# Experimental Tests for an Evolutionary Trade-Off between Growth Rate and Yield in *E. coli*

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*Submitted January 20, 2006; Accepted May 18, 2006; Electronically published July 12, 2006*

abstract: Theoretical studies have predicted a trade-off between growth rate and yield in heterotrophic organisms. Here we test for the existence of this trade-off by analyzing the growth characteristics of 12 *E. coli* B populations that evolved for 20,000 generations under a constant selection regime. We performed three different tests. First, we analyzed changes in growth rate and yield over evolutionary time for each population. Second, we tested for a negative correlation between rate and yield across the 12 populations. Finally, we isolated clones from four selected populations and tested for a negative correlation between rate and yield within these populations. We did not find evidence for a trade-off based on the first two tests. However, we did observe a trade-off based on the within-population correlation of yield and rate. Our results indicate that, at least for the populations studied here, an analysis of the within-population diversity might be the most sensitive test for the existence of a trade-off. The observation of a trade-off within, but not between, populations suggests that the populations evolved different genetic solutions for growth in the selective environment, which in turn led to different physiological constraints.

*Keywords:* trade-off, ATP production, selection for growth rate, experimental evolution.

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Am. Nat. 2006. Vol. 168, pp. 242–251. 

Adenosine triphosphate (ATP) is a key compound in the energy metabolism of cells. Its degradation into adenosine diphosphate (ADP) and phosphate is generally used to drive thermodynamically unfavorable reactions, such as active transport and biosynthesis. Thus, ATP has to be continuously regenerated for cellular growth. In heterotrophic organisms, the production of ATP is coupled to the degradation of energy-rich organic compounds. Pfeiffer et al. (2001) recently postulated that a trade-off exists between rate and yield in heterotrophic ATP production. ("Rate" refers here to units of ATP produced per unit of time and "yield" to units of ATP produced per unit of resource.) This trade-off can be observed, for example, if organisms use alternative ATP-producing pathways with opposing properties in yield and rate, such as respiration and respirofermentation (i.e., using respiration only vs. using fermentation in addition to respiration). On a more fundamental level, such a trade-off arises from thermodynamic constraints because the free energy difference between substrate and product in an ATP-producing pathway is divided into a part that is used to phosphorylate ADP to ATP and a part that is used to drive the pathway. This division causes a trade-off between yield and rate of ATP production because the larger is the part used to produce ATP, the slower is the pathway (Pfeiffer and Bonhoeffer 2002).

Under energetic limitation, the rate of cell growth and the biomass yield are determined by the rate and yield of ATP production (Bauchop and Elsden 1960; Dykhuizen and Dean 1990; Helling 2002). Because energetic limitation may be common for heterotrophic organisms under natural conditions, we expect that the properties of ATP-producing pathways have been under strong selection during evolution. The existence of a trade-off between rate and yield of ATP production thus leads to the expectation that under environmental conditions that select for an increasing rate of ATP production, a corresponding decrease should be observed in yield.

The trade-off may be measured and tested if the following two conditions are met. First, the populations must have evolved in an environment that selects primarily for

fast growth and hence for fast ATP production. Theory and experiments show that competition for shared energy resources represents such a selective environment (Vasi et al. 1994; Pfeiffer et al. 2001; Fong and Palsson 2004). Second, the populations need to have evolved for a sufficiently long time in that selective environment for the trade-off to have become manifest because organisms that are not well adapted to this selective environment might be able to improve in growth (or ATP production) rate without any associated cost in yield, as can be seen in standard laboratory strains (Fischer and Sauer 2005).

To test for the existence of the trade-off between rate and yield, we measured changes in growth rate and yield in 12 populations of *E. coli* B that have evolved independently in a long-term evolution experiment in competition for a shared energy resource (Lenski et al. 1991; Lenski and Travisano 1994; Vasi et al. 1994; Travisano et al. 1995; Elena et al. 1996; Travisano and Lenski 1996; Elena and Lenski 1997; Cooper and Lenski 2000; Cooper et al. 2003; Lenski 2004; Crozat et al. 2005; Rozen et al. 2005). In this long-term evolution experiment, the bacteria were diluted and transferred daily into fresh minimal medium, resulting in more than 20,000 generations of binary fission. During each 24-h transfer cycle, the bacteria go through a lag phase, a phase of exponential growth, and a stationary phase. The population density at stationary phase is determined by the glucose concentration in the minimal medium; in other words, the exhaustion of the energy resource glucose limits the net growth of the population.

In summary, the populations analyzed here evolved in competition for a shared, limiting energy resource. Under these experimental conditions, strong selection for growth rate is expected (Pfeiffer et al. 2001). Although evolutionary adaptations could occur in all three phases of growth, the main changes were observed toward faster growth rate and shorter lag phase (Vasi et al. 1994; Cooper et al. 2003), with selection for growth rate indeed being the strongest force based on both a priori modeling and the observed response to selection (Vasi et al. 1994). Over the course of 20,000 generations, the average growth rate that was realized in competition with the ancestor increased by some 70% (Lenski and Travisano 1994; Cooper and Lenski 2000).

The existence of a trade-off between growth rate and yield might, in theory, be tested in several ways. First, over evolutionary time, increases in growth rate could be associated with decreases in yield. However, a population that is placed into a new selective environment may not be close to the hypothetical trade-off line. Hence, it is possible that, at least initially, growth rate and yield could increase concomitantly (fig. 1*A*). Second, the adaptation of independently evolving lines might lead them to distinct points on the presumed trade-off curve (fig. 1*B*). In this case, we would expect a negative correlation between growth rate and yield across the replicate populations. Finally, a trade-off might arise within some or all of the evolving populations. In this case, we would expect to see a negative correlation between growth rate and yield among clones from individual populations. In this study, we use all three of these approaches to test for the existence of a trade-off between growth rate and yield. In closing this introduction, it should be noted that experiments with evolving *Drosophila* populations found evidence for a trade-off between growth rate and carrying capacity (Mueller et al. 1991), although the mechanistic bases for that trade-off are presumably different from those in bacterial populations that form the basis for our theory and experiments.

## Methods

### *Long-Term Serial Transfer*

We used 12 populations of *E. coli* B that were serially propagated for 20,000 generations at 37°C in Davis minimal medium supplemented with glucose at 25 $\mu$g mL$^{-1}$ (DM25). Six populations each were derived from two clones, which differed by only a single marker (arabinose utilization) that is selectively neutral in this environment (Lenski et al. 1991). Every 24 h, the 12 populations were diluted 100-fold into fresh medium, and the resulting regrowth corresponds to ~6.6 ($\log_2 100$) generations of binary fission per day. In this serial transfer regimen, the bacterial population shows a lag phase followed by a period of exponential growth and a stationary phase after the glucose is depleted. At generations 2,000, 5,000, 10,000, 15,000, and 20,000, aliquots containing population samples (i.e., whatever clones were present) were stored in 20% glycerol at −80°C. Additional information on this long-term evolution experiment, including a description of the founding *E. coli* B strain, can be found elsewhere (Lenski 2004; http://myxo.css.msu.edu/ecoli/).

### *Isolation of Clones from Generation 20,000*

Four populations that evolved high growth rates—designated Ara − 1, Ara − 2, Ara − 3, and Ara + 3—were chosen for detailed analyses of the within-population correlation between growth rate and yield. Clones were isolated from the 20,000-generation samples from each of these populations in the following manner. After overnight growth in DM25, a sample of the population was diluted and spread onto a Luria-Bertani agar plate and incubated overnight at 37°C. The next day, 92 clones were picked from the plates, inoculated into DM25, and grown overnight. The following day, these clonal cultures were stored

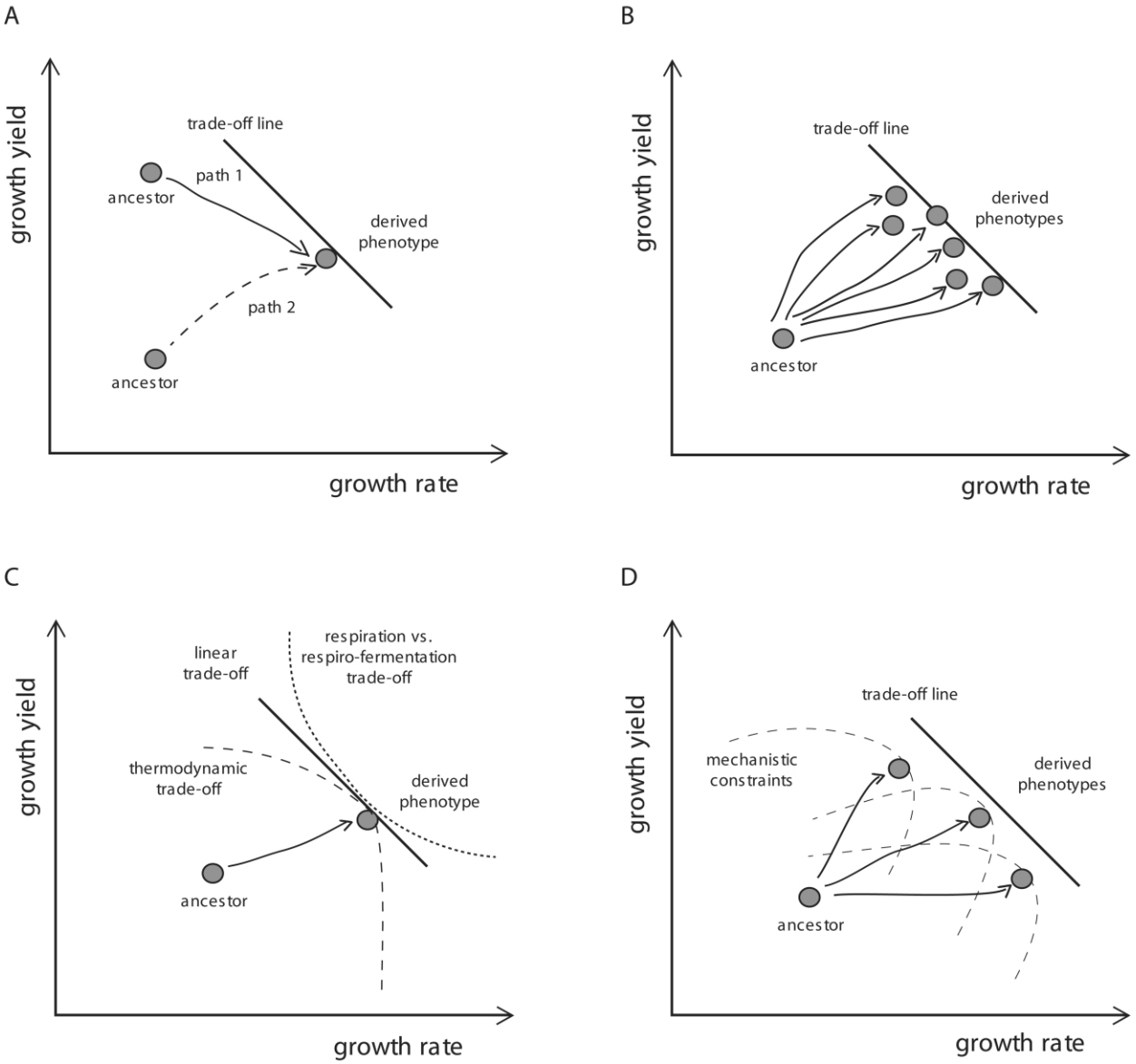


**Figure 1:** Diverse pathways of adaptation toward a hypothetical trade-off line. When a population is placed in a new environment where faster growth is selectively favored, it may initially be far from the hypothesized trade-off curve. This figure illustrates several different hypothetical scenarios for the evolutionary approach to a trade-off between growth rate and yield. *A*, Over evolutionary time, increases in growth rate could be associated with decreases in yield (*path 1*). On the other hand, if the population starts with a growth yield that is below the eventually attained yield on the trade-off line, then yield and rate may both increase over evolutionary time (*path 2*). *B*, Independently evolving populations. A trade-off between the traits measured in independently evolving populations may be observed if all of them eventually achieve states that are either on or sufficiently close to the trade-off line. *C*, Shape of the trade-off function. The trade-off between growth rate and yield is not necessarily a linear relation (*solid line*). As shown previously (Pfeiffer and Bonhoeffer 2002), the thermodynamic trade-off implies a concave shape with a maximal rate (*dashed line*). Once the phenotype has achieved this rate, it cannot further increase the rate at the cost of yield. The presence of pathways with opposing properties in rate and yield (such as complete oxidation of glucose vs. degradation into acetate) implies a convex shape (*dotted line*). Here, to observe the trade-off might be difficult because, given a sufficiently high initial rate, any increase in rate may have only small costs in yield. *D*, Mechanistic constraints. The biochemical mechanisms of ATP production may further influence the simultaneous evolution of growth rate and yield (*dashed lines*). As a consequence, replicate populations may evolve different biochemical solutions that adapt them to the same experimental environment. Thus, they may evolve toward different fitness peaks, so that each population then faces somewhat different mechanistic constraints.

in 20% glycerol at −20°C for immediate use and at −80°C for long-term keeping.

### *Measurement of Growth Kinetics*

Growth kinetics were assayed in microtiter plates in order to screen large numbers of samples (12 replicate populations from five generations + two ancestral forms + 92 clones from four populations = total of 430 samples) with substantial statistical replication (up to 229 replicates for the ancestral forms, up to 40 for each of the evolved populations, and five for each 20,000-generation clone).

Before we measured growth kinetics, all genotypes were taken from the −20°C or −80°C freezer stocks and conditioned to growth in DM25 at 37°C. To this end, the bacteria were grown overnight in 96-well microtiter plates containing Davis minimal medium supplemented with glucose at 1,000 $\mu$g mL$^{-1}$ (DM1000), then diluted via 0.85% saline solution into DM25 and grown again overnight. From these conditioned cultures, 20 $\mu$L were transferred into 180 $\mu$L of fresh DM25, incubated at 37°C, and shaken at 400 rpm (shaking radius 3 mm) in flat-bottom microtiter plates. Optical density (OD) was measured at a wavelength of 600 nm using a SpectraMax 340PC microplate spectrophotometer. About 12 OD measurements were taken during the 7–8 h before cultures reached stationary phase. Because of the low glucose concentration in DM25, only about 30% of the final OD values represent absorption of the bacterial cells at stationary phase, with the remainder due to the medium and plastic. This background adsorption was corrected as described below.

### *Numerical and Statistical Analyses*

To quantify the growth kinetics of the populations and clones, we analyzed the output files of the SpectraMax software as follows. We first subtracted the values of blanks that were individually measured for each well of a plate before inoculation. We discarded all measurements that were below the detection limit (OD < 0.002) or were above the OD value of 0.02, indicating the presence of air bubbles in the well. The log-transformed measurements were then used to fit a stepwise linear model for microbial growth for each well (Buchanan et al. 1997). This model assumes three distinct growth phases: lag phase with constant OD, log phase with exponentially increasing OD, and stationary phase with constant OD. The duration of lag phase could not be observed directly because of the low concentration of glucose in DM25, so that a population had an initial OD below the detection limit of the spectrophotometer. However, the duration could be inferred indirectly because the initial true OD should be one-tenth the final measured OD, given the known 1 : 10 dilution from the previous stationary-phase culture into the same medium. We fitted three parameters to the stepwise model, namely, the length of the lag phase (tlag), the maximum growth rate ($v$), and the final yield ($y$), which corresponds to the stationary-phase OD. We checked the quality of the fits and removed those growth curves that were based on fewer than nine measurements or that had an unacceptably high deviation between measurements and fitted model (residual sum of squares of the log-transformed data > 0.05). In total, we excluded about 5% of the fitted growth curves.

We computed the mean values of the exponential growth rate and growth yield by averaging up to 229 replicate measurements for each ancestral variant and up to 40 replicates for each of the 12 derived populations (minus the values excluded as described above). To test for the relationship between growth rate and yield across the 12 independently evolved populations, we performed a correlation analysis using the means estimated for each population at generation 20,000. For four of the populations, the within-population variance was estimated by performing an ANOVA using up to five repeated measures of rate and yield for the clones sampled from the population. To minimize the impact of a potential plate effect, the repeated measures of clones were done on different microtiter plates. Furthermore, we calculated the mean rate and yield for all sampled clones. For each of the four populations, we tested the within-population relationship between mean growth rate and yield by calculating the correlation across the sampled clones. One of these populations has evolved a stable dimorphism (Rozen and Lenski 2000; Rozen et al. 2005). We therefore partitioned the clones of this population based on their rate and yield into two corresponding clusters by applying a "partitioning around medoids" algorithm (Kaufman and Rousseeuw 1990), using Euclidean distances of standardized data (mean rates and yields of the clones). For each of the resulting clusters, we then separately calculated the correlations between mean rate and yield across the corresponding clones.

## Results

### *Evolution of Growth Rate and Yield*

To test for the possible existence of a trade-off between growth rate and yield, we first measured growth rate and yield of the ancestral strain and populations sampled from the evolved selection lines at generations 2,000, 5,000, 10,000, 15,000, and 20,000. A trade-off would be manifest if increases in growth rate were associated with decreases in yield, at least after an initial period of adaptation to the novel environment, when both rate and yield might increase. This outcome would be observed only if the path

of adaptation led over regions of higher than optimal yield (fig. 1*A*, *path 1*). If the ancestral strain was sufficiently far from the hypothetical constraint, however, then both rate and yield might increase throughout the experiment (fig. 1*A*, *path 2*). Figure 2 shows the changes in the rate and yield parameters for each of the 12 replicate populations over evolutionary time. Consistent with previous reports (Lenski et al. 1991; Vasi et al. 1994), we observed rapid increases in both rate and yield in all populations during the first 2,000 generations. After generation 2,000, the general trend (fig. 2, *dashed line*) over all 12 populations was that growth rate continued to increase, while the yield

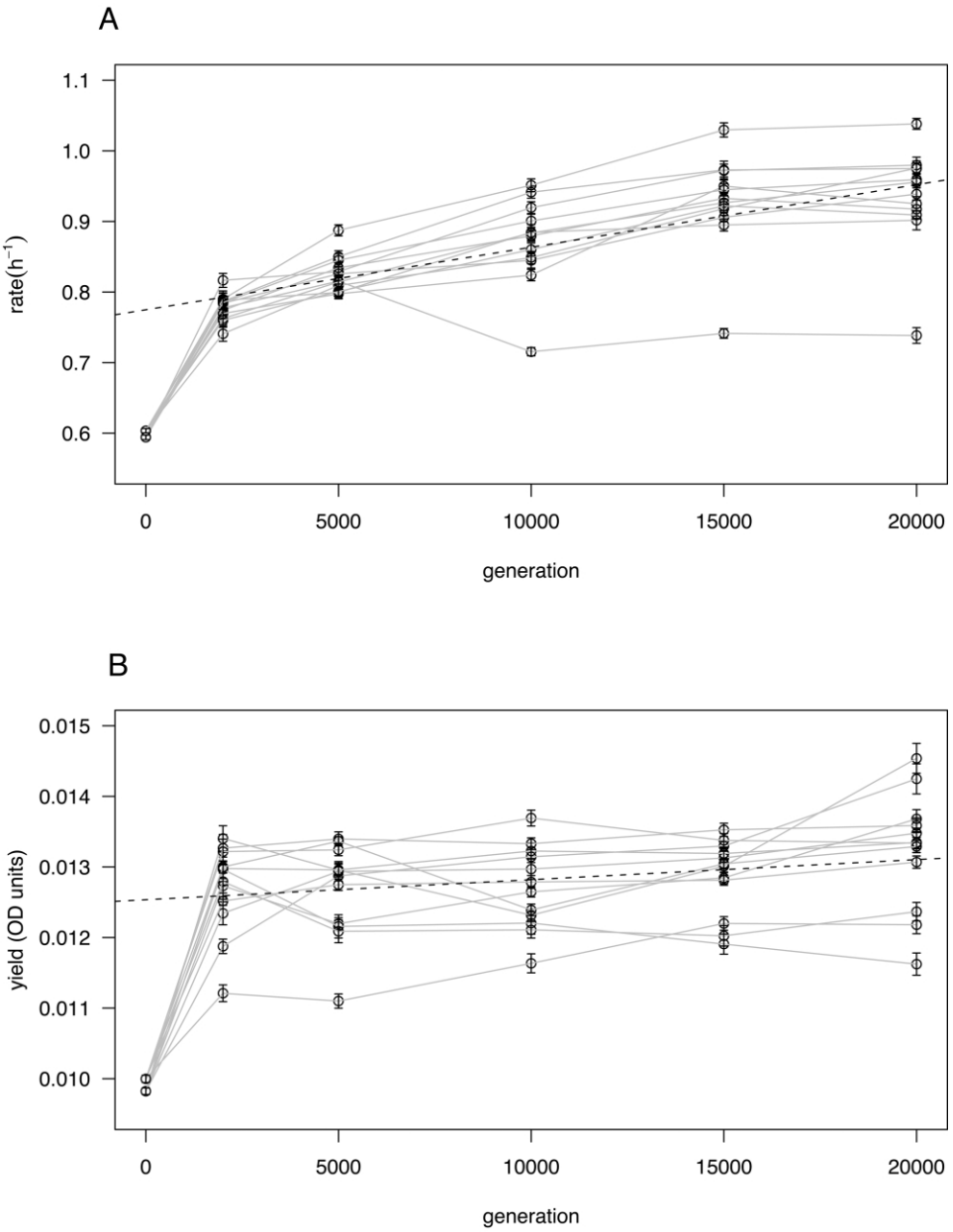


**Figure 2:** Evolution of growth rate and yield during 20,000 generations: trajectories of rate (*A*) and yield (*B*) for 12 independently evolving populations as a function of time, in generations. The two ancestral variants, which differ only by a neutral marker, are presented at generation 0. Each value is the mean of up to 40 observations, except for the two ancestral variants, each of which is the mean of approximately 200 measurements. Error bars represent standard errors. In all 12 populations, a rapid increase in both rate and yield was observed in the first 2,000 generations. Afterward, the overall trend (*dashed lines*) was that growth rate continued to increase, while growth yield remained nearly constant.

stayed more or less constant. Thus, we do not find any evidence for the hypothesized trade-off between growth rate and yield based on the net evolutionary changes in these growth characteristics over time. The temporal pattern and extent of increase in growth rate are generally consistent with the fitness gains measured for these populations during 20,000 generations (Lenski and Travisano 1994; Lenski et al. 1998), except for those of population Ara + 6 (lowest curve in fig. 2*A*). That population shows fitness gains in DM25, based on competition experiments in flasks, similar to those measured in the other populations, but it grows poorly in some conditions—for example, Ara + 6 cells form microcolonies on a certain agar medium where the ancestor and other evolved populations produce normal-sized colonies (R. E. Lenski, personal observation). Some difference between microtiter plates and flasks as growth environments, despite our using the same DM25 medium, might account for the outlier status of this population in our experiments.

### *Correlation across the Independently Evolved Populations*

A trade-off could also be manifest if the independent lines evolved toward distinct points on the presumed trade-off curve (fig. 1*B*). We therefore tested for a negative correlation between growth rate and yield across the replicate populations. Figure 3 shows the mean growth rate and mean yield of the 12 evolved populations at generation 20,000. There is no negative correlation between these two parameters; in fact, the observed correlation is weakly, but not significantly, positive ($r = 0.41$, df = 10, $P = .18$). The correlation between rate and yield becomes negative but remains nonsignificant if we exclude the Ara + 6 population that showed the anomalous decline in growth rate as measured in the microtiter plates ($r = -0.25$, df = 9, $P = .46$). Thus, we also find no evidence for the hypothesized trade-off between growth rate and yield across the independently evolved populations.

### *Evolutionary Trade-Off within the Evolved Populations*

A trade-off could also be manifested as a negative correlation between growth rate and yield among clones sampled from single populations. To look for possible trade-offs within populations that had evolved high growth rates at generation 20,000, we plot growth yield versus growth rate for 92 randomly chosen clones isolated from each of four selected populations (fig. 4). An ANOVA showed significant variation among clones in all four populations for rate (Ara − 1: $F = 2.78$, df = 91, 333; Ara − 2: $F = 10.63$, df = 88, 341; Ara − 3: $F = 6.42$, df = 90, 350; Ara + 3: $F = 5.56$, df = 89, 307; for all tests, $P < .0001$) and yield (Ara − 1: $F = 4.96$, df = 91, 333; Ara − 2: $F = 13.2$, df = 88, 341; Ara − 3: $F = 8.33$, df = 90, 350; Ara + 3: $F = 5.02$, df = 89, 307; for all tests, $P < .0001$). The ANOVA demonstrates the existence of clonal variation in

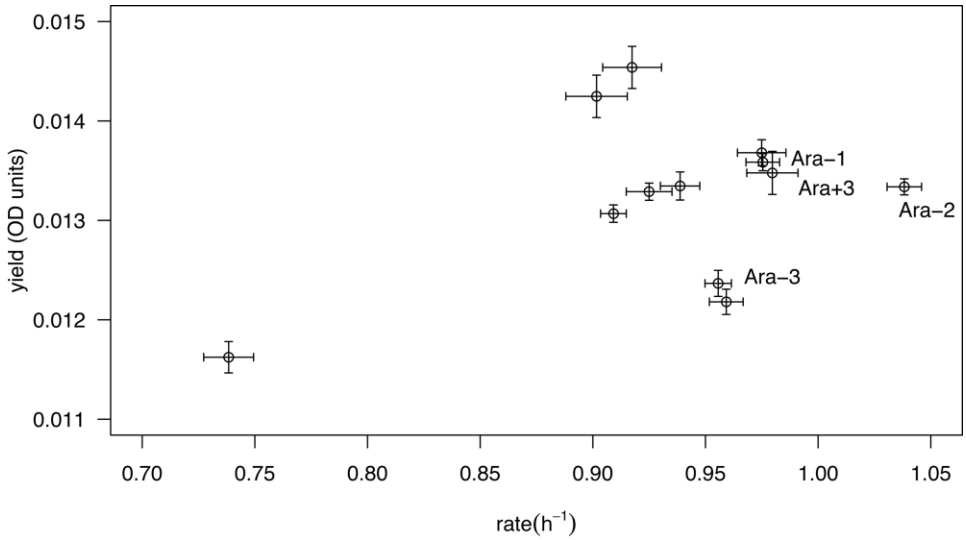


**Figure 3:** Trade-off between growth rate and yield tested across the independently evolved populations: growth yield versus rate of evolved populations at generation 20,000. Each value is the mean of up to 40 measurements of a single evolved population. Error bars represent standard errors. We tested the between-population relationship between growth rate and yield by calculating the correlation across these 12 populations. No significant correlation was observed either including or excluding the atypical population seen at the lower left; details of the statistical results are given in the text. The populations from which we subsequently isolated clones are labeled as Ara − 1, Ara − 2, Ara − 3, and Ara + 3 (see fig. 4).

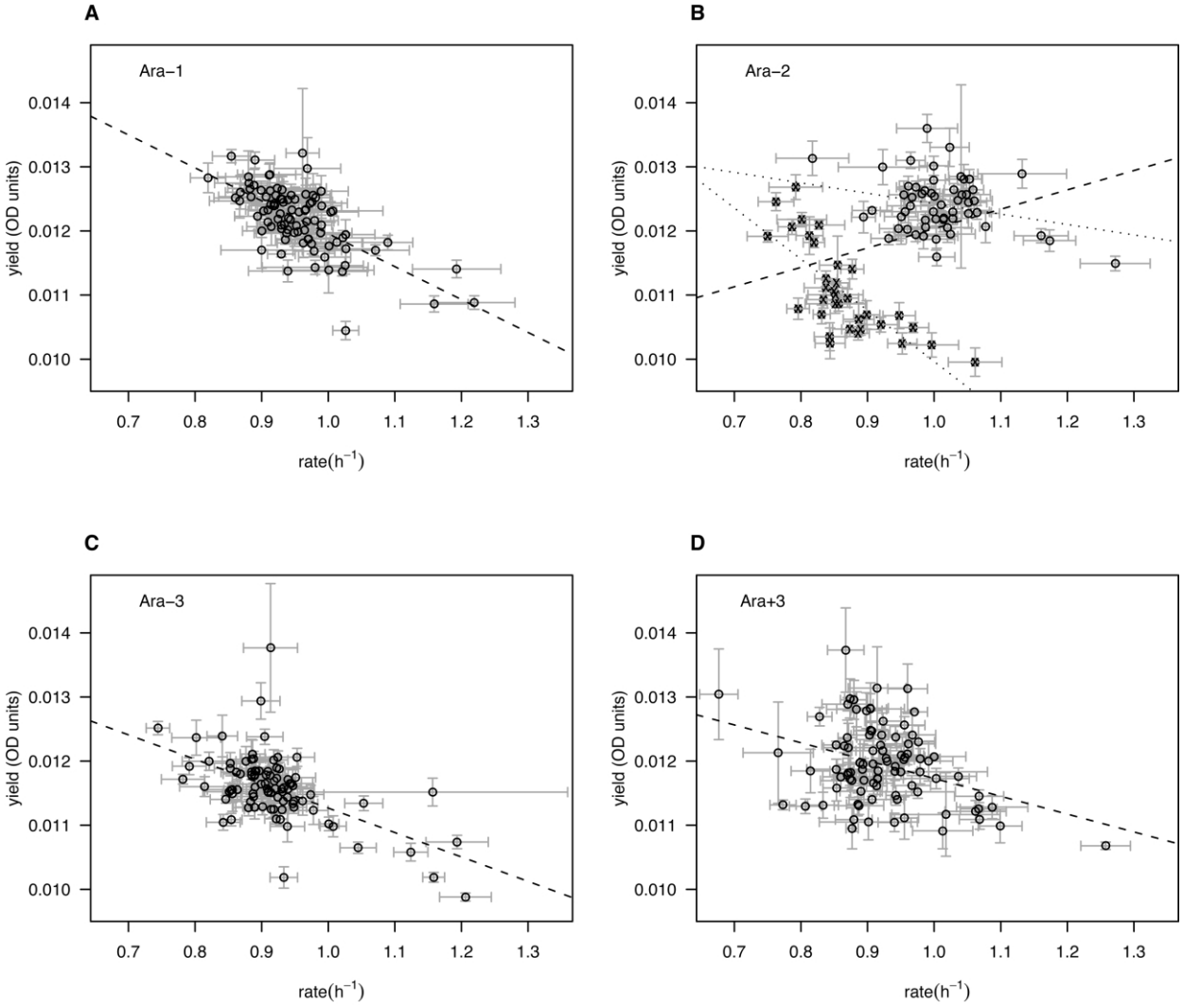


**Figure 4:** Trade-off between growth rate and yield across clones within particular evolved populations: growth yield versus rate estimated for 92 clones sampled from each of four populations at generation 20,000 (*A*, Ara − 1; *B*, Ara − 2; *C*, Ara − 3; *D*, Ara + 3). Each value is the mean of up to five measurements. Error bars represent standard errors. Three of the four populations show a highly significant negative correlation (*dashed lines*). The remaining population shows a significant positive correlation over all clones (*B*, *dashed line*), but it has been previously shown to have evolved a stable dimorphism. Based on their rate and yield data, we grouped the clones of this population into two clusters, referred to as cluster 1 (*circles*) and cluster 2 (*crosses*). Within both clusters, we observe a significant negative correlation (*dotted lines*). Details on the clustering algorithm and all statistical results are given in the text.

growth rate and yield within each population, and previous research has also demonstrated within-population genetic variability maintained by some combination of mutation-selection balance, selective sweeps including clonal interference, and frequency-dependent selection (Elena and Lenski 1997; Gerrish and Lenski 1998; Papadopoulos et al. 1999; Rozen and Lenski 2000; Rozen et al. 2005). Thus, it is meaningful to test whether growth rate and yield exhibit a negative genetic correlation at the within-population level. In three of the four populations, we observed highly significant negative correlations between growth rate and yield, although in one of the populations a significant positive correlation was found (Ara − 1: $r = -0.67$, df = 90, $P < .0001$; Ara − 2: $r = 0.35$, df = 87, $P = .0008$; Ara − 3: $r = -0.57$, df = 89, $P < .0001$; Ara + 3: $r = -0.36$, df = 88, $P = .0005$). Interestingly, the population showing a positive correlation is known to have evolved a stable dimorphism that has persisted since early in the experiment (Rozen and Lenski 2000; Rozen et al. 2005), and it also shows distinct clusters in the rate-yield plot (fig. 4*B*). We

therefore applied a clustering algorithm to partition the clones into two clusters, based on their mean rate and yield (see "Methods"). Within each of the resulting clusters (fig. 4*B*), we then observed a significant negative correlation between rate and yield ($r = -0.27$, df = 53, $P = .047$; and $r = -0.74$, df = 32, $P < .0001$, for clusters 1 and 2, respectively). Thus, in summary, we find evidence for a significant trade-off between growth rate and yield in three out of four populations. We also find support for the hypothesized trade-off within the two subpopulations, identified by the clustering algorithm, of the remaining population.

To test whether any unexpected artifacts from environmental influences or the growth-curve fitting procedure might explain these consistently negative correlations, we calculated the correlation between growth rate and yield across many replicates of the ancestral variants measured on different microtiter plates and on different days. The results of the correlation tests performed on about 200 repeated measurements of each of the ancestors show weak, but significant, positive correlations ($r = 0.16$, df = 201, $P = .018$ for the Ara− ancestor; $r = 0.19$, df = 208, $P = .007$ for the Ara+ ancestor). This pattern indicates that the negative correlations found in the tests using the evolved clones cannot be attributed to artifacts or the fitting procedure.

## Discussion

In this study, we used three different approaches to test for the existence of an evolutionary trade-off between growth rate and yield in a long-term experiment with *E. coli*. First, we analyzed changes in rate and yield over evolutionary time. Second, we tested for a trade-off across independently evolved populations at generation 20,000. Finally, we tested for a trade-off between clones within four of the evolved populations. The three approaches yield different results. Although the first two approaches do not support the trade-off hypothesis, we found a negative correlation between rate and yield in at least three out of four populations in the last analysis.

We find it difficult to believe that a trade-off that ultimately derives from fundamental thermodynamic principles should not exist. However, it is possible that this physical trade-off is of limited biological relevance because biochemical pathways may not operate close enough to the thermodynamic constraints. The absence of evidence for the trade-off in the first two approaches could be interpreted along these lines. However, one should also bear in mind that these approaches have certain limitations, which might explain their failure to support the trade-off hypothesis.

A general limitation to finding a trade-off in an experimental setting is that selection for certain other properties may indirectly affect the quantities of interest. In our case, selection to maintain survival in stationary phase, for example, could, at least in theory, lead to indirect selection for higher yield, which would thus be prevented from decreasing over the long term. This limitation would especially compromise the first approach we used to test the trade-off hypothesis. A further explanation for the absence of a negative association between growth rate and yield over 20,000 generations of experimental evolution is that the populations were initially not well adapted to the selective environment. Therefore, they may have been able to increase growth rate without having to decrease yield as they evolved toward the hypothetical trade-off line (fig. 1*A*). Furthermore, the trade-off line is not necessarily linear (fig. 1*C*). As we have shown earlier, thermodynamic considerations predict a concave trade-off (Pfeiffer and Bonhoeffer 2002). In particular, thermodynamic constraints imply that there is a maximal rate. Once a phenotype has evolved this maximal rate, then a decrease in yield cannot be traded off against any further increase in rate. In contrast, a trade-off resulting from the presence of pathways with opposing properties in rate and yield, such as complete oxidation of glucose versus partial degradation into acetate, results in a convex function (Pfeiffer and Bonhoeffer 2002). A convex shape of the trade-off implies that a population can increase the rate at a small cost in terms of yield. Thus, we cannot exclude either a convex or a concave shape of the trade-off curve, and either situation may make a trade-off more difficult to detect than in the linear case.

During 20,000 generations, many significant evolutionary adaptations have occurred (Lenski and Travisano 1994; Vasi et al. 1994; Cooper et al. 2001). Nonetheless, it is conceivable that the populations may not have had enough time to get sufficiently close to the hypothetical trade-off line, which might explain why we do not find a trade-off in the second analysis. A negative association between rate and yield across the populations is expected only if all of them are close to the trade-off line (fig. 1*B*). A further possibility is that the replicate populations climbed different fitness peaks in the adaptive landscape (fig. 1*D*). Such independent evolution of replicate populations is a consequence of chance events and is supported by evidence of sustained divergence in mean fitness between populations (Lenski and Travisano 1994) as well as by patterns of epistasis observed between at least some beneficial mutations in the evolving populations (Cooper et al. 2003). These different fitness peaks represent alternative mechanistic solutions for conversion of glucose into biomass, each of which may be subject to somewhat different biochemical constraints.

Under this scenario, we might still expect a trade-off

among clones from within individual populations rather than between populations. Indeed, in three out of four populations we find a highly significant negative correlation between growth rate and yield. This finding provides support for the existence of the trade-off. The one population that showed significant positive correlation evolved a stable dimorphism (Rozen and Lenski 2000), which may mask an underlying trade-off within each of the corresponding clades. After grouping the clones of this population into two clusters based on their rate and yield, we indeed observe a negative correlation within each cluster. The fact that we found a negative correlation between rate and yield only within populations suggests that this approach is the most sensitive test for detecting a trade-off, especially when multiple populations allow several independent tests of the trade-off, as in this study.

In summary, we used bacterial populations from a long-term evolution experiment to test for the existence of a trade-off between growth rate and growth yield. Using samples of independently evolving populations from different time points of the experiment, we had the opportunity to use three different approaches for detecting the trade-off. Careful consideration of the three approaches reveals possible obstacles in detecting trade-offs between life-history traits. In particular, the outcomes of these three approaches are likely to be differently affected by the selection regime, the properties of the ancestor that may depend on its evolutionary history, and the influence of chance events on the independently evolving populations. Indeed, the three tests yield different results in our study. We find strong support for the existence of a trade-off between growth rate and yield based only on the pattern of diversity within the evolved populations. In our view, the evidence for the trade-off in this analysis weighs more heavily than the absence of support from the other two analyses, which appear to reflect the experimental limitations discussed above. However, to settle this issue more definitively, an entirely new set of evolution experiments may need to be run, ideally using the same ancestral strain and similar environmental conditions. In particular, the potential for confounding effects resulting from indirect selection for higher yield during stationary phase could be eliminated by evolving the bacteria under constant exponential-phase growth at their maximal rate.

## Acknowledgments

We gratefully acknowledge support from an internal Eldgenössische Technische Hochschule grant to S.B. and U.S. and a grant from the National Science Foundation to R.E.L.

## Literature Cited

Bauchop, T., and S. R. Elsden. 1960. The growth of micro-organisms in relation to their energy supply. Journal of General Microbiology 23:457–469.

Buchanan, R. L., R. C. Whiting, and W. C. Damert. 1997. When is simple good enough? a comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. Food Microbiology 14:313–326.

Cooper, T. F., D. E. Rozen, and R. E. Lenski. 2003. Parallel changes in gene expression after 20,000 generations of evolution in *Escherichia coli*. Proceedings of the National Academy of Sciences of the USA 100:1072–1077.

Cooper, V. S., and R. E. Lenski. 2000. The population genetics of ecological specialization in evolving *Escherichia coli* populations. Nature 407:736–739.

Cooper, V. S., A. F. Bennett, and R. E. Lenski. 2001. Evolution of thermal dependence of growth rate of *Escherichia coli* populations during 20,000 generations in a constant environment. Evolution 55:889–896.

Crozat, E., N. Philippe, R. E. Lenski, J. Geiselmann, and D. Schneider. 2005. Long-term experimental evolution in *Escherichia coli*. XII. DNA topology as a key target of selection. Genetics 169:523–532.

Dykhuizen, D. E., and A. M. Dean. 1990. Enzyme activity and fitness: evolution in solution. Trends in Ecology & Evolution 5:257–262.

Elena, S. F., and R. E. Lenski. 1997. Long-term experimental evolution in *Escherichia coli*. VII. Mechanisms maintaining genetic variability within populations. Evolution 51:1058–1067.

Elena, S. F., V. S. Cooper, and R. E. Lenski. 1996. Punctuated evolution caused by selection of rare beneficial mutations. Science 272:1802–1804.

Fischer, E., and U. Sauer. 2005. Large-scale in vivo flux analysis shows rigidity and suboptimal performance of *Bacillus subtilis* metabolism. Nature Genetics 37:636–640.

Fong, S. S., and B. O. Palsson. 2004. Metabolic gene-deletion strains of *Escherichia coli* evolve to computationally predicted growth phenotypes. Nature Genetics 36:1056–1058.

Gerrish, P. J., and R. E. Lenski. 1998. The fate of competing beneficial mutations in an asexual population. Genetica 102–103:127–144.

Helling, R. B. 2002. Speed versus efficiency in microbial growth and the role of parallel pathways. Journal of Bacteriology 184:1041–1045.

Kaufman, L., and P. J. Rousseeuw. 1990. Finding groups in data: an introduction to cluster analysis. Wiley Interscience, New York.

Lenski, R. E. 2004. Phenotypic and genomic evolution during a 20,000 generation experiment with the bacterium *Escherichia coli*. Plant Breeding Reviews 24:225–265.

Lenski, R. E., and M. Travisano. 1994. Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations. Proceedings of the National Academy of Sciences of the USA 91:6808–6814.

Lenski, R. E., M. R. Rose, S. C. Simpson, and S. C. Tadler. 1991. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. American Naturalist 138: 1315–1341.

Lenski, R. E., J. A. Mongold, P. D. Sniegowski, M. Travisano, F. Vasi, P. J. Gerrish, and T. M. Schmidt. 1998. Evolution of competitive fitness in experimental populations of *E. coli*: what makes one genotype a better competitor than another? Antonie Van Leeuwenhoek 73:35–47.

Mueller, L. D., P. Z. Guo, and F. J. Ayala. 1991. Density-dependent natural selection and trade-offs in life history traits. Science 253: 433–435.

Papadopoulos, D., D. Schneider, J. Meier-Eiss, W. Arber, R. E. Lenski, and M. Blot. 1999. Genomic evolution during a 10,000-generation experiment with bacteria. Proceedings of the National Academy of Sciences of the USA 96:3807–3812.

Pfeiffer, T., and S. Bonhoeffer. 2002. Evolutionary consequences of tradeoffs between yield and rate of ATP production. Zeitschrift für Physikalische Chemie 216:51–63.

Pfeiffer, T., S. Schuster, and S. Bonhoeffer. 2001. Cooperation and competition in the evolution of ATP-producing pathways. Science 292:504–507.

Rozen, D. E., and R. E. Lenski. 2000. Long-term experimental evolution in *Escherichia coli*. VIII. Dynamics of a balanced polymorphism. American Naturalist 155:24–35.

Rozen, D. E., D. Schneider, and R. E. Lenski. 2005. Long-term experimental evolution in *Escherichia coli*. XIII. Phylogenetic history of a balanced polymorphism. Journal of Molecular Evolution 61: 171–180.

Travisano, M., and R. E. Lenski. 1996. Long-term experimental evolution in *Escherichia coli*. IV. Targets of selection and the specificity of adaptation. Genetics 143:15–26.

Travisano, M., J. A. Mongold, A. F. Bennett, and R. E. Lenski. 1995. Experimental tests of the roles of adaptation, chance, and history in evolution. Science 267:87–90.

Vasi, F., M. Travisano, and R. E. Lenski. 1994. Long-term experimental evolution in *Escherichia coli*. II. Changes in life-history traits during adaptation to a seasonal environment. American Naturalist 144:432–456.

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