Competition between high- and higher-mutating strains of *Escherichia coli*

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Experimental studies have shown that a mutator allele can readily hitchhike to fixation with beneficial mutations in an asexual population having a low, wild-type mutation rate. Here, we show that a genotype bearing two mutator alleles can supplant a population already fixed for one mutator allele. Our results provide experimental support for recent theory predicting that mutator alleles will tend to accumulate in asexual populations by hitchhiking with beneficial mutations, causing an ever-higher genomic mutation rate.

Keywords: mutation rate; hitchhiking; mutator; asexual populations

1. INTRODUCTION

Theories for the evolution of mutation rates have emphasized the contrasting influence of beneficial mutations in sexual and asexual populations. In sexual populations, recombination erodes linkage disequilibrium and prevents mutator alleles from hitchhiking to fixation with beneficial mutations; in asexual populations, complete linkage enables hitchhiking of mutator alleles (reviewed in [1]). Classical theories predicted that the trade-off between mutator hitchhiking and increased deleterious mutational load would enforce optimal equilibrium mutation rates in asexual populations (e.g. [2]). Recent theory, however, predicts that the genomic mutation rate of an asexual population will be evolutionarily unstable, with a strong upward bias as long as beneficial mutations are occurring, because deleterious mutational load accumulates slowly [3] whereas mutator hitchhiking is relatively rapid [4,5].

Competition experiments between isogenic wild-type and mutator strains in *Escherichia coli* and *Saccharomyces cerevisiae* have shown that mutators will fix by hitchhiking if present at sufficiently high initial frequencies [6,7]. Long-term evolution experiments have shown that spontaneously arising mutator alleles can hitchhike to fixation in wild-type

E. coli populations [8]. Mutators have also been observed at substantial frequencies in natural populations of bacteria [9–11], cancer cell populations [12] and influenza virus populations [13]. Evolved mutator phenotypes have persisted robustly for tens of thousands of generations in experimental *E. coli* populations [14,15], consistent with the idea that the increased deleterious load associated with mutators takes a considerable time to accumulate.

Here, we test whether a second mutator allele can hitchhike to fixation in an asexual population already fixed for one mutator allele, thereby raising the genomic mutation rate of the population even further. Our approach echoes earlier experiments on the fate of genotypes bearing single-mutator alleles in *E. coli* populations [6,16]. Here, however, we compete isogenic strains carrying one and two mutator alleles ('single-mutator' and 'double-mutator' strains). We assess the fate of double-mutators under contrasting regimes: selection primarily affecting growth rate (soft selection) and selection primarily affecting survival (hard selection).

2. MATERIAL AND METHODS

(a) Bacterial strains and media

An asexual (F^-) strain of *E. coli* bearing the *mutL13* allele, which confers mismatch repair deficiency, was obtained from the *E. coli* Genetic Stock Center at Yale University. (See electronic supplementary material for strains and genotypes.) The *dnaQ905* allele, which confers DNA proofreading deficiency, was cotransduced with a tetracycline resistance determinant from donor strain CSH116 [17] into the single-mutator (*mutL13*) strain to produce an isogenic double-mutator (*mutL13 dnaQ905*) strain. The point mutation rate of the single-mutator strain was approximately 100-fold higher than that of an isogenic wild-type strain, and the point mutation rate of the double-mutator strain was a further approximately 40-fold higher than that of the single-mutator strain (electronic supplementary material).

(b) Competitions under soft selection

To assess the possibility of double-mutator hitchhiking under soft selection, we carried out competitions in a relatively benign environment. We established 25 populations combining the isogenic single- and double-mutator strains in Davis minimal broth [18] supplemented with 25 mg l⁻¹ of glucose (hereafter 'DM25') and 15 similar populations in Davis minimal broth supplemented with 1 g l⁻¹ of glucose (hereafter 'DM1000'). The effective sizes of the populations propagated in DM25 and DM1000 were approximately 1.7×10^7 and approximately 6.6×10^8 . In DM25, we carried out five replicate competitions at each of the following double-mutator frequencies: high frequency (approx. 0.5), high intermediate frequency (approx. 0.3) and low intermediate frequency (approx. 0.1); we also carried out 10 replicate competitions with the double-mutator starting at a low frequency (approx. 0.03). In DM1000, we carried out five replicate competitions each with the double-mutator starting at high (approx. 0.5), high intermediate (approx. 0.3) and low intermediate (approx. 0.1) frequencies; we did not carry out competitions in DM1000 at a low double-mutator starting frequency because the results of competitions in DM1000 at the higher frequencies indicated that such competitions would be uninformative. Competitions were initiated by aliquoting appropriate numbers of cells from a single pair of parent cultures. All populations were maintained in 20 ml culture tubes at 37°C with shaking at 120g: every day, 100 µl from each overnight culture was transferred to 9.9 ml of fresh medium. During the competitions, samples from each population were diluted appropriately and plated on permissive Luria-Bertani (LB) agar [17]. After 24 h of growth, permissive plates were replica plated to LB agar supplemented with 15 µg ml⁻¹ of tetracycline to estimate double-mutator frequency.

(c) Competitions under hard selection

To assess the possibility of double-mutator hitchhiking under hard selection, we carried out competitions in an environment that imposed a series of novel lethal selective events. We established 20 populations combining the isogenic single- and double-mutator strains in DM1000 as described above. We carried out five replicate

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competitions with the double-mutator starting at an intermediate frequency (approx. 0.5), and 15 replicate competitions with the double mutator starting at low frequencies (approx. 0.03–0.1). All populations were propagated in 20 ml culture tubes at 37°C with shaking at 120g: every day for 7 days, 1 ml from each overnight culture was transferred to 9 ml of fresh medium. To impose lethal selection, we supplemented the DM1000 with a series of single antibiotics as follows: 100 µg ml⁻¹ of rifampicin on days 2 and 3, 100 µg ml⁻¹ of streptomycin on days 4 and 5 and 100 µg ml⁻¹ of novobiocin on days 6 and 7. Estimation of double-mutator frequency was carried out by replica plating as described above for the competitions under soft selection.

3. RESULTS AND DISCUSSION

Figure 1 (blue lines) shows the results of competitions under soft selection in DM25. These experiments produced outcomes consistent with the results of early work competing single-mutator and wild-type *E. coli* strains [6]. When the double-mutator began at a low intermediate frequency or higher, it always rose towards fixation. However, when the double-mutator began at a low frequency, it was always lost (data not shown). Double-mutators were detectable at the outset of the low-frequency DM25 experiments at frequencies of approximately 3 per cent; however, they fell to undetectable frequencies within a few tens of generations (electronic supplementary material). The success of the double-mutator only when initially present at an intermediate or higher frequency is best explained as a consequence of hitchhiking with beneficial mutations. The alternative interpretation—that the *dnaQ905* allele or its associated flanking DNA confers a direct fitness advantage on the double-mutator strain—would predict that the double-mutator should also prevail from a low starting frequency.

Figure 1 (red lines) shows the results of competitions under soft selection at higher effective population size, in DM1000. Here, the double-mutator was lost in all competitions except at the highest starting frequency (approx. 0.5), where some double-mutator subpopulations increased in frequency after a lag. A large effective population size increases the likelihood that single- and double-mutator subpopulations will acquire beneficial mutations contemporaneously, because the beneficial mutation supply rate of each subpopulation (the product of its beneficial mutation rate and population size) is high. Under such a circumstance, a double-mutator would have a net advantage in acquiring beneficial mutations only when at a high starting frequency, as observed in our experiments. Indeed, the dynamics of double mutators in the high-frequency DM1000 populations are quite variable, consistent with a shifting selective advantage caused by beneficial mutations arising on both backgrounds. Similar dependence of mutator hitchhiking on the beneficial mutation supply rates of competing clonal genotypes has been observed previously [19].

To address directly the possibility that intrinsic fitness differences, rather than mutator hitchhiking, could explain the results of the soft-selection experiments, we assayed maximal growth rates of the single- and double-mutator strains in DM1000 (see the electronic supplementary material for methods). Doubling times for the two strains were closely similar (single-mutator: 1.70 ± 0.05 h; double-mutator:

Figure 1. Competitions under soft selection. Blue lines: populations propagated in DM25; red lines: populations propagated in DM1000. Ten populations in DM25 in which the double mutator began at low frequency (approx. 0.03) and was lost within 70 generations are not shown.

Figure 2. Competitions under hard selection. Grey boxes indicate periods of antibiotic exposure. The number of generations reported in the figure is a minimal estimate because of sharp reductions in population size and subsequent regrowth upon exposure to antibiotics.

1.72 ± 0.03 h) and statistically indistinguishable (two-tailed, $p = 0.90$), and thus provided no evidence of intrinsic fitness differences sufficient to explain the dynamics observed in the soft-selection experiments.

Figure 2 shows the results of competitions under hard selection in which populations were exposed to a series of lethal selective events; this situation might arise, for example, in a pathogen population faced with host immune surveillance. Here, the double-mutator strain rose to apparent fixation in every experiment in which it began at an intermediate frequency (approx. 0.5). Similarly, in seven of 15 competitions in which the double-mutator began at a lower frequency (0.03–0.1), its frequency increased substantially over the course of the experiment. However, in the remaining eight competitions in which the double-mutator began at the lower frequency, the single-mutator prevailed instead. The mixed success of the double-mutator at the lower starting frequency is consistent with roughly equivalent mutation supply rates in the single- and double-mutator subpopulations: the double-mutator genotype has a 40-fold greater mutation rate than the single-mutator, but its initial frequency in these experiments was about 50-fold lower than that of the single-mutator.

Consequently, mutations conferring resistance to a particular antibiotic were equally likely to arise in the single- and double-mutator subpopulations. Overall, the results of the hard-selection experiments are similar to those of experiments in which large cultures of *E. coli* were shown to be enriched for single-mutator frequency after repeated exposure to antibiotics [16].

Our results demonstrate that there are circumstances under which recurrent mutator hitchhiking can occur in an asexual population as predicted by recent theory [4,5]. An obvious limitation of our approach is that we seeded the double-mutator genotype into populations at a higher frequency than would have applied had it arisen spontaneously, as was done in previous work with single-mutators [6]. This raises the question of how a genotype with a higher mutation rate would attain the frequency necessary for it to hitchhike in a natural population. For single-mutators, this question was answered by the observation that spontaneously arisen mutators can, over time, acquire chance associations with beneficial mutations and hitchhike [8,20]. Similar hitchhiking of spontaneously arisen double- and higher-order mutator genotypes has been predicted theoretically and detected in simulations of asexual populations [4,5], but the phenomenon has not been confirmed experimentally and remains an important subject for future research.

The observation that double-mutators can prevail over single-mutators is perhaps not surprising, because a higher mutation rate confers a *per capita* advantage in acquiring beneficial mutations. However, an extremely high mutation rate can cause extinction of even the largest asexual population, whether through erosion of genomic information [21] or excessive deleterious mutational load [22,23]. Our double-mutator strain has a high estimated genomic deleterious mutation rate of $U_d = 0.9$ (electronic supplementary material), which exceeds the value of $U_d = 0.69$ theoretically predicted to cause extinction of an asexual bacterial population in the absence of beneficial mutations [23]. Our current work is testing whether a very large population that has fixed the double-mutator genotype is destined for extinction in the relatively short term.

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