vol. 163, no. 6 the american naturalist june 2004

E-Article

Evolution of Cross-Feeding in Microbial Populations

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Submitted May 14, 2003; Accepted November 21, 2003; Electronically published May 6, 2004

abstract: Although limited by a single resource, microbial populations that grow for long periods in continuous culture (chemostat) frequently evolve stable polymorphisms. These polymorphisms may be maintained by cross-feeding, where one strain partially degrades the primary energy resource and excretes an intermediate that is used as an energy resource by a second strain. It is unclear what selective advantage cross-feeding strains have over a single competitor that completely degrades the primary resource. Here we show that cross-feeding may evolve in microbial populations as a consequence of the following optimization principles: the rate of ATP production is maximized, the concentration of enzymes of the pathway is minimized, and the concentration of intermediates of the pathway is minimized.

Keywords: cross-feeding, experimental evolution, chemostat, energy metabolism.

A number of long-term evolution studies on *Escherichia coli* in continuous culture (chemostat) demonstrate that stable polymorphism may evolve in microbial populations that are limited by a single energy resource (Helling et al. 1987; Rosenzweig et al. 1994; Turner et al. 1996; Treves et al. 1998; Rozen and Lenski 2000). Insofar as a chemostat represents a homogenous environment, these findings appear to contrast with the competitive exclusion principle that predicts, on the basis of experimental observations and mathematical models, that a homogeneous environment with a single limiting resource can support only a single competitor (Gause 1934; Hardin 1960). While there are mechanisms such as product inhibition that may lead to stable polymorphisms in microbial populations limited by a single resource (Fredrickson and Stephanopoulos 1981; Lenski and Hattingh 1986), observations that are not in line with the competitive exclusion principle deserve attention because mechanisms leading to stable coexistence of different strains in a simple environment may provide a better understanding of the processes that maintain diversity in microbial communities. In particular, the emergence of stable polymorphisms from a single strain as observed in the above long-term evolution studies may offer new insight into the evolutionary forces driving speciation in microbial populations.

Analysis of the population structure that emerged in the long-term evolution studies described above shows that coexistence of different strains can be maintained by cross-feeding: one strain shows superior growth on the limiting primary resource (glucose) but degrades it only partially and excretes product (e.g., acetate) that is used as secondary substrate by another strain (Helling et al. 1987; Rosenzweig et al. 1994; Turner et al. 1996; Treves et al. 1998; Rozen and Lenski 2000). Cross-feeding between unrelated species (i.e., syntrophy) plays an important role in a number of ecosystems, for example, in methanogenic environments (Stams 1994; Lengeler et al. 1999) and in the degradation of xenobiotic compounds (Dejonghe et al. 2003). In a cross-feeding interaction, the fate of one species may depend on the presence of the other. While the second species suffers starvation if the first is absent, the first species may require the second because it removes its waste product. These observations raise the question of what is the advantage of such an association between two partial resource degraders. Why doesn't a single competitor that completely degrades the primary resource perform better?

A model for the evolution of cross-feeding was recently proposed by Porcher et al. (2001). In our view, the evolution of cross-feeding in this model is a direct consequence of the underlying assumptions. The authors first assume that mortality increases exponentially with the catalytic activities of the enzymes involved in resource degradation and, second, that the catalytic activities can have only four discrete values ranging over four orders

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Am. Nat. 2004. Vol. 163, pp. E126–E135.

of magnitude. Apart from the lack of biological realism, the problem of these assumptions is that for the parameter values used in the model, they allow an organism to have a maximum of three highly efficient enzymes, as the authors point out. However, since, in their pathway scheme, complete degradation requires at least four enzymes, complete degradation of the primary resource with maximally efficient enzymes is impossible by a single competitor. Thus partial resource degradation emerges in the population as a direct consequence of the assumptions and in turn strongly facilitates the evolution of cross-feeding. In a more recent study, Doebeli analyzed the evolution of cross-feeding in the framework of adaptive dynamics and sympatric speciation (Doebeli 2002). In this study, the author demonstrates that cross-feeding may emerge gradually in a microbial population if there is a trade-of between the uptake efficiencies of the primary and the secondary resource and if the trade-off function has positive curvature. However, the underlying biological mechanisms for these assumptions have not been specified in this study.

Hypothesis

Here we hypothesize that the evolution and maintenance of cross-feeding interactions may result from the following three assumptions regarding the evolution of energy metabolic pathways: the rate of ATP production is maximized, the total concentration of enzymes of the pathway is minimized, and the total concentration of intermediates is minimized. The first assumption is based on the fact that many essential cellular processes (e.g., the synthesis of amino acids, proteins, and DNA) require energetic supply that is provided by ATP. It has been observed that if microbes are limited by their energetic resource, the amount of biomass formed per unit of ATP is approximately constant and independent of the mode of ATP production (Bauchop and Elsden 1960). This implies that with increasing rate of ATP production, the rate of biomass formation and thus the growth rate of an organism increases. More direct support comes from a study by Dykhuizen and Dean (1990), which showed that in an *E. coli* population in a chemostat with lactose as limiting energy resource, fitness of an organism strongly correlates to the rate of the lactose pathway. The concentration of enzymes of a metabolic pathway should be minimized because enzyme synthesis involves costs in terms of ATP, carbon, and other compounds and because the capacity of protein synthesis may be limited (Heinrich and Schuster 1996). Evidence for evolutionary forces reducing the costs of enzyme production comes from molecular evolution studies demonstrating that a negative correlation exists between biosynthetic costs of amino acids and their frequency of usage (Mazel and Marliere 1989; Baudouin-Cornu et al. 2001; Seligmann 2003). Costs and limitations for the concentrations of the intermediates arise from osmotic constraints and the limited solvent capacity of a cell and from toxic effects of intermediates. Furthermore, high intermediate concentrations may lead to increased loss through leaks (Ovádi 1991; Mendes et al. 1992; Heinrich and Schuster 1996). The negative effects of intermediates may differ. Indeed, for a number of intermediates a nonzero concentration may be optimal for the organism. For our argument, however, it is sufficient that some of the intermediates involved in ATP production have a negative impact on fitness.

Support for the hypothesis that the above optimization principles may drive the evolution of cross-feeding in microbial populations is based on theoretical studies on the optimal enzyme concentrations in simplified models of metabolic pathways (Heinrich and Schuster 1996). In these studies, the authors maximized the rate of a linear pathway with m intermediates and $m + 1$ enzymes (see fig. 1*A*) with linear irreversible kinetics under restrictions for the total concentration of enzymes ($\Sigma_{(i=1,\ldots,m+1)} E_i \leq E_{\max}$) and of intermediates ($\Sigma_{(i=1,\ldots,m)} X_i \leq X_{\max}$). Assuming that all enzymes have the same rate constant, the optimal concentration is $E_1 = E_{\max} X_{\max}/(X_{\max} + Sm^2)$ for the first enzyme of the pathway, and $E_i = SE_{\max} m/(X_{\max} + Sm^2)$ for the subsequent enzymes ($i = 2, \ldots, m + 1$), where S is the concentration of the substrate. An intuition for these

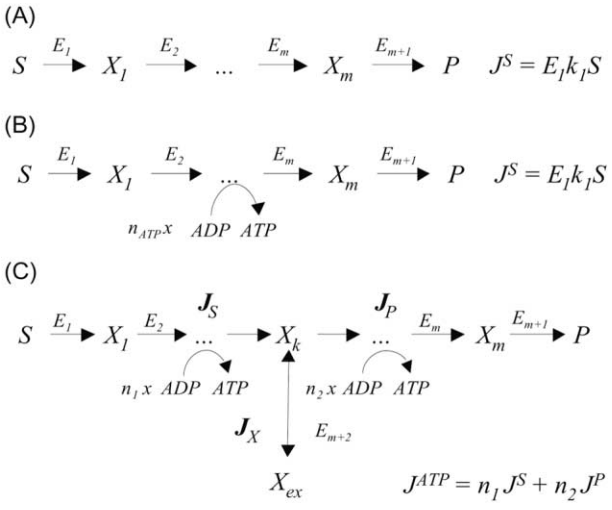

Figure 1: *A*, Unbranched biochemical pathway with m intermediates and $m + 1$ enzymes. The rate of the pathway is denoted by J^S. *B*, Unbranched ATP-producing pathway with n_{ATP} ATP-producing steps. The rate of ATP production is given by $J^{ATP} = n_{ATP} J^S$. *C*, Extended pathway scheme. An intermediate, X_k, can be excreted or imported by a reversible transporter, E_{m+2}. The rate of ATP production is given by $J^{ATP} = n_1 J^S + n_2 J^P$.

results can be obtained as follows: In an irreversible pathway, the first enzyme has complete control over the rate of the pathway. The subsequent enzymes have no impact on the rate but reduce the concentration of their substrate. This explains why these enzymes have the same optimal concentration and why it differs from the optimal concentration of the first enzyme. The quadratic dependence on the length of the pathway is obtained because by adding a new step to the pathway, a further intermediate and a further enzyme are added. When a new intermediate is added, all other intermediates have to be decreased appropriately, and thus all enzymes except for the first have to be increased. The first enzyme has to be reduced appropriately. This results in the m^2 dependence of the denominator in the expression for the optimal concentration of the first enzyme.

For an energy metabolic pathway with a number of n_{ATP} ATP-producing steps (see fig. 1*B*), the rate of ATP production of an optimized pathway is given by

$$J^{\mathrm{ATP}} = n_{\mathrm{ATP}} J_{\mathrm{S}} = \frac{n_{\mathrm{ATP}} E_{\max} X_{\max} k_1 S}{X_{\max} + S^2 m}. \quad (1)$$

It is reasonable to assume that the further a substrate is degraded, the more ATP can be produced. Thus the number of ATP-producing steps, n_{ATP}, depends on the length of the pathway, m. Equation (1) implies that an optimal pathway length, m_{opt}, exists for the ATP-producing pathway (under the biologically plausible assumption that the relation between pathway length and number of ATP-producing steps is less than quadratic). If, for example, the relation between n_{ATP} and m is linear, the optimal pathway length is given by $m_{\mathrm{opt}} = (X_{\max}/S)^{1/2}$, provided that the pathway can be broken off at any point and the corresponding intermediate is excreted without further costs. The existence of an optimal length of an energy metabolic pathway implies that it may be advantageous to degrade a substrate only partially. An optimal pathway length does not exist if there is no restriction for the total concentration of intermediates, because with $X_{\max}$ increasing to infinity, the pathway should be as long as possible. Thus all three optimization principles given above are essential for partial resource degradation being of advantage.

The above result predicts that short pathways should be preferred at high substrate concentrations, while long pathways should be preferred at low resource concentrations. This is in line with biochemical observations. Yeast (*Saccharomyces cerevisiae*), for example, uses fermentation at high resource concentrations and excretes partially degraded compounds such as ethanol, while at low resource concentrations, the substrate is completely degraded by respiration (Fiechter and Gmunder 1989; van Dijken et al. 1993). Note that the excretion of partially degraded compounds maximizes the rate of ATP production at the cost of a lower ATP yield (units of ATP per unit of resource). Thus there is a trade-off between rate and yield of ATP production. Such trade-offs could be of importance for the evolution of cooperation in microbial populations (Pfeiffer et al. 2001) and might have been a driving force in the evolution of multicellularity in heterotrophs (Pfeiffer and Bonhoeffer 2003).

The above line of reasoning suggests that a strain that partially degrades the substrate may obtain a higher growth rate than a strain that completely degrades the substrate. With partial degradation being advantageous, an essential condition for the evolution of cross-feeding is fulfilled. To specify conditions under which cross-feeding indeed evolves in a microbial population, further assumptions about the dynamics of the microbial population have to be specified. In the following, we develop a population dynamical model for the evolution of cross-feeding in continuous culture. First, we extend the pathway scheme given in figure 1*B* to allow secretion or uptake of an intermediate and specify the kinetic properties of the enzymatic reactions. Second, we specify the relation between the growth rate of a strain, its ATP production rate, and the costs for enzymes and intermediates. Third, we give a description of the dynamics of the chemostat culture using differential equations.

Methods

Extended Pathway Scheme and Enzyme Kinetics

Let us assume an ATP-producing pathway with $m + 1$ enzymes with linear irreversible kinetics. The reaction rates of the enzymes are given by $v_1 = E_1 k_1 S$, and $v_i = E_i k_i X_{i-1}$, for $i = 2, \ldots, m + 1$, where E_i is the concentration, k_i is the rate constant, and X_{i-1} is the concentration of the substrate of enzyme i. To allow for partial degradation of the substrate S, we extend the pathway scheme by assuming that one of the intermediates, X_{k}, can be transported across the cell membrane by a transporter with reversible linear enzyme kinetics. The extended pathway scheme is shown in figure 1*C*. The rate of the transporter is $v_{m+2} = E_{m+2} k_{m+2} (X_{\mathrm{k}} - X_{\mathrm{ex}})$, where E_{m+2} is the concentration of the transporter, k_{m+2} is the rate constant, X_{k} is the concentration of the intermediate inside the cell, and X_{ex} is the concentration of the intermediate outside the cell. If present, the transporter either excretes the intermediate (if $X_{\mathrm{ex}} < X_{\mathrm{k}}$) or imports it (if $X_{\mathrm{ex}} > X_{\mathrm{k}}$). Thus the transporter allows either degradation of the primary resource, S, and the excretion of X_{k} or uptake of X_{k} and degradation into the final product. The pathway scheme (fig. 1*C*) represents a minimal model to study the evolution of cross-feeding. Clearly, metabolic pathways in reality are

much more complex, and excretion and uptake routes may be different. However, by including a single reversible enzyme for the import or excretion of intermediate X_k, we use a conservative approach to study cross-feeding because both forms of partial degradation have to carry the costs for the expression of the transporter E_{m+2} and for the presence of the intermediate X_k inside the cell. In the pathway scheme (fig. 1*C*), the uptake rate of the substrate is denoted by J^S, the uptake or excretion rate of the intermediate is denoted by J^X, and the production rate of the final product P is denoted by J^P. If there are n_1 ATP-producing steps in the first part of the pathway and n_2 ATP-producing steps in the second part, the ATP production rate is given by $J^{ATP} = n_1 J^S + n_2 J^P$.

Growth Rate

In the pathway scheme (fig. 1*C*), there are $m + 2$ enzymes involved in ATP production. The concentrations of these enzymes determine the rate of ATP production and the concentrations of the intermediates in the pathway. We assume that ATP production rate, enzyme concentrations, and intermediate concentrations determine the growth rate of a strain. In contrast to the approach of Heinrich and Schuster (1996), we do not use restrictions for the total concentrations of enzymes and intermediates but assume that the intermediate concentrations and the enzyme concentrations have a negative impact on the growth rate. A simple description for the growth rate, W, that fulfills these conditions is given by

$$W = f(J^{ATP}) - \sum_{(i=1,\ldots,m+2)} (A_i E_i) - \sum_{(i=1,\ldots,m)} (B_i X_i). \quad (2)$$

The coefficients A_i and B_i describe the costs of enzyme E_i, and of intermediate X_i, respectively. The function $f(J^{ATP})$ is assumed to be saturating because with an increasing rate of ATP production, the growth rate may be increasingly limited by other factors. In the following, we use $f(J^{ATP}) = J^{ATP}/(1 + J^{ATP})$.

Chemostat Dynamics

Let N_i denote the population size of strain i, S the concentration of the substrate, and X_{ex} the concentration of the intermediate. Then the population dynamics in a chemostat with dilution rate D and a resource concentration of S_0 in the reservoir medium can be described by the following set of ordinary differential equations (Young et al. 1970):

$$\frac{dS}{dt} = D(S^0 - S) - \sum_{(i=1,\ldots,n)} (N_i J_i^S) \quad (3a)$$

$$\frac{dX_{ex}}{dt} = \sum_{(i=1,\ldots,n)} (N_i J_i^X) - DX_{ex} \quad (3b)$$

$$\frac{dN_i}{dt} = (W_i - D)N_i. \quad (3c)$$

Note that J_i^X can be positive or negative because a strain may import or excrete the intermediate. The resource uptake rate J_i^S is always nonnegative because the pathway is assumed to be irreversible.

Evolution of Enzyme Expression

In a steady state of the system (i.e., if $dS/dt = 0$, $dX_{ex}/dt = 0$, and $dN_i/dt = 0$ for all strains i) all strains have the same growth rate, which equals the dilution rate of the chemostat. A mutant can invade if its growth rate is larger than the dilution rate. After invasion the chemostat settles into a new steady state. After repeated invasion and competition, the population may evolve into a state where the resident strains generate an environment (characterized by S and X_{ex}) in which no further strain with different enzyme expression has a growth rate equal to or higher than the dilution rate. When the population is in this state, no strain can invade the population, and thus in the framework of our model, no further evolution can take place. To find such evolutionary endpoints in our model, we simulate competition between strains that differ in the expression of the enzymes $E_1, \ldots, E_{m+2}$. To reduce the space of enzyme expression from $m + 2$ to three dimensions we use analytically derived criteria for the optimal concentrations of the enzymes $E_2, \ldots, E_{k-1}$ and $E_{k+1}, \ldots, E_{m+1}$ (see appendix). Thus strains with optimal expression in the enzymes $E_2, \ldots, E_{k-1}$ and $E_{k+1}, \ldots, E_{m+1}$ can be characterized by only three properties: the expression of the first enzymes of the pathway, E_1; the expression of the first enzyme of the second branch of the pathway, E_k; and the expression of the transporter, E_{m+2}.

To study the evolution in our system, we use the following method. We start with the strain with highest growth rate under initial conditions of the chemostat ($S = S^0$, and $X_{ex} = 0$) and numerically compute the steady state of population size, resource, and intermediate. Then we determine the strain with highest growth under this condition, allow it to invade, and compute the new steady state. We repeat this procedure until no further strain can invade into the population and the evolutionary endpoint is reached. (Extensive simulation of evolution of enzyme expression with different procedures suggests that there is only a single evolutionary endpoint.) The system

is simulated for different dilution rates and different costs of the intermediates. For simplicity, we described the cost coefficients of all intermediates by a single parameter, $B = B_1 = B_2, \ldots = B_m$. The parameter values used in the simulations are given in table 1. An example run is shown in figure 2. In all simulations, invasion of a new strain always results in a stable steady state, and for all parameter values, we always obtain a state in which no further strain can invade into the population.

Results

At the evolutionary endpoint, the population consists of strains with minimal expression in one of the enzymes E_1, E_k, or E_{m+2}. (The minimal allowed expression of an enzyme in our simulations was set to 0.001.) A strain with minimal expression of enzyme E_{m+2} degrades the substrate completely ($J_X \approx 0$), a strain with minimal expression of enzyme E_k degrades it partially to X_{ex} ($J_P \approx 0$), and a strain with minimal expression of enzyme E_1 degrades X_{ex} into the final product ($J_S \approx 0$). Strains with nonminimal expression in all three enzymes were never observed to evolve. At the evolutionary endpoint, the population consists of either a single strain or two coexisting strains. Depending on dilution rate and cost for intermediates, four different types of evolutionary outcome can be observed (fig. 3). In the first type, the primary substrate is degraded completely by a single strain. Complete degradation by a single strain is obtained at low dilution rates and low costs for metabolites. In the second state, strains with complete degradation and partial degradation of the primary substrate coexist. Because of the presence of a partial resource degrader, the intermediate X_{ex} is present in the chemostat. However, it is not used as substrate. Note that the partial degrader is inhibited by its own product X_{ex} because it leads to an increased level of X_k inside of the partial degrader. This self-inhibition leads to the stable coexistence of partial and complete degradation. With increasing dilution rate and increasing costs for intermediates, we obtain a third type of population structure in the chemostat: one strain degrades the primary substrate partially into X_{ex}, and another strain degrades X_{ex} into the final product P. This state represents a cross-feeding interaction. Coexistence of the two strains is stable because the primary degrader is inhibited by its product but facilitates growth of the secondary degrader. If the dilution rate or the cost for intermediate concentration further increases, a fourth state is obtained in which the primary substrate is degraded partially by a single strain. Here, the

Table 1: List of variables and parameter values used in the simulation

Variable	Meaning	Value
m	Pathway length (number of intermediates)	3
n_{ATP}	Number of ATP-producing steps of a pathway	2
n_1	Number of ATP-producing steps in the first part of a pathway	1
n_2	Number of ATP-producing steps in the second part of a pathway	1
k	Branching point (intermediate X_k can be excreted)	2
$k_1, \ldots, k_{m+1}$	Rate constants of the irreversible enzymes	1.0
k_{m+2}	Rate constant of the reversible transporter	10.0
$A_1, \ldots, A_{m+1}$	Cost coefficients of the irreversible enzymes	.0025
A_{m+2}	Cost coefficient of the reversible transporter	.0025
$B_1, \ldots, B_m$	Cost coefficients of the intermediates	.2, …, 4.0
D	Chemostat dilution rate	.02, …, .4
S_0	Resource concentration in the chemostat reservoir medium	1.0
$E_1, \ldots, E_{m+1}$	Concentrations of the irreversible enzymes	…
E_{m+2}	Concentration of the reversible transporter	…
$X_1, \ldots, X_m$	Concentrations of the intermediate inside the cell	…
S	Resource concentration in the chemostat	…
X_{ex}	Concentration of intermediate X_k in the chemostat	…
v_i	Rate of reaction i	…
J^S	Rate of resource uptake	…
J^X	Transport rate of intermediate X_k	…
J^P	Production rate of the final product	…
J^{ATP}	Rate of ATP production	…
W	Growth rate (eq. [2])	…
E_{max}	Maximal enzyme concentration of the pathway (eq. [1])	…
X_{max}	Maximal intermediate concentration of the pathway (eq. [1])	…
m_{opt}	Optimal pathway length (see eq. [1] and the following text)	…

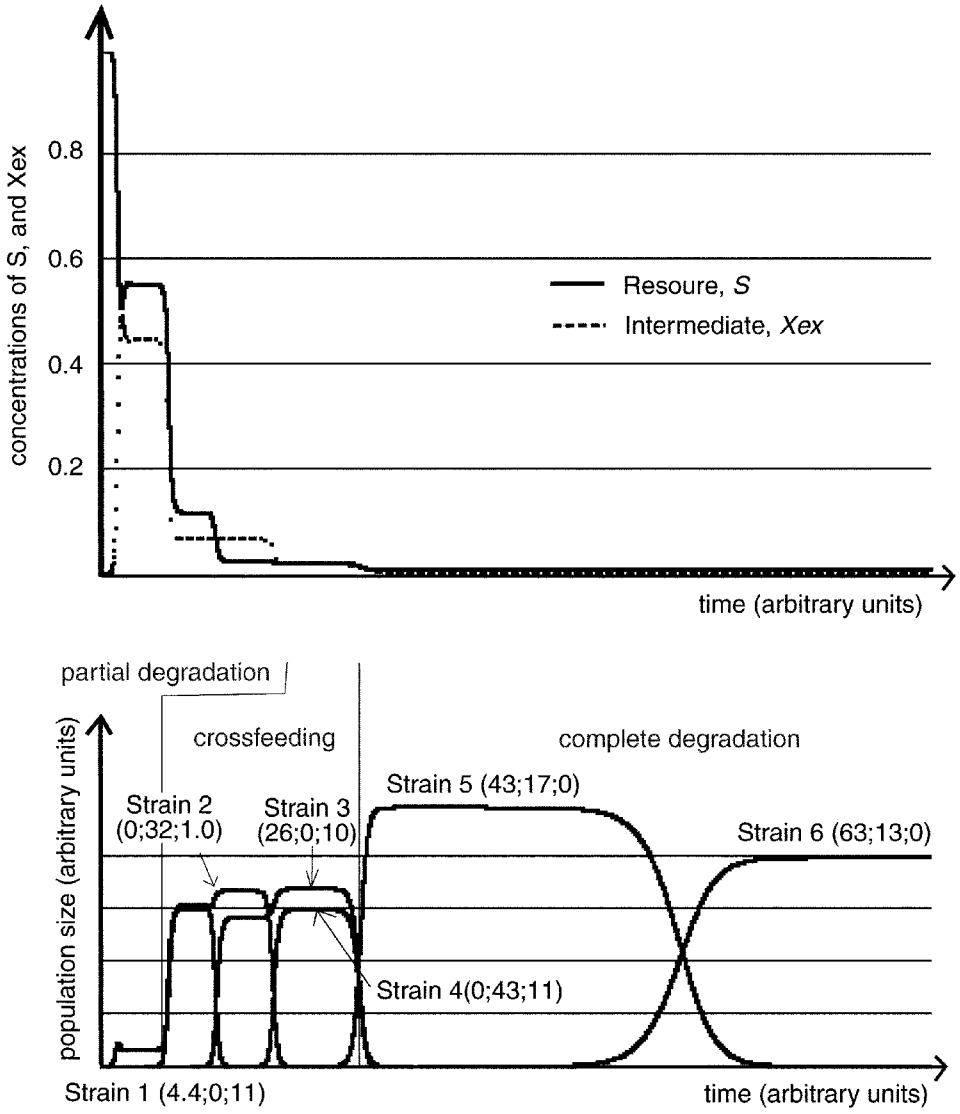

Figure 2: Time course of a simulation. The simulation is initiated with $S = S_0$ and $X_{ex} = 0$. At this condition, strain 1 has the highest growth rate. The expression of enzymes E_i, E_k, and E_{m+2} of the strains are given in parentheses. An enzyme expression of 0 stands for the minimal expression of 0.001 allowed in our simulations. Solid lines represent partial degraders of the primary resource. Dashed lines represent degraders of the intermediate. Strain 1 has minimal expression of enzyme E_k and thus degrades the primary resource partially into X_{ex}. The X_{ex} accumulates in the chemostat and allows a second strain (strain 2) to invade, which uses X_{ex} as energy resource. Both strains coexist in a cross-feeding interaction. In the following, strains that partially degrade the primary resource are replaced by other partial degraders of the primary resource, and strains that degrade the intermediate are replaced by other degraders of the intermediate. The cross-feeding interaction stays intact during the replacements. Eventually the population evolves into a state where no further replacements are possible. The parameter values of the simulation are $B = 3.0$ and $D = 0.08$.

secondary degrader cannot evolve because its resource, intermediate X_{ex}, is diluted too fast.

In summary, the simulations show that, depending on the dilution rate of the chemostat, cross-feeding consistently evolves for a large range of intermediate costs. Additional simulations with different parameter sets show generally the same patterns as the simulations described above. However, high costs and a low rate constant of the transporter (A_{m+2} and k_{m+2}, respectively) increasingly impair the evolution of cross-feeding. On the other hand, the evolution of cross-feeding is facilitated by increasing length of the ATP-producing pathway. Our simulation results are in agreement with the prediction that partial resource degradation is advantageous if the resource concentration is high or if the concentration of intermediates is restricted to a low value. In our simulations, partial

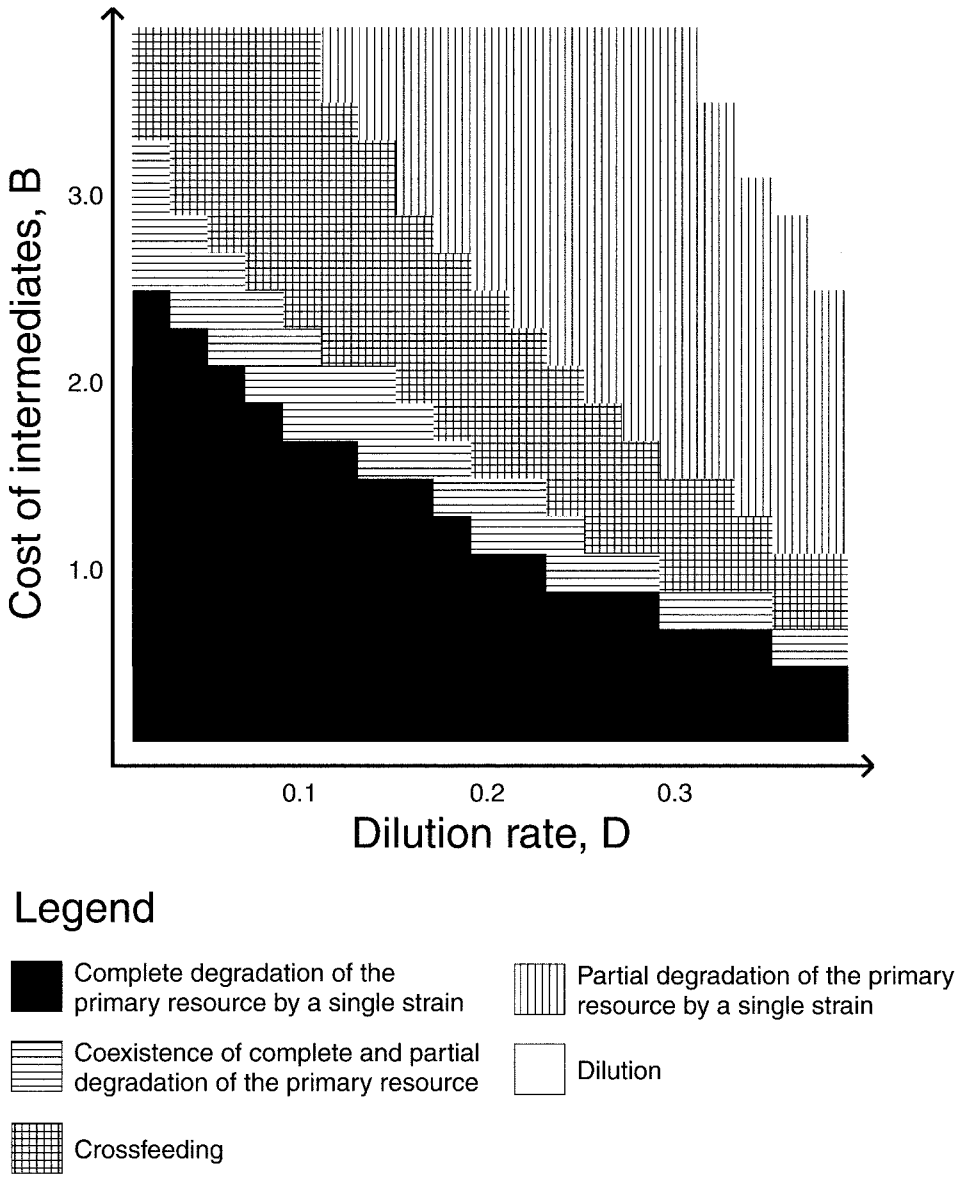

Figure 3: Final population structure for different dilution rates, *D*, and different costs of the intermediates, *B*. At low intermediate costs and low dilution rates, the resource is completely degraded by a single strain. With increasing intermediate costs and increasing dilution rate, two strains coexist. One of the strains completely degrades the primary resource; the other strain partially degrades the primary resource. If intermediate costs and dilution rate are increased further, two strains coexist in a cross-feeding interaction. At high intermediate costs and high dilution rate, the population consists of a single strain that partially degrades the primary resource. At very high intermediate costs and dilution rates, there is no strain that can establish a population in the chemostat.

resource degradation is observed at high costs for intermediates and at high dilution rates where the steady state resource concentration in the chemostat is generally higher. The coexistence of two partial degraders results in a cross-feeding interaction. Note that our model is limited to the coexistence of only two partial degraders because there is only one intermediate that can be excreted. If further intermediates can be excreted, we expect that cross-feeding between more than two species can evolve, in particular when dilution rates are high. The emergence of such cross-feeding interactions with two intermediates (acetate and glycerol) and three coexisting strains has been observed in experiments (Rosenzweig et al. 1994).

In our model, the evolution of strains with different

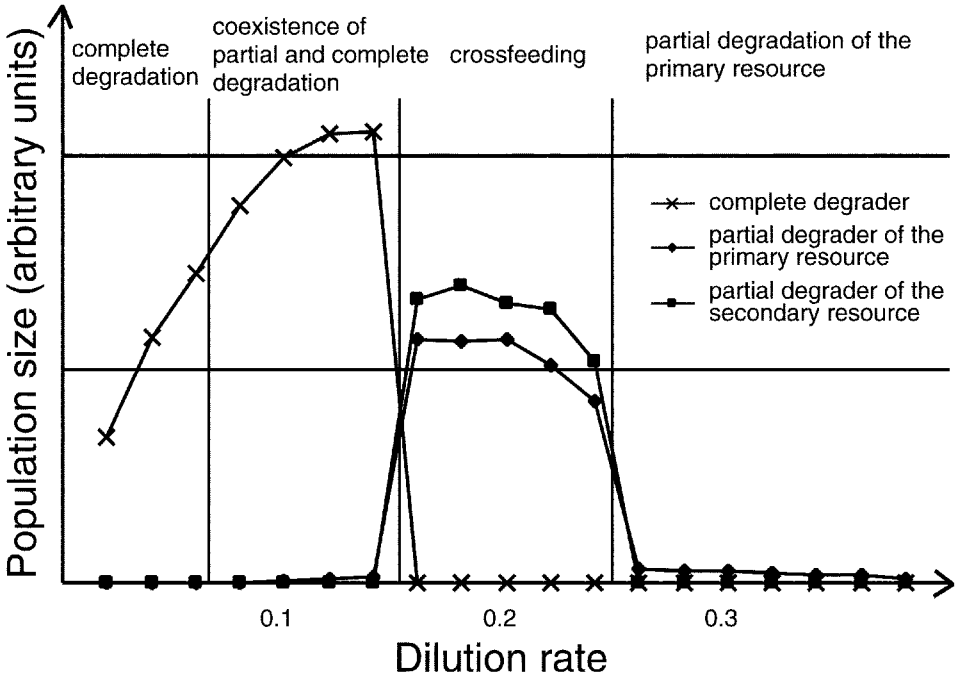

Figure 4: Composition of the final population for different dilution rate. Note that small changes in dilution rate usually have only little impact on the population structure. However, there are striking changes in the population structure if the borders of the parameter domain of cross-feeding are crossed (i.e., between $D = 0.14$–0.16 and $D = 0.24$–0.26). The intermediate costs are $B = 2.0$.

metabolic properties is based on the evolution of enzyme expression patterns. Differences in expression patterns, however, may result not only from differences in the genetic information of an organism but also from adaptation by an organism to its environment by phenotypic plasticity. Thus, based on our assumptions, we cannot distinguish whether the evolution of organisms with different metabolic properties is due to genetic differentiation or due to the ability to phenotypically switch to the optimal enzyme expression pattern. Both processes may be involved in the adaptation of microbial populations in the long-term evolution experiments described above. Considering, however, that the conditions in the chemostat are relatively constant and also that phenotypic plasticity and switching may be associated with fitness costs, we believe that it is more likely that the stable coexistence of organisms with different metabolic properties as observed for cross-feeding is due to speciation into genetically different subpopulations. Fixation of adaptive mutations has been observed in long-term evolution experiments (Helling et al. 1987).

Discussion

Our simulations show that maximizing the rate of an ATP-producing pathway while minimizing the total concentrations of enzymes and intermediates may lead to the evolution of cross-feeding in microbial populations in continuous culture (chemostat). Thus optimizing properties of ATP-producing pathways may provide a mechanism of speciation in microbial populations. Although we use a number of simplifying assumptions in our simulations, it allows us to derive testable predictions. If rate of ATP production, intermediate concentrations, and enzyme concentrations are the most important fitness-relevant properties of ATP-producing pathways, our results predict the following: First, partial degradation of energy resources generally should be used at high resource levels, and complete degradation should be used at low resource concentrations. This is in line with biochemical observations, as, for example, on yeasts (Fiechter and Gmunder 1989; van Dijken et al. 1993). However, there may be alternative explanations for these observations. To test our hypothesis, further experimental data such as intermediate concentrations and enzyme expression profiles under different environmental conditions are required. Furthermore, partial degradation is expected if high intermediate concentrations are associated with high fitness costs. This is in line with the frequent observation of cross-feeding for the degradation of xenobiotic compounds, where intermediates often are

toxic, and thus high costs associated with intermediates can be expected (Dejonghe et al. 2003). Second, in long-term evolution studies on microbial populations in chemostats, we expect, at low dilution rate, the emergence of a single strain that completely degrades the primary resource. With increasing dilution rates we expect coexistence of complete and partial degradation and coexistence of two partial degraders (cross-feeding). Coexistence of complete and partial degraders may, however, be difficult to detect because the partial degrader is expected to be present at low frequencies. At very high dilution rate, we expect a single strain that partially degrades the primary resource. To our knowledge, the long-term evolution studies described above used the same dilution rate (0.2 h^{-1}), and thus our prediction cannot be tested on the basis of these experiments. Third, in contrast to the theoretical study by Doebeli (2002), cross-feeding emerges in our simulations in two steps. First, a partial degrader invades into a population of complete degraders. The product of the partial degrader accumulates in the environment and allows invasion of a second strain that feeds on the product of the first strain. Gradual emergence of the two partial degraders from a complete degrader is not possible in our model (simulations not shown). Experimental observations support our model because for the evolution of cross-feeding, a few adaptive mutations seem to be sufficient (Helling et al. 1987). Finally, our model predicts threshold behavior for the stability of cross-feeding interactions (fig. 4). Small changes in a parameter such as dilution rate may usually have only little impact on the composition of a population. However, if the population is close to the border of the parameter domain of cross-feeding, small changes may be sufficient to trigger far-reaching changes in population composition. Such threshold behavior may have important implications for the stability of ecosystems with syntrophic species. For example, syntrophic cross-feeding species may be replaced by a single species if threshold levels in the resource influx rate are crossed. However, to determine such threshold values for a population, the costs and benefits associated with metabolic pathways would have to be known.

APPENDIX

Optimal Enzyme Expression

We assume that the intermediate concentrations of the pathway are in steady state. The steady state concentrations are given by $X_i = J^S/E_{i+1}k_{i+1}$, for $i = 1, \ldots, k-1$; $X_k = (J_1 + E_{m+2}k_{m+2}X_{ex})/(E_{k+1}k_{k+1} + E_{m+2}k_{m+2})$; and $X_i = J^S/E_{i+1}k_{i+1}$, for $i = k+1, \ldots, m$. The steady rates J^S, J^X, J^P, and J^{ATP} depend only on the concentrations of the enzymes E_1, E_k, and E_{m+2} and are given by $J^S = E_1k_1S$, $J^P = E_kk_kX_k$, $J^X = E_{m+2}k_{m+2}(X_k - X_{ex})$, and $J^{ATP} = n_1J^S + n_2J^P$. The optimal expression of an enzyme can be derived from

$$\begin{aligned}\partial W/\partial E_i &= (\partial f(J^{ATP})/\partial J^{ATP})(\partial J^{ATP}/\partial E_i) \\ &\quad - A_i - \sum_{(l=1,\ldots,m)} (B_l \partial X_l/E_l) \\ &= 0 \end{aligned} \tag{A1}$$

The enzymes $E_{2,\ldots,}E_{k-1}$ and $E_{k+1}, \ldots, E_{m+1}$ have no impact on the rate of ATP production ($\partial J^{ATP}/\partial E_i = 0$). Thus, equation (A1) yields optimal concentrations given by

$$E_i = \sqrt{\frac{J^S}{k_iA_i}}, \text{ for the enzymes } E_2, \ldots, E_{k-1}, \text{ and} \tag{A2}$$

$$E_i = \sqrt{\frac{J^P}{k_iA_i}}, \text{ for the enzymes } E_{k+1}, \ldots, E_{m+1}. \tag{A3}$$

Equations (A2) and (A3) are used to determine the optimal concentration of the enzymes $E_2, \ldots, E_{k-1}$ and $E_{k+1}, \ldots, E_{m+1}$ in the simulations.

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Associate Editor: Peter J. Morin