Constraints on microbial metabolism drive evolutionary diversification in homogeneous environments

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Abstract

Understanding the evolution of microbial diversity is an important and current problem in evolutionary ecology. In this paper, we investigated the role of two established biochemical trade-offs in microbial diversification using a model that connects ecological and evolutionary processes with fundamental aspects of biochemistry. The trade-offs that we investigated are as follows:(1) a trade-off between the rate and affinity of substrate transport; and (2) a trade-off between the rate and yield of ATP production. Our model shows that these biochemical trade-offs can drive evolutionary diversification under the simplest possible ecological conditions: a homogeneous environment containing a single limiting resource. We argue that the results of a number of microbial selection experiments are consistent with the predictions of our model.

Introduction

The *competitive exclusion principle*, which states that a simple unstructured environment containing only a single resource can support only one competitor (Gause, 1934; Hardin, 1960), is often used as a starting point for discussions of the evolution and maintenance of microbial diversity (Fredrickson & Stephanopoulos, 1981; Kassen & Rainey, 2004). A model for such a simple environment is the chemostat, a vessel with a constant influx of fresh medium and efflux of spent medium and cells (Novick & Szilard, 1950a).

Models of competition predict that diversity cannot be maintained in the chemostat (Stewart & Levin, 1973; Smith & Waltman, 1995; Pfeiffer *et al.*, 2001) unless the population is subject to product inhibition (Lenski & Hattingh, 1986; Hsu & Waltman, 1992; Pfeiffer & Bonhoeffer, 2004), cross-feeding (Pfeiffer & Bonhoeffer, 2004) or the dilution rate of the chemostat varies periodically (Stephanopoulos *et al.*, 1979; Butler *et al.*, 1985). These models, with the exception of Pfeiffer & Bonhoeffer (2004), are strictly ecological in the sense

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that they do not allow novel variants to appear during the course of competition. This is an important limitation because the large size of microbial populations ensures that novel mutants appear continuously, allowing for rapid evolution.

The evolutionary extension of the principle of competitive exclusion is the *principle of periodic selection* which states that microbial evolution in simple environments is characterized by sequential selective sweeps that replace the dominant clone in a population with a fitter descendent (Muller, 1932; Atwood *et al.*, 1951; Crow & Kimura, 1965; Dykhuizen, 1990). Although early chemostat experiments reported results that were consistent with periodic selection (Novick & Szilard, 1950b; Atwood *et al.*, 1951), diversity has been detected in a number of chemostat experiments using both culturing techniques (Adams & Oeller, 1986; Wick *et al.*, 2001; Maharjan *et al.*, 2006) and molecular population genetics (Adams & Oeller, 1986; Notley-McRobb & Ferenci, 1999a, b; Kashiwagi *et al.*, 2001; Maharjan *et al.*, 2006).

We postulate that the following well-established biochemical constraints are sufficient to allow creation and maintenance of microbial diversity in simple habitats:

1. *The rate vs. yield trade-off.* The laws of thermodynamics imply the existence of a trade-off between the rate (moles ATP/time) and yield (moles ATP/mole substrate)

of any given catabolic reaction Pfeiffer *et al.* (2001). At the level of entire pathways, trade-offs between the rate and yield of ATP production have been shown in *Candida utilis*, *Saccharomyces cerevisiae* (Weusthuis *et al.*, 1994; Otterstedt *et al.*, 2004), *Escherichia coli* (Novak *et al.*, 2006) and *Pseudomonas fluorescens* (see Appendix A)

2. *The maximal uptake rate vs. affinity trade-off.* Although the thermodynamic basis of this trade-off has not received as much attention as the rate vs. yield trade-off, there is clear evidence for a trade-off between the maximal rate and affinity of substrate transport in *S. cerevisiae* (Elbing *et al.*, 2004) and *E. coli* (Wirtz, 2002).

The role of the above trade-offs in microbial competition has been explored in Stewart & Levin (1973), Pfeiffer *et al.* (2001) and whereas Stewart & Levin (1973) considered only the rate–affinity trade-off, Pfeiffer *et al.* (2001) considered only the rate–yield trade-off. Coexistence of microbial strains was not observed in either study and one dominant strain was found to persistently outcompete all others.

In this paper, we developed a mathematical model that describes the evolution of a microbial population subject to both aforementioned biochemical trade-offs. This mathematical approach connects ecological and evolutionary dynamics and examines the way in which ecological factors influence evolution by natural selection.

Materials and methods

The model

In this section, we present a model describing the evolution of a microbial population growing on a single resource in the chemostat. The model tracks changes in phenotypic distribution of a population of micro-organisms in response to ever-changing environments and makes use of the following basic assumption:

$$
\begin{aligned}
&\text{the rate of change of resource concentration} \\
&= \text{input} - \text{resource consumption} - \text{dilution}
\end{aligned} \tag{1}
$$

$$
\begin{aligned}
&\text{the rate of change of population density} \\
&= \text{growth} + \text{phenotypic mutations} - \text{dilution}
\end{aligned} \tag{2}
$$

Resource consumption and microbial growth

In our model, cells take up an extracellular resource and convert it into ATP using a simple, unbranched metabolic pathway (see Pfeiffer & Bonhoeffer (2004) for an illustration). The rate of ATP production in the pathway is denoted by J^{ATP} and is given by

$$
J^{ATP} = n_{ATP} J^{S} \tag{3}
$$

where J^{S} denotes the rate of the pathway and n_{ATP} denotes the number of ATP molecules produced in the

pathway. As in Pfeiffer & Bonhoeffer (2004) we make a simplifying assumption that the behaviour of the entire pathway can be modelled with Michaelis–Menten kinetics of a single reaction. Therefore

$$
J^{S} = \frac{V_{max} S}{K_{m} + S} \tag{4}
$$

where V_{max} denotes maximal rate of the pathway and K_{m} the Michaelis–Menten constant. The pathway rate J^{S} represents the rate at which product is formed which in this case is the same as the rate at which substrate is consumed. Therefore, throughout this paper we refer to V_{max} as the maximal rate of resource uptake and K_{m} as the measure of affinity for a resource.

Bauchop & Elsden (1960) observed that if microbes are limited by their energetic resource, the amount of biomass formed per unit of ATP is approximately constant and does not depend on the mode of ATP production. Therefore, as highlighted by Pfeiffer and Bonhoeffer (2004) if the rate of ATP production increases, the rate of biomass formation and thus the growth rate of an organism also increases. This implies that the microbial growth rate can be represented as a linear function of the rate of ATP production, namely cJ^{ATP} where c is some proportionality constant.

We model the growth of a microbial population of density $N(t)$ at time t consuming a single limiting resource of concentration $S(t)$ at time t in the chemostat in the following way:

$$
\dot{S} = D(S_0 - S) - J^{S} N, \tag{5}
$$

$$
\dot{N} = cJ^{ATP} N - DN. \tag{6}
$$

Parameter D represents the *dilution rate* describing: (a) the rate of influx of the resource into the chemostat from an outside reservoir with the resource concentration S_0; and (b) the rate at which the content of the chemostat, including both cells and the unused resource, is removed.

Trade-offs

We assume that microbial strains differ in their values of V_{max}, which is chosen to be the evolving phenotypic trait. As there is a biologically feasible maximum to any maximal uptake rate the phenotypic trait V_{max} is assumed to reside in an interval $[a,b]$ where a and b are nonzero parameters. We also assume that evolutionary changes in V_{max} are constrained by two well-established trade-offs.

First, we assume that an increase in V_{max} leads to a decrease in n_{ATP}, an assumption that can be written in the form $n_{ATP} = g(V_{max})$ where g is a decreasing function of V_{max}. This is motivated by the rate (J^{ATP}) and yield (n_{ATP}) trade-off and was also used in Pfeiffer *et al.* (2001).

Secondly, we assume that an increase in V_{max} leads to a decrease in the affinity of the cell for its resource which

can be written as $K_m = f(V_{max})$, where f denotes an increasing function of V_{max}. For f to represent the rate–affinity trade-off we also need to ensure that for small S, J^S increases as V_{max} increases at least for some values of V_{max} in the phenotypic domain $[a,b]$ (see Appendix B for details). Taking the rate–yield and rate–affinity trade-offs into account, eqns 3 and 4 become

$$J^{ATP} = g(V_{max}) \frac{V_{max}S}{f(V_{max}) + S} \text{ and } J^S = \frac{V_{max}S}{f(V_{max}) + S'} \quad (7)$$

respectively.

Note that the model presented in this paper can easily be extended to a two-dimensional phenotypic domain where the yield n_{ATP} and the affinity K_m are the evolving phenotypes whose evolutionary changes are constrained by the same two biochemical trade-offs. In that case V_{max} would be a decreasing function of n_{ATP} but an increasing function K_m. However, for simplicity we restrict our study to a one-dimensional phenotypic domain with V_{max} as the evolving phenotype.

Phenotypic mutations

As microbes usually reproduce asexually, our model is restricted to clonal reproduction. However, if a mutation occurs during reproduction a parent cell will give rise to an offspring with a value of V_{max} different to its own.

We assume that mutations have only small phenotypic effect and we represent them in the following way. Consider a set of n values for the phenotypic trait V_{max} denoted by $a = V_{max}^1 \leq V_{max}^2 \leq \cdots \leq V_{max}^n = b$. If a mutation occurs during reproduction of a cell with phenotype V_{max}^i then there is an equal probability of $1/2$ that the phenotype of the mutant offspring will either be V_{max}^{j-1} or V_{max}^{j+1}. As V_{max}^1 and V_{max}^n are on the edge of the phenotypic domain they represent a special case whereby a parent with phenotype V_{max}^1 (V_{max}^n) can only give rise to a mutant offspring with phenotype V_{max}^2 (V_{max}^{n-1}). This is known as a *no-flux* boundary condition.

Evolutionary model

Next we explain how to include mutations from clonal reproduction described above into the ecological setting of eqns 5 and 6. Our approach is similar in nature to the adaptive dynamics method (Metz *et al.*, 1996) with a difference that here mutations are intrinsically built into the model. Contrary to this, the adaptive dynamics method considers ecological and evolutionary time scales separately and a mutant phenotype is only added into the system once the ecological interactions have reached a steady state.

Consider a microbial population with n competing strains each with a different value of V_{max} and let N_i denote the density of a strain with phenotype V_{max}^i for $i = 1,\ldots,n$. If we assume that phenotypic mutations occur at a rate ε, the ecological model 5 and 6 can be transformed into the following evolutionary model:

$$\frac{dS}{dt} = D(S_0 - S) - \sum_{i=1}^{n} J_i^S N_i, \quad (8)$$

$$\frac{dN_1}{dt} = \varepsilon(N_2 - N_1) + cJ_1^{ATP}N_1 - DN_1, \quad (9)$$

$$\frac{dN_i}{dt} = \varepsilon\left(\frac{1}{2}N_{i-1} + \frac{1}{2}N_{i+1} - N_i\right) + cJ_i^{ATP}N_i - DN_i, \quad (10)$$

for $i = 2,\ldots,n-1$

$$\frac{dN_n}{dt} = \varepsilon(N_{n-1} - N_n) + cJ_n^{ATP}N_n - DN_n \quad (11)$$

where J_i^{ATP} and J_i^S are defined as in eqn 7 with V_{max} replaced by V_{max}^i. The diversity of a population will be measured through the number of local maxima in the distribution of population densities according to their phenotype.

Note that for n sufficiently large the above system of ordinary differential eqns 8–11 can be written as a system of partial differential equations (PDEs) (see Appendix C for details). The PDE approach has its origins in the work of Fisher (1930) and Kimura (1983) and more recently it has been used to study evolution in both the mathematical (Calsina & Perell'o, 1995; Gudelj *et al.*, 2006) and biophysics (Tsmiring & Levine, 1996) literature. A feature of both eqns 8–11 and eqns 12 and 13 in Appendix C is that there is a nonzero probability that a mutation can arise anywhere in the phenotypic domain $[a,b]$ which is well suited for modelling microbial evolution.

Results

Creation and maintenance of diversity

If the dilution rate D is larger than a critical value D_0, the microbial population will not be able to persist and eventually the chemostat will only contain the resource at concentration S_0. However, if the dilution rate D is smaller than D_0, the microbial population will be able to persist on a single resource and may converge to a unique steady state $(N^*_1,\ldots,N^*_n)$ supported by a resource of concentration S^*. It can be shown that there are no other points to which the solution can be attracted; moreover, a mathematical argument shows that this steady state can be reached exponentially quickly. Therefore, in the remainder of the section we assume that $D < D_0$ and explore the phenotypic structure of the equilibrium state.

The equilibrium population $N^* = (N^*_1,\ldots,N^*_n)$ of eqns 8–11 can potentially support *any* number of phenotypic clouds, provided that f and g satisfy appropriate conditions (see Appendix D for details). This can be illustrated with the following examples.

Example 1: Straight line rate–yield trade-off

Consider f and g to be of the form illustrated in Fig. 1a and b, respectively, so that the rate–yield relationship follows a straight line whereas there is *no rate–affinity trade-off* as f does not satisfy the condition set in the Appendix B. In this case long-term diversity is not possible and the steady-state population can only support one phenotypic cloud situated either around 0.1 or $\sqrt{D/c}$ depending on the mutation rate ε and the dilution rate D, see Fig. 1c. Note that expression $\sqrt{D/c}$ is arrived at using asymptotic analyses similar in style to the one performed in Gudelj *et al.* (2006). Therefore, when $D = 0.01$, $c = 1$ and $\varepsilon = 10^{-7}$, for example, the phenotypic cloud is situated around 0.1 (see Fig. 1d for a numerically obtained solution) and when $D = 0.04$, $c = 1$ and $\varepsilon = 10^{-7}$ the phenotypic cloud is situated at 0.2 (see Fig. 1e for a numerically obtained solution).

This result can be deduced using Maynard-Smith's evolutionary theory (Maynard-Smith, 1982) which demonstrates that the evolutionary stable strategy (ESS) will be situated at $V_{max} = \sqrt{D/c}$ (see Appendix E for details). Note that in this case the strain with the highest maximal rate of resource uptake, b, will not be present in the population in the long term.

Example 2: Concave f and convex g trade-offs

Suppose that f is concave and g is convex trade-off of the form illustrated in Fig. 2a and b respectively. The number of phenotypic clouds present in the equilibrium population of eqns 8–11 depends on the mutation rate ε and the dilution rate D as illustrated in Fig. 2c. In this case, the population can support either one phenotypic cloud situated around 0.1 (see Fig. 2e) or two phenotypic clouds situated around 0.1 and 0.9 (see Fig. 2d).

Long-term diversity depends crucially on the mutation rate ε. If $\varepsilon = 0$, no mutations occur and the model eqns 8–11 reduces to an ecological system in which n strains with different phenotypes compete for a single resource. It is well known that coexistence is not possible in such systems and one strain out-competes the others (Smith & Waltman, 1995). The strain with the largest growth rate will always win the competition and in this example the winning strain is situated at $V_{max} = 0.1$.

By setting $\varepsilon > 0$ an evolutionary component is introduced into the system and when ε and D are in the region illustrated in Fig. 2c, two phenotypic clouds one around 0.1 and the other around 0.9 are able to coexist. However, if the mutation rate is sufficiently large the population will tend towards an almost uniform distribution of phenotypes.

Example 3: Staircase f and g trade-offs

If f and g are of the form illustrated in Fig. 3a and b, respectively, the population can, in the long term, support two phenotypic clouds situated around phenotypes within the interior of the domain Fig. 3c, but not at the boundary as in Example 2.

Examples 1–3 above demonstrate that the number and the location of the phenotypes will depend not only on the shape of the trade-off functions, but also on the mutation rate ε and the dilution rate D and the population can support a number of different phenotypes throughout the phenotypic domain.

Discussion

We have developed a mathematical model to study evolutionary diversification in microbial populations that is motivated by the need to integrate ecological interactions with evolutionary dynamics as highlighted by Metz *et al.* (1996) and the recognition that fundamental biochemical constraints may play an important role in microbial evolution (Pfeiffer *et al.*, 2001; Friesen *et al.*, 2004; Kreft, 2004; Pfeiffer & Bonhoeffer, 2004).
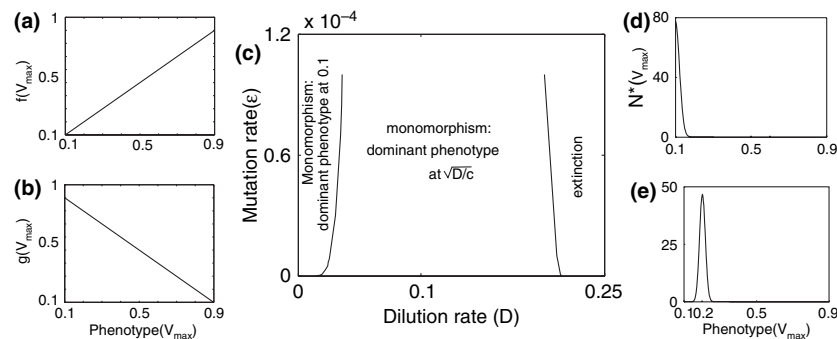

Fig. 1 Trade-offs for $V_{max} \in [0.1, 0.9]$: (a) $f(V_{max}) = V_{max}$, (b) $g(V_{max}) = 1 - V_{max}$. The structure of the steady state solution $N^* = (N^*_1, ..., N^*_n)$ of eqns 8–11 as a function of the dilution rate D and the mutation rate ε is presented in (c). A numerically computed steady state is presented in (d) with $\varepsilon = 10^{-7}$ and $D = 0.01$, and in (e) with $\varepsilon = 10^{-7}$ and $D = 0.04$. The remainder of the parameters have the following values: $V_{max}^n \in [0.1, 0.9]$ with $n = 500$, $S_0 = 3$, $c = 1$.
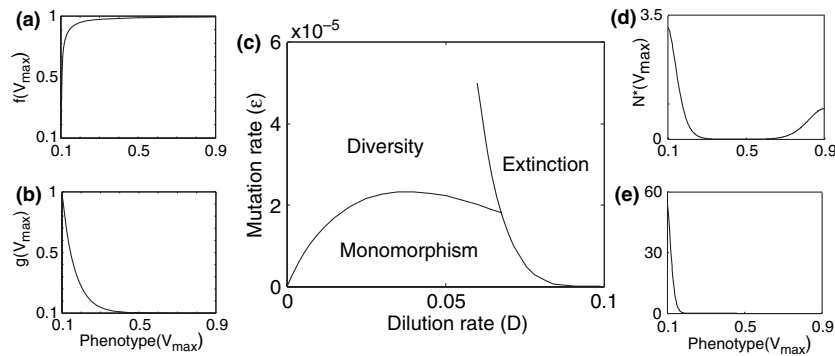
Fig. 2 Trade-offs for V_{max} in [0.1,0.9]: (a) $f(V_{max}) = \frac{V_{max} - 99 \times 10^{-3}}{5 \times 10^{-3} + (V_{max} - 99 \times 10^{-3})}$, (b) $g(V_{max}) = 1/10 + e^{-15(V_{max} - 99 \times 10^{-3})}$. The structure of the steady state solution $N^* = (N^*_1, \ldots, N^*_n)$ of eqns 8–11 as a function of the dilution rate D and the mutation rate ε is presented in (c). A numerically computed $N^*(V_{max})$ is presented in (d) for $\varepsilon = 8 \times 10^{-5}$ and $D = 0.04$, and in (e) for $\varepsilon = 10^{-5}$ and $D = 0.04$. The remainder of the parameters have the following values: $V^n_{max} \in [0.1, 0.9]$ with $n = 500$, $S_0 = 3$, $c = 1$.
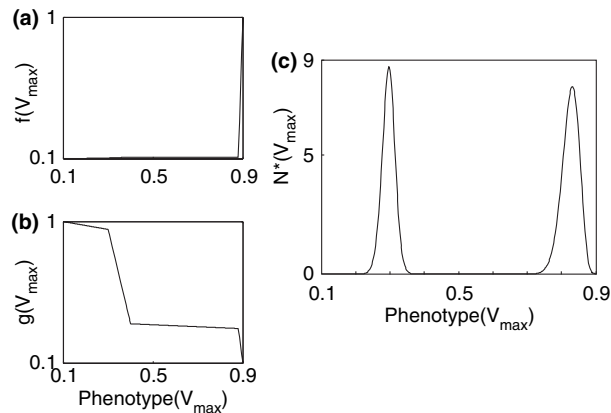

Fig. 3 Trade-offs for $x \in [0.1, 0.9]$: (a) f and (b) g. A numerically computed steady state solution $N^*(V_{max})$ of eqns 8–11 is presented in (c) for $\varepsilon = 1.1183209 \times 10^{-5}$, $D = 0.2$, $V_{max} \in [0.1, 0.9]$, $S_0 = 3$ and $c = 1$.

Our model predicts that evolutionary diversification is possible under the simplest possible ecological conditions: a homogeneous environment containing a single limiting resource.

The long-term diversity of a population will depend on an ecological, biochemical and an evolutionary component, namely the dilution rate, the geometry of the trade-off functions and the mutations that result from clonal reproduction. The simplest trade-offs that support long-term diversity are illustrated in Fig. 2a and b where the rate–yield trade-off is convex and the maximal uptake rate–affinity trade-off is concave. In this situation selection in the chemostat will ultimately result in either: (1) the dominance of a single-type gleaner that has a low maximal rate of resource uptake; or (2) the coexistence of gleaners with glutton that have a high maximal rate of resource uptake.

However, once dilution rate is fixed coexistence occurs only if the mutation rate is sufficiently high.

Let us briefly explain why this occurs by the following examination of eqns 8–11 at equilibrium. To begin with we make a note that the shape of the trade-offs in Fig. 2a and b means that a change in the biochemical pathway that leads to an increase in the maximal rate of resource uptake is initially very costly to the cell as it leads not only to a significant decrease in the affinity of the cell for the resource, but also to a significant decrease in the yield of ATP production. However, once the maximal rate of resource uptake has increased beyond a moderate value, further increases can be achieved at very little additional cost. This leads to the presence of two local fitness maxima, whereby one is a global maximum situated at the lowest value of the maximal rate of resource uptake and one a sub-optimal local maximum situated at the highest value of the maximal uptake rate. Sufficiently large mutation rate will allow sub-optimal phenotypes to be maintained alongside the fittest type. In a competition model, sub-optimal strategies would not persist in this way which is why the phenotype with the highest maximal uptake rate disappears as the mutation rate is reduced.

Unfortunately, the values of phenotypic mutation rates that lead to the coexistence of multiple phenotypic clouds in our study are difficult to compare with real rates due to a lack of available data. One way of obtaining real phenotypic mutation rates could be through mutation rates per gene which are much better documented and are known to be in a range of 10^{-6}–10^{-9} per generation (Drake, 1991). Given that multiple genes contribute to the same phenotypic trait, it would be reasonable to expect that phenotypic mutation rates will be larger than per-gene mutation rates.

In this paper mutations are assumed to have only small phenotypic effects which could be seen as overly simplistic as it is well known that mutations in micro-

organisms can also have large phenotypic effects. However, the evolutionary outcome observed in this work will not change if mutations with large phenotypic effects are introduced into the model, as long as one assumes that they arise less frequently than those with small effects. Moreover, the model could easily be adapted to take into account a wide range of mutational structures with different phenotypic effects as the appropriate data becomes available.

The exclusion of phenotypic mutations from the model that occurs on setting $\varepsilon = 0$ eliminates any chance of observing diversity. In this case microbial reproduction is perfectly clonal and eqns 8–11 reduces to a purely *competitive system* where n microbial strains compete for a single limiting resource. Moreover, in this case coexistence is not possible regardless of the shape of the trade-off functions and one phenotype always out-competes the others as observed in Stewart & Levin (1973), Smith & Waltman (1995) and Pfeiffer *et al.* (2001). This property highlights a significant difference between the application of competitive and evolutionary mathematical models.

Biochemical observations and microbial selection experiments support both the assumptions and predictions of our model. Until now a popular explanation for diversification in the chemostat has been cross-feeding, an ecological interaction whereby one clone secretes secondary metabolites derived from the exogenously supplied resource, thereby providing a secondary resource for scavenger genotypes that are inferior competitors for the primary resource (Rosenzweig *et al.*, 1994; Treves *et al.*, 1998; Pfeiffer & Bonhoeffer, 2004). However, several lines of evidence demonstrate that cross-feeding does not provide a general explanation for microbial diversification in simple environments; for instance: (1) molecular studies of adaptation in chemostat experiments have found extensive polymorphism in genes related to the utilization of primary nutrients (Notley-McRobb & Ferenci, 1999a, b; notley99b, Kashiwagi *et al.*, 2001; Maharjan *et al.*, 2006); and (2) diversification has occurred under culture conditions that are known to minimize metabolite secretion (Adams & Oeller, 1986; Weikert *et al.*, 1997).

In accordance with the outcomes of our model, many experimental studies have reported that selection in the chemostat results in the evolution of increased affinity for the limiting substrate but with the difference that any obvious diversification into metabolic variants has not been observed (Dykhuizen & Hartl, 1981; Wick *et al.*, 2001; Jansen *et al.*, 2004, 2005). Unfortunately, the power of these experiments to detect diversification is typically low because the mutations responsible for adaptation are not typically known and diversification is only recognized when it results in associated changes in colony morphology. To overcome this limitation, Kashiwagi *et al.* (2001) constructed isogenic strains of *E. coli* that varied at the glutamine synthetase locus and

then selected in chemostats containing glutamate as the sole nitrogen source. The outcome of selection was the repeated diversification into glutamine synthetase alleles with different affinities for glutamate. Critically, competition experiments established that these polymorphisms were stably maintained by negative frequency-dependent selection.

The results presented in this paper are also in agreement with a recent experimental study of Maharjan *et al.* (2006) where a clonal population of *E. coli* was grown on a single limiting resource in the chemostat. The clonal population radiated into more than five phenotypic clusters and the growth yields of the isolates on glucose varied markedly. Moreover, it was shown that a cross-feeding polymorphism was not responsible for the maintenance of the observed diversity.

We require the shape of the trade-off functions to be nonlinear for diversity to be observed. This is a plausible scenario as experimental studies have reported concave trade-offs between the maximal rate and affinity of substrate transport (Wirtz, 2002; Elbing *et al.*, 2004), whereas both straight line and nonlinear rate–yield trade-offs have been reported in yeast, depending on the pathway being used to produce ATP (Weusthuis *et al.*, 1994). In cases where sustained divergence did not occur, transient diversification was a robust outcome of our model. Although rigorous experimental demonstration of transient behaviour is a daunting task, some experimental results are consistent with this scenario (Weikert *et al.*, 1997).

Our study connects biochemical constraints, ecological interaction and evolutionary dynamics. It presents a novel result regarding mechanisms that maintain coexistence in simple environments which can help to explain the repeatable evolutionary diversification of microbial populations during evolution in the chemostat under conditions where cross-feeding between genotypes does not occur. Our model considers large and unstructured populations and as such is best suited to the study of aquatic microbes rather than those in biofilms or within eukaryotic cells.

Acknowledgments

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Supplementary Material

The following supplementary material is available for this article:

Appendix A Rate–yield trade-off in *Pseudomonas fluorescens*.

Appendix B Rate–affinity trade-off.

Appendix C The diffusion approach to modelling mutations.

Appendix D Steady states.

Appendix E Relation to ESS theory.

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