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Letter to the Editor

The Risk of Lethals for Hypermutating Bacteria in Stationary Phase

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WHEN stationary phase populations of *Escherichia coli* are subjected to intense selection for reversion of a frameshift in *lacZ* they are found to have accumulated a wide variety of other mutations. Furthermore, cells bearing multiple mutations are much more common than would be expected, and this has been attributed to the presence of a few transient hypermutators that have a greatly raised mutation rate (Hall 1990; Torkelson *et al.* 1997; Rosche and Foster 1999). Recently, Roth *et al.* (2003, p. 1483) set out to calculate the incidence of lethal mutations in these hypermutators and concluded that "selected revertants will carry an average of eight deleterious null mutants" and therefore that "temporary general mutagenesis during stress is unlikely to provide long-term selective advantage. . . ." This is a strange conclusion in view of the evident ability of *E. coli* to produce plenty of Lac$^+$ revertants when these are needed. Luckily, we are not confronted by a paradox here, because it turns out that Roth *et al.* have overestimated the probable frequency of lethals by several orders of magnitude.

Their first error was to assume that the rate of accumulation of lethal *chromosomal* mutations in essential genes is the same as that for mutation in an *episomal lacZ*. But it is known that when the *lac* allele is moved from the episome into the bacterial chromosome its mutation rate in stationary phase drops roughly 100-fold and becomes just like the rate for other chromosomal genes (Foster and Trimarchi 1995; Radicella *et al.* 1995; Rosche and Foster 1999) and the same is apparently true for *tet* (Foster 1997; Bull *et al.* 2001). If Roth *et al.* had used the rates for chromosomal genes, they would have seen that the production of successful mutants in stationary phase should not be appreciably eroded by concurrent lethal mutations, and they would then have understood how their bacteria manage to produce abundant Lac$^+$ revertants on lactose plates.

Their second error was to assume, like Torkelson *et al.* (1997), that all mutations are confined to the rare hypermutators. It seems from all published results that any given class of mutation is roughly 10 times less common in single (Lac$^+$) mutants than in double mutants (Lac$^+$ plus a second mutation; Cairns 2000), whether the *lac* allele is in an episome or the chromosome (Rosche and Foster 1999). This must mean that there are at least two classes of mutators in the population and that most single mutations are not arising in the rare hypermutators. It is wrong therefore to say that the 0.1% of the cells that are hypermutators must have a mutation rate that is 1000 times the observed overall rate for the entire population.

More interesting than the correction of these errors is the fact that the cause of hypermutators and their frequency and mutation rates were predicted many years ago. Ninio (1991) argued that errors of transcription and translation should cause ~0.1% of bacteria to lack a complete set of the proteins needed to carry out mismatch repair (MMR) and this would raise their mutation rate several hundredfold. In confirmation of his predictions, it turns out that the hypermutators of *E. coli* in stationary phase are apparently defective in MMR because an imposed genetic defect in MMR raises the overall rate of single mutations (in the general population) to the level previously found in double mutants (the hypermutators) but does not further raise the frequency of additional mutations in double mutants (the original hypermutators; Rosche and Foster 1999). The question that Roth *et al.* asked should therefore be rephrased. Has *E. coli* controlled the precision of its machinery for expressing genes and the number of copies of its various DNA repair proteins so that it produces the best frequency of hypermutators? Or is the existence of hypermutators simply the happy result of other reasons for selecting that degree of precision of gene expression and that level of redundancy of those proteins?

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