the importance of host feeding and support the general idea that host supplied nutrients are key to the ecological functioning of gut communities.

While it is already clear that the ecology of gut communities is central to human health and well-being, our understanding of this ecology remains limited. In addition to the large-scale data collection that is in progress, there

is a need for fundamental theory that attempts to dissect the key factors that affect gut communities. The field of ecology has a rich history of fundamental theory and here we have applied one of the key techniques, network theory, to the case of the gut microbiome. This reveals that two of the key features of gut communities - cooperativity and diversity - can be a problem for their stability but, equally, that host feeding may provide a mechanism to counter this threat. In this view, the host is acting as an ecosystem engineer that is constantly manipulating the system in order to promote a productive and stable symbiont community.

5.5 Box 1

We consider systems of S microbial species in which each pair interacts with probability C. A proportion P_m of these interactions are cooperative (i.e. $+/+$), P_c are competitive ($-/-$) and the remainder are of the exploitative type ($+/-$), with interaction strengths drawn from a half-normal distributions $|X|$ with standard deviation σ, mean $E(|X|) = \pm\sigma\sqrt{2}/\sqrt{\pi}$ and variance σ^2. The eigenvalues of the community matrices associated with these systems will lie on characteristic ellipses in the complex plane (Sommers et al. 1988), accompanied by a single eigenvalue that represents the average row sum of the matrix considered (Allesina & Tang 2012). It is therefore the larger value of the horizontal radius of the ellipse and the average row sum that will determine the stability of the system.

In purely cooperative systems, the horizontal radius of the eigenvalue ellipse and the average row sum of community matrix are both larger than those associated with a random system (25% of all interactions are cooperative, 25% competitive, and 50% are exploitative) with same connectivity, meaning a cooperative network will be less stable than a random one (Allesina & Tang 2012). Here we analyse more realistic scenarios for the microbiota, accounting for intermediate mixtures of interaction types, and derive in the Methods how the radius, R_e, of the ellipse that determines stability can be defined as,

$$R_e := \sqrt{S(C\sigma^2 - (C(P_m - P_c)E(|X|))^2} \; * \\ \Big(1 + \frac{E(|X|)^2(2(P_m + P_c) - 1 - C(P_m - P_c)^2)}{\sigma^2 - C(P_m - P_c)^2 E(|X|)^2}\Big),$$

and the eigenvalue associated with the average row sum, R_s, as

$$R_s := (S-1)C(P_m - P_c)E(|X|)$$

.

From the logistic term in the Lotka-Volterra equations it follows that each species has a negative effect on its own growth, which is represented on the diagonal entries of the community matrix and set to $d = -1$ unless otherwise stated. Then, the ellipses are centred at $(d, 0)$ and our criterion for stability is $max(R_e, R_s) < |d|$.

5.6 Methods

Modelling communities as networks

In accordance with previous models (Allesina & Tang 2012, Mougi & Kondoh 2012), we are using a generalisation of the classic Lotka-Volterra type model, which allows us to incorporate multiple species and interaction types. Here species are represented as densities averaging over many individuals. In this system we have a community composed of S species, which interact with one another in one of three possible ways – either in an exploitative manner, as competitors for a common resource, or in a mutually beneficial, cooperative relationship (such as through cross-feeding). The system can be generally defined through the system of non-linear, first order, ordinary differential equations, which describe how the densities of the species in the system (X_i) change over time. The rate of change of each species is dependent upon the other species with which it interacts, according to a Holling type I (linear) functional re-

sponse, such that the rate of change of species i is defined,

$$\frac{dX_i}{dt} = X_i(r_i - dX_i + \sum_{j=1}^{S} a_{ij}X_j). \tag{5.1}$$

Here r_i is the intrinsic growth rate of species i, d the self-limitation imposed by each species upon itself, and a_{ij} describes the effect species j has upon species i (assuming $a_{ii} = 0$). It is worth noting here that we are assuming each species has the same degree of self-regulation (d is the same for each $\frac{dX_i}{dt}$), although this assumption can later be relaxed. Importantly, the precise form of a_{ij} varies depending upon the nature of the relationship between species i and species j, as considered below:

1. If species i exploits species j, a_{ij} will be positive and a_{ji} will be negative.

2. If species i and j compete with one another for a common resource, the sign of both a_{ij} and a_{ji} will be negative.

3. If species i and j are in a cooperative relationship, the sign of both a_{ij} and a_{ji} will be positive.

4. If the two species do not interact with one another, both a_{ij} and a_{ji} will be zero.

The proportion of links that are present (that is, the proportion of $a_{ij} \neq 0$) is determined by the connectivity C of the system, such that in a system with $C = 1,\ a_{ij} \neq 0\ \forall\ i \neq j$, whilst in a system with $C = 0,\ a_{ij} = 0\ \forall\ i, j$. Following the same approach as in previous work we can assume the system has a positive definite equilibrium given by the $S \times 1$ vector $\mathbf{X}^*$, and thus $\underline{r} \in \mathbf{R}$ is an $S \times 1$

vector determined such that $\frac{dX_i}{dt} = 0$ when evaluated at $\mathbf{X}^*$. It is then the stability of X^* that we are interested in (Allesina & Tang 2012, Chen & Cohen 2001, Mougi & Kondoh 2012).

We begin by determining the form of the Jacobian, the matrix given by

$$M_{ij} = \frac{\partial(\frac{dX_i}{dt})}{\partial X_j}\bigg|_{X^*}.$$

By definition, at a positive definite equilibrium $r_i = dX_i^* - \sum_{j=1}^{S} a_{ij}X_j^*$, thus the diagonal entries of the Jacobian are given by $M_{ii} = -dX_i^*$ whilst the off-diagonals are given by $M_{ij} = a_{ij}X_i^*$. It is this "community matrix" M that we will now focus upon for the derivation of our stability criterion.

Derivation of stability criterion

The basis of the following work can be traced back to the seminal work by Gerschgorin, who showed for a complex matrix A with elements a_{ij}, each eigenvalue λ_i lies within a disk centred at a_{ii} with radius R_i, where $R_i = \sum_{j\neq i} |a_{ij}|$ (Gerschgorin 1931).

This work was extended by Sommers *et al* in 1988 (Sommers et al. 1988), who examined the eigenvalues of an $S \times S$ matrix A with elements drawn from a Normal distribution with mean $\mathbb{E}(A_{ij}) = 0$ and $\mathrm{Var}(A_{ij}) = \frac{1}{S}$. The authors proved that as $S \to \infty$ then the eigenvalues of A, $\lambda = x + iy$ will be distributed on an ellipse $(\frac{x}{a})^2 + (\frac{y}{b})^2 \leq 1$ centred at the origin, with $a = 1 + \tau$ and $b = 1 - \tau$ where $\tau = S\mathbb{E}(A_{ij}A_{ji})$. Further, setting each of the diagonal entries of A to $-d$ will move the centre of this ellipse to $(-d, 0)$, thus the system will be stable if the half-horizontal radius of the eigenvalue ellipse is less than d.

Here we follow a similar approach to that used by Allesina & Tang 2012, which combines this theory with various rescaling arguments to derive an equivalent eigenvalue ellipse for the Jacobian M corresponding to our Lotka-Volterra dynamical system. Specifically, we consider a community of size S and connectivity C where a proportion P_m of the interactions are cooperative (i.e. $+/+$), P_c are competitive $(-/-)$ and the remainder $(1 - P_m - P_c)$ are exploitative $(+/-)$. The diagonal entries of this matrix are each set to $-d$, whilst the values of each of the off-diagonal entries in this community matrix are taken from a half normal distribution $|X|$ with variance σ^2 and mean $E(|X|) = \frac{\sigma\sqrt{2}}{\sqrt{\pi}}$, with the signs of these then set according to the interaction type.

We can make use of the observation by Rothblum and Tan 1985 that for any $a \in \mathbb{R}$, the half horizontal radius of the eigenvalue ellipse for the matrix $N = M + (d + a)I - a.\mathbf{1}$ will have the same value as the half horizontal radius for M. In our case, we can therefore define $a = C\mathbb{E}(|X|)(P_m - P_c)$, then call $N_{ij} = M_{ij} - a$ to achieve,

$$\mathbb{E}(M_{i\neq j}) = C[P_m\mathbb{E}(|X|) - P_c\mathbb{E}(|X|)], \tag{5.2}$$

$$\mathbb{E}(N_{i\neq j}) = \mathbb{E}(M_{i\neq j}) - a = 0. \tag{5.3}$$

Thus N and any scalar multiple of this matrix will obey the first requirement of Sommers' theory – that $\mathbb{E}(cN) = 0$. We next consider the variance of N. We have $\mathrm{Var}(N_{ij}) = \mathrm{Var}(M_{ij} - a) = \mathrm{Var}(M_{ij}) = C\sigma^2 - C^2(P_m - P_c)^2\mathbb{E}(|X|)^2$ following from the definition of the distribution from which M has been drawn. We can therefore rescale setting $N^* = \frac{N}{\beta}$ with $\beta = \sqrt{SVar(N_{ij})}$ to fulfil the second requirement of Sommers' theory. That is,

$$\mathbb{E}\Big(\frac{N}{\sqrt{SVar(N)}}\Big) = 0, \tag{5.4}$$

$$Var\Big(\frac{N}{\sqrt{SVar(N)}}\Big) = \frac{1}{S}. \tag{5.5}$$

Thus all eigenvalues of N^* will be contained in the ellipse with half-horizontal radius $a^* = 1 + S\tau$ and half-vertical radius $b^* = 1 - \tau$ with $\tau = \mathbb{E}(N^*_{ij}N^*_{ji})$. Further, we note that eigenvalues are closed under scalar multiplication, thus if λ is an eigenvalue of the matrix N^*, then $\beta\lambda$ will be an eigenvalue of N. This means that the eigenvalues of N will be contained in the ellipse with half-horizontal radius $a = \beta(1 + \tau)$ and half-vertical radius $b = \beta(1 - \tau)$. This gives us a formula for the horizontal width of the eigenvalue ellipse for N, and therefore for M of,

$$R_N = \sqrt{SVar(N_{ij})}\Big(1 + \frac{S\mathbb{E}(N_{ij}N_{ji})}{SVar(N_{ij})}\Big). \tag{5.6}$$

We must then calculate $\mathbb{E}(N_{ij}N_{ji})$, which we do by noting that:

$$\mathbb{E}(N_{ij}N_{ji}) = \mathbb{E}(M_{ij}M_{ji}) - 2a\mathbb{E}(M_{ij}) + a^2 \text{ and,} \tag{5.7}$$

$$\mathbb{E}(M_{ij}M_{ji}) = C[P_m\mathbb{E}(M_{ij}M_{ji}| + /+) +$$

$$P_c\mathbb{E}(M_{ij}M_{ji}| - /-) + (1 - P_m - P_c)\mathbb{E}(M_{ij}M_{ji}| + /-)] \tag{5.8}$$

$$= C[(2(P_m + P_c) - 1)\mathbb{E}(|x|)^2]. \tag{5.9}$$

Combining (5.7) and (5.10) thus gives $\mathbb{E}(N_{ij}N_{ji}) = C\mathbb{E}(|x|)^2[2(P_m + P_c) - 1 - C(P_m - P_c)^2]$, giving us our expression for the half-horizontal radius of the eigenvalue ellipse of M as,

$$R_e := \sqrt{S(C\sigma^2 - (C(P_m - P_c)\mathbb{E}(|X|))^2} \left(1 + \frac{\mathbb{E}(|X|)^2(2(P_m + P_c) - 1 - C(P_m - P_c)^2)}{\sigma^2 - C(P_m - P_c)^2\mathbb{E}(|X|)^2}\right) \tag{5.10}$$

Finally, we note that as each of the diagonal entries of M is $-d$, this ellipse will be centred upon the point $(-d, 0)$.

However, we must also note that for systems with high levels of cooperative or competition, the eigenvalue with the largest magnitude real part will not necessarily lie within the ellipse (Allesina & Tang 2012) (this follows from Gerschgorin's work from 1931, and can also be shown with simulations). Instead, following from Gerschgorin 1931, for large matrices this eigenvalue can be approximated by the average sum of the off-diagonal entries of each row (with the equivalent eigenvector approximately $\mathbf{1}$). This can be defined analytically as,

$$R_s := (S - 1)C(P_m - P_c)\mathbb{E}(|X|) \tag{5.11}$$

From these we generate a stability criterion for a network of a mixture of P_m cooperative, P_c competitive and $1 - P_c - P_m$ exploitative interactions given by:

$$\max(R_e, R_s) < d \tag{5.12}$$

Weighting of interaction strengths

Following our same approach as previously, we now re-derive our stability criterion to enable weighting of each interaction type individually by its respective

weighting factor,

$$w_m = \text{cooperative}, \tag{5.13}$$

$$w_c = \text{competitive}, \tag{5.14}$$

$$w_e = \text{exploitative}, \tag{5.15}$$

$$w_s = \text{self-regulation}, \tag{5.16}$$

such that the average strength of a cooperative interaction will be $\frac{\mathbb{E}(|X|)}{w_m}$ and likewise for the other interaction types. The exact form of each of these weighting factors will vary, depending upon the type of weighting being employed.

Weighting by average number of interaction partners

In the first case all interactions are weighted by the average number of interaction partners, such that,

$$w_m = w_c = w_e = w_s = C * (S - 1) + 1. \tag{5.17}$$

Implementation of nutrient secretions by the host

In the subsequent weighting case, we then consider the addition of nutrients, the level of which is determined by the host-feeding parameter f. In each case, these simply act to divert interaction effort away from cooperative (or all between-species) interactions as instead of engaging in cross-feeding (or other between-species interactions), host provided nutrients are being consumed, such that in the general case, weights are defined as,

$$w_m = 1 + f, \tag{5.18}$$

$$w_c = w_e = \ w_s = \ 1, \tag{5.19}$$

whilst in the case where all between-species interactions are reduced by host-feeding, weights are,

$$w_m = 1 + f, \tag{5.20}$$

$$w_c = 1 + f, \tag{5.21}$$

$$w_e = 1 + f, \tag{5.22}$$

$$w_s = 1. \tag{5.23}$$

Derivation of the stability criterion with various weighted interaction strengths

As before, we rescale our community matrix M to generate a matrix $N = M + (d+a)I - a.\mathbf{1}$ that will have the same eigenvalue distribution as M, and also fulfil the requirements necessary to the application of Sommers' theory on eigenvalue localisation. In this case, we define $a = \mathbb{E}(M_{ij}) = C\mathbb{E}(|X|)(w_m P_m - w_c P_c)$, then call $N_{ij} = M_{ij} - a$, such that the half-horizontal radius of the ellipse containing the eigenvalues of our community matrix will be defined by,

$$R_e = \sqrt{SVar(N_{ij})}\Big(1 + \frac{S\mathbb{E}(N_{ij}N_{ji})}{SVar(N_{ij})}\Big). \tag{5.24}$$

We first note that $Var(N_{ij}) = Var(M_{ij}) = \mathbb{E}(M_{ij}^2) - \mathbb{E}(M_{ij})^2$. We can then decompose these terms into those relating to the different interaction types, such that,

$$\mathbb{E}(M_{ij}^2) = P_m\mathbb{E}(M_{ij}^2|+/+) + P_c\mathbb{E}(M_{ij}^2|-/-) + P_e\mathbb{E}(M_{ij}^2|+/-), \quad (5.25)$$

$$= C\sigma^2(w_m^2P_m + w_c^2P_c - (1-P_m-P_c)w_e^2), \quad (5.26)$$

such that

$$Var(N_{ij}) = C(\sigma^2(w_m^2P_m + w_c^2P_c - (1-P_m-P_c)w_e^2)\dots$$

$$- C\mathbb{E}(|X|)^2(w_mP_m - w_cP_c)^2). \quad (5.27)$$

We next note that $\mathbb{E}(N_{ij}N_{ji}) = \mathbb{E}(M_{ij}M_{ji}) - 2a\mathbb{E}(M_{ij})^2 + a^2$, and that,

$$\mathbb{E}(M_{ij}M_{ji}) = P_m\mathbb{E}(M_{ij}M_{ji}|+/+) + P_c\mathbb{E}(M_{ij}M_{ji}|-/-)\dots \quad (5.28)$$

$$+ P_e\mathbb{E}(M_{ij}M_{ji}|+/-), \quad (5.29)$$

$$= C\mathbb{E}(|X|)^2(P_mw_m^2 + P_cw_c^2 - (1-P_m-P_c)w_e^2), \quad (5.30)$$

which gives,

$$\mathbb{E}(N_{ij}N_{ji}) = C\mathbb{E}(|X|)^2(P_mw_m^2 + P_cw_c^2 - (1-P_m-P_c)w_e^2)\dots$$

$$- (C\mathbb{E}(|X|)(w_mP_m - w_cP_c))^2. \quad (5.31)$$

We can thus combine and simplify the terms above to get the expression for the half-horizontal radius of the eigenvalue ellipse of M as,

$$R_e := \sqrt{SC(\sigma^2(w_m^2P_m + w_c^2P_c - w_e^2(1-P_m-P_c) - C\mathbb{E}(|X|)^2(w_mP_m - w_cP_c)^2)} * \Big(1 + \frac{\mathbb{E}(|X|)^2(P_m(w_m^2+w_e^2) + P_c(w_c^2+w_e^2) - w_e^2 - C(w_mP_m - w_cP_c)^2)}{\sigma^2(w_m^2P_m + w_c^2P_c - w_e^2(1-P_m-P_c)) - C\mathbb{E}(|X|)^2(w_mP_m - w_cP_c)^2}\Big).$$

Further, we can also define the average row sum as,

$$R_s := (S-1)C(w_m P_m - w_c P_c)\mathbb{E}(|X|). \tag{5.32}$$

From these we generate the stability criterion for a weighted network is given by:

$$\max(R_e, R_s) < d \tag{5.33}$$

We can only see a short distance ahead, but we can see plenty there that needs to be done.

Alan Turing

6 Discussion and Conclusion

Each of my results chapters has its own detailed discussion and conclusion section. Here, I summarise each chapter's key findings and detail some of the general observations and conclusions that have emerged from my work.

Chapter 2: The Evolution of Quorum Sensing as a Mechanism to Infer Kinship

- Quorum sensing in biofilms can directly benefit a genotype of microbes that regulates the secretion of diffusible public goods using autoinducer concentrations as queues to initiate secretion.

- Quorum sensing is particularly powerful under strong competition with other genotypes, for example whenever a focal cell lands in an environment where nutrients are scarce and many other genotypes are present.

- During growth, spatial structure of the biofilm changes and leads to segregation of genotypes. This means that a quorum sensing genotype can infer kinship by measuring accumulating autoinducer concentration as local relatedness of cells increases with biofilm maturation.

- The advantage of quorum sensing strains over non-secretor genotypes stems from the growth-promoting effect of public goods that are now preferentially available to clonemates of the secreting cell. The advantage over constitutive secretors, however, stems from the fact that under high competition, fast growth in the early stages of biofilm growth reduces the chance of a focal cell being buried and starved by neighbouring cells. Constitutive secretors fare poorly against faster-growing non-secretors while quorum sensing strains delay secretion and grow equally fast as non-secretors, thereby maximising their chance of not getting buried. Quorum-sensing genotypes can strive where constitutive secretors fail.

Chapter 3: Adhesion as a weapon in microbial competition

- Adhesion to surfaces is a defining characteristic of microbial biofilms, but it has received little attention as a trait under natural selection. The effect of adhesion on microbial competition is dependent on the architecture of the environment. When nutrients come from above, high adhesion means that a focal genotype can be smothered by less adhesive competitors and starve. Localising at the base of a colony, however, is advantageous when nutrients are supplied from below the colony, as happens in many host environments such as the gut.

- In the gut, cells further away from the epithelium will be sloughed off, enhancing the advantage of localising near the epithelium beyond preferential access to nutrients: higher adhesiveness can help cells push less adhesive competitors off the surface and out of the colony.

- The advantage of pushing competitors off the substratum can be seen in experiments competing two strains of the human pathogen *Vibrio cholerae* growing in flow chambers. More adhesive cells push competitors aside and eventually out of the colony leading to the success of the more adhesive strain.

- Localisation within a biofilm can be key for microbes in competition with others. Our results emphasise the evolutionary importance of traits that affect key physical properties of cells that determine where a cells will localise.

Chapter 4: The Evolution of Mutualism in Gut Microbiota Via Host Epithelial Selection

- Microbial competition in the gut will lead to the selection of fast growing species instead of the most helpful microbes for a host. This poses a problem for the maintenance of a mutualistic host-microbiota relationship. Partner-choice can enforce helpfulness of microbes.

- The spatial structure in the gut environment leads to amplified selection strengths of host-secretions that control epithelium-associated microbial communities.

- Microbes selected for by epithelial secretions have the strongest advantage near the epithelium. Here, the epithelium-favoured population expands and gradually pushes any other strains up and out of the community as cells deeper in the lumen are continuously sloughed off.

- The selectivity-amplifying ratchet effect depends on growth near the epithelium and, therefore, makes selection via nutrient secretion benefitting beneficial microbes more than others a particularly powerful tool for partner-choice.

- We hypothesise that host selection via nutrients is not easily circumvented by non-helpful "cheats" because epithelium-secreted nutrients closely resemble those molecules in the human diet that the microbiota typically helps the host to digest - per definition only helpful microbes will therefore benefit from this type of epithelial nutrient secretion.

Chapter 5: A network model of the gut microbiota – stability and host control

- The populous and species-rich gut microbiota forms a stable community where microbes form cooperative cross-feeding networks that benefit the host. Cooperation, however, tends to destabilise communities and we do not know how stability in the microbiota is maintained.

- We show that additional species that compete with a focal community of cooperating species can lead to more stable systems.

- The host may allow additional competitor species to coexist in the microbiota by providing additional nutrients – this is known to occur at

the gut epithelium. As the number of additional competitors exceeds a critical number, however, further increase in competing species numbers destabilises the community.

- The destabilising effect of many species can be reduced when interactions strengths between species in general are reduced. Additional nutrients secreted by the host can achieve this if interaction efforts are diverted from between-species cross-feeding towards utilisation of host nutrients instead.

- The host may function as an ecosystem engineer that seeks to actively manage a dynamic network of microbial species in the gut. Nutrient secretion by the host epithelium emerges as apotentially powerful way to ensure microbiota stability.

6.1 General remarks and future directions

Some general points have emerged from my work regarding the importance of understanding microbe–host ecology and evolution in general, and computational and mathematical models in particular.

The role of modelling in Biology.

The success of any model should be measured by whether it provides new insight into an otherwise too-complex-to-understand problem. This means that a model and its results need to be communicated as clearly as possible. There is a trade-off between technically accurate descriptions and accessibility, but

this, therefore, needs to be a key focus of the theoretical community and has to be improved upon should the modeller's work not be ignored[1].

My work presented in this thesis has used individual-based computer models of bacteria. These models have the advantage of generating such accessible results by allowing to display the outcome of simulations in an intuitive way that resembles the real world. This is a powerful approach and there are attempts to make such models usable for biologists without training in computational modelling by creating graphical user interfaces, or through simple input files allowing a user to quickly simulate microbial communities (Lardon et al. 2011).

It will be interesting to follow where this approach leads to. A potential pitfall is that user interface will have to anticipate most of the questions that a user may ask. Therefore, the user's model already exists in principle, and novelty of generated simulations may be limited. In order to answer genuinely new questions, one will usually have to develop a new model and new code.

Biofilms on host surfaces

Here, I present a novel biofilm model of host-associated communities. Previous simulations had shown how spatial structure and nutrient concentration gradients above biofilms affect social evolution in microbes (Nadell et al. 2008, Xavier & Foster 2007). This led to a simple question: what if nutrients do not come from above?

[1]Fawcett & Higginson show that the number of equations per page negatively correlate with citation frequency in selected ecological journals (2012). This is a flawed study and has received valid criticism (Chitnis & Smith 2012, Fawcett & Higginson 2012, Fernandes 2012, Gibbons 2012, Kane 2012), but a core message is that the blame goes both ways: a reluctance of experimental scientists to embrace theoretical findings and the frequent insufficient ability of theoreticians to explain or justify the relevance of their model.

Host-associated microbes typically receive nutrients from the host surface rather than only from above the colony. Implementing a simple change in the environmental architecture allowed the investigation of how microbe-microbe interactions and microbe-host interactions affect and constrain each other in this environment. In particular, my work has shown how microbial adhesion properties, nutrient preferences and secretions can underlie unique evolutionary constraints in host environments.

Overall, my thesis focuses on host-microbe interactions that occur between humans and their gut microbiota. Improved understanding of this relationship can have immediate impact on human well-being. For example, the search for effective probiotics to improved human health have not been very successful (Borody & Khoruts 2012, Holzapfel et al. 1998, Tlaskalová-Hogenová et al. 2011) and only fecal transplants from healthy donors to severely ill patients have shown success in restoring a healthy microbiota (Borody & Khoruts 2012). My models predict that increased adhesiveness will be a powerful competitive strategy in gut microbes (chapter 3). Indeed, a recent study of the potentially probiotic *Lactobacillus rhamnosus* has attributed this strain's superior host benefits to an increased ability to persist in the gut through increased adherence to epithelial mucus compared with other probiotic candidates (von Ossowski et al. 2010). Understanding traits that allow microbes to compete well in the gut environment, therefore, will have the potential to facilitate the hunt for better probiotics. Inventing new strategies how to treat pathogen infections will be a particularly important future challenge as we are running out of effective antibiotics that can be used against pathogens. Designing more competitive

but non-pathogenic probiotic species to be administered as a treatment may have this potential.

The host as an ecosystem engineer

Apart from between-microbe interactions, my work highlights the importance of hosts as "ecosystem engineers". My models predict that secretions from the gut epithelium will be powerful tools for maintenance and control of a dynamic community of microbes. I have shown how the gut is an environment especially amenable to such host control due to its architecture which amplifies the selective strength of epithelial secretions (chapter 4). A recurring theme from my individual-based simulations as well as my more abstract ecological network model is that the secretion of growth-promoting nutrients into the microbiota may be a particularly powerful tool for the host to manipulate its microbiota (chapter 4 and 5).

This theoretical predictions can be tested. Indeed, a recent study on the *Vibrio fischeri* – bobtail squid mutualism has shown how the squid specifically provides growth advantages to mutualistic *V. fisheri* partners at the light organ's epithelium, and the authors suggest using their model in the future as a test system for our predictions that epithelial secretions can be particularly powerful (Kremer et al. 2013). Further, my models predict that individuals without the ability to secrete nutrients from the gut epithelium should be strongly impeded in their ability to maintain a beneficial microbiota. A recent experiment with mice points in that direction. Microbiota of knock-out mice lacking the Fut2 gene, and thus the ability to secrete fucose, were compared with microbiota from wild-type mice. Fucose is a carbohydrate that epithelial

cells attach to mucus polymers and is used as a food source by gut microbes (Hooper et al. 1999). In Fut2^{-} mice, diversity in the microbiota was significantly decreased compared with Fut2^{+}–mice that produced fucose (Kashyap et al. 2013). This experimental system is still quite artificial as these mice are born "germ-free" and then inoculated with an artificial, "humanised" microbiota from a healthy human donor. However, this experiment provides an ideal proof-of-principle showing that feeding can have strong effects on microbiota composition in the gut.

A key factor that is missing from my current community model is the explicit consideration of microbial evolution within a community. Rapid growth rates and the potential for phenotypic plasticity in diverse microbial communities may lead to a quick response of microbes to any attempts from a host, or a biotechnological engineer, attempting to "steer" dynamical microbial communities. Therefore, my future work will first port my current network model into a computer model that investigates permanence of communities, and then combine this with recent game-theoretical approaches allowing each individual species in a network to adjust their interactions with others in order to maximise their own fitness (Cao et al. 2011). It will be interesting to see how this explicit consideration of evolution in microbes will affect the predicted ability of a host to control diverse communities.

Engineering microbial societies

The importance of understanding microbial ecology and evolution extends beyond host-microbiota relationships. I have studied Biotechnology as an undergraduate because I was fascinated by the idea of harnessing the abilities of liv-

ing organisms to solve some of the most important[2] problems of today's world. During my undergraduate research project, I learned that without comprehending the dynamics of complex systems such as the communities of bioplastic-producing microbes I was working with (Raberg et al. 2011), Biotechnology will lag behind its potential. It is now recognised that multi-species communities of microbes can be better than monocultures at performing certain tasks, for example in biofuel production (Zuroff & Curtis 2012). Yet, maintaing stability in such multi-species communities remains difficult (Zuroff et al. 2013). Perhaps we can learn from our own bodies that evolved to live with and control complex microbial communities how to become better microbial community engineers.

[2]all of them, let's not kid ourselves

Bibliography

Absalon, C., Van Dellen, K. & Watnick, P. I. (2011), 'A communal bacterial adhesin anchors biofilm and bystander cells to surfaces.', *PLoS Pathog* **7**(8), e1002210.

Ai, H.-W., Henderson, J. N., Remington, S. J. & Campbell, R. E. (2006), 'Directed evolution of a monomeric, bright and photostable version of Clavularia cyan fluorescent protein: structural characterization and applications in fluorescence imaging.', *Biochem J* **400**(3), 531–40.

Alam, M., Sultana, M. & Nair, G. (2006), 'Toxigenic Vibrio cholerae in the aquatic environment of Mathbaria, Bangladesh', *Appl Environ Microbiol* **72**(4), 2849–2855.

Allesina, S. & Pascual, M. (2007), 'Network structure, predatorprey modules, and stability in large food webs', *Theor Ecol* **1**(1), 55–64.

Allesina, S. & Tang, S. (2012), 'Stability criteria for complex ecosystems.', *Nature* **483**(7388), 205–8.

Alpkvist, E., Picioreanu, C., van Loosdrecht, M. C. M. & Heyden, A. (2006),

'Three-dimensional biofilm model with individual cells and continuum EPS matrix.', *Biotechnol Bioeng* **94**(5), 961–79.

Amann, R., Ludwig, W. & Schleifer, K. (1995), 'Phylogenetic identification and in situ detection of individual microbial cells without cultivation', *Microbiol Rev* **59**(1), 143–169.

Atuma, C., Strugala, V., Allen, A. & Holm, L. (2001), 'The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo', *Am J Physiol-Gastr L* **280**(5), G922–929.

Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., Semenkovich, C. F. & Gordon, J. I. (2004), 'The gut microbiota as an environmental factor that regulates fat storage.', *P Natl Acad Sci USA* **101**(44), 15718–23.

Bäckhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A. & Gordon, J. I. (2005), 'Host-bacterial mutualism in the human intestine.', *Science* **307**(5717), 1915–20.

Bassler, B. L. (2002), 'Small talk. Cell-to-cell communication in bacteria.', *Cell* **109**(4), 421–4.

Bassler, B. L. & Losick, R. (2006), 'Bacterially speaking.', *Cell* **125**(2), 237–46.

Beauregard, P. B., Chai, Y., Vlamakis, H., Losick, R. & Kolter, R. (2013), 'Bacillus subtilis biofilm induction by plant polysaccharides.', *P Natl Acad Sci USA* **110**(17), E1621–30.

Belenguer, A., Duncan, S. H., Calder, A. G., Holtrop, G., Louis, P., Lobley, G. E., Harry, J. & Flint, H. J. (2006), 'Two Routes of Metabolic Cross-Feeding between Bifidobacterium adolescentis and Butyrate-Producing Anaerobes from the Human Gut Two Routes of Metabolic Cross-Feeding between Bifidobacterium adolescentis and Butyrate-Producing Anaerobes from the Human Gut', *Appl Environ Microb* **72**(5), 3593–3599.

Berk, V., Fong, J. C. N., Dempsey, G. T., Develioglu, O. N., Zhuang, X., Liphardt, J., Yildiz, F. H. & Chu, S. (2012), 'Molecular architecture and assembly principles of Vibrio cholerae biofilms.', *Science (New York, N.Y.)* **337**(6091), 236–9.

Beun, J. J., Heijnen, J. J. & van Loosdrecht, M. C. (2001), 'N-removal in a granular sludge sequencing batch airlift reactor.', *Biotechnol Bioeng* **75**(1), 82–92.

Bevins, C. L. & Salzman, N. H. (2011), 'The potter's wheel: the host's role in sculpting its microbiota.', *Cell Mol Life Sci* **68**(22), 3675–85.

Böckelmann, U., Szewzyk, U. & Grohmann, E. (2003), 'A new enzymatic method for the detachment of particle associated soil bacteria', *J Microbiol Meth* **55**(1), 201–211.

Boles, B. R., Thoendel, M. & Singh, P. K. (2004), 'Self-generated diversity produces "insurance effects" in biofilm communities.', *P Natl Acad Sci USA* **101**(47), 16630–5.

Bollinger, R. R., Barbas, A. S., Bush, E. L., Lin, S. S. & Parker, W. (2007), 'Biofilms in the normal human large bowel: fact rather than fiction', *Gut* **56**(10), 1481–1482.

Boris, S., Suárez, J. E., Vázquez, F. & Barbés, C. (1998), 'Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens.', *Infect Immun* **66**(5), 1985–9.

Borody, T. J. & Khoruts, A. (2012), 'Fecal microbiota transplantation and emerging applications.', *Nat Rev Gastroenterol Hepatol* **9**(2), 88–96.

Brown, S. (1999), 'Cooperation and conflict in hostmanipulating parasites', *Proc R Soc Lond B* **266**, 1899–1904.

Brown, S. P. & Buckling, A. (2008), 'A social life for discerning microbes.', *Cell* **135**(4), 600–3.

Brown, S. P. & Johnstone, R. A. (2001), 'Cooperation in the dark: signalling and collective action in quorum-sensing bacteria.', *Proc R Soc Lond B* **268**(1470), 961–5.

Brugman, S. & Nieuwenhuis, E. E. S. (2010), 'Mucosal control of the intestinal microbial community.', *J Mol Med* **88**(9), 881–8.

Bucci, V., Bradde, S., Biroli, G. & Xavier, J. B. (2012), 'Social Interaction, Noise and Antibiotic-Mediated Switches in the Intestinal Microbiota', *PLoS Comput Biol* **8**(4), e1002497.

Callaway, R. M., Thelen, G. C., Rodriguez, A. & Holben, W. E. (2004), 'Soil biota and exotic plant invasion.', *Nature* **427**(6976), 731–3.

Cao, L., Ohtsuki, H., Wang, B. & Aihara, K. (2011), 'Evolution of cooperation on adaptively weighted networks.', *J Theor Biol* **272**(1), 8–15.

Carter, M. Q., Brandl, M. T., Louie, J. W., Kyle, J. L., Carychao, D. K., Cooley, M. B., Parker, C. T., Bates, A. H. & Mandrell, R. E. (2011), 'Distinct acid resistance and survival fitness displayed by Curli variants of enterohemorrhagic Escherichia coli O157:H7.', *Appl Environ Microbiol* **77**(11), 3685–95.

Cash, H. L., Whitham, C. V., Behrendt, C. L. & Hooper, L. V. (2006), 'Symbiotic bacteria direct expression of an intestinal bactericidal lectin.', *Science* **313**(5790), 1126–30.

Chattopadhyay, S., Tchesnokova, V., McVeigh, A., Kisiela, D. I., Dori, K., Navarro, A., Sokurenko, E. V. & Savarino, S. J. (2012), 'Adaptive evolution of class 5 fimbrial genes in enterotoxigenic Escherichia coli and its functional consequences.', *J Biol Chem* **287**(9), 6150–8.

Chen, X. & Cohen, J. E. (2001), 'Global stability, local stability and permanence in model food webs.', *J Theor Biol* **212**(2), 223–35.

Chitnis, N. & Smith, T. A. (2012), 'Mathematical illiteracy impedes progress in biology.', *P Natl Acad Sci USA* **109**(45), E3055; author reply E3058–9.

Chuang, J., Rivoire, O. & Leibler, S. (2009), 'Simpson's paradox in a synthetic microbial system', *Science* **323**(January), 272–275.

Church, D., Elsayed, S., Reid, O., Winston, B. & Lindsay, R. (2006), 'Burn wound infections.', *Clin Microbiol Rev* **19**(2), 403–34.

Cicmanec, J. F. & Holder, I. A. (1979), 'Growth of Pseudomonas aeruginosa in normal and burned skin extract: role of extracellular proteases.', *Infect Immun* **25**(2), 477–483.

Cone, R. A. (2009), 'Barrier properties of mucus.', *Adv Drug Deliver Rev* **61**(2), 75–85.

Conrady, D. G., Wilson, J. J. & Herr, A. B. (2012), 'Structural basis for Zn 2+-dependent intercellular adhesion in staphylococcal biofilms', *P Natl Acad Sci USA* **2012**, E202–E211.

Cordero, O. X., Ventouras, L.-A., DeLong, E. F. & Polz, M. F. (2012), 'Public good dynamics drive evolution of iron acquisition strategies in natural bacterioplankton populations.', *P Natl Acad Sci USA* **109**(49), 20059–64.

Cornforth, D. M. & Foster, K. R. (2013), 'Competition sensing: the social side of bacterial stress responses.', *Nat Rev Microbiol* **11**(4), 285–93.

Cornforth, D. M., Sumpter, D. J. T., Brown, S. P. & Brännström, A. (2012), 'Synergy and group size in microbial cooperation.', *Am Nat* **180**(3), 296–305.

Costello, E. K., Lauber, C. L., Hamady, M., Fierer, N., Gordon, J. I. & Knight, R. (2009), 'Bacterial community variation in human body habitats across space and time.', *Science (New York, N.Y.)* **326**(5960), 1694–7.

Costerton, J. W., Montanaro, L. & Arciola, C. R. (2005), 'Biofilm in implant infections: its production and regulation.', *Int J Artif Organs* **28**(11), 1062–8.

Crespi, B. (2001), 'The evolution of social behavior in microorganisms', *Trends Ecol Evol* **16**(4), 178–183.

Crociani, J., Grill, J. P., Huppert, M. & Ballongue, J. (1995), 'Adhesion of different bifidobacteria strains to human enterocyte-like Caco-2 cells and comparison with in vivo study.', *Lett Appl Microbiol* **21**(3), 146–8.

Curtis, T. P., Sloan, W. T. & Scannell, J. W. (2002), 'Estimating prokaryotic diversity and its limits.', *Proceedings of the National Academy of Sciences of the United States of America* **99**(16), 10494–9.

Czárán, T., Hoekstra, R. F. & Pagie, L. (2002), 'Chemical warfare between microbes promotes biodiversity', *P Natl Acad Sci USA* **99**(2), 786–790.

Dandekar, A. A., Chugani, S. & Greenberg, E. P. (2012), 'Bacterial quorum sensing and metabolic incentives to cooperate.', *Science* **338**(6104), 264–6.

Dapa, T., Leuzzi, R., Ng, Y. K., Baban, S. T., Adamo, R., Kuehne, S. A., Scarselli, M., Minton, N. P., Serruto, D. & Unnikrishnan, M. (2013), 'Multiple factors modulate biofilm formation by the anaerobic pathogen Clostridium difficile.', *J Bacteriol* **195**(3), 545–55.

Darch, S. E., West, S. A., Winzer, K. & Diggle, S. P. (2012), 'Density-dependent fitness benefits in quorum-sensing bacterial populations.', *P Natl Acad Sci USA* **109**(21), 8259–63.

Datta, M. S., Korolev, K. S., Cvijovic, I., Dudley, C. & Gore, J. (2013), 'Range expansion promotes cooperation in an experimental microbial metapopulation.', *P Natl Acad Sci USA* **110**(18), 7354–9.

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., Ling, A. V., Devlin, A. S., Varma, Y., Fischbach, M. A., Biddinger, S. B., Dutton, R. J. & Turnbaugh, P. J. (2013), 'Diet rapidly and reproducibly alters the human gut microbiome.', *Nature* **advance on**, doi:10.1038/nature12820.

De Cruz, P., Prideaux, L., Wagner, J., Ng, S. C., McSweeney, C., Kirkwood, C., Morrison, M. & Kamm, M. A. (2012), 'Characterization of the gastrointestinal microbiota in health and inflammatory bowel disease.', *Inflamm Bowel Dis* **18**(2), 372–90.

De Vadder, F., Kovatcheva-Datchary, P., Goncalves, D., Vinera, J., Zitoun, C., Duchampt, A., Bäckhed, F. & Mithieux, G. (2014), 'Microbiota-Generated Metabolites Promote Metabolic Benefits via Gut-Brain Neural Circuits', *Cell* **156**, 1–13.

Derrien, M., van Passel, M. W., van de Bovenkamp, J. H., Schipper, R. G., de Vos, W. M. & Dekker, J. (2010), 'Mucin-bacterial interactions in the human oral cavity and digestive tract.', *Gut Microbes* **1**(4), 254–268.

Dethlefsen, L., McFall-Ngai, M. & Relman, D. A. (2007), 'An ecological and evolutionary perspective on human-microbe mutualism and disease.', *Nature* **449**(7164), 811–8.

Dethlefsen, L. & Relman, D. A. (2011), 'Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation.', *P Natl Acad Sci USA* **108 Suppl**, 4554–61.

Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., Hibberd, M. L., Forssberg, H. & Pettersson, S. (2011), 'Normal gut microbiota modulates brain development and behavior.', *P Natl Acad Sci USA* **108**(7), 3047–52.

Diggle, S. P., Gardner, A., West, S. A. & Griffin, A. S. (2007), 'Evolutionary theory of bacterial quorum sensing: when is a signal not a signal?', *Philos T Roy Soc B* **362**(1483), 1241–9.

Diggle, S. P., Griffin, A. S., Campbell, G. S. & West, S. A. (2007), 'Cooperation and conflict in quorum-sensing bacterial populations.', *Nature* **450**(7168), 411–4.

Donlan, R. M. (2002), 'Biofilms: microbial life on surfaces.', *Emerg Infect Dis* **8**(9), 881–90.

dos Santos, V. M., Müller, M. & de Vos, W. M. (2010), 'Systems biology of the gut: the interplay of food, microbiota and host at the mucosal interface', *Curr Opin Biotechnol* **21**(4), 539–550.

Dranginis, A. M., Rauceo, J. M., Coronado, J. E. & Lipke, P. N. (2007), 'A biochemical guide to yeast adhesins: glycoproteins for social and antisocial occasions.', *Microbiol Mol Biol R* **71**(2), 282–94.

Drescher, K., Nadell, C. D., Stone, H. A., Wingreen, N. S. & Bassler, B. L. (2013), 'Solutions to the Public Goods Dilemma in Bacterial Biofilms', *Curr Biol* **24**(1), 50–55.

Eberl, G. (2010), 'A new vision of immunity: homeostasis of the superorganism.', *Mucosal Immunol* **3**(5), 450–60.

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E. & Relman, D. A. (2005), 'Diversity of the human intestinal microbial flora.', *Science* **308**(5728), 1635–8.

Fabich, A. J., Jones, S. A., Chowdhury, F. Z., Cernosek, A., Anderson, A., Smalley, D., McHargue, J. W., Hightower, G. A., Smith, J. T., Autieri, S. M., Leatham, M. P., Lins, J. J., Allen, R. L., Laux, D. C., Cohen, P. S. & Conway, T. (2008), 'Comparison of carbon nutrition for pathogenic and commensal Escherichia coli strains in the mouse intestine.', *Infect Immun* **76**(3), 1143–52.

Faith, D. P. (1992), 'Conservation evaluation and phylogenetic diversity', *Biol Conserv* **61**(1), 1–10.

Faith, J. J., Guruge, J. L., Charbonneau, M., Subramanian, S., Seedorf, H., Goodman, A. L., Clemente, J. C., Knight, R., Heath, A. C., Leibel, R. L., Rosenbaum, M. & Gordon, J. I. (2013), 'The long-term stability of the human gut microbiota.', *Science* **341**(6141), 1237439.

Faith, J. J., McNulty, N. P., Rey, F. E. & Gordon, J. I. (2011), 'Predicting a human gut microbiota's response to diet in gnotobiotic mice.', *Science* **333**(6038), 101–4.

Fawcett, T. W. & Higginson, A. D. (2012), 'Heavy use of equations impedes communication among biologists.', *P Natl Acad Sci USA* **109**(29), 11735–9.

Fernandes, A. D. (2012), 'No evidence that equations cause impeded communication among biologists.', *P Natl Acad Sci USA* **109**(45), E3057; author reply E3058–9.

Fong, J. C. N., Syed, K. A., Klose, K. E. & Yildiz, F. H. (2010), 'Role of Vibrio polysaccharide (vps) genes in VPS production, biofilm formation and Vibrio cholerae pathogenesis.', *Microbiology* **156**(Pt 9), 2757–69.

Foster, J. A., Krone, S. M. & Forney, L. J. (2008), 'Application of Ecological Network Theory to the Human Microbiome', *Interdisciplinary perspectives on infectious diseases* **2008**, 839501.

Foster, K. R. (2004), 'Diminishing returns in social evolution: the not-so-tragic commons.', *J Evol Biol* **17**(5), 1058–72.

Foster, K. R. (2011), 'The sociobiology of molecular systems.', *Nat Rev Genet* **12**(3), 193–203.

Foster, K. R. & Bell, T. (2012), 'Competition, not cooperation, dominates interactions among culturable microbial species.', *Curr Biol* **22**(19), 1845–50.

Foster, K. R. & Wenseleers, T. (2006), 'A general model for the evolution of mutualisms.', *J Evol Biol* **19**(4), 1283–93.

Frank, S. A. (1994), 'Genetics of mutualism: the evolution of altruism between species.', *J Theor Biol* **170**(4), 393–400.

Friedman, L. & Kolter, R. (2003), 'Genes involved in matrix formation in Pseudomonas aeruginosa PA14 biofilms', *Mol Microbiol* **51**(3), 675–690.

Friedman, L. & Kolter, R. (2004), 'Two Genetic Loci Produce Distinct Carbohydrate-Rich Structural Components of the Pseudomonas aeruginosa Biofilm Matrix Two Genetic Loci Produce Distinct Carbohydrate-Rich Structural Components of the Pseudomonas aeruginosa Biofilm Matrix', *J Bacteriol* **186**(14), 4457–4465.

Fuqua, W. C., Winans, S. C. & Greenberg, E. P. (1994), 'Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators.', *J Bacteriol* **176**(2), 269–75.

Gans, J., Wolinsky, M. & Dunbar, J. (2005), 'Computational improvements reveal great bacterial diversity and high metal toxicity in soil.', *Science* **309**(5739), 1387–90.

Garcia, M. C., Lee, J. T., Ramsook, C. B., Alsteens, D., Dufrêne, Y. F. & Lipke, P. N. (2011), 'A role for amyloid in cell aggregation and biofilm formation.', *PloS One* **6**(3), e17632.

Gerschgorin, S. (1931), 'Über die Abgrenzung der Eigenwerte einer Matrix', *B Acad Sci USSR* **25**(1900), 750–754.

Gibbons, J. (2012), 'Do not throw equations out with the theory bathwater.', *P Natl Acad Sci USA* **109**(45), E3054; author reply E3058–9.

Gill, S. R., Pop, M., Deboy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., Gordon, J. I., Relman, D. A., Fraser-Liggett, C. M. & Nelson, K. E. (2006), 'Metagenomic analysis of the human distal gut microbiome.', *Science* **312**(5778), 1355–9.

Gilman, R. T., Nuismer, S. L. & Jhwueng, D.-C. (2012), 'Coevolution in multidimensional trait space favours escape from parasites and pathogens', *Nature* **483**(7389), 328–330.

Girardin, S. E., Boneca, I. G., Viala, J., Chamaillard, M., Labigne, A., Thomas, G., Philpott, D. J. & Sansonetti, P. J. (2003), 'Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection.', *J Biol Chem* **278**(11), 8869–72.

Gordon, J. I. & Klaenhammer, T. R. (2011), 'A rendezvous with our microbes.', *P Natl Acad Sci USA* **108 Suppl**(Supplement_1), 4513–5.

Graf, J. (1998), 'Host-derived amino acids support the proliferation of symbiotic bacteria', *P Natl Acad Sci USA* **95**(4), 1818–1822.

Grafen, A. (1990), 'Do animals really recognize kin?', *Anim Behav* **39**, 42–54.

Grice, E. A., Kong, H. H., Renaud, G., Young, A. C., Bouffard, G. G., Blakesley, R. W., Wolfsberg, T. G., Turner, M. L. & Segre, J. A. (2008), 'A diversity profile of the human skin microbiota.', *Genome Res* **18**(7), 1043–50.

Griffin, A. S., West, S. S. A. & Buckling, A. (2004), 'Cooperation and competition in pathogenic bacteria.', *Nature* **430**(7003), 1024–7.

Gudelj, I., Weitz, J. S., Ferenci, T., Claire Horner-Devine, M., Marx, C. J., Meyer, J. R. & Forde, S. E. (2010), 'An integrative approach to understanding microbial diversity: from intracellular mechanisms to community structure.', *Ecol Lett* **13**(9), 1073–84.

Hall-Stoodley, L., Costerton, J. W. & Stoodley, P. (2004), 'Bacterial biofilms: from the natural environment to infectious diseases.', *Nat Rev Microbiol* **2**(2), 95–108.

Hallatschek, O., Hersen, P., Ramanathan, S. & Nelson, D. R. (2007), 'Genetic drift at expanding frontiers promotes gene segregation.', *P Natl Acad Sci USA* **104**(50), 19926–30.

Halme, A., Bumgarner, S., Styles, C. & Fink, G. R. (2004), 'Genetic and epigenetic regulation of the FLO gene family generates cell-surface variation in yeast.', *Cell* **116**(3), 405–15.

Hamilton, W. D. (1964), 'The genetical evolution of social behaviour. I & II', *J Theor Biol* **7**(1), 1–52.

Hardin, G. (1968), 'The Tragedy of the Commons', *Science* **162**, 1243–1248.

Heilmann, C., Schweitzer, O., Gerke, C., Vanittanakom, N., Mack, D. & Götz, F. (1996), 'Molecular basis of intercellular adhesion in the biofilm-forming Staphylococcus epidermidis.', *Mol Microbiol* **20**(5), 1083–91.

Hense, B., Kuttler, C., Muller, J., Rothballer, M., Hartmann, A. & Kreft, J. (2007), 'Does efficiency sensing unify diffusion and quorum sensing?', *Nat Rev Microbiol* **5**(3), 230–239.

Hibbing, M. & Fuqua, C. (2009), 'Bacterial competition: surviving and thriving in the microbial jungle', *Nat Rev Microbiol* **8**(1), 15–25.

Holzapfel, W. H., Haberer, P., Snel, J., Schillinger, U. & Huis in't Veld, J. H. (1998), 'Overview of gut flora and probiotics.', *Int J Food Microbiol* **41**(2), 85–101.

Hooper, L. V. (2001), 'Commensal Host-Bacterial Relationships in the Gut', *Science* **292**(5519), 1115–1118.

Hooper, L. V. (2009), 'Do symbiotic bacteria subvert host immunity?', *Nat Rev Microbiol* **7**(5), 367–74.

Hooper, L. V. & Gordon, J. I. (2001), 'Glycans as legislators of host-microbial interactions: spanning the spectrum from symbiosis to pathogenicity', *Glycobiology* **11**(2), 1R–10R.

Hooper, L. V. & Macpherson, A. J. (2010), 'Immune adaptations that maintain homeostasis with the intestinal microbiota.', *Nat Rev Immunol* **10**(3), 159–69.

Hooper, L. V., Xu, J., Falk, P. G., Midtvedt, T. & Gordon, J. I. (1999), 'A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem.', *P Natl Acad Sci USA* **96**(17), 9833–8.

Hsiao, E. Y., McBride, S. W., Hsien, S., Sharon, G., Hyde, E. R., McCue, T., Codelli, J. A., Chow, J., Reisman, S. E., Petrosino, J. F., Patterson, P. H. & Mazmanian, S. K. (2013), 'Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders.', *Cell* **155**(7), 1451–63.

Hu, B., Elinav, E., Huber, S., Strowig, T., Hao, L., Hafemann, A., Jin, C., Stephanie, C., Eisenbarth, S. C. & Wunderlich, C. (2013), 'Correction for Hu et al., Microbiota-induced activation of epithelial IL-6 signaling links inflammasome-driven inflammation with transmissible cancer', *P Natl Acad Sci USA* **110**(31), 12852–12852.

Huq, A., Small, E. B., West, P. A., Huq, M. I., Rahman, R. & Colwell, R. R. (1983), 'Ecological relationships between Vibrio cholerae and planktonic crustacean copepods.', *Appl Environ Microbiol* **45**(1), 275–83.

Johansson, M. E. V., Holmén Larsson, J. M. & Hansson, G. C. (2010), 'Microbes and Health Sackler Colloquium: The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions.', *P Natl Acad Sci USA* **108**(Supplement_1), 4659–4665.

Johansson, M. E. V., Phillipson, M., Petersson, J., Velcich, A., Holm, L. & Hansson, G. C. (2008), 'The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria.', *P Natl Acad Sci USA* **105**(39), 15064–9.

Kane, A. (2012), 'A suggestion on improving mathematically heavy papers.', *P Natl Acad Sci USA* **109**(45), E3056; author reply E3058–9.

Karlsson, F. H., Nookaew, I., Petranovic, D. & Nielsen, J. (2011), 'Prospects for systems biology and modeling of the gut microbiome.', *Trends in biotechnology* **29**(6), 251–8.

Kashyap, P. C., Marcobal, A., Ursell, L. K., Smits, S. A., Sonnenburg, E. D., Costello, E. K., Higginbottom, S. K., Domino, S. E., Holmes, S. P., Relman, D. A., Knight, R., Gordon, J. I. & Sonnenburg, J. L. (2013), 'Genetically dictated change in host mucus carbohydrate landscape exerts a diet-dependent effect on the gut microbiota.', *P Natl Acad Sci USA* **110**(42), 17059–64.

Kawamoto, S., Tran, T. H., Maruya, M., Suzuki, K., Doi, Y., Tsutsui, Y., Kato, L. M. & Fagarasan, S. (2012), 'The Inhibitory Receptor PD-1 Regulates IgA Selection and Bacterial Composition in the Gut', *Science* **336**(6080), 485–489.

Keller, L. & Surette, M. G. (2006), 'Communication in bacteria: an ecological and evolutionary perspective.', *Nat Rev Microbiol* **4**(4), 249–58.

Kelly, D., Campbell, J. I., King, T. P., Grant, G., Jansson, E. A., Coutts, A. G. P., Pettersson, S. & Conway, S. (2004), 'Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA.', *Nat Immunol* **5**(1), 104–12.

Kiers, E. T., Rousseau, R. A., West, S. A. & Denison, R. F. (2003), 'Host sanctions and the legume-rhizobium mutualism.', *Nature* **425**(6953), 78–81.

Klapper, I. & Dockery, J. (2010), 'Mathematical Description of Microbial Biofilms', *SIAM Review* **52**(2), 221–265.

Korolev, K. S., Xavier, J. B., Nelson, D. R. & Foster, K. R. (2011), 'A

quantitative test of population genetics using spatiogenetic patterns in bacterial colonies.', *Am Nat* **178**(4), 538–52.

Koschwanez, J. H., Foster, K. R. & Murray, A. W. (2011), 'Sucrose utilization in budding yeast as a model for the origin of undifferentiated multicellularity.', *PLoS Biol* **9**(8), e1001122.

Kreft, J.-U. (2004), 'Biofilms promote altruism.', *Microbiology* **150**, 2751–60.

Kreft, J.-U. & Bonhoeffer, S. (2005), 'The evolution of groups of cooperating bacteria and the growth rate versus yield trade-off.', *Microbiology* **151**(Pt 3), 637–41.

Kreft, J. U., Picioreanu, C., Wimpenny, J. W. & van Loosdrecht, M. C. (2001), 'Individual-based modelling of biofilms.', *Microbiology* **147**(Pt 11), 2897–912.

Kremer, N., Philipp, E. E. R., Carpentier, M.-C., Brennan, C. A., Kraemer, L., Altura, M. A., Augustin, R., Häsler, R., Heath-Heckman, E. A. C., Peyer, S. M., Schwartzman, J., Rader, B. A., Ruby, E. G., Rosenstiel, P. & McFall-Ngai, M. J. (2013), 'Initial symbiont contact orchestrates host-organ-wide transcriptional changes that prime tissue colonization.', *Cell Host Microbe* **14**(2), 183–94.

Krivan, V. (2006), 'The ideal free distribution and bacterial growth on two substrates.', *Theor Popul Biol* **69**(2), 181–91.

Lardon, L. A., Merkey, B. V., Martins, S., Dotsch, A., Picioreanu, C., Kreft,

J. U. & Smets, B. F. (2011), 'iDynoMiCS: Next Generation of Individual-based Modelling of Biofilms', *Environ Microbiol* **13**(9), 2416–34.

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., Schlegel, M. L., Tucker, T. A., Schrenzel, M. D., Knight, R. & Gordon, J. I. (2008), 'Evolution of mammals and their gut microbes.', *Science* **320**(5883), 1647–51.

Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R. & Gordon, J. I. (2008), 'Worlds within worlds: evolution of the vertebrate gut microbiota.', *Nat Rev Microbiol* **6**(10), 776–88.

Ley, R. E., Peterson, D. A. & Gordon, J. I. (2006), 'Ecological and evolutionary forces shaping microbial diversity in the human intestine.', *Cell* **124**(4), 837–48.

Linke, D., Riess, T., Autenrieth, I. B., Lupas, A. & Kempf, V. A. J. (2006), 'Trimeric autotransporter adhesins: variable structure, common function.', *Trends Microbiol* **14**(6), 264–70.

Liu, C. H., Lee, S. M., Vanlare, J. M., Kasper, D. L. & Mazmanian, S. K. (2008), 'Regulation of surface architecture by symbiotic bacteria mediates host colonization.', *P Natl Acad Sci USA* **105**(10), 3951–6.

LoCascio, R. G., Ninonuevo, M. R., Freeman, S. L., Sela, D. A., Grimm, R., Lebrilla, C. B., Mills, D. A. & German, J. B. (2007), 'Glycoprofiling of bifidobacterial consumption of human milk oligosaccharides demonstrates

strain specific, preferential consumption of small chain glycans secreted in early human lactation.', *J Agric Food Chem* **55**(22), 8914–9.

Lozupone, C. A. & Knight, R. (2007), 'Global patterns in bacterial diversity.', *P Natl Acad Sci USA* **104**(27), 11436–40.

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K. & Knight, R. (2012), 'Diversity, stability and resilience of the human gut microbiota.', *Nature* **489**(7415), 220–30.

Lozupone, C., Faust, K., Raes, J., Faith, J. J., Frank, D. N., Zaneveld, J., Gordon, J. I. & Knight, R. (2012), 'Identifying genomic and metabolic features that can underlie early successional and opportunistic lifestyles of human gut symbionts.', *Genome Res* **22**(10), 1974–84.

Ma, L., Jackson, K. D., Landry, R. M., Parsek, M. R. & Wozniak, D. J. (2006), 'Analysis of Pseudomonas aeruginosa conditional psl variants reveals roles for the psl polysaccharide in adhesion and maintaining biofilm structure postattachment.', *J Bacteriol* **188**(23), 8213–21.

MacKenzie, D. A., Tailford, L. E., Hemmings, A. M. & Juge, N. (2009), 'Crystal structure of a mucus-binding protein repeat reveals an unexpected functional immunoglobulin binding activity.', *J Biol Chem* **284**(47), 32444–53.

Macklaim, J., Gloor, G., Anukam, K., Cribby, S. & Reid, G. (2011), 'At the crossroads of vaginal health and disease, the genome sequence of Lactobacillus iners AB-1', *P Natl Acad Sci USA* **108**(suppl. 1), 4688–4695.

MacLean, R. C. & Gudelj, I. (2006), 'Resource competition and social conflict in experimental populations of yeast.', *Nature* **441**(7092), 498–501.

Macpherson, A. J. (2000), 'A Primitive T Cell-Independent Mechanism of Intestinal Mucosal IgA Responses to Commensal Bacteria', *Science* **288**(5474), 2222–2226.

Macpherson, A. J., Geuking, M. B. & McCoy, K. D. (2005), 'Immune responses that adapt the intestinal mucosa to commensal intestinal bacteria.', *Immunology* **115**(2), 153–62.

Macpherson, A. J., McCoy, K. D., Johansen, F.-E. & Brandtzaeg, P. (2008), 'The immune geography of IgA induction and function.', *Mucosal Immunol* **1**(1), 11–22.

Macpherson, A. J. & Uhr, T. (2004), 'Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria.', *Science* **303**(5664), 1662–5.

Mah, T. F. & O'Toole, G. A. (2001), 'Mechanisms of biofilm resistance to antimicrobial agents.', *Trends Microbiol* **9**(1), 34–9.

Malik, S., Petrova, M. I., Claes, I. J. J., Verhoeven, T. L. A., Busschaert, P., Vaneechoutte, M., Lievens, B., Lambrichts, I., Siezen, R. J., Balzarini, J., Vanderleyden, J. & Lebeer, S. (2013), 'The high auto-aggregative and adhesive phenotype of the vaginal Lactobacillus plantarum strain CMPG5300 is sortase-dependent.', *Appl Environ Microb* **79**(May), 4576–85.

Martin, F.-P. J., Dumas, M.-E., Wang, Y., Legido-Quigley, C., Yap, I. K. S., Tang, H., Zirah, S., Murphy, G. M., Cloarec, O., Lindon, J. C., Sprenger,

N., Fay, L. B., Kochhar, S., van Bladeren, P., Holmes, E. & Nicholson, J. K. (2007), 'A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model.', *Mol Syst Biol* **3**, 112.

Martiny, J. B. H., Bohannan, B. J. M., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L., Horner-Devine, M. C., Kane, M., Krumins, J. A., Kuske, C. R., Morin, P. J., Naeem, S., Ovreå s, L., Reysenbach, A.-L., Smith, V. H. & Staley, J. T. (2006), 'Microbial biogeography: putting microorganisms on the map.', *Nat Rev Microbiol* **4**(2), 102–12.

Matsuo, K., Ota, H. & Akamatsu, T. (1997), 'Histochemistry of the surface mucous gel layer of the human colon.', *Gut* **40**(6), 782.

May, R. (1972), 'Will a large complex system be stable?', *Nature* **238**, 413–414.

Mazmanian, S. K., Liu, C. H., Tzianabos, A. O. & Kasper, D. L. (2005), 'An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system.', *Cell* **122**(1), 107–18.

Mazmanian, S. K., Round, J. L. & Kasper, D. L. (2008), 'A microbial symbiosis factor prevents intestinal inflammatory disease.', *Nature* **453**(7195), 620–5.

McCann, K. S. (2000), 'The diversity-stability debate.', *Nature* **405**(6783), 228–33.

McFall-Ngai, M. (2007), 'Adaptive immunity: care for the community.', *Nature* **445**(7124), 153.

McFall-Ngai, M. J. & Ruby, E. G. (1991), 'Symbiont recognition and subsequent morphogenesis as early events in an animal-bacterial mutualism.', *Science* **254**(5037), 1491–4.

McGuckin, M. A., Lindén, S. K., Sutton, P. & Florin, T. H. (2011), 'Mucin dynamics and enteric pathogens', *Nat Rev Microbiol* **9**(4), 265–278.

Meibom, K. L., Li, X. B., Nielsen, A. T., Wu, C.-Y., Roseman, S. & Schoolnik, G. K. (2004), 'The Vibrio cholerae chitin utilization program.', *P Natl Acad Sci USA* **101**(8), 2524–9.

Mitri, S. & Foster, K. R. (2013), 'The genotypic view of social interactions in microbial communities.', *Annu Rev Genet* **47**, 247–73.

Mitri, S., Xavier, J. B. & Foster, K. R. (2011), 'Social evolution in multispecies biofilms.', *P Natl Acad Sci USA* **108**(Suppl 2), 10839–46.

Mougi, A. & Kondoh, M. (2012), 'Diversity of interaction types and ecological community stability.', *Science* **337**(6092), 349–51.

Muegge, B. D., Kuczynski, J., Knights, D., Clemente, J. C., González, A., Fontana, L., Henrissat, B., Knight, R. & Gordon, J. I. (2011), 'Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans.', *Science* **332**(6032), 970–4.

Nadell, C. D. & Bassler, B. L. (2011), 'A fitness trade-off between local competition and dispersal in Vibrio cholerae biofilms.', *P Natl Acad Sci USA* **108**(34), 14181–5.

Nadell, C. D., Bucci, V., Drescher, K., Levin, S. A., Bassler, B. L. & Xavier, J. B. (2013), 'Cutting through the complexity of cell collectives Cutting through the complexity of cell collectives', *Proc R Soc B* **280**(January), 20122770.

Nadell, C. D., Foster, K. R. & Xavier, J. B. (2010), 'Emergence of spatial structure in cell groups and the evolution of cooperation.', *PLoS Comput Biol* **6**(3), e1000716.

Nadell, C. D., Xavier, J. B. & Foster, K. R. (2009), 'The sociobiology of biofilms.', *FEMS Microbiol Rev* **33**(1), 206–24.

Nadell, C. D., Xavier, J. B., Levin, S. A. & Foster, K. R. (2008), 'The evolution of quorum sensing in bacterial biofilms.', *PLoS Biol* **6**(1), e14.

Nalin, D. R., Daya, V., Reid, a., Levine, M. M. & Cisneros, L. (1979), 'Adsorption and growth of Vibrio cholerae on chitin.', *Infection and immunity* **25**(2), 768–70.

Nogueira, T., Rankin, D. J., Touchon, M., Taddei, F., Brown, S. P. & Rocha, E. P. C. (2009), 'Horizontal gene transfer of the secretome drives the evolution of bacterial cooperation and virulence.', *Curr Biol* **19**(20), 1683–91.

Nyholm, S. V. & McFall-Ngai, M. J. (2004), 'The winnowing: establishing the squid-vibrio symbiosis.', *Nat Rev Microbiol* **2**(8), 632–42.

Olmsted, S. S., Padgett, J. L., Yudin, A. I., Whaley, K. J., Moench, T. R. &

Cone, R. A. (2001), 'Diffusion of macromolecules and virus-like particles in human cervical mucus.', *Biophys J* **81**(4), 1930–7.

O'Toole, G. A. & Kolter, R. (1998), 'Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development', *Mol Microbiol* **30**(2), 295–304.

O'Toole, G., Kaplan, H. & Kolter, R. (2000), 'Biofilm formation as microbial development', *Annu Rev Microbiol* **54**, 49–79.

P. Vázquez, D., J. Melián, C., M. Williams, N., Blüthgen, N., R. Krasnov, B. & Poulin, R. (2007), 'Species abundance and asymmetric interaction strength in ecological networks', *Oikos* **116**(7), 1120–1127.

Palestrant, D., Holzknecht, Z. E., Collins, B. H., Parker, W., Miller, S. E. & Bollinger, R. R. (2004), 'Microbial Biofilms in the Gut: Visualization by Electron Microscopy and by Acridine Orange Staining', *Ultrastruct Pathol* **28**(1), 23–27.

Palmer, R. J. & White, D. C. (1997), 'Developmental biology of biofilms: implications for treatment and control.', *Trends Microbiol* **5**(11), 435–40.

Pei, Z., Bini, E. J., Yang, L., Zhou, M., Francois, F. & Blaser, M. J. (2004), 'Bacterial biota in the human distal esophagus.', *P Natl Acad Sci USA* **101**(12), 4250–5.

Peterson, D. A., McNulty, N. P., Guruge, J. L., Gordon, J. I. & McNutty, N. P. (2007), 'IgA Response to Symbiotic Bacteria as a Mediator of Gut Homeostasis', *Cell Host Microbe* **2**(5), 328–339.

Petnicki-Ocwieja, T., Hrncir, T., Liu, Y.-J., Biswas, A., Hudcovic, T., Tlaskalova-Hogenova, H. & Kobayashi, K. S. (2009), 'Nod2 is required for the regulation of commensal microbiota in the intestine.', *P Natl Acad Sci USA* **106**(37), 15813–8.

Petrova, O. E. & Sauer, K. (2012), 'Sticky situations: key components that control bacterial surface attachment.', *J Bacteriol* **194**(10), 2413–25.

Picioreanu, C., van Loosdrecht, M. C. & Heijnen, J. J. (1998), 'Mathematical modeling of biofilm structure with a hybrid differential-discrete cellular automaton approach.', *Biotechnol Bioeng* **58**(1), 101–16.

Picioreanu, C., van Loosdrecht, M. C. & Heijnen, J. J. (2001), 'Two-dimensional model of biofilm detachment caused by internal stress from liquid flow.', *Biotechnol Bioeng* **72**(2), 205–18.

Picioreanu, C., van Loosdrecht, M. C. M. & Heijnen, J. J. (1997), 'Modelling the effect of oxygen concentration on nitrite accumulation in a biofilm airlift suspension reactor', *Water Sci Tech* **36**(1), 147–156.

Popat, R., Crusz, S. A., Messina, M., Williams, P., West, S. A. & Diggle, S. P. (2012), 'Quorum-sensing and cheating in bacterial biofilms.', *Proc R Soc B* **279**(1748), 4765–71.

Poulsen, M. & Boomsma, J. J. (2005), 'Mutualistic fungi control crop diversity in fungus-growing ants.', *Science* **307**(5710), 741–4.

Pradhan, A. K., Pradhan, N., Sukla, L. B., Panda, P. K. & Mishra, B. K. (2013), 'Inhibition of pathogenic bacterial biofilm by biosurfactant

produced by Lysinibacillus fusiformis S9.', *Bioprocess Biosys Eng* pp. 10.1007/s00449–013–0976–5.

Queller, D. C., Ponte, E., Bozzaro, S. & Strassmann, J. E. (2003), 'Single-gene greenbeard effects in the social amoeba Dictyostelium discoideum.', *Science* **299**(5603), 105–6.

Raberg, M., Bechmann, J., Brandt, U., Schlüter, J., Uischner, B., Voigt, B., Hecker, M. & Steinbüchel, A. (2011), 'Versatile metabolic adaptations of Ralstonia eutropha H16 to a loss of PdhL, the E3 component of the pyruvate dehydrogenase complex.', *Appl Environ Microbiol* **77**(7), 2254–63.

Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. & Medzhitov, R. (2004), 'Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis.', *Cell* **118**(2), 229–41.

Rang, C. U., Licht, T. R., Midtvedt, T., Conway, P. L., Chao, L., Krogfelt, K. A., Cohen, P. S. & Molin, S. (1999), 'Estimation of Growth Rates of Escherichia coli BJ4 in Streptomycin-Treated and Previously Germfree Mice by In Situ rRNA Hybridization', *Clin Diagn Lab Immunol* **6**(3), 434–436.

Redfield, R. (2002), 'Is quorum sensing a side effect of diffusion sensing?', *Trends Microbiol* **10**(8), 365–370.

Reid, G., Younes, J. A., Van der Mei, H. C., Gloor, G. B., Knight, R. & Busscher, H. J. (2010), 'Microbiota restoration: natural and supplemented recovery of human microbial communities.', *Nat Rev Microbiol* **9**(1), 27–38.

Rezzonico, E., Mestdagh, R., Delley, M., Combremont, S., Dumas, M.-E., Holmes, E., Nicholson, J. & Bibiloni, R. (2011), 'Bacterial adaptation to the gut environment favors successful colonization: Microbial and metabonomic characterization of a simplified microbiota mouse model.', *Gut Microbes* **2**(6), 307–18.

Roesch, L., Fulthorpe, R. & Riva, A. (2007), 'Pyrosequencing enumerates and contrasts soil microbial diversity', *ISME J* **1**(4), 283–290.

Romero, D., Aguilar, C., Losick, R. & Kolter, R. (2010), 'Amyloid fibers provide structural integrity to Bacillus subtilis biofilms.', *P Natl Acad Sci USA* **107**(5), 2230–4.

Rothblum, U. G. & Tan, C. P. (1985), 'Upper bounds on the maximum modulus of subdominant eigenvalues of nonnegative matrices', *Linear Algebra Appl* **66**(1985), 45–86.

Round, J. L. & Mazmanian, S. K. (2009), 'The gut microbiota shapes intestinal immune responses during health and disease.', *Nat Rev Immunol* **9**(5), 313–23.

Ruby, E. G. & McFall-Ngai, M. J. (1999), 'Oxygen-utilizing reactions and symbiotic colonization of the squid light organ by Vibrio fischeri.', *Trends Microbiol* **7**(10), 414–20.

Rumbaugh, K. P., Trivedi, U., Watters, C., Burton-Chellew, M. N., Diggle, S. P. & West, S. A. (2012), 'Kin selection, quorum sensing and virulence in pathogenic bacteria.', *Proc R Soc B* **279**(1742), 3584–8.

Sachs, J. L. (2008), 'Resolving the first steps to multicellularity.', *Trends Ecol Evol* **23**(5), 245–8.

Sachs, J. L., Mueller, U. G., Wilcox, T. P. & Bull, J. J. (2004), 'The evolution of cooperation.', *Q Rev Biol* **79**(2), 135–60.

Salzman, N. (2010), 'Paneth cell defensins and the regulation of the microbiome: Détente at mucosal surfaces', *Gut Microbes* **1**(6), 401.

Salzman, N. H., Hung, K., Haribhai, D., Chu, H., Karlsson-Sjöberg, J., Amir, E., Teggatz, P., Barman, M., Hayward, M., Eastwood, D., Stoel, M., Zhou, Y., Sodergren, E., Weinstock, G. M., Bevins, C. L., Williams, C. B. & Bos, N. A. (2010), 'Enteric defensins are essential regulators of intestinal microbial ecology.', *Nat Immunol* **11**(1), 76–83.

Sandoz, K. M., Mitzimberg, S. M. & Schuster, M. (2007), 'Social cheating in Pseudomonas aeruginosa quorum sensing.', *P Natl Acad Sci USA* **104**(40), 15876–81.

Sanon, A., Andrianjaka, Z. N., Prin, Y., Bally, R., Thioulouse, J., Comte, G. & Duponnois, R. (2009), 'Rhizosphere microbiota interfers with plant-plant interactions', *Plant Soil* **321**(1-2), 259–278.

Sauer, K., Camper, A. K., Ehrlich, G. D., William, J., Davies, D. G. & Costerton, J. W. (2002), 'Pseudomonas aeruginosa Displays Multiple Phenotypes during Development as a Biofilm Pseudomonas aeruginosa Displays Multiple Phenotypes during Development as a Biofilm', *J Bacteriol* **184**(4), 1140–1154.

Schluter, J. & Foster, K. R. (2012), 'The Evolution of Mutualism in Gut Microbiota Via Host Epithelial Selection', *PLoS Biol* **10**(11), e1001424.

Sekirov, I., Russell, S. L., Antunes, L. C. M. & Finlay, B. B. (2010), 'Gut microbiota in health and disease.', *Physiol Rev* **90**(3), 859–904.

Shahid, S. A., Bardiaux, B., Franks, W. T., Krabben, L., Habeck, M., van Rossum, B.-J. & Linke, D. (2012), 'Membrane-protein structure determination by solid-state NMR spectroscopy of microcrystals', *Nat Methods* **9**(12).

Shahid, S., Markovic, S., Linke, D. & van Rossum, B. (2012), 'Assignment and secondary structure of the YadA membrane protein by solid-state MAS NMR', *Sci Rep* **2**, 803.

Shapiro, B. J., Friedman, J., Cordero, O. X., Preheim, S. P., Timberlake, S. C., Szabo, G., Polz, M. F. & Alm, E. J. (2012), 'Population Genomics of Early Events in the Ecological Differentiation of Bacteria', *Science* **336**(6077), 48–51.

Shcherbo, D., Merzlyak, E. M., Chepurnykh, T. V., Fradkov, A. F., Ermakova, G. V., Solovieva, E. A., Lukyanov, K. A., Bogdanova, E. A., Zaraisky, A. G., Lukyanov, S. & Chudakov, D. M. (2007), 'Bright far-red fluorescent protein for whole-body imaging', *Nat Methods* **4**(9), 741–746.

Sleator, R. D. (2010), 'The human superorganism - of microbes and men.', *Med Hypotheses* **74**(2), 214–5.

Smukalla, S., Caldara, M., Pochet, N., Beauvais, A., Guadagnini, S., Yan, C., Vinces, M. D., Jansen, A., Prevost, M. C., Latgé, J.-P., Fink, G. R., Foster, K. R. & Verstrepen, K. J. (2008), 'FLO1 is a variable green beard gene that drives biofilm-like cooperation in budding yeast.', *Cell* **135**(4), 726–37.

Sommer, F. & Bäckhed, F. (2013), 'The gut microbiota–masters of host development and physiology.', *Nat Rev Microbiol* **11**(4), 227–38.

Sommers, H., Crisanti, A., Sompolinsky, H. & Stein, Y. (1988), 'Spectrum of large random asymmetric matrices', *Phys Rev Lett* **60**(19), 1895–1899.

Sonnenburg, J. L., Xu, J., Leip, D. D., Chen, C.-H., Westover, B. P., Weatherford, J., Buhler, J. D. & Gordon, J. I. (2005), 'Glycan foraging in vivo by an intestine-adapted bacterial symbiont.', *Science* **307**(5717), 1955–9.

Spor, A., Koren, O. & Ley, R. (2011), 'Unravelling the effects of the environment and host genotype on the gut microbiome', *Nat Rev Microbiol* **9**(4), 279–290.

Stahl, M., Friis, L. M., Nothaft, H., Liu, X., Li, J., Szymanski, C. M. & Stintzi, A. (2011), 'L-fucose utilization provides Campylobacter jejuni with a competitive advantage.', *P Natl Acad Sci USA* **108**(17), 7194–9.

Stapper, A. P., Narasimhan, G., Ohman, D., Barakat, J., Hentzer, M., Molin, S., Kharazmi, A., Hoiby, N. & Mathee, K. (2004), 'Alginate production affects Pseudomonas aeruginosa biofilm development and architecture, but is not essential for biofilm formation', *J Med Microbiol* **53**(7), 679–690.

Stecher, B., Robbiani, R., Walker, A. W., Westendorf, A. M., Barthel, M., Kremer, M., Chaffron, S., Macpherson, A. J., Buer, J., Parkhill, J., Dougan, G., von Mering, C. & Hardt, W.-D. (2007), 'Salmonella enterica serovar typhimurium exploits inflammation to compete with the intestinal microbiota.', *PLoS Biol* **5**(10), 2177–89.

Stein, R. R., Bucci, V., Toussaint, N. C., Buffie, C. G., Rätsch, G., Pamer, E. G., Sander, C. & Xavier, J. B. (2013), 'Ecological modeling from time-series inference: insight into dynamics and stability of intestinal microbiota.', *PLoS Comput Biol* **9**(12), e1003388.

Stewart, P. S. & Franklin, M. J. (2008), 'Physiological heterogeneity in biofilms.', *Nat Rev Microbiol* **6**(3), 199–210.

Stoodley, P., Sauer, K., Davies, D. G. & Costerton, J. W. (2002), 'Biofilms as complex differentiated communities.', *Annu Rev Microbiol* **56**, 187–209.

Sutherland, I. W. (2001), 'Biofilm exopolysaccharides: a strong and sticky framework', *Microbiology* **147**(1), 3–9.

Taga, M. E. & Bassler, B. L. (2003), 'Chemical communication among bacteria.', *P Natl Acad Sci USA* **100 Suppl**, 14549–54.

Tamayo, R., Patimalla, B. & Camilli, A. (2010), 'Growth in a biofilm induces a hyperinfectious phenotype in Vibrio cholerae.', *Infect Immun* **78**(8), 3560–9.

Timmerman, C. P., Fleer, A., Besnier, J. M., De Graaf, L., Cremers, F. & Verhoef, J. (1991), 'Characterization of a proteinaceous adhesin of

Staphylococcus epidermidis which mediates attachment to polystyrene.', *Infect Immun* **59**(11), 4187–4192.

Tlaskalová-Hogenová, H., Stěpánková, R., Kozáková, H., Hudcovic, T., Vannucci, L., Tučková, L., Rossmann, P., Hrnčí, T., Kverka, M., Zákostelská, Z., Klimešová, K., Pibylová, J., Bártová, J., Sanchez, D., Fundová, P., Borovská, D., Srtková, D., Zídek, Z., Schwarzer, M., Drastich, P. & Funda, D. P. (2011), 'The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases.', *Cell Mol Immunol* **8**(2), 110–20.

Tojo, M., Yamashita, N., Goldmann, D. A. & Pier, G. B. (1988), 'Isolation and Characterization of a Capsular Polysaccharide Adhesin from Staphylococcus epidermidis', *J Infect Dis* **157**(4), 713–722.

Tolker-Nielsen, T., Brinch, U. C., Ragas, P. C., Andersen, J. B., Jacobsen, C. S. & Molin, S. r. (2000), 'Development and Dynamics of Pseudomonas sp . Biofilms', *J Bacteriol* **182**(22), 6482–6489.

Townsend, S. E., Haydon, D. T. & Matthews, L. (2010), 'On the generality of stability-complexity relationships in Lotka-Volterra ecosystems.', *J Theor Biol* **267**(2), 243–51.

Tremaroli, V. & Bäckhed, F. (2012), 'Functional interactions between the gut microbiota and host metabolism.', *Nature* **489**(7415), 242–9.

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., Sogin, M. L., Jones, W. J., Roe, B. A., Affourtit, J. P., Egholm, M., Henrissat, B., Heath, A. C., Knight, R. & Gordon, J. I. (2009), 'A core gut microbiome in obese and lean twins.', *Nature* **457**(7228), 480–4.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R. & Gordon, J. I. (2006), 'An obesity-associated gut microbiome with increased capacity for energy harvest.', *Nature* **444**(7122), 1027–31.

Vaishnava, S., Yamamoto, M., Severson, K. M., Ruhn, K. A., Yu, X., Koren, O., Ley, R., Wakeland, E. K. & Hooper, L. V. (2011), 'The Antibacterial Lectin RegIII Promotes the Spatial Segregation of Microbiota and Host in the Intestine', *Science* **334**(6053), 255–258.

Van den Abbeele, P., Van de Wiele, T., Verstraete, W. & Possemiers, S. (2011), 'The host selects mucosal and luminal associations of coevolved gut microorganisms: a novel concept.', *FEMS Microbiol Rev* **35**(4), 681–704.

Van Dyken, J. D., Müller, M. J. I., Mack, K. M. L. & Desai, M. M. (2013), 'Spatial Population Expansion Promotes the Evolution of Cooperation in an Experimental Prisoner's Dilemma.', *Curr Biol* **23**, 919–923.

Vanhoutte, T., Huys, G., Brandt, E. & Swings, J. (2004), 'Temporal stability analysis of the microbiota in human feces by denaturing gradient gel electrophoresis using universal and group-specific 16S rRNA gene primers.', *FEMS Microbiol Ecol* **48**(3), 437–46.

Varum, F. J. O., Veiga, F., Sousa, J. S. & Basit, A. W. (2012), 'Mucus thickness in the gastrointestinal tract of laboratory animals.', *J Pharm Pharmacol* **64**(2), 218–27.

Veenstra, G., Cremers, F., van Dijk, H. & Fleer, A. (1996), 'Ultrastructural organization and regulation of a biomaterial adhesin of Staphylococcus epidermidis', *J. Bacteriol.* **178**(2), 537–541.

Vidal, O., Longin, R., Prigent-Combaret, C., Dorel, C., Hooreman, M. & Lejeune, P. (1998), 'Isolation of an Escherichia coli K-12 mutant strain able to form biofilms on inert surfaces: involvement of a new ompR allele that increases curli expression.', *J Bacteriol* **180**(9), 2442–9.

Visick, K. & McFall-Ngai, M. (2000), 'An exclusive contract: specificity in the Vibrio fischeri-Euprymna scolopes partnership', *J Bacteriol* **182**(7), 1779.

Vlamakis, H., Chai, Y., Beauregard, P., Losick, R. & Kolter, R. (2013), 'Sticking together: building a biofilm the Bacillus subtilis way.', *Nat Rev Microbiol* **11**(3), 157–68.

von Ossowski, I., Reunanen, J., Satokari, R., Vesterlund, S., Kankainen, M., Huhtinen, H., Tynkkynen, S., Salminen, S., de Vos, W. M. & Palva, A. (2010), 'Mucosal adhesion properties of the probiotic Lactobacillus rhamnosus GG SpaCBA and SpaFED pilin subunits.', *Appl Environ Microbiol* **76**(7), 2049–57.

Wang, D. & Ding, X. (2001), 'Indole Can Act as an Extracellular Signal in Escherichia coli', *J Bacteriol* **183**(14), 4210–4216.

Ward, J. P., King, J. R., Koerber, A. J. & Croft, J. M. (2003), 'Mathematical Biology Early development and quorum sensing in bacterial biofilms', *J Math Biol* **55**, 23–55.

Wehkamp, J., Harder, J., Weichenthal, M., Schwab, M., Schäffeler, E., Schlee, M., Herrlinger, K. R., Stallmach, A., Noack, F., Fritz, P., Schröder, J. M., Bevins, C. L., Fellermann, K. & Stange, E. F. (2004), 'NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression.', *Gut* **53**(11), 1658–64.

Wehkamp, J., Salzman, N. H., Porter, E., Nuding, S., Weichenthal, M., Petras, R. E., Shen, B., Schaeffeler, E., Schwab, M., Linzmeier, R., Feathers, R. W., Chu, H., Lima, H., Fellermann, K., Ganz, T., Stange, E. F. & Bevins, C. L. (2005), 'Reduced Paneth cell alpha-defensins in ileal Crohn's disease.', *P Natl Acad Sci USA* **102**(50), 18129–34.

West, S. A., Diggle, S. P., Buckling, A., Gardner, A. & Griffin, A. S. (2007), 'The Social Lives of Microbes', *Annu Rev Ecol Evol S* **38**(1), 53–77.

West, S. A., Griffin, A. S., Gardner, A. & Diggle, S. P. (2006), 'Social evolution theory for microorganisms.', *Nat Rev Microbiol* **4**(8), 597–607.

West, S. A., Kiers, E. T., Simms, E. L. & Denison, R. F. (2002), 'Sanctions and mutualism stability: why do rhizobia fix nitrogen?', *Proc Biol Sci* **269**(1492), 685–94.

Williams, P., Winzer, K., Chan, W. C. & Cámara, M. (2007), 'Look who's

talking: communication and quorum sensing in the bacterial world.', *Philos T Roy Soc B* **362**(1483), 1119–34.

Wilson, D. S. (1975), 'A theory of group selection.', *P Natl Acad Sci USA* **72**(1), 143–6.

Xavier, J. B., De Kreuk, M. K., Picioreanu, C. & van Loosdrecht, M. C. M. (2007), 'Multi-Scale Individual-Based Model of Microbial and Bioconversion Dynamics in Aerobic Granular Sludge', *Environ Sci Technol* **41**(18), 6410–6417.

Xavier, J. B. & Foster, K. R. (2007), 'Cooperation and conflict in microbial biofilms.', *P Natl Acad Sci USA* **104**(3), 876–81.

Xavier, J. B., Picioreanu, C. & van Loosdrecht, M. C. M. (2005), 'A framework for multidimensional modelling of activity and structure of multispecies biofilms.', *Environ Microbiol* **7**(8), 1085–103.

Xu, J., Mahowald, M. A., Ley, R. E., Lozupone, C. A., Hamady, M., Martens, E. C., Henrissat, B., Coutinho, P. M., Minx, P., Latreille, P., Cordum, H., Van Brunt, A., Kim, K., Fulton, R. S., Fulton, L. A., Clifton, S. W., Wilson, R. K., Knight, R. D. & Gordon, J. I. (2007), 'Evolution of symbiotic bacteria in the distal human intestine.', *PLoS Biol* **5**(7), e156.

Ze, X., Duncan, S. H., Louis, P. & Flint, H. J. (2012), 'Ruminococcus bromii is a keystone species for the degradation of resistant starch in the human colon.', *ISME J* **6**(8), 1535–43.

Ze, X., Mougen, F. L., Duncan, S. H., Louis, P. & Flint, H. J. (2013), 'Some Are More Equal Than Others', *Gut Microbes* **4**(3), 236–240.

Zuroff, T. R., Barri Xiques, S. & Curtis, W. R. (2013), 'Consortia-mediated bioprocessing of cellulose to ethanol with a symbiotic Clostridium phytofermentans/yeast co-culture', *Biotechnol Biofuels* **6**, 59.

Zuroff, T. R. & Curtis, W. R. (2012), 'Developing symbiotic consortia for lignocellulosic biofuel production', *Appl Microbiol Biotechnol* **93**, 1423–1435.

7
Appendix

Chapter 2: Supplementary Materials

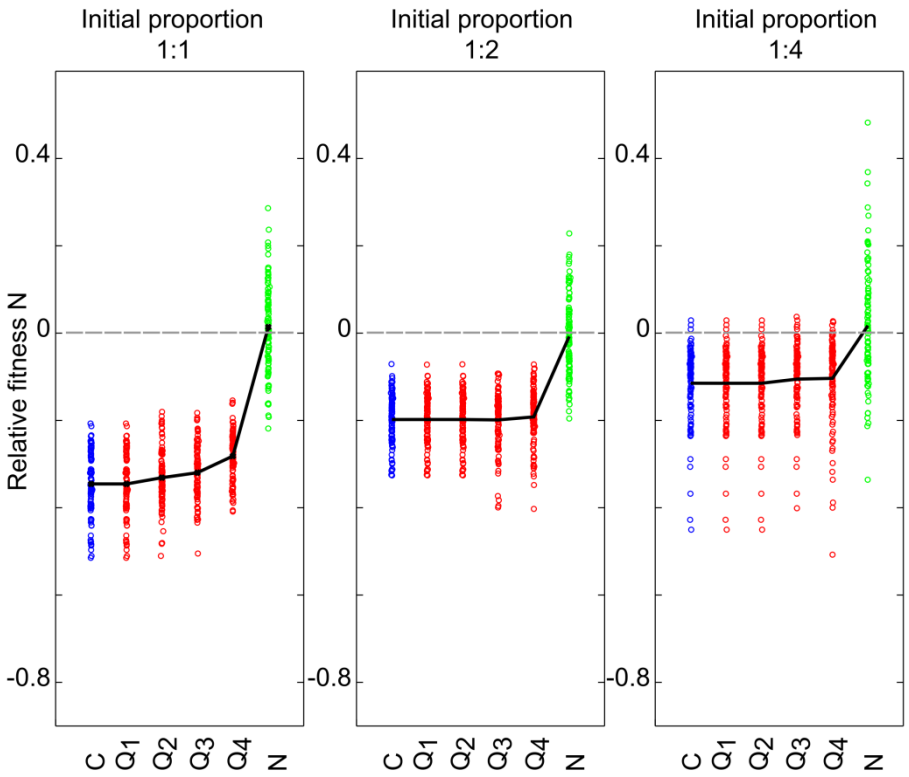


Figure S 2.1: **Relative fitness of a non-secretor genotype (N) in competition with secretor genotypes (C: constitutive, Q1-Q4: quorum sensing secretors with increasing QS thresholds**, see Table S1, N: non-secretor control). Higher genotypic diversity is reflected in lower initial proportions of the non-secretor, results of 100 independent simulations each, mean relative fitness in red.

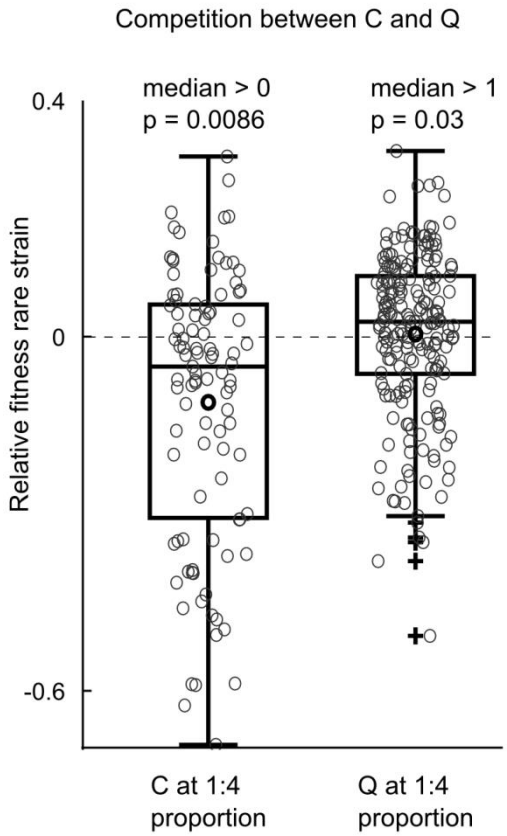


Figure S 2.2: **Competition between quorum sensing strains and constitutive secretors.** Left: Relative fitness of a rate constitutive secretor (C) in competition with quorum sensing genotypes. Right: a rare quorum sensing genotype (Q) in competition with constitutive secretors. Individual simulation results and mean (bold) in circles. p-values from non-parametric sign test for difference from zero median. While the mean relative fitness of a rare Q genotype is only slightly above 0, we show convergence of the mean in our simulations in Figure S3.

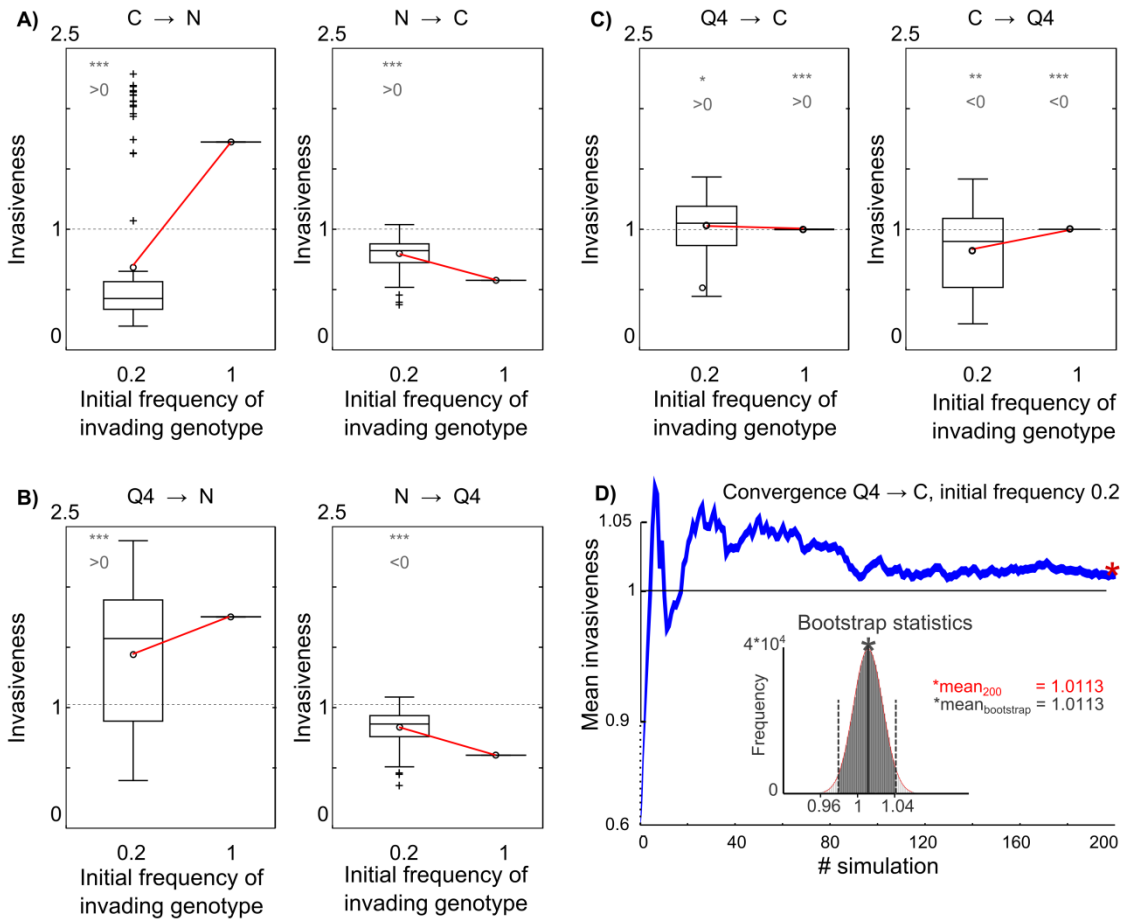


Figure S 2.3: **Invasion analysis of competitions between quorum sensing strains, constitutive secretor strains, and non-secretors.** Invasiveness values >1 indicate that a focal genotype can invade a metapopulation of the competitors under high evolutionary competition (0.2 initial frequency) or low evolutionary competition (initial frequency of rare strain: 1, this assumes that patches of strains grow separated from each other), results of 100 independent simulations, mean values connected by the red line. A-C) Pairwise reciprocal invasion analysis of constitutive secretor (C), quorum sensing strains (Q) and non-secretors (N) into metapopulations the other genotypes. Q can invade metapopulations of N and C while itself resisting invasion from both. Against C, the differences are small but significant. Some of the data is bimodally distributed meaning it is difficult to use standard statistics (we show the results of sign tests for difference of the median from 1, *:p<0.05; **: p<0.01; ***p<0.0001). We, therefore, further show an example of a convergence plot of the mean invasiveness value of Q4 invading C over 200 simulations in D). The rolling mean invasiveness shows that after about 100 simulations additional simulations do not have a strong effect and the mean converges to a value slightly above 1 (blue line). Inset: histogram of bootstrapped means (solid vertical line at 1.01) and confidence intervals (dashed vertical lines at 0.98 and 1.04) indicate good accuracy of the original simulation data (10^6 resampling events).

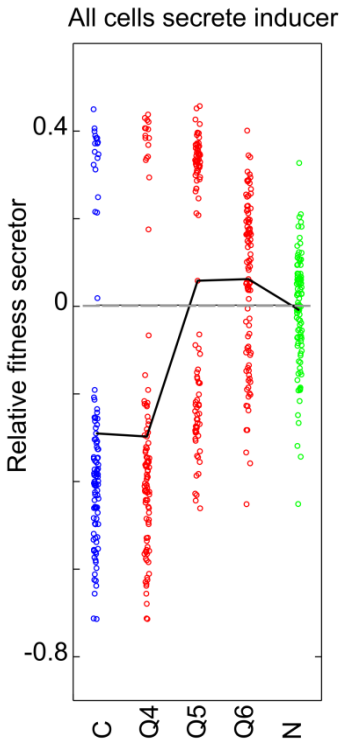


Figure S 2.4: **Relative fitness of a secretor genotype in competition with non-secretor genotypes (N) when all strains produce autoinducer.** C is constitutive, N is non-secretor control, and Q4-6 are quorum sensing genotypes with increasing autoinducer thresholds, see Table S1. Simulations assume 1:4 proportions of secretor to non-secretor genotypes, results of 100 independent simulations each, mean relative fitness in red. While C cannot outcompete its N competitors, delaying public good secretion conveys a competitive advantage to Q strains.

Table S 2.1: List of parameters and the values used in our simulation models

Symbol	Description	Dimension	Value
μ_{max}	Maximum cell growth rate of microbial species	T^{-1}	1
τ	Threshold public good concentration	$M_E L^{-3}$	4×10^{-3}
ϕ_j	Threshold autoinducer concentration for quorum sensing genotype j	$M_I L^{-3}$	$[0.02, 0.04, 0.06, 0.08, 0.16, 0.32]$
B	Growth factor increase due to the presence of public good at or above threshold concentration τ	dimensionless	3
$Z_{0,1}$	Growth factor decrease in growth rate of secretor strains due to the costly secretion of public goods	dimensionless	0: 0 or 1: -0.3
D_G	Growth substrate (nutrient) diffusivity	L^2T^{-1}	4×10^4
D_E	Extracellular secreted product diffusivity	L^2T^{-1}	3×10^5
D_I	Auto-inducer diffusivity	L^2T^{-1}	3×10^5
E_{bulk}	Bulk concentration of extracellular product	$M_E L^{-3}$	0
$[E]$	Local concentration of extracellular product	$M_E L^{-3}$	n/a
I_{bulk}	Bulk concentration of autoinducer	$M_I L^{-3}$	0
$[I]$	Local concentration of autoinducer	$M_I L^{-3}$	n/a
G_{bulk}	Bulk concentration of growth substrate (nutrient)	$M_G L^{-3}$	0.125 unless stated otherwise
$[G]$	Local concentration of growth substrate (nutrient)	$M_G L^{-3}$	n/a
K_G	Half saturation constant for growth substrate concentration	$M_G L^{-3}$	3.5×10^{-5}
	Total nutrient consumption after which fitness was calculated	M_G	10^7
$N_{x,t}$	Number of cells of strain or species x in a cell group at time t	dimensionless	n/a
R_E	Rate of secretion of extracellular product	$M_E M_X^{-1} T^{-1}$	1
w_x	Fitness of strain or species x	T^{-1}	n/a
X_i	Concentration of biomass of genotype i	$M_X L^{-3}$	n/a
Y	Yield of biomass per substrate	$M_X M_G^{-1}$	0.5

M_E represents mass of extracellular enzyme,M_I represents mass of extracellular inducer, M_G represents mass of growth substrate, M_X represents cell biomass, L represents length, and T represents time.

Table S 2.2: Stoichiometry of cell metabolism used in the simulation models

	Solutes			**Particulates**			
Reaction	G	E	I	X_C	X_Q	X_N	**Rate Expression**
C growth	$-1/Y$			$1 + Bf([E]) + B_b f([E_b])$			$\mu_{max_a} \frac{[G]}{[G]+K_G} X_C$
Q growth	$-1/Y$				$1 + Bf([E])$		$\mu_{max} \frac{[G]}{[G]+K_G} X_Q$
N growth	$-1/Y$					$1 + Bf([E])$	$\mu_{max} \frac{[G]}{[G]+K_G} X_N$
Autoinducer secreted by Q			1				1
Product secreted by C		1		$-Z_1$			$R_E \mu_{max} \frac{[G]}{[G]+K_G} X_C$
Product secreted by Q		1				$Z_0 - Z_1 \theta[I]$	$R_E \mu_{max} \frac{[G]}{[G]+K_G} X_Q$

$f([E_x])$ is a function of local extracellular product concentration, $[E]$, $f([E]) = \begin{cases} 0, [E] < \tau \\ 1, [E] > \tau \end{cases}$

$\theta[I]$ is a function of local autoinducer concentration, $[I]$, $\theta[I] = \begin{cases} 0, [I] < \phi_j \\ 1, [I] > \phi_j \end{cases}$

All other symbols are defined in Table S 2.1.

Chapter 3: Supplementary Materials

Cell cluster volume expansion can offset the cost of adhesiveness

Our results predict that adhesion is a strategy whose success depends on the localisation of the nutrient source; on the other hand, cell cluster volume expansion is generally beneficial whenever cells live in a structured, nutrient limited environment in which such volume expansion can lead to preferential access to nutrients. Extracellular polymers can either confer adhesiveness to secreting cells (Ma et al. 2006; Vlamakis et al. 2013; Kierek & Watnick 2003) or, conversely, bind to adhesive cell-surface molecules and thus reduce the adhesiveness of secreting cells (Hay et al. 2009; Orgad et al. 2011). These observations suggest that adhesiveness and cell cluster volume expansion can be separately selected over evolutionary time. Our simulations allow us to disentangle the fitness effects of adhesiveness and cell cluster volume expansion, clarifying the separate evolutionary dynamics of these two phenotypic characteristics.

We first consider competition of an adhesive and volume-expanding EPS-producer versus a non-producer. Adhesion and volume expansion were shown to independently confer a competitive advantage when nutrients diffuse into the biofilm from the substratum. As expected, these advantages are preserved when the two traits are combined (Figure S3.1). When nutrients diffuse from above the biofilm, however, our simulations show that the competitive advantage of volume expansion can, depending on position in parameter space, outweigh the potential costs of adhesiveness, and the combined-trait genotype outcompetes a non-producer (Figure S3.1).

In some cases, EPS secretion may reduce cell adhesiveness: for example, alginate secretion *by Pseudomonas aeruginosa* is thought to cover adhesive polymers on the surface of secreting cells (Orgad et al. 2011). To model this potential effect of EPS secretion, we compete the adhesive and volume-expanding genotype against a new genotype *C* that produces volume-expanding EPS but is not adhesive. These simulations recapitulate our previous findings: when nutrients diffuse from above, genotype *C* outcompetes genotype *A* because it can expand towards the nutrient source more readily. When nutrients diffuse into the biofilm from below, adhesiveness allows strain *A* to gain preferential access to nutrients. These results confirm that the evolution of adhesiveness should be strongly influenced by the environment cells are occupying. Whenever limiting nutrients are acquired from the substratum on which biofilms are growing, we predict that cells should evolve to become more adhesive. However, secretion of volume-expanding EPS can help to maintain adhesiveness when nutrients diffuse from above, where adhesiveness alone would be detrimental.

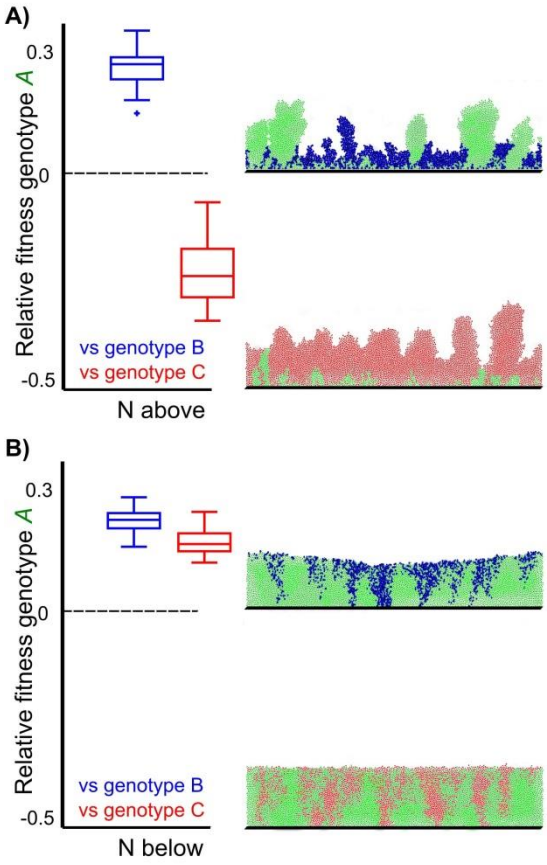


Figure S 3.1: **Volume expansion can offset the cost of adhesiveness.** Cells of strain *A (*adhesive and volume-expanding due to EPS production) are competed against strain *B* (non-adhesive, non-EPS secreting) and species *C* (non-adhesive, but volume expanding due to EPS secretion). A) Nutrients diffuse from above, B) Nutrients diffuse from below. Snapshots of simulations at t = 120h.

Long term evolutionary dynamics of adhesiveness

In the main text, we focus primarily on conditions in which cells grow for a limited period of time defined by a fixed initial supply of nutrients. However, microbes also occupy environments in which nutrients are continuously replenished. Plausible examples include communities on plant roots whose exudates provide nutrition to the resident microbes (Nardi et al. 2000; Narula et al. 2009), riverbeds to which nutrients are constantly supplied from upstream, and the mammalian gut in which large amounts of complex carbohydrates are secreted by the epithelium to feed and potentially select beneficial microbial species (Bevins & Salzman 2011; Hooper et al. 1999). We modified our model to better capture such communities by allowing communities to grow over much longer time scales, and we also implemented a sloughing mechanism whereby cells are lost from the biofilm once they reach a defined height. Our findings are unaffected by these conditions: EPS production is competitively advantageous whenever concentrations of nutrients are low. On the other hand, the fitness effect of adhesiveness still depends on the direction from which nutrients diffuse into the biofilm: highly

adhesive cells outcompete non-adhesive cells when nutrients diffuse from the substratum, but highly adhesive cells are outcompeted when nutrients diffuse from above the biofilm (Figure S3.2).

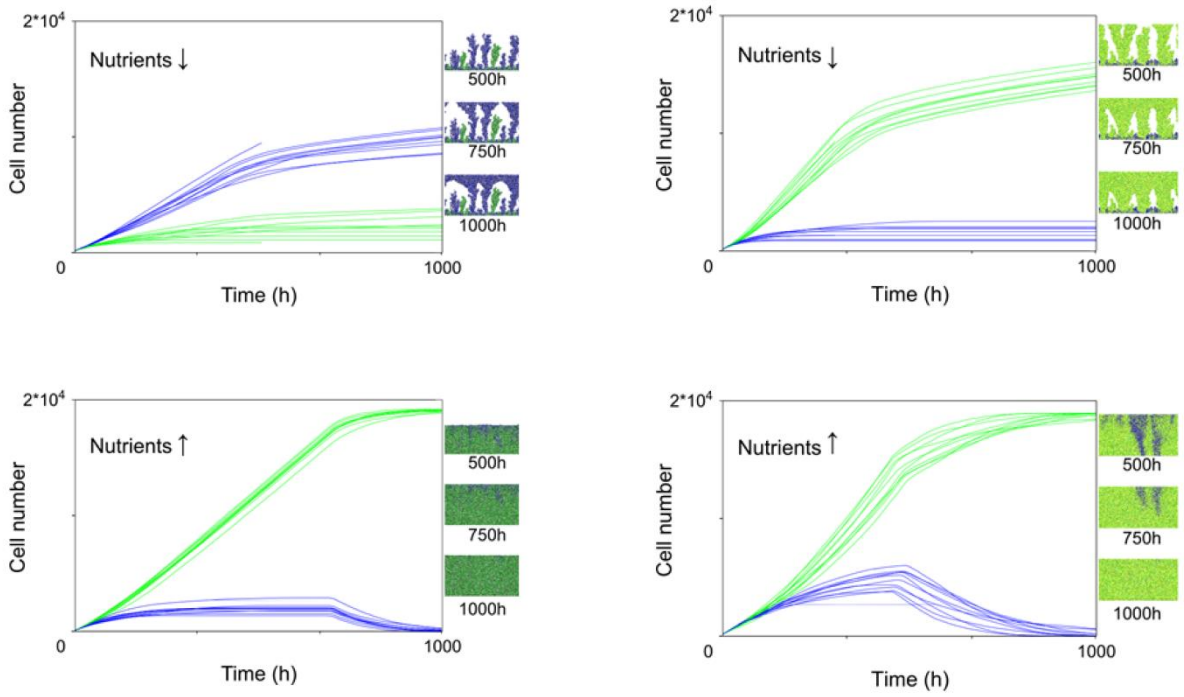


Figure S 3.2: **Adhesion and volume expansion in long term microbial communities**. Left: adhesive genotype *A* (green) competes with non-adhesive genotype *B* (blue). Right: EPS-secreting species *A* competes against non-producing genotype *B.* Plots show cell numbers over time and representative snapshots of three time points. Cells are sloughed from the biofilm surface at a defined height of 150 µm.

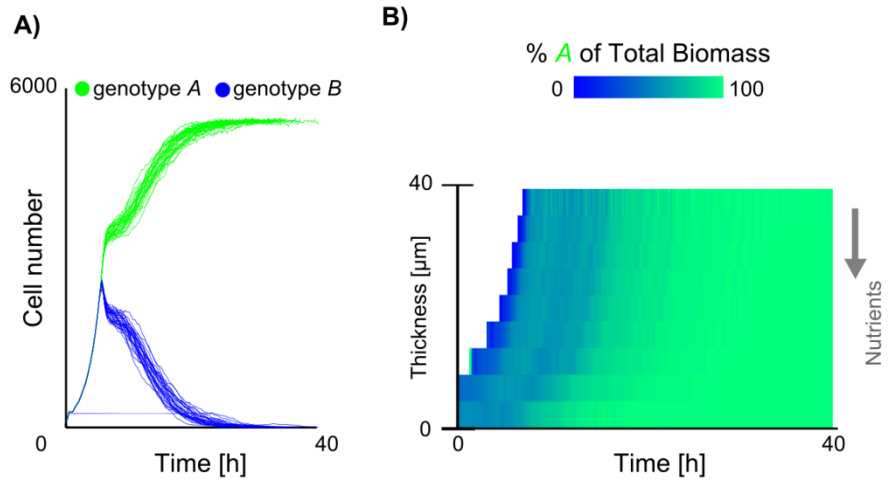


Figure S 3.1: **Competition in a model of a nutrient saturated biofilm with saturating nutrients diffusing into the colony from above.** A) Cell number over time; initiated with equal cell numbers; genotype *A* outcompetes *B* over time. B) The heat map shows the biomass distribution of the two genotypes averaged over the width of a single simulated biofilm community. Cells grow protected from sloughing in a 40µm thick layer beyond which cells are lost from the biofilm, nutrient concentration N = 4.

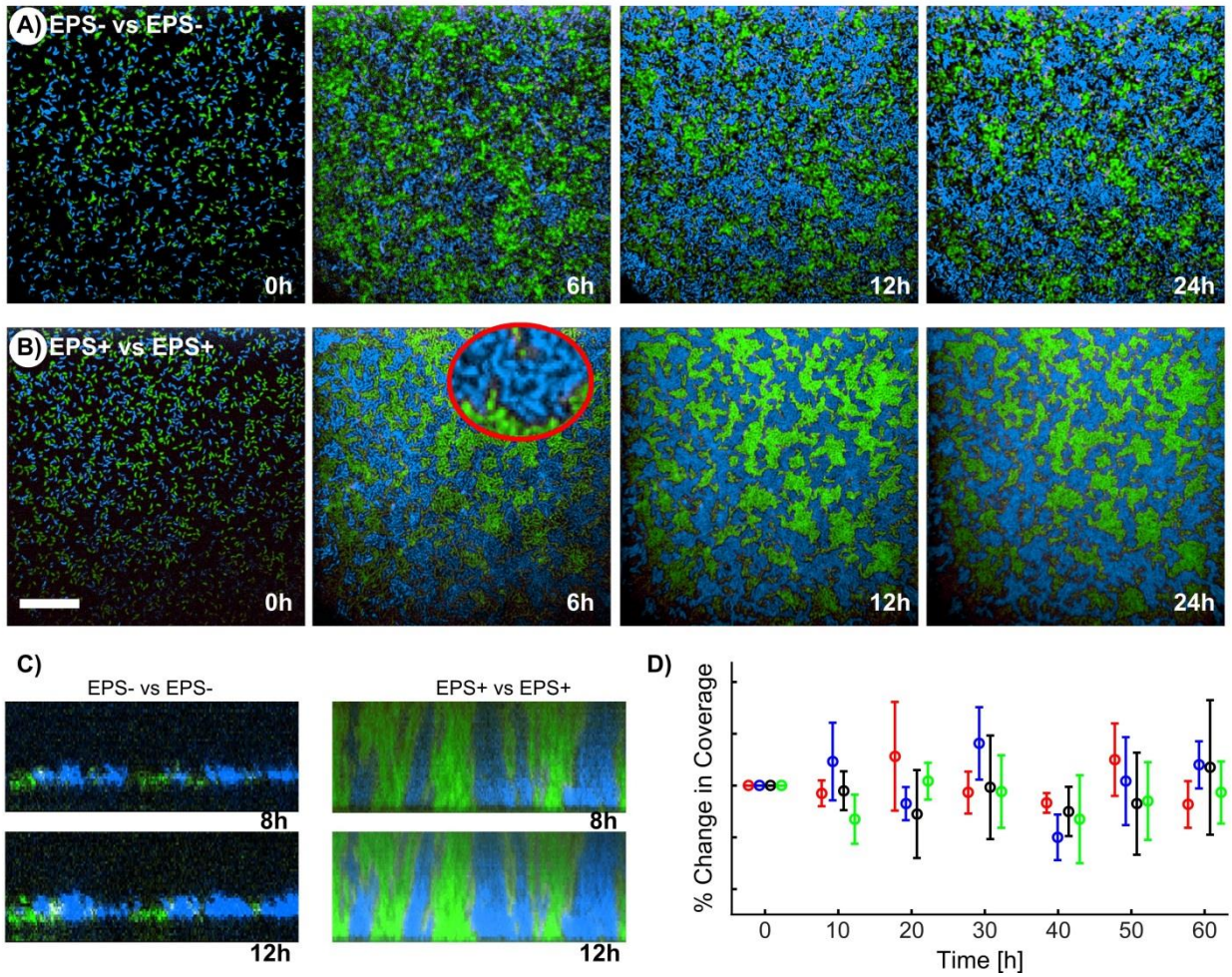


Figure S 3.4: **Control experiments.** A) A time series for the bottom layer of EPS^- (green) competing against other EPS^- cells (blue). B) A time series for the bottom layer of EPS^+ (green) competing against other EPS^+ cells. Inset at 6h: a digital zoom shows tight packing of cells in EPS^+ colonies. C) Cross-sections of EPS^- (blue) vs. EPS^- (green) and EPS^+ (blue) vs. EPS^+ (green) show no clear displacement. D) Resistance to shear by monocultures of EPS^+ and EPS^- cells. Glass slides were colonized with either strain and exposed to two different flow rates, and surface coverage was measured at 10 min intervals for 1 h. Analysis of surface coverage by EPS^+(red: 400 µm /sec flow; blue: 20 µm /sec flow) and EPS^- cells (black: 400 µm /sec flow, green: 20 µm/sec flow) indicates that both are capable of forming and maintaining monolayers in the presence of flow.

Table S 3.1) Simulation parameters. Length (L), mass (M), time (T).

Symbol	Description	Dimen-sion	Value	Units	References
Max. colony thickness gut simulations		L	40	μm	(Schluter & Foster 2012)
Max. cell radius before division		L	1	μm	(Mitri et al. 2011)
Width of simulated colony		L	350	μm	
Initial cell number (A+B), unless stated otherwise			280		
Boundary layer thickness		L	100	μm	
$\boldsymbol{\mu}$	maximum growth rate	T^{-1}	1	h^{-1}	(Mitri et al. 2011; Rang et al. 1999)
$\boldsymbol{K_N}$	Half saturation constant for growth on nutrient N and L	ML^{-3}	$3.5*10^{-5}$	gl^{-1}	(Mitri et al. 2011; Nadell et al. 2010)
$\boldsymbol{D}$	Diffusion coefficient of solutes	L^2T^{-1}	$5.76*10^4$	μm^2h^{-1}	(Nadell et al. 2010)
$\boldsymbol{N}$	Bulk nutrient concentration	ML^{-3}	$5*10^{-4}$	gl^{-1}	(Nadell et al. 2010)
$\boldsymbol{\rho_X}$	Density of biomass	ML^{-3}	220	gl^{-1}	(Mitri et al. 2011)
$\boldsymbol{\rho_{EPS}}$	Density of EPS	ML^{-3}	As a fraction of ρ_X	gl^{-1}	(Mitri et al. 2011)
$\boldsymbol{Y}$	Yield of biomass per substrate		0.5		(Mitri et al. 2011)
$\boldsymbol{f_{EPS}}$	Fraction of growth diverted into EPS production		0.25		
$\boldsymbol{\sigma}$	Adhesion parameter		2 when adhesive, 1 otherwise	dimen-sionless	
$\boldsymbol{r}$	Sloughing parameter		0: no sloughing 0.1: weak sloughing 20: strong sloughing		
	Maximum allowed biomass in nutrient limited simulations	M	10^{-8}	g	

Table S 3.2) Stoichiometry of microbial growth and EPS secretion

Reaction	**Solute**	**Biomass**		**Rate Expression**
	N	**Cell biomass (X)**	**EPS**	
growth	-1/Y	$1 - f_{EPS}$	f	μ [N] / ([N] + K_N) X

Chapter 4: Supplementary Materials

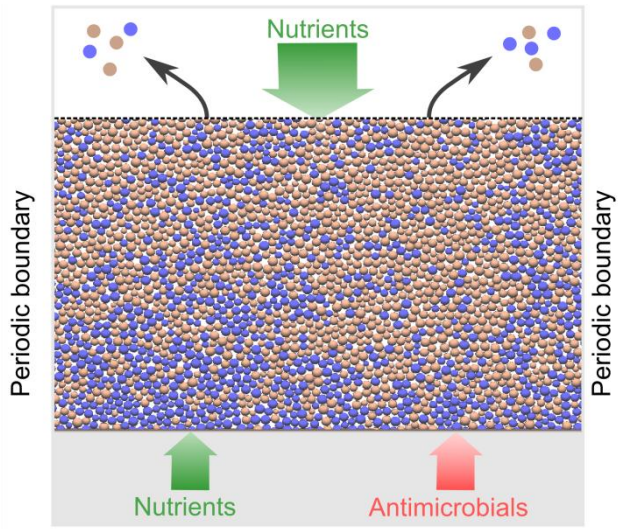


Figure S 4.1: **Diagram of the simulation setup.** Cells live and divide on an impenetrable host epithelium. Growth-promoting nutrients can diffuse into the bacterial colony from the lumen (top) and/or the epithelium (bottom) where they are utilized by the cells. In some simulations the epithelium also releases antimicrobials that kill cells. The direction of fluxes is indicated by arrows (nutrients, green; antimicrobials, red). Periodic boundaries at the sides simulate continuous space. Cells moving beyond the maximum thickness are removed simulating sloughing (dashed line, the location is a parameter that we vary). doi:10.1371/journal.pbio.1001424.s001

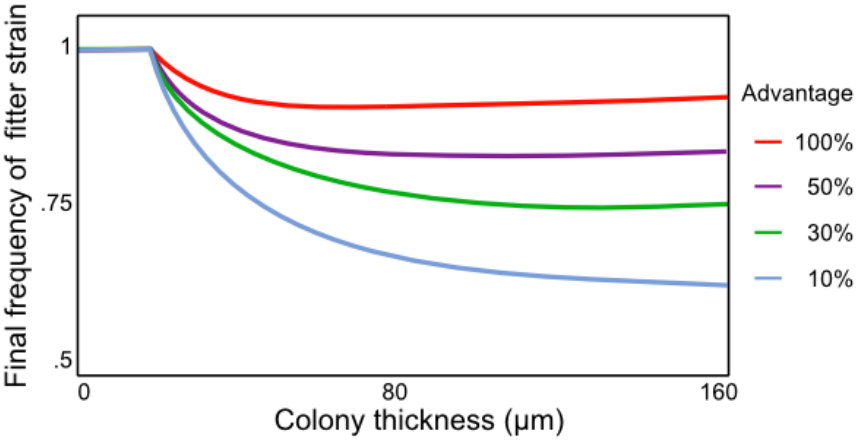


Figure S 4.2: **Final frequencies of faster growing strain B in a simplified ordinary differential equation model.** A minimum final frequency of the faster growing strain is found for intermediate microbial community thickness; the exact location depends on the growth functions of the two strains (see Text S1). doi:10.1371/journal.pbio.1001424.s002

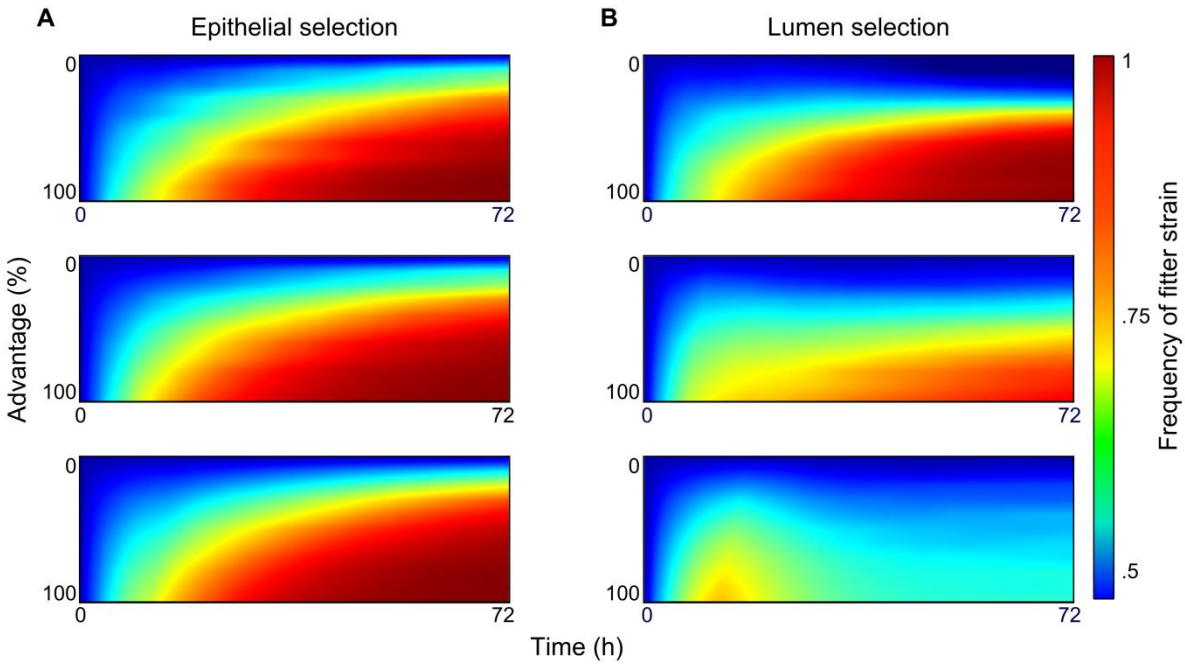


Figure S 4.3: **The effect of non-selective nutrients from one direction on selectivity from the other direction**. We show the biomass development over time beginning with cells at low densities (125 cells each). (A) Host-secreted nutrients provide a growth rate advantage and neutral nutrients diffuse into the biofilm from the lumen. (B) Lumen nutrients provide a growth rate advantage and the host secretes neutral nutrients. Points of sloughing are 20, 40, and 80 µm. For 80 µm, lumen selection is strongly impeded by the presence of neutral host nutrients, whereas host selection is unaffected by additional neutral lumen nutrients (initially the favoured species outgrows the other but upon reaching the capacity will be sloughed off more frequently, leading to a decrease in frequency compared with the maximum at ~10 h).doi:10.1371/journal.pbio.1001424.s003

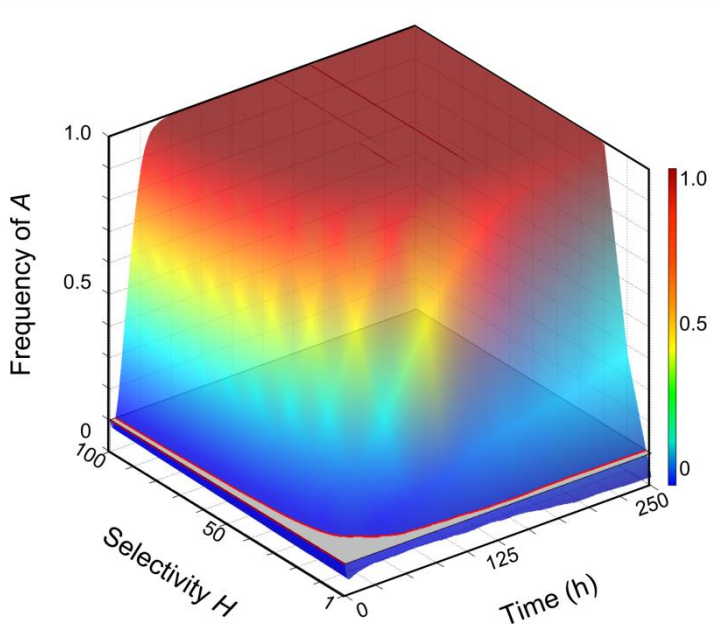


Figure S 4.4: **Selection amplification of an initially rare strain by the host epithelium**. Weak epithelial selection dominates strong lumen selection. Strain B has a 100% growth rate advantage on nutrients from the lumen, and lumen nutrients are five times the concentration as epithelial nutrients. Host nutrients provide growth rate advantages to an initially rare strain A (initial frequency 0.1, grey plane) ranging from 1% to 100%.doi:10.1371/journal.pbio.1001424.s004

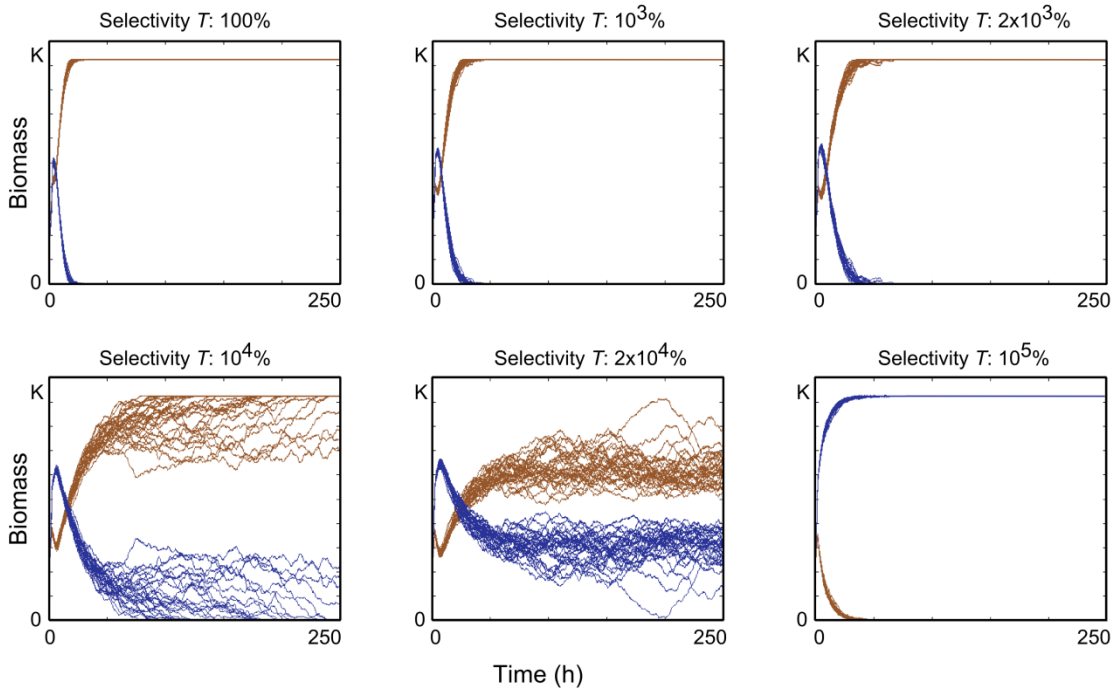


Figure S 4.5: **Selection with antimicrobials.** Selection via host antimicrobial secretion in the absence of host nutrient secretion is possible only when lumen nutrients are available throughout the bacterial colony. The host can select for slow growing strain A despite antimicrobials being available at relevant concentrations only in a fraction of the overall bacterial colony (near the epithelium) when selectivities of antimicrobials in favour of the strain A (SB<SA) are sufficiently high. In the majority of the bacterial colony, cells of strain B have a net growth rate advantage due to the high concentration of lumen nutrients, which favour B. Capacity K, maximum biomass. doi:10.1371/journal.pbio.1001424.s005

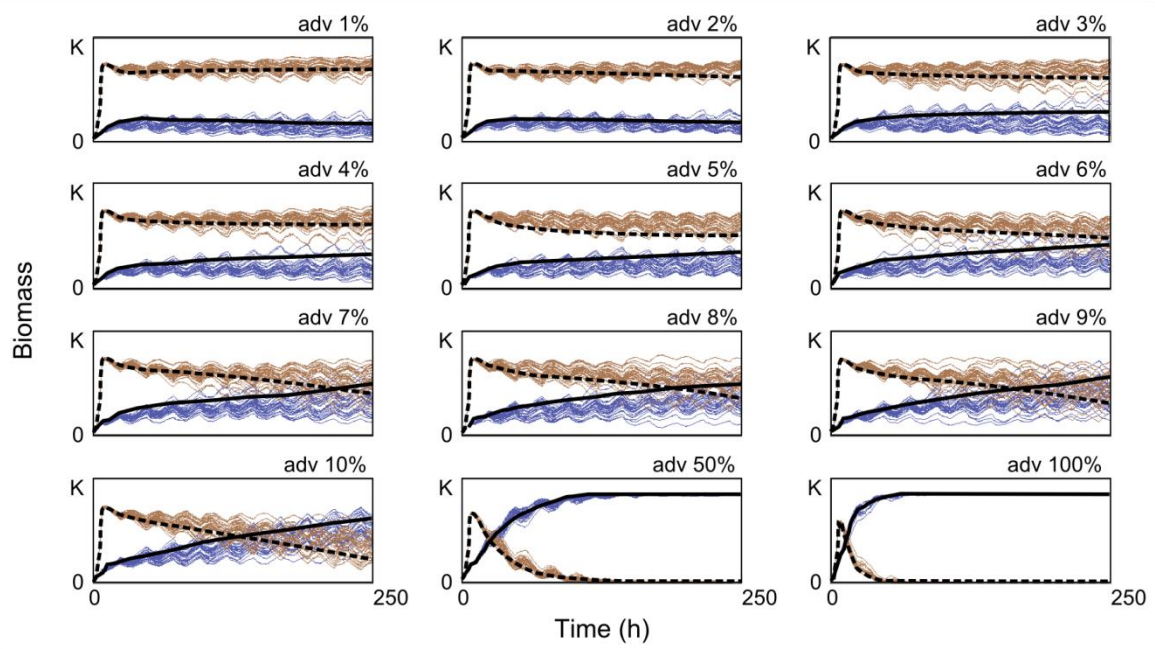


Figure S 4.4: **Fluctuations in lumen nutrient concentrations do not affect predictions.** Each figure shows the biomass development of the two strains in 30 independent simulations (brown, strain B; blue, strain A) with discontinuously available nutrients in the lumen. Feast-famine periods last 8 h each. For comparison, thick black lines show the mean biomass from 30 simulations under identical selection strengths but with continuously available nutrients for stain B (dashed) and strain A (solid). Host nutrients provide varying growth rate advantages to strain A as indicated in the figure. The mean nutrient concentration in the lumen is five times higher than nutrients from the host, and strain B has a 100% growth rate advantage over A on these nutrients. Mean nutrient concentrations in the continuous and discontinuous case are identical. Capacity K, maximum biomass. doi:10.1371/journal.pbio.1001424.s006

Table S 4.1) Simulation parameters. Length (L), mass (M), time (T).

Symbol	Description	Dimension	Value	Units	References
	Max. colony thickness	L	10-160	µm	(Atuma, Strugala, Allen, & Holm, 2001)
	Max. cell radius before division	L	1	µm	(Mitri, Xavier, & Foster, 2011)
	Width of simulated colony	L	250	µm	
	Initial cell number (*A*+*B*)		250		
	Boundary layer thickness	L	25	µm	(Mitri et al., 2011)
$\mu_{i,N/L}$	maximum growth rates of strain *i* on nutrient *N* or *L*	T^{-1}	$0.5 \leq \mu \leq 1$	h^{-1}	(Mitri et al., 2011; Rang et al., 1999)
$K_{N,L}$	Half saturation constant for growth on nutrient *N* and *L*	ML^{-3}	$3.5*10^{-5}$	gl^{-1}	(Mitri et al., 2011; Nadell, Foster, & Xavier, 2010)
D	Diffusion coefficient of solutes	L^2T^{-1}	$4*10^4$	μm^2h^{-1}	(Mitri et al., 2011; Nadell et al., 2010)
L	Bulk lumen nutrient concentration	ML^{-3}	0.8, 4 or non-limiting (const. at 4)	gl^{-1}	Based on *high nutrient concentration* in (Mitri et al., 2011)
H	Maximum host nutrient concentration near the epithelium. The secretion rate of nutrients from host epithelial cells is based on total fluxes from the lumen such that energy values can be compared, i.e. five times more from the lumen in Figure 4.	ML^{-3}	≤ 0.8	gl^{-1}	
S	Susceptibility defined as concentrations at which cell death within one hour occurs with a probability of 50%. Values based on maximum antimicrobial concentration (*T*) such that cell growth is possible.	ML^{-3}	0.065-0.26	gl^{-1}	
T	Maximum concentration of antimicrobial near the epithelium	ML^{-3}	0.005	gl^{-1}	
ρ	Density of bacterial biomass	ML^{-3}	150	gl^{-1}	(Mitri et al., 2011)
Y	Yield of biomass per substrate		0.5		(Mitri et al., 2011)

Text S1

We simplify the individual based simulations where nutrients exclusively come from the lumen by considering two non-spatial, coupled ordinary differential equations describing the biomass development of strain *A* and *B* over time. Starting from an initial monolayer of cells, the bacterial colony in the individual based model increases in thickness due to cell division, eventually excluding cells below from access to nutrients. The region of active growth where nutrients are available at sufficient concentrations moves upwards with the growing colony towards the source of nutrients in the lumen. When the maximum height (which we vary) where cells are sloughed off is reached, a region of actively dividing cells exists, which we will call final active layer. Here, cells are removed constantly through sloughing and replenished by bacterial growth. We can describe the competition between strain *A* and *B* in the active layer with

$$\frac{dA}{dt} = \mu_{maxA}\left(1 - \frac{A+B}{\alpha}\right)A - \varsigma A \quad (1)$$

$$\frac{dB}{dt} = \mu_{maxB}\left(1 - \frac{A+B}{\alpha}\right)B - \varsigma B \quad (2),$$

where $\mu_{maxB} > \mu_{maxA} > \varsigma > 0$. Under these conditions, the frequency of *B* tends towards 1 as constant removal of a fraction of cells due to sloughing (ς) and faster replenishing of *B* will lead to successful competition for the limited capacity (α) of the final active layer. Before the colony has reached it's final thickness, regions below the final active layer are subject to competition between the two strains simplified as

$$\frac{dA}{dt} = \mu_{maxA}\left(1 - \frac{A+B}{K}\right)A \quad (3)$$

$$\frac{dB}{dt} = \mu_{maxB}\left(1 - \frac{A+B}{K}\right)B \quad (4),$$

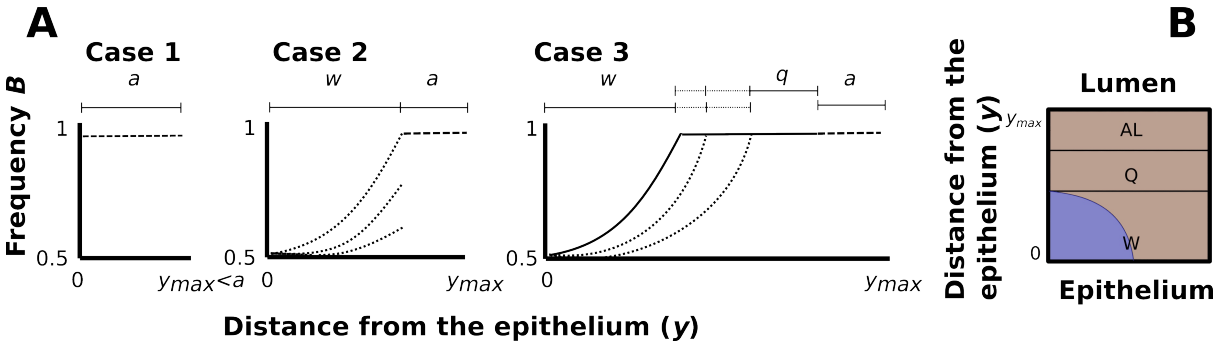
with capacity of the system *K*, which is solved until $t^* = 72$ days equivalent to the individual

based simulations. The final frequency $R(t^*)$ of B (shown in Figure S2) is calculated taking into account the frequency of B below the final active layer at t^* and the fraction of the total colony that lies within the final active layer where the frequency of B will be 1 (eqs. 1 and 2). Hence,

$$R(t^*) = \frac{B(t^*)}{A(t^*) + B(t^*)} \frac{K - \alpha}{K} + 1\frac{\alpha}{K} \quad (5),$$

where K is the overall capacity of the colony which increases with the thickness and α is the capacity of the final active layer which is constant and $K \geq \alpha$. Hence the contribution to the final frequency of B from α in the last term in equation 5 is larger for smaller K, i.e. in thinner colonies. The values for K and α are based on the values of total biomass found in the individual based simulations for different points of sloughing. For values of K smaller than α (thin colonies), the final ratio is 1, as the entire bacterial colony is subject to constant selection because nutrients are available everywhere, i.e. the entire bacterial colony resides within the final active layer.

These considerations allow us to distinguish three compartments of a steady state bacterial community: the final active layer (*AL*) which will exist for any sloughing height, and two other compartments below *AL*, which can arise depending on where sloughing occurs. In the early stages, strains A and B exist in a 50:50 ratio. Then, in these simulations, because $\mu_{maxB} > \mu_{maxA}$, strain B begins to outgrow strain A and will eventually take over the surface of the growing colony entirely (Sketch, B). Hence, all cells with access to nutrients will be of strain B. Immediately below the final active layer, there thus may then be a region which is comprised only of cells of strain B (non-dividing as no nutrients can reach this region). We call this compartment Q. Below Q at the base of the colony, another stagnant region exist which unlike Q is comprised of both strains (compartment W).



Sketch: A) Graphs show the frequency of strain *B* as we move away from the epithelium. Lower case *a*, *q*, *w* indicate the location of the compartments *AL*, *Q*, *W*. Case 1) In thin bacterial colonies, nutrients are available everywhere and the entire colony [0 *y*] lies within the final active layer (*a*). Case 2) The final active layer is located at the top of the colony below which a compartment where strain *B* and *A* coexist arises (*w*). Case 3) In thick colonies, a third compartment arises (*q*) which harbours stagnant cells of strain *B* only. Different curves result from different growth functions and growth rate advantages for *B*. B) Cartoon demonstrating the biomass distribution in a two dimensional colony for case 3 (strain *A* blue, *B* brown). Compartments AL, Q and W arise: *AL*, a stagnant region only containing *B* cells: *Q*, and at the base a stagnant region containing a mixture of *A* and *B* cells: *W*.

How does altering the colony thickness (y) affect final total ratios? We call the distance from the epithelium of the three compartments a, q and w. For sloughing points very close to the epithelium, nutrients will always penetrate the entire system ($y \leq a$) and hence the entire colony is subject to continuous selection and will thus tend to be composed entirely of the faster growing strain B (Case 1). Colony thicknesses for which case 1 holds will result in a final frequency of B of 1. When compartmentalisation due to nutrient depletion at the base of the colony arises ($y > a$), we declare $w = y - a$. Competition between the two strains leads to an increase in the frequency of the faster growing strain B. As we move away from the epithelium where the initial frequency is 0.5, we can describe the increase in frequency of B as a function $g(y)$ of the distance (y) from the epithelium between 0 and w (from the epithelium to the active layer). Case 2 arises when $0 < w <= y^*$, where $y^* := g(y) = 1$. Hence y^* is the distance from the epithelium at which B has taken over entirely. For case 2, the

final ratio is given by:

$$R_{C2} = 1 - \frac{0.5w - \int_0^w g(y)dy}{w + a} \quad (6).$$

Case 3 finally arises for $y - a > y^* = w$. In case 3, we find all three compartments described above and the final frequency of *B* is given by

$$R_{C3} = 1 - \frac{0.5y^* - \int_0^{y^*} g(y)dy}{y^* + q + a} \quad (7)$$

The colony thickness for which the frequency of *B* is minimal will be intermediate as it has to be larger than *a* and (7) tends to 1 again for very large y_{max} (and thus very large *q*). The exact location of the minimum will depend on the function *g(y)*.

Chapter 5: Supplementary Materials

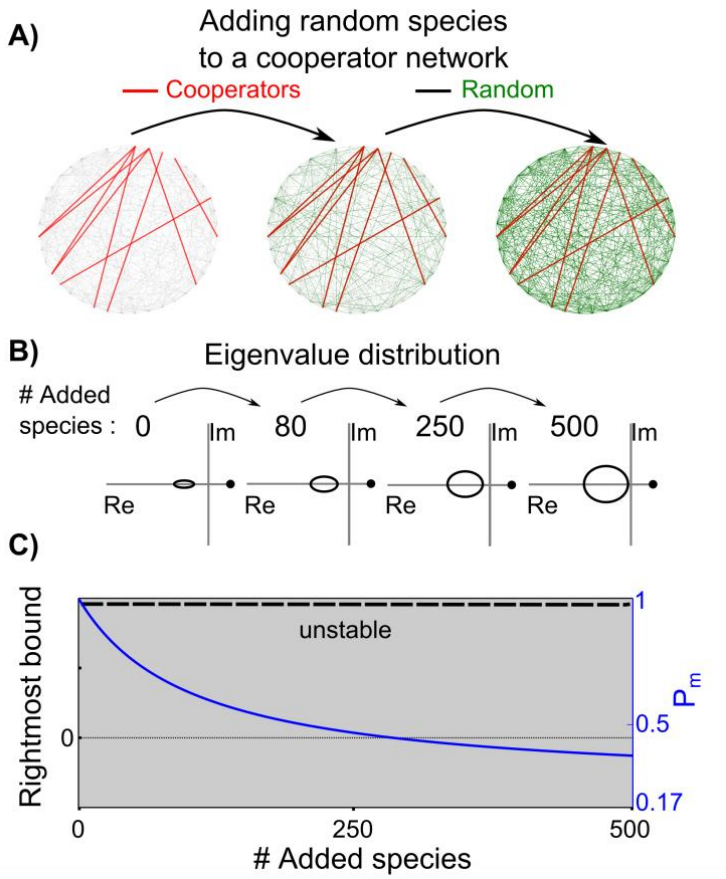


Figure S 5.1: **Introducing random species does not increase stability.** A) Cartoon demonstrating how a network of cooperating species (red) is embedded in larger networks of species (green) that interact randomly either cooperatively (25%), competitively (25%) or exploitative (50%) with the focal group of cooperating species. B, C) We add random species to a focal community of 100 cooperating species. Stability is unaffected because the initial system of 100 cooperators is unstable and the stability-determining eigenvalue corresponding to the average row-sum (dot) is unchanged when random species are added. Addition of more species increases the widths of the ellipses which leads to lower likelihood of stable communities.

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The Evolution of Mutualism in Gut Microbiota Via Host Epithelial Selection

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Abstract

The human gut harbours a large and genetically diverse population of symbiotic microbes that both feed and protect the host. Evolutionary theory, however, predicts that such genetic diversity can destabilise mutualistic partnerships. How then can the mutualism of the human microbiota be explained? Here we develop an individual-based model of host-associated microbial communities. We first demonstrate the fundamental problem faced by a host: The presence of a genetically diverse microbiota leads to the dominance of the fastest growing microbes instead of the microbes that are most beneficial to the host. We next investigate the potential for host secretions to influence the microbiota. This reveals that the epithelium–microbiota interface acts as a selectivity amplifier: Modest amounts of moderately selective epithelial secretions cause a complete shift in the strains growing at the epithelial surface. This occurs because of the physical structure of the epithelium–microbiota interface: Epithelial secretions have effects that permeate upwards through the whole microbial community, while lumen compounds preferentially affect cells that are soon to slough off. Finally, our model predicts that while antimicrobial secretion can promote host epithelial selection, epithelial nutrient secretion will often be key to host selection. Our findings are consistent with a growing number of empirical papers that indicate an influence of host factors upon microbiota, including growth-promoting glycoconjugates. We argue that host selection is likely to be a key mechanism in the stabilisation of the mutualism between a host and its microbiota.

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Abbreviations: IgA, Immunoglobulin A; ODE, ordinary differential equation

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Introduction

Many microbial species live on or are associated with epithelia of multicellular organisms. Examples range from plants and soil bacteria interactions in the rhizosphere where plant secretions affect the composition of bacterial communities [1,2], through the light organs of marine animals in which specialised symbiotic bacteria are cultivated by the host [3–5] to many surfaces of the mammalian body [6].

Every human is home to roughly 100 trillion bacterial cells, collectively called the microbiota. The majority of these cells reside in the human gastrointestinal tract and, in particular, in the large intestine [7]. Here, bacteria can have beneficial effects such as the digestion of complex carbohydrates, colonisation resistance against invading pathogens, maturation of the adaptive mucosal immune system and immune cells, and the production of secondary metabolites, including vitamins [8–10]. However, these activities are not performed by all species, and the species composition of the microbiota in a healthy human is clearly distinct from bacterial communities in other environments [11]. Moreover, various diseases correlate with disturbances in the species composition of the microbiota [6,10]. It is clear then that the gut community has the ability to both help and harm the host. Despite the potential for harmful effects of the gut microbiota, the major class of interaction with the host appears to be one of mutualism, whereby both sides benefit from the interaction. The evidence for host benefits comes both from our understanding of the metabolic services that the gut microbiota provides and studies of germ-free animal models [6,12–18].

There is a growing literature on the evolution of mutualisms among species, both theoretical and empirical, which emphasizes a number of key factors required for the evolutionary stability of mutualisms [19–22]. Most relevant for the gut microbiota is the issue of having multiple genetically different individuals on one side of the mutualism (microbes) involved in a single interaction with the other (host) [19,20,22]. On the side with multiple genotypes, this can lead to the loss of helpful mutualistic genotypes, whenever non-helpful genotypes are more competitive. How is such potential conflict among partner species resolved in other systems? Theory predicts a central role for partner choice: the selection of the best mutualistic partners by a focal species [22]. Moreover, partner choice is widespread in nature with evidence from many different systems [21,22] including leaf cutter ants and their fungus [23], legumes and rhizobia [24], and the mutualism between the bobtail squid and the luminescent bacterium *Vibrio fischeri* [25]. The predominance of partner choice mechanisms in other systems begs the question: What is the role of partner choice in the mammalian gut?

Author Summary

The cells of our bodies are greatly outnumbered by the bacteria that live on us and, in particular, in our gut. It is now clear that many gut bacteria are highly beneficial, protecting us from pathogens and helping us with digestion. But what prevents beneficial bacteria from going bad? Why don't bacteria evolve to shirk on the help that they provide and simply use us as a food source? Here we explore this problem using a computer model that reduces the problem to its key elements. We first illustrate the basic problem faced by a host: Whenever beneficial bacteria grow slowly, the host will lose them to fast-growing species that provide no benefit. We then propose a solution to the host's problem: The host can use secretions—nutrients and toxins—to control the bacteria that grow on the epithelial cell layer of the gut. In particular, our model predicts that the epithelial surface acts as a "selectivity amplifier". The host can thereby maintain beneficial bacteria with only small amounts of weakly selective secretions. Our model fits with a growing body of experimental data showing that hosts have diverse and important influences on their gut bacteria.

The sheer diversity of microbial species in the mammalian gut shows that hosts do not select for one or two partner species, as occurs in some mutualisms. In addition, there is a clear environmental effect on microbial species composition in the form of host nutrient intake [26,27]. Nevertheless, there are also a range of mechanisms by which vertebrate hosts affect their microbes more directly. In particular, the intestinal epithelium produces a wide range of secretions that help to maintain the barrier between the gut lumen and host tissues [9,28–31]. Central to this barrier is mucus secretion [32–35] that limits the direct access of bacteria to the epithelium [36]. The mucus becomes less dense, however, as it moves upwards away from the epithelium and bacteria grow in the upper layers that can feed on carbohydrates such as fucose, which the host adds to the mucus proteins [37–40].

The host also secretes a range of antimicrobials into the mucus, including defensins. Mucosal community composition has been studied in mice that lack an enzyme required for murine alpha-defensins but secrete human alpha-defensin [31]. The observed changes in community composition, in combination with other studies, led to the conclusion that defensins are essential regulators of intestinal microbial ecology (for a review, see [41]). More work is now required to understand the exact role of defensins as a selective agent of the microbiota. In particular, the defensins of the small intestine have been the primary focus of research, and the effect of defensins in the large intestine is less well understood. Moreover, studies have shown that production and activation of defensins can themselves be dependent on the resident microbiota [42,43], which opens the way for feedback loops between the host and its microbiota. In addition to defensins, the adaptive immune system also has the potential for selective effects. B-cell-derived immunoglobulin A (IgA) is considered the most likely host secretion to affect the localization, growth, and composition of the microbiota [29,44,45].

While it is clear that epithelial secretions can affect the microbiota, the primary role is often assumed to be as a simple barrier between the lumen and host tissues [46,47]. However, there is evidence that epithelial secretions differentially affect different strains and species. Sugars like fucose are more easily utilized by some microbial species than others [37,38,40], and defensins and IgA have biased effects on the microbiota [14,29,31,45]. Such findings suggest that host secretions might help to control the composition of the resident microbiota [41,48]. Indeed, it has even been suggested that control over a wide array of non-pathogenic microbes is the primary reason why adaptive immunity first evolved [49]. Despite this, we understand very little about how the host might in practice select for particular microbial strains or species.

Here we build a model to evaluate the potential of a host to select their microbiota. Ecologies like the mammalian gut are extremely complex dynamical systems and will require a central role for theoretical approaches if we are to dissect their complexity [50,51]. We have, therefore, developed a new model of host-associated microbial communities with the goal of bringing an evolutionary perspective to the study of host–microbiota interaction. Our model is relatively complex in that it includes realistic features such as mechanistic interactions among cells, spatial structure, and chemical gradients. However, it greatly simplifies the full complexity of the gut and is not intended as a complete description. We hope to show, nevertheless, that one can gain new understanding by the application of such simplifications to the problem of the host–microbiota interaction. In particular, our study reveals three key findings. First, we demonstrate the problem of multiple genotypes on one side of a mutualistic partnership, which renders the host–microbiota mutualism intrinsically fragile. Second, we show that a solution to this fragility is host selection: The epithelium–microbiota interface acts as a selectivity amplifier that can quickly shift the composition of the microbiota at the interface. Finally, we show that central to the selectivity is the provision of nutrients, and not just antimicrobial factors, by the host. Our results suggest a host's epithelium is a remarkable environment for partner choice, which is well suited to control bacterial community composition.

Results

We model a bacterial community containing two strains, which is growing on the host epithelium (Figure 1) where cells are represented by spheres that consume nutrients, grow, and divide (Materials and Methods, Table S1, Figure S1).

Host–Microbiota Mutualism Is Fragile

Our first goal is to evaluate the potential effects of differences in growth rates between strains under the simplest of conditions, and then build in increasing complexity in order to understand the key factors at play. We denote two bacterial competitors A and B, where B divides more rapidly than A (Figure 1B). These two strains can either represent two members of one species that differ only in their interaction with the host or two different species that differ in other ways. As such, the model can be viewed from either an evolutionary (genotypes within a species) or ecological (species within a community) perspective. We return to the differences between these two scenarios in the Discussion. While we only model two strains, the model also approximates more diverse communities in which there is selection for a set of beneficial ecotypes where each "strain" would then represent multiple strains with similar phenotypes.

These simple models show the potential power of competition in a host-associated microbial community. Figure 1C shows the increase in frequency of the fittest species over time in the epithelial community. Here and in the majority of subsequent figures, we show time as an axis. One reason we do this is because it is impractical to run all simulations until the final frequencies of the two strains have been established, especially for very small

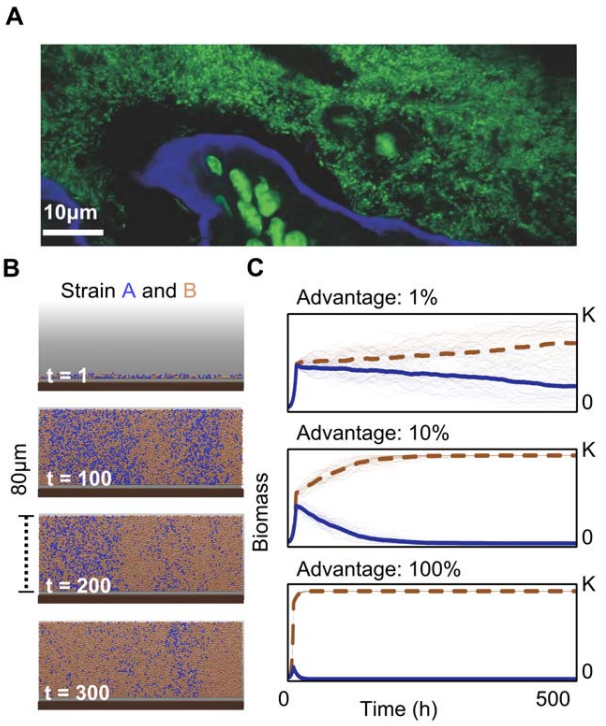

Figure 1. Microscopic image and simulations of microbial growth near a host epithelium. (A) Confocal fluorescence image of bacteria growing in the lumen on top of host epithelial cells. Sample taken from the cecum of a laboratory mouse, where there has been no intentional manipulation of the animal's microbiotia. Epithelial and bacterial cells in green (DNA stained with Sytox green), and the epithelial border brush in blue (actin stained with Alexa-647-phalloidin) from [83]. (B) Simulation of bacterial growth on host epithelium; brown bacterial cells (strain *B*) have a 1% growth rate advantage over blue bacterial cells (strain *A*). Even with a modest growth rate advantage, strain *B* succeeds as strain *A* is slowly washed out. (C) Thirty independent simulations of bacterial competition. Development of biomass of strain *B* (brown dashed) and *A* (blue) with growth rate advantages for strain *B* of 1%, 10%, and 100% and environmental capacity K. The thick lines are mean values.
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differences in fitness. Nevertheless, we expect in the majority of cases that one strain will ultimately dominate the system (see following section and Text S1 for exceptions). Indeed, even for a modest difference in the growth rate among strains (e.g., 10%), a faster growing strain rapidly reduces the slower growing strain to negligible frequency in tens of generations (Figure 1C). This corresponds to a few days for species like *E. coli* in a mammalian gut [52]. The constant removal of cells leads to thinning out and eventual eradication of the slower growing strain *A* near the epithelium (Figure 1B,C). For larger difference in growth rate, such as *B* doubling at twice the rate of *A*, the eradication of *A* occurs in a few generations.

This demonstrates the fundamental problem faced by a host when having multiple possible genotypes competing for a niche where a mutualistic species could exist. Whenever the most beneficial bacteria do not grow the fastest, competition between bacterial genotypes will lead to the loss of mutualistic strains within the host and thus a suboptimal microbiota composition (Figure 2). But is it possible that mutualistic species are, without exception, intrinsically faster growing than non-mutualist species? If anything, the reverse is expected. Recent phylogenetic work shows that species from healthy guts tend to cluster with species from complex and relatively slow-growing communities [53]. By contrast, bacteria of infants and unhealthy guts tend to cluster with bacteria from fast-growing pioneer communities. In an entirely neutral host that does not exert any control over the bacterial composition, therefore, our model predicts that the mutualism between bacteria and a host is intrinsically fragile.

Epithelial Selection Dominates Lumen Selection

So far, there is little spatial structure in our model, and we confirmed that our first results correspond to a well-mixed (no spatial resolution) ordinary differential equation model of evolutionary competition (Text S1, Figure S2). We next extend the simulations to introduce more realism and calculate nutrient levels as a function of space and time. As cells divide, they use up nutrients such that nutrient concentration is depressed as one moves away from the nutrient source and into a group of dividing cells. These solute gradients are known to be important in natural bacterial groups and can have strong influences on community structure and composition [54–56]. In our case, there is the potential for two solute gradients, one from the lumen direction and one from the host epithelium direction. Our question is then: How do selective compounds from the epithelium and from the lumen influence the composition of this bacterial community?

Compared to the well-mixed case, the ability of nutrients to select for one strain over the other is reduced in the presence of solute gradients because not all cells have access to nutrients. With less reproduction, natural selection is less powerful. However, more striking is that lumen nutrients exert a much weaker selective effect than epithelial nutrients. This suggests a bias that may empower the host to affect the microbial communities growing on the epithelial surface (Figure 3). What causes this difference? When the epithelium secretes nutrients, growth occurs at the base of the bacterial colony, which can affect the whole bacterial community. By contrast, lumen selection from the opposite direction preferentially affects cells that are about to be sloughed off, which limits the effect of lumen nutrients on cells at the base of the bacterial community.

The inhibition of lumen selection only occurs beyond a certain thickness of the bacterial community (Text S1, Figure S2). While it is difficult to measure the thickness of these bacterial communities in vivo, the range of thicknesses used in our model are consistent with the outer mucus layer of mice and rats [32]. A corollary of these results is that selection from the lumen should be weakened by growth near the epithelium. Hence, we further show that the addition of non-selective nutrients at the epithelium strongly inhibits lumen selection (Figure S3B). By contrast, additional non-selective lumen nutrients do not affect the ability of epithelial nutrients to select for one strain over the other.

Our model predicts that the physical layout of the gut epithelium environment allows host secretions to have disproportionately strong effects. We next test this by pitting the two sources of nutrients against one another. We assume that epithelial nutrients select for strain *A*, whereas lumen nutrients select for strain *B*, simulating a scenario in which the slow growing strain *A* would be lost without host selection. We present a conservative case in which epithelial nutrients are both less abundant and less selective. Specifically, lumen nutrient concentrations are five times higher than epithelial nutrients and the growth rate advantage of strain *B* on lumen nutrients (100%) is always higher than or equal to the (varied) growth rate advantage of strain *A* on epithelial nutrients (Figure 4A).

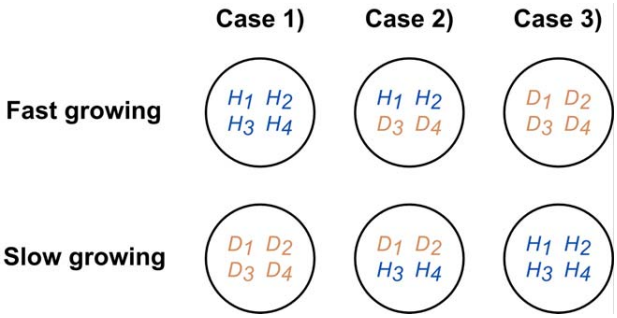

Figure 2. Cartoon to illustrate the potential problem faced by a host. Three scenarios are shown for four helpful strains (*H*) and four detrimental strains (*D*) that occupy four different niches, 1 to 4. Two extreme cases exist: beneficial strains grow faster in all niches (case 1) or all detrimental stains grow faster in all niches (case 3). In the first case, no partner choice is required, as natural selection favours the beneficial strain throughout all niches. However, any deviation (case 2 or 3) from this means that the host will experience a sub-optimal microbiota.
doi:10.1371/journal.pbio.1001424.g002

Initially, strain *B* outgrows strain *A* as the former's overall growth rate advantage from the nutrients is much greater than that of strain *A*. However, the advantage of strain *B* diminishes as the microbial community grows and the effects of lumen nutrients and epithelial nutrients separate into distinct regions. This allows strain *A* to establish itself at the epithelial surface, and for all but the weakest selection by the host, strain *B* is eliminated eventually. In fact, in this example, the host need only provide a 5% growth rate advantage to strain *A* to counter the 100% growth rate advantage and five times higher concentrations that lumen nutrients provide to strain *B*. In summary, we find that a fast growing strain, which would rapidly replace slow growing strains in a well-mixed environment, can be eliminated by moderate counter-selection at the gut epithelium. This process is also effective when strain *A* is initially rare (Figure S4). Host selection at the epithelium, therefore, can effectively operate on an initially rare strain or species that is a minor member of a diverse community.

Nutrients Are Often Critical to Host Epithelial Selection

We next tested the effects of epithelial selection using antimicrobials that tend to harm strain *B* more than strain *A*. In our model, selection with antimicrobials is slower than with nutrients, because the antimicrobials kill both strains, which reduces the rate at which one strain outgrows the other. Antimicrobials could, in principle, select more quickly than nutrients if they could instantly kill only one of the two strains. In the absence of such extreme selectivity, however, nutrient selection is more powerful. Indeed, for a wide range of conditions, we find that it is critical that the host also supplies nutrients (Figure 4B). These do not need to be selective if selective antimicrobials are secreted. However, nutrients are required because the selective effects of antimicrobials will not permeate up through the community unless there is net positive growth at the epithelial surface. With antimicrobials alone, cell death can easily outweigh the birth of new cells at the epithelial surface because lumen nutrients are at their lowest concentrations. This means that although the host kills more cells of strain *B* than of strain *A* (depending on the specificity of the antimicrobial), if growth is limited by nutrients at the epithelium, no net positive growth of strain *A* will occur either. For this reason, providing nutrients at the epithelial surface greatly widens the range of

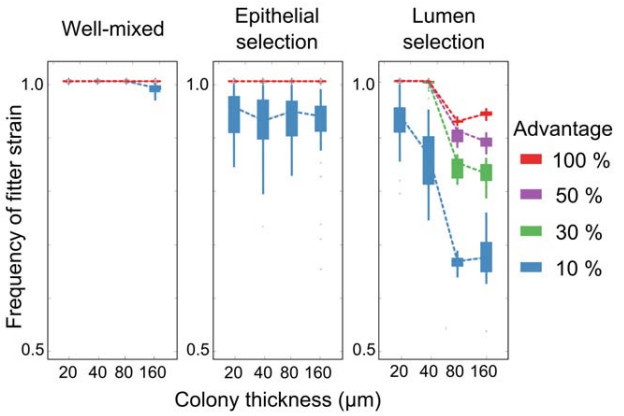

Figure 3. Epithelial nutrients have more effect on a bacterial community than lumen nutrients. Box plots show the final frequency of a faster growing strain after 12 d as a function of microbial community thickness, where the growth rate advantages of the fitter strain range from 10% to 100%. Well-mixed: No gradients of nutrients exist (Figure 1B,C). Epithelial selection: Nutrients exclusively diffuse into the colony from the host epithelium. Lumen selection: Nutrients exclusively diffuse into the colony from the lumen. Dashed lines connect mean values of 30 independent simulations. The total nutrient influx into the system from the host or the lumen is kept identical. Results agree with a steady-state solution of a simplified ODE model (Figure S2).
doi:10.1371/journal.pbio.1001424.g003

conditions under which antimicrobials can be used as a selectivity mechanism by allowing sufficient growth in this critical region.

One challenging case for the host is when lumen nutrient levels are so great as to remove all nutrient gradients in the bacterial community and hence nutrients are available at high concentrations throughout the colony. However, even here, the host can use the epithelium as a selectivity amplifier (Figures 5, S5). Selectivity amplification occurs whenever the host can maintain a thin region next to the epithelium that favours strain *A* over strain *B* and allows for net positive growth. With this, strain *A* will eventually take over the community even though it is counter-selected in the vast majority of the community (Figure 5). As a control, we show in Figure 5 how the same amount of solutes evenly distributed throughout the system would strongly select against *A*, which contrasts with the selectivity amplification seen when solute gradients are present. Finally, our results are robust to fluctuations in lumen nutrient concentrations, which are inevitable in organisms that have discontinuous food intake. As our model predicts, the effects of epithelial secretions are strongest during starvation periods, because lumen nutrient concentrations are highest after feeding [37]. However, implementing a feast–famine cycle that increases the variance in lumen nutrient concentration (but does not affect the mean) suggests that the net effect of these cycles is modest (Figure S6).

Discussion

The gut is a competitive environment where the potential for high growth rates and population turnover means that slower-growing bacterial strains can be rapidly lost. This presents a problem for hosts. Natural selection of microbial phenotypes based upon intrinsic growth rate will disadvantage any microbes that grow more slowly (Figures 1, 2). Our model predicts that a host can compensate for this effect using epithelial secretions that promote relatively slow-growing strains. Importantly, these effects

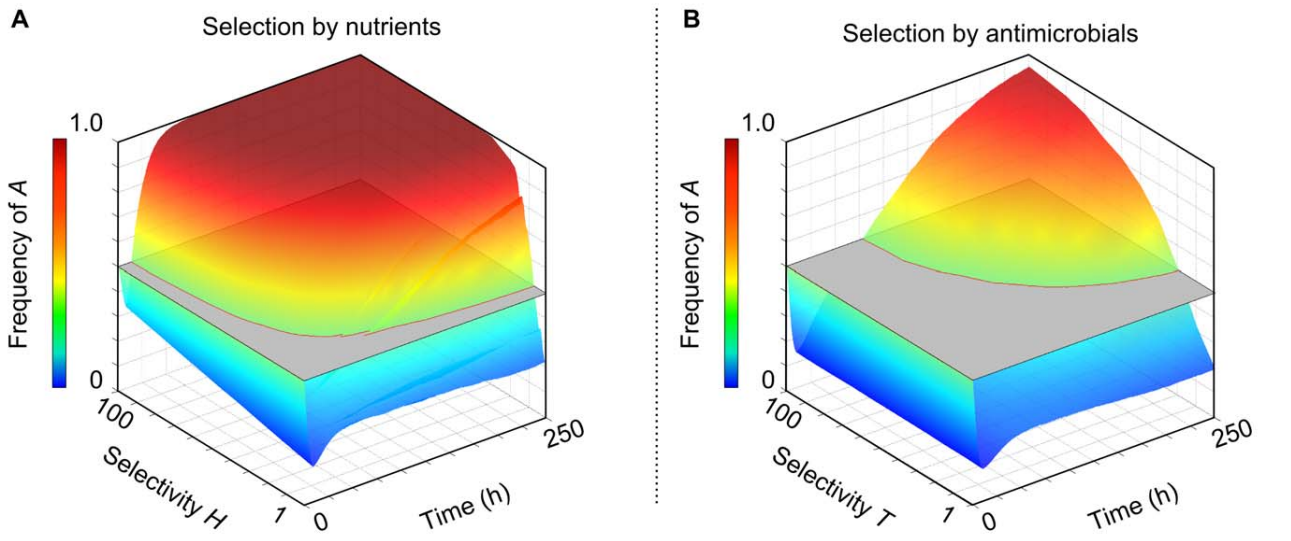

Figure 4. Selectivity amplification by the host epithelium. Weak epithelial selection dominates strong lumen selection. Strain *B* has a 100% growth rate advantage on nutrients from the lumen, and lumen nutrients are five times the concentration as epithelial nutrients. Grey planes mark the starting frequency of the two strains (0.5). (A) Host nutrients provide growth rate advantages to strain *A* ranging from 1% to 100%. (B) The host secretes antimicrobials that preferentially kill strain *B*; susceptibility advantages for strain *A* range from 1% to 100%. Host-secreted nutrients are also provided that neutral. In (A) and (B) strain *A* outcompetes strain *B* for all but the smallest selective advantages.
doi:10.1371/journal.pbio.1001424.g004

do not require a highly specific selection mechanism akin to the full force of adaptive immunity. In our model, moderate selectivities that allow poorly growing strains to grow 5% to 10% faster at the epithelial surface are sufficient to reverse their fate.

Epithelial selection may occur either through growth-promoting secretions or toxic growth inhibitors, but we find that growth promotion is often critical because selectivity amplification requires net growth of the microbial community near the epithelial surface. In this context, it is interesting that host epithelial secretions include growth promoters, particularly mucosal glycans [57,58], in addition to the growth inhibitors of the immune system.

Positive growth at the epithelium surface is important because it causes a flow of microbial cells towards the lumen that limits the effects of lumen nutrients on the community. Cells nearest the lumen are least likely to persist due to detachment and sloughing deeper in the lumen. In our model, this motion is driven by pushing and shoving of dividing bacterial cells. In the mammalian gut, the flow towards the surface is likely to be further promoted by the constant release of mucin polymers from the epithelial surface [57,59]. Furthermore, the diffusion of IgA—a key secretion known to influence the microbiota—is inhibited by mucins [34]. Our work suggests that this diffusion limitation will maximize not only the residence time of IgA in the gut but particularly the residence time close to the epithelium, where IgA will have an amplified effect.

Our model requires that a host has mechanisms to differentially affect the net growth rate of different bacterial strains or species. Are such effects always possible, particularly in the face of bacterial coevolution to evade the negative effects of host selection? The greatest challenge for host selection will occur when the strains involved are variants of a single species that differ only in their cooperativity towards the host (as opposed to different species that differ in many ways). However, even here, host selection is possible if the host can select directly on the beneficial phenotype in the bacteria [60,61]. This appears to occur in the mutualism between bioluminescent *Vibrio fischeri* bacteria and the bobtail squid. It is thought that the squid creates an oxidizing environment in the light organ that selects for cells using the luminescence reaction because this reaction uses up oxygen [4].

We believe comparable mechanisms to those seen in the bobtail squid may exist in the gut. Mammalian cells produce glycoconjugates of a remarkable structural complexity and diversity, which are known to favour, or disfavour, the attachment and growth of different microbial species [62]. These compounds may represent an evolutionarily stable way to select for bacteria, like *Bacteroides thetaiotaomicron*, which are carbohydrate specialists that convert complex carbohydrates for the host: *B. thetaiotaomicron* has over five times the number of glycoside hydrolases as species like *Salmonella enterica* or *Shigella flexneri* [63]. Indeed, human milk contains polysaccharides that cannot be digested by the infant, suggesting that mothers may also be exercising this simple but effective form of selection [64]. But is host secretion of complex carbohydrates vulnerable to exploitation by a variant that receives benefits but does not provide any help to the host? The use of complex carbohydrates as a selective mechanism is likely to greatly constrain the evolutionary options for bacterial species by demanding that bacteria use the glycoside hydrolases that also help the host with digestion. Of course, these species might still attempt to invade the epithelial layer. Our model is not intended to capture direct attacks by pathogens, but the detection of tissue damage is a relatively simple problem for a host as compared to selecting among more or less metabolically useful symbionts. And we know that hosts possess mechanisms to counter direct attacks, such as the inflammation response.

However effective, host selection will not preclude bacterial coevolution in the gut. Indeed, long-term bacterial evolution in the

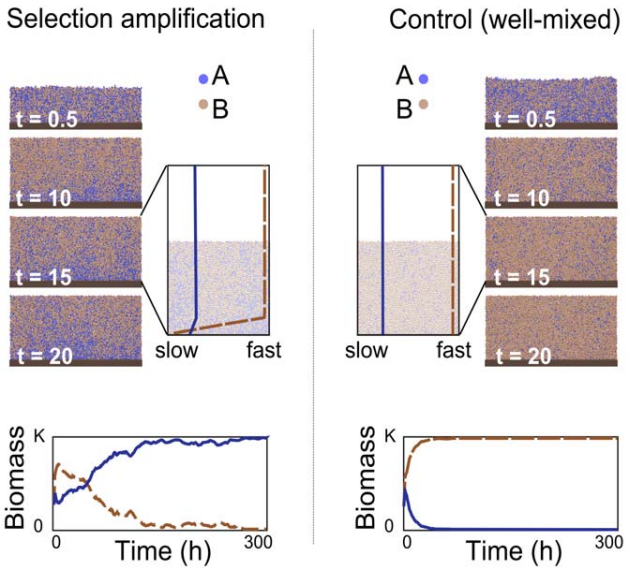

Figure 5. The host need only influence a thin layer of a microbial community to exert control. Selection amplification: To illustrate, we apply constant distributions to all solutes in the simulation (no gradient for lumen nutrients, steep gradient for host secretions) to create a thin layer in which the strain *A* (blue) outgrows strain *B* (brown). The snapshots show the progression of a representative simulation with the expanded snapshot showing the growth rates of the two strains throughout the community. Strain *A* only grows better very close to the surface of the epithelium. Well-mixed: Control simulation with identical total amounts of solutes but without spatial differences in solute concentrations and growth rates of the two strains. In such an environment, strain *A* is out-competed by strain *B*; environmental capacity K.
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gut may allow mutualists to achieve gains in competitiveness both in the presence and absence of host selection. Consistent with such adaptation, *B. thetaiotaomicron* can induce carbohydrate secretion by the host [37]. Coevolution also brings the potential for arms-races with pathogens that adapt and use host-provided nutrients or evade host-secreted antimicrobials. For example, l-Fucose utilization provides *Campylobacter jejuni* with a competitive advantage [65]. More generally, the possibility of bacterial counter-adaptation to host selection mechanisms leaves interesting questions to be answered. These include the issue of how antimicrobial secretions can remain selective when bacteria are known to rapidly develop resistances to many antimicrobials. In addition, the fitness of a bacterial cell will be influenced by cells that possess different secretion, motility, or adhesion phenotypes [66]. We do not yet understand how the potential for complex social interactions among cells will influence host selection. In sum, hosts may be forced to modify or increase their exact selection criteria, either during the life of the host via adaptive immunity or over evolutionary time. Interestingly, recent work has shown how the use of multiple selective mechanisms can allow a host to stay ahead in evolutionary arms races with parasites [67].

Can multiple strains coexist within the epithelial community? We do not find evidence that coexistence is a stable state in our model in the sense that multiple strains will persist indefinitely. This can be seen in Figure 5, where despite lumen selection being much stronger than epithelial selection, the lumen-favoured strain does not persist. The reason for this is that epithelial selection generates a ratchet-like effect whereby the epithelial-favoured population expands and gradually pushes any other strains up and out of the community. If host selection is weak and/or growth in the community is slow, however, favoured and disfavoured strains may both persist for long periods. Moreover, a number of other processes in the gut will counter any winnowing by host selection and help to maintain bacterial diversity. This includes the existence of multiple niches, both at different positions along the epithelial surface but also within the lumen proper. Community diversity will be further influenced by the influx rates of different species [68] and diet [26,27].

Host epithelial selection is not the only process that influences the microbial species composition of the gut. Nevertheless, our model predicts that the control of epithelium-associated microbial communities is much easier for a host than expected from unstructured environments. Selection of particular microbial species and strains at this position is likely to pay dividends both metabolically but also in terms of the competitive exclusion of undesirable species. Furthermore, epithelium-associated communities are relatively unlikely to be washed out and may represent a stable source community for the rest of the gut. We conclude that host influence on the composition of microbiota is both likely and likely to be powerful.

Materials and Methods

The study centres upon an individual-based simulation framework that captures bacterial growth and the concentration gradients of solutes, such as nutrients, that originate from bacterial activity while they are growing near to an epithelial host layer. While the model can capture a wide range of conditions, our analysis focuses upon a relatively nutrient-rich environment where cells grow rapidly (Table S1) and slough off at a fixed height above the epithelial surface, which is intended to reflect microbial growth in an animal intestine [32,52]. In the mammalian gut, these cells will typically grow in the loose upper mucus layer of the epithelial surface, which continually detaches and sloughs off into the lumen [32,36,57]. We do not explicitly model the effects of these mucin polymers but implicitly include the protection from sloughing they provide for adherent bacteria in the loose layer. Note that we are only explicitly modelling the bacteria at the surface of the epithelium and not those in the lumen. Of course, selection at the epithelial surface will influence the lumen to some degree (discussion), but we do not explicitly model this process.

The model is an extension of an established framework that has been developed and tested over the last 15 years to understand and predict the behaviour of bacterial communities growing on inert surfaces [54,69–73]. While originally developed for problems in bioengineering, it has most recently been applied to understand the evolution and ecology of microbial groups [55,66,73–75]. Subsequent empirical validation of these models has demonstrated the ability of the framework to both describe bacterial communities and identify new biological mechanisms [76,77]. The model assumptions, justifications, and implementation are extensively discussed elsewhere [69,70,72,78]. In brief, bacterial cells are modelled as solid spheres that metabolise nutrients in a continuous concentration field. At each iteration, the concentration field is updated solving the two- or three-dimensional reaction-diffusion equations using multigrid solvers. This takes into account local sinks, such as a bacterium utilising the solutes around it as a nutrient source or local sources, such as secretions from a cell. Cells increase in diameter and eventually divide pushing aside neighbouring cells.

The model focuses upon the resident bacterial communities that grow in the loose upper mucus layer at the interface of the lumen and epithelium, which are most likely to be affected by host selection [79]. We inoculate our simulations with a total of 250 cells in varying frequencies. This is a simplification as initial

assembly of the microbiota has been shown to be more complex and may depend on interbacterial cross-talk as well as other yet unknown factors [80]. Bacteria reside above a layer of host cells that secrete solutes at varying rates. We assume that this epithelial layer and the dense mucus layer immediately above it is impenetrable to the bacterial cells [32,81]. This is supported by data on the healthy gut with a few notable exceptions, such as segmented filamentous bacteria in mice that live in the dense mucus layer [41]. Accordingly, we do not consider host responses to invasion of a pathogen or breach of the mucus layer, such as inflammation (but see Discussion). The bacteria grow and divide utilising nutrients diffusing in from the lumen or the epithelium. At a certain height above the epithelium, cells are sloughed and excluded from the simulation. Bacteria utilise nutrients (N) and convert them into biomass at the rate μ following Monod-kinetics:

$$\mu = \mu_{max} \frac{N}{N + K_s},$$

where K_s is the Monod constant. Competing strains in our simulations differ in their maximum growth rates, μ_{max}. Bacteria may switch between different substrates, ensuring that the maximum growth rate cannot be exceeded, where switching is based upon a recent analysis of optimal foraging in microbes [82]. Death of cells through antimicrobials is modelled using a similar equation as for growth:

$$p = \frac{T}{T + S},$$

where p is the probability of death for a cell, T is the local concentration of the antimicrobial, and S the concentration at which cell death within 1 h occurs with a probability of 50%. Different strains may have different susceptibilities to the antimicrobial and hence different probabilities for cell death at a given concentration. Most of our understanding of host-secreted antimicrobials stems from secretions of the epithelium in the small intestine, whereas secretions in the larger intestine are less well understood [41,48].

Supporting Information

Figure S1 Diagram of the simulation setup. Cells live and divide on an impenetrable host epithelium. Growth-promoting nutrients can diffuse into the bacterial colony from the lumen (top) and/or the epithelium (bottom) where they are utilized by the cells. In some simulations the epithelium also releases antimicrobials that kill cells. The direction of fluxes is indicated by arrows (nutrients, green; antimicrobials, red). Periodic boundaries at the sides simulate continuous space. Cells moving beyond the maximum thickness are removed simulating sloughing (dashed line, the location is a parameter that we vary).
(TIFF)

Figure S2 Final frequencies of faster growing strain B in a simplified ordinary differential equation model. A minimum final frequency of the faster growing strain is found for intermediate microbial community thickness; the exact location depends on the growth functions of the two strains (see Text S1).
(TIFF)

Figure S3 The effect of non-selective nutrients from one direction on selectivity from the other direction. We show the biomass development over time beginning with cells at low densities (125 cells each). (A) Host-secreted nutrients provide a growth rate advantage and neutral nutrients diffuse into the biofilm from the lumen. (B) Lumen nutrients provide a growth rate advantage and the host secretes neutral nutrients. Points of sloughing are 20, 40, and 80 μm. For 80 μm, lumen selection is strongly impeded by the presence of neutral host nutrients, whereas host selection is unaffected by additional neutral lumen nutrients (initially the favoured species outgrows the other but upon reaching the capacity will be sloughed off more frequently, leading to a decrease in frequency compared with the maximum at ~10 h).
(TIFF)

Figure S4 Selection amplification of an initially rare strain by the host epithelium. Weak epithelial selection dominates strong lumen selection. Strain B has a 100% growth rate advantage on nutrients from the lumen, and lumen nutrients are five times the concentration as epithelial nutrients. Host nutrients provide growth rate advantages to an initially rare strain A (initial frequency 0.1, grey plane) ranging from 1% to 100%.
(TIFF)

Figure S5 Selection with antimicrobials. Selection via host antimicrobial secretion in the absence of host nutrient secretion is possible only when lumen nutrients are available throughout the bacterial colony. The host can select for slow growing strain A despite antimicrobials being available at relevant concentrations only in a fraction of the overall bacterial colony (near the epithelium) when selectivities of antimicrobials in favour of the strain A ($S_B < S_A$) are sufficiently high. In the majority of the bacterial colony, cells of strain B have a net growth rate advantage due to the high concentration of lumen nutrients, which favour B. Capacity K, maximum biomass.
(TIFF)

Figure S6 Fluctuations in lumen nutrient concentrations do not affect predictions. Each figure shows the biomass development of the two strains in 30 independent simulations (brown, strain B; blue, strain A) with discontinuously available nutrients in the lumen. Feast-famine periods last 8 h each. For comparison, thick black lines show the mean biomass from 30 simulations under identical selection strengths but with continuously available nutrients for stain B (dashed) and strain A (solid). Host nutrients provide varying growth rate advantages to strain A as indicated in the figure. The mean nutrient concentration in the lumen is five times higher than nutrients from the host, and strain B has a 100% growth rate advantage over A on these nutrients. Mean nutrient concentrations in the continuous and discontinuous case are identical. Capacity K, maximum biomass.
(TIFF)

Table S1 Simulation parameters. L, length; M mass; T, time.
(DOC)

Text S1 Simplified ordinary differential equation model. The model shows the occurrence of a minimum influence of the lumen on the outcome of competition in the individual-based simulations for intermediate bacterial colony thicknesses.
(PDF)

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Author Contributions

The author(s) have made the following declarations about their contributions: Conceived and designed the experiments: JS KRF. Performed the experiments: JS. Analyzed the data: JS KRF. Wrote the paper: JS KRF.

References

1. Sanon A, Andrianjaka ZN, Prin Y, Bally R, Thioulouse J, et al. (2009) Rhizosphere microbiota interfers with plant-plant interactions. Plant Soil 321: 259–278.
2. Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. Nature 427: 731–733.
3. Ruby EG, McFall-Ngai MJ (1999) Oxygen-utilizing reactions and symbiotic colonization of the squid light organ by *Vibrio fischeri*. Trends Microbiol 7: 414–420.
4. Visick KL, McFall-Ngai MJ (2000) An exclusive contract: specificity in the *Vibrio fischeri-Euprymna scolopes* partnership. J Bacteriol 182: 1779.
5. Graf J (1998) Host-derived amino acids support the proliferation of symbiotic bacteria. Proc Natl Acad Sci U S A 95: 1818–1822.
6. Dethlefsen L, McFall-Ngai M, Relman DA (2007) An ecological and evolutionary perspective on human-microbe mutualism and disease. Nature 449: 811–818.
7. Ley RE, Peterson DA, Gordon JI (2006) Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 124: 837–848.
8. Holzapfel WH, Haberer P, Snel J, Schillinger U, Huis in't Veld JH (1998) Overview of gut flora and probiotics. Int J Food Microbiol 41: 85–101.
9. Hooper LV, Macpherson AJ (2010) Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol 10: 159–169.
10. Sekirov I, Russell SL, Antunes LCM, Finlay BB (2010) Gut microbiota in health and disease. Physiol Rev 90: 859–904.
11. Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI (2008) Worlds within worlds: evolution of the vertebrate gut microbiota. Nat Rev Microbiol 6: 776–788.
12. Bäckhed F, Ley RE, Sonnenburg JL, Peterson D a, Gordon JI (2005) Host-bacterial mutualism in the human intestine. Science 307: 1915–1920.
13. Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, et al. (2004) The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 101: 15718–15723.
14. Cash HL, Whitham CV, Behrendt CL, Hooper LV (2006) Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science 313: 1126–1130.
15. Kelly D, Campbell JI, King TP, Grant G, Jansson EA, et al. (2004) Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol 5: 104–112.
16. Martin F-PJ, Dumas M-E, Wang Y, Legido-Quigley C, Yap IKS, et al. (2007) A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. Mol Syst Biol 3: 112.
17. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 118: 229–241.
18. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL (2005) An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 122: 107–118.
19. Frank SA (1994) Genetics of mutualism: the evolution of altruism between species. J Theor Biol 170: 393–400.
20. West SA, Kiers ET, Simms EL, Denison RF (2002) Sanctions and mutualism stability: why do rhizobia fix nitrogen? Proc Biol Sci 269: 685–694.
21. Sachs JL, Mueller UG, Wilcox TP, Bull JJ (2004) The evolution of cooperation. Q Rev Biol 79: 135–160.
22. Foster KR, Wenseleers T (2006) A general model for the evolution of mutualisms. J Evol Biol 19: 1283–1293.
23. Poulsen M, Boomsma JJ (2005) Mutualistic fungi control crop diversity in fungus-growing ants. Science 307: 741–744.
24. Kiers ET, Rousseau RA, West SA, Denison RF (2003) Host sanctions and the legume-rhizobium mutualism. Nature 425: 78–81.
25. Nyholm SV, McFall-Ngai MJ (2004) The winnowing: establishing the squid-vibrio symbiosis. Nat Rev Microbiol 2: 632–642.
26. Muegge BD, Kuczynski J, Knights D, Clemente JC, González A, et al. (2011) Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science 332: 970–974.
27. Faith JJ, McNulty NP, Rey FE, Gordon JI (2011) Predicting a human gut microbiota's response to diet in gnotobiotic mice. Science 333: 101–104.
28. Salzman N (2010) Paneth cell defensins and the regulation of the microbiome: Détente at mucosal surfaces. Gut Microbes 1: 401.
29. Peterson DA, McNulty NP, Guruge JL, Gordon JI, McNutty NP (2007) IgA response to symbiotic bacteria as a mediator of gut homeostasis. Cell Host Microbe 2: 328–339.
30. Vaishnava S, Yamamoto M, Severson KM, Ruhn KA, Yu X, et al. (2011) The antibacterial lectin RegIII promotes the spatial segregation of microbiota and host in the intestine. Science 334: 255–258.
31. Salzman NH, Hung K, Haribhai D, Chu H, Karlsson-Sjöberg J, et al. (2010) Enteric defensins are essential regulators of intestinal microbial ecology. Nat Immunol 11: 76–83.
32. Atuma C, Strugala V, Allen A, Holm L (2001) The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. Am J Physiol-Gastr L 280: G922–G929.
33. Brugman S, Nieuwenhuis EES (2010) Mucosal control of the intestinal microbial community. J Mol Med 88: 881–888.
34. Olmsted SS, Padgett JL, Yudin AI, Whaley KJ, Moench TR, et al. (2001) Diffusion of macromolecules and virus-like particles in human cervical mucus. Biophys J 81: 1930–1937.
35. Cone RA (2009) Barrier properties of mucus. Adv Drug Deliver Rev 61: 75–85.
36. Johansson MEV, Holmén Larsson JM, Hansson GC (2010) Microbes and Health Sackler Colloquium: the two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions. Proc Natl Acad Sci U S A 108: 4659–4665.
37. Hooper LV, Xu J, Falk PG, Midtvedt T, Gordon JI (1999) A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. Proc Natl Acad Sci U S A 96: 9833–9838.
38. Fabich AJ, Jones SA, Chowdhury FZ, Cernosek A, Anderson A, et al. (2008) Comparison of carbon nutrition for pathogenic and commensal *Escherichia coli* strains in the mouse intestine. Infect Immun 76: 1143–1152.
39. Hooper LV (2001) Commensal host-bacterial relationships in the gut. Science 292: 1115–1118.
40. Sonnenburg JL, Xu J, Leip DD, Chen C-H, Westover BP, et al. (2005) Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science 307: 1955–1959.
41. Bevins CL, Salzman NH (2011) The potter's wheel: the host's role in sculpting its microbiota. Cell Mol Life Sci 68: 3675–3685.
42. Petnicki-Ocwieja T, Hrncir T, Liu Y-J, Biswas A, Hudcovic T, et al. (2009) Nod2 is required for the regulation of commensal microbiota in the intestine. Proc Natl Acad Sci U S A 106: 15813–15818.
43. Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, et al. (2003) Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 278: 8869–8872.
44. Macpherson AJ, Uhr T (2004) Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science 303: 1662–1665.
45. Kawamoto S, Tran TH, Maruya M, Suzuki K, Doi Y, et al. (2012) The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. Science 336: 485–489.
46. Wehkamp J, Harder J, Weichenthal M, Schwab M, Schäffeler E, et al. (2004) NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. Gut 53: 1658–1664.
47. Wehkamp J, Salzman NH, Porter E, Nuding S, Weichenthal M, et al. (2005) Reduced Paneth cell alpha-defensins in ileal Crohn's disease. Proc Natl Acad Sci U S A 102: 18129–18134.
48. Van den Abbeele P, Van de Wiele T, Verstraete W, Possemiers S (2011) The host selects mucosal and luminal associations of coevolved gut microorganisms: A novel concept. FEMS Microbiol Rev 35: 681–704.
49. McFall-Ngai M (2007) Adaptive immunity: care for the community. Nature 445: 153.
50. dos Santos VM, Müller M, de Vos WM (2010) Systems biology of the gut: the interplay of food, microbiota and host at the mucosal interface. Curr Opin Biotechnol 21: 539–550.
51. Gudelj I, Weitz JS, Ferenci T, Claire Horner-Devine M, Marx CJ, et al. (2010) An integrative approach to understanding microbial diversity: from intracellular mechanisms to community structure. Ecol Lett 13: 1073–1084.
52. Rang CU, Licht TR, Midtvedt T, Conway PL, Chao L, et al. (1999) Estimation of growth rates of *Escherichia coli* BJ4 in streptomycin-treated and previously germfree mice by in situ rRNA hybridization. Clin Diagn Lab Immunol 6: 434–436.
53. Lozupone C, Faust K, Raes J, Faith JJ, Frank DN, et al. (2012) Identifying genomic and metabolic features that can underlie early successional and opportunistic lifestyles of human gut symbionts. Genome Res 22: 1974–1984.
54. Kreft J-U (2004) Biofilms promote altruism. Microbiology 150: 2751–2760.
55. Nadell CD, Foster KR, Xavier JB (2010) Emergence of spatial structure in cell groups and the evolution of cooperation. PLoS Comp Biol 6: e1000716. doi:10.1371/journal.pcbi.1000716
56. Kreft J-U, Bonhoeffer S (2005) The evolution of groups of cooperating bacteria and the growth rate versus yield trade-off. Microbiology 151: 637–641.
57. Varum FJO, Veiga F, Sousa JS, Basit AW (2012) Mucus thickness in the gastrointestinal tract of laboratory animals. J Pharm Pharmacol 64: 218–227.
58. Matsuo K, Ota H, Akamatsu T, Sugiyama A (1997) Histochemistry of the surface mucous gel layer of the human colon. Gut 40: 782.
59. McGuckin MA, Lindén SK, Sutton P, Florin TH (2011) Mucin dynamics and enteric pathogens. Nat Rev Microbiol 9: 265–278.
60. Foster KR (2004) Diminishing returns in social evolution: the not-so-tragic commons. J Evol Biol 17: 1058–1072.
61. Foster KR (2011) The sociobiology of molecular systems. Nat Rev Genet 12: 193–203.
62. Hooper LV, Gordon JI (2001) Glycans as legislators of host-microbial interactions: spanning the spectrum from symbiosis to pathogenicity. Glycobiology 11: 1R–10R.
63. Hooper LV (2009) Do symbiotic bacteria subvert host immunity? Nat Rev Microbiol 7: 367–374.
64. LoCascio RG, Ninonuevo MR, Freeman SL, Sela DA, Grimm R, et al. (2007) Glycoprofiling of bifidobacterial consumption of human milk oligosaccharides demonstrates strain specific, preferential consumption of small chain glycans secreted in early human lactation. J Agric Food Chem 55: 8914–8919.

65. Stahl M, Friis LM, Nothaft H, Liu X, Li J, et al. (2011) L-fucose utilization provides *Campylobacter jejuni* with a competitive advantage. Proc Natl Acad Sci U S A 108: 7194–7199.
66. Nadell CD, Xavier JB, Foster KR (2009) The sociobiology of biofilms. FEMS Microbiol Rev 33: 206–224.
67. Gilman RT, Nuismer SL, Jhwueng D-C (2012) Coevolution in multidimensional trait space favours escape from parasites and pathogens. Nature 483: 328–330.
68. Bucci V, Bradde S, Biroli G, Xavier JB (2012) Social interaction, noise and antibiotic-mediated switches in the intestinal microbiota. PLoS Comp Biol 8: e1002497. doi:10.1371/journal.pcbi.1002497
69. Xavier JB, Picioreanu C, van Loosdrecht MCM (2005) A framework for multidimensional modelling of activity and structure of multispecies biofilms. Environ Microbiol 7: 1085–1103.
70. Xavier JB, De Kreuk MK, Picioreanu C, van Loosdrecht MCM (2007) Multi-scale individual-based model of microbial and bioconversion dynamics in aerobic granular sludge. Environ Sci Technol 41: 6410–6417.
71. Picioreanu C, van Loosdrecht MC, Heijnen JJ (1998) Mathematical modeling of biofilm structure with a hybrid differential-discrete cellular automaton approach. Biotechnol Bioeng 58: 101–116.
72. Kreft JU, Picioreanu C, Wimpenny JW, van Loosdrecht MC (2001) Individual-based modelling of biofilms. Microbiology 147: 2897–2912.
73. Nadell CD, Xavier JB, Levin SA, Foster KR (2008) The evolution of quorum sensing in bacterial biofilms. PLoS Biol 6: e14. doi:10.1371/journal.-pbio.0060014
74. Mitri S, Xavier JB, Foster KR (2011) Colloquium Paper: social evolution in multispecies biofilms. Proc Natl Acad Sci U S A 108: 10839–10846.
75. Xavier JB, Foster KR (2007) Cooperation and conflict in microbial biofilms. Proc Natl Acad Sci U S A 104: 876–881.
76. Korolev KS, Xavier JB, Nelson DR, Foster KR (2011) A quantitative test of population genetics using spatiogenetic patterns in bacterial colonies. Am Nat 178: 538–552.
77. Nadell CD, Bassler BL (2011) A fitness trade-off between local competition and dispersal in *Vibrio cholerae* biofilms. Proc Natl Acad Sci U S A 108: 14181–14185.
78. Lardon LA, Merkey BV, Martins S, Dotsch A, Picioreanu C, et al. (2011) iDynoMiCS: next generation of individual-based modelling of biofilms. Environ Microbiol 13: 2416–2434.
79. Spor A, Koren O, Ley R (2011) Unravelling the effects of the environment and host genotype on the gut microbiome. Nat Rev Microbiol 9: 279–290.
80. Rezzonico E, Mestdagh R, Delley M, Combremont S, Dumas M-E, et al. (2011) Bacterial adaptation to the gut environment favors successful colonization: microbial and metabonomic characterization of a simplified microbiota mouse model. Gut Microbes 2: 307–318.
81. Johansson MEV, Phillipson M, Petersson J, Velcich A, Holm L, et al. (2008) The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc Natl Acad Sci U S A 105: 15064–15069.
82. Krivan V (2006) The ideal free distribution and bacterial growth on two substrates. Theor Popul Biol 69: 181–191.
83. Stecher B, Robbiani R, Walker AW, Westendorf AM, Barthel M, et al. (2007) *Salmonella enterica* Serovar Typhimurium exploits inflammation to compete with the intestinal microbiota. PLoS Biol 5: 2177–2189. doi:10.1371/journal.-pbio.0050244