Evolution of r- and K-selected species of Virtual Microbes: a case study in a simple fluctuating 2-resource environment

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Introduction:

Among other microbes, bacteria have coped with a huge variety of fluctuating and unfavorable environments throughout billions of years of evolution. Adapting to these fluctuations has molded bacteria such as *Escherichia coli* to have really high growth rates when conditions are favorable, but also to survive when conditions are harsh. While *E. coli* can switch between these two modes, these different growth modes are classically used to categorize species as being either r- or K-selected. An r-selected species invests mostly in producing as many offspring as possible, whereas K-selected species live close to carrying capacity and investing all their efforts in surviving while producing fewer offspring. Strikingly, most models of microbial evolution define fitness solely in terms of reproduction probability, while (not) dying is obviously part of "survival and reproduction". Here, we aim to better understand when r- and K-selected growth evolves, and wish to learn something about how *E. coli* has learned to switch between the two.

Contrary to the fluctuating and unpredictable environments *E. coli* evolved in, the Long Term Evolutionary Experiment (LTEE, Lenski *et al.,* 1991) has offered a constant and very predictable environment for *E. coli* to adapt to. After 65.000 generations of evolution, the bacteria show a greatly reduced lag-phase, bigger cells, and outcompete the initial ancestor in competition experiments. Among others, genes related to starvation become expressed less and less (Hindré *et al.,* 2016), suggesting a trend towards more r-selected growth. Furthermore, the bacteria might even anticipate the predictable resource cycle of the experiment. Are the results from the LTEE an expected effect of transferring a complex wild-type into a constant and predictable environment? Is *E. coli* a special case, or could we have expected the same results for any other microbe? We attempt to tackle these questions by using the Virtual Microbe Model (Cuypers *et al.,* 2016) to evolve complex wildtypes, and transferring them to an *in silico* equivalent of the LTEE.

Methods:

The Virtual Microbe Model is an *in silico* model of microbial evolution, where cells convert resources into biomass by taking up and converting influxed metabolites. This implicit fitness is ideal for the questions above, because the cells are selected for their ability to grow as well as their ability to survive in fluctuating environmental conditions, and can freely shape their regulatory network and genome to do so. To evolve complex wildtypes, we initialize a simple 2-resource system in the Virtual Microbe Model (for more details, see table 1). One of these resources (B) is of high energetic value, and can be broken down into the other (less energetic) resource A, and energy (E). Energy is required to transcribe proteins, and to pump resources into the cell. In order to grow, the cells must combine the resources into a third metabolite (C), which is then autonomously invested in cell-growth. The A and B resources flux in at different rates, both temporally and spatially. When/where resources are rare, the cells might not grow sufficiently, which could lead to local extinction. When resources are abundant, cells can grow very rapidly, but these high concentrations also increase the risks of toxicity. Toxicity increases the probability of dying, which is otherwise very minimal (10e-3). Ideally, the Virtual Microbe could have high growth rates by efficiently converting the resources to biomass, without leading to toxic effects. Here we report on a pilot study, where we evolved 3 wildtypes using the same conditions, but with different initial populations.

Results:

For 2 of the simulations, r-selected wildtypes have evolved. These wildtypes are short-lived generalists that take up both resources, dividing frequently while ignoring effects of toxicity. These wildtypes appear to continuously exploit the environment and are only found where resource concentrations are relatively high (less than half of the grid is occupied). These observations of high growth and low population size are typical for r-selected species. Both of these r-selected wild-types appear to have no conserved gene regulatory network.

One of the simulations evolved wild-types that are very different more like K-selected species, which have minimized their death and survive even under very harsh resource conditions. This K-selected species subsequently diverges into two separate specialists, which have partitioned the resources A and B. The A-consumer, seems to be specialized in surviving at low nutrient concentrations, while the B-consumer grows much quicker when empty space is available. In other words, while the common ancestor of these two species appears K-selected, the B-consumer lineage is more r-selected than the A-consumer. Strikingly, after 3000 generations of the B-species, the lineage of the A-consumer only has reproduced for less than 2000 generations. Other than the aforementioned wildtypes, these two species both appear to have functional gene regulatory networks (*e.g.* the B-consumer only imports resource B when the internal concentrations of B decline). The transcription factors for this regulation show high expression, and show a strongly conserved binding profile. These regulatory network could help to cope with fluctuations during the relatively long lives of these cells, and could potentially also be of use to switch from starvation to growth, much like *E. coli* does in the lab when recovering from the lag-phase. In summary, we have shown that the evolution of r- and K-selected species can come about merely as a historical contingency, and can even stably coexist in a small ecosystem.

How do all the evolved wildtypes respond to the periodic protocol of the LTEE? How do they adapt after long term evolution? We continued all populations with the same parameters they evolved in, but periodically transfer a maximum of 300 cells to a new medium. We furthermore disabled Horizontal Gene Transfer (HGT) between cells, to more closely mimic the LTEE. One representative r-selected wildtype (figure 1A) quickly grows out in response to this treatment, and exhausts all the resource before the medium is refreshed. After long-term evolution, it adapts to exploit the resource even quicker (figure 1A, right side), and an average of 5% of the population makes it into the next cycle. The total amount of resources used within one cycle remains more or less the same.

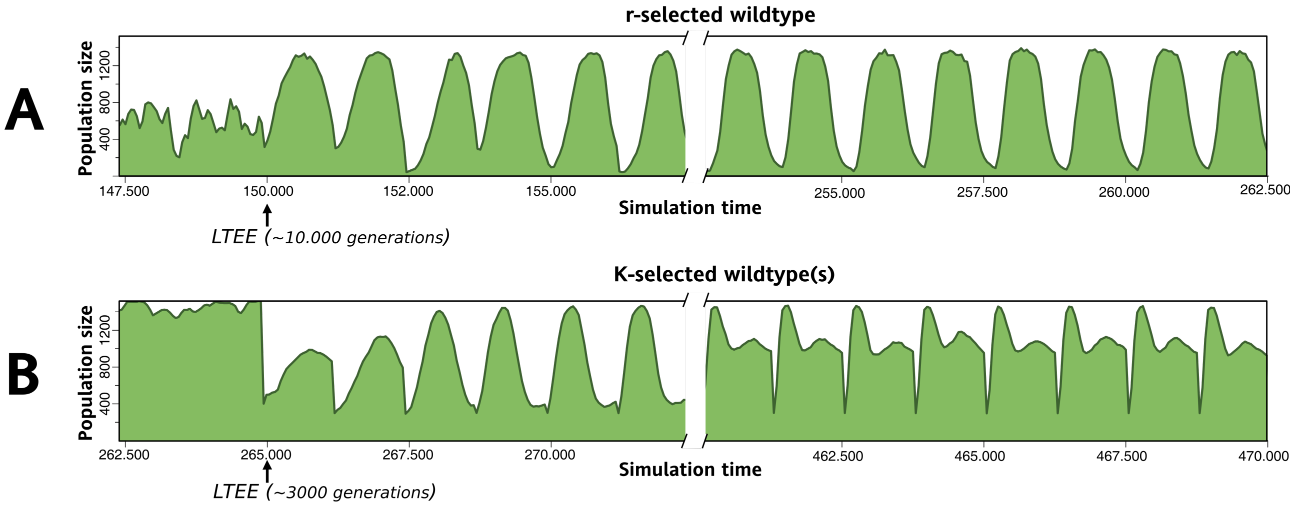

Figure 1: r- and K-selected species adapting to the LTEE. *The population size of the evolved r- and K-selected wildtypes are plotted during the transition to the LTEE, and after long-term evolution (thousands of generations).*

We gave the K-selected species the same treatment. Surprisingly, and despite the small population and strong bottlenecks, both the A-consumer and the B-consumer survive the LTEE for the entire duration of the experiment. The B-consumer shows adaptation similar to the r-selected species, and adapts to more quickly grow after new resources are applied. The A-consumer grows after the B-consumer peaked, and shows a peak of its own after long-term evolution (figure 1B). Evidently, the coexistence in the K-selected regime is very robust. Only when HGT between cells is allowed, we see the emergence of a generalist species that consumes both the A- and B-resource in the LTEE, but not in the native environment.

Discussion:

We have shown that from the same initial conditions, vastly different species of microbes can evolve. The r-selected species are continuously in growth-mode, and ignores the high probability of death because of toxic effects. These species do not seem to evolve conserved gene regulatory networks. The K-selected species shows a much lower growth-rate, and furthermore show conserved regulatory networks. These long-lived cells also show speciation by resource partitioning, with two clearly specialized lineages. In the LTEE, these species keep coexisting, where one species is adapted to quickly exploit the abundant resource after transfer, while the other is adapted to grow much alter, when resources are sparse. The striking difference between the r-selected and K-selected species when adapting to the LTEE shows how evident historical contingency can be, even when the initial conditions are the same.

One could wonder why a generalist that consumes both resource A and B does not emerge after the K-selected species have diverged. A potentially answer is that the species show very optimized internal dynamics, showing near-toxic resource concentrations. Potentially, a generalist can only emerge by first going through a fitness-valley entailed by increasing risks of toxicity. Nevertheless, in the LTEE a generalist does very quickly emerge, as the transition to more r-selected growth modes (*i.e.* with more toxicity*)* is already favored.

In this pilot study we have shown that when reproduction AND survival are both evolvable, both r- and K-selected species can evolve. Moreover, we also show that this historical contingency leads to a clear difference in the population and evolutionary dynamics of the two species, yielding different results when adapting to the LTEE. However, both species adapt to the LTEE by picking up regularity of the protocol, and *E. coli* seems to do the same.

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Refences:

1. Richard E. Lenski, Miacheal R. Rose, Suzanne C. Simpson and Scott C. Tadler *The American Naturalist* Vol. 138, No. 6 (Dec., 1991) pp. 1315-1341
2. Thomas Hindré *et al.,* 2016 (unpublished discussion EvoEvo meeting)
3. Thomas Cuypers *et al.,* 2016 (unpublished Virtual Microbes Model)]

Table 1: Enzymatic reactions in a simple 2-resource system. *A and B are influxed molecules, while C and E (energy) have to be produced by the cells. Cells can discover genes / exchange genes for these reactions via HGT with very low rates. Parameters such as binding constants and Vmax are parameters that are evolvable per gene. The investment of C into cell-growth is an autonomous reaction, and cannot be evolved.*

Enzymatic reactions in 2-resource system	
$A + B \rightarrow C$	$C \rightarrow A + 5E$
$2A + 2E \rightarrow C$	$C \rightarrow 2A + 2E$
$2A \rightarrow B$	$1E \rightarrow$ *pump A, B, C (in or out of the cell)*
$B \rightarrow A + 3E$	[$C \rightarrow$ *Growth of cell*]