JOURNAL OF BACTERIOLOGY, Sept. 1996, p. 5333–5334 Vol. 178, No. 17
0021-9193/96/$04.00+0

Experimental Rejection of a Nonadaptive Explanation for Increased Cell Size in *Escherichia coli*

JUDITH A. MONGOLD* AND RICHARD E. LENSKI

Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824-1325

Received 25 March 1996/Accepted 20 June 1996

Populations of *Escherichia coli* that have been serially propagated for thousands of generations in glucose minimal medium show heritable increases in both cell size and growth rate. We sought to test the hypothesis that the increased cell size of the derived genotypes could be explained solely by their faster growth. The regression of cell size on growth rate differed significantly between populations having ancestral and derived genotypes, with the latter producing larger cells over almost the entire range of growth rates. Thus, the physiological coupling between cell size and growth rate has been evolutionarily altered.

Populations of *Escherichia coli* have proven to be very useful in experimental studies of evolutionary adaptation. Lenski et al. (7) propagated 12 replicate lines of *E. coli* from a common ancestor for 2,000 generations (300 days) by daily serial transfer in glucose minimal medium. By the process of natural selection, the derived lines became genetically adapted to the experimental environment so that after 2,000 generations they had, on average, a 35% advantage in competition with their ancestor. Vasi et al. (11) investigated the growth characteristics of the lines having derived genotypes and found that increases in maximum growth rate contributed substantially to these fitness gains. In addition, they observed that all 12 derived lines produced significantly larger cells than the ancestor.

It is tempting to assume that the increase in cell size is somehow advantageous in the experimental environment and to speculate on its possible adaptive significance. In recent years, however, evolutionary theorists have emphasized the critical need for rigorous tests to distinguish between adaptive and nonadaptive explanations of evolutionary change in particular organismal traits (5). In one important class of nonadaptive explanation, a change in trait *Y* may be considered solely a correlated response to selection on trait *X*, thereby obviating the need to invoke any specific adaptive significance of the change in trait *Y*. In the case of cell size, bacterial physiologists have repeatedly demonstrated a positive correlation between growth rate and cell size when bacteria are grown in batch cultures on a variety of different media (1, 2, 8). Thus, a simple nonadaptive explanation of the observation that the evolutionarily derived genotypes have larger cells is that the genotypes have been selected to grow faster in the experimental environment, so that the larger cell size is merely a correlated response to selection on growth rate (and requires no further explanation).

Fortunately, the nonadaptive hypothesis can be rigorously tested in this case. We can measure the average sizes of cells with the ancestral and derived genotypes over a range of growth rates, which we set by manipulating the dilution rate in chemostat culture; we can then compare the regression of cell size on growth rate that is obtained for each genotype. If the larger cell size of the derived lines during exponential growth (11) is simply a correlated response to their higher rate of growth, then the difference in size between cells of the ancestral and derived genotypes should disappear when both types of cells are forced to grow at the same rate. However, a significant difference in either the slope or the intercept of the regression between populations with the two genotypes would allow us to reject this nonadaptive hypothesis.

The growth rate of bacteria in chemostats is governed by the rate at which the culture medium is diluted with fresh medium flowing into the chemostat. By adjusting this rate, we were able to vary the growth rate of the bacteria without changing any other environmental factor, including medium composition. (For a description of the chemostat apparatus, see reference 3.) Individual chemostats were inoculated from overnight batch cultures of cells of either the ancestor or one of the derived genotypes. The medium used for both the overnight cultures and the chemostats was a minimal salts medium (7) supplemented with glucose at a concentration of 1 mg/ml. The chemostat cultures were first allowed to grow to maximum turbidity with aeration but without flow of medium through the vessels. The flow was then started and adjusted to the desired rate. These cultures were allowed to grow for 5 to 14 volume turnovers before sampling. Samples for the cell size measurements were taken on each of the following 2 days. After a final sample was taken, we measured the actual dilution rate for each vessel by directly measuring the flow of medium through the tubing (in milliliters per hour) and dividing this value by the volume (in milliliters) of that culture. The chemostats were then resterilized and set up for the next batch of replicates having a different growth rate. Cells of the ancestral and derived genotypes (ancestor, REL606; derived, REL1206) have been previously described (7, 11). The highest growth rate used in this study ($\sim$0.6 h^{-1}) is just below the maximum growth rate of the ancestor (0.77 h^{-1}). During the evolution experiment, which produced the derived genotype, the bacterial population grew at or very near its maximum rate of growth each day (11). Average cell sizes were estimated with a particle counter and Channelyzer (Coulter Electronics, Inc.), which measure the volume displaced by a particle.

Figure 1 shows the average cell sizes for populations having each genotype as a function of their growth rates. Each point represents the average cell size, measured in duplicate samples, from a separate chemostat culture. Thus, all observations are statistically independent. The lines indicate the least-squares linear regression of cell size on growth rate. For both genotypes, there is a highly significant relationship between cell size and growth rate (ancestor, $r = 0.794$, $n = 27$, $P < 0.0001$; derived, $r = 0.633$, $n = 27$, $P = 0.0004$). The slopes and y-intercepts of the cell size versus growth rate for the two

* Corresponding author.

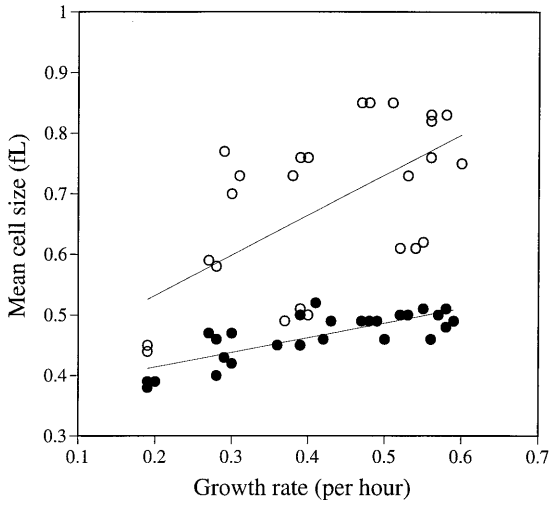

FIG. 1. Mean cell size versus growth rate for cultures having the ancestral (●) and derived (○) genotypes. Each point represents the mean cell size obtained for an independent chemostat culture. The measurement for each chemostat culture was averaged over two samples obtained on consecutive days.

genotypes were then compared by using two-tailed t tests (6). For the ancestral genotype, the slope and y-intercept (means ± standard errors) were 0.246 ± 0.038 and 0.364 ± 0.016, respectively. The corresponding values for the derived genotype were 0.663 ± 0.162 and 0.400 ± 0.070, respectively. The difference in slope between cells of the two genotypes is highly significant (t = 2.483, 50 df, P = 0.016), but there is no significant difference in their y-intercepts (t = 0.497, 50 df, P = 0.622). Thus, the two genotypes have similar cell sizes at very low growth rates but the difference between their cell sizes becomes progressively greater at higher growth rates. It is noteworthy that there appears to be much more variation in the measurements of cell size for the derived genotype than for the ancestor. It may be that the derived genotype is more sensitive to small variations in the experimental environment or starting conditions. Nonetheless, the fact that the derived genotype leads to larger cells than its ancestor cannot be explained simply by a difference in cell growth rate.

There are probably many hypotheses that might explain the evolution of bacterial cell size. For the case described here, we were able to directly test and reject the nonadaptive hypothesis that cell size increased solely as a consequence of the evolution of higher growth rate. Cells of the derived genotype were larger even when they were grown at the same rate as those of the ancestor. This experiment also demonstrates that the physiological coupling between cell size and growth rate has been evolutionarily altered.

What alternative explanations, either adaptive and nonadaptive, might there be for this evolutionary change? During the evolution of this line in serial batch culture, the cells experienced lag, exponential growth, and stationary phases on a daily basis. In the exponential growth phase, the cells are growing at their maximal rate and the size difference is significant. This size difference apparently carries over even into the stationary phase (11). Therefore, one possibility is that larger cells have more reserves and hence greater transcriptional and translational capacity so that they can respond more quickly to a shift-up in nutrients. Consistent with this alternative hypothesis, Vasi et al. (11) showed that the derived genotypes had significantly shorter lag phases than their ancestor when they were transferred from spent to fresh medium.

Working with the same lines, Travisano et al. (9, 10) studied the correlated effects of adaptation to minimal glucose medium on growth on other carbon sources. Their results strongly implicate the involvement of glucose transport functions in the adaptive changes. It is unclear, a priori, how changes in transport functions could affect cell size, if at all. One complication in the analysis of these lines is the fact that multiple genetic changes have undoubtedly occurred during the evolutionary period studied. These changes could have opposing or epistatic (nonadditive) effects on cell size and fitness. Elena et al. (4) have recently performed a fine-scale analysis of the changes in cell size over time in the evolving population that gave rise to the 2,000-generation isolate that we used here. They identified several short intervals over which cell size increased significantly, and these increases are likely the results of single mutations. Given that a full explanation of the significance of the changes in cell size may require the identification of the mutations that are responsible, these data may help to narrow the search.

We hope from these studies to learn more about the interactions between such fundamental processes as resource transport, growth rate control, and morphogenesis. Does a mutation that affects any one of these processes almost inevitably affect the others through various feedback mechanisms? Or are the controls over these processes largely distinct so that each can be modified during evolution independently of the others? We are pursuing work to address these basic questions.

We thank Al Bennett, Vaughn Cooper, Santiago Elena, Larry Forney, Tom Schmidt, Mike Travisano, and Farida Vasi for helpful discussions.

This work was supported by the NSF Center for Microbial Ecology (grant BIR-9120006) and an NSF grant (IBN-9421237) to R.E.L.

REFERENCES

1. **Åkerlund, T., K. Nordström, and R. Bernander.** 1995. Analysis of cell size and DNA content in exponentially growing and stationary-phase batch cultures of *Escherichia coli*. J. Bacteriol. **177:**6791–6797.
2. **Bremer, H., and P. P. Dennis.** 1987. Modulation of chemical composition and other parameters of the cell by growth rate, p. 1527–1542. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
3. **Chao, L., B. R. Levin, and F. M. Stewart.** 1977. A complex community in a simple habitat: an experimental study with bacteria and phage. Ecology **58:**369–378.
4. **Elena, S. F., V. S. Cooper, and R. E. Lenski.** 1996. Punctuated evolution caused by selection of rare beneficial mutations. Science **272:**1802–1804.
5. **Gould, S. J., and R. C. Lewontin.** 1979. The spandrels of San Marco and the panglossian paradigm: a critique of the adaptationist programme. Proc. R. Soc. Lon. Ser. B **205:**581–598.
6. **Kleinbaum, D. G., and L. L. Kupper.** 1978. Applied regression analysis and other multivariable methods. Duxbury Press, North Scituate, Mass.
7. **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. Am. Nat. **138:**1315–1341.
8. **Schaechter, M., O. Maaløe, and N. O. Kjelgaard.** 1958. Dependence on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium*. J. Gen. Microbiol. **19:**592–606.
9. **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.
10. **Travisano, M., F. Vasi, and R. E. Lenski.** 1995. Long-term experimental evolution in *Escherichia coli*. III. Variation among replicate populations in correlated responses to novel environments. Evolution **49:**189–200.
11. **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. Am. Nat. **144:**432–456.