

## **Evolutionary Consequences of Tradeoffs between Yield and Rate of ATP Production**

By Thomas Pfeiffer and Sebastian Bonhoeffer\*

Ecology & Evolution, ETH Zürich, ETH Zentrum, NW, CH-8092 Zürich, Switzerland

*Dedicated to Prof. Dr. Peter Schuster  
on the occasion of his 60th birthday*

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### ***Energy Metabolism / Evolution / ATP Production / Evolutionary Game Theory***

Adenosine triphosphate (ATP) is a key compound in the energy metabolism of cells and is required to drive vital biochemical reactions. In heterotrophic organisms ATP production is coupled to the degradation of energy-rich organic material taken up from the environment. In the transfer of the environmental energy to cellular processes heterotrophs face a tradeoff, since the conversion of the environmental energy into ATP cannot be both maximally fast and efficient. Here we show how tradeoffs between rate and yield of ATP production arise firstly from thermodynamical principles, and secondly for the ATP production by respiration and fermentation. Using methods derived from game theory and population dynamics we investigate the evolutionary consequences for both tradeoffs. We show that spatially structured environments enable the evolution of efficient pathways with high yield. The strategies of ATP production realized in a population, however, depend on the quantitative properties of the tradeoffs.

### **1. Introduction**

Many biochemical processes in living organisms require supply of energy. In particular the synthesis of biomass for growth and reproduction requires large amounts of energy. The energy to drive these biochemical reactions is generally obtained from environmental resources such as sunlight or energy-rich organic material. Inside a cell the energy obtained from the environment is transiently converted into ATP, which is in turn used to balance energetically unfavourable reactions, such as those involved in transport processes, cellular motility, and synthesis of biomass. ATP is used for the transfer, but not the storage of en-

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\* Corresponding author. E-mail: bonhoeffer@eco.umw.ethz.ch

ergy. It is generally present only in small amounts and needs to be continuously regenerated according to the energetic requirements of the cell.

Depending on the ecological properties of the habitat, the availability of energy resources can be an important limiting factor. In this case, growth of an organism is mainly determined by the production of ATP [1, 11]. Hence, the specific properties of ATP production, such as rate (unit of ATP per unit of time) and yield (unit of ATP per unit of resource), likely have a strong impact on the fitness of an organism.

## 2. Quantitative formulation of the tradeoffs between rate and yield of ATP production

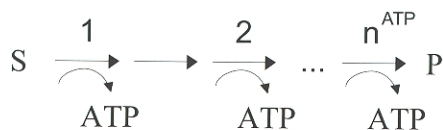
### 2.1 Thermodynamic tradeoff

In heterotrophic organisms ATP production is coupled to the degradation of a substrate S into a product P with a lower free energy (Fig. 1). The net free energy difference for an ATP-producing pathway is given by

$$\Delta G^{\text{NET}} = \Delta G^{S \rightarrow P} - n^{\text{ATP}} \Delta G^{\text{ATP}} \quad (1)$$

where  $\Delta G^{S \rightarrow P}$  is the free energy difference between substrate S and product P,  $n^{\text{ATP}}$  is the yield of ATP production, and  $\Delta G^{\text{ATP}}$  is the amount of energy conserved per mol of ATP. The maximal ATP yield,  $n^{\text{MAX}}$ , is obtained if the entire free energy difference between substrate and product is conserved as ATP. In this case the pathway is in thermodynamic equilibrium (*i.e.*  $\Delta G^{\text{NET}} = 0$ ). Thus the maximal yield is given by  $n^{\text{MAX}} = \Delta G^{S \rightarrow P} / \Delta G^{\text{ATP}}$ . At thermodynamic equilibrium the rates of substrate degradation,  $J^S$ , and ATP production,  $J^{\text{ATP}} = n^{\text{MAX}} J^S$ , vanish. However, at yields lower than  $n^{\text{MAX}}$ ,  $\Delta G^{\text{NET}}$  is negative and drives the reaction. Consequently both  $J^S$  and  $J^{\text{ATP}}$  become positive. Hence with decreasing yield, the rate of ATP production increases. In other words, there is a tradeoff between rate and yield of ATP production. Importantly, this tradeoff is a direct consequence of fundamental thermodynamic principles and is present in any ATP producing pathway irrespective of its specific properties.

A quantitative relation between rate and yield of ATP production has been derived by Waddell *et al.* [20]. Assuming a linear relation between the rate  $J^S$



**Fig. 1.** Schematic illustration of an ATP-producing pathway. The degradation of a substrate S into a product P is coupled to the production of  $n^{\text{ATP}}$  mol of ATP per mol of substrate. The net free energy difference of the pathway depends on the yield of ATP production (see Eq. (1)).

and the net free energy  $\Delta G^{\text{NET}}$  of an ATP-producing pathway the rates of substrate degradation and ATP production are given by

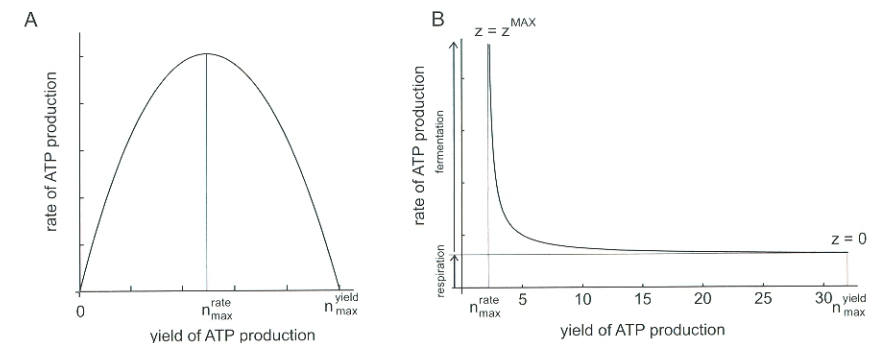
$$\begin{aligned} J^S &= L \Delta G^{\text{NET}} = L (\Delta G^{S \rightarrow P} - n^{\text{ATP}} \Delta G^{\text{ATP}}) \\ &= k_1 (n^{\text{MAX}} - n^{\text{ATP}}) \end{aligned} \quad (2)$$

and

$$\begin{aligned} J^{\text{ATP}} &= n^{\text{ATP}} J^S = L n^{\text{ATP}} (\Delta G^{S \rightarrow P} - n^{\text{ATP}} \Delta G^{\text{ATP}}) \\ &= k_1 n^{\text{ATP}} (n^{\text{MAX}} - n^{\text{ATP}}) \end{aligned} \quad (3)$$

with the constant  $k_1 = L \Delta G^{\text{ATP}}$ . The relation between rate and yield of ATP production is shown in Fig. 2a. The maximal rate of ATP production is obtained at a yield of  $n^{\text{ATP}} = n^{\text{MAX}}/2$ , *i.e.* if half the energy is conserved as ATP and half is used to drive the reaction. A tradeoff between rate and yield is only present at ATP yields higher than  $n^{\text{MAX}}/2$ , since for smaller yields the rate increases with increasing yield.

The above line of reasoning applies in principle to all ATP-producing pathways. However, the assumption of a linear relation between flux ( $J^S$ ) and force ( $\Delta G^{\text{NET}}$ ) is valid only for systems close to thermodynamic equilibrium and is not necessarily compatible with the commonly used mass-action rate law [8]. Clearly the above general thermodynamic description is a simplification, because it does not reflect the mechanistic details of any particular ATP-producing pathway. On the other hand it has been shown that approaches that include an explicit kinetic description of the mechanisms of ATP production lead to similar results [15]. Furthermore, it has been suggested that



**Fig. 2.** Relation between rate and yield of ATP production. (A) For the thermodynamic tradeoff (Eq. (3)) the maximal rate of ATP production is obtained at half maximal ATP yield ( $n^{\text{ATP}} = n^{\text{MAX}}/2$ ). At higher yields, the rate of ATP production decreases resulting in a tradeoff between rate and yield. (B) For the fermentation tradeoff (Eqs. (4) and (5)) the maximal yield of  $n^{\text{ATP}} = 32$  is obtained if respiration is used exclusively ( $z = 0$ ). With increasing rate in fermentation,  $z$ , the total yield of ATP production decreases to a value of about  $n^{\text{ATP}} = 2$  (at  $z = z^{\text{MAX}}$ ).

a linear relation between flux and force can be applied to the ATP production in oxidative phosphorylation [16], which is certainly the most important ATP-producing pathway in eukaryotes. Therefore we argue that the above thermodynamic description allows to derive fundamental conclusions for ATP-producing pathways in general. For the detailed analysis of a specific pathway, mechanistic details would need to be included.

## 2.2 Tradeoff for ATP production from sugars

A tradeoff between rate and yield may also arise for the production of ATP from sugars. In the presence of oxygen, many organisms are in principle capable of using two pathways for ATP production from sugars, namely fermentation and respiration. Both pathways have opposing properties in terms of rate and yield: Fermentation of glucose to lactate or ethanol yields only 2 mol of ATP per mol of glucose. However, the rate of ATP production in fermentation can be high, because the required enzymes can be present in large amounts in the cytosol. In contrast, the complete oxidation of glucose to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  in the respiration pathway results in a much higher yield of about 32 mol of ATP per mol of glucose. However, in respiration, ATP is produced at much lower rates and gets rapidly saturated at high resource levels or limiting oxygen supply [5, 14, 18, 19]. This is probably because in contrast to fermentation respiration involves processes that are confined to membranes, where dense packing of large amounts of enzymes may be more restrictive than in the cytosol. When respiration is saturated, an organism may increase its rate of ATP production by using fermentation in addition to respiration. Again this leads to a tradeoff between yield and rate of ATP production: Due to the low ATP yield of fermentation, increasing the rate of ATP production by using fermentation in addition to respiration occurs at the cost of a lower total yield.

The tradeoff between rate and yield resulting from the additional use of fermentation can be described quantitatively. Assume that the rate in respiration is saturated and constant,  $J^R = k_2$ , and that the rate of fermentation is  $J^F = zJ^R$ , where  $z$  is the relative rate of fermentation. Thus the resulting rates of resource consumption and ATP production are given by:

$$\begin{aligned} J^S &= J^R + J^F = k_2(1+z) \\ J^{\text{ATP}} &= 32J^R + 2J^F = k_2(32+2z) \end{aligned} \quad (4)$$

where  $z$  ranges between 0 and some physiologically maximal value  $z^{\text{MAX}}$ . The yield of ATP production is given by

$$n^{\text{ATP}} = J^{\text{ATP}}/J^S = (1+z)/(32+2z). \quad (5)$$

The resulting relation between rate and yield of ATP production is shown in Fig. 2b. The maximal yield of 32 mol of ATP per mol of substrate is obtained

if respiration is used exclusively ( $z = 0$ ), while the maximal rate is obtained if fermentation is used in addition to respiration at maximal rate ( $z = z^{\text{MAX}}$ ). With increasing rate in fermentation the yield asymptotically becomes 2.

In summary, there are two different tradeoffs between rate and yield of ATP production. Firstly, there is a tradeoff that arises for fundamental thermodynamic reasons, which in principle applies to any ATP-producing pathway (thermodynamic tradeoff). Secondly there is a tradeoff for ATP production from sugars if fermentation is used in addition to respiration (fermentation tradeoff). With appropriate assumptions, both tradeoffs can be described quantitatively. A strategy of ATP production is described by a single continuous parameter,  $n^{\text{ATP}}$  and  $z$ , respectively. Note that the parameter  $z$  is not identical to the ATP yield but describes it implicitly.

## 3. Yield versus rate in ATP production

Provided that energetic limitation plays an important role in the life history of an organism in its natural environment, the presence of a tradeoff between rate and yield leads to the following important question: Under which conditions does evolution favor efficient but slow rather than inefficient but fast ATP production? To illustrate the significance of a specific strategy of ATP production, we consider that an organism exclusively uses a limited amount of resource,  $S$ . We define the payoff,  $P$ , of a strategy as the total amount of ATP produced from a given amount of resource. The payoff is given by  $P(n^{\text{ATP}}) = tJ^{\text{ATP}}(n^{\text{ATP}})$ , where  $t$  is the time taken until the resource is exhausted. This time is given by  $S = tJ^S(n^{\text{ATP}})$ . Combining both equations one obtains for the payoff

$$P(n^{\text{ATP}}) = S \frac{J^{\text{ATP}}(n^{\text{ATP}})}{J^S(n^{\text{ATP}})} = S \cdot n^{\text{ATP}}. \quad (6)$$

Hence the payoff for a given strategy is determined only by its yield but not its rate of ATP production. A high yield results in a high payoff, if the organism has exclusive access to a resource.

However, if there is competition for a shared resource, the payoff for a given strategy  $n^{\text{ATP}}$  depends on the strategies of its competitors:

$$P(n^{\text{ATP}}) = S \frac{J^{\text{ATP}}(n^{\text{ATP}})}{J^S(n^{\text{ATP}}) + J^C} \quad (7)$$

where  $J^C = J^S(n_1^{\text{ATP}}) + J^S(n_2^{\text{ATP}}) + \dots + J^S(n_k^{\text{ATP}})$  denotes the total resource consumption rate of all  $k$  competitors.  $J^C$  can be interpreted as a measure of resource competition. Increasing the ATP yield,  $n^{\text{ATP}}$ , leads to a decrease of both the numerator and the denominator in Eq. (7). Whether the payoff,  $P(n^{\text{ATP}})$ , increases as a result depends on  $J^C$  and the exact relation between rate and yield of ATP production. Generally, for small values of  $J^C$  the payoff gets

large if  $J^S(n^{\text{ATP}})$  is small. In other words, a strategy with a high yield in ATP production is of advantage, if the resource is shared among a small number of competitors or with competitors that use an efficient, but slow strategy of ATP production. (If no competitors are present ( $J^C = 0$ ), Eq. (7) is equivalent to Eq. (6)). However, if  $J^C$  is large, increasing  $n^{\text{ATP}}$  does not significantly reduce the denominator, but more significantly reduces the numerator of Eq. (7). Thus if the total resource consumption of the competitors is high, strategies with higher rates, but lower yields of ATP production obtain a higher payoff.

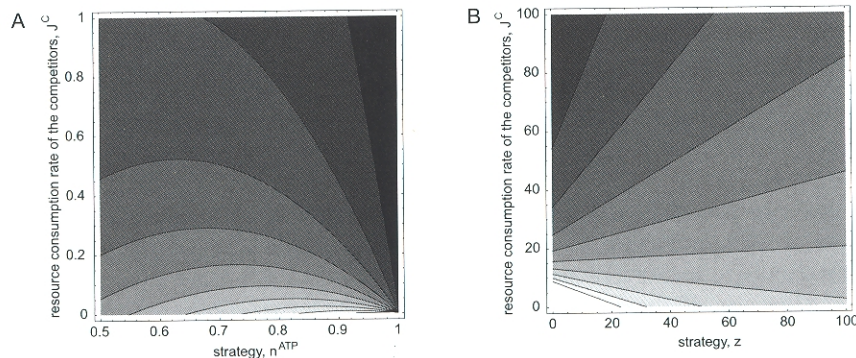
The payoff given in Eq. (7) can be specified by using the quantitative description of the tradeoffs (Eqs. (2)–(5)). We obtain

$$P(n^{\text{ATP}}) = S \frac{k_1 n^{\text{ATP}} (n^{\text{MAX}} - n^{\text{ATP}})}{k_1 (n^{\text{MAX}} - n^{\text{ATP}}) + J^C} \quad (8)$$

for the thermodynamic tradeoff and

$$P(z) = S \frac{k_2 (32 + 2z)}{k_2 (1 + z) + J^C} \quad (9)$$

for the fermentation tradeoff. The relation between the fitness of a strategy and the total resource consumption rate of the competitors,  $J^C$ , is shown in Fig. 3. For both tradeoffs, efficient strategies (characterized by high  $n^{\text{ATP}}$ ) have larger



**Fig. 3.** The payoff of a strategy in dependence of the total rate of resource consumption by the competitors,  $J^C$ . (A) Payoff of a strategy with yield  $n^{\text{ATP}}$  for the thermodynamic tradeoff (Eq. (8));  $S = 1$ ,  $n^{\text{MAX}} = 1$ ,  $k_1 = 1$ ). (B) Payoff of a strategy with relative fermentation rate  $z$  for the fermentation tradeoff (Eq. (9));  $S = 1$ ,  $k_2 = 1$ . Increasing levels of gray indicate decreasing levels of payoff. For small values of  $J^C$ , *i.e.* if there is low resource competition, efficient strategies (characterized by high values of  $n^{\text{ATP}}$  for the thermodynamic tradeoff and low values of  $z$  for the fermentation tradeoff) have higher payoff. For large values of  $J^C$ , *i.e.* at high levels of competition, strategies with low yield but fast ATP production have higher payoff. For the thermodynamic tradeoff the resulting yield is about half of the maximal yield ( $n^{\text{ATP}} \approx 0.5$ ). For the fermentation tradeoff the resulting rate of fermentation is given by  $z = z^{\text{MAX}}$ .

payoff for small values of  $J^C$ , and fast strategies (characterized by low  $n^{\text{ATP}}$ ) have large payoff for high values of  $J^C$ .

Essentially, fast resource consumption is favorable because all users of the resource can exploit it for the same time. An organism individually benefits from a high rate of ATP production, since it produces a higher amount of ATP within this time. However, the disadvantage of the fast and inefficient resource exploitation is shared equally by all users of the resource. Hence evolution favors fast and inefficient ATP production if there is a high level of competition for a shared resource, although the payoff for an individual is much smaller compared to a situation, where all users of a resource use an efficient way of ATP production. Efficient resource use for ATP production can be seen as cooperation between organisms, which is, however, vulnerable to a non-cooperative, inefficient but fast resource use. This is equivalent to Hardin's Tragedy of the Commons [7], a framework widely used in evolutionary game theory to describe the evolution towards an inefficient use of a shared resource.

#### 4. Spatial structure

It has been shown that a cooperative resource use may evolve in a spatially structured environment [4, 12]. We have recently shown that in a reaction-diffusion system organisms using respiration exclusively may outcompete cells that use fermentation additionally to obtain a higher rate of ATP production at the cost of a lower yield [13]. However, the high computational demands of the model restricted a detailed study of the evolutionary dynamics with a high number of different strategies. In the following, we present a model for the evolution of ATP production with a continuous set of strategies as described by Eqs. (2)–(5).

We assume that cells are repeatedly in competition for spatially isolated resources. In each round, the payoff of a cell determines its number of offspring in the next round. For the calculation of the payoffs in the local competition, Eq. (7) is used. Cells are distributed randomly to new resources in each round. The number of cells per resource (referred to as population density) is thus Poisson distributed. Depending on the average population density a fraction of cells may use a local resource exclusively, while others share a resource with competitors. According to the arguments outlined in the previous section, efficient resource use pays off, if a cell uses a resource exclusively or shares it with other cooperative users. On the other hand, if a cell faces intense competition for a shared resource it pays off to use a fast, but inefficient strategy of ATP production. The model contains a feedback between population dynamics and ATP production strategies: On the one hand the population density depends on the strategies used in the population. Efficient resource use results in higher population densities. On the other hand, the population density determines the success of the strategies. At high population densities, competition for shared resources is stronger and fast resource use is favored. Hence, the success of

a strategy depends not only on other strategies present in the population, but also on the population density itself.

To analyze the dynamics of the system described above, we first calculate the steady state population density  $N$  for a homogeneous population of cells using a strategy that is characterized by the ATP yield,  $n^{\text{ATP}}$ . Since the payoff of a cell determines the number of offspring in the next round, at steady state of the system the mean payoff,  $P_m(n^{\text{ATP}})$ , is 1. Assume that a cell has access to a resource. The probability that it shares the resource with  $i$  competitors is Poisson distributed and then given by  $p_i = N^i \exp(-N)/i!$ . Hence one obtains for the mean payoff

$$P_m(n^{\text{ATP}}) = \sum_{i=0}^{\infty} \frac{p_i S J^{\text{ATP}}(n^{\text{ATP}})}{J^S(n^{\text{ATP}}) + i J^S(n^{\text{ATP}})} = S n^{\text{ATP}} \sum_{i=0}^{\infty} e^{-N} \frac{N^i}{(i+1)!} = 1. \quad (10)$$

From this equation, the steady state population density can be derived. It is implicitly given by

$$N = S n^{\text{ATP}} (1 - e^{-N}). \quad (11)$$

A strategy  $k$  is evolutionary stable, if at the corresponding steady state population density,  $N_k$ , a rare mutant using any other strategy,  $s \neq k$ , has a mean payoff against strategy  $k$ ,  $P_m(n_s^{\text{ATP}}, n_k^{\text{ATP}})$ , which is smaller than that of the resident strategy  $k$  against itself and hence is smaller than one. The mean payoff for the invading strategy,  $s$ , is given by

$$P_m(n_s^{\text{ATP}}, n_k^{\text{ATP}}) = \sum_{i=0}^{\infty} e^{-N_k} \frac{N_k^i}{i!} \frac{S J^{\text{ATP}}(n_s^{\text{ATP}})}{J^S(n_s^{\text{ATP}}) + i J^S(n_k^{\text{ATP}})}. \quad (12)$$

Using Eqs. (2)–(5), the payoff can be determined explicitly and is given by

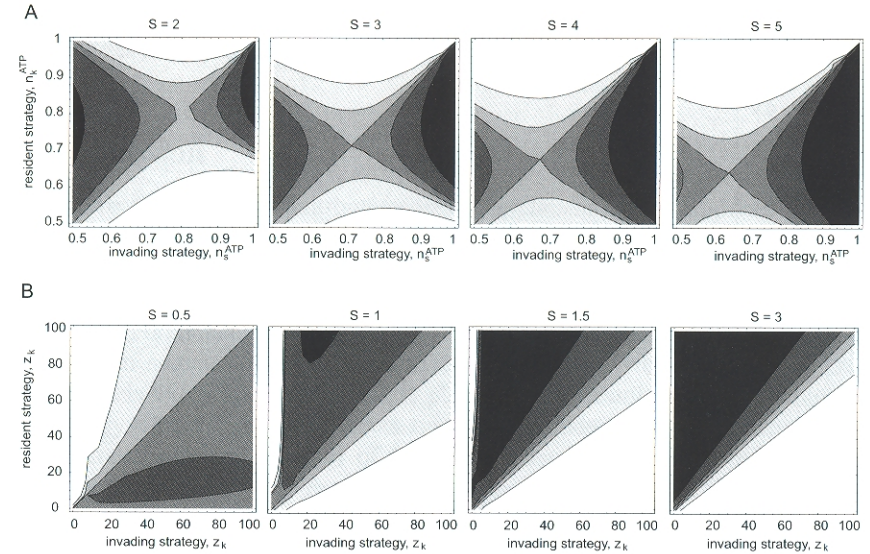
$$P_m(n_s^{\text{ATP}}, n_k^{\text{ATP}}) = \sum_{i=0}^{\infty} e^{-N_k} \frac{N_k^i}{i!} \frac{S n_s^{\text{ATP}} (n^{\text{MAX}} - n_s^{\text{ATP}})}{n^{\text{MAX}} - n_s^{\text{ATP}} + i (n^{\text{MAX}} - n_k^{\text{ATP}})} \quad (13)$$

for the thermodynamic tradeoff, and

$$P_m(z_s^{\text{ATP}}, z_k^{\text{ATP}}) = \sum_{i=0}^{\infty} e^{-N_k} \frac{N_k^i}{i!} \frac{S (32 + 2z_s^{\text{ATP}})}{1 + z_s^{\text{ATP}} + i (1 + z_k^{\text{ATP}})} \quad (14)$$

for the fermentation tradeoff.

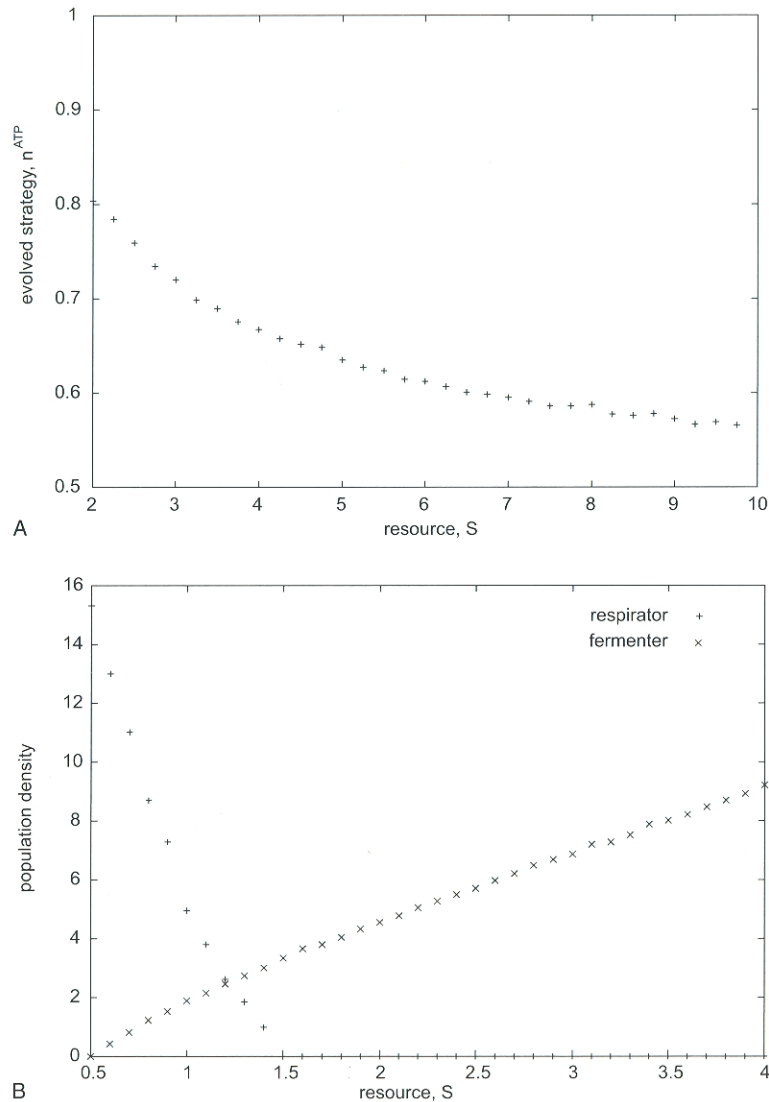
Fig. 4 shows contour plots of the payoff functions for different values of the local resource  $S$ . For the thermodynamic tradeoff (Eq. (13) and Fig. 4a) the graphs show that a single evolutionary stable strategy exists, which depends on the resource parameter  $S$ . Fig. 5a shows the evolutionary stable strategy as a function of  $S$  based on computational simulations of the evolutionary process



**Fig. 4.** Mean payoff of a rare mutant strategy in a resident population for a different resource influx,  $S$ , (A) for the thermodynamic tradeoff (Eq. (12),  $n^{\text{MAX}} = 1$ ,  $k_1 = 1$ ) and (B) for the fermentation tradeoff (Eq. (13),  $z^{\text{MAX}} = 100$ ,  $k_2 = 1$ ). The mean fitness of the resident strategy is 1. Light gray indicates fitness values higher than 1, dark gray indicates smaller values. A resident strategy,  $n_k^{\text{ATP}}$  is evolutionary stable if the fitness,  $P(n_k^{\text{ATP}}, n_s^{\text{ATP}})$  of a rare mutant strategy  $n_s^{\text{ATP}}$ , is smaller than one for all strategies  $s \neq k$ . An evolutionary stable strategy exists for the thermodynamic tradeoff (at  $n_k^{\text{ATP}} \approx 0.8$  for  $S = 2$ ;  $n_k^{\text{ATP}} \approx 0.72$  for  $S = 3$ ;  $n_k^{\text{ATP}} \approx 0.68$  for  $S = 4$ ;  $n_k^{\text{ATP}} \approx 0.64$  for  $S = 5$ ). For the fermentation tradeoff, an evolutionary stable strategy exists for  $S = 0.5$  (at  $z_k = 0$ ), and for  $S = 3$  (at  $z_k = z^{\text{MAX}}$ ). For intermediate values of  $S$ , no evolutionary stable strategies exist. High fitness values can be obtained for  $z = 0$  and for  $z = z^{\text{MAX}}$ . However, a resident strategy with minimal rate in fermentation ( $z_k = 0$ ) can be invaded by a strategy that uses fermentation at a high level ( $z_k \approx z^{\text{MAX}}$ ) and vice versa. A simulation of the system shows that at intermediate values of  $S$ , both strategies coexist.

(see appendix). At low values of  $S$ , strategies with high yields are favored. With increasing  $S$ , the yield of the evolutionary stable strategy decreases. This is because with increasing  $S$ , the population density increases. As a consequence, local competition increases and strategies with fast but inefficient resource use have a selective advantage.

Fig. 4b shows the payoff function for the fermentation tradeoff. Here, a single evolutionary stable strategy exists only, if the amount of the locally shared resource is small or high. At small values of  $S$ , the strategies that exclusively uses respiration ( $z = 0$ ) outcompetes all other strategies. At high values of  $S$ , the strategy which uses fermentation at the maximal rate ( $z = z^{\text{MAX}}$ ) outcompetes all other strategies. At intermediate values of  $S$ , no single evolutionary stable strategy exists. Computer simulations show that at intermediate



**Fig. 5.** Computational simulation of the evolutionary dynamics as described in the appendix. (A) For the thermodynamic tradeoff a single evolutionary stable strategy evolves ( $0.5 < n^{ATP} < 1$ ) for all values of the resource,  $S$ . With increasing values of  $S$  the evolutionary stable strategy has decreasing ATP yield and thus increasing rate of ATP production. (B) For the fermentation tradeoff, the evolving strategies always have close to maximal ( $z > 90$ ) or close to minimal ( $z < 0.1$ ) levels of fermentation. Strategies with intermediate levels never evolved. However, for some levels of resource,  $S$ , both strategies coexist. Plot (B) shows the population densities of both type of strategies, the maximally fermenting (fermenter) and the minimally fermenting (respirator), at the endpoint of the simulations. For a detailed description of the simulation see the appendix.

values, two coexisting strategies evolve, in which all individuals use fermentation either at minimal or at maximal rate (see appendix and Fig. 5b).

## 5. Discussion

We have shown above that heterotrophic organisms face a tradeoff between rate and yield of ATP production. This tradeoff arises generally for any heterotrophic ATP-producing pathway, since it is an immediate consequence of fundamental thermodynamic principles. An equivalent tradeoff also arises specifically in organisms that can use both fermentation and respiration to produce ATP from sugars. Given the central role of ATP in vital biochemical processes, it is likely that such tradeoffs played an important role in the evolution of energy metabolism. Our models suggest that under different environmental conditions different strategies of ATP production are favored. Irrespective of the specific properties of the tradeoff our models predict that if an organism has exclusive access to a resource, a slow but efficient resource use is expected. Conversely, if organisms are in direct competition for a shared resource, the models predict fast, but inefficient resource use. This can be seen in the use of respiration and fermentation across different organisms.

Moreover our theory predicts that if an organism produces ATP from internal energy resources, such as storage metabolites or ingested food items in higher organisms, then the corresponding metabolic pathway maximizes yield rather than rate of ATP production. The exclusive use of respiration by higher animals supports our line of reasoning. The occasional use of fermentation-like processes in higher animals, as can be observed for example in muscle cells, is not in conflict with our hypotheses, since they are only used in conditions when high rates of ATP production are essential. In contrast to higher animals, many unicellular organisms grow on shared resources. For these the theory predicts that ATP production is rather optimized for high rates of ATP production. This is supported by the observation that microorganisms frequently use fermentation even in the presence of oxygen.

Other differences in the use of energy resources are manifest in the specific design of ATP-producing pathways and may be direct evolutionary consequences of the thermodynamic tradeoff. For example, there is considerable variability in the design of oxidative phosphorylation in different organisms. Microorganisms often obtain smaller yields in oxidative phosphorylation than higher organisms [10, 17, 18]. This may imply that in microorganisms oxidative phosphorylation is designed to produce ATP at a higher rate at the cost of a lower yield. Moreover it has been reported that some microorganisms may be able to vary rate and yield of ATP production and therefore adjust their strategy of ATP production to environmental conditions [3, 6, 17].

Since ATP production with high yield can be seen as cooperative resource use we further expect that it can be observed wherever cells that share a local

resource are rather selected at the level of the group rather than on the level of the individual cell. This is certainly the case for the cells of a multicellular organism but may under certain conditions also apply to groups of unicellular organisms as for example bacterial colonies. Moreover the more than tenfold reward in overall ATP production, that is the consequence of a cooperative use of the resource by respiration rather than fermentation, may have played a key role for early steps in the evolutionary transition from unicellular to multicellular organisms. It is a widely accepted hypothesis that the benefits of cooperation between cells were an important driving force in the evolutionary transition from single cells to multicellular organisms [2, 9]. This is exemplified by the benefits arising from the division of labour between highly specialized cells in a multicellular organism. However, resource consumption with a slow, but efficient ATP-producing pathway rewards cooperation between identical cells and might thus have played a role for the evolution of early, undifferentiated forms of multicellularity.

## Appendix

To determine numerically the evolutionary stable strategies for both the thermodynamic and the fermentation tradeoffs we performed the following simulation. In each step cells were randomly distributed to 4900 isolated locations, containing an amount of resource  $S$ . The payoff for each cell was calculated as a function of the other cells also present at this resource (using Eq. (12) with  $n^{\text{MAX}} = 1$  and Eq. (13) with  $z^{\text{MAX}} = 100$  for the respective tradeoffs). The payoff then directly gives the mean number of offspring in the next round, whereby we use Poisson distributed random numbers. The simulations were started with 4900 cells. For the thermodynamic tradeoff we started with an initial strategy of  $n^{\text{ATP}} = 0.7$ . For the fermentation tradeoff we started with  $z = 0.5$ . In the simulation the above steps were iterated 50 000 times for the thermodynamic tradeoff and 10 000 times for the fermentation tradeoff. In addition in each step cells were subject to mutation with a probability of  $10^{-3}$  per step. For the thermodynamic tradeoff mutations were performed by increasing or decreasing the strategy  $n^{\text{ATP}}$  with a maximal step size of 0.01 in the first 25 000 steps, and 0.001 in the last 25 000 steps. For the fermentation tradeoff, mutations were performed by changing the rate of fermentation at a maximal step size of 1 in the first 5000 steps and 0.1 in the last 5000 steps. Additionally, in the first 5000 steps, mutations were performed by assigning random values to cells with a probability of  $10^{-4}$ .

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