Siderophore production and the evolution of investment in a public good: an adaptive dynamics approach to kin selection.

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Abstract

Like many other bacteria, *Pseudomonas aeruginosa* sequesters iron from the environment through the secretion, and subsequent uptake, of iron-binding molecules. As these molecules can be taken up by other bacteria in the population than those who secreted them, this is a form of cooperation through a public good. Traditionally, this problem has been studied by comparing the relative fitnesses of siderophore-producing and non-producing strains, but this gives no information about the fate of strains that do produce intermediate amounts of siderophores. Here, we investigate theoretically how the amount invested in this form of cooperation evolves. We use a mechanistic description of the laboratory protocols used in experimental evolution studies to describe the competition and cooperation of the bacteria. From this dynamical model we derive the fitness following the adaptive dynamics method. The results show how selection is driven by local siderophore production and local competition. Because siderophore production reduces the growth rate, local competition decreases with the degree of relatedness (which is a dynamical variable in our model).

Our model is not restricted to the analysis of small phenotypic differences and allows for theoretical exploration of the effects of large phenotypic differences between cooperators and cheats. We predict that an intermediate ESS level of cooperation (molecule production) should exist. The adaptive dynamics approach allows us to assess evolutionary stability, which is often not possible in other kin-selection models. We found that selection can lead to an intermediate strategy which in our model is always evolutionarily, yet can allow invasion of strategies that are much more cooperative. Our model describes the evolution of a public good in the context of the ecology of the microorganism, which allows us to relate the extent of production of the public good to the details of the interactions.

Introduction

Cooperation can take several forms. Individuals can help each other directly in pairwise interactions, but also indirectly, for instance, by investing in traits that improve the environment so as to increase population growth. If such a modification of the environment benefits all members of a population, it is referred to in the biological literature as a public good (Rankin et al., 2007). It is well-known that public good production can evolve despite the possibility of cheaters or free-loaders, if there is spatial structure or some other form of assortment (Lion and van Baalen, 2008; Fletcher and Doebeli, 2009). However, it is not always clear how to relate these quite general models to specific cases, in particular when aspects such as relatedness are not constants but depend on the local and global dynamics. Here, we will explore how cooperation via a public good can evolve in the specific instance of siderophore production in parasitic bacteria.

Virtually all bacteria need iron for various metabolic functions (Andrews et al., 2003). Parasitic bacteria, in particular, live inside host organisms where free iron is scarce but also many other bacterial habitats have little free iron available. In order to take up iron many bacteria excrete siderophores, which are molecules that bind to iron with high affinity. A siderophore-iron complex, once formed, can be taken up by any cell with appropriate siderophore receptors (Ratledge and Dover, 2000; Griffin et al., 2004; Wandersman and Delepelaire, 2004). This system has been particularly well studied for the bacterium *Pseudomonas aeruginosa*, an opportunistic pathogen which produces the siderophore pyoverdine. Experimental work has shown that when iron is limiting, pyoverdine producing strains reach higher densities than strains which do not produce pyoverdine, yet when put in competition the pyoverdine producers are outcompeted, which shows that there is a cost associated with siderophore production (Griffin et al., 2004; Ross-Gillespie et al., 2007). Thus, because the production of these siderophores is costly, strains that produce less, or altogether no siderophores should be advantaged when competing against high siderophore producers, and cooperation should not evolve if selection is dominated by local competition (West and Buckling, 2002; Kümmerli et al., 2009, 2010).

The evolution of siderophore use depends on how strains interact, both locally and globally. Bacterial populations that produce more siderophores reach higher densities and thus contribute more to the next generation. But these strains are vulnerable to cheater strains that are better local competitors (as these do not pay the cost yet benefit from siderophores). Bacterial strains that produce siderophores thus face a trade off between their ability to compete locally (siderophore production reduces growth rate), and productivity (siderophores allow higher densities). The balance between these two forces has been shown to affect the evolution of siderophore production (Griffin et al., 2004; Kümmerli et al., 2009, 2010).

There has been considerable debate whether siderophore production can invariably be seen as a public good (Zhang and Rainey, 2013; Kümmerli and Ross-Gillespie, 2014; Ghoul et al., 2014). Zhang and Rainey (2013) argued, on the basis of an experimental approach, that under some conditions siderophore production can be outright counterselected, in which case non-producers cannot be considered to be 'cheats.' Whether siderophores are a common good thus depends on the conditions. This debate shows that fitness functions should be *derived* from the full underlying dynamics and for arbitrary trait complexes (siderophore production and use) (see also Alizon (2013)).

This argument extends to evolution in general, but in particular to the evolution of pathogens. Pathogen evolution, for instance in the development of resistance, depends on the epidemiological interaction between host and bacterium, bacterial genetics as well and the molecular mechanisms of antibiotic action. To predict and understand such evolutionary processes we need models that integrate all these aspects (MacLean et al., 2010a,b; Metcalf et al., 2015). Here we show that it is possible, and practically feasible, to derive fitness expressions from models that include a detailed description of the dynamics. This allows us to apply an inclusive fitness perspective to evolutionary problems using an adaptive dynamics approach. Although this paper focuses on siderophore production, the approach can, and should, be used for a much wider class of evolutionary problems.

Evolutionary models for siderophore production have tended to focus on scenarios in which the local and global interactions are described in a highly stylised form, either based on the model framework developed by Frank (1998) (e.g., West and Buckling, 2002; Brown et al., 2009) or models based on *a priori* chosen, stylised fitness functions (Ross-Gillespie et al., 2009, 2007; Cornforth et al., 2012). The construction of such models appears to be based on simply filling in the components of Hamilton's Rule by choosing plausible functions. This may give insight in the potential for kin selection, but this methodology can easily miss essential feedback mechanisms. This is particularly relevant if one goes beyond marginal fitness considerations and one needs to know the fitness to a higher degree of approximation than a first degree. This is important if larger mutational steps are studied and if one wants to know where the long-term evolutionary process will end (i.e if the endpoint is evolutionarily stable or whether branching will occur). As we wish to develop an understanding of how ecological and evolutionary processes interact in shaping the evolution of common goods, we here derive the fitness from a model based on simple but plausible assumptions about experimental protocol and the local bacterial ecology. Because we base our model on explicit mechanisms, we can assess how the relevant costs and benefits (as well as the relatedness parameter) depend on the ecological details, instead of having to assume they are constants, as it is usually done. In doing so we go beyond agent-based simulation models in which such realism is included, but for which it is normally not possible to interpret the results in terms of inclusive fitness theory, or to identify cost and benefits. Without identifying costs and benefits it is hard to generalise the results from models and to form an integrative understanding of the evolutionary process.

To analyse our model we have applied a separation of timescales. This is based on the observation that while bacteria grow fast, competition between strains (in a local population) is much slower. This analysis allows us to easily identify fitness costs and benefits. Furthermore, this approach enables us to assess how the degree of assortment in a metapopulation, as measured by relatedness, depends on local and global processes. We will study how this feedback affects the evolutionary end result using an adaptive dynamics approach (Metz et al. 1992). This not only allows us to work out the optimum compromise between cooperation and cheating, but also to assess whether this optimum is an evolutionarily stable strategy, that is, whether natural selection will favour a monomorphic population with a unique strategy or whether it will favour a heterogeneous population with divergent strategies (Geritz et al. 1998). Such approaches have been developed and are frequently used in ecologically inspired models of evolution, but have rarely been applied to the evolution of social interactions (but see Ohtsuki (2010).)

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

The model

The model framework is inspired by the Haystack model (Maynard Smith, 1964) and its mathematical analysis follows Jansen and Mulder (1999) and Jansen (2011). We consider a collection of many subpopulations inhabiting identical environments, such as the wells on a plate, which are referred to as patches (as in e.g. Dumas et al., 2013). Our model is inspired by experimental protocols in which wells are inoculated with bacteria by pipetting a fixed volume into each well, grown for an incubation period, harvested and the contents redistributed to a new plate of wells (e.g. Griffin et al. 2004; Kümmerli et al. 2009, 2010; Livingston et al. 2012; Dumas and Kümmerli 2012) (Figure 1). This gives two levels of dynamics: the local dynamics (what happens inside a patch or well) and the global dynamics (what happens at the metapopulation, or plate, level). The local interactions are based on similar assumptions as in Brown et al. (2009).

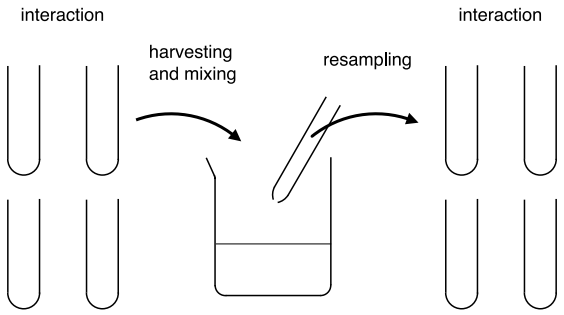


Figure 1: Illustration of the model. Patches are represented by tubes, which could be wells on a multi-well plate. First, the bacteria compete in each tube for a fixed time τ (causing local competition among strains present in the patch). Then, the solutions are harvested and mixed. A fixed volume μ is taken and transferred to new tubes (causing global competition among strains for places in the inoculum). The cycle starts anew after a time τ.

Within-patch dynamics

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

$$\frac{dS}{dt} = \sum_i b_i Q_i - uFS \tag{1}$$

where $\sum_i b_i Q_i$ is the total amount of siderophores produced and uFS the rate with which siderophores bind with iron.

The dynamics of bound siderophores is given by

$$\frac{dS_F}{dt} = uFS - \theta S_F \sum_i Q_i \tag{2}$$

where $\theta S_F \sum_i Q_i$ is the assimilation of bound siderophores by bacteria.

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

$$\frac{dQ_i}{dt} = Q_i \left(r\left(1 - \kappa Q\right) - \alpha b_i + \epsilon \theta S_F - \lambda \right) \tag{3}$$

where $r(1 - \kappa Q)$ is the rate of density-dependent reproduction and where Q is the total density of bacteria $Q = \sum_i Q_i$, αb_i the cost of siderophore production,

Symbol	Description	Value used
N	Average number of bacteria in the inoculum	
S	Density of free siderophores	
S_F	Density of bound siderophores	
Q_i	Density of strain i	
Q	Density of the total population	
b_i	Rate of siderophore production of strain i	0 to 40
F	Iron concentration	10^{-2}
u	Affinity of siderophore to iron ions	10^{3}
λ	Bacteria mortality	10^{-2}
θ	Rate of assimilation of siderophores	10^{-2}
ϵ	Increase in growth rate due to siderophore assimilation	1
α	Decrease in growth rate due to siderophore production	10^{-2}
r	Base-line growth rate	1
κ	Density-dependence in growth rate	10^{-2}
μ	Volume transferred to seed a patch	10^{-2}
τ	Duration of local interaction	10

Table 1: Table of symbols, with the value for parameters used in the figures (unless otherwise indicated).

$\epsilon\theta S_F$ the increase in growth through the assimilation of bound siderophores, and λ the mortality rate. Together with the initial conditions for these equations this completely describes the within-patch dynamics.

For a single strain the within patch dynamics allow for, at most, one positive equilibrium that is always stable. If there are more strains, numerical simulations for a range of parameters showed that the dynamics always go to a stable equilibrium. The total bacterial numbers tend to settle quickly, after which the different strains compete, leading to a slow process of replacement of all strain by the strain with the lowest value of b_i. We could not derive explicit solutions for this non-linear system of ODEs, but in what follows we will derive a close approximation to the dynamics.

To approximate the dynamics, following the rationale given in Jansen and Mulder (1999), we first describe the dynamics in terms of the total local bacterial density Q, and the fraction f_i of the population of strain i, where $f_i = Q_i/Q$. We can now rewrite the dynamics as

$$\frac{dS}{dt} = Q\bar{b} - uFS \tag{4}$$

$$\frac{dS_F}{dt} = uFS - \theta S_F Q \tag{5}$$

$$\frac{dQ}{dt} = Q\left(r\left(1-\kappa Q\right) - \alpha\bar{b} + \epsilon\theta S_F - \lambda\right) \tag{6}$$

$$\frac{df_i}{dt} = \alpha f_i(\bar{b} - b_i) \tag{7}$$

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

It is easy to check that

$$f_{i,t} = \frac{f_{i,0} e^{-\alpha t b_i}}{\sum_j f_{j,0} e^{-\alpha t b_j}} \tag{8}$$

is a solution of (7). We thus find that the average siderophore production is a function of time, given by

$$\bar{b}_t = \frac{\sum_i b_i f_{i,0} e^{-\alpha t b_i}}{\sum_j f_{j,0} e^{-\alpha t b_j}}. \tag{9}$$

If this change in the proportion of the different strains of bacteria is slow compared to the change in the total bacterial population we can apply a time scale separation. The fast change in the total bacterial and siderophore densities will lead them to quasi-steady states (denoted with tildes) given by

$$\widetilde{S}(\bar{b}_t) = \frac{\bar{b}_t \widetilde{Q}(\bar{b}_t)}{uF} \tag{10}$$

$$\widetilde{S}_F(\bar{b}_t) = \frac{\bar{b}_t}{\theta} \tag{11}$$

where the stable quasi-steady state of the bacteria $\widetilde{Q}(\bar{b}_t)$ is

$$\widetilde{Q}(\bar{b}_t) = \frac{1}{r\kappa}(r + (\epsilon - \alpha)\bar{b}_t - \lambda). \tag{12}$$

If there is only one strain present, with siderophore production rate b, then $\widetilde{Q}(b)$ is simply the equilibrium density. The approximate rate of approach of the variables S, S_F and Q to the quasi steady state is, respectively, uF, $\bar{b}\widetilde{Q}$ and $r\kappa\widetilde{Q}$. In contrast, the fraction of the bacterial population f_i changes with rate $\alpha(\bar{b}_t - b_i)$. Therefore quasi-steady state approximation above requires

$$uF, \bar{b}\widetilde{Q}, r\kappa\widetilde{Q} \gg \alpha|\bar{b}_t - b_i|, \tag{13}$$

or in words, the rate of binding of siderophores to iron, the siderophore production rate and the maximum growth rate of the bacterial population should be much bigger than the change in the relative dominance of individual strains. If the time scales separate the quasi-steady state $\widetilde{Q}(\bar{b}_t)$ changes slowly over time with $\bar{b}_t$, which depends on time through equation (9) (Figure 2). The time scale separation works particularly well for a population that is largely monomorphic and in which strains with marginally different traits appear occasionally.

Global population dynamics and fitness

In the previous section, we considered the dynamics of a collection of strains with different siderophore production rates within a patch. In this section we will

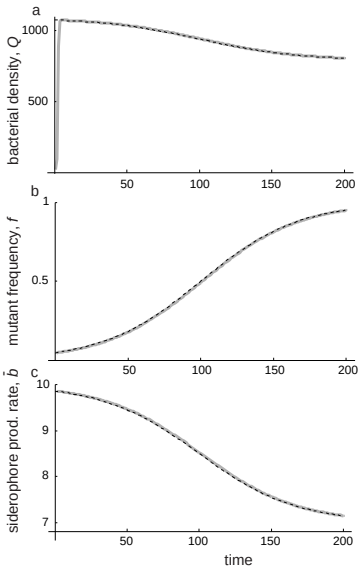


Figure 2: Example of the dynamics in a patch being invaded by a cheat. The predominant strain, produces siderophores at rate b_1. A second strain, which produces siderophores at rate b_2, can invade because it produces siderophores at a lower rate ($b_1 > b_2$), and could thus be seen as a 'cheat'. The grey drawn lines are the result of numerical integration of the model for the within patch dynamics, the dashed lines are the approximation that we obtained through a separation of time scales. In most places the two lines overlap, meaning that the approximation is very close. The top panel (a) shows the density of bacteria in the patch. The middle panel (b) shows the frequency of the mutant ('cheater' strain). The lower panel (c) shows the mean production of siderophores. As the competitor increases in frequency, the density of bacteria decreases because there are less siderophores produced. Note the difference in timescales: the bacterial density very quickly goes up to its quasi equilibrium. Once that has happened the balance between the two strain changes at a much slower time scale. Parameters as in Table 1, with in addition $b_1 = 10$, $b_2 = 7$.

focus on the evolution of this production rate at the metapopulation level, and ask whether there is an evolutionary stable strategy: an unbeatable siderophore production rate, such that populations which produce siderophores at this rate cannot be invaded by strains which produce slightly more or less siderophores. To find this unbeatable rate we will establish which strains are evolutionarily stable by applying the approach used in adaptive dynamics, based on whether a strain with a given siderophore production rate can be invaded by a rare mutant with a different siderophore production rate.

As outlined above, we consider a discrete-time metapopulation of infinitely many patches, where in every cycle the patches are inoculated with a random sample taken from the previous growth cycle of pooled subpopulations and then allowed to grow for a time τ. In reality the number of patches will be finite, but our assumption can be justified if this number is sufficiently large. For instance, for a lab based protocol based on plates with 96 wells (e.g Livingston et al. (2012)), the variation in the average size of the inoculum that the finite number of wells causes in an experiment using several 96 well plates is unlikely to be a significant factor.

We assume that the number of cells in the inocula is a random variable, i, which follows a Poisson distribution, $\mathcal{P}(i, N)$, with mean N. This describes a distribution of the number of cells in fixed volume as would result from pipetting a small volume from the same solution (Livingston et al., 2012, V. Calcagno, pers. comm.). No further migration occurs until the end of the interaction within the patches, when all subpopulations are pooled again. We assume that the amount of siderophores that is transferred with the inoculum is negligible.

We will start with the description of a global population consisting only of a resident strain with siderophore production rate b. In all inoculated patches, the production rate is therefore $\bar{b} = b$ (we assume that the length of the interaction, τ is sufficiently long for the local populations to converge to their equilibrial densities). These patches will thus produce $\widetilde{Q}(b)$ bacteria. There is obviously no output if a patch receives no inoculum, which happens with a probability $\mathcal{P}(0, N_T) = e^{-N_T}$ where N_T is the average number of bacteria in the inoculum. Therefore, when at the end of a cycle all patches are pooled together, the concentration of bacteria in the pool is $\sum_{i=1}^{\infty} \widetilde{Q}(b)\mathcal{P}(i, N_T) = \widetilde{Q}(b)(1 - \mathcal{P}(0, N_T))$. If a volume μ is taken from the pool to inoculate each new patch, the average number of bacteria in the next cycle is

$$N_{T+1} = \mu\widetilde{Q}(b)(1 - \mathcal{P}(0, N_T)) \tag{14}$$

which defines the dynamics of the average inoculum size N_T and the global population dynamics. The average will converge to an equilibrium, given by the solution of

$$\overline{N} = \mu\widetilde{Q}(b)\left(1 - \mathcal{P}(0, \overline{N})\right). \tag{15}$$

Next, we consider a mutant appearing in the population, which produces siderophores at a rate b^*. The output of this mutant at the next cycle depends on how many of its propagules arrive in every patch. The distribution of mutant

inocula, is also random and given by the Poisson distribution $\mathcal{P}(j, N_T^*)$, where N_T^* is the average number of mutants in the inocula in growth cycle number T and j is the actual number of mutants. Note that while N_T^* may be an arbitrarily small real number, j is a non-negative integer, typically either zero or one (if the probability of two or more mutants arriving in the patch is vanishingly small). Even if the mutant is globally rare, in the few patches where it does arrive it may form a significant proportion of the local population. As illustrated in Figure 2, the introduction of a new competitor in a patch will set off a process of competition and partial replacement, which will affect the overall bacterial density. The global dynamics are given by

$$N_{T+1} = \mu \sum_{i=1}^{\infty} \sum_{j=0}^{\infty} \mathcal{P}(i, N_T) \mathcal{P}(j, N_T^*) \widetilde{Q}(\bar{b})(1 - f_\tau) \tag{16}$$

$$N_{T+1}^* = \mu \sum_{i=0}^{\infty} \sum_{j=1}^{\infty} \mathcal{P}(i, N_T) \mathcal{P}(j, N_T^*) \widetilde{Q}(\bar{b}) f_\tau \tag{17}$$

where i and j respectively count the number of residents and mutants in the inoculum and f_τ and $1 - f_\tau$ denote the fractions of mutants and residents respectively, at the end of the interaction, when $t = \tau$. The average siderophore production in a patch with mutants at the end of the interaction is given by $\bar{b}_\tau = f_\tau b^* + (1 - f_\tau) b$, and the total amount of bacteria in the patch is then $\widetilde{Q}(\bar{b}_\tau)$ (note that this depends on the resident and mutant traits, b and b^*, through $\bar{b}_\tau$). The fractions relate to the number of bacteria in the inoculum through (8); the initial fraction of mutants is given by $f_0 = \dfrac{j}{i + j}$.

Next, we make the assumption that the mutant is globally very rare, so that the resident's (global) dynamics is not affected by the mutant (Metz et al., 1992; Geritz et al., 1998) which will thus settle at the equilibrium value $\overline{N}$ associated with b. As the mutant is rare it is very unlikely to have a patch inoculated with more than one mutant (for small N, $\mathcal{P}(1, N) = Ne^{-N} \approx N$ and $\mathcal{P}(i, N) \approx 0$ for $i > 1$). The expression for the global dynamics of the mutant then simplifies to

$$N_{T+1}^* \approx \mu N_T^* \sum_{i=0}^{\infty} \mathcal{P}(i, \overline{N}) \widetilde{Q}(\bar{b}_\tau) f_\tau. \tag{18}$$

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

$$W(b^*, b) = \mu \sum_{i=0}^{\infty} \mathcal{P}(i, \overline{N}) \widetilde{Q}(\bar{b}_\tau) f_\tau. \tag{19}$$

Using equilibrium condition (15) to eliminate μ and, using the fact that for Poisson distributions it holds that $\mathcal{P}(i, \overline{N})\overline{N} = \mathcal{P}(i + 1, \overline{N})(i + 1)$, this can be

rewritten as

$$W(b^*, b) = \frac{\sum_{i=0}^{\infty} \mathcal{P}(i+1, \overline{N}) \widetilde{Q}(\bar{b}_\tau)(i+1) f_\tau}{(1 - \mathcal{P}(0, \overline{N})) \widetilde{Q}(b)} \tag{20}$$

where it should be remembered that the functions $\bar{b}_\tau$ and f_τ depend on both b and b^* (under mild assumptions a similar result can be derived for other distributions than Poisson (Jansen, 2011)). If the mutant's trait is the same as that of the resident the fitness is unity, that is $W(b, b) = 1$. This follows from the fact that if $b^* = b$ then $\widetilde{Q}(\bar{b}_\tau) = \widetilde{Q}(b)$ and $(i+1) f_\tau = 1$.

Because our model describes the full dynamics between any pair of traits, the fitness function is valid for any pair of trait values. We can therefore construct a pairwise invasibility plot (Figure 3a), which shows which mutants with trait b^* can invade a resident population with trait b (Metz et al., 1992; Geritz et al., 1998). From this, we can also construct the mutual invasibility plot, showing the areas where both resident and mutant both have a positive fitness when rare and thus where combinations of strains with these traits can coexist (Figure 3b). The diagram also indicates that there is an evolutionarily stable trait; a population of individuals carrying this trait cannot be invaded by strains with similar traits.

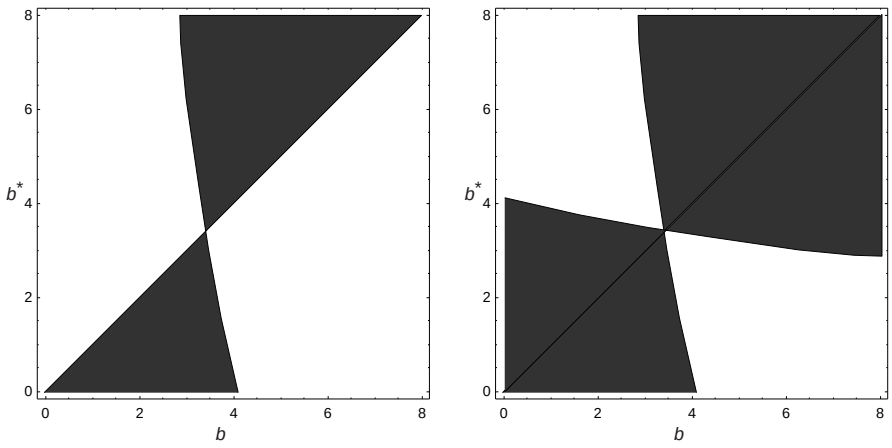
Marginal fitness and Hamilton's rule

The pairwise invasibility plot (Figure 3a) shows that an evolutionarily stable point can exist. To characterise and interpret the trait value for this point we will derive the marginal fitness, which is the change of fitness with a small change of siderophore production rate, which is proportional to $\frac{\partial W}{\partial b^*}$. To do so we first observe that if there are only two traits, those of the mutant and the resident, the dynamics of f_τ obeys logistic growth. It follows from (8) that, if the mutant differs marginally from the resident, the fraction of mutants at time τ changes with the trait as:

$$\left.\frac{df_\tau}{db^*}\right|_{b^*=b} \approx -\alpha \tau f_0 (1 - f_0), \tag{21}$$

and from (9) that

$$\left.\frac{d\bar{b}_\tau}{db^*}\right|_{b^*=b} \approx f_0. \tag{22}$$



(a) Pairwise invasibility plot

(b) Mutual invasibility plot

Figure 3: Invasion diagrams. (a) Pairwise invasibility plot. Areas where the fitness of the mutant is positive are white and areas where the fitness of the mutant is negative are black. (b) Mutual invasibility plot. White areas designate combinations of trait values that are mutually invasible. Parameters as in Table 1.

Because we only need to consider patches with one mutant in the inoculum we have $f_0 = 1/(1+i)$. Putting this all together gives for the marginal fitness

$$\begin{aligned} \left.\frac{\partial W(b^*,b)}{\partial b^*}\right|_{b^*=b} &= \frac{\sum_{i=0}^{\infty}\mathcal{P}(i+1,\overline{N})\left[\widetilde{Q}'(b)f_0 - \alpha\tau\widetilde{Q}(b)(1-f_0)\right]}{(1-\mathcal{P}(0,\overline{N}))\widetilde{Q}(b)} \\ &= \frac{\sum_{i=1}^{\infty}\mathcal{P}(i,\overline{N})\left[\widetilde{Q}'(b)\frac{1}{i} - \alpha\tau\widetilde{Q}(b)(1-\frac{1}{i})\right]}{(1-\mathcal{P}(0,\overline{N}))\widetilde{Q}(b)}, \end{aligned} \tag{23}$$

where the prime stands for the derivative of a function with respect to its argument.

Inspection of this expression reveals that marginal fitness depends on the relatedness (Grafen, 1985; Queller and Goodnight, 1989) given by

$$R = \frac{\sum_{i=1}^{\infty}\mathcal{P}(i,\overline{N})\frac{1}{i}}{1-\mathcal{P}(0,\overline{N})}. \tag{24}$$

If the mutant is globally rare, the fraction $1/i$ is the probability to randomly pick a mutant bacterium from a patch that contains mutants. Because for neutral mutants the local siderophore concentration is proportional to the mutant cell density, this is also the probability that a rare, selectively neutral mutant individual retrieves a siderophore that was produced by itself or a fellow mutant. It can be shown that the expression (24) also carries the interpretation of relatedness as the probability to pick two individuals of the same type from the same patch over and above the probability of picking two individuals of the same type from the global population, relative to the probability of picking the same pair of types in the population at large (Queller and Goodnight, 1989; Jansen, 2011). For the latter interpretation the mutant does not have to be rare; also note that this result and interpretation can be generalised to other distributions than a Poisson (Jansen, 2011).

Thus we can rewrite the marginal fitness as

$$\left.\frac{\partial W(b^*,b)}{\partial b^*}\right|_{b^*=b} = \frac{\widetilde{Q}'(b)}{\widetilde{Q}(b)}R - \alpha\tau(1-R) \tag{25}$$

In the first term ($\frac{\widetilde{Q}'(b)}{\widetilde{Q}(b)}R = \frac{(\epsilon-\alpha)}{r\kappa\widetilde{Q}(b)}R$) we recognise that if there is a net benefit to siderophore production ($\epsilon > \alpha$) a mutant which produces more siderophores gains a benefit from the change of output from the patch: the share of the benefit is proportional to the relatedness. The second term $\alpha\tau(1-R)$ is the cost this mutant will pay through the decreased competitive ability, from its reduction in growth rate that results from siderophore production. As competition amongst the mutants is neutral, this cost is proportional to the fraction of unrelated individuals $(1-R)$ (see also Jansen and Vitalis, 2007; Bryden and Jansen, 2010). The marginal fitness thus has three contributing elements: the change in the output of a patch, the change in competitive ability, and thirdly the

relatedness. The first two elements are associated with local behaviour and can be quantified by straightforward experimentation. The relatedness results from the global dynamics and associated redistribution. It can be assessed through the global bacterial density, or through the fraction of patches that are occupied (see Fig. 4).

An alternative, and perhaps more familiar, way of writing the marginal fitness is

$$\left.\frac{\partial W(b^*,b)}{\partial b^*}\right|_{b^*=b} = R\left(\frac{\widetilde{Q}'(b)}{\widetilde{Q}(b)} + \alpha\tau\right) - \alpha\tau \tag{26}$$

in which we recognise the structure of Hamilton's rule (Hamilton, 1964),

$$\left.\frac{\partial W(b^*,b)}{\partial b^*}\right|_{b^*=b} = RB - C \tag{27}$$

The important result to notice here is that we do recover Hamilton's Rule (Hamilton, 1964), but with costs and benefits that are compound functions of both physiological and environmental parameters. For instance, the overall benefit ($B = \widetilde{Q}'(b)\widetilde{Q}(b)^{-1} + \alpha\tau$) consists of the increase of the output of a patch plus the benefit of not suffering from competition with related individuals. Deriving costs and benefits from the local dynamics thus allows to gain insight in the mechanisms causing frequency and density dependence. As we will discuss later in some more detail, note that the cost and benefits are not constant but depend on the length of the interaction.

Evolutionarily Stable Strategies

We can now calculate the candidate evolutionary stable siderophore production rates from the equation $RB - C = 0$. To show that solutions are evolutionary stable strategies, however, we have to demonstrate that they are evolutionary and convergence stable. It can be seen from the sign structure in the pairwise invasibility plot that the candidate points are always convergence stable (Metz et al., 1992). The calculation of the evolutionary stability requires that the second derivative of the fitness function is negative (Geritz et al., 1998). We find that production rates are only evolutionary stable if

$$\left(\frac{\widetilde{Q}''}{\widetilde{Q}} - 2\frac{\widetilde{Q}'^2}{\widetilde{Q}^2}\right)R - \alpha^2\tau^2\left(2\frac{(1-R)^2}{R} - (1-R) + 2\left(R - \frac{\sum_{i=1}^{\infty}\mathcal{P}(i,\overline{N})\left(\frac{1}{i^2}\right)}{1-\mathcal{P}(0,\overline{N})}\right)\right) < 0. \tag{28}$$

In the appendix we show that this condition is always fulfilled and that this is independent of the way the patches are inoculated. For the model presented here $Q'' \leq 0$; however if $Q'' > 0$ it is possible the singular point is not evolutionarily stable, depending on the magnitude of the second derivative. In this case disruptive selection and evolutionary branching would be possible. Note that the condition above contains information about the population structure that goes beyond relatedness. The term in $\frac{1}{i^2}$ suggests that for evolutionary stability

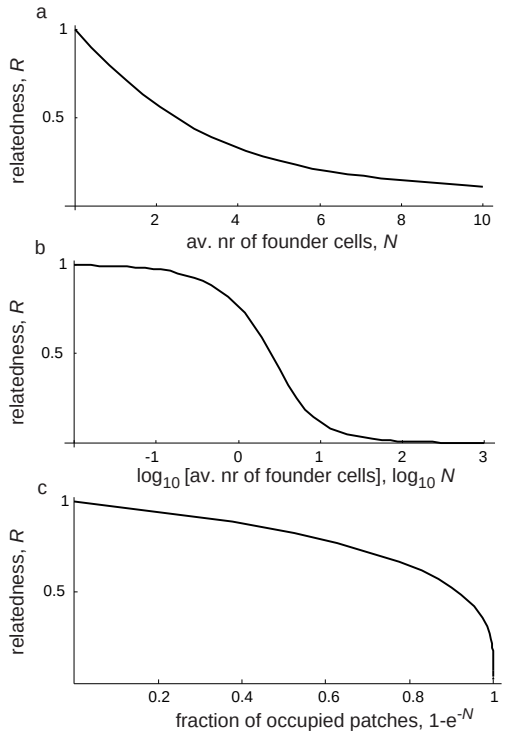


Figure 4: The relatedness as a function of (a) the global bacteria density measured through the average number of cells per patch at the beginning of the cycle (N), and (b) as a function of the 10 log of the average number of cells per patch ($\log_{10} N$). Note that there is a relatively small range of values for which the relatedness takes on values bounded away from 0 or 1. This is roughly the range for which $10^{-1} < N < 10^{1}$. Panel (c) gives the relatedness as a function of the fraction of patches that are occupied. This is a measure that is easy to assess experimentally. To get a relatedness that is bounded away from zero not all patches

the chance for a mutant to encounter 2 other mutants is an important factor (see also Ohtsuki 2010)

For our model all singular points are thus evolutionarily stable. Figure 4 shows the values of b at the ESS as a function of the length of the interaction.

The ESS investment in siderophore production depends on all ecologically relevant parameters. Of particular interest is the transfer volume μ: if μ increases more individuals will arrive in a patch to start a new cycle, reducing the relatedness and giving kin selection less potential. Figure 6a shows that this is indeed the case. The decrease of the ESS value is caused by the decrease in relatedness (Figure 6b). An increase in μ will cause an increase in $\overline{N}$, which in turn will cause the relatedness (given by Eq. 24) to decreases with μ (Figure 6b). This result is interesting as transfer volume, μ, can be easily manipulated experimentally.

All singular points in this model are always evolutionarily stable. However, evolutionary stability is a local form of stability, which tells us that strains that are marginally different cannot invade. It is possible that at the ESS strains with very different siderophore production rate can invade. For suitably chosen parameters, the pairwise invasiblity plot in Figure 7 shows that it is indeed possible that strains with a much larger siderophore production rate can invade. This is possible, because these strains can survive in patches that are left empty by the strain at ESS. If the siderophore production rate is very large, these strains can persist in the restricted number of patches to which they have sole access, even though they are eliminated in patches already occupied by the strain at ESS. Such ultra-cooperators are likely to evolve to their own ESS value, and in this way a diversity of strains can coexist in an evolutionarily stable manner (Jansen and Mulder, 1999).

Discussion

We found that for some parameter combinations, siderophore producer ('cooperator') strains can indeed stably coexist with non-producer ('cheater') strains, confirming the results of earlier studies (Ross-Gillespie et al., 2007). However we also found that typically such producer, non-producer pairs are not evolutionarily stable and can be invaded by phenotypically similar intermediate strains, that is, strains that do produce siderophores but at a slightly different rate. Such intermediates are often predicted by adaptive dynamics analyses, where phenotypes can be drawn from a continuum instead of from a discrete, predefined set (Metz et al., 1992) and may also arise in cooperative interactions (van Baalen and Rand, 1998; Le Galliard et al., 2003). The evolutionary end result is therefore an intermediate production rate rather than a mixture of producers and non-producers.

Theory predicts that under certain conditions populations may diverge to produce a pair of evolutionarily stable daughter populations (or more) (Metz et al., 1992; Geritz et al., 1998), but this will not occur with the fitness functions

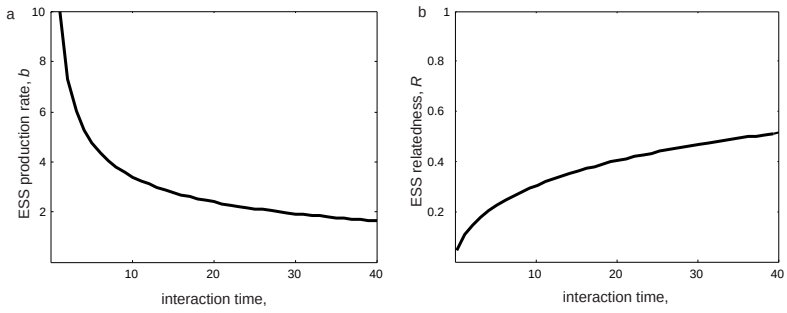


Figure 5: (a) Siderophore production at ESS and (b) Relatedness at the ESS as a function of the duration of the local interaction, τ. Parameters as in Table 1.

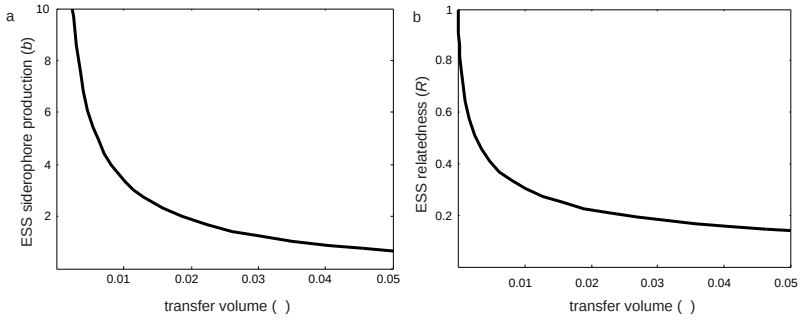


Figure 6: (a) Siderophore production at ESS and (b) Relatedness at the ESS as a function of transfer volume μ. The relatedness is given by $R = \frac{\sum_{i=1}^{\infty} \mathcal{P}(i,\overline{N})\frac{1}{i}}{1-\mathcal{P}(0,\overline{N})} = \frac{\mathrm{Ei}(\overline{N})-\gamma-\ln \overline{N}}{e^{\overline{N}}-1}$ where $\gamma \approx 0.5772$ is the Euler-Mascheroni constant and Ei the exponential integral. The relatedness depends on μ through $\overline{N}$, which is a solution of $\overline{N} = \mu\widetilde{Q}(b)(1-e^{-\overline{N}})$. This has to be evaluated for the value of b that satisfies $\left.\frac{\partial W(b^*,b)}{\partial b^*}\right|_{b^*=b} = 0$. In practice, this plot is easiest constructed as a parametric plot, by calculating, for a given value of $\overline{N}$, the corresponding R, then work out the ESS value for b, and with this information calculate μ from $\mu = \overline{N}(\widetilde{Q}(b)(1-e^{-\overline{N}}))^{-1}$. Parameters as in Table 1.

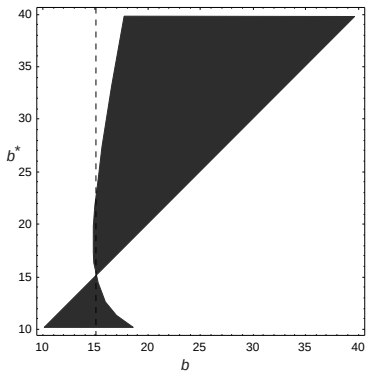


Figure 7: Pairwise Invasability plot in which the ESS is further invadable. Areas where the fitness of the mutant is positive are white and areas where the fitness of the mutant is negative are grey. In the figure the position of the ESS can be found at the point where the two invasion boundaries cross. As the dashed line indicates, in the vicinity of the ESS no mutant strains can invade. If strains are substantially different, further invasion at the ESS is possible. Parameters as in Table 1, but with $\tau = 20$ and $\mu = 10^{-3}$.

that we derived. A necessary condition for divergence is that the equilibrium bacterial density is a convex function of the siderophore production rate. That said, it is possible that further strains can invade, if they produce substantially more siderophores. These strains typically persist in the patches that are left unoccupied. Because such strains are competitively weak, they do not have much impact on the ESS value of the most competitive strain (Jansen and Mulder, 1999). This mechanism can lead to evolutionary stable diversity in which strains with moderate levels of cooperation coexist with strains with very high levels of cooperation. In how far the existence of such ultra-cooperators is realistic we do not know, although also in other studies they have been predicted on theoretical grounds (Brown et al., 2009). As hypermutable strains of siderophore producing bacteria are found in the wild (Oliver et al., 2000), it is plausible that large phenotypic effects can occur through mutation.

In agreement with other studies (West and Buckling, 2002; Ross-Gillespie et al., 2007; Kümmerli et al., 2009, 2010) the outcome can be understood in terms of kin selection. If all members of the population are fully related siderophore production would be maximal; but the fact that the population infecting a host typically results from multiple ancestors (either because the infective dose is a mixture, or because of multiple infection events), reducing the average relatedness and, with it, the incentive to cooperate. In contrast with previous approaches, however, in our analysis the components of Hamilton's Rule, (that is, benefits, costs and relatedness) are not fixed constants but emerge from the underlying within- and between-host dynamics. A consequence is that fitness costs and benefits are compound variables that do not simply depend on the physiological costs and benefits as is usually assumed (cf. Eqn (26)).

One can incorporate considerable ecological detail in evolutionary models by using agent based simulation studies. Such simulation models have been made in the context of siderophore production and the evolution of public goods (see, e.g. Xavier and Foster (2007); Dobay et al. (2014)). Although such models are useful for investigating the effect of mechanism, we argue that there is merit in the expression and interpretation of fitness functions in a form compatible with inclusive fitness theory. The advantage is that this identifies the components that separate the local interaction (the cost and benefits) from the effect of global redistribution and assortment (relatedness). This allows, firstly, to reduce fitness expressions to simpler components which, in principle, are measurable. Relatedness can be measured using standard methods based on neutral variants. In principle, the costs and benefits can be estimated in experiments after manipulation of the relatedness (using a linear regression of marginal fitness on relatedness), but this is difficult without an analytical derivation and interpretation of the results. A second conceptual advantage is that, once the cost and benefits have been established, results can then be generalised to more complicated spatial arrangements of the local populations.

An important novel aspect of our analysis is that it shows how the dynamical processes involved in the use of public goods integrate to produce inclusive fitness. This allows us to interpret the fitness elements in terms of observable and measurable quantities: here the increased output that comes with increased

siderophore production, the decreased competitive ability and the relatedness. To do this requires models that include relevant details of the ecology and experimental protocol, but that at the same time are mathematically tractable so that the fitness can be teased out (Rousset and Ronce, 2004). These models need to lie between the strategic models based on arguments of plausibility and convenience and highly detailed simulation models. Here, we do this by approximating the local dynamics by a separation of time scales; a technique which is applicable well beyond the simple ecology dealt with here (see, for instance, Jansen and Mulder (1999); Bryden and Jansen (2010); Jansen (2011)).

An important additional advantage of such an approach is that it not only allows us to gain insight in the short-term evolutionary dynamics (whether a given mutant can invade, as given by Hamilton's Rule) but also where the long-term evolutionary process will end (at an ESS characterised by an intermediate production rate). Approaches that limit the analysis to two fixed types (e.g., 'cooperators', 'cheaters') cannot make such predictions.

We have shown that the effect of competition is modulated through the length of the local interaction. If the interaction is long-lasting, then competition dominates and the ESS level for public good production is low. If the interaction is for a short period of time, cooperation is promoted and high levels of public good production arise. This suggests a way to experimentally vary the importance of competition through variation of the time of interaction. The relative importance of competition has previously been investigated through manipulation of the "scale of competition" (Frank, 1998), which describes the extent to which patches can export the benefits from cooperation (Grafen, 1984). Varying the duration of the interaction provides an alternative for this manipulation that is arguably of closer to the ecology of natural populations than imposed variations of the scale of competition. An alternative method of experimentation, in particular to demonstrate the role of kin selection, is leaving the patch duration constant but varying the transfer volume μ: if μ is small, mutants will face little competition with residents whereas if μ becomes larger there will be more residents in the inoculum and hence competition with non-kin will become more important. A change in μ will affect the distribution of immigrants, and through this, the relatedness, R, without affecting other elements of the marginal fitness. To obtain interesting results, relatedness values should be neither too low nor too high; the average number of bacteria per patch should roughly be between 0.1 and 10. Note that the occurrence of empty patches in the experiment is an unavoidable consequence if one aims for experiments with relatively high relatedness.

Our model is obviously a very simple one that mimics an experimental setup. More than that, it incorporates all important aspects of bacteria and their interaction with the environment. For instance, we assume that the system is well-mixed at the patch level, so that locally all bacteria profit equally from the siderophores that have been released. This will change if one would incorporate diffusion of siderophores, either locally at a small scale, or at the larger inter-patch scale (see Allen et al. (2013); Dobay et al. (2014)), but this is an aspect that we did not include in our model for the sake of mathematical tractability.

However, the method that we have outlined here can, in principle, also be used to assess where siderophores sit on the continuum between common and private goods and how this depends on the details of the reassortment between cycles. For such more complicated situations we anticipate that the calculation of the relatedness measure will become cumbersome, but that the other elements of the marginal fitness (changes in output and competitive ability) will remain qualitatively unaffected.

An important aspect that is missing from most models (including the one we studied here) is the fact that siderophore production is not a fixed constant, but dependent on the conditions. Not surprisingly, bacteria do not produce siderophores if there is sufficient iron available (Kümmerli et al., 2009; Harrison, 2013). However, as Alizon (2013) points out, because not enough is known about the mechanisms it may be premature to consider this plasticity as an adaptation to the presence of cheaters. An important next step would thus be to use our approach to predict how production *strategies* evolve rather than just production *rates*, as a function of various aspects of the bacterial environment.

Our model does predict that the evolutionary dynamics are dominated by an optimum compromise between cheating and cooperation, but that at such evolutionarily stable strategies highly cooperative strains can invade — if the bacteria are limited to using one standard type of siderophore. However, it should be noted that bacteria have potentially the means to exclude other groups from using the public good and to reserve the benefits to themselves and their kin (Dionisio and Gordo, 2007). What happens if, rather than changing the *rate* of production, mutations affect the *type* of siderophore that is produced is investigated in a previous study, in which we found that cheaters can regulate the diversity of siderophore types: even if they rarely appear in the population, they can stabilise the balance of the different types of siderophore by episodically counteracting genetic drift (Lee et al., 2012). A further theoretical possibility is that coexisting pairs of cheaters and cooperators strains coexist with other such pairs that use different types of siderophore. This can result in a coexistence equilibrium or complex chromodynamics: the coexistence of different recognition markers under non-equilibrium dynamics (Jansen and van Baalen, 2006). Our results suggest that it is possible that the coexistence of cheaters and cooperators, together with the dynamics that go with such recognition systems (Lee et al., 2012; Jansen and van Baalen, 2006), may emerge if both production rate and type can evolve simultaneously.

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Appendix

1. Evolutionary Stability

To find out if our candidate ESS points are evolutionarily stable we need to assess whether

$$\left.\frac{\partial^2 W(b^*,b)}{\partial {b^*}^2}\right|_{b^*=b} < 0$$

To do so we first derive from (8) that

$$\left.\frac{d^2 f_\tau}{d{b^*}^2}\right|_{b^*=b} \approx \alpha^2\tau^2 f_0(1-f_0)(1-2f_0), \tag{29}$$

and from (9) that

$$\left.\frac{d^2 \bar{b}_\tau}{d{b^*}^2}\right|_{b^*=b} \approx -2\alpha\tau f_0(1-f_0). \tag{30}$$

The second derivative takes the form

$$\begin{aligned}
\left.\frac{\partial^2 W(b^*,b)}{\partial {b^*}^2}\right|_{b^*=b} &= \frac{\sum_{i=0}^{\infty}\mathcal{P}(i+1,\overline{N})\left[\widetilde{Q}'' f_0 - 4\alpha\tau\widetilde{Q}'(1-f_0) + Q(b)\alpha^2\tau^2(1-f_0)(1-2f_0)\right]}{(1-\mathcal{P}(0,\overline{N}))\widetilde{Q}(b)} \\
&= \frac{\sum_{i=1}^{\infty}\mathcal{P}(i,\overline{N})\left[\widetilde{Q}''\frac{1}{i} - 4\alpha\tau\widetilde{Q}'(1-\frac{1}{i}) + \widetilde{Q}\alpha^2\tau^2(1-\frac{1}{i})(1-2\frac{1}{i})\right]}{(1-\mathcal{P}(0,\overline{N}))\widetilde{Q}(b)} \\
&= \frac{\widetilde{Q}''}{\widetilde{Q}}R - 4\alpha\tau\frac{\widetilde{Q}'}{\widetilde{Q}}(1-R) + \alpha^2\tau^2\frac{\sum_{i=1}^{\infty}\mathcal{P}(i,\overline{N})(1-\frac{1}{i})(1-\frac{2}{i})}{1-\mathcal{P}(0,\overline{N})}
\end{aligned}$$

using that at the ES point $\frac{\widetilde{Q}'}{\widetilde{Q}} = \alpha\tau\frac{1-R}{R}$, and $\alpha\tau = \frac{\widetilde{Q}'}{\widetilde{Q}}\frac{R}{1-R}$ this can be written as

$$\left.\frac{\partial^2 W(b^*,b)}{\partial {b^*}^2}\right|_{b^*=b} = \left(\frac{\widetilde{Q}''}{\widetilde{Q}} - 2\frac{\widetilde{Q}'^2}{\widetilde{Q}^2}\right) R - \alpha^2\tau^2\left(2\frac{(1-R)^2}{R} - \frac{\sum_{i=1}^{\infty}\mathcal{P}(i,\overline{N})(1-\frac{1}{i})(1-\frac{2}{i})}{1-\mathcal{P}(0,\overline{N})}\right).$$

Note that

$$\frac{\sum_{i=1}^{\infty}\mathcal{P}(i,\overline{N})\left(1-\frac{1}{i}\right)\left(1-\frac{2}{i}\right)}{1-\mathcal{P}(0,\overline{N})} = \frac{\sum_{i=1}^{\infty}\mathcal{P}(i,\overline{N})\left(1-\frac{3}{i}+\frac{2}{i^2}\right)}{1-\mathcal{P}(0,\overline{N})}$$

can be written as

$$1-3R+2\frac{\sum_{i=1}^{\infty}\mathcal{P}(i,\overline{N})\left(\frac{1}{i^2}\right)}{1-\mathcal{P}(0,\overline{N})}$$

Because $2-4R \geq 1-3R$ for $0 \leq R \leq 1$, therefore

$$2\frac{1-2R}{R} \geq 1-3R$$

for $0 \leq R \leq 1$. Because $R = \frac{\sum_{i=1}^{\infty}\mathcal{P}(i,\overline{N}))\frac{1}{i}}{1-\mathcal{P}(0,\overline{N})} \geq \frac{\sum_{i=1}^{\infty}\mathcal{P}(i,\overline{N}))\frac{1}{i^2}}{1-\mathcal{P}(0,\overline{N})}$ with equality if $R=1$ (which implies that $\overline{N}=0$) it follows that

$$\begin{aligned} 2\frac{(1-R)^2}{R} - \frac{\sum_{i=1}^{\infty}\mathcal{P}(i,\overline{N})(1-\frac{1}{i})(1-2\frac{1}{i})}{1-\mathcal{P}(0,\overline{N})} &= \\ 2\frac{1-2R}{R} - (1-3R) + 2R - 2\frac{\sum_{i=1}^{\infty}\mathcal{P}(i,\overline{N})\left(\frac{1}{i^2}\right)}{1-\mathcal{P}(0,\overline{N})} &\geq 0 \end{aligned}$$

if $\overline{N} \geq 0$, with equality if $R=1$ and $\overline{N}=0$.

Because $Q''(b)=0$ we have always that

$$\frac{\widetilde{Q}''}{\widetilde{Q}} - 2\frac{\widetilde{Q}'^2}{\widetilde{Q}^2} < 0.$$

It follows that if we define b_{ES} as

$$\left.\frac{\partial W(b^*,b_{ES})}{\partial b^*}\right|_{b^*=b_{ES}} = 0$$

if $\widetilde{Q}(b_{ES}) > 0$ (which implies $\epsilon > \alpha$) then

$$\left.\frac{\partial^2 W(b^*,b_{ES})}{\partial {b^*}^2}\right|_{b^*=b_{ES}} < 0.$$

This means the candidate evolutionary stable siderophore production rates are indeed always evolutionarily stable, provided $\widetilde{Q} > 0$ at the ESS. This result holds for all inoculum distributions.