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Evolution: Bacterial Mutation in Stationary Phase

Dispatch

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A recent study indicates that the genomic mutation rate of the gut bacterium *Escherichia coli* is substantially higher in nongrowing than growing cultures. These findings are important in the light of the ongoing controversy over the generality and robustness of stationary phase mutagenesis and its evolutionary implications.

The genomic mutation rate is a fundamental evolutionary parameter of any population, determining the rate of influx of new deleterious and beneficial alleles. Because most mutations are likely to be harmful to fitness, DNA repair and proofreading systems have probably evolved so as to minimize rates of mutation [1]. Even the microbial extremophiles that normally inhabit harsh and potentially mutagenic environments seem to have low genomic mutation rates [2], suggesting that selection almost always puts a premium on the faithful maintenance and transmission of genetic information. Nonetheless, geneticists have long known that some environmental extremes can elevate mutation rates; indeed, this is the basis for the use of DNA damaging agents to induce mutations for study.

What has remained unclear is whether the range of natural environmental stresses encountered by organisms can also have a strong effect on mutation rates. Bacteria, in particular, may often find themselves in environments where cell division is arrested by resource limitation, and this raises the interesting possibility that exposure to such environments elevates the bacterial genomic mutation rate. A recent study by Loewe *et al.* [3] supports this idea by finding that genomic mutation rates are higher when *Escherichia coli* cultures are held under prolonged growth arrest than when they are actively dividing.

To assay mutation rates in nongrowing *E. coli* cultures, Loewe *et al.* [3] used the 'mutation accumulation' approach developed from quantitative genetic theory [4,5]. In contrast to classical genetic methods that estimate rates of mutation by tracking changes at specific loci, mutation accumulation experiments use measurements of fitness or a phenotypic character closely related to fitness to estimate the rate at which deleterious mutations arise genome-wide. A mutation accumulation experiment is conducted by maintaining a set of initially isogenic replicate populations at low effective population size, so that new mutations persist regardless of their fitness effects. Because almost all mutations are deleterious to fitness, absolute measures of mean fitness in the replicate populations decrease over time; because different numbers and combinations of mutations accumulate by chance in different replicate populations, the variance in fitness increases. The mean change in fitness over all populations, and the increase in the variance of fitness, can be used to estimate the deleterious genomic mutation rate, denoted U_{del}.

Using data from a mutation accumulation experiment with *E. coli* cultures that were kept growing by daily transfers to fresh medium, Kibota and Lynch [6] had previously estimated U_{del} in *E. coli* as ~0.0002 per generation. Working with the same starting *E. coli* strain as Kibota and Lynch [6], Loewe *et al.* [3] took the novel approach of allowing mutations to accumulate in populations that were not receiving fresh medium but were, instead, maintained in a depleted liquid medium for 92 days. Loewe *et al.* [3] then compared the mean and variance in maximal growth rates that these replicate populations exhibited in fresh medium to those measured before mutation accumulation. As in other, more typical mutation accumulation experiments, the mean in this estimate of fitness declined and the variance rose. Analysis of the growth data indicated that ~0.03 deleterious mutations arose per genome per day during the 92 days of growth stasis.

To compare U_{del} of ~0.03 per day estimated by Loewe *et al.* [3] to that of ~0.0002 per generation estimated under growth conditions [6], one must consider the maximum number of generations that could plausibly have occurred per day in the cultures that Loewe *et al.* [3] maintained in depleted medium. Bacterial cultures maintained under such conditions are said to be in 'stationary phase', meaning that the total number of cells is stable or even declining. Nonetheless, subpopulations of cells can be dividing actively within such cultures [7], giving rise to mutations in a generation-dependent manner. As Loewe *et al.* [3] note, however, even an implausibly high 25 cell divisions per day in stationary phase at U_{del} ~0.0002 per generation would yield a U_{del} per day that is less than one-fifth their observed value. A more realistic two generations per day in stationary phase yields U_{del} per day that is more than an order of magnitude lower than their observed value. Given these considerations, it is hard to escape the conclusion that mutagenesis increased during stationary phase in the experiment carried out by Loewe *et al.* [3].

To guard against the possibility that prolonged stationary phase merely altered gene expression patterns rather than mutation rates, Loewe *et al.* [3] reconditioned their populations after the experiment by putting them through freezer storage followed by several generations of growth on fresh medium; only then did they measure the accumulated effect of stationary phase on maximal growth rates. This seems like an adequate control in the absence of evidence that gene expression patterns established during stationary phase can persist after a return to active cell division. It would be interesting to know, however, whether extended propagation on fresh medium would

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lead to a return of pre-stationary phase growth rates in the populations, at a rate consistent with selection on reversions of acquired deleterious mutations.

Loewe *et al.* [3] also investigated the possibility that constitutive mutator alleles were substituted into the populations by 'hitchhiking' during stationary phase; such mutator hitchhiking events have been documented repeatedly in experimental populations of bacteria [8–11]. To test for this, Loewe *et al.* [3] measured the rate of a specific point mutation in the founder strain and in three randomly chosen mutation accumulation lines using fluctuation tests [12]. No difference was found, suggesting that mutator alleles are unlikely to have been an important source of increased mutation in stationary phase.

Loewe *et al.* [3] are by no means the first to report that stationary phase elevates the mutation rate in bacteria. This subject has a controversial recent history beginning with an uproar in the late 1980s and 1990s over claims that bacteria can escape starvation stress by manufacturing specifically needed adaptive mutations in a neo-Lamarckian manner [13]. This 'directed mutation' hypothesis was later rejected in favor of the notion that generally elevated mutagenesis in at least some cells can lead to the appearance of directed mutation in a population under starvation stress.

This 'hypermutable state' hypothesis has been studied in great depth in the case of a specific *E. coli* strain — unrelated to the one studied by Loewe *et al.* [3] — that is unable to utilise lactose (Lac^-) without the reversion of a frameshift mutation. A variety of mechanistic models for how genomic mutation rates might increase when this strain is starved have been investigated and debated [14–16]. Careful recent experimental and theoretical analyses by Roth and collaborators [17], however, have seriously called into question the necessity to invoke increased mutagenesis at all in order to explain the observed accumulation of Lac^+ mutations in this strain under starvation in the presence of lactose. Overall, both the robustness and generality of stationary phase mutagenesis in bacteria remain to be firmly established. For example, whereas a recent study of *E. coli* strains isolated from a wide range of animal species found that the frequency of mutants is elevated in aging colonies of many (but not all) isolates [18], no evidence of starvation-induced mutagenesis has been detected in *Salmonella typhimurium* cultures deprived of a carbon source [19].

Where elevated mutagenesis does occur in stationary phase — as is apparently the case in the system investigated by Loewe et al. [3] — its evolutionary significance is debatable. It is an undeniable biological fact that vast numbers of *E. coli* and other gut bacteria are expelled into the external environment daily by animals. Once outside their hosts, these cells are likely to be faced with prolonged growth arrest, and perhaps eventual death, unless they can somehow adapt to their straitened circumstances. The capacity to increase mutation rates in stationary phase could thus be viewed as an evolved mechanism whose purpose is to increase adaptability [14–16,18,20]. However, several considerations suggest that increased mutagenesis may be an unavoidable epiphenomenon of starvation with greater evolutionary costs than benefits; the most obvious of these is the fact that an increase in the genomic mutation rate will result in far more deleterious mutations than beneficial mutations [13,17].

If stationary phase mutagenesis has evolved in order to promote adaptability, then any given bacterial lineage that exhibits it must have had a history of repeated adaptation to stationary phase. For gut bacteria such as *E. coli*, this implies that cell lineages that leave the gut, mutate at high rates, and adapt to the oligotrophic external environment must somehow regularly find their way back into the gut without being outcompeted by the lineages that have persisted there all the while. Whether this occurs is an empirical question that has yet to be addressed. There is certainly plenty of room for further research in the lively area of bacterial mutation and adaptation.

References

1. Drake, J.W., Charlesworth, B., Charlesworth, D., and Crow, J.F. (1998). Rates of spontaneous mutation. Genetics *148*, 1667-1686.
2. Grogan, D.W., Carver, G.T., and Drake, J.W. (2001). Genetic fidelity under harsh conditions: Analysis of spontaneous mutation in the thermoacidophilic archaeon *Sulfolobus acidocaldarius*. Proc. Natl. Acad. Sci USA *98*, 7928-7933.
3. Loewe, L., Textor, V., and Scherer, S. (2003). High deleterious mutation rate in stationary phase of *Escherichia coli*. Science *302*, 1558-1560.
4. Mukai, T. (1964). The genetic structure of natural populations of *Drosophila melanogaster.* I. Spontaneous mutation rate of polygenes controlling viability. Genetics *50*, 1-19.
5. Bateman, A.J. (1959). The viability of near-normal irradiated chromosomes. Int. J. Rad. Biol. *1*, 170-180.
6. Kibota, T.T., and Lynch, M. (1996). Estimate of the genomic mutation rate deleterious to overall fitness in *E. coli*. Nature *381*, 696.
7. Finkel, S.E., and Kolter, R. (1999). Evolution of microbial diversity during prolonged starvation. Proc. Natl. Acad. Sci USA *96*, 4023-4027.
8. Boe, L., Danielson, M., Knudsen, S., Petersen, J.B., Maymann, J., and Jensen, P.R. (2000). The frequency of mutators in populations of Escherichia coli. Mut. Res. *448*, 47-55.
9. Giraud, A., Matic, A., Tenaillon, O., Clara, A., Radman, M., Fons, M., and Taddei, F. (2001). Costs and benefits of high mutation rates: Adaptive evolution of bacteria in the mouse gut. Science *291*, 2606-2608.
10. Notley-McRobb, L., and Ferenci, T. (2000). Experimental analysis of molecular events during mutational periodic selections in bacterial evolution. Genetics *156*, 1493-1501.
11. Shaver, A.C., Dombrowski, P.G., Sweeney, J.Y., Treis, T., Zappala, R.M., and Sniegowski, P.D. (2002). Fitness evolution and the rise of mutator alleles in experimental *Escherichia coli* populations. Genetics *162*, 557-566.
12. Luria, S.E., and Delbrück, M. (1943). Mutations of bacteria from virus sensitivity to virus resistance. Genetics *28*, 491-511.
13. Sniegowski, P.D., and Lenski, R.E. (1995). Mutation and adaptation: the directed mutation controversy in evolutionary perspective. Annu. Rev. Ecol. Syst. *26*, 553-578.
14. Rosenberg, S. (2001). Evolving responsively: adaptive mutation. Nat. Rev. Genet. *2*, 504-514.
15. Foster, P.L. (2000). Adaptive mutation: implications for evolution. BioEssays *22*, 1067-1074.
16. Kivisaar, M. (2003). Stationary phase mutagenesis: mechanisms that accelerate adaptation of microbial populations under stress. Environ. Microbiol. *5*, 814-827.
17. Roth, J.R., Kofoid, E., Roth, F.P., Berg, O., Seger, J., and Andersson, D. (2003). Regulating general mutation rates: Examination of the hypermutable state model for Cairnsian adaptive mutation. Genetics *163*, 1483-1496.
18. Bjedov, B., Tenaillon, O., Gérard, B., Souza, V., Denamur, E., Radman, M., Taddei, F., and Matic, I. (2003). Stress-induced mutagenesis in bacteria. Science *300*, 1404-1409.
19. Hughes, D., and Andersson, D.I. (1997). Carbon starvation of *Salmonella typhimurium* does not cause a general increase of mutation rates. J. Bacteriol. *179*, 6688-6691.
20. Radman, M., Matic, I., and Taddei, F. (1999). Evolution of evolvability. Ann. NY Acad. Sci. *870*, 146-155.