

Evolving biological systems: Evolutionary Pressure to Inefficiency

Dominique Chu¹ and David Barnes¹

¹School of Computing, University of Kent, CT2 7NF, Canterbury
dfc@kent.ac.uk

Abstract

The evolution of quantitative details (i.e. “parameter values”) of biological systems is highly under-researched. We use evolutionary algorithms to co-evolve parameters for a generic but biologically plausible topological differential equation model of nutrient uptake. In our model, evolving cells compete for a finite pool of nutrient resources. From our investigations it emerges that the choice of values is very important for the properties of the biological system. Our analysis also shows that clonal populations that are not subject to competition from other species best grow at a very slow rate. However, if there is co-evolutionary pressure, that is, if a population of clones has to compete with other cells, then the fast growth is essential, so as not to leave resources to the competitor. We find that this strategy, while favoured evolutionarily, is inefficient from an energetic point of view, that is less growth is achieved per unit of input nutrient. We conclude, that competition can lead to an evolutionary pressure towards inefficiency.

Introduction

Much is now known about biological systems at the molecular level. There are countless databases that contain gigabytes of detailed information about biochemical networks, reactions, gene regulation, protein-protein interactions and much more. As far as biochemical reaction networks are concerned, most of the available information is about structural properties of these networks, i.e. which molecules react with which molecule, which protein represses/activates which gene and so on. At the same time, very little is known about the quantitative details of these reactions, i.e. how fast reactions proceed, how strong a gene is repressed or at what rate genes are expressed.

Recently, a large scale analysis of topological data has led to important insights into the design principles of living systems. The discovery of so-called network motifs (Alon, 2007; Kashtan and Alon, 2005; Mangan and Alon, 2003), i.e. over-represented local connectivity patterns of gene regulatory networks is but one example. These motifs were found to be not only statistically over-represented but also functionally significant (Alon, 2006). While much research effort has been expended to understand the significance of

these topological features, very little research has been done to understand quantitative details of biochemical reaction networks (Chu, 2013).

One of the conditions for being able to gain insight into the topological design principles of biological systems was the wealth of empirical available about them. Since there is not a comparable amount of information available about the parameter values of biochemical networks, it is only natural that much less is known about the quantitative design principles of natural systems. At the same time, it is likely that the values of parameters of biological systems contain very much biologically valuable information. They are a product of natural evolution and as such have to be assumed to reflect the adaptive pressures to which the system has been exposed and as such encode valuable biological information.

In order to understand the principles that guide the evolution of quantitative parameter values, it is not necessary to know the actual values of biological systems. Instead, a different approach based on synthetic evolution using evolutionary algorithms can be used. In this article we will take this approach. To do this we focus on a generic topological model of nutrient uptake, i.e. a model that only contains the structure of the biochemical reactions, but not their numerical parameters. We then use evolutionary algorithms to evolve parameters for specific conditions. Comparisons of a large number of runs will then enable us to draw some conclusions as to how parameters evolve. The hope is that these conclusions are valid beyond the specifics of the particular model we have chosen and provide insight about natural biological systems as well.

Our model does not describe any specific biological system, but it is a biologically plausible generic representation of nutrient uptake in bacteria and contains topological features that are widely used by bacteria. An important way for bacteria to take up nutrient is by importing nutrient molecules through specialised openings at the cell surface—so called *porins*. These porins are proteins and they tend to be specific to a particular nutrient type. So, a porin for one nutrient cannot be used to take up a different type of nutrient. In bacteria, these porins whose production requires energy

are only expressed by the cell when the relevant nutrient is actually present in the environment. A typical way for the cell to achieve this is to use the nutrient as an activator for the expression of the porin. Once imported into the cell the nutrient stimulates the expression of the gene coding for the porin (indeed, often it represses the repression of the gene, which amounts to stimulation). This very general scheme of porin activation is reflected in our model.

A typical feature of bacterial uptake system is that expression is demand driven and porins are only produced when they are needed. The evolutionary rationale for this is that gene expression requires resources that could be invested otherwise, for example to fuel growth. Moreover, there is finite space on the cell surface which limits the number of porins that can be expressed at any one time. It is also commonly observed that over- or under-expressing a gene often decreases the growth of the mutant strains. So, apparently, for many proteins there is an optimal rate of porin expression. At the same time, evolution has the ability to tune the rate of some biochemical reactions, including the rate of gene expression. It is therefore likely that the particular rates of gene expression and that of other bio-chemical reactions are fine-tuned by evolution.

This motivates the research question to be addressed in this contribution: How do the parameters of generic bacterial uptake systems depend on the adaptive pressures that led to their emergence. Moreover, given a set of adaptive pressures, is it possible to predict the parameters, or vice versa, given a set of parameters, is it possible to understand what adaptive pressures led to them? Finally, can the results obtained from the generic biologically plausible model provide any insight that is relevant for the real world.

To address these questions, we performed two types of artificial evolution experiments. Firstly, we evolved parameters (i.e. “solutions”) on their own. We found that this results in uptake mechanisms that could turn most of the nutrient on offer into growth using a very low number of porins resulting in slow nutrient uptake and growth. We found this to be the most efficient mode of growth because it allows the cell to channel most nutrient into growth while minimising the amount of energy spent on the uptake mechanisms. In a further set of experiments we then evolved new solutions in competition with a previously evolved one.

The solutions obtained from these co-evolutions were different from the solutions evolved without competition. Rather than taking up nutrients slowly with a low number of porins, they evolved towards increasingly rapid uptake of nutrient (although not necessarily rapid growth). While this allowed them to grow fast it also means, as we will discuss below, that they grow inefficiently. Specifically, we found a clear trend that co-evolved solutions are less efficient than the original solutions that evolved without a competitor. However, within the chain of evolved solutions there was no clear further trend toward inefficiency. Hence, rather

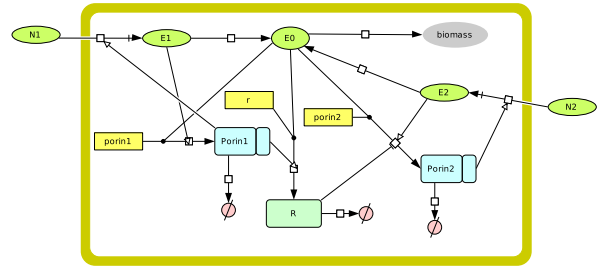


Figure 1: A schematic representation of the model.

than getting more efficient by competition, we found that co-evolution leads to less efficient solutions, which is a counter-intuitive at first. We will argue below that this pattern towards inefficiency is universal, in the sense that it does not depend on the specifics of the particular model, but would be true for a large class of nutrient uptake systems, including those of real organisms.

Furthermore, in our simulations we presented the simulated cells with two different types of nutrients of differing quality. We also added a structural motif into the model that would allow the cells to suppress take-up of the less valuable nutrient 2 in favour of the other. Indeed, we observed the evolution of the suppression of nutrient 2 uptake. However, surprisingly to us, the solutions did not use the motif offered, but instead came up with a different way of regulating the uptake of the less efficient nutrient.

The basic model

We present here a generic model of a bacterial uptake/metabolic system (see figure 1). The idea is that there are two sources of nutrients N_1 and N_2 . Uptake of these sources of nutrients requires specific porins, namely P_1 and P_2 respectively. Once taken up into the cell the nutrient becomes an internal source of energy (E_1 and E_2) which can be converted into actual energy (or ATP), which we denote by E_0 . We assume that the uptake and conversion of nutrient follows Hill kinetics (Chu et al., 2011). The internal energy is converted either into porins (i.e. porin 1 and porin 2 abbreviated as P_1 , P_2) or into biomass (bm) which represents the results of bacterial growth.

We only determined the topology of this model which is designed such that the expression of porin 1 and 2 is activated by the presence of nutrient 1 and 2 within the cell (i.e. E_1 and E_2 respectively). The model topology does not by itself specify how strong this activation is. The strength of the activation depends on the parametrisation, which needs to be evolved. Indeed, there are many parameters that would effectively turn off the activation. The same is true for all other regulatory functions in the model.

An important feature of the model is that the expression of nutrient and the production of biomass require energy.

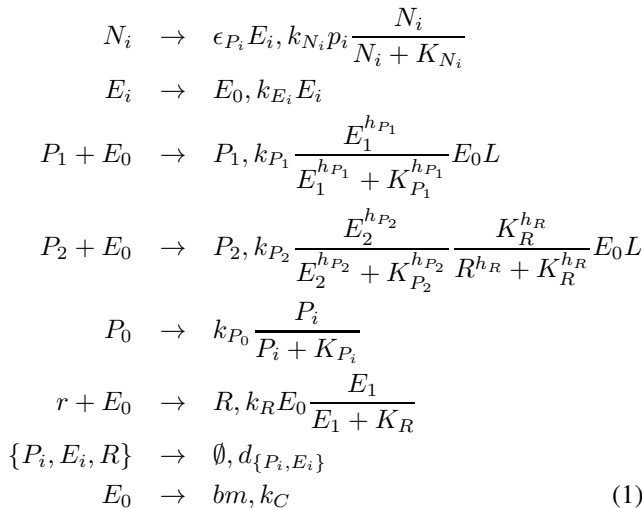
Hence, the (*a priori* unspecified) parameter values for the expression rates of porins and the growth rate decide to what extent the resources (i.e. nutrient) is used to fuel growth and to what extent it is used to maintain the cellular uptake machine, i.e. how much is allocated to porin production.

It appears that there is an optimal allocation of resource to growth and the uptake mechanism. If the cell allocates no energy to uptake but all to growth, it will not be able to use any of the nutrients and hence it will not grow at all. On the other hand, if the cell allocates all of its nutrients into uptake, but none into growth, then it will be rich in nutrients, but never grow and hence never divide. In-between these two extremes there is one (or possibly several) optimal allocation. While it is clear from this argument that such an optimum exists, we do not know where it is and what it depends on.

Another important feature of the model is that the total number of porins in the system is limited. Porins in bacteria are located at the cell surface. They build openings there and selectively let molecules in and out of the cell. In real cells there is limited space on the surface to accommodate porins. This limitation is represented in our model by the term L (see below). It is a repressing term that reduces the expression of porins 1 and 2 as a (Hill-repressor) function of the sum of the concentration of both.

Finally, the model also features a repressor motif. The molecule R is expressed when there is porin 1 available in the cell and its sole purpose in the model is to repress the expression of porin 2. This sort of regulatory motif whereby a repressor is activated by some part of the system and represses another part of the system is commonly found in gene regulatory networks. The idea of introducing this motif is to enable the cell to evolve a repression mechanism for nutrient 2 when the (better) nutrient 1 is available.

The topology of the model can be summarised by these chemical equations:



where L is the space-limit which represents the fact that

there is limited space at the surface of cells to accommodate porins, given by

$$\frac{K_L}{(P_1 + P_2) + K_L}$$

The quality factor ϵ_{P_i} determines the quality of a nutrient and we set it to 1 for P_1 and 0.5 for P_2 . This means that one unit of nutrient 2 gives only 1/2 unit of biomass. Uptake and gene expression are assumed to follow Hill kinetics. While this is an approximation, in reality it has been found that Hill kinetics is a good description of the reactions described here. It is also widely used to model them and is a fairly simple approach. In all simulations reported here we keep the Hill exponent fixed at a value of 2, which is biologically plausible.

Evolving the system

In this article we evolve parameters for the topological model described by equation 1. Concretely this means that we evolve values for the kinetic parameters determining the system, including the Hill-constants (i.e. K_{N_i} and dynamic constants such as k_{P_i}). Note that we do not evolve the decay rate d which we keep fixed at 0.1, the Hill exponents (i.e. $h_x = 2$), the relative value of ϵ_{P_i} (which we keep fixed at 1 and 0.5 respectively) and K_L which determines how much space there is for the porins in the cell. This latter parameter we set to 1. All other parameters are evolved and we allow them to take values between 0 and 15. In all simulations reported here the model is implemented as a system of differential equations. As a solver we use the general purpose numeric differential equation solver of the Maple computer algebra system version 16 for Linux.

The model was implemented as a co-evolutionary system, that is we have two different solutions compete for the same nutrient pool of N_i . This represents two different species of bacteria co-existing in the same environment. In practice this means that we used two sets of differential equations with two sets of the variables E_i, P_i, R, bm representing two different cell-types. Each set had their own kinetic parameters, yet their dynamics depended on one another via the shared nutrient pool. Of the two competing solutions, we ever only evolved one of those solutions, while keeping the other one fixed. Initially, we use as the fixed solution an ‘‘unfit standard solution’’ with all parameters set to 1. This solution supports no growth beyond the start-up allocation which is equivalent to 1 unit of biomass. Co-evolution is achieved by using previously evolved solutions as fixed solutions (i.e. ‘‘incumbents’’) in further evolutionary runs. In all simulations we set as the initial condition all variables to zero except for $P_i = 0.001, E_0 = 1$. This means that any solution can support a maximum of 1 unit of biomass even if it does not take up a single unit of nutrient.

During each evolutionary run only one of the solutions is evolved, while the other one is kept fixed at user-defined pa-

rameters. Co-evolution was achieved in a sequential manner, that is, one solution was evolved against a fixed solution. The evolved solution was then subsequently used as the fixed incumbent in a further evolutionary run. Co-evolutionary chains were obtained as follows;

1. Evolve a first solution against an un-evolved base solution (all parameters set to 1).
2. Once the first solution is obtained, evolve a second solution against the first solution (which is kept fixed).
3. Create a third solution by evolving against the second solution (which is now also kept fixed).
4. Continue in this manner until no more solutions evolve.

To evolve the system we used a genetic algorithm with elitism. Individual solutions were represented as an array of real numbers in the range $[0, 15]$. The population size was set to 50. The initial population consisted of random parameters within the range $[0, 15]$ sampled from a uniform distribution. As a fitness function we chose the biomass after 500 units of time. We found that 500 time units was large compared to the transient periods of the system, i.e. increasing this time did not change the results of the evolution.

As a selection algorithm we chose a fitness proportional selection. However, in every generation the best solution and a mutated version of it was allowed to proceed to the next population. The mutation and crossover rate was set to 0.8. Mutation was done by changing a random parameter by up to ten percent of its current value. If a mutation resulted in a value lower than 0.00001 or greater than 15 then the parameter was set to 0.00001 and 15 respectively. The amount of available nutrient was set to 10 for both nutrient types. The GA was implemented in Perl, but the fitness function was evaluated using Maple. Both the relevant Maple script and the Perl source code are available from the authors upon request.

We performed two different types of experiments. Firstly, we performed a simple evolution without competition (i.e. with the standard unit solution as competitor). Subsequently, we used the results of those evolutionary simulations to initiate a co-evolutionary chain, as described above. In practice we found that after a number of iterations no more fit solutions were found, in the sense that the total biomass produced for the evolving solution did not substantially exceed 1, i.e. evolution could not find solutions to outperform the incumbent. In this situation it was helpful to evolve a new solution by seeding the new evolutionary solution with the incumbent parameters, rather than starting from a random solution. However, even in this case, the co-evolutionary potential was limited.

Individual evolutionary runs were stopped either after 5000 generations or when a plateau of high fitness with no apparent further increases over time was reached, whatever

happened first. The presence of such plateaus was determined by visual inspection. In practice, it turned out to be a clear-cut case. A typical evolution would show rapid increases of the fitness at first, followed by fitness stagnation.

Results

Unconstrained evolution

We evolved a number of solutions without competitor. Figure 2 illustrates three typical results obtained from unconstrained evolution. It shows the amount of biomass over time obtained by simulating in Maple the best solution of the final population in the GA. It is part of the set-up that there is a limited amount of nutrient of 20 units divided across two types of nutrients. Since the second nutrient gives only half the growth of the first, at best the available resource can be converted into a biomass of 15 units under ideal conditions; the solutions also get a start-up energy equivalent to 1 biomass. Hence, in total the maximum they can reach is 16 biomass units.

It is apparent from figure 2 that most solutions evolved come close to the maximum attainable biomass, although there is some variation. Occasionally, we have also observed that solutions got stuck on a local minimum and did not discover the second nutrient source. This resulted in cells that would not take up any of the nutrient 2 and achieve only a level of about 10 units of biomass (data not shown). This indicates that the solutions were able to channel most nutrients into growth rather than using them for enzyme production. This high level of conversion was made possible by a very low assumed degradation rate of enzymes that allowed the solutions to grow at a slow rate.

The figure shows that the time required for achieving the maximal growth varies somewhat from solution to solution. The three example solution shown in figure 2 are representative for the range observed in all unconstrained evolutionary runs. Generally, we observed that these evolved solutions take up nutrient over a time period of 20 to 150 time units. There is a wide variation between the solutions that we obtained.

Co-evolution

Co-evolution changes the nature of the solution obtained in very specific ways. The system as a whole offers a finite amount of resources and both solutions need to compete for the same two pots of nutrients. Hence, competition is not a zero sum game.

At the beginning of a co-evolutionary run the competitor will have random parameters and not be able to compete well against the incumbent. However, as new solutions are discovered the competitor evolves to outperform the incumbent. One way to do this is to consume the available nutrients faster than the incumbent. Ideally, the new solution has used up all of the nutrients before the incumbent can

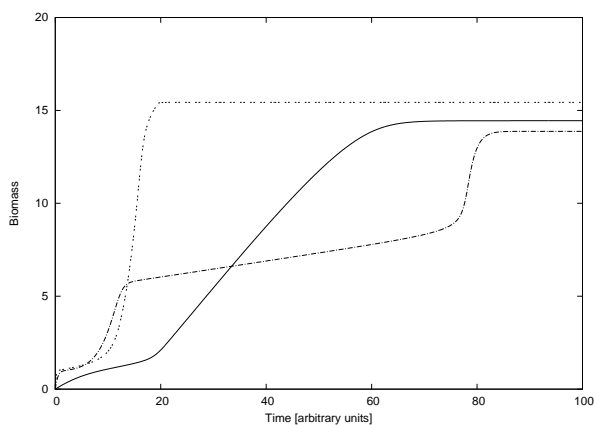


Figure 2: Three solutions of simple evolutions where the competitor is the maximally unfit solution.

do this preventing the latter from growing. Indeed, throughout all co-evolutionary runs we performed, this speed strategy emerged as one important way for solutions to undermine their competitors' abilities to grow. Co-evolution led to a sequence of increasingly fast solutions until a limit was reached and no more increases were possible. Note, however, that increased speed does not necessarily mean an increased growth-rate. Indeed, we observed a number of cases where growth was slower (i.e. occurred later) in the new competitor than in the incumbent but its nutrient uptake was still faster.

Figure 3 shows a typical co-evolutionary interaction. The first solution, which has been evolved against the unfit set of parameters, takes up nutrients slowly. This particular solution requires more than 100 time units to reach the final biomass. In contrast, the second solution is much faster and reaches its final biomass within 15 time units. Interestingly, the third solution, which is evolved against the second one, grows slower. Yet, a closer inspection shows that, while it produces biomass slower, its nutrient uptake is faster than that of the second solution. Hence, it leaves no nutrient to the second.

In all evolutionary experiments we performed we never found a case where a solution evolved to co-exist with its competitors, in the sense that *both* the incumbent and the competitor were able to take-up nutrient and grow. Instead, in all cases we considered, one solution came to dominate the other. However, there are cases where we observed the dominated solution to have some minimal growth, i.e. less than 1 biomass unit above the start-up energy.

Connected to this minimal growth of the dominated competitor we observed an interesting phenomenon. Figure 4 shows two simulations of a solution that we had obtained as a third solution during one of our co-evolutionary chains. Note that the graph does *not* show the evolution experiment, but an evaluation of the solution obtained from one of the

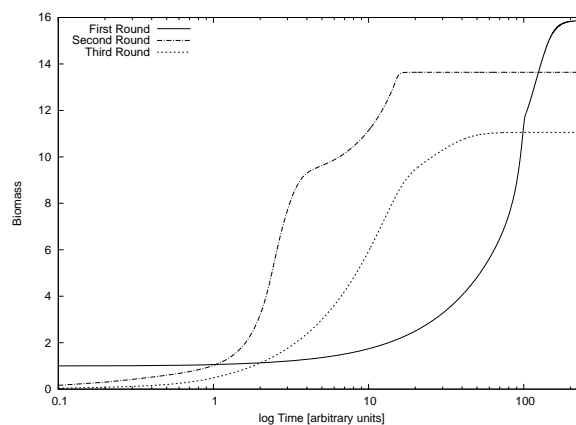


Figure 3: Three co-evolutionary solutions. The three curves show the growth of the solutions as a function of time. The curve labelled "First" is the original solution evolved against unfit parameters. The line "Second Round" was evolved in competition with the first and similarly the "Third Round" was evolved against the second. Time is shown in log scale to improve readability.

evolution experiments. The difference between the two runs in figure 4 is the competitor with which the evolved third solution competes. In one curve it is the standard unfit solution (which does not consume any nutrient) and in the other it is the second solution, i.e. the solution against which the third solution was evolved. In this particular case the second solution takes up a small amount of nutrient when competing against the third solution and grows roughly by 0.6 (data not shown) above its initial endowment. On the other hand the unfit solution, where all parameters are set to 1, does not take up any nutrient in competition with the third solution. Hence, one would assume that the growth of the third solution when competing with the second is *lower* than when competing with the standard solution. However, in reality, the third solution leads to a *higher* biomass in combination with the second solution than against the standard unfit solution. This is shown in figure 4. Increased nutrient uptake requires a higher level of investment into the metabolic machinery compared to the

Upon closer inspection this effect can be related to the usage of the second (less efficient) nutrient. In competition with the standard unfit solution the third solution does not use up all of the less efficient nutrient, but in competition with the second solution it does. This hints at the explanation for the observed effect. When competing with the standard unfit parameters the third solution has more nutrient 1 available. This additional nutrient leads to a higher production of porin 1 than when competing with the second solution. Note that there is a limit to the total number of porins for nutrient 1 and 2. Hence, if there is more porin for nutrient 1 produced then this means that less porin for

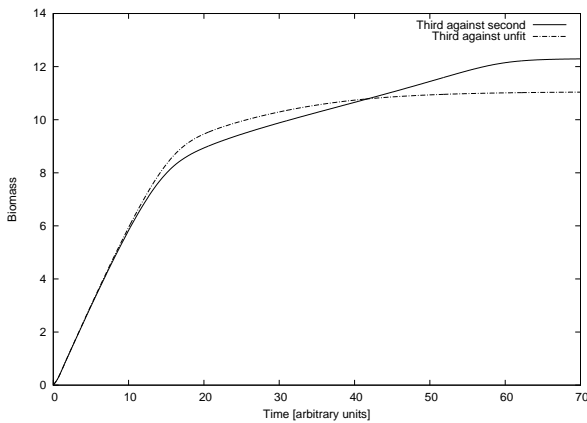


Figure 4: Comparing the third solution when evaluated against the unfit solution and against the second solution. Surprisingly, it does better against the (evolved) second solution than it does against the unfit standard solution.

nutrient 2 can be produced. Indeed, in the competition with the standard solution the porin for nutrient 2 is lower and tends to zero before all of the nutrient 2 can be taken up. In competition with solution two, on the other hand, the overall amount of porin 1 is lower which allows more porin 2 to be produced. The effect of this is that sufficient amounts of the porin can be produced to take up all of the available nutrient 2. Altogether, this leads to higher growth.

Based on this, one would expect that less biomass is produced by solution three in competition with the second solution than with the unfit parameters when the limit on the total number of porins is removed. To check this, we performed simulations where we removed the limitation (i.e. removed the factor L from equation 1). A comparison of solution three under these two different conditions then shows that indeed it develops more biomass when paired with the unfit solution than when with solution two (data not shown).

A comment on switching

In real bacteria there is a phenomenon called “diauxic growth.” When bacteria are presented with two nutrient sources of different quality then they take up the good quality source first. Only when this one is exhausted will they take up the secondary source. From an adaptation point of view it is quite straightforward to make sense of this. Those cells that take up the good quality nutrient faster will be able to produce more offspring (because they have the better quality nutrient) and hence out-compete the others while at the same time leave less for their competitors. By the same reasoning, we expected to observe the emergence of diauxic growth in our artificial evolution experiments. Hence, we included a simplified mechanism to allow cells to suppress production of porin 2 when porin 1 is present in the cell. We specified that the regulator R has a suppressing effect on the

expression of porin 2, but requires porin 1 to be expressed itself. Given the right parameters, it should then be possible for diauxic growth to emerge.

In our simulations we found that the evolved solutions universally favoured porin 1 over porin 2, but they did not use a switching mechanism based on the regulator R . Instead the cells evolved other mechanisms to ensure that nutrient 1 is always taken up before nutrient 2.

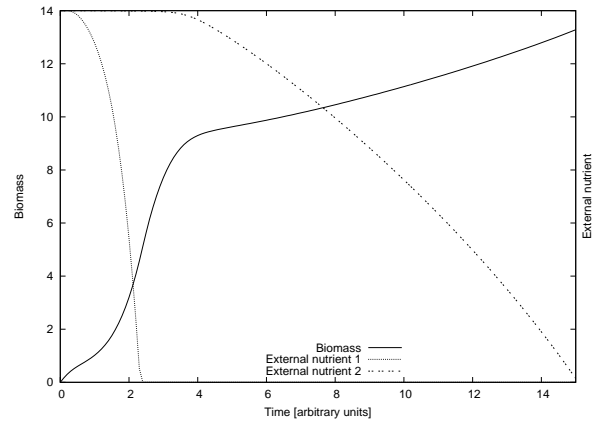


Figure 5: A second round solution and the amount of external nutrient available. Nutrient one is exhausted after around 2.5 time units. Only then the consumption of nutrient 2 starts. The control mechanism relies on limited space for porins. See main text for details.

We observed a small number of solutions that did not take up nutrient 2 at all. Amongst those solutions that did take up nutrient 2 a subset did not have any apparent regulation mechanisms, but simply took up nutrient 2 at a slow rate compared to nutrient 1, i.e. produced porin 2 at a low rate. This is only a mechanism in the most trivial sense. A more advanced, true mechanisms that frequently evolved was based on the limit on the total number of porins via the factor L in equation 1. The idea is as follows: If the porins for nutrient 1 are expressed at a higher rate than those for porin 2, then this leads to a higher rate of uptake of nutrient 1, further stimulating expression of porin 1. Since there is limited space, once a certain amount of porin is expressed, further expression of *any* type of porin is suppressed. Altogether, this allows porin 1 to increase its advantage and to crowd out porin 2 which is expressed at a low rate only. Yet, once nutrient 1 runs out, porin 1 is no longer produced and then porin 2 can be expressed.

While this mechanism effectively repressed porin 2, it limits by design the speed with which porin 2 can be expressed and hence it limits the uptake speed of nutrient 2. The ideal scenario for a bacterial cell would be to take up nutrient 1 rapidly, then switch and take up nutrient 2 rapidly. However, the simple regulatory mechanism via L relies on the production of porin 2 to be slower than that of porin 1

and therefore does not allow efficient repression of nutrient 2 uptake while nutrient 1 is still present *and* rapid uptake of nutrient 2.

This begs the question as to why the system does not accept the repressor R for the regulation of nutrient 2. The repression topology we used is a common gene regulatory motif in biology to control the expression of genes. Yet still, in none of the simulations that we performed it was used to regulate the expression of porin 2. We suspect that this simple regulatory motif is not effective in the regulation of porin expression. We conjecture that the underlying reason for the failure to evolve has to do with the difficulty of removing the repressor once nutrient 1 has run out. Further investigations are required to understand why this regulatory system is not effective.

The effects of competition

From the above analysis of the solutions it becomes clear that co-evolutionary pressure changes the nature of the solutions. The first solution, that is evolved against an unfit competitor tends to take up nutrient over a long time. Subsequent co-evolved solutions tend to take up nutrients, especially nutrient 1, over a much shorter time. The question is now why in the absence of competition solutions tend to evolve towards slow uptake. One possible explanation could be that there simply are more solutions (i.e. combinations of parameters) that take up nutrient slowly than there are solutions that take them up fast. Hence, in the absence of co-evolutionary pressure, evolution is more likely to discover slow solutions than fast ones.

Another interpretation, that does not necessarily preclude the first explanation, is that there is a functional significance to the slow speed with which nutrient is taken up. To understand whether this is the case, we considered the growth efficiency of solutions. To do this we defined a simple measure of efficiency given by the biomass divided by the total nutrient usage. According to this measure, a solution is more efficient if it requires less nutrient to grow to a given size.

In order to gain an insight into the nature of the solution we plotted the efficiency over time; see figure 6. A clear pattern emerged. The first solution that evolved against the unfit standard solution was always more efficient than subsequent solutions. For subsequent solutions, however, there is no clear trend towards further inefficiency. So, the fourth solution may or may not be less efficient than the third solution from the same co-evolutionary chain. Figure 6 shows the efficiency of three consecutively evolved solutions as an example.

There are again two ways to interpret this finding. One could assume that this trend towards inefficiency is merely an artefact of the particular modelling choices made, or that it is a more general phenomenon that is relevant for a large class of systems including real systems. We believe the latter is the case. Within our model, nutrient can only be converted

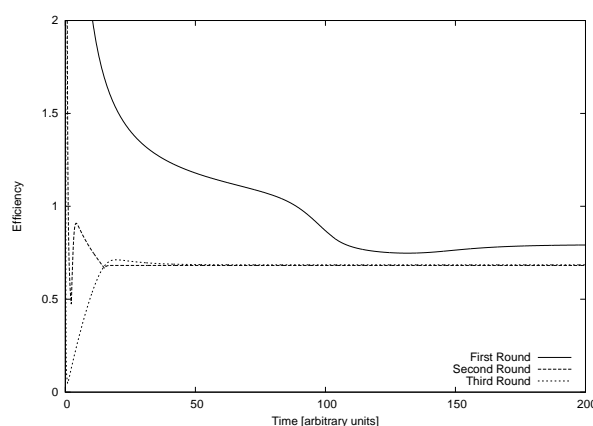


Figure 6: Comparing the efficiency of solutions during subsequent rounds. Efficiency is the amount of growth achieved for each unit of nutrient consumed.

into either biomass or into porins. The latter are necessary in order to take up nutrient. As long as there is no time-constraint on the system, it is sufficient for solutions to produce a small number of porins. It will take a long time to absorb all the available nutrient, but the investment into the metabolic machinery is low, so altogether the cell can grow efficiently. The major limiting factor here is the decay of nutrient which requires a certain production rate of porins to replace lost ones and keep the uptake stream constant. Up to that limit, slow growth is more efficient.

However, if a cell needs to compete with another one for resources, fast uptake is required, because otherwise the competitor takes up all the nutrient and nothing is left for the cell. Hence, competing cells need to take up nutrient rapidly. This is, however, inefficient. Uptake can only be achieved by a large number of porins concentrated into a small amount of time which entails a corresponding energy investment. Once the nutrient is used up, the porins no longer fulfil a function, and there is no return on their investment. Altogether, this results in an inefficient use of resources. Hence, fast nutrient uptake is inefficient independently of the specifics of the model assumptions, simply because it requires diversion of resources into porins.

Discussion and Conclusion

The current model makes a number of assumptions and simplifications. For example, the “infinite population” assumption implicit in the use of differential equations is of limited relevance for biological systems which are known to exhibit substantial noise at the molecular level. A deeper analysis of the system presented here would have to take into account stochastic fluctuations originating from the discrete nature of biochemistry. Yet, simulating such discrete systems is much more difficult than solving differential equations. Hence, for a first analysis differential equations provide a good trade-

off between feasibility and accuracy.

By using our model we found that taking up nutrients slowly is most efficient, but not necessarily the best strategy. Yet, in the absence of competitors the slowest possible growth is the most efficient one. In the hypothetical case of a continuous system with no protein breakdown an infinitely slow take-up rate corresponding to an infinitely slow expression rate of porin would be ideal. In more realistic models that include decay of components, there is an optimal rate of porin creation rate which depends on the rate of porin breakdown. The conclusion is that a group of clonal cells does best when growing very slowly, because then it expends the least amount of energy on maintaining the uptake machine. Only in competition with other cells will faster uptake rates be beneficial.

Not included in the above picture is the cost and the speed of computation. If we allow dynamically changing environments in the model then the picture changes. Environmental changes need to be sensed by the cell which then has to make internal adjustments based on the sensed changes. In the simplest case this is simply the presence and absence of nutrients. It can be shown that the speed with which these adjustment can be made depends directly on the breakdown rate and the speed of uptake. It has been shown recently (Chu et al., 2011) that slow uptake entails a limited ability to adjust to external conditions. On the other hand, faster uptake and growth is required to “compute” changes in the external environment effectively. Doing so comes at a cost in terms of additional nutrient that needs to be expended. Moreover, a hypothetical cell with no breakdown of components is not able to switch to a new state, simply because it is not able to forget its previous state. Say, at some point there are only porins of the first type in the system and these porins occupy all of the available surface, so that no more porins can be created. If then the nutrient of the first type is used up, the cell cannot express any other porins. As such it would miss out on growth opportunities. Similarly, if it can break down porins only slowly, then it will only be able to react slowly to changes in the environment. The conclusion from this is that extremely slow growth is only realistic for populations that live in constant environments that do not require any regulation.

So, in many ways the assumptions that we made in this contribution are somewhat unrealistic with respect to real biological system. Yet still, we think that the conclusions we reached are relevant. While ultra-slow speed will not be achievable in real systems, it is still likely the case that slower growing cells would be more efficient than faster growing ones simply because they will have lower rates of resource wastage. Yet, when in competition with other cells, then the slowest growth rate is no longer feasible and the cell has to invest a high amount of resource for growth. While the details of the evolutionary dynamics will be more complicated in real cells, and the particular trade-offs will be

more involved, the underlying fact that competition requires fast growth and that fast growth is inefficient is likely of very wide general applicability and relevant for our artificial cells and real biological cells alike.

Biological systems are commonly thought of as being optimal. The reasoning is that intense competition between cells will drive biosystems over time to fine-tune their internal processes to a point where resource usage and allocation is most “efficient.” There are a number of well known problems of this optimality assumption. The best known one is that in evolving systems non-optimal, even slightly detrimental traits may piggy-back on advantageous traits and establish themselves in that way. Or, even in very simple fitness landscapes, constant mutational pressure will push the population away from any theoretical optimum generating a *quasi-species* (Eigen and Schuster, 1979). As a result of these and other similar effects biological systems cannot be assumed to be tuned perfectly to an optimum.

Our experiments show an additional biological driver towards inefficiency based on competitive co-evolution. Our results contradict the intuition that competition leads to efficiency. Under some circumstances biological systems are driven away from their most optimal mode of operation. One can now speculate whether or not the same effect applies in other competitive systems, such as in economics where it is routinely argued that competition to the most efficient allocation/use of resources. At least with respect to bacterial growth, our experiments seem to indicate that this is not necessarily so, but competition could lead to less efficient solutions rather than more efficient ones.

References

- Alon, U. (2006). *An Introduction to Systems Biology: Design Principles of Biological Circuits*. Chapman & Hall.
- Alon, U. (2007). Network motifs: theory and experimental approaches. *Nature Review Genetics*, 8(6):450–461.
- Chu, D. (2013). Evolving parameters for a noisy bio-systems. In *2013 IEEE Symposium series on Computational Intelligence*.
- Chu, D., Zabet, N., and Hone, A. (2011). Optimal parameter settings for information processing in gene regulatory networks. *BioSystems*, 104:99–108.
- Eigen, M. and Schuster, P. (1979). *The Hypercycle : A Principle of Natural Self-Organization*. Springer-Verlag, Berlin, Heidelberg and New York.
- Kashtan, N. and Alon, U. (2005). Spontaneous evolution of modularity and network motifs. *Proceedings of the National Academy of Science USA*, 102(39):13773–13778.
- Mangan, S. and Alon, U. (2003). Structure and function of the feed-forward loop network motif. *Proceedings of the National Academy of Science USA*, 100(21):11980–11985.
- New, A., Cerulus, B., Govers, S., Perez-Samper, G., Zhu, B., Boogmans, S., Xavier, J., and Verstrepen, K. (2014). Different levels of catabolite repression optimize growth in stable and variable environments. *PLoS Biol*, 12(1):e1001764.