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The evolution of siderophore production as a competitive trait

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Microbes have the potential to be highly cooperative organisms. The archetype of microbial cooperation is often considered to be the secretion of siderophores, molecules scavenging iron, where cooperation is threatened by "cheater" genotypes that use siderophores without making them. Here, we show that this view neglects a key piece of biology: siderophores are imported by specific receptors that constrain their use by competing strains. We study the effect of this specificity in an ecoevolutionary model, in which we vary siderophore sharing among strains, and compare fully shared siderophores with private siderophores. We show that privatizing siderophores fundamentally alters their evolution. Rather than a canonical cooperative good, siderophores become a competitive trait used to pillage iron from other strains. We also study the physiological regulation of siderophores using in silico long-term evolution. Although shared siderophores evolve to be downregulated in the presence of a competitor, as expected for a cooperative trait, privatized siderophores evolve to be upregulated. We evaluate these predictions using published experimental work, which suggests that some siderophores are upregulated in response to competition akin to competitive traits like antibiotics. Although siderophores can act as a cooperative good for single genotypes, we argue that their role in competition is fundamental to understanding their biology.

KEY WORDS: **Bacteria, competition, cooperation, fitness trade-off, microbial interaction, phenotypic regulation, public good, sharing, siderophores, specificity, xenosiderophores.**

Iron limits the growth of many microorganisms making it a key determinant of evolutionary fitness and ecological competition. To cope with iron limitation, 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 (strain) have matching siderophores and siderophore receptors such that the siderophores produced by a cell are shared with other cells of the same strain (Griffin et al. 2004). Because siderophores can also carry metabolic costs

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(Griffin et al. 2004), siderophores have been identified as a microbial public good (West et al. 2006, 2007; Nadell et al. 2009), with the key corollary that a nonproducer (cheater) mutant may outcompete a producer by using its siderophores without paying the production cost (Griffin et al. 2004; Ross-Gillespie et al. 2007; Brown et al. 2009).

Although it is clear that siderophores have the potential to act as public goods between cells of a single genotype, this perspective lacks a key piece of siderophore biology. The different strains and species that commonly meet in natural communities 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). Importantly, experiments have shown that this specificity can 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 privatization 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; Oliveira et al. 2014).

How does siderophore privatization affect its social role and the evolution of siderophore production? To answer these questions, we developed a novel ecoevolutionary model of siderophore production. Our theory is centered upon an explicit mechanistic model of siderophore scavenging (Fgaier and Eberl 2010; Lee et al. 2016), which we extend to allow different levels of interstrain 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 privatization leads to a major shift in the regulation of siderophore production. Although public siderophores are downregulated in the presence of competitors, partly privatized siderophores evolve to be upregulated instead. We use published experimental work (Traxler et al. 2013) to test between these two regulatory responses and argue that the role of siderophores in ecological competition is fundamental to both their evolution and regulation.

Materials and Methods

MODEL OVERVIEW

Our goal is to understand how siderophore privatization affects 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. 2009aa, 2014; Lee et al. 2016) and we model the processes explicitly using ordinary differential equations (ODEs). Although 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), where 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, importantly, the level of privatization 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 nonproducers (West and Buckling 2003; Eberl and Collinson 2009; Inglis et al. 2011). By contrast, 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 nonproduction of siderophores. The number of different strains that interact in a single patch is given by n ($n = 1, 2, 3, \ldots$). 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 interstrain 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. Although bacterial genotypes have the potential to produce multiple siderophores at the same time (Cornelis et al. 1989; Carson et al. 1994; Cornelis 2010; Dumas et al. 2013), we follow previous models (West and Buckling 2003; Lee et al. 2012) and assume that each strain can produce at most one siderophore. Production of multiple public goods has been studied elsewhere (Oliveira et al. 2014). Each strain expresses the cognate receptor for its siderophore but may also take up siderophores produced by other strains

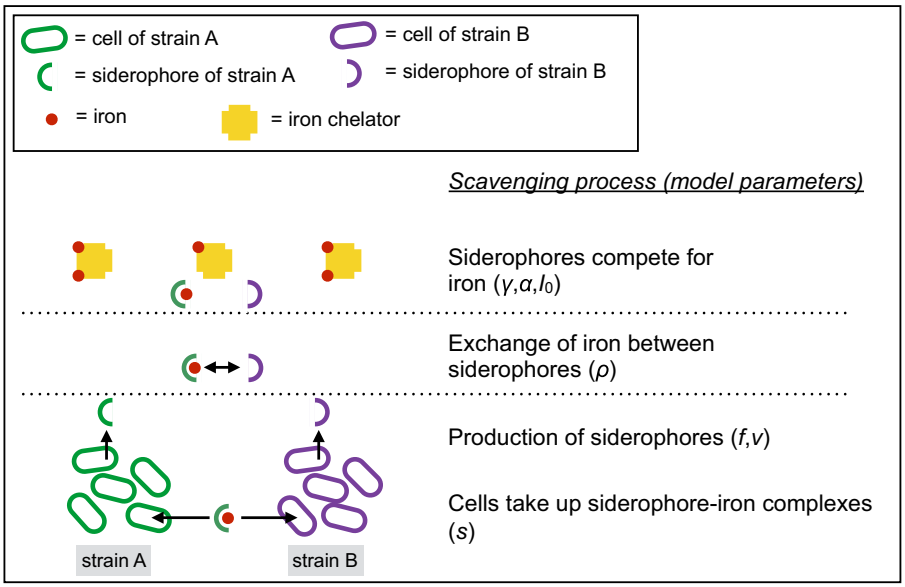

Figure 1. **A schematic view of siderophore scavenging divided into its key component processes. Iron scavenging begins with the secretion of siderophores by cells with a specified level of investment and yield. Siderophores (half-circles) then bind the environmental iron (red dots on yellow shapes, 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 (l_0). Siderophores and iron form complexes according to the siderophore affinity γ. Siderophores can also "steal" or strip iron away 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 process is shown separately in this cartoon, but in our model, all processes occur continuously in one well-mixed patch.**

(xenosiderophores). Uptake of xenosiderophores may happen either because a strain's siderophore receptor has affinity for other siderophores (Crowley et al. 1991) or because it coexpresses multiple siderophore uptake systems (Cornelis and Matthijs 2002; Cornelis and Bodilis 2009). Our model captures both single and multiple uptake systems, and we make the 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. Conceptually, this parameter can be linked to the proportion of siderophores retained for personal use in the model considered by Kümmerli & Ross-Gillespie (2014).

Siderophores are secreted molecules and iron scavenging occurs outside the cells through several processes. For clarity, we capture scavenging as four key processes, which we incorporate into our model to occur simultaneously (Fig. 1):

1. Siderophore secretion: We assume the production of siderophores to be metabolically costly (Griffin et al. 2004) and to depend on the level of energy invested (f), which is traded-off against the energy allocated to growth (Lee et al. 2016). The total amount of energy available to the cells depends on the uptake of a nonlimiting nutrient (N) which is not considered explicitly in the model.
2. 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).
3. Stealing iron: Siderophores can strip iron away from other siderophore-iron complexes with rate ρ. The extent of this exchange will depend on the quantity and the affinity of the siderophore that is stealing the iron and the quantity of iron-complexes by other siderophores (Kraemer 2004; Khan et al. 2006).
4. 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 take up 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). Although there is the potential to extend our model to capture such effects explicitly, we decide to focus here on 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

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

where $C(t)$ is the cell density 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. We model cellular metabolism such that cellular growth relies both on iron and on other sources of energy, such as carbohydrates, lipids or protein. Iron is often limiting due to low concentrations of its soluble form (Wandersman and Delepelaire 2004), and we assume that other energy sources are abundant. From these assumptions, we show in the Supporting Information Materials and Methods how we can go from a form where non-iron nutrients are explicitly captured to equation (1) where these nutrients are captured implicitly. We also assume that the production of siderophores does not require significant iron, which is consistent with their chemistry (Hider and Kong 2010).

Cells proliferate with a rate that depends on a maximum growth rate μ, the investment of energy into cell growth, and the iron available per cell. The available iron depends on the uptake of iron-siderophore complex (P) and iron-uptake through siderophore-independent mechanisms (ε) (Wandersman and Delepelaire 2004). Cells invest a fraction $1 - f$ of their energy into building up cell biomass, and we assume here the presence of an abundant energy source that is captured in β of the Monod function with saturation constant K (see Supporting Information Materials and Methods). The acquisition of iron through uptake of siderophore-iron complexes also follows a Monod function with constant K_P. Cells die at a constant per capita rate d_C. The free siderophore concentration $S(t)$ changes over time due to cellular siderophore production, which is proportional to the amount of energy that the cells invest into siderophore production (f) and to the siderophore production yield (v). We assume that the siderophores are instantaneously recycled from iron-siderophore complexes: the rate of siderophore recycling is equal to the rate of iron-siderophore complex uptake multiplied by a recycling efficiency constant q. In an extended version of the model (Supporting Information Results), we consider a trade-off between this recycling efficiency parameter and the affinity of siderophores for iron, assuming that the stronger siderophores bind iron, the more difficult to recycle they are. A sweep over the range of the recycling parameter reflects this trade-off, where at intermediate values of recycling and affinity strains can invest less into production (Fig. S1). Siderophores are lost from the system through diffusion (d_S).

Our model follows chemostat dynamics with a permanent input of iron as well as loss of siderophores and siderophore-iron complexes. The external concentration of iron is I_0, and there is a reflux of iron into the patch at reflux rate a. 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), due to the uptake by cells, and due to loss (d_P). Finally, the concentration of available iron follows chemostat dynamics with a reflux of external iron and depletion through the formation of iron-siderophore complexes.

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:

$$\begin{cases} \frac{dC_1}{dt} = \mu \frac{(1-f_1)}{(1-f_1)+\beta} \left(\frac{P_1 + sP_2}{P_1 + sP_2 + K_P} + \epsilon I \right) C_1 - d_C C_1 \\ \frac{dS_1}{dt} = v f_1 C_1 + q \frac{(1-f_1)}{(1-f_1)+\beta} \frac{P_1 + sP_2}{P_1 + sP_2 + K_P} C_1 - \gamma S_1 I - d_S S_1 + \rho (S_2 P_1 - S_1 P_2) \\ \frac{dP_1}{dt} = \gamma S_1 I - \frac{(1-f_1)}{(1-f_1)+\beta} \frac{P_1}{P_1 + sP_2 + K_P} C_1 - \frac{(1-f_2)}{(1-f_2)+\beta} \frac{sP_1}{P_2 + sP_1 + K_P} C_2 - d_P P_1 + \rho (S_1 P_2 - S_2 P_1) \\ \frac{dC_2}{dt} = \mu \frac{(1-f_2)}{(1-f_2)+\beta} \left(\frac{P_2 + sP_1}{P_2 + sP_1 + K_P} + \epsilon I \right) C_2 - d_C C_2 \\ \frac{dS_2}{dt} = v f_2 C_2 + q \frac{(1-f_2)}{(1-f_2)+\beta} \frac{P_2 + sP_1}{P_2 + sP_1 + K_P} C_2 - \gamma S_2 I - d_S S_2 + \rho (S_1 P_2 - S_2 P_1) \\ \frac{dP_2}{dt} = \gamma S_2 I - \frac{(1-f_2)}{(1-f_2)+\beta} \frac{P_2}{P_2 + sP_1 + K_P} C_2 - \frac{(1-f_1)}{(1-f_1)+\beta} \frac{sP_2}{P_1 + sP_2 + K_P} C_1 - d_P P_2 + \rho (S_2 P_1 - S_1 P_2) \\ \frac{dI}{dt} = a(I_0 - I) - \gamma (S_1 + S_2) I - \epsilon (C_1 + C_2) I \end{cases} \quad (2)$$

We assume that siderophores can strip iron away 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. Importantly, the siderophore produced by a bacterial strain can to some extent also be used by other strains. The sharing parameter s determines how much a siderophore can be used by the other strains that did not produce this siderophore. The number of strains (n) gives the strength of ecological competition that is intensified with increasing number of strains.

We assume that the chemical properties of siderophores (amount of sharing, recycling efficiency, affinity, loss, yield) are identical for all interacting strains. In reality, siderophores can differ in their chemical properties (Cornelis and Matthijs 2002), but with this assumption we can study the isolated effect of siderophore privatization on its evolution. Diversity in the other properties such as affinity and yield will have additional effects on siderophore evolution.

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 Runge-Kutta-Fehlberg 4–5 method (Fehlberg 1970) with adaptive step-size steps to solve the equations numerically using the ODE solver gsl-odeiv-step-rkf45 from GSL (GNU Standard Library) library version 2.1 in C++ (http://www.gnu.org/software/gsl). The C++ code of our model is available at via http://zoo-kfoster.zoo.ox.ac.uk. The duration of competition in a local patch is determined by the integration time of the ODE τ. The ecological equilibrium is not necessarily reached. We study the effect of this competition span in Fig S3 G–I. We summarize the biological significance and default values of the parameters used in our model in the Table S1.

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, given through

$$f = f_{bas} + \frac{(f_{act} - f_{bas})}{(1 + exp\,(100\,(x - T)))}, \qquad (3)$$

Under this functional form, f approximately takes the "activated" value f_{act} when the signal x is above the threshold T, and the "basal" value f_{bas} otherwise. 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 i 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). We model quorum sensing without an explicit signal. However, by implementing an evolvable cell density threshold where quorum sensing activates siderophore production, our model captures a quorum threshold effect that is consistent with an autoinducing molecule. Finally, we consider competitor cell density ($\sum_{j=1}^{n} C_j - C_i$), which can be detected by any compound that is specific to the competitor. This detection might include nonself quorum sensing autoinducers but also sensing the damage from antibiotics or bacteriocins of the competing strain (competition sensing as defined in Cornforth & Foster 2013).

In our optimizing 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 identical for all strains, and then we iteratively 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 model is based on previously published work and assumes an infinite number of local patches that are linked through the dynamics of a simple microbial life cycle (Cremer et al. 2012; Oliveira et al. 2014):

1. Seeding: An empty patch is seeded with a certain number n of different strains with initially small density ($C_{\text{i}}(0) = 1$). 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 given in equations (1) and (2). Interactions are simulated for a fixed amount of time (that can be varied as a parameter).
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 coevolving strategies that allows one to avoid explicitly modeling each step in a life cycle (Nowak and Sigmund 2004). 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, because it is rare, is determined by its local competition with other strains that have the resident strategy, $w_{\text{inv}} = w(f_{\text{inv}}|f_{\text{res}})$. 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 cell density 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),

$$I_{inv} = \frac{w_{inv}}{w_{res}} = \frac{w\left(f_{inv} \vee f_{res}\right)}{w\left(f_{res} \vee f_{res}\right)}. \quad (4)$$

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 an 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. Although multiple ESS are theoretically possible in game theory, we always found a unique ESS for each analysis.

Results

OVERVIEW

We can use our ecoevolutionary model to study how siderophore production evolves across different scenarios and, in particular, in response to siderophore privatization. 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 use this to identify the evolutionarily stable investment into siderophore production for each situation. We then study the physiological regulation of siderophore production and find that the regulatory strategies that evolve also depend strongly on how much siderophores are shared. Specifically, with siderophore privatization our model predicts that production should increase when strains encounter competitors.

PRIVATIZATION STRONGLY AFFECTS THE EVOLUTION OF SIDEROPHORE PRODUCTION

Evolutionary studies typically treat siderophores as a canonical public good that benefits all cells in an environment equally, where nonproducers (cheaters) can thrive in the presence of siderophore producers (Griffin et al. 2004; West et al. 2006, 2007; Ross-Gillespie et al. 2007; Brown et al. 2009; Nadell et al. 2009). The potential for one genotype to exploit another, however, rests upon strains mixing such that nonproducers 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 ($n = 1$, Fig. 2, black diamond) and in the presence of increasing numbers of competitor strains. The single-strain case serves here as the baseline to compare the optimal siderophore production in monoculture with cases of competition. When siderophores are entirely shared ($s = 1$) and their benefit returns to the producer as much as to other cells, we recapitulate the classic social evolution prediction that optimal investment into siderophores decreases for higher numbers of competing strains in a patch (Fig. 2, green dots). Strains now produce fewer siderophores relative to the monoculture case to reduce sharing with their competitors.

We then ask how this relationship is affected by limited siderophore sharing between genotypes ($s < 1$). The privatization 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 (Fig. 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; Oliveira et al. 2014; Mitri et al. 2015; Nadell et al. 2016), the effect of increasing competition is to *increase* siderophore

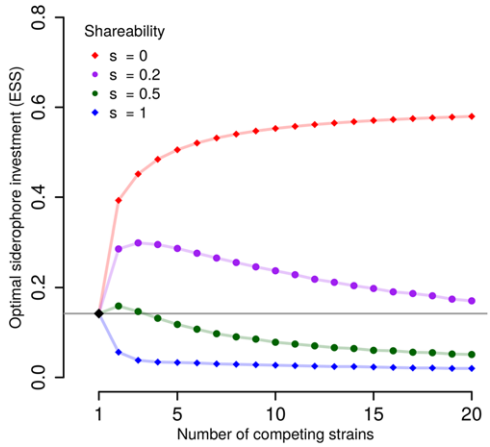

Figure 2. The effect of competition and siderophore sharing on siderophore production. Using an adaptive dynamics framework we find the evolutionarily stable level of siderophore production for different levels of competition and siderophore sharing. We plot the optimal investment into siderophores (f^*) against different numbers of competitors (n) per local competition. In the absence of competition between strains, where siderophore sharing does not occur, the optimal investment into siderophore production is shown by the black diamond on the far left. This serves as baseline for comparison with scenarios with competition, and it is marked with a horizontal gray line and highlighted by slightly smoothed lines joining the points. 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). For this figure only, $v = 7$, $a = 0.1$, and $\tau = 30$.

investment relative to the monoculture baseline (Fig. 2). This additional investment is explained by a competitive benefit from hoarding iron away from the competitor. At ESS production strains optimize both harvesting and hoarding iron, and these two siderophore functions—while conceptually distinguishable—occur instantaneously in our model.

Even though siderophores are still a public good with respect to the cells of a single genotype, therefore, privatization shifts them to behaving as a mechanism of exploitative competition, which is used to deplete and steal the iron of competitors.

THE EFFECTS OF PRIVATIZATION ARE ROBUST FOR A WIDE RANGE OF CONDITIONS

We have shown that privatization can have strong effects on the evolution of siderophore production. In particular, privatization 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 (Fig. 3, Figs. S2–S5).

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 the evolution of siderophore production are in some cases also relatively complex. For example, a decreased iron concentration commonly causes an increased siderophore production on physiological timescales (Schmitt and Holmes 1991; Rodriguez et al. 2002; Chakraborty et al. 2013) (see section "Privatization is Critical to the Evolution of Siderophore Regulation"), but this is not necessarily true for an ESS level of constitutive production. This is because the evolved level is also affected by changes in growth dynamics: when iron availability is very low, cells grow poorly and so benefit less from producing siderophores (Fig. 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 (Fig. 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 (Fig. 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, in the vast majority of sweeps, for private siderophores we never see a decrease, and for public siderophores, we never see an increase in production when we move from the clonal group to the mixed-genotype group (Fig. 3, but see e.g., Fig. S3D–F).

Conditions of intermediate sharing ($s = 0.5$) are again intermediate in their behaviour and we see that, dependent on parameters, strain mixing can drive an increase or decrease in the evolutionarily stable level of siderophore production. In conditions where cells cannot use siderophores to exploit competitors (high iron, low production yield and/or low cell density), cross-feeding effects dominate and production reduces with strain mixing. But when siderophores are most effective (low iron, high production yield, and/or high cell density) the benefit of exploiting can overcome the cost of cross-feeding (Fig. 3; crossing

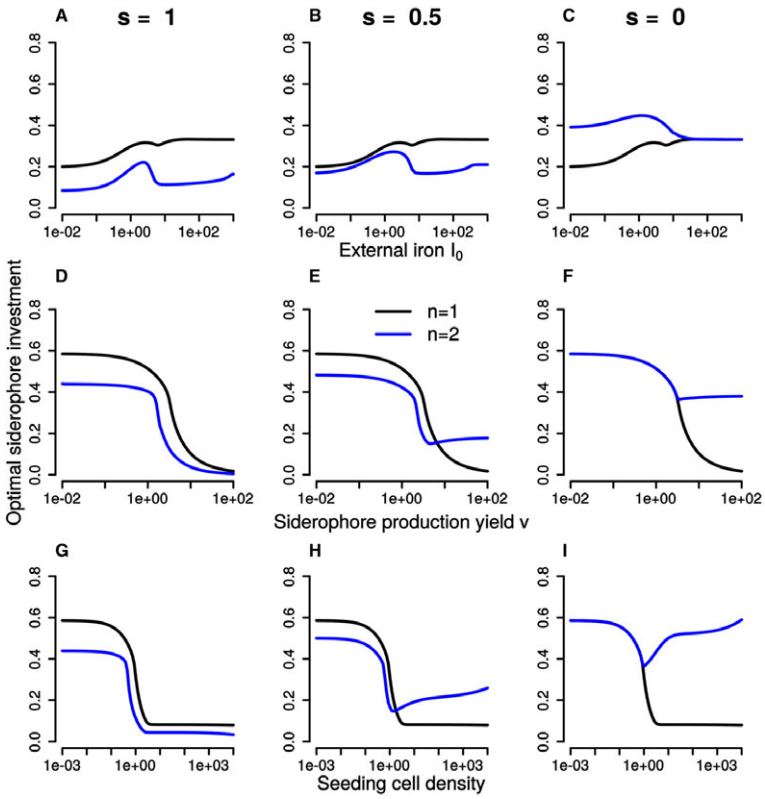

Figure 3. **Wide-ranging parameter sweeps show consistent effects of privatization on siderophore evolution (see also Figs. S2–S5). We study the effect of iron concentration, siderophore yield, and seeding cell density on ESS production of siderophores. We plot the optimal siderophore investment with 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 increases (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 cells cannot use siderophores to improve their growth rate (high iron, low production yield, and/or low cell density) the cross-feeding effect dominates and production is reduced with strain mixing. But when siderophores become effective (low iron, high production yield, and/or high cell density) then their exploitative potential means that strains increase production in strain mixes.**

of black and blue line) so that production increases with strain mixes.

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

PRIVATIZATION IS CRITICAL TO THE EVOLUTION OF SIDEROPHORE REGULATION

As is typical of previous theoretical work on siderophore evolution (West and Buckling 2003; Lee et al. 2012, 2016), 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 are often strongly regulated in response to environmental conditions (Harrison et al. 2008; Kümmerli et al. 2009b; Traxler et al. 2013). 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 (Ratledge and Dover 2000; Wandersman and Delepelaire 2004), density of clone mate cells (e.g., quorum-sensing) (Waters and Bassler 2005), and density of competitor cells (Abrudan et al. 2015) (e.g., competition sensing (Cornforth and Foster 2013)) (section Materials and Methods). For each type of sensing, we can then follow the evolution of strategies as before and identify the evolutionarily stable strategies of regulation (section Materials and Methods, Fig. 4).

Siderophore regulation in response to iron level and own cell density evolves in a consistent way, irrespective of the degree of privatization. The responses evolve such that low iron and

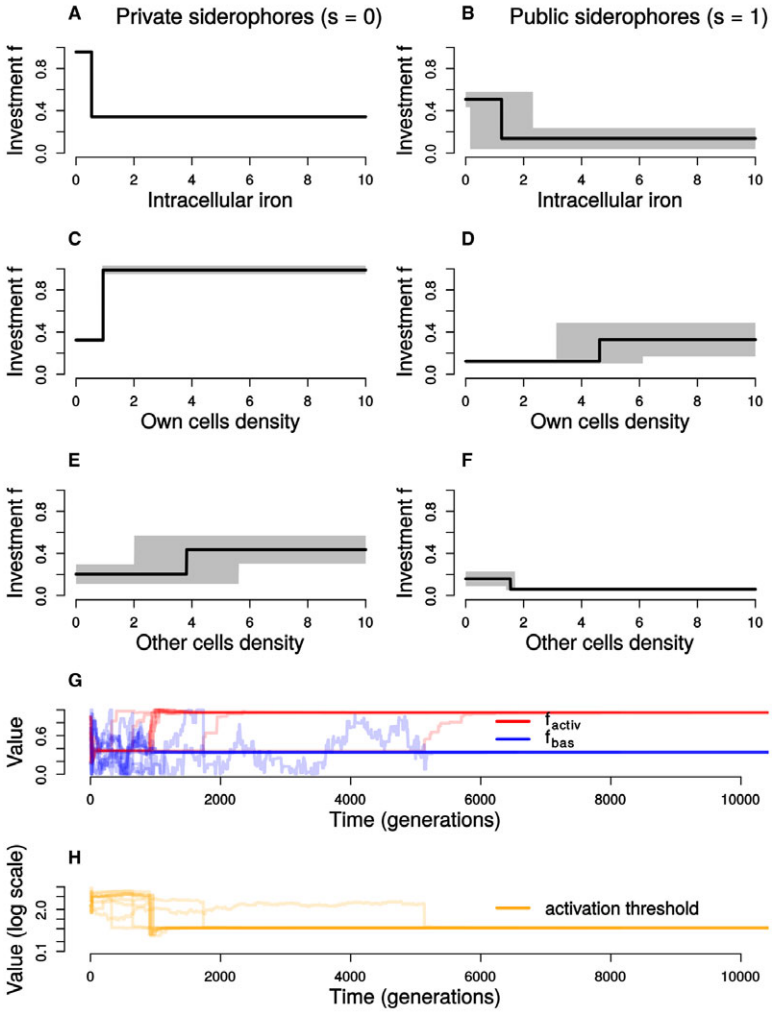

Figure 4. The evolution of physiological siderophore regulation. We evolve the sensing function for siderophore production in pairwise competitions ($n = 2$) using the evolutionary algorithm outlined in Supporting Information Methods and Results. We study three cases that each captures a different source of information: intracellular iron, own cell density, and competitor cell density. The plots show the evolved response in siderophore production as a function of the sensed signal, as a mean from 30 runs of the simulation. The gray area shows the standard deviation (SD). Siderophores evolve to be upregulated at low iron, independent of the shareability (A, B), consistent with siderophores being most valuable when iron is low. Siderophores evolve to be upregulated for high quorum (C, D) consistent with their efficiency being highest at high cell density. 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 (G, H) show illustrative time plots from the evolutionary algorithm optimizing iron sensing, given $s = 0$, showing how the initial, and the activated siderophore investment (G) and the activation threshold (H) evolve. We show 20 realizations of the algorithm.

high quorum both favour the release of siderophore. There is a clear post-hoc logic to 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 recapitulates the typical interpretation of the evolutionary function of quorum sensing. Quorum sensing allows a group of cells to only release a secreted product once the cell density of the focal strain is high enough to generate and receive an effective concentration of the product (Schluter et al. 2016). Consistent with this prediction, 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 (Fig. 4E, F).

The evolution of the regulation of public siderophores leads to a response where production is downregulated 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 privatized, we predict the opposite pattern. Now, siderophores respond as expected for a competitive trait, such as the release of an antibiotic that kills another strain (Cornforth and Foster 2013), with secretion upregulated in response to the presence of the competing strain.

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, we see the same predictions for private and public siderophores so the data are unable to distinguish between the two models. 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 privatization for the evolution of siderophore use.

Does siderophore production, therefore, increase or decrease upon strain mixing? Empirical work suggests that siderophore production typically increases in the presence of unrelated strains and species. Specifically, coculture experiments between *Pseudomonas aeruginosa* with *Staphylococcus aureus* found that *P. aeruginosa* makes more siderophores in the presence of *S. aureus* (Harrison et al. 2008). 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). Caution is required as the underlying mechanisms driving the upregulation of siderophore production is not yet clear and the responses may be driven by multiple factors, including iron limitation. Nevertheless, the data in both cases are most consistent with the upregulation of siderophores upon competition with other species, not the downregulation expected for a public good.

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. 2009a; Ross-Gillespie et al. 2009; Luján et al. 2015). In mixed cultures with a wild-type producer strain, siderophore null mutants (cheaters) can thrive and outcompete the wild-type, which is consistent with the idea that siderophores act as canonical public goods in microbial communities. This view was recently emphasized 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. 2012; 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 siderophores (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 the typical public goods model. With privatization, strains that face a lot of competition from other genotypes evolve to increase their investment in siderophores (Fig. 2), rather than the decrease expected from a public good. The effects of privatization 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 privatized, regulation evolves to increase production in the presence of competing strains. Siderophores then function as a way to compete with other genotypes (Fig. 4); they become mediators of exploitative competition (Hibbing et al. 2010).

One of our simplifying assumptions is that each strain only produces a single siderophore. However, many bacteria produce multiple types of siderophores (Cornelis 2010) and recent theoretical work has shown that this can lead to the evolution of dependencies between strains for public goods (Oliveira et al. 2014). Despite the potential for such complexities, we can use our model to make some predictions for such scenarios. In particular, if a strain produces two siderophores, one private and one public with otherwise identical properties, we predict that in the face of competition, this strain would downregulate the public siderophore and upregulate the private one (Fig. 4E, F). Another complexity would be the scenario where strains, instead of making siderophores, invest in pirating siderophores as seems to occur for example in pseudomonads (Cornelis and Matthijs 2002). Our framework can easily be extended to study such strategies.

The existing data on siderophore regulation suggest that siderophore production is upregulated in the presence of other strains, consistent with it being used in competition (Harrison et al. 2008; Traxler et al. 2013). More generally, there is growing evidence that bacteria are capable of regulating a wide range of traits based upon the presence of different strains. Competing 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. 2015). 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 competitive responses has so far focused on bacterial warfare and the upregulation of toxins and type VI secretion systems in response to ecological competition (Basler and Mekalanos 2012; Basler et al. 2013; Cornforth and Foster 2013; Majeed et al. 2013). Siderophores are another important way to compete with other genotypes, as is biofilm formation, which was also recently found to be upregulated in response to competition (Oliveira et al. 2015). Some studies have previously stressed that the public good role of siderophores can disappear in certain environments (Griffin et al. 2004; Zhang and Rainey 2013). Here, we provide a framework that demonstrates how siderophores can be agents of both competition and cooperation.

In summary, siderophores can function as a public good when all cells have the same receptors. This effect may dominate ecoevolutionary dynamics whenever competition is primarily between cells with a recent common ancestor, as may occur in lung infections in patients with cystic fibrosis (Andersen et al. 2015). However, the ecology of many bacterial species centers upon competition in diverse communities, where strain-specific siderophores limit between genotype sharing (Joshi et al. 2006; Khan et al. 2006). We have shown that these conditions strongly affect how siderophores function in nature. Siderophores should no longer be considered a simple public good. Instead, siderophores become a competitive phenotype that, like antibiotics, is upregulated to overcome other strains.

AUTHOR CONTRIBUTIONS

R.N., A.P., and K.R.F. conceived the model, A.P. performed the computational work, R.N. wrote the first draft, and all authors discussed the results and contributed to manuscript corrections.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Biological significance and default values of the parameters used in the model.

Figure S1. The effect of the recycling efficiency on the ESS production of siderophores, for three shareability scenarios ($s = 1$, $s = 0.5$, $s = 0$), with a trade-off between recycling efficiency and the iron affinity of siderophores.

Figure S2. The effect of the cell mortality, free siderophore, and complex siderophore decay terms on the ESS production of siderophores for three shareability scenarios ($s = 1$, $s = 0.5$, $s = 0$).

Figure S3. The effect of the siderophore uptake saturation constant, recycling efficiency, and competition duration on the ESS production of siderophores, for three shareability scenarios ($s = 1$, $s = 0.5$, $s = 0$).

Figure S4. The effect of the siderophore iron binding rate, iron bioavailability (how much iron cells can take up without siderophores), and iron turnover on the ESS production of siderophores, for three shareability scenarios ($s = 1$, $s = 0.5$, $s = 0$).

Figure S5. The effect of the cell maximum growth rate, ligand exchange rate, and external iron nutrient saturation on the ESS production of siderophores, for three shareability scenarios ($s = 1$, $s = 0.5$, $s = 0$).