

Response to Comment on "High Deleterious Genomic Mutation Rate in Stationary Phase of *Escherichia coli*"

It did not escape our attention that fixation times for mutations would be excessive in our experiment, if effective population size (N_e) was similar to census population size. Therefore, we pointed to fixation of adaptive mutations (e.g., growth advantage in stationary phase, or GASP mutations) that reduce N_e drastically (1–5): One cell that can grow in stationary phase, while all others cannot, will sweep through the population, fixing all its slightly deleterious mutations (SDMs). GASP experiments suggest several sweeps in 100 days (6, 7), leading to repeated bottlenecks. The resulting tiny N_e in our experiment justifies the mutation accumulation (MA) design if we assume that a reasonable number of sites can accumulate SDMs mild enough not to interfere with GASP-sweeps, and if the sweep-causing advantageous mutations themselves have no negative side effects on exponential growth. de Visser and Rozen (8) claim that there is enough evidence for antagonistic pleiotropy (AP) to doubt the second assumption. However, their postulated high variability of nonselected pleiotropic effects seems rather unlikely with repeated quasi-deterministic sequences of GASP mutations assayed in constant environments.

AP always implies that the same mutations that promote growth in stationary phase, slow growth during log phase. Without detailed genetic analysis, these effects of AP are hard to separate (9, 10) from other SDMs on the same nonrecombining genome (predicted by MA). Thus, circumstantial evidence must decide whether MA or AP contributed more to our observations.

de Visser and Rozen cite Vasi and Lenski (11), who reported growth patterns similar to ours, but provided no data to conclusively distinguish AP from MA. Cooper and Lenski (9) attempted to differentiate between AP and MA, but did not carry out a formal genetic analysis of the mutants. They based their test largely on a presumptive log-linear decline in performance under MA versus a log-curvilinear decline under AP. This does not have a strong theoretical basis, especially if N_e changes or mutants inactivating crucial pathways are occasionally fixed under AP (9). Moreover, a detailed analysis suggests

that the statistical power of their data is insufficient to detect deviations from log-linearity. A subsequent genetic analysis (12) of the most eroded catabolic function found in (9) is suggestive of AP, but not conclusive. Unfortunately, the genes involved showed extraordinarily high mutation rates (12) and only a few-fold higher increase (1) is necessary to explain the observed parallel evolution by MA.

de Visser and Rozen (8) suggested measuring growth rates in stationary phase (13), but this would simply provide evidence for the presence of GASP mutations. Furthermore, it is problematic to derive static assay conditions from an arbitrary snapshot of stationary phase, which is highly dynamic (6, 14–17). However, two other approaches might help quantify the contributions of MA and AP. First, comparisons of DNA sequences could reveal increased mutation rates predicted by MA, as in the studies which found adaptive mutations in stationary phase to be accompanied by transiently high mutation rates in many unselected genes (18–23). These studies inspired the interpretation of our experiment. A prominent role for transposable elements (TE) in MA is suggested by the similarity of mutational effects found in Bateman-Mukai analyses of data in (1, 9, 24) to the effects of TE mutagenesis (25). Confirmation comes from similar catabolic decay rates (9) and TE mutation rates (26) between mutators and non-mutators. Point mutations appear less important for Lenski's lines (27). Second, functional characterization of GASP mutations at the molecular level, combined with high-precision growth curves when placed in an isogenic background, can quantify their effect on fitness during exponential growth. The extensive double checks needed to exclude mutations generated by the molecular methods used (12, 28) appear within reach for some well-studied GASP mutations [e.g., (12–14)].

Should GASP mutations regularly have deleterious side effects (AP), our findings present bacteria with an evolutionary dilemma: To either avoid extinction by acquiring GASP mutations, or avoid collecting excess GASP mutations because removing side-effects would require improbable back-mutations. GASP mutations without negative

side effects will survive better over the long term, once growth resumes. Thus, frequencies of stationary phase mutators (that survive by adapting) and nonmutators (that preserve quality genes) may indeed be associated with a complex evolutionary fitness tradeoff (11) that depends heavily on horizontal gene transfer (29, 30). The mutators may not "cheat" (13), but rather "cooperate" with nonmutators to generate frequent adaptive mutations without degrading adapted genes in the species. Such "cooperative evolution" may be pivotal for the success of bacteria—AP would prevent it.

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