Evolution on the microbial battlefield

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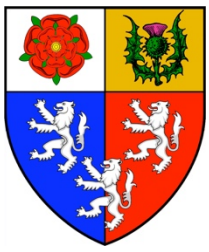

Statement of Originality

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

Rene Niehus, Hilary term 2016

For Ilona and Kalle

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Publications and statement of authorship

The majority of each chapter in this thesis is my own work but I have received help from collaborators. Below I mention published parts and clarify the contribution of others to my chapters.

Chapter 4 has been published as:

> Niehus, Rene, Sara Mitri, Alexander G. Fletcher, and Kevin R. Foster. "Migration and horizontal gene transfer divide microbial genomes into multiple niches." Nature communications 6 (2015).

Alexander Fletcher has helped with the modelling work, Kevin Foster was involved in the data analysis, and all co-authors commented on the manuscript.

The unpublished chapters 2,3 and 5 are my own work but have benefitted from help by others. Kevin Foster has given comments on all my chapters and has helped to analyse results. In Chapter 2, Aurore Picot has helped with the modelling work and was involved in the data analysis. Nuno Oliveira performed the experiments. Both commented on the manuscript.

I have received permission to present each chapter in journal format. As such, each chapter has its own introduction, conclusion, and sometimes supplementary material. I include the published version of Chapter 4 in the Appendix of this thesis.

Abstract

Evolution on the microbial battlefield

Rene Niehus, Pembroke College

Hilary term 2016

Microbes live in dense communities where strains and species compete for space and nutrients. Cells within these communities produce a large array of secretions, such as toxins or scavenging molecules, that kill and inhibit other strains. While competition is common and important for microbes, its impacts on their communities remains poorly understood. In this thesis, I study the impacts of competition on (1) the evolution of secretions and (2) horizontal gene transfer. Using a wide range of approaches, including game theory, population genetics, differential equations and individual-based models, I investigate the evolution and ecology of diverse microbial communities. First, I study the production of iron-scavenging siderophores–a trait often assumed to be cooperative–and show how they can function as a competitive trait that harms other strains by starving them of iron. My competitive model predicts that siderophores should be upregulated in response to competitors and I show this fits with both published and new experimental data (Chapter 2). I next study the logic of bacterial warfare proper: the evolution of strategies to produce antibiotics and bacteriocins to kill other strains. I show that the most robust strategy for using antibiotics is to attack only when detecting the attack of others, a negative form of tit-for-tat (Chapter 3). The second half of my thesis studies how competition influences another major bacterial trait, horizontal gene transfer. Firstly, I show how competition and migration combine to enable single genes to sweep horizontally through microbial communities, thus providing an explanation for a large body of data that shows that these sweeps are both common and important (Chapter 4). Finally, I ask how genetic transfer can evolve in microbial communities, despite the potential for high individual costs from taking up foreign DNA. As for the evolution of sex in eukaryotes, I show that epistasis for fitness can promote horizontal transfer under certain conditions. However, by studying regulated gene transfer, I argue that key to horizontal transfer evolution in bacteria is their ability to actively upregulate transfer in response to stress (Chapter 5). My thesis helps to map out the rules of interaction in microbial communities, and how these rules affect major phenotypes, including siderophores, antibiotics and horizontal genetic transfer. To close, I discuss how the effects of clinical antibiotics mirrors many of the effects of natural competition between microbial strains. I argue that only by understanding natural competition can we understand how the use, and overuse, of clinical antibiotics affects microbes.

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1 Thesis Introduction

It is now recognised that microbes commonly live in dense and diverse communities where strains and species compete for both nutrients and space (Hibbing et al. 2010; Foster and Bell 2012; Mitri and Foster 2013) (Figure 1a). Moreover, cells in these communities have evolved an array of draconian mechanisms in order to kill and inhibit neighbouring cells (Be'er et al. 2009; Pukatzki et al. 2013; Johnstone and Nolan 2015). Despite this, we still understand relatively little of how competition affects the evolution and ecology of microbes. In my thesis, I study two major ways in which microbes have adapted to competition: by releasing factors that inhibit other strains; and by rapid adaptation through horizontal uptake of novel genes.

1.1 Mechanisms of microbial competition

Microbial strains interact with other strains via a range of factors that they release into the environment. These can take the form of molecules that help nutrient uptake–such as secreting molecules to scavenge or digest nutrients–or molecules

that actively harm other strains, such as the release of antibiotics. These are known "social" traits in evolutionary biology, and they are both extremely common and also known to be central to their ability to compete with other strains (Chao and Levin 1981; Griffin et al. 2004; Mideo 2009; Hibbing et al. 2010), as well as host virulence (Meyer et al. 1996; Zhang et al. 2000; Harrison et al. 2006). However, while considerable attention has been paid to the importance of cooperation for microbial interactions (West et al. 2006; Mitri and Foster 2013; Claessen et al. 2014), much less attention has been paid to the evolution of traits for competitive interactions (Nadell et al. 2016).

Recent work sheds some light on how we can study and understand the effects of competition on gene regulation using tools from social evolution theory (Foster 2011; Cornforth and Foster 2013). From this work the "competition sensing" hypothesis emerged, which suggests that microbes use stress-response pathways to detect competition from other genotypes and respond appropriately. In this way, microbes can physiologically detect and respond to competitors by upregulating their competitive phenotypes (Cornforth and Foster 2013). This hypothesis has support from a large literature review (Cornforth and Foster 2013) and a number of recent experiments (Traxler et al. 2013; Abrudan et al. 2015; Oliveira et al. 2015).

However, there are many ways in which microbes can detect and respond to competition, and this complexity demands new theory to dissect the logic underlying competitive responses. Understanding the effects of competition is more than an interesting evolutionary problem, it is also central to understanding how bacteria make and use antibiotics, which is a major health concern with the rising threat of antibiotic resistance (Harris et al. 2010; Boucher et al. 2011;

Stokes and Gillings 2011). In the first half of my thesis, I study how strain competition shapes two major microbial traits: the production of siderophores, and the production of toxins such as antibiotics and bacteriocins.

Siderophores are molecules made by many and diverse microbial species whose primary function is to scavenge iron from the environment. The evolution of siderophores has been intensively studied. However, the focus has been on how siderophores evolve in a single strain background in the face of "cheater" mutants that do not make siderophore (Griffin et al. 2004; West et al. 2006; West et al. 2007; Ross-Gillespie et al. 2009; Lee et al. 2016). This neglects to consider how siderophore production evolves in diverse communities containing many strains and species (Figure 1b). A key biological feature of these community settings is that any the different strains will often have different siderophore receptors. This reduces the ability for one strain to use the siderophore of others. The focus of my second chapter is how such privatisation of siderophores affects the evolution of their production.

The clearest examples of bacterial competition come from the diverse forms of weaponry that cells employ against other strains and species (Stein 2005; Cascales et al. 2007; Ghequire & De Mot 2014) (Figure 1c). These include the well-known antibiotics (Wiener 1996; Slattery et al. 2001), but also narrow-spectrum bacteriocins (Riley and Wertz 2002a; Vetsigian et al. 2011) and the poisoned molecular spears of type VI secretion systems (Maldonado et al. 2004; Abrudan et al. 2015). It is also clear that these attacks mechanisms of bacteria are costly to a genotype and highly regulated to ensure that they are only expressed at certain times (Cornforth and Foster 2013; Choi et al. 2015). In Chapter 3, I study

how strain competition shapes bacterial strategies of attack and I study the importance of different environmental cues for this regulation.

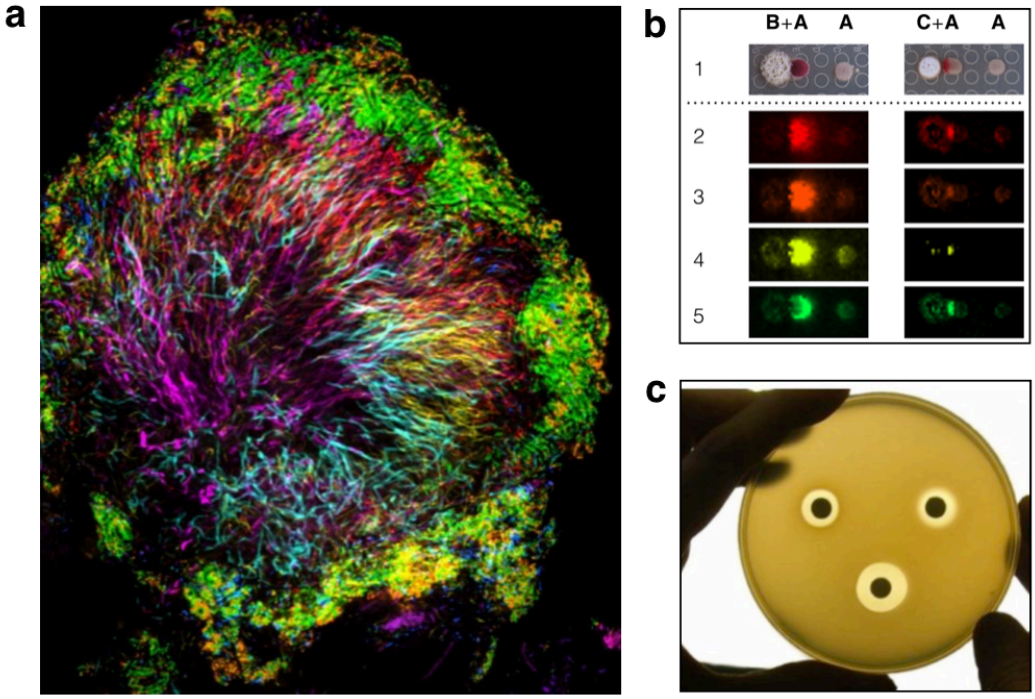


Figure 1 | Interacting microbes. (**a**) Image showing dental biofilm using fluorescence in situ hybridization. Different colours indicate a high number of different bacterial species. Image adapted from (Ferrer and Mira 2016) (**b**) Bacteria react when they are close to a competitor. A colony of *Stremptomyces coelicolor* (strain A) is grown in proximity of a strain of *Amycolatopsis* (B, left-hand side column) and a strain of *Streptomyces* (C, right-hand side column). The upper row shows the colonies as micrographs, while the remaining rows show mass spectrometry signals of different siderophores that are produced by the colonies. The presence of another strain evoke clear and specific siderophore responses in *S. coelicolor.* compared to the when *S. coelicolor* is grown on its own (right-hand side of each column). Images adapted from (Traxler et al. 2013) (**c**) Soaked disks with antimicrobial compounds (visible as black dots) cause clear zones of inhibited cell growth in their surrounding. The top-left disk contains antibiotic, the top-right disk contains bacteriocin, and the bottom one a combination of both. Credits to Margaret Riley.

1.2 Bacterial sex

In many sexual organisms, two parental genomes merge every generation to form a recombined offspring genome, and this recombination is considered central to the evolution and ecology of eukaryotes. Somewhat analogously, microbes have the ability to strongly alter their genomes by taking up genes from a foreign cell and integrating it into the genome (Nakamura et al. 2004; Gogarten and Townsend 2005; Chen and Novick 2009). One of the clearest examples of adaptation by horizontal transfer is the evolution of antibiotic resistance encoded by enzymes that target and break down antibiotics. Indeed, the transfer of antibiotic genes has led to the evolution of multiply-resistant strains that are a major public health concern (Levy and Marshall 2004). Other examples of frequently transferred genes are virulence factors such as host-specific toxins (Friesen et al. 2006), heavy metal pumps (Martinez et al. 2006) or heat shock proteins (Gogarten and Townsend 2005). Horizontal transfer, therefore, has long been understood to have important effects. However, its impacts on any one microbial community was often thought to be relatively modest because transfer events are extremely rare (Vos and Didelot 2009).

Microbes primarily reproduce asexually, meaning that a new genome is made by copying a single parent genome. This asexual competition appeared to put great importance on the ability of a particular lineage to outcompete its parent strain by asexual (vertical) reproduction, leaving little role for horizontal transfer to shape microbial evolution or genomics (Levin 1981; Cohan 1994; Cohan 1995). However, large-scale sequencing studies reveal a different picture. Microbial genomes are mosaics built from genetic pieces with different

phylogenetic origin (Lawrence and Ochman 1997; Levin and Bergstrom 2000). On geological time-scales almost every gene in prokaryotic genomes has, at some point in time, moved between clonal lineages (Bapteste et al. 2009). Moreover, it is clear that transfer can occur, albeit rarely, between distantly related lineages of bacteria (Boucher et al. 2011; Kav et al. 2012). These observations have underlined the importance of horizontal genetic transfer for the evolution and ecology of microbes.

We can now see a strong fingerprint of horizontal gene transfer in natural microbial populations, where horizontal gene transfer and selection can sweep ecologically important genes across diverse genomes (Kav et al. 2012; Cordero et al. 2012b) (Figure 2a). With the low rates of transfer, existing models have been unable to explain how these sweeps are possible, and how horizontal transfer exerts such strong effects on microbial communities (Cohan 1994; Cohan 1995) (Figure 2a, b). In Chapter 4, I develop a theoretical framework to show how horizontal genetic transfer can cause single-gene sweeps of ecologically important genes. While some species relatively commonly engage in horizontal gene transfer, however, others show few genetic traces of genetic exchange (Touchon et al. 2009; Lobkovsky et al. 2016). This begs the question of how natural selection favours or disfavours horizontal genetic transfer. In Chapter 5, I develop a model to understand when gene transfer can benefit a focal cell.

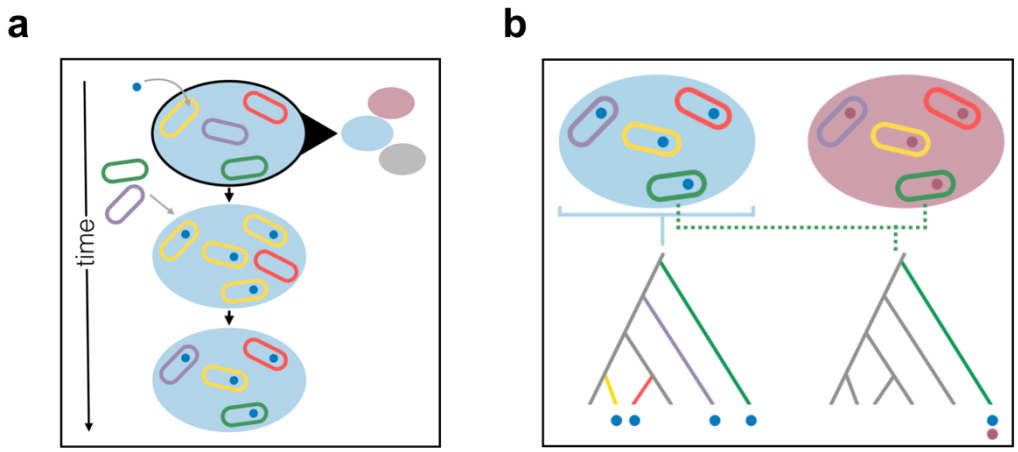


Figure 2 | The effect of horizontal genetic transfer on population genomics. **a**) An increasing number of studies indicate that horizontal gene transfer occurring between cells in a single niche (oval shapes) can sweep an adaptive allele (blue dot) through diverse background genomes (differently coloured rod-shapes). **b**) Because of horizontal gene transfer, different parts of microbial genomes can follow different phylogenies: a transferred and locally selected allele (blue dot) can be carried by different background genomes of the phylogenetic tree (left-hand side phylogeny). At the same time can adaptive loci of different origin be part of the same genome (right-hand side phylogeny). This demonstrates that a tree-like representation for prokaryotic evolution is often not sufficient.

1.3 Thesis outline

The focus of my thesis is microbial competition within natural microbial communities. In particular, I study how inter-strain competition shapes the use of secretions by microbes, and the ecology and evolution of horizontal genetic transfer.

Chapter 2: The production of iron-scavenging siderophores is a key microbial trait that can determines their ability to grow and harm a host. Because siderophores are secreted and can then be shared with other cells, siderophore producers can be

outcompeted by strains that exploit this cooperative behaviour. But how does this change when siderophore-producing strains meet genotypes that do not express the same siderophore receptors? In this chapter, I develop a modelling framework that studies the production of iron-scavenging siderophores and that explicitly captures the biochemical dynamics within a community. Using this framework, I show how siderophore production can be competitive by stealing iron from others more than it benefits them by cross-feeding. I also study how competition affects both the investment into siderophores and their regulation in real time. I show that privatisation selects for upregulation of siderophore in response to competitors, analogous to other competitive traits such as production of antimicrobials.

Chapter 3: The key weapons of bacterial competition are antibiotics and bacteriocins, which kill cells of other strains and species. These weapons are highly regulated by environmental cues and here I study the evolutionary logic underlying this regulation. In particular, I focus on the hypothesis that bacterial cells have evolved to sense their surrounding in order to attack in an efficient and reactive fashion. I compare the evolutionary benefit of different environmental cues for toxin regulation strategies and I show that sensing opponents' toxins allows for the most robust toxin counterattack strategy.

Chapter 4: Horizontal genetic transfer is central to the ability of microbes to adapt to novel environments, including clinical antibiotics. This horizontal gene transfer, together with positive selection, seems to be able to decouple single genes from the remaining background genome, leading to single-gene sweeps.

Here I develop a modelling framework to show how genetic transfer, even when rare, can achieve such dramatic effects on the population genomics of microbes. I apply a modelling framework that starts with a simple compartment model and then step-wise I add genetic and ecological detail via a coalescent model and an individual-based model. I use this to recapitulate existing theory that suggest that asexual reproduction will overpower horizontal transfer and limit its effects. However, I then show how accounting for migration between patches greatly increases the rates, and impacts, of horizontal gene transfer. In this way, genetic transfer can remove diversity only at a locus under selection while background genomes remain diverse.

Chapter 5: The importance of horizontal transfer for the ecology and evolution of microbes is clear. However, taking up foreign DNA is risky and can carry strong costs to a focal cell. How then can horizontal gene transfer evolve under natural selection? Here I use a large eco-evolutionary simulation to study conditions where horizontal transfer can evolve. This underlines the problem with horizontal genetic transfer. Cells will, on average, take up DNA from competitors that are no better adapted than them. However, I show that accounting for fitness epistasis between loci, and regulated transfer in particular, leads to evolutionary benefits to transfer. I conclude that horizontal transfer can evolve in microbes as an active process that enables them to deal with their changing and competitive environment.

2 The evolution of siderophore production as a competitive trait

2.1 Abstract

Many microbes rely on molecules called siderophores to obtain enough iron to grow. Siderophores have emerged as the archetype of a "public good" in microbes where cooperation by producer cells is threatened by non-producer "cheater" genotypes that use siderophores without making them. However, this view neglects a key piece of biology: siderophores are imported by specific receptors that can prevent their use by competing strains. Here we study the effect of this specificity in an eco-evolutionary model of siderophore production. We show that privatising siderophores fundamentally alters how they evolve. Rather than a simple public good, siderophores become a competitive trait that strains use to pillage iron from others. We study the physiological regulation of siderophores under *in-silico* long-term evolution. As expected for a canonical cooperative

phenotype, shared siderophores evolve to be downregulated in the presence of a competitor. However, privatisation reverses this prediction. Now siderophores evolve to be upregulated, analogous to other competitive traits such as antibiotics. We test our predictions using published data and experiments with the pathogen *Pseudomonas aeruginosa*, and show that siderophores are upregulated in response to competition. While siderophores can act as a public good for single genotypes, we argue that their role in competition is fundamental to understanding their biology.

2.2 Introduction

Iron limits the growth of many microorganisms making it a key determinant of evolutionary fitness and ecological competition. To cope with iron limitation, many microbes secrete siderophores into the environment (Ratledge and Dover 2000; Chakraborty et al. 2013). These molecules chelate insoluble iron and allow it to be taken up via siderophore receptors (Wandersman and Delepelaire 2004). Cells of one genotype have matching siderophores and siderophore receptors such that the siderophores produced by a cell are shared with other clones (Griffin et al. 2004). Because siderophores can also carry metabolic costs (Griffin et al. 2004), siderophores have been identified as a microbial public good (West et al. 2006), with the key corollary that a non-producer ("cheater") mutant can outcompete a producer by using its siderophores without paying the production cost (Griffin et al. 2004). The success of one production strategy over another will however strongly depend on the effects of siderophores on the producer and on others, and

these will likely change between environments (Harrison et al. 2008; Ghoul et al. 2014).

While it is clear that siderophores have the potential to act as public goods in experimental systems, this perspective lacks a key piece of siderophore biology. Different strains and species possess a large diversity of both siderophores and siderophore receptors (Miethke and Marahiel 2007; Hider and Kong 2010) and many receptors bind siderophores in a highly specific manner (Braun 2001; Hantke 2001). This specificity has the potential to greatly limit siderophore cross-feeding between competing strains (Joshi et al. 2006; Khan et al. 2006). Siderophores may then act as public goods within a strain but they can be *private* goods between different strains (Joshi et al. 2006). This privatisation is further amplified when bacteria grow in clonal patches, which is common in many environments (Mitri et al. 2015; Stacy et al. 2015), because limited diffusion then means that siderophores tend to remain close to the strain that released them (Nadell et al. 2010; Julou et al. 2013; Kümmerli et al. 2014).

How does siderophore privatisation affect its social role and the evolution of siderophore production? To answer these questions, we developed a new eco-evolutionary model of siderophore production. Our theory is centred upon an explicit mechanistic model of siderophore-scavenging (Fgaier and Eberl 2010; Lee et al. 2016), which we extend to allow different levels of inter-strain sharing of siderophores. When siderophores are private, or partially private, we find they evolve as an exploitative strategy that functions to steal iron from competitors. Moreover, our model predicts that privatisation leads to a major shift in the regulation of siderophore production. While public siderophores are downregulated in the presence of competitors, partly privatised siderophores

evolve to be upregulated. We use published data and experiments with *Pseudomonas aeruginosa* to test between these two regulatory responses and argue that the role of siderophores in ecological competition is fundamental to their evolution and regulation.

2.3 Methods

Model Overview

Our goal is to understand how different ecological conditions affect the evolution of siderophore production and regulation. The core of our approach is based upon the biochemical mechanisms of iron scavenging via siderophores, a well-studied process that includes secretion of siderophore molecules, their binding to iron and subsequent formation of siderophore-iron complexes, the uptake of these complexes via siderophore receptors, and the loss of siderophores through diffusion (Winkelmann et al. 1987; Winkelmann 1991; Andrews et al. 2003). All of these processes affect the evolutionary costs and benefits of siderophore production (Kümmerli et al. 2009; Kümmerli et al. 2014; Lee et al. 2016) and we model the processes explicitly using ordinary differential equations (ODEs). While this leads to relatively large systems of equations, this allows us to make full use of the detailed experimental work on siderophore production (Boukhalfa and Crumbliss 2002; Mey et al. 2004; Hider and Kong 2010). In addition, as we will show, the relative complexity that comes with this realism does not prevent us from extracting clear and testable predictions from our model. We embed the model within an implicit meta-population framework (Cremer et al. 2012; Oliveira et al. 2014). We study sets of strains that grow, interact and compete over

iron in local patches before dispersing and seeding new patches. With this, we can study the evolutionary fate of strains that differ in their siderophore production as a function of the ecology and, in particular, the level of privatisation of siderophores that limits their use to a single strain.

Local dynamics

We study strains that migrate to and interact in a focal patch, which could represent for example a small neighbourhood within a structured community, or a host organism. Most theory to date has focussed on the interaction between producers and non-producers (West and Buckling 2003; Eberl and Collinson 2009; Inglis et al. 2011). However, in our model all strains have the potential to produce siderophores, although they may evolve not to produce any. We study selection on the investment into siderophore production (f), which can take any value in the range [0, 1], where 0 corresponds to non-production of siderophores. The number of different strains that interact in a single patch is given by n (n = 1, 2, 3, …). This number determines the strength of ecological competition in the patch: when a single strain seeds a patch (n = 1) there is no competition between genotypes; when two or more strains seed a patch there is inter-strain competition for iron; and this competition increases with the number of competing strains.

Each strain in the focal patch is a distinct genotype that originates from the external ecological landscape. For simplicity, we assume that each strain can produce at most one siderophore. Each strain expresses also the cognate receptor for its siderophore but may also take up siderophores produced by other strains, either because its receptor has affinity for other siderophores (Crowley et al. 1991) or because it co-expresses multiple siderophore receptors (Cornelis and

Matthijs 2002; Cornelis and Bodilis 2009). We make this between-strain sharing a tuneable parameter ($s \in [0, 1]$) of our model, where $s = 0$ means that only the producer strain benefits and $s = 1$ means that all strains benefit equally.

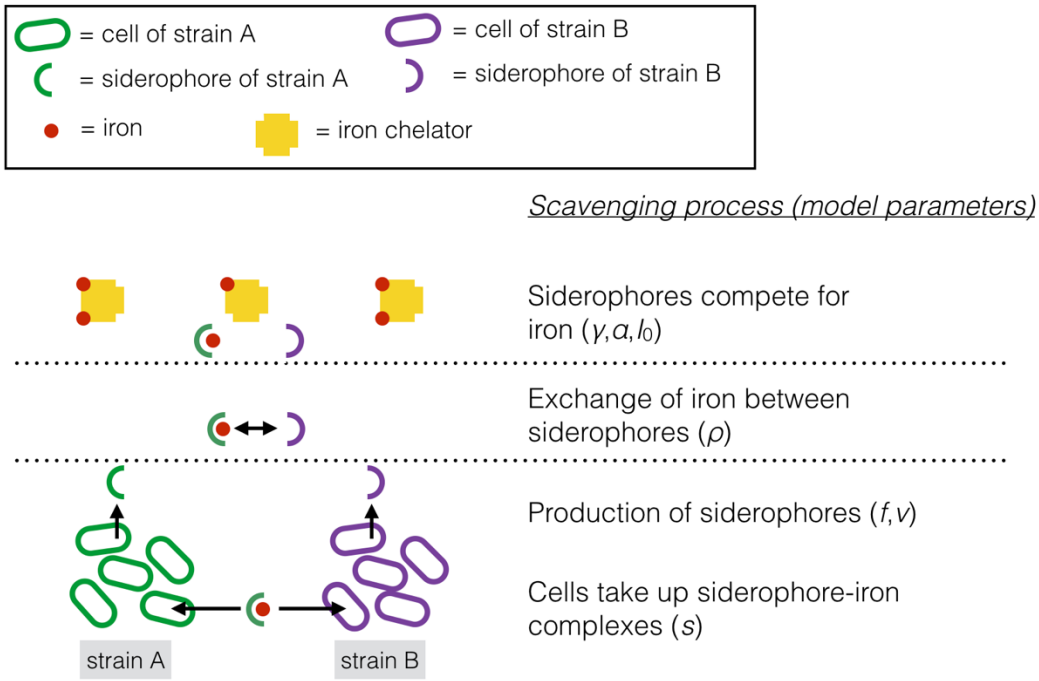


Figure 1 | A schematic view of siderophore scavenging divided into its key component steps. Iron scavenging begins with the secretion of siderophores by cells with a specified level of investment and yield. Siderophores (half-circles) then bind the environmental iron (red dots on yellow shapes, representing for example other iron chelators), that is present in the patch according to its reflux rate (α) and its concentration in the external environment (I_0). Siderophores and iron form complexes according to the siderophore affinity γ. Siderophores can also 'steal' or strip away iron from existing complexes at a rate that depends on the iron exchange parameter ρ. Finally, different strains compete for the uptake of siderophore-iron complexes according to the extent to which siderophores are shared between strains (s). For clarity, each step is shown separately in the cartoon, but in our model all processes occur continuously in one well-mixed patch.

Siderophores are secreted molecules and iron scavenging occurs outside the cells through several processes. For clarity, we divide this scavenging process into three key steps, which we incorporate into our model (Figure 1):

1) **Binding iron**: Excreted siderophores bind the environmental iron with a certain affinity (γ), thereby making this iron unavailable for other siderophores (Boukhalfa and Crumbliss 2002; Hider and Kong 2010).
2) **Stealing iron**: Siderophores can strip away iron from other siderophore-iron complexes with rate ρ. The extent of this exchange will depend on the quantity of the siderophore that is stealing the iron and the quantity of iron-complexes by other siderophores (Kraemer 2004; Khan et al. 2006).
3) **Cellular uptake**: Cells take up their own type of siderophore-iron complexes via siderophore receptors. And strains may, as discussed above, also be able to uptake siderophores from other strains (Joshi et al. 2006; Khan et al. 2006). For simplicity, we do not explicitly model siderophore diversity and receptor affinities, but we capture the between-strain sharing through a single parameter s ($s \in [0,1]$), which gives the fraction of a siderophore concentration that can be used by strains other than the producer strain. We also assume that cells carry a limited amount of siderophore receptors, giving a saturating siderophore uptake response. Siderophore sharing may also be affected by spatial arrangement of the different bacterial strains (Nadell et al. 2010; Julou et al. 2013; Kümmerli et al. 2014). While there is the potential to extend our model to capture such effects explicitly, we decide to focus here a well-mixed case.

We can then capture the dynamics of siderophore scavenging within a single patch for a single-strain (n = 1) by the ODE system

$$\begin{cases} \dfrac{dC}{dt} = \mu\left(\dfrac{(1-f)N}{(1-f)N+K}\left(\dfrac{P}{P+K_P}+\epsilon I\right)\right)C - d_C C \\ \dfrac{dS}{dt} = VfNC + \dfrac{(1-f)N}{(1-f)N+K}\dfrac{P}{P+K_P}C - \gamma SI - d_S S \\ \dfrac{dP}{dt} = \gamma SI - \dfrac{(1-f)N}{(1-f)N+K}\dfrac{P}{P+K_P}C - d_P P \\ \dfrac{dI}{dt} = a(I_0 - I) - \gamma SI - \epsilon CI, \end{cases} \quad (1)$$

where $C(t)$ is the biomass of the focal strain, $S(t)$ is the concentration of its siderophores, $P(t)$ is the concentration of iron-siderophore complex, and $I(t)$ is the concentration of available iron at time t. Our model follows chemostat dynamics with a constant input of iron as well as loss of cells, siderophores and complexes. The external concentration of iron is I_0, and there is a reflux of iron into the patch at reflux rate a. The dynamics of the focal strain biomass are due to two processes: cell death, which proceeds with a constant per capita rate d_C; and cell proliferation, whose rate depends on a maximum growth rate μ, a non-limited and therefore constant nutrient (N), the available iron-siderophore complex (P) and iron uptake through siderophore-independent mechanisms (ε). Cells invest a fraction 1 - f of their energy into biomass accumulation and the conversion of nutrient into biomass is given by a saturating Monod function with saturation constant K. The acquisition of iron through uptake of siderophore-iron complexes also follows a Monod function with constant K_P. The free siderophore concentration S changes over time due to cellular siderophore production, which is proportional to the fraction of non-limited nutrients that the cells allocate to siderophore production (fN) and to the siderophore production yield (V).

Siderophores are recycled from iron-siderophore complexes and are lost from the system through diffusion (d_S). The concentration of iron-bound siderophores changes over time due to the formation of such complexes (at a rate γ, that represents the affinity of siderophore for iron), on the uptake by cells, and on a loss term with loss parameter d_P. Finally, the concentration of available iron follows a chemostat dynamics with a reflux of external iron and depletion through the formation of iron-siderophore complexes.

As the level of non-limited nutrient N is constant, we can reduce the system by introducing the parameter $\beta = \frac{K}{N}$, giving

$$\begin{cases} \dfrac{dC}{dt} = \mu\left(\dfrac{(1-f)}{(1-f)+\beta}\dfrac{P}{P+K_P} + \epsilon I\right)C - d_C C \\ \dfrac{dS}{dt} = \dfrac{v}{\beta} fC + \dfrac{(1-f)}{(1-f)+\beta}\dfrac{P}{P+K_P}C - \gamma SI - d_S S \\ \dfrac{dP}{dt} = \gamma SI - \dfrac{(1-f)}{(1-f)+\beta}\dfrac{P}{P+K_P}C - d_P P \\ \dfrac{dI}{dt} = a(I_0 - I) - \gamma SI - \epsilon CI \end{cases} \quad (2)$$

When multiple strains interact in a patch ($n > 1$) we extend the number of equations accordingly. We illustrate this here with the extended system for two strains, to demonstrate the implementation of between-strain sharing of siderophores and well as of ligand exchange:

$$
\begin{cases}
\dfrac{dC_1}{dt} = \mu\left(\dfrac{(1-f_1)}{(1-f_1)+\beta}\dfrac{P_1+sP_2}{P_1+sP_2+K_P}+\epsilon I\right)C_1 - d_C C_1 \\
\dfrac{dS_1}{dt} = \dfrac{v}{\beta}f_1 C_1 + \dfrac{(1-f_1)}{(1-f_1)+\beta}\dfrac{P_1+sP_2}{P_1+sP_2+K_P}C_1 - \gamma S_1 I - d_S S_1 + \rho(S_2P_1 - S_1P_2) \\
\dfrac{dP_1}{dt} = \gamma S_1 I - \dfrac{(1-f_1)}{(1-f_1)+\beta}\dfrac{P_1}{P_1+sP_2+K_P}C_1 - \dfrac{(1-f_2)}{(1-f_2)+\beta}s\dfrac{P_1}{P_2+sP_1+K_P}C_2 - d_P P_1 \\
\qquad +\rho(S_1P_2 - S_2P_1) \\
\dfrac{dC_2}{dt} = \mu\left(\dfrac{(1-f_2)}{(1-f_2)+\beta}\dfrac{P_2+sP_1}{P_2+sP_1+K_P}+\epsilon I\right)C_2 - d_C C_2 \\
\dfrac{dS_2}{dt} = \dfrac{v}{\beta}f_2 C_2 + \dfrac{(1-f_2)}{(1-f_2)+\beta}\dfrac{P_2+sP_1}{P_2+sP_1+K_P}C_2 - \gamma S_2 I - d_S S_2 + \rho(S_1P_2 - S_2P_1) \\
\dfrac{dP_2}{dt} = \gamma S_2 I - \dfrac{(1-f_2)}{(1-f_2)+\beta}\dfrac{P_2}{P_2+sP_1+K_P}C_2 - \dfrac{(1-f_1)}{(1-f_1)+\beta}s\dfrac{P_2}{P_1+sP_2+K_P}C_1 - d_P P_2 \\
\qquad +\rho(S_2P_1 - S_1P_2) \\
\dfrac{dI}{dt} = a(I_0 - I) - \gamma(S_1+S_2)I - \epsilon(C_1+C_2)I
\end{cases}
\tag{3}
$$

Here the sharing parameter *s* determines how much each strain can use up the other strain's siderophores. No sharing ($s = 0$) means that siderophores can only be used by their producing strain. Complete sharing ($s = 1$) means that siderophores are available to all strains equally, which is equivalent to a single pool of siderophores. We assume that siderophores can strip away iron from other iron-siderophore complexes, according to the reactions $S_1 + P_2 \rightarrow S_2 + P_1$ and $S_2 + P_1 \rightarrow S_1 + P_2$, which proceed with mutual rate constant ρ.

To isolate the effect of siderophore production, we assume that the chemical properties of siderophores (amount of sharing, affinity, loss, yield) are identical for all interacting strains. We show a schematic overview of the siderophore-scavenging process in Figure 1. To solve the system of ODEs we impose initial conditions ($C(t{=}0) = 1$, $S(t{=}0) = 0$, $P(t{=}0) = 0$, $I(t{=}0) = I_0$) and we use the finite difference method with constant grid spacing and implicit Euler stepping with constant time steps to solve the equations numerically using the standard ODE solver from GSL (Gnu Standard Library) in C++.

Physiological regulation of siderophores

Our first models studied strains that invest a fixed proportion of their resources into siderophores. We next study the evolution of regulated siderophore production. We replace the fixed investment f by a sigmoid, quasi-step function that represents a simple sensory trigger function that responds to a signal x. We consider three possible sources of information for the regulation of siderophores for a focal strain i. The first is intracellular iron concentration, which is known to strongly regulate siderophore production in some species (Schmitt and Holmes 1991; Ratledge and Dover 2000; Rodriguez et al. 2002; Chakraborty et al. 2013), which will be proportional to the iron-siderophore complexes that a focal strain can use, given as $[(1-s)P_i + s\sum_{j=1}^{n} P_j]$. The second source of information is clonemate density (C_i), which can be detected by quorum sensing or another product specific to the focal strain (Stintzi et al. 1998; Lewenza et al. 1999; Mok et al. 2003). Finally, we consider competitor biomass ($(\sum_{j=1}^{n} C_j - C_i)$), which can be detected by any compound that is specific to the competitor. This detection might include non-self quorum sensing autoinducers but also sensing the damage from antibiotics or bacteriocins of the competing strain (competition sensing, Cornforth & Foster 2013). In each case, the investment function takes the form

$$f = f_{bas} + \frac{(f_{act} - f_{bas})}{\Big(1 + exp\big(100(x - T)\big)\Big)}, \qquad (4)$$

where T is the activation/de-activation threshold. Under this functional form, f approximately takes the 'activated' value f_{act} when the signal x is above the threshold, and the 'basal' value f_{bas} otherwise. In our optimising algorithm, the

three parameters that define the shape of the trigger function (f_{act}, f_{bas}, and T) will initially be selected at random and be the same for all strains, and then we interactively test the invasion of a new strategy with either of the parameters changed. Note that while we use the term 'activated' for above threshold, the strains are free to evolve either an increase or decrease in the production of siderophores upon activation.

Meta-population dynamics

We embed our model of local competition between strains within a meta-population to study how different strategies evolve over time. Our meta-population consists of a large number of patches that are linked through the dynamics of a simple microbial life-cycle (Cremer et al. 2012):

1) **Seeding:** An empty patch is seeded with a certain number n of different strains with initially small density ($C_i(0) = 1$). The strategy for each strain is determined according to the frequency of the strategies in the entire meta-population.
2) **Competition:** Strains grow and interact within each patch of the meta-population according to the local dynamics model.
3) **Mixing:** Cells from all patches disperse and mix, leading to a new seeding episode.

We then assess the evolutionary fate of new strategies that appear in the meta-population. To do this, we use invasion analysis, which is based upon the logic of evolutionary game theory (Maynard Smith 1982). When it can be used, invasion analysis is a powerful way to study co-evolving strategies that allows one to avoid explicitly modelling each step in a life-cycle (Nowak and Sigmund 2004a).

Specifically, to follow the evolution of new siderophore production strategies, we study how a rare mutant or immigrant with the new strategy performs in a meta-population where all other strains perform another strategy. We can then ask whether this immigrant will successfully invade the resident strategy population, or instead go extinct.

We calculate invasion ability from the fitness of the new mutant strain (w_{inv}) and the fitness of the resident strategy (w_{res}). The fitness of the invader, since it is rare, is determined by its local competition with other strains that have the resident strategy, $w_{inv} = w(f_{inv}|f_{res})$. The fitness of the resident strategy, which is very common and will therefore nearly always meet itself–is determined by local competition with strains with this strategy so that $w_{res} = w(f_{res}|f_{res})$. If strains in the local patch do not meet any other strain ($n = 1$), then the resident and migrant strategy's fitnesses are determined by their autonomous growth, following a single set of ODEs (Eq. 2). We define the fitness of a strain as its biomass at the end of a competition phase, which is after a fixed amount of competition time t_{end}. We then compute the invasion index for an invading strategy as defined by Mitri *et al*. (2011),

$$I_{inv} = \frac{w_{inv}}{w_{res}} = \frac{w(f_{inv}|f_{res})}{w(f_{res}|f_{res})}. \quad (5)$$

When the invasion index of a new strategy is larger than one ($I_{inv} > 1$) the migrant strategy will increase its meta-population frequency from its initial appearance to the next mixing step. When the strategy's invasion index is smaller than one ($I_{inv} < 1$), it will go extinct. An important nuance of evolutionary invasion analysis is that a strategy's evolutionary success is not determined solely by its local

competitive success: a migrant strategy that wins in a local patch against the resident strategy could still go extinct from the meta-population if the fitness of the resident strategy is high ($I_{inv} < 1$). For example, a really aggressive strategy might win locally but harm itself so much in the process that it cannot outcompete the other patches in the meta population (Hauert et al. 2002).

We use the invasion index to follow the evolution of siderophore production and find optimal strategies. A successfully invading strategy will become the new resident strategy. This will occur repeatedly until we find a evolutionarily stable strategy (ESS, Maynard Smith 1982), which cannot itself be replaced by other strategies. Specifically, the ESS is a strategy that, if adopted by the entire population, cannot be invaded by any other strategy. We find ESSs by following the gradual evolution of strategies as they compete with others that are different to themselves. We test invading strategies that are locally-similar as well as strategies from the full range of possible strategies. By combining local and global searches in this way, we identify strategies that are evolutionarily stable in the face of a vast range of possible competing strategies. While multiple ESS are theoretically possible in game theory, we always found a unique ESS for each analysis.

Coculture experiments in Pseudomonas aeruginosa

We next study the link between between-strain competition, via bacteriocins, and siderophore production. We mixed strains of *P. aeruginosa* and measured the production of siderophores. We studied five different strains of *P. aeruginosa*: – PAK, PAO1, strain 4, PA14, and its mutant PA14 *prtR.* The mutant PA14 *prtR* has a constitutively active suppressor of its bacteriocins (pyocins) (Oliveira et al.

2015), which allows us to test the role of bacteriocins on siderophore production. Previous work has shown strain 4 and PAK are susceptible to the bacteriocins produced by PAO1 and PA14 (Oliveira et al. 2015).

We set-up pairwise strain mixtures with different starting frequencies of two strains (yielding the ratios 25:75, 50:50, and 75:25), including monocultures of both strains. To initiate mixtures, we dilute overnight cultures of each strain and grow these to obtain exponential phase cells and we then inoculate mixes with different proportions of each strain. After 20 hours of incubation of the mixtures, we determine the per-cell concentration of culture pyoverdine, a siderophore produced by strain PAO1 and PA14 that can be measured through its fluorescent properties (Wendenbaum et al. 1983; Meyer 2000). Specifically, we measure absorbance of cultures at 600 nm, which gives the optical density of the cells and an estimate of cell density. In addition, we assess pyoverdine concentration, following a standard protocol (Jiricny et al. 2010), by measuring the relative fluorescence units of the cell cultures at excitation wavelength of 400 nm and an emission wavelength of 460 nm. Finally, we determine the pyoverdine index, which is the amount of pyoverdine per optical density.

To study whether siderophore response is specifically induced by competition sensing to cell damage from bacteriocins, we tested the two strains known to be susceptible to pyocins (strain 4 and PAK) in cocultures with the mutant strain PA14 *prtR,* which does not produce pyocins. We repeated each coculture experiment eight times. To confirm that the mutation in strain PA14 *prtR* does not directly affect siderophore production and our results, we also studied how a focal strain (strain 4) responds to cell-free supernatant of strain

PA14 and the mutant strain PA14 *prtR*. Each supernatant experiment was repeated six times.

Finally, another potential confounding factor is that bacteriocin-induced cell lysis of susceptible individuals could passively release siderophores, which we would then measure as a response. We, therefore, performed control experiments where we lyse strains 4 and PAO1 using a standard sonication protocol. We followed cell lysis via optical density and the pyoverdine concentration per cell as before (pyoverdine index), which revealed that cell lysis does not increase siderophore levels (Results).

2.4 Results

Overview

We use our eco-evolutionary model to study how siderophore production evolves across different ecological scenarios and, in particular, in response to siderophore privatisation. This allows us to test a range of ecologies where differing number of strains meet and compete for resources and we can vary the extent to which the different strains can share each other's siderophores. We then identify the evolutionarily stable investment into siderophore production for each situation. In the second part, we study siderophore regulation and find that the regulatory strategies that evolve also depend strongly on how much siderophores are shared. Specifically, with siderophore privatisation our model predicts that production should increase when strains encounter competitors. Finally, we test this prediction with co-culture experiments with the pathogen *Pseudomonas aeruginosa*.

Privatisation strongly affects the evolution of siderophore production

Many studies treat siderophores as a canonical public good that benefits all cells in an environment equally, where non-producers (cheaters) can thrive in the presence of siderophores producers. The potential for one genotype to exploit another, however, rests upon strains mixing such that non-producers have access to the siderophore producers. As such, a key prediction of the standard social evolution model of siderophores is that the evolution of production will decrease with an increasing number of strains mixing in local competition (Harrison et al. 2008). In social evolution terminology, the investment into cooperation will evolve to decrease under conditions of decreased relatedness (Hamilton 1964; Frank 1998).

We compare the evolutionarily stable siderophore production in the absence of competitors ($n = 1$, Figure 2, black diamond) and in the presence of increasing numbers of competitor strains. When siderophores are entirely shared, we recapitulate the classic social evolution prediction that optimal investment into siderophores decreases for higher numbers of competing strains in a patch (Figure 2, green diamond shapes). We then ask how this relationship is affected by the degree of siderophore sharing between genotypes ($s \in [0, 1]$). When $s = 1$, siderophores are entirely shared and their benefit returns to the producer as much as to other cells. And $s = 0$ corresponds to private siderophores that are only taken up by the producer.

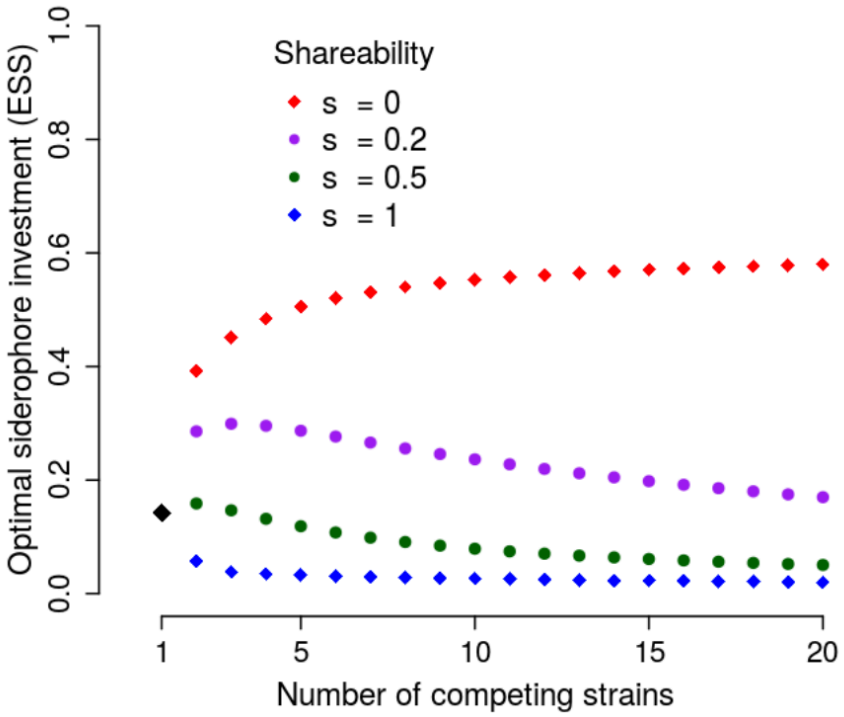


Figure 2 | The effect of competition and siderophore sharing on siderophore production. We determine the optimal (ESS) investment into siderophore production (f^*) using adaptive dynamics and we plot this optimal investment against different numbers of number of competitors (n) per local competition. In the absence of competition, where siderophore sharing does not occur, the optimal investment into siderophore production is shown by the large black diamond on the far left. The effect of added competition on investment (f^*) is qualitatively different for different levels of between-strain sharing of siderophores. When the number of competing strains increases, the production of an entirely shared siderophore is reduced (blue diamonds), while the production of a private siderophore is increased (red diamonds). At intermediate levels of sharing siderophore production first increases and then decreases with increasing competition (purple and green dots).

The privatisation of siderophores fundamentally changes the effects of strain mixing on production. When siderophores are private to the producer strain, increasing the intensity of competition between strains (increasing n) results in an increase in siderophore production (Figure 2). When siderophores are partly private and partly public, we see that the effect of increasing competition has elements of both the purely public and private evolutionary responses. Importantly, for relatively low levels of strain mixing, which may often be

common due to spatial structuring in communities (Hallatschek et al. 2007; Nadell et al. 2010), the effect of increasing competition is to *increase* siderophore investment (Figure 2). Siderophores are evolving as competitive traits. Even though siderophores are still a public good with respect to the cells of a single genotype, therefore, privatisation shifts them to behaving as a mechanism of exploitative competition, which is used to deplete and steal the iron of competitors.

The effects of privatisation are robust for a wide range of conditions

We have shown that privatisation can have strong effects on the evolution of siderophore production. In particular, privatisation means that siderophores evolve as a competitive rather than a cooperative trait, with investment increasing under conditions of high strain mixing (low relatedness). How robust is this effect? Our model contains a number of parameters that can be used to study how key ecological and biological factors influence the evolution of siderophore production. We performed sweeps of these parameters and studied in each case how strain mixing affects the evolved level of siderophore production (Figure 3).

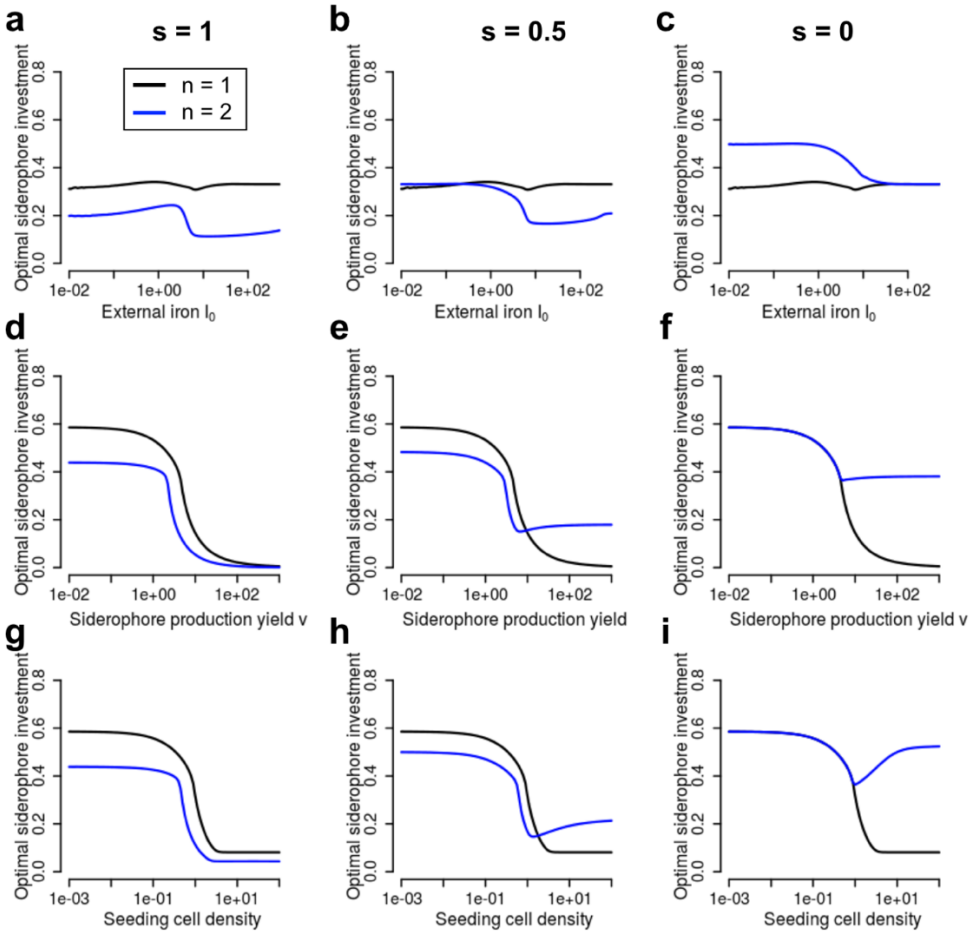


Figure 3 | The effect of iron concentration, siderophore yield, and seeding cell density on ESS production of siderophores. We plot the optimal siderophore investment with and without competition ($n = 2$, $n = 1$, respectively) over a range of external iron concentrations (**a-c**), and over a range of siderophore production yields (**d-f**), and over a range of seeding cell densities (**g-i**). We find that when siderophores are fully shared between strains ($s = 1$), adding local competition always decreases the evolutionarily stable level of siderophore production (**a,d,g**; blue line below black line). By contrast, for entirely private siderophores ($s = 0$), the production level decreases (**c,f,i**; blue line above black line). Under intermediate sharing ($s = 0.5$) we observe regions of both decreased and increased siderophore production (**b,e,h**; crossings of blue and black line). When siderophores are mainly powerless to exploit the environment (high iron, low production yield and/or cell density) the cross-feeding effects dominates and production is reduced with strain mixing. But when siderophores become effective (low iron, high production yield and/or cell density) then their exploitative potential means that strains increase production in strain mixes.

Our model is relatively complex in that it combines a dynamic model of local competitions between strains, with global competition in a meta-population and search algorithms that identify the evolutionarily stable strategy for each set of parameters. The predicted effects of parameters on siderophore production are in some cases also relatively complex, with regions where the ESS level of production decreases before increasing again (Figure 3). Nevertheless, we observe clear and consistent effects of strain mixing on production level. When siderophores are fully public ($s = 1$), introducing local competition between strains (increased mixing) always decreases the evolutionarily stable level of siderophore production (Figure 3; blue line below black line).

By contrast, with no sharing of siderophore between strains ($s = 0$), the investment level increases with adding a competitor (Figure 3; blue line above black line). For some parameters, this decrease is minor, particularly when siderophore production becomes minimal (low production yield or seeding cell density) or when iron is abundant, because under these conditions siderophores have little effect on competitors and are produced similarly to the single strain case. However, critically, for private siderophores we never see a decrease, and for public siderophores, we never see an increase in production with strain mixing, when this mixing is with few strains, such as $n = 2$.

Conditions of intermediate sharing ($s = 0.5$) are again intermediate in their behaviour and we see that, dependent on parameters, for all three parameters strain mixing can drive an increase or decrease in the evolutionarily stable level of siderophore production. Essentially, in conditions where siderophores are powerless to exploit competitors (high iron, low production yield and/or cell density) the effect of cross-feeding siderophore dominates and, hence, its

production is reduced with strain mixing. But when siderophores are efficient (low iron, high production yield and/or cell density) the benefit from exploiting can overcome the effect of cross-feeding (Figure 3; crossing of black and blue line) so that production is increased in strain mixes.

In summary, privatisation has strong effects on the evolution of siderophore production that are robust for a wide range of ecological and biological parameters.

Privatisation is critical to the evolution of siderophore regulation

As is typical of previous theoretical work on siderophore evolution, we have so far treated siderophore production as a constitutive trait where each cell invests a fixed proportion of its resources (*f*) into siderophores. However, in reality siderophores can be strongly regulated in response to environmental conditions. We therefore extend our model to consider regulation of siderophore production and evolution of this regulation. We study regulation based on three sources of information in the environment, which are known to affect bacterial regulatory networks for multiple traits: iron concentration, density of clone mate cells, and density of competitor cells (*Methods*). For each type of sensing, we can then follow the evolution of strategies as before and identify the evolutionarily stable strategies of regulation (*Methods*, Figure 4).

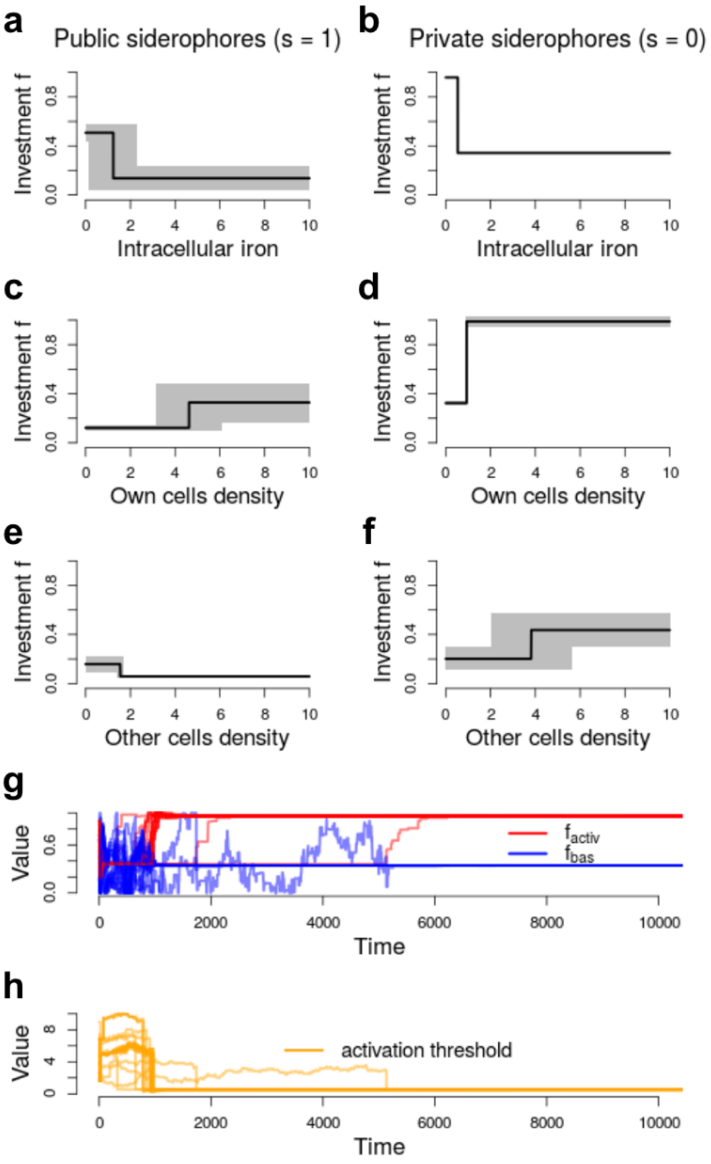


Figure 4 | The evolution of physiological siderophore regulation. We optimise the sensing function for siderophore production for pairwise competitions (n = 1) using the evolutionary algorithm outlined in *Supplementary Methods and Results* and perform this optimisation for three different sources of information: intracellular iron, own cell density and competitor cell density. The plots show the evolved response as siderophore production as a function of the sensed signal, as a mean from 30 runs of the simulation. The grey area shows the standard deviation. Siderophores evolve to be upregulated at low iron, independent of the shareability (**a,b**). This is because siderophores become most valuable when iron is low. Siderophore evolve to be upregulated for high quorum (**c,d**) because at high cell density production becomes most efficient. However, when siderophores are regulated based upon the density of competing cells, they are downregulated when siderophores are shared (s = 1) and upregulated when siderophores are private (s = 0) (**e,f**). The bottom two plots show illustrative time-plots from the evolution algorithm, showing how the initial, and the activated siderophore investment (**g**) and the activation threshold (**h**) evolve in 30 realisations of the algorithm. The examples shown are for the evolution of quorum sensing and s = 0.

Because our model framework combines local dynamics with global competitions and an evolutionary algorithm, some model parameters have a complex effect on the evolved strategies. Nonetheless, for a range of parameters we observe a clear pattern. Siderophore regulation in response to iron level and own cell density then evolves in a consistent way, irrespective of the degree of privatisation. The responses evolve such that low iron and high quorum both favour the release of siderophore. There is a clear logic behind these forms of regulation. Most simply, siderophores are most valuable when iron is low and our predicted regulation is well supported by empirical work that shows that in a number of different species siderophore production can be strongly upregulated by low iron (Schmitt and Holmes 1991; Ratledge and Dover 2000; Rodriguez et al. 2002; Chakraborty et al. 2013). The quorum-based regulation is consistent with the typical interpretation of the evolutionary function of quorum sensing. Quorum sensing allows a group of cells to only release a secreted product once cell density is high enough to generate an effective concentration of this product (Schluter et al. 2016). In addition, as with iron based regulation, there is evidence that siderophore production increases at high cell density for a number of species (Stintzi et al. 1998; Lewenza et al. 1999; Mok et al. 2003).

When we allow cells to evolve siderophore regulation based upon the density of competing cells, we see a different pattern. Now, whether cells upregulate or downregulate production depends upon whether siderophores are public or private (Figure 4e, f). The evolution of the regulation of public siderophores leads to a response where production is down regulated in response to increased numbers of the competing strain. This is because under these conditions, increased competition means that there is a greater threat of

siderophore piracy from the competitors and so downregulation benefits the focal strain. When siderophores are privatised, we predict the opposite pattern. Now, siderophores respond as expected of a competitive trait, such as the release of an antibiotic (Cornforth and Foster 2013), with secretion upregulated in response to the presence of the competing strain.

Competition increases siderophore production in coculture experiments. Our predictions on the evolution of siderophore regulation are well supported by known regulatory responses to iron level (Schmitt and Holmes 1991; Ratledge and Dover 2000; Rodriguez et al. 2002; Chakraborty et al. 2013) and quorum sensing (Stintzi et al. 1998; Lewenza et al. 1999; Mok et al. 2003). However, for these responses, our models also predict that regulation should be qualitatively identical for private or public siderophores. By contrast, the predicted regulation based upon the level of competition with other strains changes depending on whether siderophores are private or public. This latter form of regulation, therefore, lends itself to testing the importance of privatisation for the evolution of siderophore use.

Two previous studies have found evidence that siderophore production can increase in the presence of unrelated strains and species. Coculture experiments between *P. aeruginosa* with *Staphylococcus aureus* found that *P. aeruginosa* makes more siderophores in the presence of *S. aureus* (Harrison et al. 2008). However, the experimental design started co-culture experiments with a higher total cell density than the monoculture experiments. Rather than *P. aeruginosa* directly responded to the addition of *S. aureus*, therefore, the authors argued that the upregulation may simply be a response to increased iron limitation (Figure 4a,

b). More recently, Traxler *et al.* (2013) placed colonies of *Streptomyces* species either alone, or next to a colony of a different species. When next to a foreign colony, the *Streptomyces* strain increased secretion of a number of siderophores (Traxler et al. 2013). While this response may be driven by multiple factors–including iron limitation–the data is consistent with *Streptomyces* upregulating siderophores upon detection of competition from other species, possibly via the detection of competitors' siderophores (Traxler et al. 2013).

Recent work found that *P. aeruginosa* strains respond strongly to the presence of other strains of their own species. In particular, co-culture experiments revealed that the presence of a competing strain can drive increased biofilm formation in a focal strain (Oliveira et al. 2015). Moreover, the increase in biofilm formation was found to rest upon a response to the bacteriocins of competing strains ("competition sensing", Cornforth and Foster 2013). Given that *P. aeruginosa* has the capacity for such strong responses to competition from other strains, we sought to test the prediction of our model using the same system. We, therefore, compared pure cultures and mixed cultures of *P. aeruginosa* strains in a setup analogous to Oliveira *et al.* (2015) and asked how siderophore production changed. Importantly, in all experiments, the total number of inoculated cells was constant. This design ensures that there is *not* a decrease in the iron available per cell in co-culture relative to monoculture (*Methods*). We find that coculture leads to a striking increase in siderophore production per unit of optical density across all strain combinations (Figure 5).

As for biofilm response in *P. auruginosa* (Oliveira et al. 2015), we also find evidence that the increase in siderophore production occurs in response to antibiotic competition between strains. When one of the strains in coculture has a

constitutively active repressor of bacteriocin (*prtR*) the siderophore response is greatly decreased (Figure 5b, d blue data points). We can show this effect is not due to the *prtR* mutation affecting siderophore production in the engineered strain. Specifically, we can induce siderophore production in a focal strain by using the cell free supernatant of a pyocin-producing wildtype strain, but this effect is absent when the cell free supernatant is made from the mutant strain with supressed bacteriocin production (Figure 5g). Finally, we confirm that the observed effect of bacteriocins on pyoverdin production was not simply caused by siderophores being released from lysed cells: lysing cells causes a negligible effect on siderophore levels (Figure S3).

The association between the action of bacteriocins and siderophore upregulation is important. Not only does it raise the possibility that siderophores and biofilm formation act as co-regulated phenotypes, the link to cell damage suggests that the siderophore response is not simply a by-product of increased iron limitation in co-culture. Instead, our data suggest that siderophores are upregulated as a response to sensing the presence of other strains, where the detection of cell damage is the proxy for competition (Cornforth and Foster 2013). In sum, we find that the regulation of siderophores is consistent with them functioning as a privatised and competitive trait in bacterial communities.

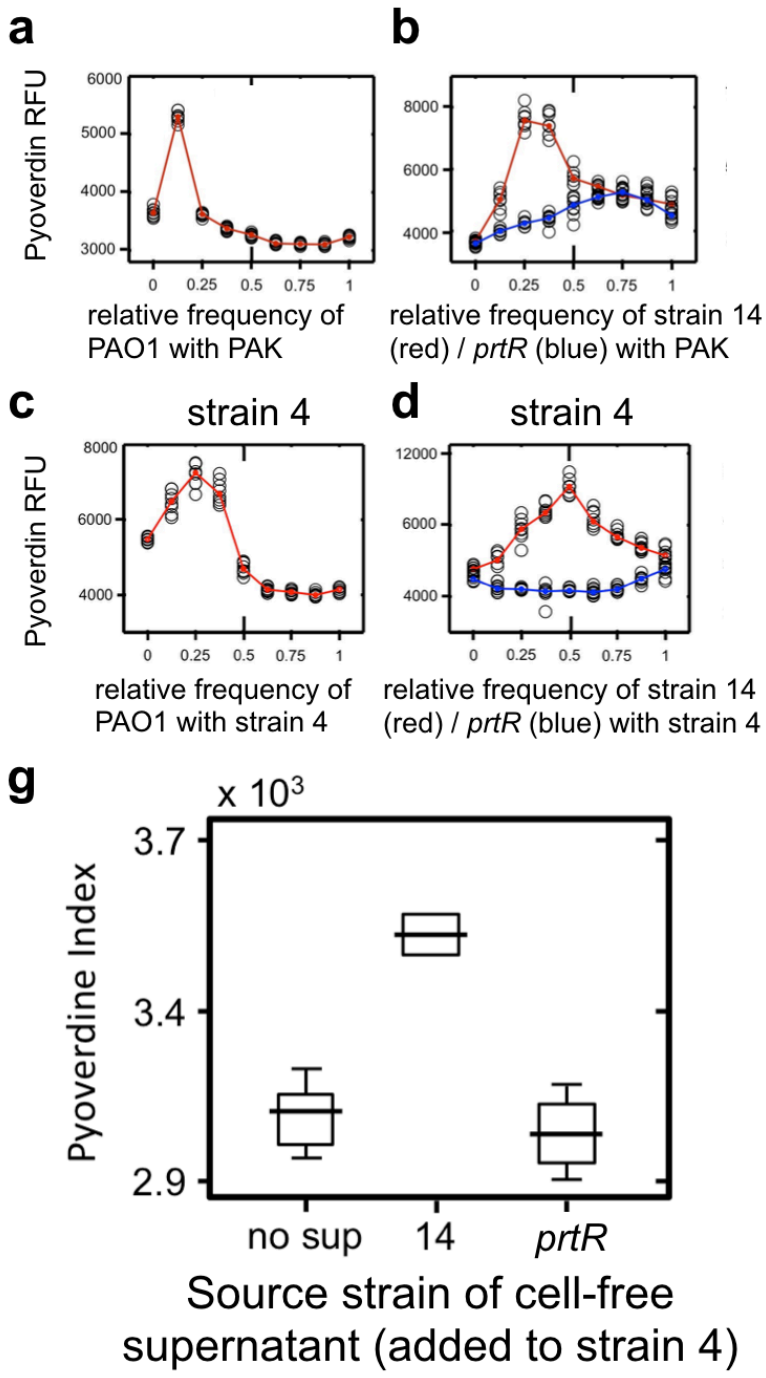


Figure 5 | Siderophore induction in response to competitor strains. Strain mixing induces the pyoverdine production (measured as pyoverdine per optical density, RFU) in all four pairwise strain combinations: PAK with PAO1 (**a**), PAK with PA14 (**b**), strain 4 with PAO1 (**c**), and strain 4 with PA14 (**e**). This siderophore response disappears when we mix a pyocin-negative PA14 mutant ('PA14 prtR') with the pyocin-sensitive strains PAK or strain 4, indicating that bacteriocins are an important factor to trigger siderophore response. To test whether the mutation causes decreased siderophore production, we induce siderophore production in pyocin-sensitive strain 4 by using cell free supernatant of PA14 and also from PA14 *prtR* (**g**). While the supernatant of PA14 increases siderophore production in strain 4, the supernatant from the mutant does not induce production.

2.5 Discussion

Siderophores have emerged as a powerful model system to understand microbial sociality (Griffin et al. 2004; Ross‐Gillespie et al. 2007; Buckling and Brockhurst 2008; Kümmerli et al. 2009; Ross-Gillespie et al. 2009; Luján et al. 2015). In mixed cultures with a wildtype producer strain, siderophore null mutants (cheaters) can thrive and outcompete the wildtype. Such experiments mean that siderophores are often viewed as a canonical public good in microbial communities. This view was recently emphasised in discussions of "black queen" evolution (Oliveira et al. 2014; Morris 2015). Microbes may commonly lose genes, including those for siderophores, when they can be complemented by other strains and species in their diverse communities (Cordero et al. 2012a; Andersen et al. 2015). However, it is also clear that the siderophores of one strain are often not fully shared with other strains, because of the use of specific receptors to import siderophore (Joshi et al. 2006; Khan et al. 2006; Lee et al. 2012), and limited diffusion (Nadell et al. 2010; Julou et al. 2013; Kümmerli et al. 2014).

Here we have shown how limited siderophore sharing between strains has fundamental effects on their ecology and evolution, which are missed in a pure public goods model. With privatisation, species that face a lot of competition from other genotypes are expected to evolve to increase their investment in siderophores (Figure 2). The canonical social evolution prediction for a cooperative trait–that investment will *decrease* with decreased relatedness (Hamilton 1964)–is no longer seen. We also find that the effects of privatisation are mirrored in the evolution of siderophore regulation. When siderophores are fully shared, our model predicts that cells will evolve to downregulate production

when competing strains are detected. By contrast, when siderophores are privatised, regulation evolves to increased siderophore in the presence of competing strains: siderophores function as a way to compete with other genotypes (Figure 4). By studying the regulation, therefore, one can assess whether siderophores have evolved as a mechanism of competition versus purely being a way to cooperate with other cells.

There is growing evidence that bacteria are capable of regulating a wide range of traits based upon the presence of different strains and species. Other genotypes can be detected by quorum sensing autoinducers or other molecules released into the environment (Keller and Surette 2006; Cornforth and Foster 2013; LeRoux et al. 2015b). Another way to achieve detection is via competition sensing, in particular via stress responses that detect the cell damage caused by the toxins of competing strains (Basler and Mekalanos 2012; Cornforth and Foster 2013). The discussion of such responses has so far focused on bacterial warfare and the upregulation of toxins and type VI secretion systems in response to ecological competition. However, biofilm formation also appears to respond to competition and, most relevant here, work on *Streptomyces* bacteria found evidence that both antibiotics and siderophores are upregulated in the presence of other species (Traxler et al. 2013). In addition, we found that siderophore production increases when different strains of *P. aeruginosa* are mixed (Figure 5). Moreover, as previously observed for biofilm formation in *P. aeruginosa* (Oliveira et al. 2015), at least part of this response can be attributed to the effects of the bacteriocins (pyocins) of one strain on a responding strain. Data from both gram-positive and gram-negative bacteria, therefore, suggest that siderophores are upregulated in response to competition.

Siderophores can function as a public good with respect to the cells of one genotypes. This effect may dominate eco-evolutionary dynamics whenever competition is primarily between cells with a recent common ancestor, as may occur in chronic diseases like cystic fibrosis (Andersen et al. 2015). However, the ecology of many species centres upon competition in diverse communities, where strain-specific siderophores limit between genotype sharing. We have shown that these conditions strongly affect how siderophores function in nature. Siderophores are no longer a simple public good. Instead, siderophores become a competitive phenotype that, like antibiotics, is upregulated in order to overcome other strains and species.

2.6 Supplementary Methods and Results

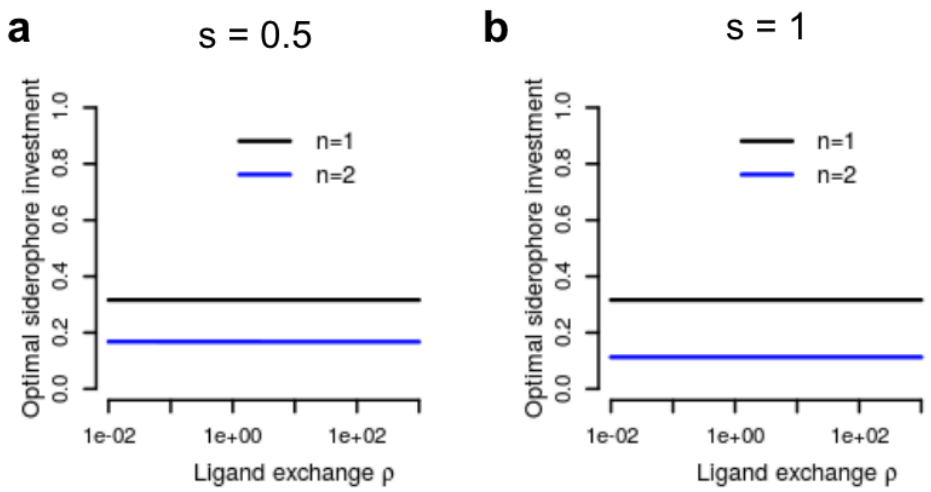
In this supplementary material, we analyse and discuss the potential for iron to be stolen from one iron-siderophore complex by another siderophore; and we present a supplementary figure showing that cell lysis does not increase siderophore concentrations.

The effect of stealing iron from other complexes

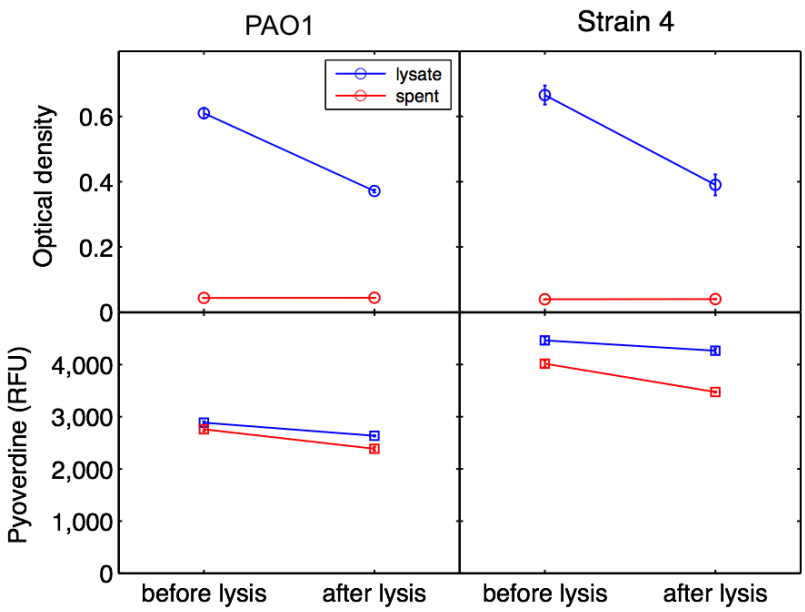
Our model captures the process by which a free siderophore can strip away iron form another siderophore, according to the following mechanisms: $S_1 + P_2 \rightarrow S_1 + P_2$; $S_1 + P_2 \rightarrow S_1 + P_2$, where S denotes a free siderophore and P denotes an iron-siderophore complex. We introduce a parameter ρ that gives the rate at which this stealing can occur. It is known, for example, that siderophores can strip iron away from mammalian host proteins that chelate iron (Meyer et al. 1996; Kirienko et al. 2013).

We expect that the ability of siderophores to steal iron from siderophores produced by another strain should give an additional benefit to producing a large amount of siderophore in the competition for iron. However, we find that the stealing parameter ρ does not affect the optimal siderophore production (Supplementary Figure S1). How can we explain this? The term for stealing iron by siderophores of strain 1 in our model is given by $\rho(S_1P_2 - S_2P_1)$, and this term decreases S_1 and it increases S_2. This then means that free siderophores of strain 2 can steal iron back from strain 1, and because of this negative feedback the stealing term becomes smaller from the process of stealing. This negative feedback is the reason for the diminishing effect of stripping away iron. In

particular, in the process of getting close to the evolutionarily stable state we compare strategies with very similar siderophore concentrations, so that the effect of stealing becomes even smaller.



Supplementary Figure S1 | Exchange of iron between chelators does not affect siderophore production. We plot the optimal investment into siderophores over different ligand exchange rates ρ, for different levels of competition (n = 1, and n = 2) and for different levels of siderophore sharing (s = 0.5 and s = 1). Ligand exchange does not affect siderophore production because we assume that siderophores have identical affinity of iron, so that stealing iron will always be counteracted by stealing back iron.



Supplementary Figure S3 | Cell lysis through sonication does not increase siderophore concentrations. We lyse cells of strain PAO1 and strain 4 using standard sonication and we measure cell concentrations through optical density and the pyoverdine index (RFU) both in the lysate and in the spent. The sonication lyses cells, visible as the decrease optical density of the lysate (blue lines, upper panels), but this cell lysis does not increase the pyoverdine index (lower panels). In fact, we see a marginal decline, which is likely due to sonication-induced siderophore break-down.

3 The evolution of warfare in bacteria

3.1 Abstract

Bacteria kill and inhibit one another with a diverse array of armaments, including antibiotics. These attacks are highly regulated but we lack a clear understanding of the logic underlying this regulation. Here we combine game theory and differential equation modelling to study the rules of bacterial engagement. We model strategies that employ quorum sensing or bacterial stress responses to regulate antibiotics (autoinducer concentration, nutrient depletion, and damage from competitor's toxins). We then pit strategies against each other in massive tournaments and ask: who would win in a fight? Despite the vast range of possible strategies, we find clear rules of competitive supremacy. Regulated antibiotic production by any means dominates over constitutive strategies. Moreover, the most common winning strategy across a diversity of opponents is to attack when a

competitor's toxin is detected. This prediction is supported by empirical work suggesting that bacteria engage in "competition sensing" whereby cells produce antibiotics in response to cell damage. As in the classical results of game theory, our work suggests that reciprocation is a fundamental principle of bacterial warfare. However, in contrast to the peaceful resolution of classical results, our work suggests that reciprocity does not evolve in bacteria to avoid conflict. Instead, reciprocity evolves because it allows aggressive strains to rapidly return to a passive state once a competitor is eliminated.

3.2 Introduction

Bacteria commonly live in dense and diverse communities where competition for space and nutrients can be intense (Hibbing et al. 2010; Kim et al. 2014). In response, bacteria have evolved a wide range of competitive traits, including contact-dependent inhibition (Hayes et al. 2010; Ghequire and De Mot 2014), type VI secretion systems (Pukatzki et al. 2013; Russell et al. 2014), narrow-spectrum bacteriocins and broad-spectrum antibiotics (Riley and Wertz 2002b; Bérdy 2005). We aim to understand the evolutionary logic underlying all of these different traits, and so we refer to them collectively as bacterial 'toxins'. These systems are used to kill and inhibit other strains, and can prevent competing strains from invading a niche (Wiener 1996), kill off coexisting strains (Chao and Levin 1981; Gordon and Riley 1999; Majeed et al. 2011), or help strains to invade new niches (Wiener 1996; Kommineni et al. 2015). Mechanisms of attack are also extremely widespread. Toxin genes are found in almost all major bacterial

lineages (Riley and Wertz 2002b) and individual species commonly make use of multiple toxins and diverse means of attack (Michel-Briand and Baysse 2002).

The production and regulation of bacterial toxins have been studied for decades because many toxins have potential as clinical antibiotics (Slattery et al. 2001; Lewis 2013). This work has revealed that toxin production is often highly regulated. Indeed, it is thought that many new antibiotics remain undetected because they are only activated under certain conditions (Maldonado et al. 2003; Traxler et al. 2013; Abrudan et al. 2015). A trigger for some antibiotics and bacteriocins is quorum sensing (Manefield et al. 2000; Redfield 2002; Navarro et al. 2008), which is thought to ensure toxin production occurs at the right cell density (Hibbing et al. 2010). Many other factors regulate bacterial toxin production, including diverse stress responses and particular nutrient conditions. This has led to the argument that bacteria may also engage in "competition sensing" whereby they use nutrient stress and cell damage to detect ecological competition (Cornforth and Foster 2013). However, we lack a formal evolutionary analysis of the different potential strategies that bacteria use to attack and overcome their competitors.

Here we build a general model of bacterial competition via toxins and use evolutionary game theory to study the evolution of bacterial warfare. Motivated by data on the regulation of bacteriocin and antibiotics, we compare four major classes of potential strategy: constitutive toxin production, and regulation by nutrient level, quorum sensing and a competitor's toxin. All three types of regulated strategies carry benefits, but our work suggests that directly sensing attacks and responding in kind is the most robust offensive strategy across a diversity of competitive scenarios. As we will show, a major evolutionary benefit

to this reciprocation comes from the ability to turn off an attack once a competitor is defeated; thereby saving the energy that would be lost in needless aggression.

3.3 Results and Discussion

Overview

We are interested in how competition between strains and species of bacteria shapes the evolution of toxin regulation. We use ordinary differential equations to capture competitions between bacteria, and pit different strategies of attack against one another to study which strategies are victorious. We first study strains that lack regulation to understand how key factors affect how much a strain should invest in attacking others. We then extend our model and allow strains to regulate their toxin production in response to the environment according to three different cues: nutrient depletion, competitor toxin, and the density of clone-mates (quorum sensing). We put these strategies into massive evolutionary tournaments against constitutive toxin producers and other versions of their own strategy. Finally, we compete the regulatory strategies against each other and against a range of constitutive producers in order to identify the most robust winning strategy across a wide diversity of opponents.

The evolution of unregulated toxin production

We first study the evolution of constitutive toxin production and ask what promotes or inhibits investment in toxin production. This allows us to identify the general principles underlying attack strategies and to form a baseline from which to compare regulated strategies. We use a general model of competition between

strains based upon a simple system of differential equations (Bucci et al. 2011) (Figure 1a), which allows us to capture the temporal dynamics of strain interactions and, later, toxin regulation.

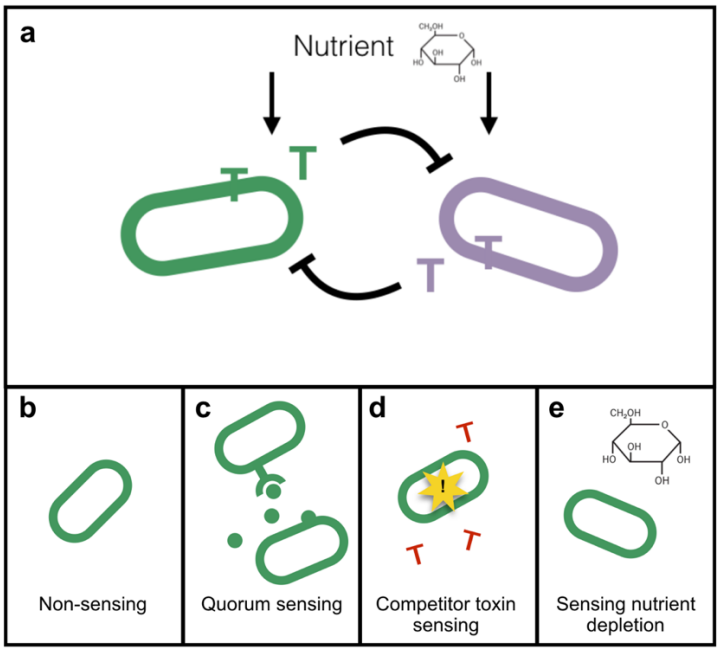


Figure 1 | A schematic of the toxin competition model. (**a**) The differential equation model captures the interaction of two strains (here represented by two single cells in green and in purple) in a well-mixed patch. Both strains consume nutrients from a shared pool, and each strain can produce a toxin that inhibits the other strain (represented as 'T's). (**b-e**) The four major classes of toxin production strategies tested in the model. We start our analysis with cells without ability to sense their surrounding (**b**). We then allow cells to sense the density of own cells (quorum sensing) (**c**), damage by the competitor toxin (**d**), or nutrient depletion (**e**).

In the model, we can follow nutrient concentration and cell numbers over time as the strains engage with each other (Figure 2). We focus on competitions between two strains that each possess a toxin that does not harm the producer but does harm the other strain. In reality, strains may carry multiple toxins and resistances (Gordon et al. 1998; Cordero et al. 2012b) and our framework can be extended to include such complexity. However, for simplicity, we focus here on a single toxin produced by each strain. We embed these competitions within a broader

framework of evolutionary game theory (Maynard Smith 1982; Weibull 1997; Mitri et al. 2011). This asks whether a particular, initially rare strategy could successfully invade a population of another strategy (*Methods*). By studying large numbers of competitions, we can categorise strains by their ability to invade others, and thereby identify the evolutionarily stable investment into toxin production (f^*). We then seek the optimal level of toxin production, which cannot be invaded by any mutant strategy, but can invade all others.

Table 1 | Model parameters and their effect on optimal investment.

Model parameter	**Parameter description**	**Standard value [Unit]**	**Effect on optimal toxin investment f^* when increasing model parameter**
$C(t=0)$	Initial cell biomass of each strain	0.1 [gC]	⬆
$N(t=0)$	Initial pool of nutrients	1 [gN]	(see Supplementary Fig.S1)
K_N	Saturation constant for nutrient uptake	5 [gN]	⬆
μ_{max}	Maximum growth rate	10 [1/h]	⬇
k	Killing efficiency of the toxin	20 [1/gT*1/h]	⬆
l_T	Toxin loss rate	0.1	⬇

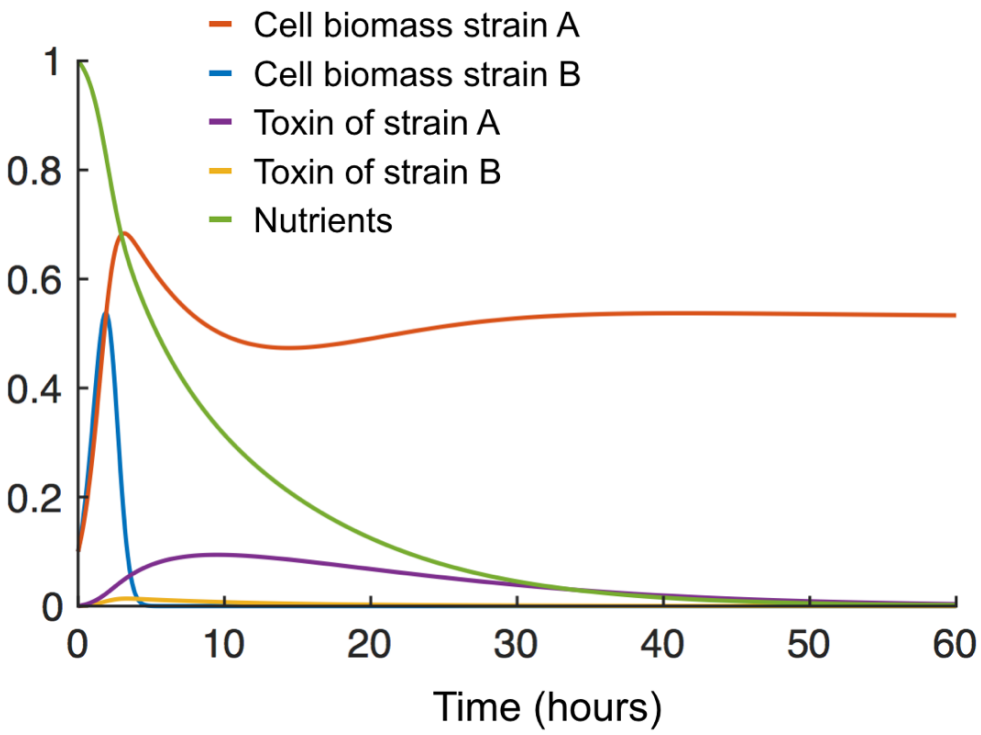


Figure 2 | Example of the temporal dynamics of a competition between two strains. Most competitions follow a similar temporal pattern where cells initially grow with high growth rate (blue and red line). Then, toxins (yellow and purple line) become sufficient to kill off cells and finally nutrients (green line) are depleted so that growth and toxin production stops. Here strain A wins the competition by investing more into toxin production ($f_A = 0.3$) than strain B ($f_B = 0.1$). All other parameters take the standard values given in Table 1.

What determines the optimal level of toxin investment? Intuitively, we find that cells evolve to invest more in attacking their competitors when toxins are efficient at killing the competitor and/or stable in the environment (Table 1). In addition, the investment in toxin depends strongly on the relative benefits of investing in cell division rather than attacking others. When bacteria enter a competition at low density and resources are abundant, there is a great potential for cells to divide rapidly. Under these conditions, cells evolve to invest relatively little in toxin production; energy is instead better invested in rapid growth to win a competition by outgrowing, rather than killing, another strain. This prediction

agrees with the "resource availability hypothesis" that states that natural selection favours high growth rates and low levels of attack under high nutrient conditions (Ianora et al. 2006). However, when growth is limited and toxins are effective, the opposite is the case and cells benefit from investing heavily in warfare. Indeed, for highly effective toxins, we find that strains will engage in an arms race that escalates to the point where populations can go extinct (Figure 3). Such "evolutionary suicide" is known from a wide range of conflict scenarios in biology (Rankin and López-Sepulcre 2005). Our prediction is also mirrored in the idea that atomic warfare can lead to mutually-assured destruction (Ringmar 2002).

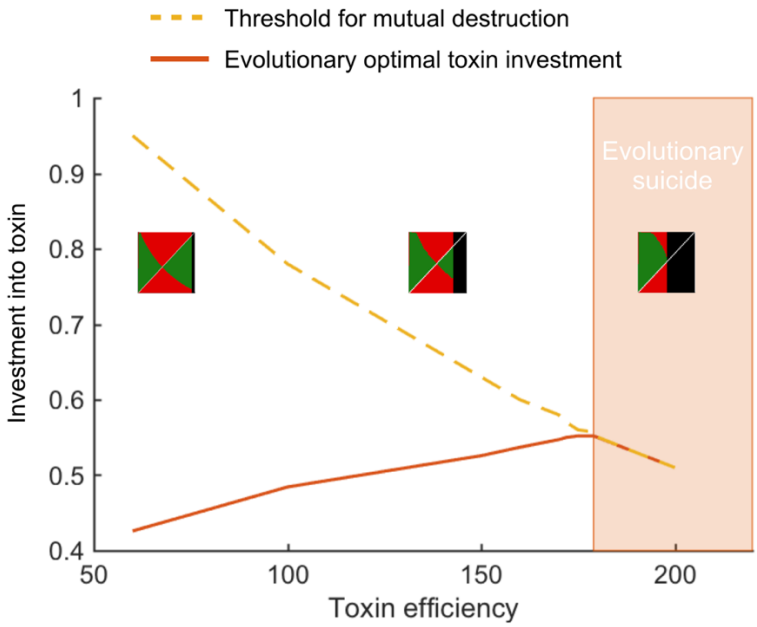


Figure 3 | At high toxin efficiency the evolutionary arms-race drives populations toward extinction. We plot the optimal toxin investment f^* (red line) and the investment threshold for mutual destruction f_{kill} (orange line) over different toxin efficiencies (k). Insets show the pairwise-invasibility plot (as in Supplement Figure S1) for low, medium, and high toxin efficiency. We see that above a certain toxin efficiency (k = 179) co-evolution causes toxin strategies to produce amounts of toxins that are deadly to both competitors. The toxin arms-race causes an evolutionary suicide of the population, analogous to mutually-assured destruction. All other model parameters take standard value as given in Table 1.

The evolution of regulated attack strategies

We next investigate what happens when cells are able to regulate their level of toxin production in response to environmental cues. It is clear that the production of antibiotic and bacteriocins is commonly highly regulated by a variety of environmental cues. As discussed above, these cues can be broadly divided into three major classes based upon known bacterial regulatory networks. The first is detection of cell density by canonical quorum sensing or related means (LeRoux et al. 2015b). In addition, bacteria are highly responsive to both nutrient stress and cell-damage associated stress, which both can detect the level of ecological competition in the environment ("competition sensing", Cornforth & Foster 2013).

We first compare the evolution of regulation by quorum, nutrient level and the level of the competitor's toxin when each are in competition with constitutive strains (see *Methods*, Figure 1). Specifically, we pit all possible forms of each regulated strategy against all possible versions of the fixed strategy in a vast tournament. We then use invasion analysis, as before, to look for regulated strategies that can invade all unregulated strategies.

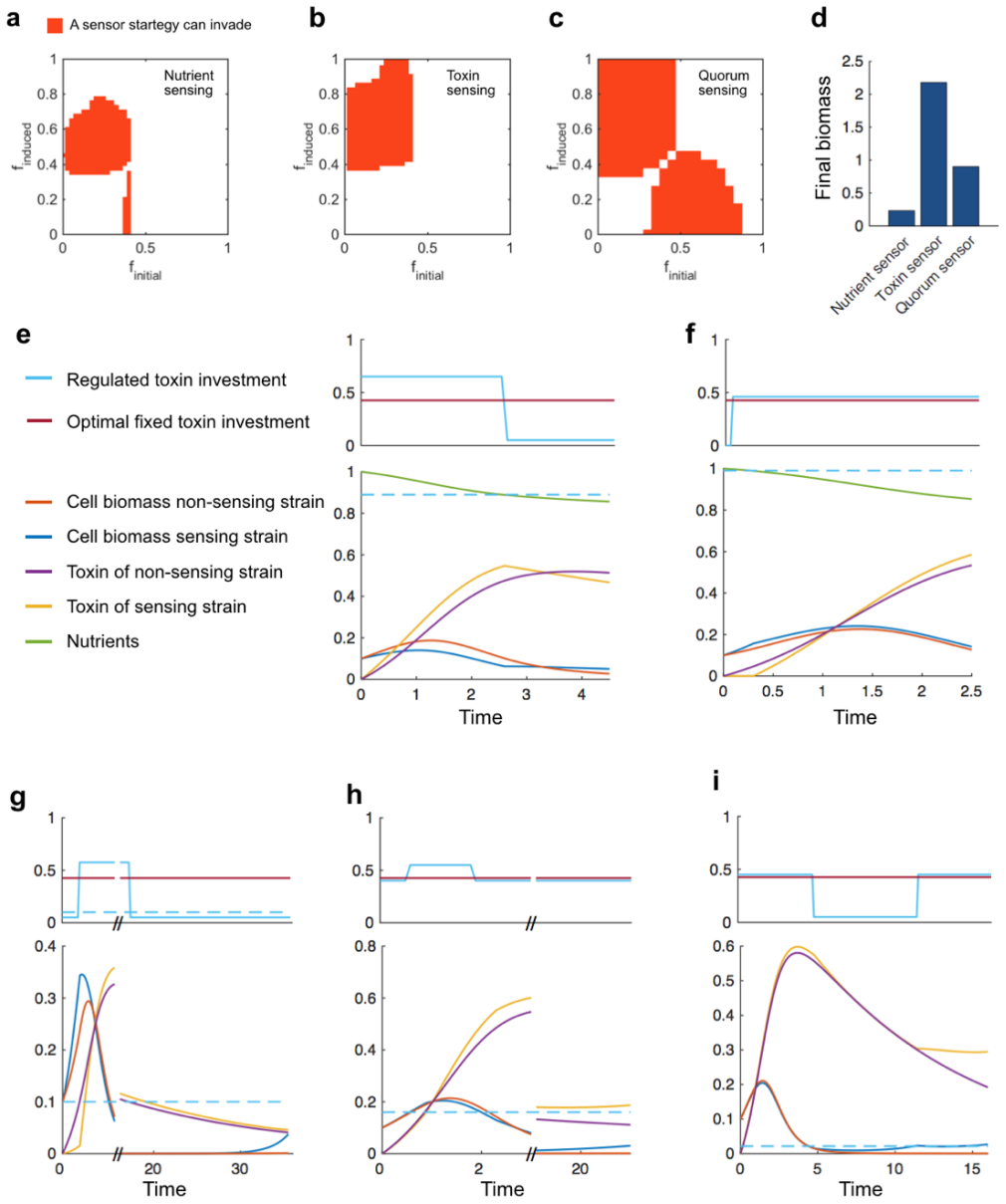


Figure 4 | Regulated toxin production outcompetes and evolutionarily replaces constitutive toxin production. We find nutrient sensing, toxin sensing and quorum sensing strategies that can invade the entire range of non-regulated producer strategies (**a-c**, red areas). The toxin sensing strain achieves the highest biomass in direct competition with the optimised constitutive producer, indicating that it is most energy efficient (**d**). Illustrative competitive dynamic for the nutrient sensing strategies with deactivating (**e**) and activating strategies (**f**), a toxin sensing strain (**g**), and two quorum sensing strains (**h,i**) against the optimal constitutive toxin strategy. To show dynamics and steady states, broken axes are used in places and the toxin levels are shown at 20-fold actual values. Regulation allows strains to use toxins more efficiently and effectively than constitutive producers. However, only the toxin sensing strain has the ability to defeat a producing strain and then switch back to near-zero toxin production (**g**). All parameters take standard value as given in Table 1.

Our work identifies versions of each regulated strategy that can outcompete all possible constitutive strategies (Figure 4a-c). This is expected and confirms the basic intuition that a well-regulated trait can generally outcompete an unregulated one (Cornforth and Foster 2013). How though do the different regulatory strategies achieve this? For the great majority of cases, successful strains evolve to only attack after a delay, either based on the detection of low nutrients, high quorum or high levels of the competitor's toxin (Figure 4f-h). We also discovered winning strategies that function by turning *off* toxin production after a delay (Figure 4e, i). For nutrient-based regulation, these strategies begin aggressively with the expression of toxin and then turn it off when nutrients are limited. Some quorum sensing strategies also start with high toxin investment. However, these strategies are more complex. They will downregulate toxins and invest in growth if losing too much ground but they will also reactivate their attack if they then recover sufficiently (Figure 4i). However, regulation based upon the competitor's toxin is the most efficient in terms of the final biomass achieved after competition with the constitutive producer (Figure 4d). Here, winning strategies activate toxin production after a delay and can also deactivate the toxin once the competitor is killed off (Figure 4g).

We find that well-regulated strategies of each type can outcompete non-regulated strategies, with toxin sensing being the most efficient strategy. We next compare the best performers of each of the regulated strategies. We first focus on how each regulated strategy performs against constitutive toxin-producing opponents, before pitting regulated strategies against each other (next section). We consider a population where focal regulated strains engage in tournaments with different sets of unregulated opponents. For each set of opponents, we then

identify the regulated strategies that obtain high overall competitive fitness using an evolutionary algorithm that searches for strategies that achieve the highest mean biomass across a tournament (*Methods*).

When opponents all have the same strategy, all three regulation strategies perform equally (Figure 5a). However, in cases where opponents are diverse and a focal strain experiences both passive and aggressive strains, the three types of regulation no longer perform equally well. Now, regulation based upon competitors toxin is the strategy that most effectively deals with competitors (Figure 5a, b). The superiority of this toxin sensing holds for a range of parameters, including different toxin efficiencies, toxin loss rates, and nutrient concentrations (Figure 5c). There is a clear *post hoc* intuition to this result. A strain that only attacks when attacked will be best able to deal with a range of strategies that differ in their propensity to engage. In addition, as seen in the last section, such a strain will also inactivate toxin production after a weak opponent is eliminated, which employs the toxin efficiently. We can directly demonstrate the importance of this toxin inactivation by shortening the duration of the strain competitions such that toxin sensing strains do not have the opportunity to switch off toxin production (Figure 5d). For short competition times, we see that all three sensing strategies are equal in their performance.

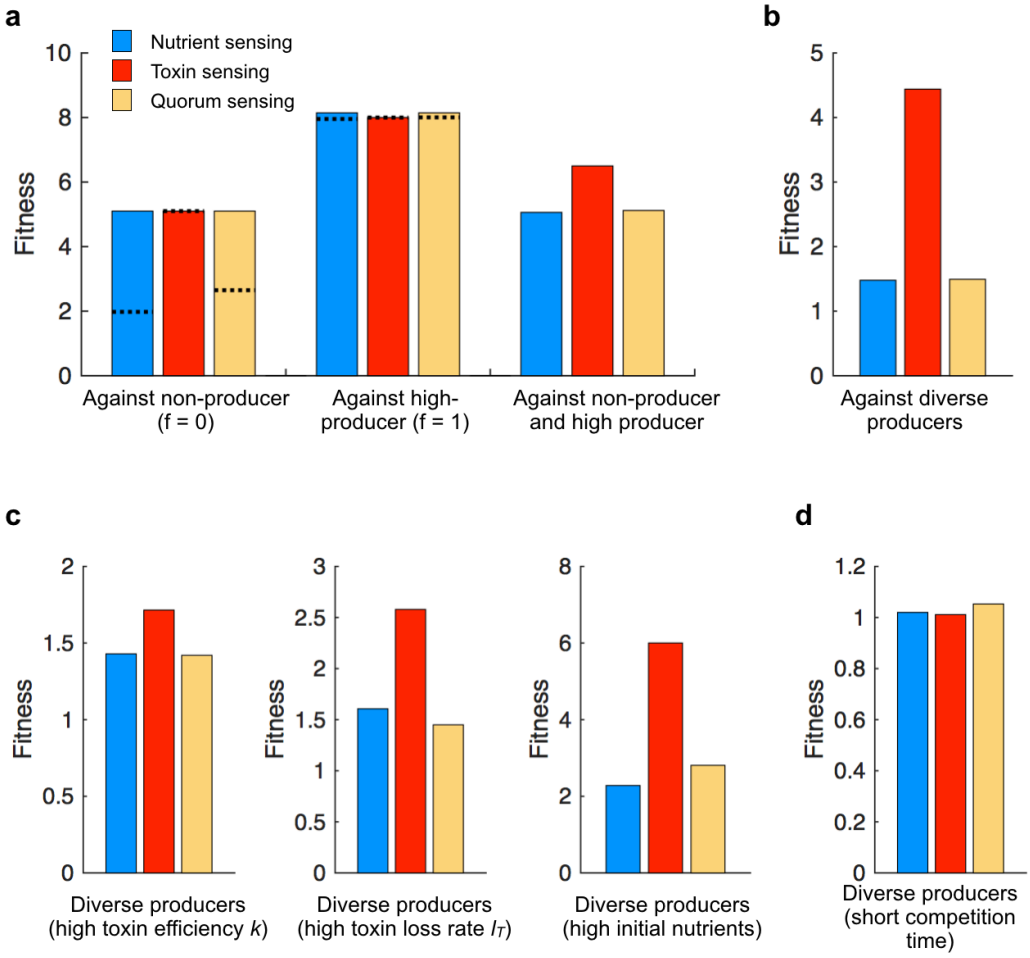


Figure 5 | Toxin sensing is the most successful strategy against a range of different competitor strategies. a) We optimised each of the sensing strategies against a non-producer (f=0), a high producer (f=1), and against a mix of these two strategies. While all three sensing strategies perform equally well against pure strategies, a toxin sensing strain perform the best against a mixture of strategies. Dotted lines show fitness of sensing strategies optimised against mixed-strategy competitors, and then competed against pure strategies. **b**) Fitness (biomass) when regulated strategies were optimised against a wide range of fixed toxin producers (f = 0, 0.1, 0.2, …,1). Again, toxin sensing provides the most robust strategy against the diverse competitors. All parameters take standard value as given in Table 1. **c**) We repeated the optimisation shown in b for several conditions, including higher toxin efficiency (k = 50), toxin loss rate (l_T = 0.3), and nutrient concentrations ($N(t=0)$ = 5), to show that toxin sensing robustly outperforms the other forms of regulation. d) Short competition time (t_{end} = 6h) removes the benefit of toxin sensing for the scenario shown in **b**. Other parameters are as given in Table 1.

We have so far focussed on bacterial competitions that run until the nutrients in the patch run out. However, we have just seen that shortening the duration of competition can affect the relative performance of the different strategies (Figure 5d). To investigate this effect more systematically, we next vary competition time and ask how this affects the best performing strategies. In nature, the duration of competition will vary depending on the rates of dispersal to new patches. A short competition time implies more rapid group turn over and that a strain will meet a new competitor more often. Short competitions, therefore, mean more time facing aggression than long competitions where a victorious strain can spend a significant time alone. We find that while competition time only weakly affects constitutive toxin production (Figure 6a), it strongly influences the best performing sensing strategies. Short competition times select for an increased baseline of aggression in sensing strategies, because it becomes important to overcome a competitor as quickly as possible (Figure 6b). The frequency of competitive events faced by a strain, therefore, will affect the degree to which it relies on preemptive versus regulated attack strategies. However, even for short competition times, a degree of regulation remains beneficial (Figure 6c).

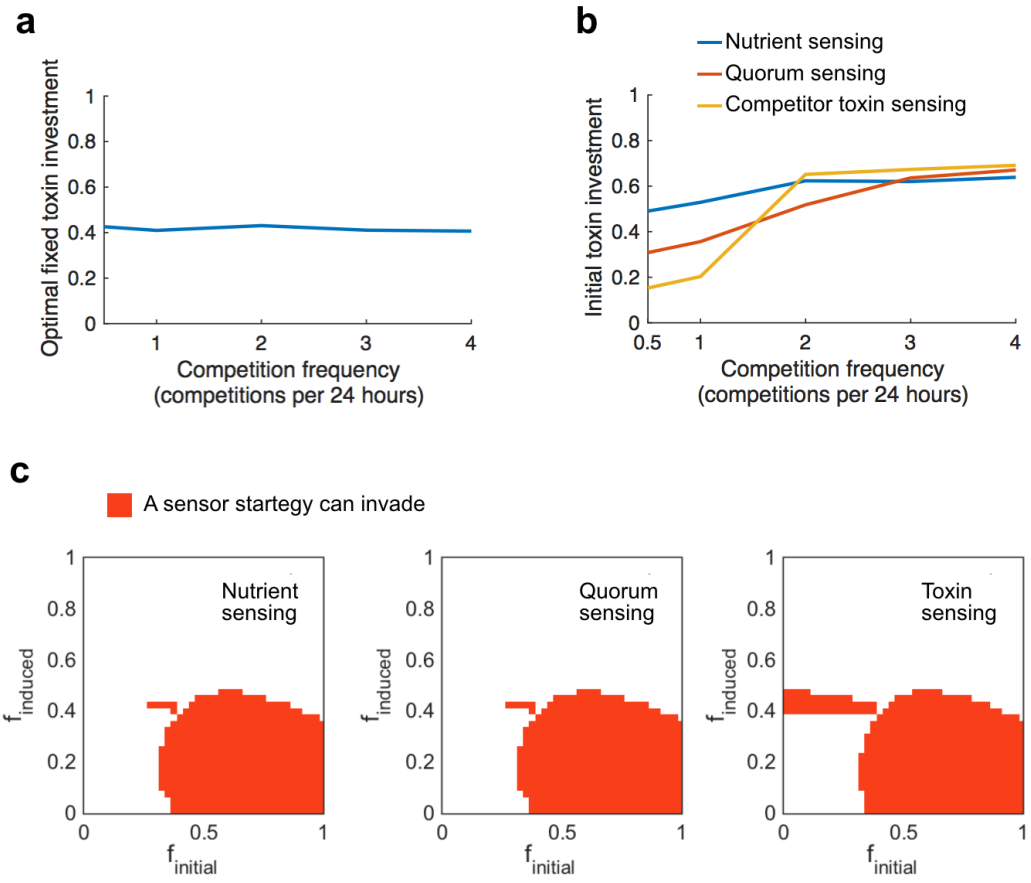


Figure 6 | Frequent competitions favour the evolution of preemptive attack. a) Increasing the frequency of new competitions has little effect on the evolution of constitutive toxin production. **b**) The initial investment in regulated toxins increases strongly with more frequent competition events, favouring pre-emptive attacks. However, even at short competition times (6h), regulated toxin strategies of all three sensing types can invade and evolutionarily replace all constitutive producers (**c**). All parameters take standard values as given in Table 1.

The coevolution of regulated attack strategies

We have considered how regulated attack strategies perform in the face of fixed strategies that simply vary in their level of aggression. What happens if regulated strategies compete against one another? In particular, for species that interact primarily with other members of their species, the typical competition will be against another strain that has a similar attack strategy. Such within-species competition appears to be fundamental to the evolution of many species because many use bacteriocins, which are narrow spectrum antibiotics that preferentially target members of the same species (Riley and Wertz 2002b; Be'er et al. 2010). We next explore the effects of within-species competition on the regulation of toxin production by competing variants of a single strategy against each other. For the three types of regulation, we perform a large number of competitions as before and search for the evolutionarily stable strategy (see *Methods*).

How do the evolutionarily stable strategies of the three types of regulation compare? All increase toxin production during the competition ($f_{\text{initial}} < f_{\text{induced}}$) (Figure 7). Moreover, the responses to nutrient depletion and to quorum are similar with relatively high initial toxin investment compared to the increase with the response. The toxin sensing strategy is different. It invests very little toxin at the start of the competition, but responds very strongly if the competitor attacks ($f_{\text{induced}} = 0.71$). Interestingly, the corollary of this is that when it meets an identical toxin strategy, both remain passive and reach a very high biomass. This outcome has close analogies to the success of "tit-for-tat", a reciprocal cooperating strategy in the classic evolutionary game theory tournament of Axelrod and Hamilton (1981). Importantly, however, while this reciprocity allows such strategies to remain peaceful, when facing other strategies it means that they

can defeat an attacking competitor before reverting to a peaceful state (Figures 3-5).

In addition to competition within species, bacteria face competition from a range of species (Watve et al. 2001). The tournaments between regulated and unregulated strategies suggest that regulation based upon detecting incoming attacks is the most robust way to deal with the variability that between-species competition will bring (Figure 6). What happens when different regulated strategies compete against each other? Competing all possible regulated strategies against each other is computationally unfeasible owing to the large number of parameters and, therefore, possible competitions. However, we can use the fact that the study of within-species competition identified an evolutionarily stable strategy for each class of regulation. With these three optimised strategies, we can ask how each perform in head-to-head competitions with one another.

Nutrient sensing is the poorest performer because it acts as a constitutive strategy once toxins are upregulated. As discussed above, this contrasts with quorum sensing, which down regulates toxin production if cell biomass drops too far (Figure 7c, Toxin vs. quorum). However, consistent with our previous results, we find that the toxin sensing strategy is the ultimate victor, which wins in competition against both the nutrient and quorum sensing strategy (Figure 7c). Starting off passively, a toxin sensing strain launches a stronger attack than both competitors (Figure 7b), killing them off before returning back to passive state. Across a wide range of competitors, therefore, the most robust competitive strategy is to detect incoming attacks and respond in kind.

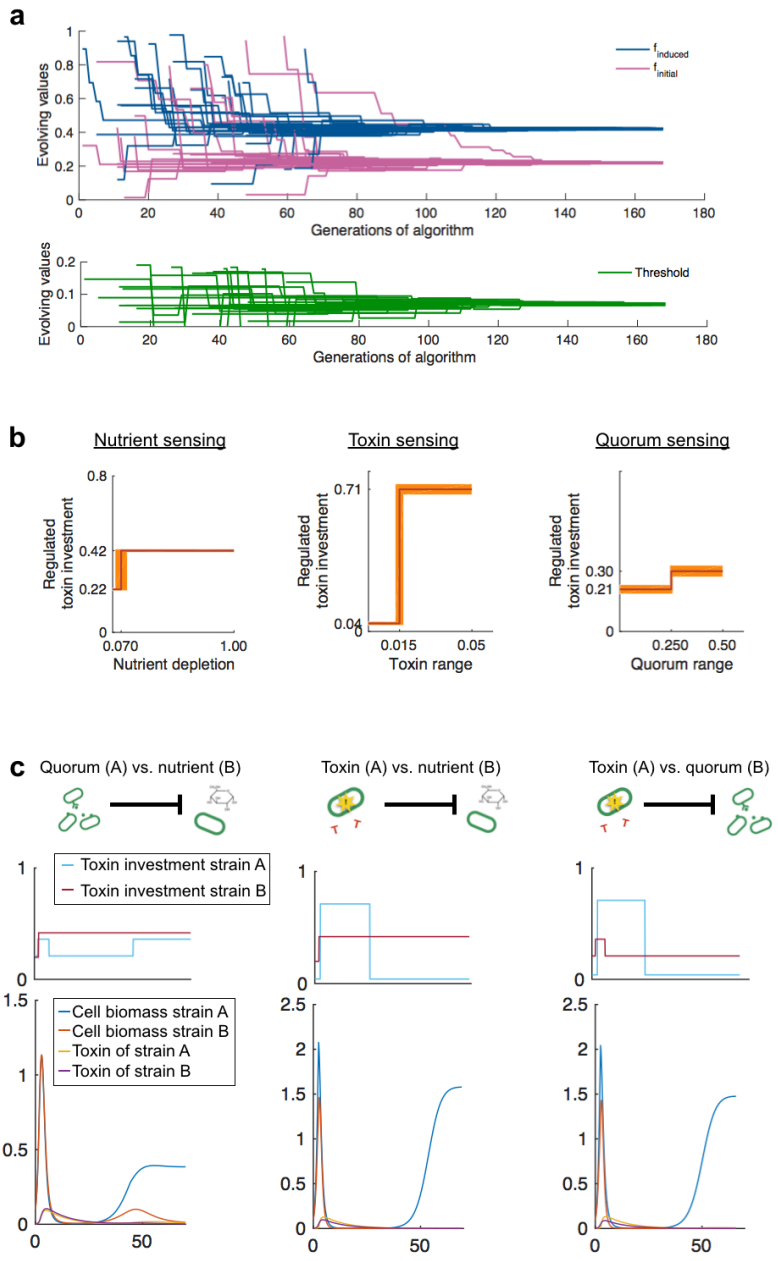


Figure 7 | Co-evolution of the sensing strategies. For each of the three sensing classes we search the evolutionarily stable strategy using a co-evolutionary algorithm. **a**) Parameter trajectories for 30 runs of our algorithm that, starting from a random first strategy, incrementally moves toward the parameter combination that cannot be replaced by another strategy. **d**) Evolutionarily stable strategies all increase toxin production in response to the signal, but the toxin sensing strain has the strongest response and invests relatively little into initial toxin. **c**) We pit the optimal strategies from each of the three regulatory classes against each other. Quorum versus nutrient sensing: The quorum sensing strain wins because it can, unlike nutrient sensing, downregulate toxin after an initial attack and thereby save energy. Toxin versus nutrient sensing: Toxin sensing wins. And toxin versus quorum sensing: Toxin sensing wins also against nutrient sensing, by launching the strongest toxin attack of all three strategies. All parameters take standard value as given in Table 1.

3.4 Conclusion

Bacteria use a wide variety of weaponry to harm other strains and species. A large body of work shows that toxins and other forms of microbial attack are under tight regulation (Michel-Briand and Baysse 2002; Stein 2005; Cascales et al. 2007; Ghequire and De Mot 2014). How bacteria employ their mechanisms of attack is central to understanding why particular species can robustly invade and persist in communities, while others cannot (Kommineni et al. 2015). There is already considerable evidence that cells are strategic in how they engage and attack their competitors (Cornforth and Foster 2013; Cornforth and Foster 2015; LeRoux et al. 2015b). Here we have explored the evolutionary logic underlying the strategies of bacterial attack. We find that well-regulated attacks can consistently outcompete strategies that lack regulation. The benefit of employing a toxin can change both between competitions and within a competition over time. Regulation allows a strain to better follow the optimal investment at any given situation. We also find that the three major classes of bacterial regulatory network are not always equivalent ways to control attacks. Across a diverse range of potential competitors, responding directly to incoming attacks is the most robustly successful strategy. These strains only attack when faced with relatively aggressive opponents, but also because they will turn off attacks once an attacking competitor is defeated.

Our results suggest that sensing competitor toxin should be a widespread way to regulate toxins and other modes of attack. This is supported by a large number of examples where bacteria upregulate attack mechanisms via stress responses that detect cell damage (Cornforth and Foster 2013). The examples

include many bacteriocins and antibiotics but also mechanisms like type VI secretion systems. These molecular spears deliver a toxin directly to the surface of another cell and, in *P. aeruginosa*, the assembly of the type VI system will occur in direct response to an incoming type VI attack (Basler and Mekalanos 2012; Basler et al. 2013). In addition, type VI secretion systems are also upregulated in response to cell lysate (LeRoux et al. 2015a). Responding to the damage of other cells is another way to reciprocate an attack ("danger sensing" LeRoux *et al.* 2015b).

While we find evidence that bacteria respond to direct attacks, bacterial attack mechanisms are also upregulated in other ways, including via nutrient depletion and quorum sensing (Cascales et al. 2007; Ghequire and De Mot 2014). If detecting damage is the best basis for attack, why do bacteria use other forms of regulation? When we model regulation by quorum or nutrients, we typically find that attacks evolve to be activated at high quorum or limited nutrients, which is consistent with the typical directionality of the regulation observed in nature. Moreover, for a number of scenarios, we observe that all three regulatory strategies are largely equivalent, particularly when strains face a consistent and relatively predictable set of opponents. The evolution of toxin regulation will often occur by co-opting a pre-existing regulatory system. When the different systems are largely equivalent, therefore, the evolutionary path to one form of regulation may largely be determined by which pre-existing regulatory systems are available for co-option (Cotter and DiRita 2000; Hockett et al. 2015).

There may also be conditions where regulation based upon the detection of cell damage is either impossible or costly. The detection of cell damage can be achieved by a diversity of mechanisms and it has been argued that oxidative

stress, in particular, might be a way to detect a diversity of attacks via one system (Goh et al. 2002; Dwyer et al. 2014; Dong et al. 2015). However, the detection of cell damage can carry the potential cost that when it is detected, the damage has already been done. Moreover, there is also the potential for 'silent' toxins. For example, these can compromise the inner cell membrane and prevent formation of a membrane potential, and which cannot be detected by any of the major stress responses (Yang and Konisky 1984). Indeed such toxins may be favoured by natural selection, specifically because they do not elicit strong and costly reciprocation in other cells (Majeed et al. 2013).

Whenever detection of cell damage occurs too late or not at all, therefore, regulation by quorum or nutrient limitation will be a more favourable strategy. In addition, there is the potential for benefits to these forms of regulation that are not captured by our model. One potential benefit for using nutrient limitation to activate toxins is the notion of "metabolic prudence", where cells only make a molecule when they cannot use the precursors for growth (Xavier et al. 2011; Mellbye and Schuster 2014; Boyle et al. 2015). When limited by iron, for example, a cell might redirect carbon and nitrogen to make toxins without having an impact on its growth rate. Finally, a cell may employ multiple regulators to control any one antibiotic or bacteriocin. The use of quorum sensing, in addition to a particular stress response, may allow cells to better tune their responses to a particular competitor or situation.

Bacteria use a diverse set of regulatory networks in order to attack and overcome competitors (Stein 2005). We are far from fully understanding how and why these rich and complex networks have evolved. Nevertheless, here we have identified simple and general principles of bacterial warfare that relate well to

known biology. There are great benefits in timing an attack; both to minimise its cost and maximise its effect on an opponent. We also find that reciprocation is a powerful rule of engagement. If cells only attack when attacked, they invest their energy where and when it is most needed: against aggressive opponents. This prediction is supported by a growing body of evidence that bacteria upregulate their attacks in response to cell damage (Basler and Mekalanos 2012; Majeed et al. 2013; Basler et al. 2013; Cornforth and Foster 2013). Our findings are also mirrored in the classical predictions from the game theory developed during the cold war (Freedman 1989; Ringmar 2002; Sokolski 2004), which again suggested that reciprocation is a powerful strategy. However, in contrast to the lessons from the cold war, reciprocation in microbes is not about avoiding conflict; it is about being peaceful only once a competitor is eliminated.

3.5 Methods

A differential equation model of bacterial warfare

We build a general model of competition between strains (Figure 1). We employ differential equations, which are ideally suited to capture the temporal dynamics of strain interactions and, later, toxin regulation. Following Bucci et al. (2011), we study a competition between two strains that each possess a toxin that does not harm the producer but does harm the other strain. In reality, strains may carry multiple toxins and resistances (Gordon et al. 1998; Cordero et al. 2012b) and the evolution of multiple mechanisms of attack and defence is an interesting question in its own right. However, we focus here on a single toxin produced by each strain. We also follow the nutrients and cell numbers in a well-mixed

environment. The interaction of cells, nutrients and toxins can be then described by the system of ordinary differential equations (ODEs):

$$\begin{aligned}
\frac{dC_A(t)}{dt} &= (1-f_A)\mu_{max}\left(\frac{N}{N+K_N}\right)C_A(t) - kT_B(t)C_A(t),\\
\frac{dC_B(t)}{dt} &= (1-f_B)\mu_{max}\left(\frac{N}{N+K_N}\right)C_B(t) - kT_A(t)C_B(t),\\
\frac{dT_A(t)}{dt} &= f_A\left(\frac{N}{N+K_N}\right)C_A(t) - l_T T_A(t),\\
\frac{dT_B(t)}{dt} &= f_B\left(\frac{N}{N+K_N}\right)C_B(t) - l_T T_B(t),\\
\frac{dN(t)}{dt} &= -\left(\frac{N}{N+K_N}\right)[C_A(t)+C_B(t)],
\end{aligned} \tag{1}$$

where $C_A(t)$ and $C_B(t)$ are the cell biomasses of strain A and B, respectively. Cell strain A produces toxin A ($T_A(t)$) and strain B produces toxin B ($T_B(t)$), and both cell strains compete for growth-limiting nutrients ($N(t)$). The equations are satisfied over a range of $t \in [0,t_{end}]$, where t_{end} is the time for one strain interaction. We consider a pool of nutrient that is depleted by the cells. Similarly to Nadell *et al.* (Nadell et al. 2008) we describe the energy that is available to the cells by the Monod equation, in which K_N is the nutrient saturation constant. The maximum growth rate is given by μ_{max}. Toxins kill with efficiency k and are lost at a rate l_T. We assume that all toxins have identical loss and killing rates in order to remove biochemical differences between strains and focus our analysis on the effects of different production strategies.

For constitutive toxin production, the strategy of a strain is given simply by a fixed f ($f \in [0,1]$), which capture the investment into toxin production

relative to cell biomass. The production of antibiotics and bacteriocins can have significant metabolic costs and can even require a cell to lyse, as occurs with colicins and pyocins (Nakayama et al. 2000; Cascales et al. 2007). We model the cost of toxin production on cellular growth as a linear allocative trade-off function in the growth term (Bucci et al. 2011). A strain then that invests $f = 0.1$ into its toxin will only reach 90% of its maximal growth rate.

We solve the system of ODEs using a finite difference method in order to assess which of any two strains wins in direct competition (Methods). We use constant grid spacing and implicit Euler stepping with constant time steps to solve the equations numerically using MATLAB. Our implementation solves the equations until a time point where changes in the state variables become negligible (steady-state).

Invasion analysis to identify the evolutionary optimal level of toxin production

In order to study the evolution of different strategies of attack, we employ game theory and, in particular, invasion analysis to find the best strategies (Nowak and Sigmund 2004b), where the best strategy is one that, if adopted by the whole population, cannot be invaded by any other strategy. These strategies are also called evolutionarily stable strategies (Maynard Smith 1982). For constitutive toxin producers, we find the optimal investment in toxin production f^* by pairing strains with all possible values of f to determine which strategies can invade which.

For this imply a standard microbial life cycle (Cremer et al. 2012) that consists of a seeding step where local patches are seeded with two competing

strains, a competition step where strains grow and interacts according to the differential equations Eq. (1), and a mixing step where cells from all patches disperse and mix, leading to a new seeding episode. Without explicitly modelling this life-cycle, we ask whether a particular strain when rare f_{inv} can invade a population dominated by another strategy f_{res} (the resident, (Weibull 1997)). To answer this, we calculate the fitness of the resident strategy (w_{res}) and the fitness of the invading strategy (w_{inv}). The fitness of the resident is determined by the competition between two strains with resident strategy so that $w_{\mathrm{res}} = w(f_{\mathrm{res}}|f_{\mathrm{res}})$ and the fitness of the rare invader is determined by the competition between invader and resident strategy, $w_{\mathrm{inv}} = w(f_{\mathrm{inv}}|f_{\mathrm{res}})$. We define fitness of a strain as its biomass at the end of a competition. We then calculate the invasion index for an invading strategy according to Mitri *et al*. (Mitri et al. 2011) as

$$I_{inv} = \frac{w_{inv}}{w_{res}} = \frac{w(f_{inv}|f_{res})}{w(f_{res}|f_{res})}. \qquad (2)$$

When the invasion index I_{inv} is larger than one, the rare strategy can invade the resident strategy; when the index is smaller than one, the rare strategy cannot invade and it disappears. We implement strain competitions solving the system of ODEs given in Equation (1) as described above. By calculating the invasion index for a large number of invading strategy-resident strategy pairs, we obtain a pairwise invasibility plot (Brannstrom and Festenberg 2013) (Supplement Figure S1). Using this plot we find a single evolutionarily stable strategy f^* that can invade all strategy and that cannot be invaded by any other strategy. We determine this globally optimal strategy using the algorithm outlined in the

Supplementary Methods and Results. We can then ask how the key parameters in the model affect the evolution of toxin investment (Table 1).

A model of regulated toxin attack

In our sensing model, toxin production of bacterial strain A is either a function of nutrient depletion, toxin of strain B, or of quorum sensing (given as cell biomass of strain A). Each signal triggers toxin production via a simple on-and-off switch so that the toxin production of strain A is given through the one of the equations

$$f_A = f_{initial} + (f_{induced} - f_{initial})H\big[\big(N(t=0) - N(t)\big) - U_N\big], \quad (3)$$

$$f_A = f_{initial} + (f_{induced} - f_{initial})H(T_B(t) - U_{TB}), \quad (4)$$

$$f_A = f_{initial} + (f_{induced} - f_{initial})H(C_A(t) - U_{QS}), \quad (5)$$

where H is the Heaviside step functions given as

$$H(x) = \begin{cases} 0, x < 0 \\ 1, x \geq 0 \end{cases} \quad (6)$$

and where

$f_{initial} \in [0,1]$ and $f_{induced} \in [0,1]$

These equations of regulated toxin production each comprise the initial investment into toxins (f_{initial}) when the trigger term is deactivated and the trigger term. This trigger term contains a Heaviside step function and becomes active

when the signal increases over the sensing threshold ($U_N/U_{TB}/U_{QS}$). When activated, the trigger term changes the toxin investment to become the induced toxin investment ($f_{induced}$). We allow the induced toxin investment to be smaller (when the signal is a repressor) or larger than the initial toxin investment (when the signal is an activator).

Using invasion analysis, we search among the sensing strategies for those that can invade and replace all strategies of fixed toxin investment. Our search is a parameter grid-search that tests a large number of sensing strategies (stepping: $\Delta f_{initial}/f_{induced} = 0.02$ and $\Delta U = 0.002$) against the range of blind strategies. Also for the blind strategies we select from a fine grid spacing that also includes the optimal blind strategy ($f_{fixed} = [0.00, 0.01, 0.02, \ldots, 1.00]$).

Evolutionarily stable strategy of sensing strategies

For each type of regulation–through nutrient sensing, toxin sensing, and quorum sensing–we perform a large number of competitions and search for the evolutionarily stable strategy in an evolutionary algorithm. In this algorithm, the three parameters that define the sensing strategy will initially be selected at random, and this will be first resident strategy and its resident fitness is determined. The algorithm then tests for each iteration novel strategies to find higher quality strategies that invade and replace the current resident strategy. Novel strategies are generated either by small changes in one strategy parameters (for 50% of the iterations) or by replacing one parameter with a random number (for the other 50% of iterations). At the start of the algorithm parameter steps are relatively large ($\Delta 0.1$). But when the algorithm hits a resident strategy that cannot be replaced by the surrounding strategies, or entirely randomly generated

strategies, then it decreases the step size and searches again. Better strategies will invade, giving a succession of resident strategies (Figure 6a). Iterations are repeated until the algorithm reaches our preferred precision step size ($\Delta 0.001$) and a strategy, that cannot be invaded for another 1000 iterations. We call this the evolutionarily stable strategy. We determine this evolutionarily stable strategies for 30 instances of our algorithm. These strategies are very similar, yet they are not exactly identical because the relative fitness landscape very close to a stable strategy is shallow (de Mazancourt and Dieckmann 2004), which explains the standard deviation in Figure 6a.

3.6 Supplementary Methods and Results

Developing the ordinary differential equation model

Here we show how we develop our model of the competition between toxin producing strains. We start with a system of differential equations that builds on previous models of bacterial toxin competition (Bucci et al. 2011; Cornforth and Foster 2013). The model describes two cell strains that interact through producing toxins and competing for nutrients. In our model each strain produces a toxin that kills cells of the competitor strain. We assume that competing cell strains have identical biochemical properties, such as the cell yield and toxin efficiency, in order to focus our analysis on the effect of toxin production strategies. Our first system of ordinary differential equations (ODEs) is given through:

$$\begin{aligned}
\frac{dC_A(t)}{dt} &= (1-f_A)\mu_{max}\left(\frac{N}{N+K_N}\right)C_A(t) - \tau\alpha T_B(t)C_A(t),\\
\frac{dC_B(t)}{dt} &= (1-f_B)\mu_{max}\left(\frac{N}{N+K_N}\right)C_B(t) - \tau\alpha T_A(t)C_B(t),\\
\frac{dT_A(t)}{dt} &= \varepsilon f_A\left(\frac{N}{N+K_N}\right)C_A(t) - l_T T_A(t),\\
\frac{dT_B(t)}{dt} &= \varepsilon f_B\left(\frac{N}{N+K_N}\right)C_B(t) - l_T T_B(t),\\
\frac{dN(t)}{dt} &= -\frac{1}{\gamma}\left(\frac{N}{N+K_N}\right)[C_A(t)+C_B(t)],
\end{aligned} \tag{S1}$$

where $C_A(t)$ and $C_B(t)$ are the cell biomasses of strain A and B, respectively. Cell strain A produces toxin A ($T_A(t)$) and strain B produces toxin B ($T_B(t)$), and both cell strains compete for growth-limiting nutrients ($N(t)$). Analogous to Nadell et *al*. (Nadell et al. 2008) we model consumption of nutrients

according to the Monod equation with the saturation constant K_N. Nutrients are consumed inversely proportional to γ, and proportional to the yield of the cells, the nutrient uptake and the total cell biomass. Cells can then invest energy either into cell mass (growth) or toxin. Toxin production is given through the strain specific strategy parameter f (f_A for strain A and f_B for strain B) and the yield parameter ε. Growth is given through (1-f) and the maximum growth rate μ_{max}. Toxins kill according to the mass action principle, proportionally to their affinity to target cells, and proportionally to τ, their killing efficiency, and toxin are lost at a rate l_T. The system of ODEs is satisfied over a range of $t \in [0,T]$ where T is the time for one strain interaction.

We then simplify the system of equations to reduce the number of model parameters. We multiply the equation for the change in nutrients by the consumption yield γ and can remove the yield parameter. We collapse the parameters k, α, and ε into a single compound parameter k, that gives the quality of a toxin. The simplified system of differential equation is given through:

$$\begin{aligned}
\frac{dC_A(t)}{dt} &= (1-f_A)\mu_{max}\left(\frac{N}{N+K_N}\right)C_A(t) - kT_B(t)C_A(t),\\
\frac{dC_B(t)}{dt} &= (1-f_B)\mu_{max}\left(\frac{N}{N+K_N}\right)C_B(t) - kT_A(t)C_B(t),\\
\frac{dT_A(t)}{dt} &= f_A\left(\frac{N}{N+K_N}\right)C_A(t) - l_T T_A(t),\\
\frac{dT_B(t)}{dt} &= f_B\left(\frac{N}{N+K_N}\right)C_B(t) - l_T T_B(t),\\
\frac{dN(t)}{dt} &= -\left(\frac{N}{N+K_N}\right)[C_A(t)+C_B(t)],
\end{aligned} \tag{S1}$$

with the model parameters given in Table 1.

Invasion analysis

Among the possible toxin strategies, we search for the evolutionarily stable strategy, that is defined as a strategy that, if adopted by the entire population, cannot be invaded by any other strategy. We use invasion analysis to test if a strategy f_{inv} can invade a strategy f_{res}. This analysis makes simplifying assumptions based on the following microbial lifecycle:

Consider a diverse population of bacterial strains, in which each strain i produces its unique toxin T_i with strategy f_i. We imagine a standard microbial life-cycle of three phases (Cremer et al. 2012; Oliveira et al. 2014): In phase 1 ('Seeding') two strains are seeded to compete with each other. Their toxin strategies are chosen according to the strategy frequencies in the entire population. In phase 2 ('Competition') these strains compete following the Eq. (S2). In phase 3 ('Mixing') all strains are mixed into one pool and the new frequencies of strategies determine their abundance in the seeding phase. We assume a large and diverse population, so that a strain with unique toxin T_i is always rare, while the frequency of its adopted strategy f_i in the population might become significant. Instead of simulating the entire microbial population, we can use the method of invasion analysis to perform simplified tests for strategy invasion.

For this, we assume that the population is dominated by a single strategy, given by f_{res} and we consider a rare invader strategy f_{inv} that arrives in the population through migration. We consider migration rather than mutation, because in natural populations it is migration that brings in new, diverse toxic strains. To determine the potential of strategy f_{inv} to invade f_{res} we calculate the fitness of the resident strategy (w_{res}) and of the invader strategy (w_{inv}). In general,

we define the fitness of a strain A in competition with strain B as the biomass of strain A at the end of this competition ($w_A = w(f_A|f_B)$). The fitness of the resident strategy is then $w_{res} = w(f_{res}|f_{res})$ and the fitness of the invader strategy is $w_{inv} = w(f_{inv}|f_{res})$. We solve the system of ODEs given in Eq. (S1) until the system reaches steady-state, using finite difference method with constant grid spacing and implicit Euler stepping with constant time steps to solve the equations numerically using MATLAB. The code for the numerical solution is given below in Code 1.

We calculate the invasion index according to Mitri et *al*. (Mitri et al. 2011) as

$$I_{inv} = \frac{w_{inv}}{w_{res}} = \frac{w(f_{inv}|f_{res})}{w(f_{res}|f_{res})}. \quad \text{(S2)}$$

When this invasion index I_{inv} is larger than one, the rare strategy can invade the resident strategy and when I_{inv} is smaller than one the rare strategy cannot invade and it disappears. By pairing a range of different toxin investment strategies in the roles of resident and rare invader strategy we obtain the pairwise invasibility plot, that helps to visualise the evolutionarily stable strategy f^* (Supplement Figure S1). We then implement an efficient algorithm to identify the optimal strategy f^*. This algorithm is outlined in Code 2.

Code 1 | MATLAB Code for numerically solving Equation (S1).

```
function [t, X] =
my_own_ode46K_sensALL(a_strat,b_strat,param,InitC,TIME,dt,endtimewarning,
plotting)
%my_own_ode45 Solving my ODEs with fixed timestep
StartTime = TIME(1); Endtime = TIME(2);
t = StartTime:dt:Endtime;     % time discretization
N = length(t);  % number of time points
X = zeros(7,N); % initialise X

% Initialise X
X(1:5,1) = InitC; % Ca, Cb, Ta, Tb, N
[fa,fb] = my_own_fafb02K_sensALL(a_strat,b_strat,X(1:5,1));
X(6:7,1) = [fa;fb];
stead_state_reached = 0;
i = 1; % initiate step counter
dSV = inf; % initiate change condition
while sum(abs(dSV)) > 1e-8 && t(i) < Endtime
    i = i + 1;
    dSV = ODE_amSee04K_NiehusToxinODEmonod_sensALL(X(:,i-1),param)*dt; %
delta state variables
    X(1:5,i) = X(1:5,i-1) + dSV;
    [fa,fb] = my_own_fafb02K_sensALL(a_strat,b_strat,X(1:5,i));
    X((X(:,i) < 1/1000000),i) = 0; % correct so that state variables are
never < 0
    X(6:7,i) = [fa;fb];
end
if t(i) == Endtime && endtimewarning == 1
    disp('Steady-state not reached!!')
end
t = t(:,1:i);
X = X(:,1:i);
end % end of function

function dXdt = ODE_amSee04K_NiehusToxinODEmonod_sensALL(X,param)
Ca=X(1); Cb=X(2); Ta=X(3); Tb=X(4); N=X(5);
fa = X(6); fb = X(7);
%Cell Density A
% (N/(N+param.KN)) = N
dCadt =(1-fa)*param.mu*(N/(N+param.KN))*Ca - param.kay*Tb*Ca;
%Cell Density B
dCbdt =(1-fb)*param.mu*(N/(N+param.KN))*Cb - param.kay*Ta*Cb;
%Toxin A Concentration
dTadt = fa*param.q*(N/(N+param.KN))*Ca - (param.l + param.D)*Ta;
%Toxin B Concentration
dTbdt = fb*param.q*(N/(N+param.KN))*Cb - (param.l + param.D)*Tb;
% explicit Nutrients
dNdt = -param.gamma*(N/(N+param.KN))*(Ca + Cb) +
param.beta*param.kay*(Tb*Ca + Ta*Cb);
%%%% put them all together
dXdt=[dCadt;dCbdt;dTadt;dTbdt;dNdt];
```

```
end % end of function
```

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Code 2 | Pseudo code to find the evolutionarily stable strategy of fixed toxin production

Initialization: Find the initial resident fresini (for example, start with the highest average resident biomass)

forward = 1 *indicates if the next migrant will have a higher or lower toxin investment than the resident. Can be 1 or -1.*

previousforward = 1 *record the direction of strategy change from the previous iteration*

previousfres = 0 *record the resident from the previous iteration*

step = 0.1 *initial step for the strategy change (initially coarse to localize the ESS)*

minstep = 0.0001 *the precision we want for the strategy value*

singular = 0 *boolean saying if a singular strategy is localised*

nbflip = 0 *record the number of consecutive flips in direction*

fres = fresini

newres = 0 *boolean saying if there is a new resident*

while (!singular) {*do this while no singular strategy is localised*

if (newres) {

res vs N res competition. *Compute the resident against the resident. Record the resident average strategy: wresav.*

}

fmut = fres + forward*step *pick an invader that differs from resident according to direction*

mut vs N res competition. *Compute mutant vs resident competition. Record the mutant biomass (wmut) at the end of the competition.*

previousforward = forward *update the previous direction*

previousfres = fres *update the previous resident before the competition*

if (wmut < wresav) {*resident stays the resident.*

forward = - forward *change the direction, to test a migrant with lower f value at the next step*

}

if (wmut > wresav) { *the mutant invades and replaces the resident*

fres = fmut *the new resident will take the migrant value*

newres = 1 *we have a new resident, we'll have to compute*

its resident fitness at the next iteration

}

if (forward != previousforward) { *compare the direction with previous direction*

nbflip = nbflip + 1

}

if (forward == previousforward) { *compare the direction with previous direction*

nbflip = 0

}

this will allow to localise an ESS. We want fres to stay identical for 2 consecutive time steps, but we want to make sure that the higher and lower strategies have been checked. Indeed if the mutant that we just tested goes extinct, the resident will stay resident but that is not enough to say it is an ESS. We also want the number of flips in direction to be > 1.

if (previousfres == fres & nbflip > 1) { *singular strategy localised*

if (step <= minstep) {*precision is high enough*

singular = 1 *we have found the optimal strategy*

}

if (step > minstep) { *will redo the whole procedure with tinier step*

step = step/10 *increase precision 10 times*

nbflip = 0 *reset the number of consecutive flips*

}

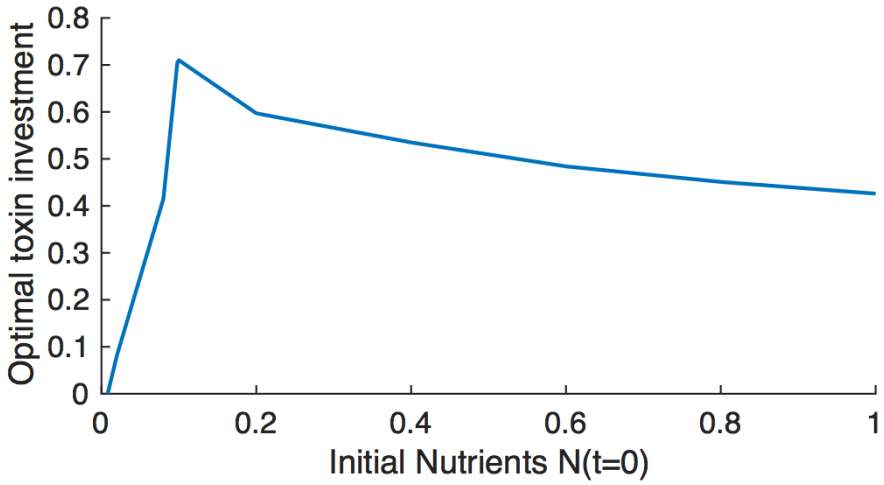
}

}

Record the final fres (ESS).

Finally, we test whether this fres (ESS) is a GLOBAL optimum by competing it against the range of strategies given through f = [0, 0.01, 0.02, ….1].

Supplementary Figure



Supplementary Figure S1 | The effect of nutrient availability on optimal toxin investment. We plot the evolutionary stable investment into toxin over a range of different initial levels of nutrient. The optimal investment is highest for an intermediate amount of nutrients. Other parameters of the model take the standard values given in Table 1.

4 Migration and horizontal gene transfer divide microbial genomes into multiple niches

4.1 Abstract

Horizontal gene transfer is central to microbial evolution because it enables genetic regions to spread horizontally through diverse communities. However, how gene transfer exerts such a strong effect is not understood. Here we develop an eco-evolutionary model and show how genetic transfer, even when rare, can transform the evolution and ecology of microbes. We recapitulate existing models, which suggest that asexual reproduction will overpower horizontal transfer and greatly limit its effects. We then show that allowing immigration completely changes these predictions. With migration, the rates and impacts of horizontal transfer are greatly increased, and transfer is most frequent for loci under positive natural selection. Our analysis explains how ecologically important loci can sweep through competing strains and species. In this way, microbial

genomes can evolve to become ecologically diverse where different genomic regions encode for partially overlapping, but distinct, ecologies. Under these conditions, ecological species do not exist because genes, not species, inhabit niches.

4.2 Introduction

Microbes survive and reproduce in an extremely wide range of environments, from hydrothermal vents (Jannasch and Mottl 1985), through marine snow (Azam and Long 2001) and soil (Torsvik et al. 1990), to host associations such as the human microbiome (Ley et al. 2006). Within and between such environments, microbial genomes differ widely both in terms of allelic diversity and gene content (Torsvik and Ovreos 2002; Thompson et al. 2005; Dethlefsen et al. 2006). At the heart of this genetic diversity is the ability of microbes to gain both homologous and non-homologous DNA via horizontal gene transfer. This process occurs through one of three different mechanisms: uptake of naked DNA from the environment into the cell and incorporation into the genome (transformation), transfer of DNA through phages between phage-susceptible cells, and finally transfer of plasmids or conjugative elements between neighbouring cells through a pilus (conjugation). These transfers appear to occur in almost all prokaryotic lineages and have significant impacts on both bacterial and archaeal genomes (Koonin et al. 2001).

Horizontal transfer is considered central to the ability of cells to adapt to new ecological conditions, including clinical or environmental settings that contain antibiotics (Cordero et al. 2012b; Ojala et al. 2014). Recent empirical

work suggests that these transfers can spread a single beneficial allele horizontally through a microbial community–where the allele can either represent a single gene or a small group of genes–with the result that an otherwise diverse microbial community becomes genetically identical in a certain genomic region (Whitaker et al. 2005; Boucher et al. 2011; Shapiro et al. 2012; Cordero et al. 2012b; Shapiro and Polz 2014; Cordero and Polz 2014). However, the rates at which gene transfers occur are thought to be extremely low, with asexual reproduction a much faster process. Competition between strains and species within a patch, therefore, will mean that a beneficial allele can spread much more quickly via whole-genome vertical transmission than horizontal transmission, which should prevent horizontal sweeps (Cohan 1994; Cohan 1995; Gevers et al. 2005; Shapiro et al. 2009). One way that genes can transfer horizontally is if they hop between ecologically-distinct populations that do not compete (Majewski and Cohan 1999; Cohan 2001; Kopac and Cohan 2012). However, the prevalence of competition within microbial communities (Foster and Bell 2012; Mitri and Foster 2013) suggests that vertical sweeps should remain a barrier to horizontal sweeps. And yet, the experimental evidence for horizontal sweeps comes from communities of phylogenetically-related strains (Whitaker et al. 2005; Boucher et al. 2011; Shapiro et al. 2012; Cordero et al. 2012b; Kumar et al. 2015) where ecological competition is likely to be significant.

A major question then is how horizontal transfer can so strongly impact microbial communities and cause the observed horizontal sweeps. Answering this question is necessary to understand microbes and how they evolve, both in nature and in the clinic. In a recent study, Takeuchi et al. (2015) provide an explanation for horizontal sweeps via negative frequency-dependent selection acting on other

loci in the genome. Frequency-dependent selection can prevent full genome-wide selective sweeps and allow more time for genetic transfer. Here we show that horizontal sweeps can occur without the need for negative frequency-dependent selection. We develop a series of models of a microbial community in which all cells compete for the same resource. We use these models to study both the rate of horizontal gene transfer and the impact that this transfer has on the genomics of the focal community. Our work reveals a missing ingredient that can explain horizontal sweeps: migration. When including immigration in our models, we find that the highest rates of horizontal transfer will occur for ecologically-important traits that are under positive natural selection. The result is a genome where selected regions become partially decoupled from the ecology of the remaining loci such that different parts of the genome map to different niches.

4.3 Results

Overview

We are interested in understanding how a beneficial trait can spread horizontally through a microbial community. We build on a previously modelled scenario (Levin 1981; Shapiro et al. 2009) that considers a community of diverse strains that compete and that will have the opportunity for genetic transfer (Smillie et al. 2011; Kav et al. 2012). Although we use the word 'community' throughout, our model can also capture a set of strains from a single species, commonly referred to as a 'population'. We follow the fate of a novel beneficial allele that is able to transfer horizontally between the genotypes in our community. Existing theory suggests that very little gene transfer should occur because, once a beneficial trait

is picked up horizontally, the carrier will rapidly outcompete the other strains in the patch before it has a chance to transfer the trait to other genomes (Levin 1981; Cohan 1994; Cohan 1995; Shapiro et al. 2009). However, previous models neglect the possibility that fixation of a trait can be delayed or prevented by migration of new cells into the community. We therefore begin by recapitulating previous predictions that competition suppresses gene transfer and we then show how making a single change, allowing immigration, can explain how horizontal genetic sweeps occur.

Without migration adaptation by horizontal transfer is rare

We consider a community that lives in a focal patch, which can either be literally a single isolated patch or a set of similar patches that are themselves well-connected by migration. At first we consider that this patch is largely isolated from the external environment, such that there is negligible migration between the community of the patch and the world at large. This scenario could correspond, for example, to communities living in hosts where there is limited super-infection with new strains and species over time (Marvig et al. 2014). We focus on those strains in the patch that are ecologically interchangeable in the sense that they compete for a common limiting resource. Under these conditions, horizontal transfer has been predicted to be almost powerless compared with vertical selective sweeps (Levin 1981; Cohan 1994; Cohan 1995; Shapiro et al. 2009)

Our model follows the fate of a rare beneficial allele that first appears in a small subset of the community. While non-carriers have fitness $w = 1$, carriers of the adaptive allele have increased fitness $w = 1 + s$, where s is the local benefit of the trait (b) minus the cost of carrying it (ε). We assume that the benefit of the

trait in the focal patch is always larger than its cost, so that $s > 0$. Any cell in the community lacking the gene has a chance of picking it up horizontally, and it is the overall rate of this process in the community that we are interested in. Not all loci can transfer their phenotypes horizontally (Jain et al. 1999; Thomas and Nielsen 2005; Wiedenbeck and Cohan 2011), and we focus here on those loci where horizontal transfer is possible. In addition, genetic transfer is typically only relevant for phenotypes that lie outside the range of physiological responses or short term evolution by de-novo mutation (Jain et al. 2003; Earl and Deem 2004). Examples of such phenotypes–transferable and otherwise hard to achieve–include toxin resistance genes (Nakamura et al. 2004), virulence factors (Chen and Novick 2009), and heat shock proteins (Gogarten and Townsend 2005).

We want to capture the horizontal spread of an allele in a community of microbes. Although there is relatively little theoretical work on genetic transfer and its impact, there is a long theoretical tradition of modelling the horizontal spread of infectious diseases, along with associated empirical tests (Baker 1985; Recker et al. 2007). These models, often known as compartment models, have proved to be a powerful way to capture the key processes underlying the horizontal spread of a focal trait. We therefore begin here with a simple compartment model (Fig. 1a) that allows us to identify the conditions that maximize horizontal genetic transfer, before extending and developing our predictions using other modelling approaches.

We study a focal community of constant size N that contains two sub-communities, adaptive gene 'carriers' and 'non-carriers'. Applying a deterministic continuum approach, the relative community size in each sub-community and the flux between them can be described by the ordinary differential equation (ODE)

$$\frac{dC(t)}{dt} = rC(t)\big(1 - C(t)\big) + \frac{s}{1+sC(t)}C(t)\big(1 - C(t)\big), \quad (1)$$

where $C(t)$ is the fraction of carriers in the community at time t, 1 - $C(t)$ is the fraction of non-carriers, and r is the rate of gene transfer from carriers to non-carriers (Fig. 1a). The growth of compartment C due to selection is given through the term $s/(1 + sC(t))$. The term $rC(t)(1 - C(t))$ captures the transition of cells from being a non-carrier to carrier through horizontal gene transfer. The proportion of trait carriers has two steady-states (C^*) when s or r are non-zero, given by

$$C^* = 0\,, C^* = 1, \quad (2)$$

with $C^* = 0$ being an unstable and $C^* = 1$ a stable equilibrium (see *Supplementary Methods*). Therefore, for any non-zero initial number of carriers ($C(0) > 0$) the fraction $C(t)$ will increase until fixation of the focal beneficial allele ($C^* = 1$) at steady-state. A key model parameter for our further analysis is the probability that a carrier cell transfers the focal locus to a non-carrier in any generation, r. Gene transfer rates in natural communities remain largely unknown. Previous theoretical work used a relatively low and conservative estimate for transfer rate of 10^{-6} per gene per generation (Wiedenbeck and Cohan 2011), which corresponds to our transition rate ($rC(t)(1 - C(t))$). However, recent studies suggest that the rates of horizontal transfer can be much higher than such estimates (Overballe-Petersen et al. 2013; McCarthy et al. 2014). We therefore consider a range of rates, $10^{-6} < r < 10^{-4}$, which goes either side of 10^{-6} per gene per generation (using $r = 10^{-6}$ guarantees that the transition rate $rC(t)(1 - C(t))$ is

less than 10^{-6}). Genetic transfer can occur both by homologous and non-homologous recombination. While we phrase our results here in terms of the former, our conclusions should apply to both mechanisms.

What then defines the rate of horizontal gene transfer in the whole patch? We can calculate this rate by multiplying the transition rate from carriers to non-carriers, $rC(t)(1 - C(t))$, by the community size N to obtain the community-level rate of gene transfer, which we term horizontal gene flux ($rC(t)(1 - C(t))N$). This horizontal gene flux spreads the beneficial gene without removing cells, while the vertical gene flux, given through $sC(t)(1 - C(t))$, spreads the gene by removing non-carrier cells.

As the selective sweep for the focal trait proceeds, this horizontal gene flux initially increases up to a maximum at a 1:1 ratio of donors and recipients ($C(t) = 1 - C(t) = 0.5$) before decreasing back down to zero at allele fixation (Fig. 1b). The overall impact of this process can be quantified from the integral of the gene flux, which gives the expected number of horizontal gene transfer events over time (cumulative gene flux, *Methods*). How is this cumulative gene transfer affected by the strength of positive selection? Plotting cumulative gene transfer against selection pressure shows that strong selection regimes minimize the effects of horizontal transfer because the time window during which this transfer can occur is short (Fig. 2b, inset). Our model then recapitulates previous conclusions that, given a small rate of genetic transfer, only weakly selected traits can undergo significant horizontal transfer by slowly sweeping through a community (Shapiro et al. 2009). However, weakly selected traits are, by definition, relatively unimportant for the ecology and evolution of their carriers. In contrast, many successful horizontally-transferred traits, such as antibiotic

resistance genes, appear to be both functionally important and under significant positive natural selection (Bergstrom et al. 2000; Rankin et al. 2011; Kav et al. 2012) and these are the traits that we are interested in here.

Table 1 | Parameters used in the compartment model.

Parameter	Range	Description
N	*Positive integers*	Carrying capacity of the community
C_N	[0,N]	Total number of trait carriers in the focal patch
C	[0,1]	Faction of carriers in the focal patch
r	[0,1]	Rate of gene transfer between trait carriers and non-carriers per generation
s	[0,∞]	Strength of positive selection, given as the fitness increase of allele carriers in the focal patch
m	[0,1]	Migration rate per generation time, given as fraction of the focal patch

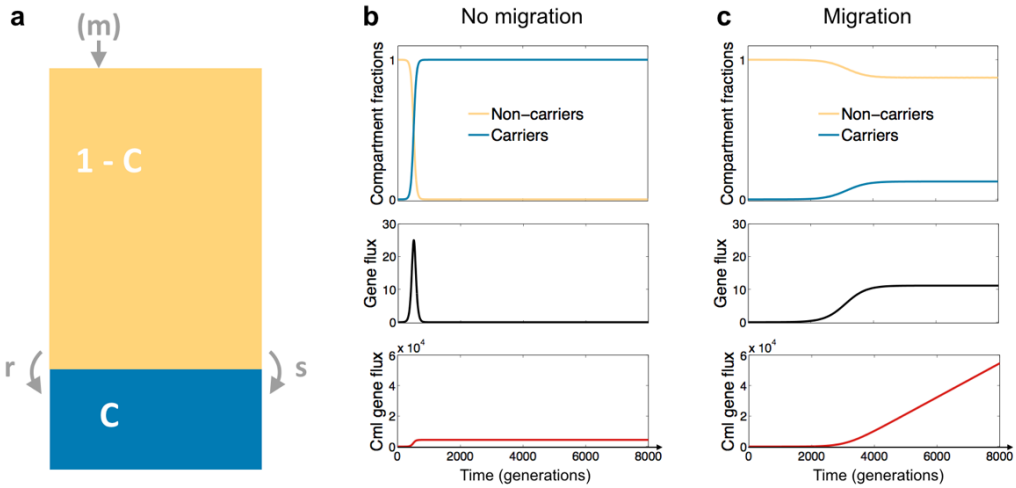


Figure 1 | Migration greatly increases the potential for horizontal genetic transfer of a beneficial allele. (**a**) The compartment model. Sub-communities C (blue) and 1-C (yellow) represent the fractions of allele carriers and non-carriers in the community, respectively. In the absence of migration, positive selection (s) causes a replacement of non-carriers by carriers and gene transfer (r) converts non-carriers into carriers. In the presence of migration, non-carriers continuously arrive at the patch in addition to the processes of positive selection and gene transfer. (**b**) No migration: The beneficial allele is rapidly fixed, so that the horizontal gene flux becomes zero and the total amount of horizontal transfers since beginning of the sweep, the cumulative gene flux (cml gene flux), stays constant. (**c**) With migration: the immigration supplies the system with non-carriers, resulting in ongoing gene transfer at a constant rate. Other parameters are $N = 10^8$, $s = 0.025$, $r = 10^{-6}$, $m = 0$ (**b**) and $m = 0.02$ (**c**).

With migration adaptive traits transfer horizontally

Previous models of genetic transfer have not considered a key feature of microbial life, migration, which has the potential for important effects on population dynamics (Hall-Stoodley and Stoodley 2005; Grossart et al. 2010). We next introduce migration into our model, and we study its effect on our predictions. We now assume that our focal patch is separated, but not completely isolated, from its external environment, so that there will be a limited but on-going exchange of cells. For example, this focal patch could be a nutrient particle in ocean water, a mammalian host, or tree hole. As discussed for our no-migration model, the "patch" can also represent a set of connected patches that all select for the same

horizontally-transferred trait i.e. a set of particles, hosts or tree holes. The key is that we now allow there to be immigration from other regions that do not select for the focal trait. Accordingly, we assume that all immigrating cells that arrive from outside the focal patch (or patches) lack the trait (but we relax this assumption below). For example, the focal locus might provide resistance to a toxin that is specific to the focal patch. Some immigrating cells will be unable to establish themselves in the focal community due to a mismatch with general ecological characters, such as nutrient conditions, temperature and alike. Other migrants might not be compatible with the selected locus and are unable to adapt. However, both of these effects will lead to fast extinction of these migrants and we can account for both by varying the migration rate, where an increase in the frequency of non-viable strains corresponds to a reduced migration rate. Extending our model to include migration gives

$$\frac{dC(t)}{dt} = rC(t)\big(1 - C(t)\big) + \frac{s}{1+sC(t)}C(t)\big(1 - C(t)\big) - mC(t), \qquad (3)$$

where m is the migration rate, given as the fraction of cells that is replaced through migrators landing and replacing them per unit time, and $mC(t)$ is the replacement of trait carriers only (Fig. 1a). If the basic rate of genetic transfer is very small ($r \approx 0$), the steady-state proportions of cells carrying the focal trait (C^*) are given approximately by

$$C^* = 0 \ and \ C^* = \frac{s - m}{(m + 1)s}, \qquad (4)$$

with $C^*= 0$ defining an unstable equilibrium and $C^* = (s - m)/((m + 1)s)$ a stable equilibrium (see *Supplementary Methods*). Therefore, given an initial, non-zero number of carriers, the system will reach the second equilibrium ($C^* = (s - m)/((m + 1)s)$). A key implication of this expression is that with non-zero migration ($m \neq 0$) the selected trait will now reach a steady-state before it has been fixed in the community ($C^* < 1$), because migration continuously brings new genotypes into the system. These migrators mean that opportunities for genetic transfer remain after the initial selective sweep has occurred. Migration stops the selective sweep before it can complete as a classic selective sweep (Burke 2012) and instead there is a second longer-lasting incomplete sweep. Indeed, horizontal transfer now occurs as long as the community persists, which greatly increases its potential effects (Fig. 1c and Fig. 2a). Migration rates are commonly considered to be high in natural microbial communities as cells can be so easily dispersed, but exact rates are difficult to assess. In these first models, we use a relatively high rate of $m = 0.02$, which corresponds to 2% of cells being replaced by incoming cells each generation. However, we show in the next section that our conclusions are robust for a range of possible migration rates, just so long as migration does not overpower natural selection ($m < s$).

With migration in the model, the relationship between the strength of natural selection and the cumulative gene transfer is fundamentally changed. Now, horizontal transfer peaks for traits under intermediate selection pressure (Fig. 2), whereas without migration it peaks at minimum selection strength (Fig. 2b, inset). This means that migration greatly increases the potential for horizontal sweeps of ecologically-important traits that are associated with significant positive selection pressures, e.g. a trait that provides a fitness advantage of more

than 10% (Fig. 2). The sweep occurs in spite of the fact that vertical flux remains the dominant mode of transmission in the community; even modest rates of natural selection ($s > 10^{-3}$) are much greater than the expected rates of gene transfer ($10^{-6} < r < 10^{-4}$, Wiedenbeck and Cohan 2011).

We have assumed so far that incoming migrators lack the adaptive trait. However, there are clearly cases where new cells may be pre-adapted and carry the focal trait. When will this occur and how does it change our predictions about horizontal gene flux? To investigate this, we consider an extended model that explicitly captures the external environment as an additional compartment where the focal trait is disfavoured by natural selection (Supplementary Fig. 1). Cells from the focal patch can leave and enter the surrounding environment and, equally, cells can return from the surrounding environment into the focal patch. As before, our 'focal patch' can also represent a set of connected patches that all select for the same focal trait, which are surrounded by the wider external environment that does not favour the trait.

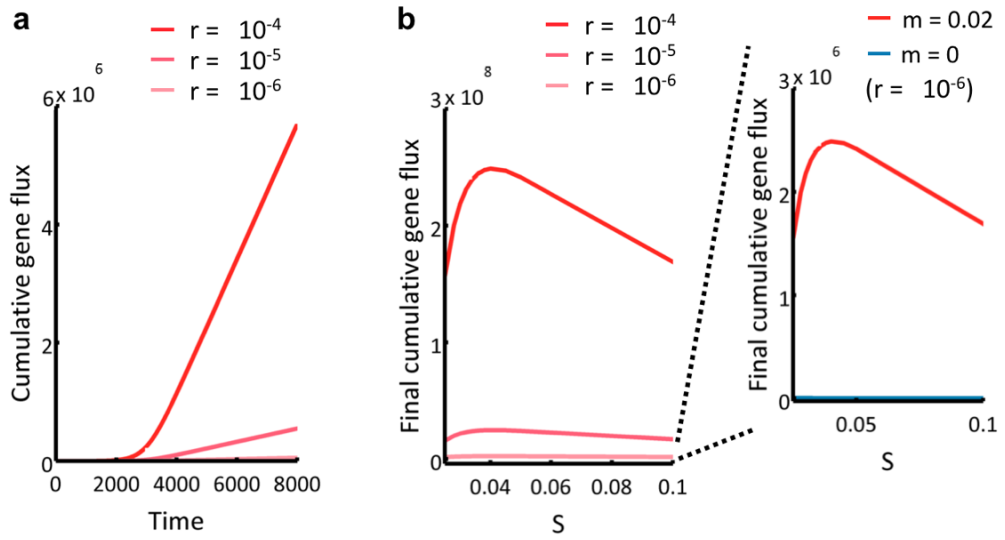


Figure 2 | Migration allows horizontal transfer even with significant positive natural selection for a transferred trait. (**a**) Gene transfer events accumulate over time when immigration is possible, with increased gene transfer rates increasing the horizontal gene flux. (Selection pressure $s = 0.025$) (**b**) Cumulative horizontal gene flux measured at $t = 10^5$ across different selection pressures (left hand side plot) and for different rates of migration (right hand side inset). With migration (left hand plot, $m = 0.02$), the cumulative gene flux peaks for intermediate selection pressure ($s = 0.04$ with ~2.5×10^6 transfer events) and remains significant even for stronger positive selection ($s = 0.1$ with ~1.7×10^6 transfer events). Without migration (blue line in right hand side plot, $r = 10^{-6}$), gene transfer remains extremely infrequent and peaks at the lowest selection pressure with only around 4,000 transfer events. In all plots, we calculate cumulative gene flux for $N = 10^8$, and $C(0) = 1000/N$.

Our extended model makes the same predictions as our original model with migration whenever the external environment is large relative to the focal patch (Supplementary Figs 2 and 3). This is intuitive: a large external environment means that the focal gene is likely to be lost outside the focal patch before a cell returns such that few or no immigrants will possess the focal trait. By contrast, when the external environment is itself a small patch, the focal patch and the external environment converge to act as a single patch in which the focal gene reaches fixation with limited horizontal gene transfer (as seen in the no-migration model above, Supplementary Figs 2 and 3). More specifically, the size of the

external environment and its selection against the focal locus are a proxy for the rarity of the focal niche: a large external environment that selects against the focal locus means that the focal niche is relatively rare. For the rest of the study, we focus on this case where a focal niche is rare relative to the environment from which immigrants arrive, such as a niche that selects for resistance to a specific antibiotic (Riley and Wertz 2002a). Under these conditions, the great majority of immigrants will be non-adapted.

Horizontal transfer divides the genome into distinct niches

We have shown that migration from outside of a focal patch greatly increases the potential for gene transfer in microbial communities. But is this increased transfer important for the ecology and evolution of microbes? Specifically, we are interested in whether the rate of horizontal transfer is sufficient to generate a horizontal selective sweep whereby a particular allele moves horizontally through a diverse community of microbes. To address this question, we next investigate how gene transfer with migration affects genomic diversity, at both the horizontally transferred and the non-transferred regions of the microbial genomes. While compartment models allow us to follow the dynamics of horizontal transfer and identify the population processes driving a horizontal sweep, these models are not well suited to follow genomic effects. We therefore next develop a coalescence model to capture the genetic effects of horizontal transfer, selection, and migration probabilistically.

Our new model assesses the impact of genetic transfer in terms of how much it can decouple evolution at the genetic locus of the beneficial allele (focal locus) from the rest of the genome (background genome). We determine this

effect by comparing the diversity at the focal locus (D_f) to the diversity at the background genome (D_{bg}), in the diversity ratio DR = D_{bg}/D_f (for details see *Methods*). Without migration, the diversity ratio changes little over time and remains close to one (Fig. 3a-c). Consistent with the predictions of our first model, we see very little effect of horizontal transfer when there is no migration. Genetic transfer is largely powerless to evolve the focal locus independently of the background genome and the two remain locked together in a vertical selective sweep that purges diversity in both genetic regions.

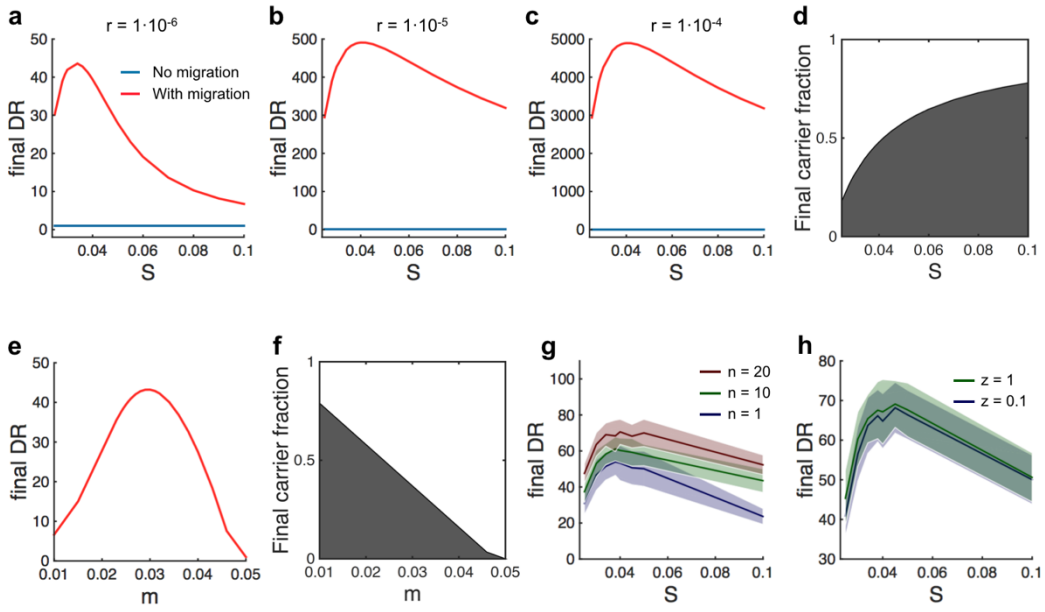


Figure 3 | Migration enables horizontal genetic sweeps. We plot for $r = 10^{-6}$ (**a**), $r = 10^{-5}$ (**b**) and $r = 10^{-4}$ (**c**) the effect of horizontal transfer on genetic diversity in the coalescence model, with and without migration. With migration, the diversity ratio (*DR*), which gives the strength of the selective sweep, peaks at intermediate selection pressure ($s \approx 0.04$), while (**d**) the fraction of cells carrying the same adaptive gene increases monotonically with selection pressure, reaching about 80% for all three transfer rates. Parameters are $t = 5\text{x}10^6$, $N = 10^8$, and $C(0) = 1000/N$, and either $m = 0$ or $m = 0.02$. (**e**) The effect of horizontal gene transfer on the diversity ratio and (**f**) the final fraction of gene carriers over a range of migration rates ($s = 0.05$, $t = 5\text{x}10^6$, $N = 10^8$, and $C(0) = 1000/N$). (**g**) The individual-based model shows the effect of ecologically different migrants for an increasing number of ecological niches ($n = 1$, 10, 20 and $z = 0.2$). (**h**) We also plot (for $n = 20$) the diversity ratio against selection pressure for two different levels of ecological specialization: cells competing twice as much in their own niche than in other niches (blue line), and cells that compete entirely in their own niche (green line). Other parameters are $N = 10^6$, $t_{end} = 10^5$, $r = 10^{-4}$, $C(0) = 1000/N$. Results are averaged over 50 simulations, and the standard deviation is given by the transparent areas.

We next consider the case where there is immigration into the focal patch. Now, the behaviour of the model is very different. We find a wide range of parameter values for which the combination of positive natural selection, migration and horizontal transfer largely purge diversity at the focal locus while leaving significant variability in the background genomes (high DR, Fig. 3a-c,e). The result is that the majority of cells carry the same adaptive allele (high fraction of carriers, Fig. 3d,f), while their background genomes remain diverse. The relative proportion of adaptive gene carriers increases monotonically with a stronger selection pressure (Fig. 3d) and with a decreasing migration rate (Fig. 3f). The DR is maximal when migration rate and positive selection are in a balanced regime. That is, some immigration is needed for an effective horizontal sweep to occur but, as seen in results from classical population genetics (Felsenstein 1976), if migration is too strong ($m > s$) then it will overpower selection and prevent adaptive evolution (Fig. 3e,f). As a result, the horizontal gene sweep is most effective at intermediate positive selection pressures (Fig. 3a-c).

This result has major implications for the evolution of microbial communities in the face of horizontal transfer. Positive natural selection is no longer a barrier to the horizontal spread of a trait. Instead, the impacts of genetic transfer are greatest for ecologically-important traits that are under positive natural selection. In this way, a horizontally-transferred trait with its own specific ecology is able to move through a diverse set of strain backgrounds. As we discuss below, a key implication of this uncoupling is that genomes can become ecologically diverse in the sense that the ecology of the focal locus and the rest of the genome are overlapping but distinct.

Ecological division of the patch promotes horizontal sweeps

We have so far focussed on an ecologically-cohesive community because the potential for diverse genotypes to compete ecologically is clear (Hibbing et al. 2010; Foster and Bell 2012; Mitri and Foster 2013), and also because previous work suggests that competitive exclusion in a community is the worst case scenario for gene-specific horizontal sweeps (Levin 1981; Cohan 1994; Cohan 1995; Shapiro et al. 2009). For this reason the above results should be conservative in their estimates of how migration promotes horizontal sweeps. However, we can use an individual-based model to relax our assumption about competition within the community and study the consequences for gene sweeps and genomic diversity.

We introduce ecological differences between genotypes by introducing n different background niches in our focal patch, which could represent specialization on different resources. Each incoming cell then belongs to one background niche but also partly competes within the other niches. We denote the extent to which a cell competes within its assigned niche as z, whose value lies between 0 and 1, where $z = 1$ corresponds to a cell competing purely in its own niche and $z = 1/n$ corresponds to a cell competing equally in all niches (see *Methods*). Our simulations show that the genetic effect of horizontal gene transfer increases with an increasing number of distinct niches (n) in the community (Fig. 3g) and gene transfer is also increased by a stronger separation of the cells into separate niches (increasing z, Fig. 3h). This result is intuitive: the ecological subdivision reduces competition between cells in the different niches and thus reduces the loss of diversity in the background genome during the selective sweep. This effect of ecological subdivision agrees with the study of Majewski

and Cohan (Majewski and Cohan 1999) who found that gene transfer has greater impact if communities were subdivided into a number of completely non-competing lineages (or 'ecotypes', Cohan 2001). Another way to view the effect of 'niches' in our model is in terms of negative frequency dependent selection that prevents any one genotype from completely dominating the focal community (Cordero and Polz 2014), which was the subject of a recent study by Takeuchi *et al.* (Takeuchi et al. 2015)

In summary, our results suggest that an influx of diverse and non-adapted migrator genotypes can greatly increase the effect of genetic transfer on microbial communities. It does so in at least two ways. First, migration adds new gene recipients that, even after the beneficial trait is established, enable continued gene flux. Second, migration may introduce new genomes that increase ecological subdivision ($n > 1$). Both of these processes constrain the impact of vertical selective sweeps, but within ecologically-cohesive communities (low n), it is the addition of new gene recipients that is critical for horizontal sweeps. The result is that most species and strains can evolve to be identical at the locus under positive selection while the rest of the genomes are highly diverse.

4.4 Discussion

Our models explain how horizontal sweeps of small stretches of DNA can occur in ecologically-cohesive communities of microbes. The strains and species that compete within such communities are ideal candidates for horizontal transfer, because they live in close proximity, and they can induce lysis in one another releasing DNA for uptake (Steinmoen et al. 2003; Vos 2009). However, previous

work suggests that transfer of a beneficial gene within competing communities should be limited by selective sweeps that propagate the allele vertically to fixation before significant horizontal transfer can occur (Levin 1981; Cohan 1994; Cohan 1995; Shapiro et al. 2009) (Fig. 4a). Here we have shown that this prediction does not hold when one includes the possibility of immigration. Migration is a significant process in microbial ecology (Hall-Stoodley and Stoodley 2005; Grossart et al. 2010) and allowing migration in our model results in large amounts of horizontal transfer that has the power to transform the genomics of the community.

Our models then provide an evolutionary explanation for the increasing number of sequencing studies showing that otherwise diverse microbial communities possess regions of the genome that contain very little diversity (Whitaker et al. 2005; Allen et al. 2007; Boucher et al. 2011; Shapiro et al. 2012; Cordero et al. 2012b). Further evidence of the processes we describe comes from the recent observation that mobile genetic elements can be enriched in their own niches, largely independently of their bacterial host (Smillie et al. 2011; Kav et al. 2012; Kumar et al. 2015). Horizontal transfer then has the potential to make diverse and competitive strains coherent in an ecologically-important phenotype, including key traits such as resistances to toxins (Cordero et al. 2012b). While our models explain how horizontal sweeps can occur, they also predict that the timescale required for a sweep is likely to be on the order of months to years (e.g. 10^4 to 10^6 generations for a 30-minutes generation time), based on current estimates of genetic transfer rates (Wiedenbeck and Cohan 2011; Overballe-Petersen et al. 2013; McCarthy et al. 2014). A key prediction then is that horizontal sweeps are relatively slow compared to the canonical vertical sweep

often seen in the laboratory (Imhof and Schlotterer 2001; Rozen et al. 2005). Nevertheless, the timescales of horizontal sweeps remain extremely short compared to phylogenetic timescales, and fit with data showing that genetically-coherent microbial communities persist for years in the face of migration (Szabo et al. 2013) and vertical sweeps (Croucher et al. 2011). Recent work also suggests that the basic rates of gene transfer can sometimes be much higher than typically assumed (Fernandez-Lopez et al. 2014; McCarthy et al. 2014), which in our model will significantly reduce the timescales required for horizontal sweeps. This prediction contrasts with the horizontal gene sweep scenario described by Takeuchi *et al.* (Takeuchi et al. 2015), in which gene transfer rates need to remain low ($< 10^{-6}$), for single-gene sweeps to occur.

We have emphasized here how horizontal gene transfer can remove diversity at one locus relative to the rest of the genome in a microbial community. How is this result reconciled with the notion of horizontal transfer as a way to generate diversity, in particular in the form of the much-discussed accessory genome (Allen et al. 2007; Simmons et al. 2008; Boucher et al. 2011; Cordero and Polz 2014)? Our analyses explain how genetic transfer can be seen to generate diversity in some studies, while removing diversity in others. This effect can be illustrated by considering two contrasting examples. First, if an experimenter samples in a specific patch that, as in our model, selects strongly for a particular horizontally-transferred trait, then the data may show evidence of the horizontal sweep that removed variability at the focal locus relative to the rest of the genome (*horizontal gene sampling*, Fig. 4c) (Whitaker et al. 2005; Allen et al. 2007; Shapiro et al. 2012). In contrast, if an experimenter samples one species such as *Escherichia coli* across different locations, then its background genome is likely

to cross many niches for different horizontally-acquired loci. In this case, horizontal transfer will be a process that mostly generates diversity relative to the core genome (*background genome sampling*, Fig 4c) (Kettler et al. 2007; Lukjancenko et al. 2010). Arguably then, what is considered the 'accessory' region of a genome will depend upon the ecological basis for sampling (Shapiro and Polz 2014).

Our work speaks to the fundamental question of how microbial genotypes map to ecology (Gogarten et al. 2002; Doolittle and Papke 2006; Cordero and Polz 2014). A key result from our model is that the highest rates of transfer occur for loci that are under significant positive natural selection: loci that are important for the ecology of a cell. Horizontal transfer, therefore, can enable a particular locus to accumulate in a local environment to which it is evolutionarily adapted, without the rest of the genome evolving in the same way. An interesting corollary is that a single cell carrying such loci will become ecologically diverse, in the sense that its genome can evolve to become a community of genetic regions with multiple partially-overlapping, but distinct, ecologies. This idea of distinct 'gene ecologies' (Shapiro 2014) has recently been discussed in light of the microbial species question (Burke et al. 2011; Kav et al. 2012; Polz et al. 2013; Shapiro 2014; Kumar et al. 2015). Our model explains how distinct gene ecologies are possible, as well as identifying the conditions required for them to occur (Fig. 4c). We show that, with sufficient migration, an ecologically-important trait can readily decouple itself from any one genetic background via horizontal transfer. When this occurs, a microbial niche is defined at the sub-genomic scale so that ecological species concepts will no longer map to the whole organism but rather to a subset of any one genome.

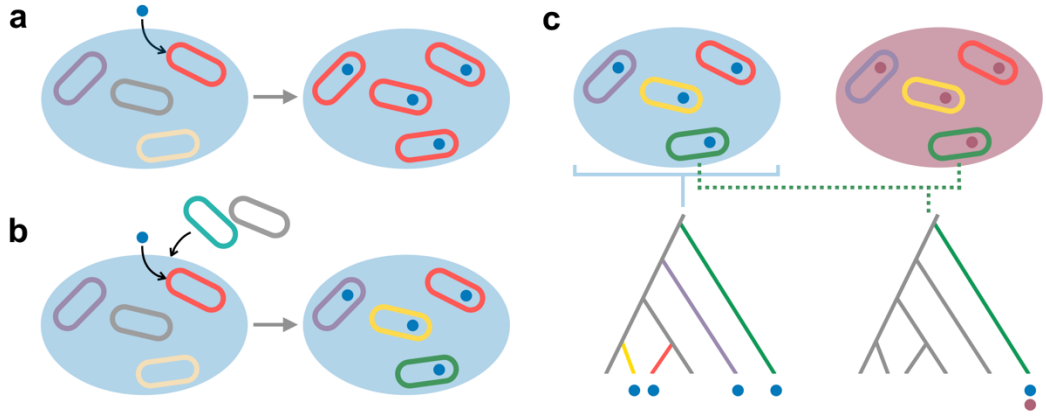


Figure 4 | Horizontal transfer creates multiple ecologies within one microbial genome. (**a**) The ecotype model (Cohan 2006). A selected trait (blue dot) causes a selective sweep of the red genome in which it first appears, wiping out other genomes if selection strength (*s*) for the trait is much larger that its gene transfer rate (*r*). (**b**) Divided-genome model. Immigration of new genotypes causes horizontal genetic sweeps. (**c**) Genome parts can display high or low diversity depending on the ecological basis of sampling. The two circles containing the cells represent patches with loci-specific sweeps. Sampling from a single patch that selects for a horizontally transferred locus (dots) will capture cells that are diverse in their background genome phylogeny but homogeneous at the transferred locus (*horizontally transferred gene sampling*, left hand phylogeny). In contrast, sampling from a single background genome will capture cells that are diverse in their horizontally transferred loci (*background genome sampling*, right hand phylogeny).

4.5 Methods

Continuous model

We model the dynamics of a selective sweep with opportunity for genetic transfer and migration using an ordinary differential equation (ODE). This equation describes the community in our selective patch where there are two sub-communities: carriers and non-carriers of the selected trait. The community is assumed to have a constant number of cells (N) with varying fractions of beneficial gene carriers (C) and non-carriers ($1 - C$). For simplicity, we assume

the community to be well-mixed and we do not consider stochastic effects (we relax this later). The dynamics and steady-state levels of carriers in the community are described by equations 1-3 in the main text. To capture the gene flux through horizontal transfer in a given community, we define a composite parameter that is the rate of transfer of the selected trait in the whole community:

$$HGT_{flux} = NrC(t)(1 - C(t)). \quad (5)$$

This horizontal gene flux is maximized at equal number of donors and recipients ($C = 0.5$). At steady-state of the system the gene flux is given by:

$$HGT^{*}_{flux} = rN\frac{m(s+1)(s-m)}{(sm+s)^2}. \quad (6)$$

Fig. 1b,c show plots of C, 1-C and the horizontal gene flux over time with and without migration. Gene transfer events accumulate over time and ultimately cause sweeps of single loci or small groups of loci. As the horizontal gene flux gives the rate of gene transfer events, the integral of this flux over a time interval gives the expected number of transfer events in this time. To obtain the numerical solutions of the cumulative gene flux plotted in Fig. 1 and Fig. 2, we employ the rectangle-rule in MATLAB. Table 1 provides a summary of the parameters present in this model and a more detailed description and analysis of the model are given in the *Supplementary Methods*.

Coalescence model

We use a coalescence approach to model the genomes in our focal patch under influence of the selective sweep in combination with horizontal transfer and migration. We first simulate the fraction of selected gene carriers (C) in the time interval $t \in [0, t_{end}]$ using the ODE of our continuous model. With the simulated values of $C(t)$ we then compute the coalescence process of two homologous loci to determine the expected diversity in their genome site. For the diversity in the background genome, we consider two random background loci at time $t = t_{end}$ and for the diversity in the focal locus we consider two focal loci at $t = t_{end}$. We then go backward in time until $t = 0$, while updating the probabilities of the two loci being in a given state. The loci can take the following states:

State *11*: Both loci are in two distinct individuals that are both carriers.

State *00*: Both loci are in two distinct individuals that are both non-carriers.

State *01*: Both loci are in two distinct individuals where one is a carrier and the other one a non-carrier.

State *1*: The two loci are coalesced in one individual that is a carrier.

State *0*: The two loci are coalesced in one individual that is a non-carrier.

State m: At least one of the loci is in a migrating individual outside the patch.

We simulate the change of all six probabilities backward in time, until $t = 0$, by solving a set of coupled ordinary differential equations given in the Supplementary Methods. We obtain the diversity in the focal locus D_f and the diversity of the background genome D_{bg}. We measure the power of the horizontal gene sweep using the ratio of the diversity in the background genome over the diversity in the focal locus, and we call this the diversity ratio DR, given by:

$$DR = \frac{D_{bg}}{D_f}. \quad (7)$$

Individual-based model

We develop an individual-based model of our selective patch to confirm the predictions of our coalescence model and to be able to change the ecological details of the patch. The simulated patch contains a fixed number of cells (N), where each individual cell is described by a set of three numbers representing the focal locus (transferrable), the genotype of the remaining background genome (non-transferrable) and the niche/resource association of the genotype. The background genotype can take any positive integer, which matches the focal locus for cells at the beginning of the simulation and for migrating cells. The focal locus can take the adapted state 1 or alternatively any other positive integer for non-adapted cells. We simulate the ecological competition of the cells in n different niches in the patch similar to the *symsim* model by Friedman et al. (Friedman et al. 2013). Each cell obtains resources from an assigned niche, but also from the remaining niches. Thus, for a given cell i there is a vector of length n giving the cell's ecological fit to each niche. A cell's fit to its assigned niche is denoted by $z \in [0,1]$, where $z = 1$ means that a cell only competes in its assigned niche, and $z = 1/n$ means that a cell competes in all niches equally. We then define the competitive weight ω of a cell i in niche j as the product of its fitness f_i ($f = 1 + s$ for carriers and $f = 1$ for non-carrier cells) times its association with niche j, so that $\omega_{ij} = f_i \sigma_{i,1}$. Each of the n niches holds a resource share of N/n in each generation, so that a cell obtains resources from niche j proportionally to its relative competitive weight in this niche according to:

$$R_{ij} = \frac{N}{n} \frac{f_i \sigma_{ij}}{\Omega_j}, \tag{8}$$

where R_{ij} is the amount or resources obtained by cell i from niche j and Ω_j is the summed competitive weight in niche j given by:

$$\Omega_j = \sum_{i=1}^{N} f_i \sigma_{ij}. \quad (9)$$

The total amount of resources obtained by cell i per generation is then given by:

$$R_i = \frac{N}{n} \sum_{j=1}^{n} R_{i,j} = \frac{N}{n} \sum_{j=1}^{n} \frac{f_i \sigma_{ij}}{\sum_{i=1}^{N} f_i \sigma_{ij}}. \quad (10)$$

The resources of a cell determine the reproduction of a cell and we can use R_i as the mean number of offspring of a cell. We update the cell numbers stochastically using a Poisson distribution following a discrete time Wright-Fisher process (Kingman 1982). Then, cells that lack the selected trait have a chance of acquiring the trait with a probability $C(t)r$. We implement the simulations using MATLAB and measure the horizontal gene flux and the diversities in different parts of the genome. The diversity is calculated as:

$$D = {}^{1}/_{\sum_{i=1}^{n} (p_i)^2}, \quad (11)$$

where n is the number of different locus variants present in the community and p_i is their respective proportion. This calculation is analogous to the effective number of species in a community (of order 2, Hill 1973). We measure the

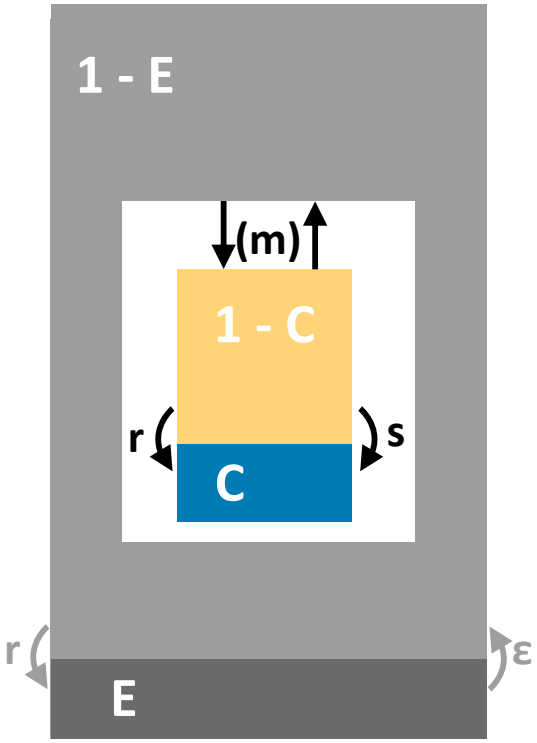
genetic effect of the horizontal sweep as in the coalescence model using the diversity ratio DR given by:

$$DR = \frac{D_{bg}}{D_f}, \quad (12)$$

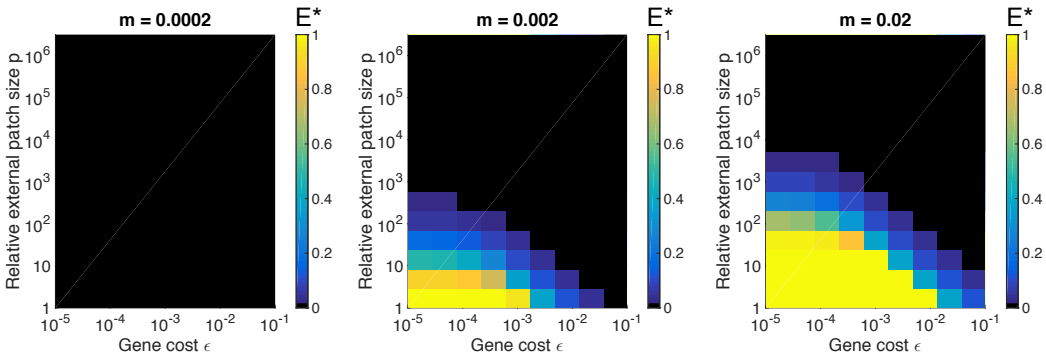
where D_{bg} is the diversity in the background genome and D_f is the diversity in the focal locus. More details of this simulation are given in the Supplementary Methods. We show that the results of our individual-based model and coalescence model match quantitatively, despite being fundamentally different models (Supplementary Fig. 4). The MATLAB code of our individual-based model is available online via http://zoo-kfoster.zoo.ox.ac.uk.

4.6 Supplementary Methods and Results

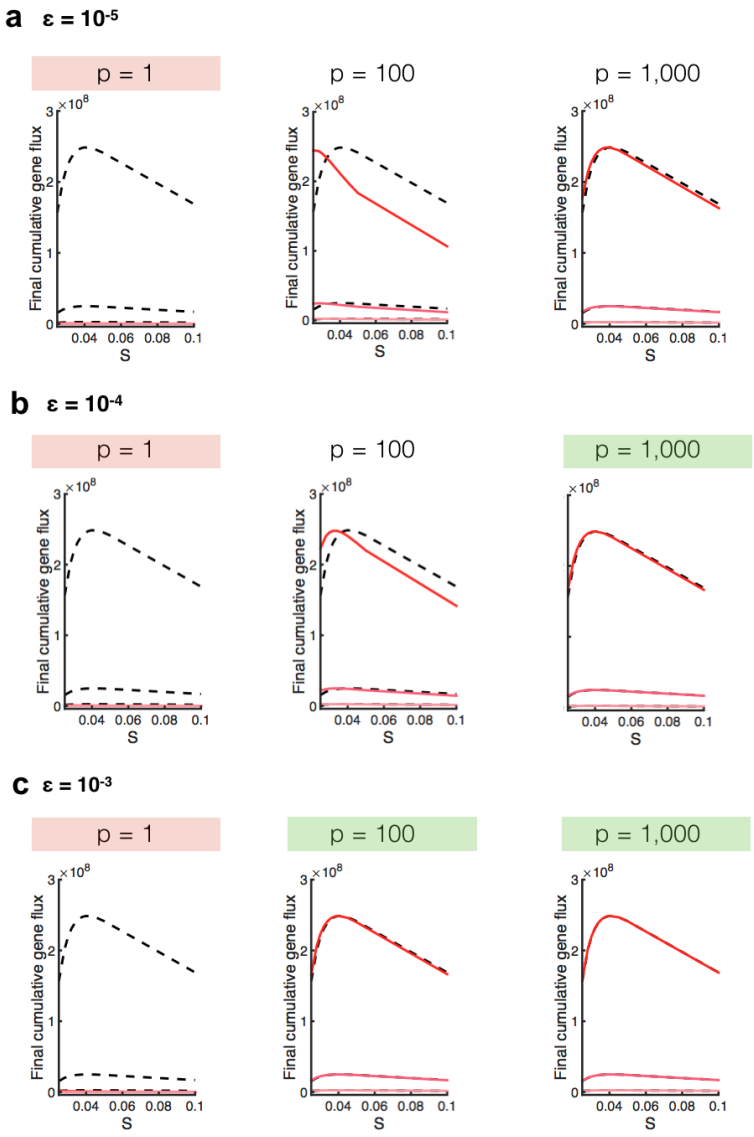
Supplementary Figures.



Supplementary Figure 1 | The extended compartment model. Sub-compartment *C* (blue) and 1-*C* (yellow) represent the fractions of allele carriers and non-carriers in the focal patch, respectively, and sub-compartment *E* (dark grey) and 1-*E* (light grey) represent the fractions of allele carriers and non-carriers in the surrounding environment, respectively. Gene transfer (*r*) converts non-carriers into carriers. Positive selection (*s*) causes a replacement of non-carriers by carriers in the focal patch, while the cost (ε) causes a replacement of carriers by non-carriers in the external environment. Migration (*m*) exchanges cells between focal patch and external patch.

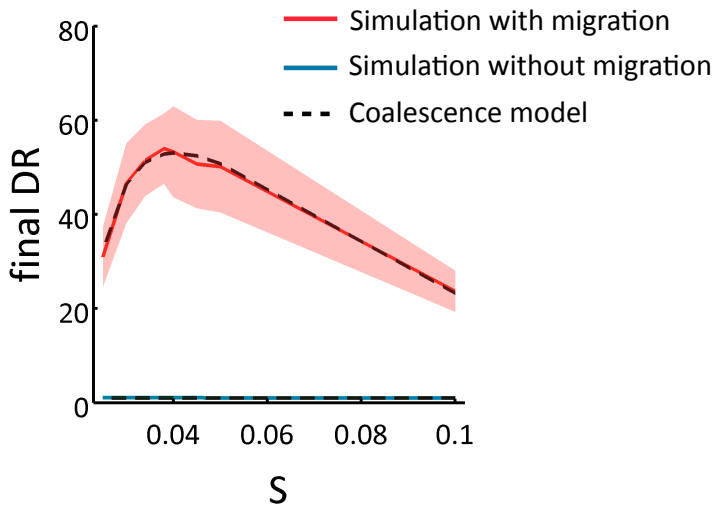


Supplementary Figure 2 | The focal trait is lost in large environments. Final fractions of gene carriers in the external environment calculated for different relative external patch sizes (p), gene costs (ε), and migration rates (m). The final fraction of gene carriers E^* is given as a colour from yellow (high E^*) to blue (small E^*) and with very small values ($E^* < 0.0001$) indicated in black. The plots show that the amount of external gene carriers becomes vanishingly small for $p > 10^4$. For lower values of p ($p < 10^4$) one observes that E^* becomes lower for higher gene costs ε and for lower migration rates m. The other parameters are, as in main-text Figure 1 and Figure 2, $N = 10^8$, and $C(0) = 1000/N$, $E(0)=0$, $r = 10\text{-}6$ and for each panel $s = 2m$.



Supplementary Figure 3 | Comparison of the simplified model (Fig. 1a) and the extended compartment model (Supplementary Fig. 1). Graphs showing the final cumulative horizontal gene transfer over a range of positive selection pressures, as shown in Figure 2b. Here we show the results of the simplified compartment model (assuming $E(t) = 0$) in black and the results of the extended compartment model as red dotted lines for a range of p and ε. There are 3 different outcomes: for low values of p and of ε, gene flux is very limited in the extended model (plots highlighted in red). For intermediate values of p and ε the gene flux of the extended model peaks at a different selection pressure than the simplified model, but with the same peak value (non-highlighted plots). For high values of p and of ε the gene flux of the two models are almost identical (gene flux of the extended model differs less than 1% from the simplified model, plots highlighted in green). The cumulative gene transfer is measured at $t = 10^5$ and the other parameters are $N = 10^8$, and $C(0) = 1000/N$

.



Supplementary Figure 4 | The Wright-Fisher simulations match the coalescence model. Graphs showing the final diversity ratio over a range of positive selection pressures calculated using the coalescence model (dotted black lines) and calculated using the individual-based model (red and blue lines). Simulation results are averaged over 50 simulations, and the standard deviation is given by the transparent area. The two different models show the same results. ($N = 10^6$, $t_{end} = 10^5$, $r = 10^{-4}$, $C(0) = 1000/N$, and either $m = 0$ or $m = 0.02$).

Supplementary Methods.

Here we present an extended version of the methods presented in the paper.

Continuous model. Here we introduce an extended version of the compartment model presented in the main text. This extended model captures how a focal gene spreads both inside a focal patch where it is under positive selection, and outside this patch. Outside the focal patch the cells do not benefit from the focal gene but pay a fitness cost when they carry it. For simplicity, we capture the external environment as a single patch that is connected to the focal patch by cell migration.

Our model is analogous to an SIR (Sensitive, Infected, Resistant) model, often known as a “compartment model”, which is commonly used to describe the dynamics of an epidemic by representing the flow between sub-communities ("compartments"). Our model contains two patches, the focal patch and the environment patch, each comprising two compartments: carriers ("infected") and non-carriers ("sensitive") of the selected trait. In the focal patch there are a constant number of cells (N) and varying fractions of beneficial gene carriers (C) and non-carriers ($1-C$), outside the patch there is a p-times larger number of cells (pN) and varying fractions of focal gene carriers (E) and non-carriers ($1-E$). For simplicity, each patch is well-mixed and we do not consider spatial effects. We also do not consider stochastic effects in this model but our later models do. The dynamics within both patches are determined by the following three processes, which are considered to occur continuously in time, where one time unit is one cell generation:

1. Selection: A cell's fitness depends on whether it carries the focal gene or not and whether it lives in the focal patch or not. The general fitness term of a cell is $w = 1 - \varepsilon + \beta$, where ε is the cost that all trait-carriers experience and β is the benefit that only trait-carriers within the focal patch experience. We assume that the benefit of the focal trait in the selective patch is larger than its cost ($\beta > \varepsilon$) so that the net change in fitness is positive ($s = \beta - \varepsilon$, $s > 0$). This leads to the following fitnesses:

Carriers in focal patch: $w = 1 + s$, non-carriers in focal patch: $w = 1$

Carriers in environment patch: $w = 1 - \varepsilon$, non-carriers in environment patch: $w = 1$

2. Horizontal gene transfer: We let r be the probability that a carrier transmits the focal gene to a non-carrier per cell generation and we assume that horizontal transfer occurs according to the mass action principle: the transmission rate is proportional to both the fraction of recipients (1-C or 1-E) and the fraction of donors (C or E).

3. Migration: Migration works as an exchange of cells between focal patch and environment patch. The migration rate is specified through m, the fraction of the cells in the focal patch that is replaced randomly by individuals from the external patch landing in the focal patch per unit time. In the external patch cells from the focal patch replace a fraction of m/p per unit time.

The evolution of our compartment model can be described using two coupled ordinary differential equations (ODEs). We let $C_N(t)$ and $E_N(t)$ be the number of carriers in the focal patch and the environmental patch, respectively, at time t. Due to the constant carrying capacities of the patches, the number of non-carriers is given through $N - C_N(t)$ for the focal patch and through $pN - E_N(t)$ for the

environment patch. We can then describe the dynamics of the whole system by considering sub-communities C and E only. The rate of transitions from sub-compartment 1-C to C via horizontal gene transfer is given by $v_{\mathrm{HGT}} = rC_{\mathrm{N}}(t)\,(N - C_{\mathrm{N}}(t))/N$. Similarly, in the environment patch the rate of gene transitions is given by $\omega_{\mathrm{HGT}} = rE_{\mathrm{N}}(t)\,(pN - E_{\mathrm{N}}(t))/(\mathrm{p}N)$. The rate of increase in size of carrier-compartment C due to positive selection is given by $v_{\mathrm{selection}} = s/(1 + sC_{\mathrm{N}}(t)/N)C_{\mathrm{N}}(t)\,(N - C_{\mathrm{N}}(t))/N$. In the environment patch the carrier-compartment E is reduced by selection at rate $\omega_{\mathrm{selection}} = \varepsilon/(1 - \varepsilon E_{\mathrm{N}}(t)/(pN))E_{\mathrm{N}}(t)\,(pN - E_{\mathrm{N}}(t))/(pN)$. Finally, the rate at which individuals in C are replaced through migration is given by $v_{\mathrm{migration}} = mNC_{\mathrm{N}}(t)(pN\text{-}E_{\mathrm{N}}(t))/(pN) - mNE_{\mathrm{N}}(t)/(pN)(N\text{-}C_{\mathrm{N}}(t))/N = mN(C_{\mathrm{N}}(t)/N\text{-}E_{\mathrm{N}}(t)/(pN))$. In the external patch, migration increases the carrier-compartment E at rate $\omega_{\mathrm{migration}} = mN(C_{\mathrm{N}}(t)/N\text{-}E_{\mathrm{N}}(t)/(pN))$.

In summary, the system evolves according to the system of ODEs

$$\frac{dC_N(t)}{dt} = v_{HGT} + v_{selection} - v_{migration},$$
$$\frac{dE_N(t)}{dt} = \omega_{HGT} - \omega_{selection} + \omega_{migration}, \qquad \text{(A1)}$$

or

$$\frac{dC_N(t)}{dt} = rC_N(t)\frac{N-C_N(t)}{N} + \frac{s}{1+sC_N(t)/N}C_N(t)\frac{N-C_N(t)}{N} - mN\left(\frac{C_N(t)}{N} - \frac{E_N(t)}{pN}\right),$$
$$\frac{dE_N(t)}{dt} = rE_N(t)\frac{pN-E_N(t)}{pN} - \frac{\varepsilon}{1-\varepsilon E_N(t)/(pN)}E_N(t)\frac{pN-E_N(t)}{pN} + mN\left(\frac{C_N(t)}{N} - \frac{E_N(t)}{pN}\right). \qquad \text{(A2)}$$

Dividing the first equation of equation system (A2) throughout by N and dividing the second equation throughout by pN yields, for the carrier-fractions $C(t) = C_N(t)/N$ and $E(t) = E_N(t)/(pN)$, the coupled equations:

$$\frac{dC(t)}{dt} = rC(t)\big(1 - C(t)\big) + \frac{s}{1+sC(t)}C(t)\big(1 - C(t)\big) - m(C(t) - E(t)),$$

$$\frac{dE(t)}{dt} = rE(t)\big(1 - E(t)\big) - \frac{\varepsilon}{1-\varepsilon E(t)}E(t)\big(1 - E(t)\big) + \frac{m}{p}(C(t) - E(t)). \qquad \text{(A3)}$$

For a visualisation of our extended compartment model see Supplementary Figure S1. In the external patch, trait carriers are added through migration from the focal patch, and the trait spreads through horizontal transfer and is removed due to positive selection. Given a very small gene transfer rate, the key parameters that determine to what extend the trait is maintained in the external patch are the cost ε and p, the size of the external patch relative to size of the focal patch. Because microbial genome evolution by gene gain and loss is biased toward loss of unnecessary genes (Mira et al. 2001; Moran 2002), we assume that ε is larger than the gene transfer rate r ($\varepsilon > r$). We explore in our model an according range of values for ε ($10^{-5} < \varepsilon < 10^{-1}$ in Supplementary Fig. 2 and $10^{-5} < \varepsilon < 10^{-3}$ in Supplementary Fig. 3). The migration parameter m determines the amount of cell exchange between the patches, and when m is very high it causes the two patches to behave close to a single, well-mixed patch. Because we assume that the focal patch and its environment are spatially separated, we consider $m = 0.02$ as a high migration rate and choose this to be the upper limit. We plot the fraction of carriers in the external patch at equilibrium (E^*) for a range of parameters ε and p (see Supplementary Fig. 2). We determine E^* by solving equations (A3), in

MATLAB until equilibrium is reached, with initial conditions $C(0) = 0.001$, $E(0) = 0$ and for different migration rates m and a selection pressure of $s = 2m$ so that the focal trait is fixed in the selective patch. Supplementary Figure S2 shows that when the external patch is much larger than the focal patch ($p > 10^4$), then the focal trait is extremely rare outside the focal patch ($E^* < 0.0001$). We begin by studying this case in detail by setting $E(t) = 0$:

$$\frac{dC(t)}{dt} = rC(t)\big(1 - C(t)\big) + \frac{s}{1+sC(t)}C(t)\big(1 - C(t)\big) - mC(t), \quad \text{(A4)}$$

The steady-states of this system for $r \approx 0$ is given approximately by:

$$C^* = 0 \ and \ C^* = \frac{s-m}{(m+1)s}, \quad \text{(A5)}$$

with $C^* = 0$ defining an unstable equilibrium and $C^* = (s - m)/((m + 1)s)$ a stable equilibrium, given $s > 0$, according to linear stability analysis. Here and throughout, we use an asterisk to denote the steady-state value of a variable. Table 1 provides a summary of the parameters present in this model.

Horizontal gene flux. To capture the gene flux through genetic transfer in our focal community, we define a composite parameter that is the rate of transfer of the selected trait in the whole community:

$$HGT_{flux} = NrC(t)(1 - C(t)). \quad \text{(A6)}$$

This horizontal gene flux spreads the beneficial gene without removing migrant cells, while the vertical gene flux, given through $sC(t)(1 - C(t))$, spreads the gene with removing migrants. The horizontal gene flux is maximized at equal numbers of donors and recipients ($C = 0.5$), and it thus depends on the patch conditions, including the initial number of trait carriers. At steady-state of the system, the horizontal gene flux then only depends on the selection pressure, the migration rate and the rate of gene transfer. In this case the horizontal gene flux is calculated by substituting equation (A5) for the steady-state fraction of non-carriers into equation (A6), giving

$$HGT^{*}_{flux} = rN\frac{m(s+1)(s-m)}{(sm+s)^2}. \quad \text{(A7)}$$

Analyzing this expression for the horizontal gene flux at steady-state, we find that there is no gene flux in the absence of migration ($HGT_{\text{flux}} = 0$ for $m = 0$). We also see that, for a given migration rate, the gene flux is highest at an intermediate selection pressure ($s = 2m/(1 - m)$).

Cumulative gene flux. Gene transfer events can accumulate over time and may ultimately cause sweeps of the focal locus. Because the horizontal gene flux gives the rate of gene transfer events, the integral of the flux over a time interval gives the expected number of transfer events in this time. To obtain the numerical solutions of the cumulative gene flux plotted in Fig. 1 and Fig. 2, we employ the midpoint rule.

Explicitly modelling immigration. We now move to explicitly modelling immigrating cells to relax the assumption that migrants never arrive pre-adapted in the focal patch and test how this changes our results. For this we return to equations (A3) and we use these equations to repeat the calculations of the final cumulative gene flux using different values for p and ε, and we compare the new results with our results from Figure 2b (Supplementary Fig. 3). Very small fractions of external carriers (Supplementary Fig. 2, black areas) then mean that the system with external patch (Eq. A3) behaves like the simplified system (Eq. A4) and the latter is a good approximation of the former. In our next models, we explore the effects of the horizontal gene flux observed when migrants lack the adaptive trait ($E(t) = 0$) which corresponds to the case were the outside environment is much larger than the focal patch.

Coalescence model. We use a coalescence approach to model the genomes of our community under influence of a selective sweep in combination with genetic transfer and migration. We ask how these processes influence the evolution of genetic diversity in the focal locus and in the background genome. To answer this question we first simulate the fraction of selected gene carriers (C) in the time interval $t \in [0, t_{end}]$ using equation (A3). With the simulated values of $C(t)$ we can then compute the coalescence process for two homologous loci in order to determine the expected diversity in their genome site. For the diversity in the background genome, we consider two random background loci at time $t = t_{end}$ and for the diversity in the focal locus we consider two focal loci at $t = t_{end}$. We then go backward in time until $t = 0$, while updating the probabilities of the loci being in a given state.

The loci can take the following states:

State *11*: Both loci are in two distinct individuals that are both carriers

State *00*: Both loci are in two distinct individuals that are both non-carriers

State *01*: Both loci are in two distinct individuals where one is a carrier and the other one a non-carrier

State *1*: The two loci are coalesced in one individual that is a carrier

State *0*: The two loci are coalesced in one individual that is a non-carrier

State m: At least one of the loci is in a migrating individual outside the patch.

We first explain how we apply the coalescence model to the background genome. When taking two random loci of the background genome at $t = t_{end}$, we know that these loci can be found in one of the first 3 states: *11* (with probability $P_{11}(t_{end}) = C(t_{end})^2$), *00* (with probability $P_{00}(t_{end}) = (1 - C(t_{end}))^2$), or *01* (with probability $P_{01}(t_{end}) = 2C(t_{end})(1-C(t_{end}))$). The probabilities for the remaining states are zero ($P_1(t_{end}) = 0$, $P_0(t_{end}) = 0$, $P_m(t_{end}) = 0$). We use these probabilities as our initial condition and simulate the change of all six probabilities backward in time, until $t = 0$, by solving the following set of coupled ODEs:

$$\frac{dP_{11}(t)}{dt} = -(p_{11\to01} + p_{11\to00} + p_{11\to0} + p_{11\to1})P_{11}(t),$$

$$\frac{dP_{00}(t)}{dt} = -(p_{00\to0} + p_{00\to m})P_{00}(t) + p_{11\to00}P_{11}(t) + p_{01\to00}P_{01}(t),$$

$$\frac{dP_{01}(t)}{dt} = -(p_{01\to00} + p_{01\to0} + p_{01\to m})P_{01}(t) + p_{11\to01}P_{11}(t),$$

$$\frac{dP_{1}(t)}{dt} = +p_{11\to1}P_{11}(t),$$

$$\frac{dP_{0}(t)}{dt} = p_{11\to0}P_{11}(t) + p_{01\to0}P_{01}(t) + p_{00\to0}P_{00}(t),$$

$$\frac{dP_{m}(t)}{dt} = p_{10\to m}P_{10}(t) + p_{00\to m}P_{00}(t), \qquad \text{(A7)}$$

where we use the following probabilities of the loci to change their state at time t:

$$p_{11\to01} = 2 \cdot \frac{f_a(1-m)C(t-1)}{C(t)} \cdot \frac{C(t) - f_a(1-m)C(t-1)}{C(t)},$$

$$p_{11\to00} = \left[\frac{C(t) - f_a(1-m)C(t-1)}{C(t)}\right]^2 \cdot \left(1 - \frac{1}{N \cdot (1 - C(t-1))}\right),$$

$$p_{11\to0} = \left[\frac{C(t) - f_a(1-m)C(t-1)}{C(t)}\right]^2 \cdot \frac{1}{N \cdot (1 - C(t-1))},$$

$$p_{11\to1} = \left[\frac{f_a(1-m)C(t-1)}{C(t)}\right]^2 \cdot \frac{1}{N \cdot C(t-1)},$$

$$p_{00\to0} = \left[\frac{1 - C(t) - m}{1 - C(t)}\right]^2 \cdot \frac{1}{N \cdot (1 - C(t-1))},$$

$$p_{00\to m} = 1 - \left[\frac{1 - C(t) - m}{1 - C(t)}\right]^2,$$

$$p_{01\to00} = \left[\frac{1 - C(t) - m}{1 - C(t)}\right] \cdot \frac{C(t) - f_a(1-m)C(t-1)}{C(t)} \cdot \left(1 - \frac{1}{N \cdot (1 - C(t-1))}\right),$$

$$p_{01\to0} = \left[\frac{1 - C(t) - m}{1 - C(t)}\right] \cdot \frac{C(t) - f_a(1-m)C(t-1)}{C(t)} \cdot \frac{1}{N \cdot (1 - C(t-1))},$$

$$p_{01\to m} = \left[\frac{f_a(1-m)C(t-1)}{C(t)} + \frac{C(t) - f_a(1-m)C(t-1)}{C(t)}\right] \cdot \frac{m}{1 - C(t)}, \quad \text{(A8)}$$

where f_a is the relative fitness of a carrier cell at time t - 1, given by:

$$f_a = \frac{1+s}{1+C(t-1)\cdot s}. \quad \text{(A9)}$$

Here we make the typical assumptions of a coalescence model (Otto and Day 2007) that generations are discrete and non-overlapping and that the community size (N) is constant throughout time.

After running the coalescence simulation from $t = t_{end}$ to $t = 0$, we obtain the unconditional probabilities for the two background loci (i) to have coalesced

within the simulated time interval ($P_1 + P_0$), or (ii) to be derived from two distinct cells that have either been in the community since $t = 0$, or that have migrated into the patch ($P_{11} + P_{10} + P_{00} + P_m$). In case (i) the two loci are identical and in case (ii) the two loci are different. Only, however, when the two loci descended from 2 distinct cells at $t = 0$ that are carriers (with probability $P_{11}(0)$), we assume that the loci are identical, because the genotype that initially carries the beneficial gene comes into the patch as a clonal group. We can calculate the probability of the two background loci being identical as:

P(2 background loci are identical) = $P_1(0) + P_0(0) + P_{11}(0)$.

The probability of two randomly chosen gene sites to be identical is the Simpson's index of diversity (Simpson 1949). Here we use the inverse of the Simpson's index to measure diversity, which is the effective number of species in a community (of order 2, Hill 1973). The diversity in the background genome is then given by:

$$D_{bg} = \frac{1}{P(2\ background\ loci\ are\ identical)}. \quad \text{(A10)}$$

Similarly, we can calculate the probability of two focal loci to be identical. Note that two randomly sampled focal loci at $t = t_{end}$ are identical when they are in two carrier cells (state 11) and different when only one locus is in a carrier cell (state 01). We define the diversity of the focal locus as:

$$D_f = \frac{1}{P(2\ focal\ loci\ are\ identical)}. \quad \text{(A11)}$$

We measure the power of the horizontal sweep using the ratio of the diversity in the background genome over the diversity in the focal locus, and we call this ratio the diversity ratio *DR*, given by:

$$DR = \frac{D_{bg}}{D_f}. \quad \text{(A12)}$$

Individual-based model. We develop an individual-based model of our selective patch to confirm our coalescence model prediction and to be able to change the ecological scale of the patch by subdividing the patch into multiple niches. This model allows us to describe an ecologically homogeneous patch where cells live from a single resource, or to model an ecologically heterogeneous patch in which different genotypes are specialized in eating different resources. Alternatively, the ecological subdivision may represent negative frequency-dependent selection of genotypes, for example through phage predation (Rodriguez-Valera et al. 2009). Our simulated patch contains a fixed number of cells (*N*), where each individual cell is described by a set of three numbers representing the focal locus (transferable), the genotype of the remaining background genome (non-transferable) and the niche/resource association of the genotype. The background genotype can take any positive integer, which matches the focal locus for cells at the beginning of the simulation and for migrating cells. The focal locus can take the adapted state 1 or any other positive integer for non-adapted cells.

We start each simulation with a diverse community of cells so that every cell carries a different background genotype (and matching focal locus) with randomly assigned niche association. The initial community then also contains a small clonal group of adapted cells. For our simulations we choose a large

community size that is possible to simulate in considerable time ($N = 10^6$) and we choose an initial number of adapted cells so that the frequency process of the adapted type is expected to behave deterministically (C(0) > 5/(Ns), Kaplan *et al.* 1989, $C(0) = 0.001$). We then update the cell numbers for each following generation according to a discrete time Wright-Fisher process (Sigwart 2014). At each generation the fitness of each cell is determined according to their adaptive state and their competition within the n ecological niches. We simulate the ecological competition similar to the *symsim* model by Friedman et al. (Friedman et al. 2013).

Each cell obtains resources from its assigned niche, but also from the remaining niches. Thus, for a given cell i there is a vector of length n giving the cell's ecological fit to each niche. A cell's competitive strength in its niche is denoted by $z \in [0,1]$, where $z = 1$ means that a cell only competes in its own niche, and $z = 1/n$ means that a cell competes in all niches equally. For example, a cell that is specialized in resources of niche 1 with $z = 0.8$ would compete in each of the n niches (here $n = 5$) according to the vector σ_i = [0.8 0.05 0.05 0.05 0.05]. We then define the competitive weight ω of a cell i in niche j is as the product of its fitness f_i ($f = 1 + s$ for carriers and $f = 1$ for non-carrier cells) times its association with niche j, so that $\omega_{ij} = f_i\sigma_{i,1}$. For example, the cell specialized in niche 1 has in niche 1 a competitive weight of 0.8 if does not carry the trait, or $0.8(1 + s)$ if it does carry the trait. Each of the n niches holds a resource share of N/n in each generation, so that a cell obtains resources from niche j proportionally to its relative competitive weight in this niche according to:

$$R_{ij} = \frac{N}{n}\frac{f_i\sigma_{ij}}{\Omega_j}, \tag{A13}$$

where R_{ij} is the amount or resources obtained by cell i from niche j and Ω_j is the summed competitive weight in niche j given by:

$$\Omega_j = \sum_{i=1}^{N} f_i \sigma_{ij}. \quad \text{(A14)}$$

The total amount of resources obtained by cell i per generation is then given by:

$$R_i = \frac{N}{n} \sum_{j=1}^{n} R_{i,j} = \frac{N}{n} \sum_{j=1}^{n} \frac{f_i \sigma_{ij}}{\sum_{i=1}^{N} f_i \sigma_{ij}}. \quad \text{(A15)}$$

The resources of a cell determine the reproduction of a cell. Because the resource share per niche is scaled to N/n, the mean amount of resources per cell per generation is 1. Therefore, we can use R_i directly as the mean number of offspring of a cell. We update the cell numbers stochastically using a Poisson distribution. Then, cells that lack the selected trait have a chance of acquiring the trait with a probability $C(t)r$. We use a rate of gene transfer of $r = 10^{-4}$, which is higher than conservative estimates ($10^{-6} \leq r \leq 10^{-5}$, Wiedenbeck & Cohan 2011), in order for simulations to run in a reasonable computational time. However, the qualitative effects of niche separation that we observe should generalize to lower rates of genetic transfer. Moreover, recent studies suggest that rates of gene transfer may be orders of magnitude larger than these conservative estimates (Overballe-Petersen et al. 2013; McCarthy et al. 2014). In simulations with migration, we model this process by replacing existing cells at random with new incoming

genotypes (and matching focal locus). Each new genotype is also assigned to an associated niche at random.

Each simulation (implemented in MATLAB) is run for $t_{end} = 10^5$ time steps, which is equivalent to about 6 years of microbial evolution given a generation time of 30 minutes (Ulitzur 1974). We show that the results of our individual-based model match our coalescence model (see Supplementary Fig. 4). We track the background genomes and focal loci of all individuals in the community over time and we also measure the horizontal gene flux by counting the number of gene transfer events. From there we determine the parameters of interest, such as the fraction of carrier cells *C*, or diversities in different parts of the genome. The diversity of a genome is calculated as:

$$D = {}^{1}\!/_{\sum_{i=1}^{n}(p_i)^2}, \tag{A16}$$

where n is the number of different locus variants present in the community and p_i is their respective proportion. This calculation is analogous to the effective number of species in a community (of order 2, Hill 1973). We measure the power of the horizontal sweep using the ratio of the diversity in the background genome over the diversity in the focal locus, and we call this ratio the diversity ratio *DR*, given by:

$$DR = \frac{D_{bg}}{D_f}, \tag{A17}$$

where D_{bg} is the diversity in the background genome and D_f is the diversity in the focal locus. A diversity ratio of 5, for example, means that effectively 5 different background genomes per single focal locus appear in the genomes of the community.

This diversity ratio can also be calculated for compartment C and compartment 1-C separately. For the compartment of non-carriers (1-C) the background genomes match their focal locus, giving a diversity ratio of 1. For the compartment of carriers (C), gene transfer allows for an increase of diversity in the background genome against the homogeneous focal locus. Thus, the diversity ratio in C is directly linked to the cumulative gene flux. The diversity ratio of the whole community lies between the diversity ratios of compartments C and 1-C, depending on the size of C. It therefore behaves similarly, but not exactly like the cumulative gene flux with respect to a changing selection pressure.

5 The evolution of horizontal gene transfer in microbes

5.1 Abstract

Microbes incorporate small stretches of DNA from other strains and species into their genomes. These genetic transfers are fundamental to the biology of microbes and their ability to adapt to new challenges, including antibiotics. However, incorporating foreign DNA can be costly and we lack a clear understanding of why genetic transfer evolves. Here we develop an eco-evolutionary model to study horizontal genetic transfer. We first demonstrate a barrier to benefitting from horizontal transfer: DNA typically comes from strains that are, on average, no better adapted. As a result, transfer is only beneficial to a focal cell for a specific form of fitness epistatis between loci. Specifically, transfer will only evolve with positive epistasis for fitness, where each subsequent loci that is locally adapted contributes disproportionately to fitness. However, we show that these limitations are removed by introducing a key additional piece of the biology

of horizontal transfer: regulation. If cells only take up DNA when stressed and poorly adapted, they incorporate DNA that is, on average, a better fit to their current environment than their own genome. We discuss a number of examples that are consistent with our predictions. Our work supports the view that microbes evolved regulated horizontal genetic transfer in order to cope with their ever-changing environment.

5.2 Introduction

Many microbes pick up DNA and incorporate it into their genome by horizontal genetic transfer (HGT). Next-generation sequencing has revealed that HGT has had major impacts on the evolution of microbes, particularly bacteria, where for most genomes almost every gene has been subject to transfer at some stage in evolutionary history (Hanage et al. 2005; Bapteste et al. 2009; Kloesges et al. 2011). Horizontal genetic transfer (HGT) allows cells to acquire ecologically-important genes and rapidly adapt to new environments (Gogarten et al. 2002; Dobrindt et al. 2004; Anantha et al. 2004; Doolittle and Papke 2006), including antibiotics, and this makes HGT a major health concern (Schlüter et al. 2007; Norberg et al. 2011; Stokes and Gillings 2011; McCarthy et al. 2014). Despite its importance, it is not clear why a cell will evolve horizontal transfer.

Taking up foreign DNA can easily damage a well-evolved genome by introducing sequences that interrupt critical functions and do not themselves function well (Bell 1982; Chou et al. 2011; Park and Zhang 2012; Baltrus 2013; San Millan et al. 2015).

One explanation for HGT is that recipient cells can be powerless to prevent it. Phages can introduce novel DNA into host genomes via transduction as a byproduct of infection and lysogeny. Plasmids are under strong natural selection to conjugate, even if this is costly to a recipient cell, which can occur when plasmids encode cooperative traits that benefit the population but not the individual cell (Nogueira et al. 2009; Rankin et al. 2011; Mc Ginty et al. 2013). However, natural selection on phages and plasmids to spread is not sufficient to explain the evolution of HGT. Many microbes actively bring novel DNA into the cell. Some DNA uptake may be nutritional (Redfield 1993; Finkel and Kolter 2001) but, importantly, uptake is often co-regulated with the active incorporation of DNA into the genome. In bacteria, uptake typically occurs via type 4 pili that bind double-stranded DNA outside the cell. The DNA is then imported, converted to single-strand DNA, and bound by the conserved protein, DprA, which loads it onto RecA for homologous recombination (Solomon and Grossman 1996; Johnston et al. 2014b). This process, known as natural competence, is thought to be a major driver of HGT in natural and clinical populations (Engelmoer and Rozen 2011a; Morita et al. 2013; Johnston et al. 2014a) and, yet, we do not know why it evolves.

Here we use large-scale eco-evolutionary simulations to study the evolution of horizontal genetic transfer by microbes. Our models capture both competition between genotypes and migration to new environments where genotypes are poorly adapted (Earl and Deem 2004). We first identify an evolutionary conundrum: DNA from competing cells is, on average, no better adapted to the environment than the DNA of a focal cell. There is, therefore, no natural selection to take up DNA. However, the literature from the evolution of sex in eukaryotes

offers a solution to this conundrum. When there is positive epistasis for fitness between loci, HGT can be beneficial because it allows cells to create strongly beneficial gene combinations that are lost due to migration. Moreover, we find that HGT is universally beneficial when upregulated under stress, which appears to commonly occur in nature (Solomon and Grossman 1996; Meibom 2005; Prudhomme et al. 2006; Johnsborg et al. 2007). Our analyses suggest that microbes evolve HGT to obtain the best genotypic combinations for their changing and challenging environments.

5.3 Methods

Model Overview

We study a simple but realistic ecology where microbes compete in local patches and migration moves genotypes to patches where they are not well adapted (Figure 1). For example, a new patch may have different metabolic requirements or contain an antibiotic or toxin that was not in the previous patch (McDaniel et al. 2010; Kav et al. 2012; Biernaskie et al. 2013). When transfer occurs, it changes a focal cell's genotype at a particular locus to the genotype of one of its neighbouring strains. In nature, genetic transfer can occur via both homologous and non-homologous recombination. We focus on the former here, which primarily causes allelic replacement, but our conclusions should apply to both processes whenever they are under the control of the focal cell. The study of HGT evolution requires one to follow the ecology, evolution, and genomics of microbial populations (Earl and Deem 2004; Jones et al. 2007; Lobkovsky et al. 2016). In order to do this, we use an individual-based simulation that can

explicitly follow a large number of individual genotypes that each have a specific gene transfer rate, which is the trait we are focussing on. These simulations capture the stochastic processes when genotypes immigrate, compete ecologically and replace one another in a focal patch due to natural selection on particular genotypic combinations. With these processes in place, we can then ask under what conditions HGT evolves (Bell 2005).

Local competition

We implement our model using an adaptation of the Gillespie algorithm that simulates stochastic cell events continuous in time (Allen and Dytham 2009). We focus on a single environmental niche that is connected to a large number of other niches. The focal niche contains a fixed population of K = 1,000 cells and, as implied for each other niche, poses a unique selection pressure on the local cells. The genome of each local cell i contains n ecological gene loci. Each locus can take one of four different adaptive states, e, which denote whether the locus contains: a non-adapted gene (e = 0); a non-adapted gene that is calibrated to fit its background genome (e = 1); an ecologically-adapted gene (e = 2); or an adapted gene that is calibrated (e = 3). The adaptive state of a cell is then defined by

$$a_i = (\sum_{j=1}^{n} e_j)/(3n), \qquad (1)$$

so that the adaptive state is between 0 (non-adapted) and 1 (fully adapted). This is a linear function between locus states and cell adaptation and, thus, assumes that

the gene loci are non-interacting. We can relax this assumption and allow for both positive and negative epistasis for fitness. For this we change our adaptive state function into

$$a_i = \left[\left(\sum_{j=1}^{n} e_j \right) / (3n) \right]^u , \qquad (2)$$

where u is the gene-interaction parameter. When $u > 1$ there is positive epistasis, meaning that positive effects of the loci enhance each other. When $u < 1$ there is negative epistasis, with diminishing positive effects. Positive natural selection in the focal niche means that maladapted cells die and are replaced by other cells. Each cell i has an associated cell death parameter given by $(1 - a_i)s$, where s is the selection pressure, plus a base-line death-rate d (for 'drift') at which cells die independently of their adaptation. During a cell death event, one cell is chosen with probability proportional to its death parameter divided by the population mean of this parameter, and it is replaced through replication of a random cell. Time-steps in our simulations are scaled such that the per-cell rate of selection is one per time-step.

Cell immigration

Microbes migrate widely and frequently to new environments (Hall-Stoodley & Stoodley 2005; Grossart *et al.* 2010). A key process in our simulations, therefore, is migration. Cells from external niches replace local cells with a per-cell rate m. A migration rate of 0.01, for example, means that at each time step ~1% of the local cells are replaced through immigrants. Because we assume that cells in other niches have evolved similar properties to the local cells, we implement migration

by retaining a cell's gene uptake rate but removing a fraction z of the cell's adaptive loci, where z quantifies how different the ecological niches are. In this process a gene's adaptation to the genomic background is preserved (i.e. $e = 2$ becomes $e = 0$, and $e = 3$ becomes $e = 1$). The value $z = 0$ means that ecological niches are identical so that adaptive loci stay adaptive after migration, while $z = 1$ corresponds to entirely different niches so that all genes are non-adaptive after migration.

Cell migration in our model could also represent environmental change in the absence of cell movement, since our core assumption is that cells find themselves in changing environments. In the same way, a "migration" event in our model could also represent DNA damage events that decrease the adaptation of a strain to the environment. Here, horizontal transfer will functions to re-adapt a cell by repairing the genome. This repair function has been found to be important when stress leads to high rates of DNA damage (Engelmoer and Rozen 2011b).

Mutation and horizontal gene transfer

We assume that niche adaptation through mutation alone is impossible on the short evolutionary timescales allowed by migration between patches. We are, therefore, focused on traits that are relatively complex where evolutionary adaptation can only be achieved by expressing a protein or set of proteins that a cell initially lacks, which can occur for heat shock (Gogarten and Townsend 2005), virulence (Chen and Novick 2009), or antibiotic resistance (Nakamura et al. 2004). Mutation can occur, however, and can improve horizontally-transferred loci within a particular cell lineage. In particular, we assume that genes become

calibrated to their genomic background at a stochastic rate r_{mut}, at which $e = 0$ becomes $e = 1$, and $e = 2$ becomes $e = 3$.

In gene transfer events, a cell takes up an external gene and swaps it with one of its own genes. We assume that a gene that integrates will not be adapted well to its new genome and it will have state $e = 0$ or $e = 2$. We first investigate two different scenarios for the source of transferred genes: In the first scenario the transferred genes come from a fixed gene pool of adapted genes (Earl and Deem 2004), so that each transfer event brings in an adapted gene ($e = 2$). In the second scenario genes come from other cells within the population. This is implemented as follows: At each transfer event we select a random gene from the population. This gene, with position j in its donor cell genome, will copy itself into the same gene position j of the recipient cell through homologous transfer. In this process, the gene loses its within-genome calibration that it might have achieved through mutation ($e = 3$ becomes $e = 2$, and $e = 1$ becomes $e = 0$).

Importantly, we also explicitly model the modifier locus for the rate of gene uptake, which has its own position in the genome ($n + 1$), and we allow this modifier to also be subject to horizontal transfer. With a probability of $1/(n + 1)$ a cell does not take up one of the loci that affects adaptations to the niche, and instead the cell takes up another cell's gene uptake rate. Cells also evolve in their ability to acquire genes (E) by mutation. A cell i changes its gene uptake ability E_i at a rate q. We implement this evolution by allowing E_i to take a Gaussian random walk (mean $\mu = 0$ and a small standard deviation $sd = 0.001$). At initialization of our simulations, we set the gene uptake rates (E) of each cell at a value that is sampled from a uniform random distribution between 0 and 1. We set the initial adaptive states of the genome loci to $e = 0$ with probability z, and otherwise to $e =$

2. We simulate our evolutionary model over a time that allows 1,000 migrations per cell (for example 10,000 time-steps for $m = 0.1$) and we track the average per-cell gene uptake rate in the population over time. For all parameter values that we consider this rate progresses toward a steady-state value over time. We determine the steady-state value by averaging the mean population rate over the final 50 time-steps of the simulation. For any given set of model parameters, we repeat our simulations 5 times because the model is time-expensive to simulate.

Regulated gene uptake

In our first models, the gene uptake rate of a cell (E_i) only changes through evolution. This is unrealistic as competence is often strongly regulated by stress responses (Lee and Morrison 1999; Hamoen et al. 2003; Kjos et al. 2016). We, therefore, extend our model to allow cells to change their uptake rate physiologically in response to stress and we assume that poorly adapted cells (e.g. cells that susceptible to an antibiotic in the patch) experience the most stress. We implement conditional gene transfer by using a linear function, but other functions could be considered. Gene transfer rate E_i of a cell i takes the value $C_{i,1}$ if the cell is non-adapted, and the value $C_{i,2}$ if it is fully adapted:

$$E_i = C_{i,2} + (C_{i,1} - C_{i,2}) * (1 - a_i), \qquad (3)$$

where a_i is the adaptive state of the cell i (given through Eq. 1). Instead of evolving gene uptake rate (E_i), cells now evolve the parameters $C_{i,1}$ and $C_{i,2}$ via mutation (a random walk at a rate q, mean $\mu = 0$ and a small standard deviation $sd = 0.001$). We run larger simulations of the evolution of this regulated gene

transfer (to reach the steady-state of the two parameters requires 100,000 migrations per cell) and we find that $C_{i,2}$ for all tested parameter combinations evolves to become zero (Figure 4a) so that $C_{i,1}$ evolves to be larger than $C_{i,2}$. This confirms what we know from cellular stress responses, that competence is commonly upregulated in the face of stress (Prudhomme et al. 2006; Claverys et al. 2006; Engelmoer and Rozen 2011b). We can therefore reduce simulation time by simplifying the function for conditional gene transfer into

$$E_i = C_i * (1 - a_i), \qquad (4)$$

with a single function parameter C_i that determines the strength of the response of a cell *i* to a state of maladaptation. Figure 1 provides a schematic of the model processes, and Table 1 gives a list of parameters and standard values used.

Table 1 | Key parameters of our eco-evolutionary model.

Model parameter	Parameter description	Standard value
K	Carrying capacity of the patch	1,000
n	Number of loci for local adaptation	10
m	Cell migration rate	[0,1]
s	Strength of selection	0.9
d	Amount of random cell death (drift)	0.1
u	Type of epistasis between gene loci	[0.5, 1, 2]
z	Ecological difference between niches	0.2
r_{mut}	Mutation rate	0.01
q	Rate of gene uptake evolution	0.1

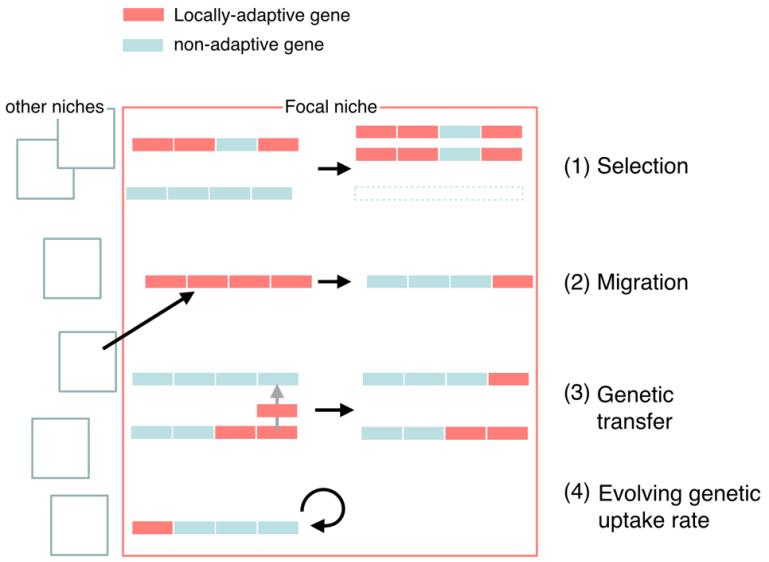


Figure 1 | Schematic of our eco-evolutionary model. The focal niche (red box) contains cells that are defined by their genome (here shown as four loci). Red loci are locally-adapted, blue loci are non-adapted. Our model combines four key processes. 1) Selection: maladapted cells compete poorly in the niche and are more often replaced than well-adapted cells. 2) Migration: a well-adapted genotype moves to a new patch and becomes a poorly adapted immigrant. We capture this effect by making genotypes in our focal patch poorly adapted with a certain probability. 3) Genetic transfer: cells take up genes either from a remote gene-pool of well-adapted genes, or from competing cells, and 4) Evolution of the cell-specific rate of gene uptake.

5.4 Results

A barrier to HGT: competitors' DNA is no better

We begin by recapitulating the findings of a previous simulation model of HGT evolution (Earl and Deem 2004). This model provides a good baseline as it shows how HGT can be beneficial whenever it brings in DNA that is better adapted to a local environment than the DNA of a focal cell. When we incorporate the assumptions of this model into our framework, we recapitulate the prediction that frequent changes in a cell's environment (migration) favours the evolution of high rates of gene exchange (Figure 2a) (Earl and Deem 2004).

However, this result rests upon a strong and unrealistic assumption that the transferred DNA always comes from an ecologically-undefined pool of DNA that is well-adapted to the current environment. In reality, DNA exchange typically occurs between competing cells with similar ecology (Boucher et al. 2011; Smillie et al. 2011; Kav et al. 2012), because they live in close proximity, secrete antibiotics that lyse other cells, and have similar sequences for homologous recombination (Steinmoen et al. 2003; Vos 2009). We, therefore, modify our model such that genetic transfer occurs between the cells that co-occur in a patch, rather than coming from a fixed well-adapted gene pool.

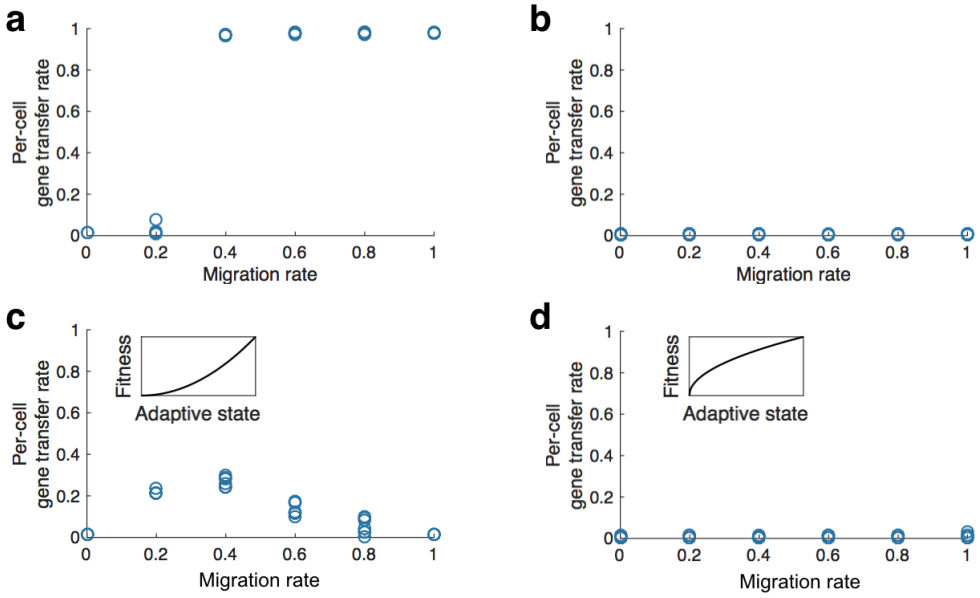


Figure 2 | Horizontal gene transfer only evolves for positive fitness epistasis between loci. We show the final evolved per-cell gene transfer rate over the range of migration rates for 5 instances of the simulation. **a**) When HGT brings DNA from a fixed gene pool containing well-adapted genes, frequent migration favours the evolution of horizontal gene transfer. **b**) When horizontal transfer occurs between cells within the community, gene transfer does not evolve. **c**) Horizontal transfer can evolve for local DNA uptake when there is positive epistasis for fitness between loci. This means that each additional well-adapted locus obtained has a larger benefit than the last (inset). Under these conditions, intermediate rates of migration favour the evolution of genetic transfer because too much migration makes all strains poorly adapted and limits the potential for natural selection. **d**) When genetic loci diminish each others adaptive effect on fitness (negative epistasis, inset), genetic transfer does not evolve. All model parameters take the standard values given in Table 1.

This simple change in assumption undermines the benefit to horizontal transfer (Figure 2b). Now, the gene pool sampled by HGT evolves as a result of the ecological and evolutionary processes in the niche. The result is fatal for the evolution of transfer because, on average, the DNA of competitors has the same average level of evolutionary adaptation as the genome of a focal cell. This removes the adaptive benefit of HGT.

HGT evolves with positive epistasis for fitness

The evolution of horizontal genetic transfer in bacteria has similarities to the evolution of sexual recombination in eukaryotes, where there is a large theoretical literature (Williams 1975; Bell 1982; Frank 1998). Importantly, this literature suggests that there can be evolutionary benefits to gene exchange, even when it occurs between similar individuals (Williams 1975; Young 1981; Maynard-Smith and Szathmáry 1997). Recombination with other individuals creates offspring with both maladapted genotypes and well-adapted genotypes. While recombination cannot increase the number of adapted alleles in a population, it can change the distribution of these alleles between individuals. This can lead to fitness benefits if recombination creates very high-fitness individuals that can then sweep through the population. However, the theory from eukaryotes predicts that for this to occur, there must be epistasis for fitness between loci (de Visser and Elena 2007).

We therefore extended our model to allow for positive epistasis, where beneficial effects of different gene loci enhance each other, and negative epistasis, where beneficial loci diminish each other's effect. This reveals that positive epistasis will allow the evolution of HGT by natural selection on microbial cells (Figure 2c). There is an intuitive logic to this finding. With positive epistasis, well-adapted individuals have disproportionate fitness value and, therefore, horizontal transfer can be beneficial when it allows a genotype to increase the probability of generating these fittest individuals. Consistent with this, HGT does not evolve for negative epistasis for fitness (Figure 2d). In this case it becomes more difficult to improve fitness by recombination, but relatively easy to create very low fitness progeny. In sum, as for the evolution of sex, we find that epistatic

relationships between adaptive genes can be important for the evolution of horizontal genetic transfer.

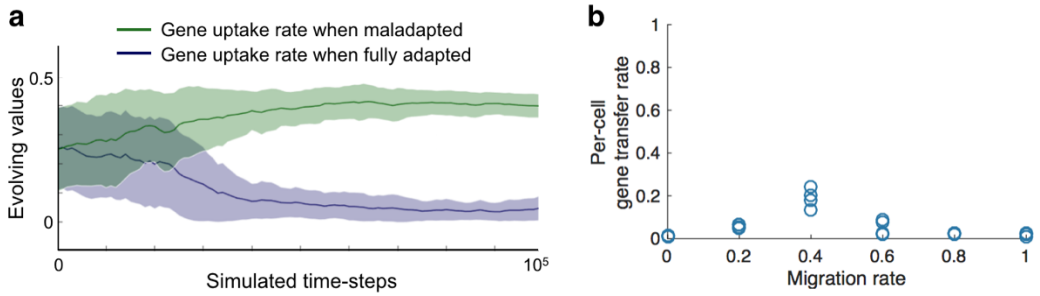


Figure 3 | Gene transfer evolves when it is regulated under stress. (**a**) The evolution of regulated DNA uptake over time. We asked what happens when cells are free to evolve transfer rate in response to their level of adaptation. Here we plot the evolved rates over time as a function of being maladapted (C_1) or fully adapted (C_2). Consistent with data on regulation of competence, cells evolve to have a near zero gene uptake rate when fully adapted and a positive uptake rate when maladapted. Simulations are initialized by drawing C_1 and C_2 for each cell from a uniform random distribution between 0 and 0.5. Other parameters take the standard values given in Table 1. (**b**) The evolution of regulated horizontal genetic transfer. Here, we allow cells to evolve regulated competence as a function of C_1 only and we measure the actual per-cell gene transfer rate at steady-state for a range of migration rates (*Methods*).

Regulated gene uptake evolves readily

Our model predicts that horizontal gene transfer can only evolve when there is positive epistasis for fitness between different loci. While this may indeed occur under some conditions, other relationships are possible and indeed likely (Otto and Feldman 1997; de Visser and Elena 2007). This suggests that horizontal transfer may often be counter selected. However, our models have ignored a major piece of the biology of natural competence, by which bacteria take up extracellular DNA and incorporate it into their genome. Competence is known to be highly regulated across strains and species, particularly in response to stress

(Lee and Morrison 1999; Hamoen et al. 2003; Kjos et al. 2016). We therefore extend our model to allow for the possibility of regulated genetic uptake (*Methods*). In order to capture the effects of stress, we ask how the gene uptake rate evolves as a function of a cell's adaptive state. This is based on the observation that well-adapted cell–such as one that is resistant to a local antibiotic–will experience less environmental stress than a poorly adapted (antibiotic susceptible) cell (Gustavsson et al. 2002; Maiques et al. 2006).

We first ask what happens when a fully adapted cell has a gene uptake rate C_2 that can change independently of cells that are not perfectly adapted, which have an uptake rate of C_1 (*Methods*). This shows, as expected, that cells evolve gene uptake of zero when they are fully adapted, but non-zero when they are maladapted (Figure 3a), which is consistent with induced natural competence in bacteria where gene uptake increases under stress (Prudhomme et al. 2006; Claverys et al. 2006; Engelmoer and Rozen 2011b). With C_2 set to zero, we next studied how the level of C_1 evolves over time (*Methods*), as before, as a function of different migration rates (Figure 3b). Now, despite the assumption of no epistasis for fitness, we find that horizontal transfer can readily evolve. Regulated competence allows a cell to engage in gene transfer only when it is most beneficial: when its genome is less adapted than other genomes. By contrast, when cells are fully adapted they can shut down gene uptake and protect their genomes from damaging changes.

5.5 Conclusion

A key process of microbial adaptation is their ability to modify their genomes using horizontally acquired DNA (Lawrence 1999; Ochman et al. 2000; Gogarten et al. 2002). Horizontal gene transfer enables cells to take up beneficial traits, such as antibiotic resistant genes (Schlüter et al. 2007; Norberg et al. 2011; Stokes and Gillings 2011; McCarthy et al. 2014). However, transfer can also carry significant costs for recipient cells, which can be metabolic and also genetic when existing functions are compromised (Bell 1982; Chou et al. 2011; Park and Zhang 2012; Baltrus 2013; San Millan et al. 2015). Here we use an eco-evolutionary model to study horizontal gene transfer and we find significant barriers to its evolution. In particular, because transfer will occur between neighbouring cells, imported DNA will, on average, be no better adapted than the genome of a focal cell. This effect can prevent the evolution of horizontal genetic transfer (Figure 2).

The study of the evolution of sex in eukaryotes also finds significant barriers to its evolution (Bell 1982). A growing body of theory suggests that fitness epistasis between loci can drive benefits to recombination (Otto and Feldman 1997; de Visser and Elena 2007). Incorporating fitness epistasis in our model leads to an advantage to horizontal transfer under conditions of positive fitness epistasis (Figure 2). Positive fitness epistasis puts great value on the best-adapted genotypes. These genotypes are continually eroded in our model due to migration, which puts genotypes in a new environment where they are poorly adapted. Horizontal gene transfer allows cells to recreate these valuable genotypes and, therefore, it can be positively selected. As for the evolution of sex then, these results predict that the evolution of horizontal transfer will be dependent of

specific epistatic relationships between adapted loci. While epistatic interactions between gene loci are indeed common (MacLean et al. 2010), it appears, however, that for beneficial mutations negative epistasis is most common (MacLean et al. 2010; Chou et al. 2011; Khan et al. 2011), leading to the question, how then can horizontal gene transfer evolve?

Our first models neglect a key feature of horizontal genetic transfer, its regulation. Allowing cells to regulate their horizontal transfer based upon their level of adaptation resulted in its evolution, in the absence of epistasis (Figure 3). While microbes cannot directly sense their level of adaptation, cells sense their environment via sophisticated stress responses that detect damage to DNA, proteins, the envelope and other cellular components (Gustavsson et al. 2002; Maiques et al. 2006). These responses regulate many traits including natural competence (Claverys et al. 2006) and can be brought about through conditions including antibiotics (Prudhomme et al. 2006). Indeed, poorly adapted cells that face a novel antibiotic are known to upregulate competence, and gene uptake is central to the evolutionary rescue of bacteria treated with antibiotics (Ojala et al. 2014). This phenomenon has interesting parallels in induced mutagenesis, where cells display stress-induced increases of mutation rates (Tenaillon et al. 2004). These high rates of mutation temporarily increase the "evolvability" of cells that are struggling for survival under environmental stress conditions (Bjedov et al. 2003).

Horizontal genetic transfer can occur for multiple reasons, including the inability of host cell to suppress the transmission of phages and plasmids (Vos et al. 2015). While a phage will benefit greatly from transfer, however, incorporating foreign DNA can be very costly to microbial cells. Our models underline the

potential for barriers to the evolution of genetic transfer by microbes, but we also find conditions where there is positive natural selection to take up novel DNA. Our work suggests that cells evolve horizontal genetic transfer in order to cope with their competitive and ever-changing environments.

6 Discussion and Conclusion

6.1 Summary

While each of my chapters has its own discussion, I summarise here the key findings of each chapter and lay out some general conclusions that emerge from my thesis as a whole. Finally, I discuss caveats and future directions of this work.

Chapter 2: The evolution of siderophore production as a competitive trait

Siderophores are key for microbial survival and virulence. The goal of Chapter 2 was to understand how the production of siderophores evolves in natural communities. Siderophores are often studied as a classic public good[1] that is shared within a cell community. I used differential equation modelling to study the effect of specific receptors that limit sharing of siderophores among genotypes. I showed that this privatisation is a key parameter for siderophore ecology and evolution because it determines whether production benefits or harms competitors. When siderophores are fully shared between genotypes, competition

[1] A public good is a secreted product that can be used by others, including cheaters that benefit without paying the cost of production.

between strains drives down the evolutionarily stable level of production, because this limits siderophore piracy by competing strains. This matches the prediction for a canonical cooperative phenotype. By contrast, when siderophores are private, they function to competitively pillage iron away from others, and therefore production levels evolve to increase with increase competition.

I find that the evolution of *regulation* of siderophore production follows the same rules as the evolution of production. When siderophores are entirely shared, strains evolve to downregulate production with strain-mixing in order to reduce cross-feeding of others. When siderophores are private, they are instead upregulated in the presence of competitors, as expected of a competitive trait such as antibiotic production. Data from multiple species support this latter prediction that siderophores regulation responds as a competitive trait. Siderophores may be public goods at the scale of a single genotype, but my work suggests that their role in competition strongly shaped their evolution. Considering both the cooperative and competitive side of siderophores will be important to understand this key microbial trait.

Chapter 3: The evolution of warfare in bacteria

Bacteria inhibit and kill each other using a diverse array of toxic secretions, which are often strongly regulated in response to environmental conditions. The goal of Chapter 3 was to analyse and understand this regulation. In particular, I asked: what is the most effective way for a bacterial strain to attack? The first results showed that regulating attacks is a good thing. A well-regulated toxin response always has an evolutionary benefit over fixed toxin production. Regulation allows a producer strain to better follow the optimal toxin investment at any given time.

This is true whether a cell responds to other based upon any one of our three tested forms of regulation: nutrient depletion, quorum sensing, or competitor toxins.

However, the three classes of sensing are not equivalent: I showed that regulating toxin production via the levels of a competitor's toxin is the most robustly successful strategy against a diverse range of other toxin strategies. This is because these strains only attack when faced with a toxin producing opponent, but also because they can turn off costly toxin production once the competitor is defeated.

My model predictions are supported by a growing body of evidence that bacteria upregulate their attacks in response to cell damage ('competition sensing') and this also mirrors predictions from game theory, that reciprocation is a powerful strategy. However, reciprocation in microbes is not always to avoid conflict but rather to enter an efficient peacetime once a competitor has been eliminated.

Chapter 4: Migration and horizontal gene transfer divide microbial genomes into multiple niches

Microbes rapidly adapt to environmental challenges, such as antibiotics, by incorporating foreign DNA into their genomes. However, theory has failed to explain how horizontal transfer exerts such dramatic effects on communities of microbes. In Chapter 4 I sought to understand what allows high rates of horizontal gene transfer that can transform the ecology, evolution and genomics of communities. I found that a single gene can sweep through a microbial community via horizontal gene transfer and positive natural selection, whenever

there are large numbers of maladapted immigrants into the communities. These migrants prevent fixation of the selected trait, and will struggle in the new environment until they pick up the key gene that is needed to survive. This process allows continual transfer to occur and enables the horizontal transfer of ecologically important genes through whole communities.

I also showed that single-gene sweeps can strongly change the genomics of local populations. Within a patch, sweeping genes remove diversity at one locus relative to the remaining background genome. In contrast, across patches that select for different transferrable genes, the fingerprint of horizontal gene transfer will be that it generates diversity in transferrable loci. This can explain how horizontal gene transfer is able to remove locus diversity in some studies and create locus diversity in others. Horizontal gene transfer, with the support of migration, can enable ecologically-important genes to accumulate in local environments to which they are adapted, without the rest of the genome evolving in the same way. Thereby, single genes can have an own specific ecology or niche. This led to my key conclusion that the niche of a microbe will often be defined at a sub-genomic scale, with any one genome mapping to multiple independent but overlapping niches.

Chapter 5: The evolution of horizontal gene transfer in microbes

Microbes can adapt through uptake of DNA from other strains and species. This ability is fundamental for microbial evolution and their ability to acquire antibiotic resistance. However, we lack understanding of how this horizontal gene transfer evolves. In Chapter 5, I developed an eco-evolutionary model of horizontal gene transfer. This model captures the evolution of cells that constantly

face new adaptive challenges. I first showed an important barrier to the evolution of horizontal gene transfer: DNA that is taken up from other cells is often not better than the genome of a focal cell. I then tested different forms of fitness epistasis between the gene loci, and I showed that horizontal gene transfer can evolve, but only with positive epistasis for fitness, where adapted loci enhance each other's effect on fitness. Finally, I showed that these limitations to evolution of gene transfer are removed when cells can regulate gene uptake. If cells only take up DNA when they are poorly adapted and stressed, they will incorporate DNA that is mostly better adapted to their current environment than their own genome. My results support the view that microbes have evolved regulated gene transfer to cope with environmental changes, including those caused by cell migration.

6.2 Insights, clarifications and future work

In this final section I bring together some key emerging topics and themes that run through the different chapters of my thesis. This allows me to draw general conclusions from my work and highlight interesting future work.

The functional complexity of microbial secretions

My work has investigated the evolution of both competitive and cooperative factors released by microbial cells. Indeed, I have shown how the evolution of siderophores can respond simultaneously to both cooperative and competitive effects of a single trait. This underlines the potential functional complexity of microbial secretions and the general point that, in order to understand a social

trait, one must understand its evolutionary function. Assigning evolutionary function to any trait can be challenging (Gould and Lewontin 1979), but this is particularly true of microbial factors whose effects can change between different situations (Ghoul et al. 2014; Kümmerli et al. 2014). For example, some siderophores are involved in cell-cell signaling (Roux et al. 2009), others, such as sideromycins, are both siderophores and a toxin into a single "Trojan horse" molecule that gets taken up by competitors (Braun et al. 2009). An important extension of my work will be to study how these other potential effects of siderophores affect the evolution of production and regulation.

The secretion of toxins, such as antibiotics, also has the potential for functional complexity. My work has focus on the typical function for these molecules as microbial weapons, since their potential to inhibit other strains is clear (Hibbing et al. 2010; Abrudan et al. 2015). However, there are many potential alternative functions for antibiotic molecules, including the coordination of other phenotypes such as biofilm formation or plasmid transfer (Andersson and Hughes 2014). One key alternative function of toxins is auto-induction, where toxins (bacteriocins) are known to act as their own inducer once cell density is sufficient (Kleerebezem and Quadri 2001; Kleerebezem 2004). An intriguing possibility is that this self-induction may be used to signal the threat of a competitor to clone-mates that have not yet detected the threat. I expect that this will be particularly important in spatially-organized communities like colonies competing on a surface, where cells on the edge are the first to sense the presence of a competitor (Be'er et al. 2010; Traxler et al. 2013). A colony may, in this way, generate an early warning signal that travels through it faster than the threat (the competing toxin) itself (Daniel Cornforth, *pers. comm.*).

Like siderophores then, antibiotics can function as a cooperative trait with respect to the producing genotype but as a competitive trait relative to other genotypes. Considering the vast diversity of microbial toxins (Riley 1998; Watve et al. 2001; Bérdy 2005), it is likely that the exact nature of their role in competition will differ between diverse microbial species. My study of toxin regulation demonstrates how one can map the mechanistic effects of toxins onto the evolution of regulation. This opens the way for further modelling that combines experimental knowledge of toxin regulation (Gottesman 1984; Worobo et al. 2000; Kjos et al. 2016), with different putative functions to better understanding their evolution.

The importance of competition for microbial communities

A key theme throughout my thesis is the importance of competition for understanding the biology of microbes. This is nowhere more clear than for the case of microbial regulatory networks. Microbial regulation has been intensively studied for many years as it is central to their ability to resist antibiotics and many other stresses. However, only recently has there been the suggestion that stress responses can be understood through the lens of social evolution thinking (Foster 2011; Cornforth and Foster 2013). In particular, the competition sensing hypothesis (Cornforth and Foster 2013) links the key social function of toxins–to harm others–to their molecular regulation. It states that microbes use DNA damage from antibiotics, and other forms of biotic stress, as an internal cue to detect competitors and respond with producing toxins. This allows bacteria to attack back when they are themselves attacked, analogous to the classic "tit-for-tat" response from evolutionary game theory (Axelrod and Hamilton 1981).

In the first part of my thesis I added two new pieces to the puzzle of microbial regulatory networks. I showed that siderophores, commonly studied as a public good, can mediate competition. This means that siderophore regulation evolves like toxin regulation, with upregulation in response to competition sensing. I have also studied toxin regulation itself, comparing different possible different environmental cues that can be used to launch an attack. Via large evolutionary tournaments, I found that regulating toxins via an incoming toxin attack is the most robust strategy.

However, it is also clear that many microbes regulate their siderophores and toxins using forms of regulation other than cell damage, including quorum-sensing or nutrient stress. Further work is required to understand the variability in regulatory networks between microbial species and ecologies. For example, how does informational noise, delay or the metabolic cost of sensing affect the evolution of regulation? Both noise and delay will make information less reliable, which should favour the use of other forms of information. Or, cells might evolve to integrate different cues, and such a combination may be more robust to situations where, for example one cue cannot be detected. There is a growing potential to study the evolution of complex cellular networks, with multiple sources of information, using *in silico* neuronal networks (Gross and Blasius 2008). Further work should also consider how spatially structured environments affect the evolution of sensing, because optimised strategies of toxin production and iron scavenging will be different in the middle and at the edge of a colony. More broadly still, understanding the regulation of competitive traits is important for engineering and manipulating multi-strain communities, either to disrupt dangerous communities or enhance beneficial ones (Saeidi et al. 2011). This can

come in the form of engineering the regulation of a strain to achieve a particular function (Zuroff and Curtis 2012), or in terms of understanding the complex effects of antibiotics on communities, which I discuss next.

The effects of clinical antibiotics of regulatory networks

A key prediction of both my siderophore and toxin work is that both traits may often be upregulated in direct response to competitors (Chapter 2 and 3). A major way that cells can detect competitors is by detecting the cell damage caused by their antibiotics (competition sensing). This raises the interesting possibility that the use of clinical antibiotics will have previously neglected effects, by making microbes act as though they are under attack from a competitor. Do clinical antibiotics induce production of toxins and siderophores? Antibiotics modulate various bacterial secretions, including toxins, in a dose-dependent manner (Fajardo and Martínez 2008). Sub-lethal doses of antibiotics are known to trigger toxin production in different bacterial species including *E. coli* (Zhang et al. 2000; Bano et al. 2013), *Streptococcus pneumonia* (Kjos et al. 2016), and *Pseudomonas* (Ghequire and De Mot 2014). My work suggests that this induction is explained by the cells misfiring and releasing toxins that evolved to be used against a microbial competitor. Little is known about the effects of clinical antibiotics on siderophores. However, recent work suggests that antibiotics can change iron-metabolism in microbes (Méhi et al. 2014), and preliminary data from our lab indicates that siderophores might indeed be induced at sublethal doses of clinical antibiotics (Nuno Oliveira *pers. comm*.).

Current data then support the view that we should interpret microbial responses to clinical antibiotics under the conceptual framework of evolved

competition between strains. What are the implications of these effects? Both toxins and siderophores have been shown to induce damage to host organisms (Reimmann et al. 1997; Zhang et al. 2000; Harrison et al. 2006), suggesting that antibiotic treatment could indeed have adverse effects when triggering these virulence factors. A dramatic example comes from a study in mice infected with Shiga toxin-producing *Escherichia coli.* When the mice are treated with antibiotics the bacterial toxin is induced, and instead of being cured from infection, the mice die (Zhang et al. 2000). More typically, the upregulation of competitive phenotypes via antibiotic treatment may be felt first within microbial communities themselves. Bacteriocins often induce production of other bacteriocins (Majeed et al. 2013; Abrudan et al. 2015), and siderophores can induce production of toxins as well as other siderophores (Traxler et al. 2013). Intensifying competition between strains with antibiotics, therefore, might help to disrupt microbial communities by promoting the expression of destructive phenotypes. However, counter intuitive effects are also possible. Recent work suggests that competition between strains, while inefficient, can be central to creating ecologically-stable communities (Coyte et al. 2015).

In sum, clinical antibiotics have diverse effects on communities of microbes beyond killing of susceptible strains, and we are only now starting to understand these effects. My work suggests that we must consider how warring bacteria have adapted over millions of years in order to understand the effects of clinical antibiotics on microbial communities today

The effects of clinical antibiotics on horizontal genetic transfer

The use of clinical antibiotics also impacts another major topic in my thesis: horizontal genetic transfer. The widespread application of antibiotics in clinical settings and in the food industry (Cabello 2006; Silbergeld et al. 2008) creates intense natural selection and creates novel adaptive challenges for microbes. My models predict that this variable and strong selection can favour an increased rate of horizontal genetic transfer over evolutionary time. Such an effect is already known in real time. Some antibiotics are known to induce horizontal transfer, which might evolve as a way for a susceptible genotype to evolve resistance against competitors (Maiques et al. 2006). Any increase in transfer is worrying because it will accelerate the adaptability of microbes observed in clinical settings (Lipsitch and Samore 2002; Ojala et al. 2014), as well as allow resistance genes to more easily jump between isolated genetic pools, for example between soil microbes and pathogens (Forsberg et al. 2012; Djordjevic et al. 2013). And, within any one community, I have shown how prolonged antibiotic treatment may drive a single-gene sweep that transforms the genomics of the community.

It is clear that the selective pressures imposed by clinical antibiotics can have strong and complex effects on microbial communities (Sundberg et al. 2016). We urgently need to understand these effects if we are to tackle the rising threat of antibiotic resistance. As I hope to have illustrated here, there is an important role for modelling. During my postdoctoral position with the Mahidol-Oxford Tropical Medicine Research Unit in Bangkok, I will continue and extend my work on modelling horizontal genetic transfer. My goal will be to understand the within-patient and between-patient spread of antimicrobial resistance genes occurring under the influence of antimicrobial treatments, using a 20-year

database. With my work I hope to contribute to our understanding of the accelerating spread of antimicrobial resistances in Southeast Asian countries (Song et al. 2004; Song et al. 2011).

Concluding remarks

The study of microbes has historically focused on clonal populations. I hope to have shown the importance of the genetic diversity within microbial communities and, in particular the competition that this brings between strains and species. It is this competition that can drive the secretion of both siderophores and toxins, which are microbial traits of major clinical concern. Competition is also fundamental to the evolution of horizontal genetic transfer, and how this transfer can become so powerful as to ecologically decouple genes from the genome that contains them.

In closing, I find it particularly fascinating that organisms as "simple" as microbial cells have clearly evolved sophisticated regulatory systems to cope with unfriendly neighbours. This regulation allows them to make informed decisions on their microscopic battlefields, and to achieve the measured use of their weapons against competitors. Moreover, when in dire straits, they can even engage horizontal transfer in an apparent attempt to steal the weapons and defences of competitors. Microbes are undeniably social but it is perhaps their antisocial behaviour that will, in the end, teach us most about their biology.

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8 Appendix: Published work

nature COMMUNICATIONS

ARTICLE

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Migration and horizontal gene transfer divide microbial genomes into multiple niches

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Horizontal gene transfer is central to microbial evolution, because it enables genetic regions to spread horizontally through diverse communities. However, how gene transfer exerts such a strong effect is not understood. Here we develop an eco-evolutionary model and show how genetic transfer, even when rare, can transform the evolution and ecology of microbes. We recapitulate existing models, which suggest that asexual reproduction will overpower horizontal transfer and greatly limit its effects. We then show that allowing immigration completely changes these predictions. With migration, the rates and impacts of horizontal transfer are greatly increased, and transfer is most frequent for loci under positive natural selection. Our analysis explains how ecologically important loci can sweep through competing strains and species. In this way, microbial genomes can evolve to become ecologically diverse where different genomic regions encode for partially overlapping, but distinct, ecologies. Under these conditions ecological species do not exist, because genes, not species, inhabit niches.

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Microbes survive and reproduce in an extremely wide range of environments, from hydrothermal vents[1] through marine snow[2] and soil[3], to host associations such as the human microbiome[4]. Within and between such environments, microbial genomes differ widely both in terms of allelic diversity and gene content[5–7]. At the heart of this genetic diversity is the ability of microbes to gain both homologous and non-homologous DNA via horizontal gene transfer. These transfers appear to occur in almost all prokaryotic lineages and have significant impacts on both bacterial and archaeal genomes[8].

Horizontal transfer is considered central to the ability of cells to adapt to new ecological conditions including clinical or environmental settings that contain antibiotics[9,10]. Recent empirical work suggests that these transfers can spread a single beneficial allele horizontally through a microbial community—where the allele can either represent a single gene or a small group of genes—with the result that an otherwise diverse microbial community becomes genetically identical in a certain genomic region[9,11–15]. However, the rates at which gene transfers occur are thought to be extremely low, with asexual reproduction a much faster process. Competition between strains and species within a patch, therefore, will mean that a beneficial allele can spread much more quickly via whole-genome vertical transmission than horizontal transmission, which should prevent horizontal sweeps[16–19]. One way that genes can transfer horizontally is if they hop between ecologically distinct populations that do not compete[20–22]. However, the prevalence of competition within microbial communities[23,24] suggests that vertical sweeps should remain a barrier to horizontal sweeps. And yet, the experimental evidence for horizontal sweeps comes from communities of phylogenetically related strains[9,11–13,25] where ecological competition is likely to be significant.

A major question then is how horizontal transfer can so strongly impact microbial communities and cause the observed horizontal sweeps. Answering this question is necessary to understand microbes and how they evolve, both in nature and in the clinic. In a recent study, Takeuchi *et al.*[26] provide an explanation for horizontal sweeps via negative frequency-dependent selection acting on other loci in the genome. Frequency-dependent selection can prevent full genome-wide selective sweeps and allow time for genetic transfer. Here we show that horizontal sweeps can occur without the need for negative frequency-dependent selection. We develop a series of models of a microbial community in which all cells compete for the same resource. We use these models to study both the rate of horizontal gene transfer and the impact that this transfer has on the genomics of the focal community. Our work reveals a missing ingredient that can explain horizontal sweeps: migration. When including immigration in our models, we find that the highest rates of horizontal transfer will occur for ecologically important traits that are under positive natural selection. The result is a genome where selected regions become partially decoupled from the ecology of the remaining loci such that different parts of the genome map to different niches.

Results

Overview. We are interested in understanding how a beneficial trait can spread horizontally through a microbial community. We build on a previously modelled scenario[18,27] that considers a community of diverse strains that compete, and that will have the opportunity for genetic transfer[28,29]. Although we use the word 'community' throughout, our model can also capture a set of strains from a single species, commonly referred to as a 'population'. We follow the fate of a novel beneficial allele that is able to transfer horizontally between the genotypes in our community. Existing theory suggests that very little gene transfer should occur, because once a beneficial trait is picked up horizontally the carrier will rapidly outcompete the other strains in the patch before it has a chance to transfer the trait to other genomes[16–18,27]. However, previous models neglect the possibility that fixation of a trait can be delayed or prevented by migration of new cells into the community. We therefore begin by recapitulating previous predictions that competition suppresses gene transfer and we then show how making a single change, allowing immigration, can explain how horizontal genetic sweeps occur.

Without migration adaptation by horizontal transfer is rare. We consider a community that lives in a focal patch, which can either be literally a single isolated patch or a set of similar patches that are themselves well connected by migration. At first we consider that this patch is largely isolated from the external environment, such that there is negligible migration between the community of the patch and the world at large. This scenario could correspond, for example, to communities living in hosts where there is limited superinfection with new strains and species over time[30]. We focus on those strains in the patch that are ecologically interchangeable in the sense that they compete for a common limiting resource. Under these conditions, horizontal transfer has been predicted to be almost powerless compared with vertical selective sweeps[16–18,27].

Our model follows the fate of a rare beneficial allele that first appears in a small subset of the community. Although non-carriers have fitness $w=1$, carriers of the adaptive allele have increased fitness $w=1+s$, where s is the local benefit of the trait (b) minus the cost of carrying it (c). We assume that the benefit of the trait in the focal patch is always larger than its cost, so that $s>0$. Any cell in the community lacking the gene has a chance of picking it up horizontally and it is the overall rate of this process in the community that we are interested in. Not all loci can transfer their phenotypes horizontally[31–33] and we focus here on those loci where horizontal transfer is possible. In addition, genetic transfer is typically relevant only for phenotypes that lie outside the range of physiological responses or short-term evolution by *de-novo* mutation[34,35]. Examples of such phenotypes—transferable and otherwise hard to achieve—include toxin resistance genes[36], virulence factors[37] and heat shock proteins[38].

We want then to capture the horizontal spread of an allele in a community of microbes. Although there is relatively little theoretical work on genetic transfer and its impact, there is a long theoretical tradition of modelling the horizontal spread of infectious diseases, along with associated empirical tests[39,40]. These models, often known as compartment models, have proved to be a powerful way to capture the key processes underlying the horizontal spread of a focal trait. We therefore begin here with a simple compartment model (Fig. 1a) that allows us to identify the conditions that maximize horizontal genetic transfer, before extending and developing our predictions using other modelling approaches.

We study a focal community of constant size N that contains two sub-communities, adaptive gene 'carriers' and 'non-carriers'. Applying a deterministic continuum approach, the relative community size in each sub-community and the flux between them can be described by the ordinary differential equation (ODE)

$$\frac{dC(t)}{dt}=rC(t)(1-C(t))+\frac{s}{1+sC(t)}C(t)(1-C(t)), \qquad (1)$$

where $C(t)$ is the fraction of carriers in the community at time t, $1-C(t)$ is the fraction of non-carriers and r is the rate of gene

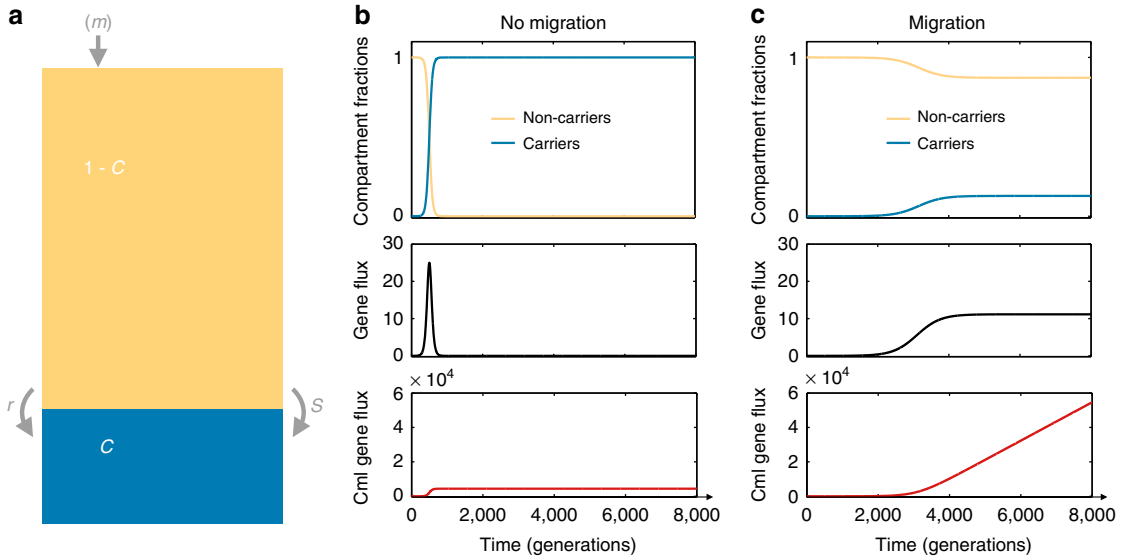

Figure 1 | Migration greatly increases the potential for horizontal genetic transfer of a beneficial allele. (**a**) The compartment model. Sub-communities C (blue) and $1-C$ (yellow) represent the fractions of allele carriers and non-carriers in the community, respectively. In the absence of migration, positive selection (s) causes a replacement of non-carriers by carriers and gene transfer (r) converts non-carriers into carriers. In the presence of migration, non-carriers continuously arrive at the patch in addition to the processes of positive selection and gene transfer. (**b**) No migration: the beneficial allele is rapidly fixed so that the horizontal gene flux becomes zero and the total amount of horizontal transfers since the beginning of the sweep, the cumulative gene flux (cml gene flux), stays constant. (**c**) With migration: the immigration supplies the system with non-carriers, resulting in ongoing gene transfer at a constant rate. Other parameters are $N=10^8$, $s=0.025$, $r=10^{-6}$, $m=0$ (**b**) and $m=0.02$ (**c**).

transfer from carriers to non-carriers (Fig. 1a). The growth of compartment C due to selection is given through the term $s/(1+sC(t))$. The term $rC(t)(1-C(t))$ captures the transition of cells from being a non-carrier to carrier through horizontal gene transfer. The proportion of trait carriers has two steady states (C^*) when s or r are non-zero, given by

$$C^*=0,\ C^*=1, \qquad (2)$$

with $C^*=0$ being an unstable and $C^*=1$ being a stable equilibrium (see Supplementary Methods). Therefore, for any non-zero initial number of carriers ($C(0)>0$) the fraction $C(t)$ will increase until fixation of the focal beneficial allele ($C^*=1$) at steady state. A key model parameter for our further analysis is the probability that a carrier cell transfers the focal allele to a non-carrier in any generation, r. Gene transfer rates in natural communities remain largely unknown. Previous theoretical work used a relatively low and conservative estimate for transfer rate of 10^{-6} per gene per generation[33], which corresponds to our transition rate ($rC(t)(1-C(t))$). However, recent studies suggest that the rates of horizontal transfer can be much higher than such estimates[41,42]. We therefore consider a range of rates, $10^{-6}\leq r\leq 10^{-4}$, which goes either side of 10^{-6} per gene per generation (using $r=10^{-6}$ guarantees that the transition rate $rC(t)(1-C(t))$ is $<10^{-6}$). Genetic transfer can occur both by homologous and non-homologous recombination. Although we phrase our results here in terms of the former, our conclusions should apply to both mechanisms.

What then defines the rate of horizontal gene transfer in the whole patch? We can calculate this rate by multiplying the transition rate from carriers to non-carriers, $rC(t)(1-C(t))$, by the community size N to obtain the community-level rate of gene transfer, which we term horizontal gene flux ($rC(t)(1-C(t))N$). This horizontal gene flux spreads the beneficial gene without removing cells, whereas the vertical gene flux, given through $sC(t)(1-C(t))$, spreads the gene by removing non-carrier cells.

As the selective sweep for the focal trait proceeds, the horizontal gene flux initially increases up to a maximum at a 1:1 ratio of donors and recipients ($C(t)=1-C(t)=0.5$) before decreasing back down to zero at allele fixation (Fig. 1b). The overall impact of this process can be quantified from the integral of the gene flux, which gives the expected number of horizontal gene transfer events over time (cumulative gene flux, see Methods). How is this cumulative gene transfer affected by the strength of positive selection? Plotting cumulative gene transfer against selection pressure shows that strong selection regimes minimize the effects of horizontal transfer, because the time window during which this transfer can occur is short (Fig. 2b, inset). Our model then recapitulates previous conclusions that, given a small rate of genetic transfer, only weakly selected traits can undergo significant horizontal transfer by slowly sweeping through a community[18]. However, weakly selected traits are, by definition, relatively unimportant for the ecology and evolution of their carriers. In contrast, many successful horizontally transferred traits, such as antibiotic resistance genes, appear to be both functionally important and under significant positive natural selection[29,43,44] and these are the traits that we are interested in here.

With migration adaptive traits transfer horizontally. Previous models of genetic transfer have not considered a key feature of microbial life, migration, which has the potential for important effects on population dynamics[45,46]. We next introduce migration into our model and we study its effect on our predictions. We now assume that our focal patch is separated, but not completely isolated, from its external environment so that there will be a limited but ongoing exchange of cells. For example, this focal patch could be a nutrient particle in ocean water, a mammalian host or tree hole. As discussed for our no-migration model, the 'patch' can also represent a set of connected patches that all select for the same horizontally transferred trait, that is, a set of

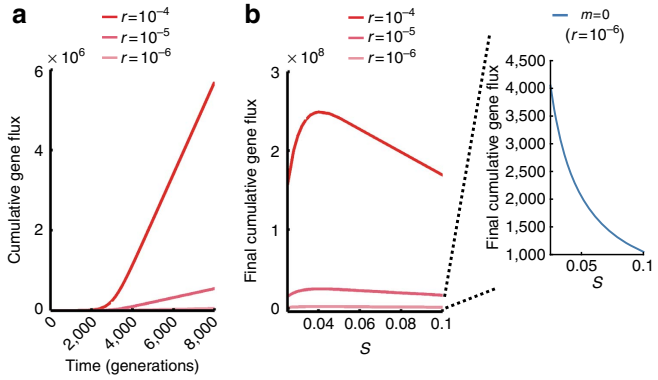

Figure 2 | Migration allows horizontal transfer even with significant positive natural selection for a transferred trait. (**a**) Gene transfer events accumulate over time when immigration is possible, with increased gene transfer rates increasing the horizontal gene flux. (Selection pressure $s=0.025$). (**b**) Cumulative horizontal gene flux measured at $t=10^5$ across different selection pressures (left-hand side plot) and for different rates of migration (right-hand side inset). With migration (left-hand plot, $m=0.02$), the cumulative gene flux peaks for intermediate selection pressure ($s=0.04$ with $\sim 2.5\times10^6$ transfer events) and remains significant even for stronger positive selection ($s=0.1$ with $\sim 1.7\times10^6$ transfer events). Without migration (inset plot, $r=10^{-6}$), gene transfer remains extremely infrequent and peaks at the lowest selection pressure with only around 4,000 transfer events. In all plots, we calculate cumulative gene flux for $N=10^8$ and $C(0)=1{,}000/N$.

particles, hosts or tree holes. The key is that we now allow there to be immigration from other regions that do not select for the focal trait. Accordingly, we assume that all immigrating cells that arrive from outside the focal patch (or patches) lack the trait (but we relax this assumption below). For example, the focal allele might provide resistance to a toxin that is specific to the focal patch. Some immigrating cells will be unable to establish themselves in the focal community due to a mismatch with general ecological characters, such as nutrient conditions, temperature and alike. Other migrants might not be compatible with the selected locus and are unable to adapt. However, both of these effects will lead to fast extinction of these migrants and we can account for both by varying the migration rate, where an increase in the frequency of non-viable strains corresponds to a reduced migration rate. Extending our model to include migration gives

$$\frac{dC(t)}{dt} = rC(t)(1-C(t)) + \frac{s}{1+sC(t)}C(t)(1-C(t)) - mC(t), \quad (3)$$

where m is the migration rate, given as the fraction of cells that is replaced through migrators landing and replacing them per unit time, and $mC(t)$ is the replacement of trait carriers only (Fig. 1a). If the basic rate of genetic transfer is very small ($r \approx 0$), the steady-state proportions of cells carrying the focal trait (C^*) are given approximately by

$$C^* = 0 \text{ and } C^* = \frac{s-m}{(m+1)s}, \quad (4)$$

with $C^*=0$ defining an unstable equilibrium and $C^*=(s-m)/((m+1)s)$ a stable equilibrium (see Supplementary Methods). Therefore, given an initial non-zero number of carriers, the system will reach the second equilibrium ($C^*=(s-m)/((m+1)s)$). A key implication of this expression is that with non-zero migration ($m \neq 0$) the selected trait will now reach a steady state before it has been fixed in the community ($C^* < 1$), because migration continuously brings new genotypes into the system. These migrators mean that opportunities for genetic transfer remain after the initial selective sweep has occurred. Migration stops the selective sweep before it can complete as a classic selective sweep[47] and instead there is a second longer-lasting incomplete sweep. Indeed, horizontal transfer now occurs as long as the community persists, which greatly increases its potential effects (Figs 1c and 2a). Migration rates are commonly considered to be high in natural microbial communities, as cells can be so easily dispersed, but exact rates are difficult to assess. In these first models, we use a relatively high rate of $m=0.02$, which corresponds to 2% of cells being replaced by incoming cells in each generation. However, we show in the next section that our conclusions are robust for a range of possible migration rates, just so long as migration does not overpower natural selection ($m<s$).

With migration in the model, the relationship between the strength of natural selection and the cumulative gene transfer is fundamentally changed. Now, horizontal transfer peaks for traits under intermediate selection pressure (Fig. 2), whereas without migration it peaks at minimum selection strength (Fig. 2b, inset). This means that migration greatly increases the potential for horizontal sweeps of ecologically important traits that are associated with significant positive selection pressures, for example, a trait that provides a fitness advantage of $>10\%$ (Fig. 2). The sweep occurs in spite of the fact that vertical flux remains the dominant mode of transmission in the community; even modest rates of natural selection ($s>10^{-3}$) are much greater than the expected rates of gene transfer ($10^{-6}<r<10^{-4}$).

We have assumed so far that incoming migrators lack the adaptive trait. However, there are clearly cases where new cells may be pre-adapted and carry the focal trait. When will this occur and how does it change our predictions about horizontal gene flux? To investigate this, we consider an extended model that explicitly captures the external environment as an additional compartment where the focal trait is disfavoured by natural selection (Supplementary Fig. 1). Cells from the focal patch can leave and enter the surrounding environment and, equally, cells can return from the surrounding environment into the focal patch. As before, our 'focal patch' can also represent a set of connected patches that all select for the same focal trait, which are surrounded by the wider external environment that does not favour the trait.

Our extended model makes the same predictions as our original model with migration whenever the external environment is large relative to the focal patch (Supplementary Figs 2 and 3). This is intuitive: a large external environment means that the focal gene is likely to be lost outside the focal patch before a cell returns, such that few or no immigrants will possess the focal trait. By contrast, when the external environment is itself a small patch, the focal patch and the external environment converge to act as a single patch in which the focal gene reaches fixation with limited horizontal gene transfer (as seen in the no-migration model above, Supplementary Figs 2 and 3). Another way to view the size of the external environment is as a proxy for the rarity of the focal niche: a large external environment that selects against the focal allele means that the focal niche is relatively rare. For the rest of the study, we focus on this case where a focal niche is rare relative to the environment from which immigrants arrive, such as a niche that selects for resistance to a specific antibiotic[48]. Under these conditions, the great majority of immigrants will be non-adapted.

Horizontal transfer divides the genome into distinct niches. We have shown that migration from outside of a focal patch

greatly increases the potential for gene transfer in microbial communities. However, is this increased transfer important for the ecology and evolution of microbes? Specifically, we are interested in whether the rate of horizontal transfer is sufficient to generate a horizontal selective sweep whereby a particular allele moves horizontally through a diverse community of microbes. To address this question, we next investigate how gene transfer with migration affects genomic diversity, at both the horizontally transferred and the non-transferred regions of the microbial genomes. Although compartment models allow us to follow the dynamics of horizontal transfer and identify the population processes driving a horizontal sweep, these models are not well suited to follow genomic effects. We therefore next develop a coalescence model to capture the genetic effects of genetic transfer, selection and migration probabilistically.

Our new model assesses the impact of genetic transfer in terms of how much it can decouple evolution at the genetic locus of the beneficial allele (focal locus) from the rest of the genome (background genome). We determine this effect by comparing the diversity at the focal locus (D_f) to the diversity at the background genome (D_{bg}), in the diversity ratio $DR = D_{bg}/D_f$ (for details, see Methods). Without migration, the DR changes little over time and remains close to one (Fig. 3a–c). Consistent with the predictions of our first model, we see very little effect of horizontal transfer when there is no migration. Genetic transfer is largely powerless to evolve the focal locus independently of the background genome and the two remain locked together in a vertical selective sweep that purges diversity in both genetic regions.

We next consider the case where there is immigration into the focal patch. Now, the behaviour of the model is very different. We find a wide range of parameter values for which the combination of positive natural selection, migration and horizontal transfer largely purge diversity at the focal locus, while leaving significant variability in the background genomes (high DR, Fig. 3a–c,e). The result is that the majority of cells carry the same adaptive allele (high fraction of carriers, Fig. 3d,f), while their background genomes remain diverse. The relative proportion of adaptive gene carriers increases monotonically with a stronger selection pressure (Fig. 3d) and with a decreasing migration rate (Fig. 3f). The DR is maximal when migration rate and positive selection are in a balanced regime. That is, some immigration is needed for an effective horizontal sweep to occur but, as seen in results from classical population genetics[49], if migration is too strong ($m > s$) then it will overpower selection and prevent adaptive evolution (Fig. 3e,f). As a result, the horizontal gene sweep is most effective at intermediate positive selection pressures (Fig. 3a–c).

This result has major implications for the evolution of microbial communities in the face of horizontal transfer. Positive natural selection is no longer a barrier to the horizontal spread of a trait. Instead, the impacts of genetic transfer are greatest for ecologically important traits that are under positive natural selection. In this way, a horizontally transferred trait with its own specific ecology is able to move through a diverse set of strain backgrounds. As we discuss below, a key implication of this uncoupling is that genomes can become ecologically diverse in the sense that the ecology of the focal locus and the rest of the genome are overlapping but distinct.

Ecological division of the patch promotes horizontal sweeps. We have so far focussed on an ecologically cohesive community,

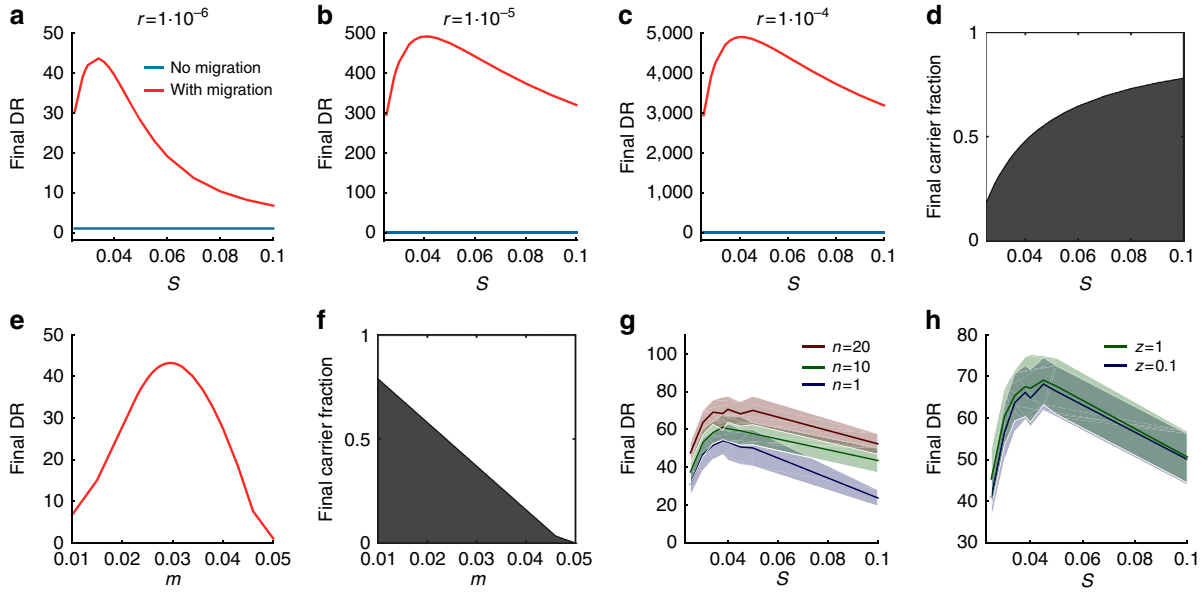

Figure 3 | Migration enables horizontal genetic sweeps. We plot the diversity ratio (DR) as a function of the strength of natural selection (s) for $r = 10^{-6}$ (**a**), $r = 10^{-5}$ (**b**) and $r = 10^{-4}$ (**c**) as calculated from the coalescence model both with and without migration. With migration, the DR, which gives the strength of the selective sweep, peaks at intermediate selection pressure ($s \approx 0.04$), whereas (**d**) the fraction of cells carrying the same adaptive gene increases monotonically with selection pressure, where for the different transfer rates the curve of the final carrier fraction is approximately identical, reaching about 80% for all three transfer rates. Parameters are $t = 5 \times 10^6$, $N = 10^8$ and $C(0) = 1{,}000/N$, and either $m = 0$ or $m = 0.02$. (**e**) The effect of migration rate (m) on the diversity ratio (DR) and (**f**) the effect of migration rate on the fraction of the community that carry the trait, for all three transfer rates ($s = 0.05$, $t = 5 \times 10^6$, $N = 10^8$ and $C(0) = 1{,}000/N$). (**g**) The individual-based model shows the effect of ecologically different migrants for an increasing number of ecological niches ($n = 1$, 10 and 20, and $z = 0.2$). (**h**) We also plot (for $n = 20$) the DR against selection pressure for two different levels of ecological specialization: cells competing twice as much in their own niche than in other niches (blue line) and cells that compete entirely in their own niche (green line). Other parameters are $N = 10^6$, $t_{end} = 10^5$, $r = 10^{-4}$, $C(0) = 1{,}000/N$. Results are averaged over 50 simulations and the s.d. is given by the transparent areas.

because the potential for diverse genotypes to compete ecologically is clear[23,24,50] and also because previous work suggests that competitive exclusion in a community is the worst-case scenario for gene-specific horizontal sweeps[16–18,27]. For this reason the above results should be conservative in their estimates of how migration promotes horizontal sweeps. However, we can use an individual-based model to relax competition within the community and study the consequences for gene sweeps and genomic diversity.

We introduce ecological differences between genotypes by introducing n different background niches in our focal patch, which could represent specialization on different resources. Each incoming cell then belongs to one background niche but also partly competes within the other niches. We denote the extent to which a cell competes within its assigned niche as z, whose value lies between 0 and 1, where $z=1$ corresponds to a cell competing purely in its own niche and $z=1/n$ corresponds to a cell competing equally in all niches (see Methods). Our simulations show that the genetic effect of horizontal gene transfer increases with an increasing number of distinct niches (n) in the community (Fig. 3g) and gene transfer is also increased by a stronger separation of the cells into separate niches (increasing z, Fig. 3h). This result is intuitive: the ecological subdivision reduces competition between cells in the different niches and thus reduces the loss of diversity in the background genome during the selective sweep. This effect of ecological subdivision agrees with the study of Majewski and Cohan[20] who found that gene transfer has greater impact if communities were subdivided into a number of completely non-competing lineages (or 'ecotypes'[21]). Another way to view the effect of 'niches' in our model is in terms of negative frequency-dependent selection that prevents any one genotype from completely dominating the focal community[14], which was the subject of a recent study by Takeuchi *et al.*[26]

In summary, our results suggest that an influx of diverse and non-adapted migrator genotypes can greatly increase the effect of genetic transfer on microbial communities. It does so in at least two ways. First, migration adds new gene recipients that, even after the beneficial trait is established, enable continued gene flux. Second, migration may introduce new genomes that increase ecological subdivision ($n > 1$). Both of these processes constrain the impact of vertical selective sweeps, but within ecologically cohesive communities (low n) it is the addition of new gene recipients that is critical for horizontal sweeps. The result is that most species and strains can evolve to be identical at the locus under positive selection, while the rest of the genomes are highly diverse.

Discussion

Our models explain how horizontal sweeps of small stretches of DNA can occur in ecologically cohesive communities of microbes. The strains and species that compete within such communities are ideal candidates for horizontal transfer, because they live in close proximity and they can induce lysis in one another releasing DNA for uptake[51,52]. However, previous work suggests that transfer of a beneficial gene within competing communities should be limited by selective sweeps that propagate the allele vertically to fixation before significant horizontal transfer can occur[16–18,27](Fig. 4a). Here we have shown that this prediction does not hold when one includes the possibility of immigration. Migration is a significant process in microbial ecology[45,46] and allowing migration in our model results in large amounts of horizontal transfer that has the power to transform the genomics of the community.

Our models then provide an evolutionary explanation for the increasing number of sequencing studies showing that otherwise diverse microbial communities possess regions of the genome that contain very little diversity[9,11–13,53]. Further evidence of the

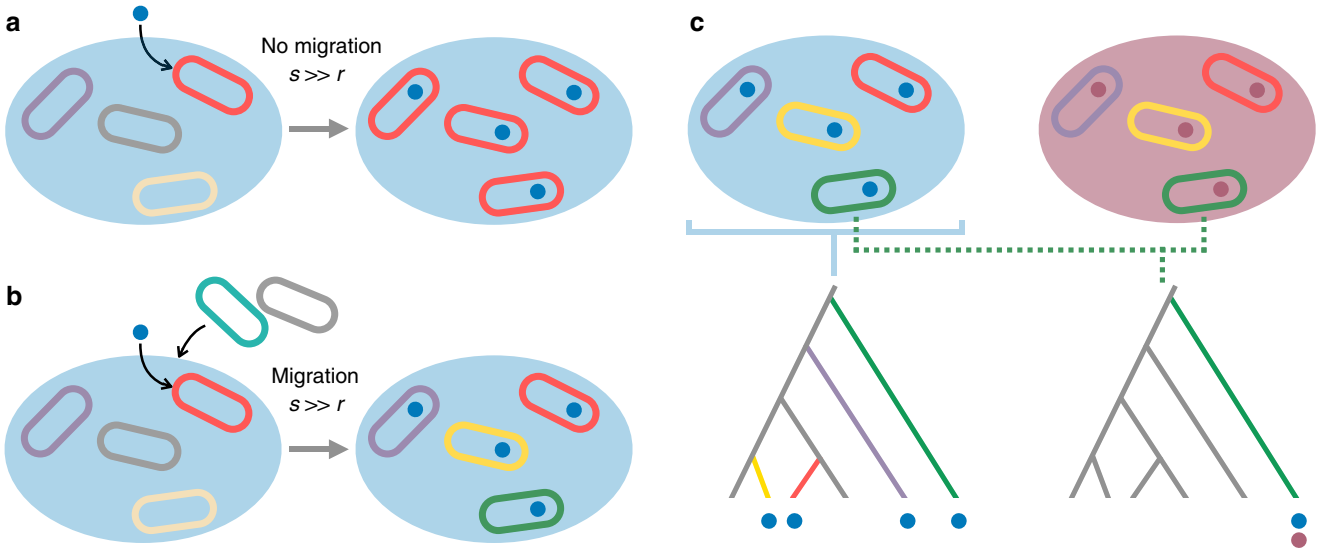

Figure 4 | Horizontal transfer creates multiple ecologies within one microbial genome. (**a**) The ecotype model[70]. A selected trait (blue dot) causes a selective sweep of the genome in which it first appears (red cell), wiping out other genomes if selection strength (s) for the trait is much larger than its gene transfer rate (r). (**b**) Divided-genome model. Immigration of new genotypes causes a horizontal genetic sweep because incoming genotypes can pick up the selected trait. The trait is now found in diverse genomic backgrounds, which can have ecologies that are distinct from both the focal locus and from each other. (**c**) Genomic regions can display high or low diversity depending on the ecological basis of sampling. The two circles containing the cells represent patches with loci-specific sweeps. Sampling from a single patch that selects for a horizontally transferred locus (blue dots) will capture cells that are diverse in their background genome phylogeny but homogeneous at the transferred locus (horizontal gene sampling, left-hand phylogeny). In contrast, sampling from a single background genome (green cells) will capture cells that are diverse in their horizontally transferred loci (background genome sampling, right-hand phylogeny).

processes we describe comes from the recent observation that mobile genetic elements can be enriched in their own niches, largely independently of their bacterial host[25,28,29]. Horizontal transfer then has the potential to make diverse and competitive strains coherent in an ecologically important phenotype, including key traits such as resistances to toxins[9]. Although our models explain how horizontal sweeps can occur, they also predict that the timescale required for a sweep is likely to be on the order of months to years (for example, 10^4 to 10^6 generations for a 30-min generation time), based on current estimates of genetic transfer rates[33,41,42]. A key prediction then is that horizontal sweeps are relatively slow compared with the canonical vertical sweep often seen in the laboratory[54,55]. Nevertheless, the timescales of horizontal sweeps remain extremely short compared with phylogenetic timescales, and fit with data showing that genetically coherent microbial communities persist for years in the face of migration[56] and vertical sweeps[57]. Recent work also suggests that the basic rates of gene transfer can sometimes be much higher than typically assumed[41,58], which in our model will significantly reduce the timescales required for horizontal sweeps. This prediction contrasts with the horizontal gene sweep scenario described by Takeuchi *et al.*[26], in which gene transfer rates need to remain low ($<10^{-6}$), for single-gene sweeps to occur.

We have emphasized here how horizontal gene transfer can remove diversity at one locus relative to the rest of the genome in a microbial community. How is this result reconciled with the notion of horizontal transfer as a way to generate diversity, in particular in the form of the much-discussed accessory genome[13,14,53,59]? Our analyses explain how genetic transfer can be seen to generate diversity in some studies, while removing diversity in others. This effect can be illustrated by considering two contrasting examples. First, if an experimenter samples in a specific patch that, as in our model, selects strongly for a particular horizontally transferred trait, then the data may show evidence of the horizontal sweep that removed variability at the focal locus relative to the rest of the genome (horizontal gene sampling, Fig. 4c)[11,12,53]. In contrast, if an experimenter samples one species such as *Escherichia coli* across different locations, then its background genome is likely to cross many niches for different horizontally acquired loci. In this case, horizontal transfer will be a process that mostly generates diversity relative to the core genome (background genome sampling, Fig. 4c)[60,61]. Arguably then, what is considered the 'accessory' region of a genome will depend on the ecological basis for sampling[15].

Our work speaks to the fundamental question of how microbial genotypes map to ecology[14,62,63]. A key result from our model is that the highest rates of transfer occur for loci that are under significant positive natural selection: loci that are important for the ecology of a cell. Horizontal transfer, therefore, can enable a particular locus to accumulate in a local environment to which it is evolutionarily adapted, without the rest of the genome evolving in the same way. An interesting corollary is that a single cell carrying such loci will become ecologically diverse, in the sense that its genome can evolve to become a community of genetic regions with multiple partially overlapping, but distinct, ecologies. This idea of distinct 'gene ecologies'[64] has recently been discussed in light of the microbial species question[25,29,64–66]. Our model explains how distinct gene ecologies are possible, as well as identifying the conditions required for them to occur (Fig. 4c). We show that, with sufficient migration, an ecologically important trait can readily decouple itself from any one genetic background via horizontal transfer. When this occurs, a microbial niche is defined at the sub-genomic scale so that ecological species concepts will no longer map to the whole organism but rather to a subset of any one genome.

Methods

Continuous model. We model the dynamics of a selective sweep with opportunity for genetic transfer and migration using an ODE. This equation describes the community in our selective patch where there are two sub-communities: carriers and non-carriers of the selected trait. The community is assumed to have a constant number of cells (N) with varying fractions of beneficial gene carriers (C) and non-carriers ($1-C$). For simplicity, we assume the community to be well-mixed and we do not consider stochastic effects (we relax this later). The dynamics and steady-state levels of carriers in the community are described by equations (1–3) in the main text. To capture the gene flux through horizontal transfer in a given community, we define a composite parameter that is the rate of transfer of the selected trait in the whole community:

$$HGT_{\text{flux}} = NrC(t)(1-C(t)). \tag{5}$$

This horizontal gene flux is maximized at equal number of donors and recipients ($C=0.5$). At steady state of the system, the gene flux is given by:

$$HGT^{*}_{\text{flux}} = rN\frac{m(s+1)(s-m)}{(sm+s)^2}. \tag{6}$$

Figure 1b,c show plots of C, $1-C$ and the horizontal gene flux over time with and without migration. Gene transfer events accumulate over time and ultimately cause sweeps of single loci or small groups of loci. As the horizontal gene flux gives the rate of gene transfer events, the integral of this flux over a time interval gives the expected number of transfer events in this time. To obtain the numerical solutions of the cumulative gene flux plotted in Figs 1 and 2, we employ the rectangle rule in MATLAB. Table 1 provides a summary of the parameters present in this model and a more detailed description and analysis of the model are given in the Supplementary Methods.

Coalescence model. We use a coalescence approach to model the genomes in our focal patch under influence of the selective sweep in combination with horizontal transfer and migration. We first simulate the fraction of selected gene carriers (C) in the time interval $t\in[0, t_{\text{end}}]$ using the ODE of our continuous model. With the simulated values of $C(t)$ we then compute the coalescence process of two homologous loci, to determine the expected diversity in their genome site. For the diversity in the background genome, we consider two random background loci at time $t=t_{\text{end}}$ and for the diversity in the focal locus we consider two focal loci at $t=t_{\text{end}}$. We then go backward in time until $t=0$, while updating the probabilities of the two loci being in a given state. The loci can take the following states:

State 11: Both loci are in two distinct individuals that are both carriers.
State 00: Both loci are in two distinct individuals that are both non-carriers.
State 01: Both loci are in two distinct individuals where one is a carrier and the other one a non-carrier.
State 1: The two loci are coalesced in one individual that is a carrier.
State 0: The two loci are coalesced in one individual that is a non-carrier.
State m: At least one of the loci is in a migrating individual outside the patch.

We simulate the change of all six probabilities backward in time, until $t=0$, by solving a set of coupled ODEs given in the Supplementary Methods. We obtain the diversity in the focal locus D_{f} and the diversity of the background genome D_{bg}. We measure the power of the horizontal gene sweep using the ratio of the diversity in the background genome over the diversity in the focal locus and we call this the DR given by:

$$\text{DR} = \frac{D_{bg}}{D_f}. \tag{7}$$

Individual-based model. We develop an individual-based model of our selective patch to confirm the predictions of our coalescence model and to be able to change the ecological details of the patch. The simulated patch contains a fixed number of cells (N), where each individual cell is described by a set of three numbers representing the focal locus (transferrable), the genotype of the remaining

Table 1 | Parameters used in the compartment model.

Parameter	Range	Description
N	*Positive integers*	Carrying capacity of the community
C_{N}	$[0,N]$	Total number of trait carriers in the focal patch
C	$[0,1]$	Fraction of carriers in the focal patch
r	$[0,1]$	Rate of gene transfer between trait carriers and non-carriers per generation
s	$[0,\infty]$	Strength of positive selection, given as the fitness increase of allele carriers in the focal patch
m	$[0,1]$	Migration rate per generation time, given as a fraction of the patch community

background genome (non-transferrable) and the niche/resource association of the genotype. The background genotype can take any positive integer, which matches the focal locus for cells at the beginning of the simulation and for migrating cells. The focal locus can take the adapted state 1 or alternatively any other positive integer for non-adapted cells. We simulate the ecological competition of the cells in n different niches in the patch similar to the *symsim* model by Friedman *et al.*[67]. Each cell obtains resources from an assigned niche and from the remaining niches as well. Thus, for a given cell i there is a vector of length n giving the cell's ecological fit to each niche. A cell's fit to its assigned niche is denoted by $z \in [0,1]$, where $z=1$ means that a cell only competes in its assigned niche and $z=1/n$ means that a cell competes in all niches equally. We then define the competitive weight ω of a cell i in niche j is as the product of its fitness f_i ($f=1+s$ for carriers and $f=1$ for non-carrier cells) times its association with niche j, so that $\omega_{ij}=f_i\sigma_{i,1}$. Each of the n niches holds a resource share of N/n in each generation, so that a cell obtains resources from niche j proportionally to its relative competitive weight in this niche according to:

$$R_{ij} = \frac{N}{n}\frac{f_i\sigma_{ij}}{\Omega_j}, \tag{8}$$

where R_{ij} is the amount or resources obtained by cell i from niche j and Ω_j is the summed competitive weight in niche j given by:

$$\Omega_j = \sum_{i=1}^{N} f_i\sigma_{ij}. \tag{9}$$

The total amount of resources obtained by cell i per generation is then given by:

$$R_i = \frac{N}{n}\sum_{j=1}^{n} R_{ij} = \frac{N}{n}\sum_{j=1}^{n}\frac{f_i\sigma_{ij}}{\sum_{i=1}^{N} f_i\sigma_{ij}}. \tag{10}$$

The resources of a cell determine the reproduction of a cell and we can use R_i as the mean number of offspring of a cell. We update the cell numbers stochastically using a Poisson distribution following a discrete time Wright–Fisher process[68]. Then, cells that lack the selected trait have a chance of acquiring the trait with a probability $C(t)r$. We implement the simulations using MATLAB and measure the horizontal gene flux and the diversities in different parts of the genome. The diversity is calculated as:

$$D = 1\Big/\sum_{i=1}^{n}(p_i)^2, \tag{11}$$

where n is the number of different locus variants present in the community and p_i is their respective proportion. This calculation is analogous to the effective number of species in a community (of order 2 (ref. 69)). We measure the genetic effect of the horizontal sweep as in the coalescence model using the DR given by:

$$\mathrm{DR} = \frac{D_{bg}}{D_f}, \tag{12}$$

where D_{bg} is the diversity in the background genome and D_f is the diversity in the focal locus. More details of this simulation are given in the Supplementary Methods. We show that the results of our individual-based model and coalescence model match quantitatively, despite being fundamentally different models (Supplementary Fig. 4).

Code availability. The MATLAB code of our individual-based model is available online via http://zoo-kfoster.zoo.ox.ac.uk.

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Author contributions

R.N. and K.R.F. designed research. R.N. designed the models and performed the research. A.G.F. helped with the modelling work. R.N. and K.R.F. analysed data. R.N. wrote the first draft of the manuscript and all authors contributed substantially to revisions.

Additional information

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