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Evolutionary biology

Evolution of mutation rates in hypermutable populations of *Escherichia coli* propagated at very small effective population size

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Mutation is the ultimate source of the genetic variation—including variation for mutation rate itself—that fuels evolution. Natural selection can raise or lower the genomic mutation rate of a population by changing the frequencies of mutation rate modifier alleles associated with beneficial and deleterious mutations. Existing theory and observations suggest that where selection is minimized, rapid systematic evolution of mutation rate either up or down is unlikely. Here, we report systematic evolution of higher and lower mutation rates in replicate hypermutable *Escherichia coli* populations experimentally propagated at very small effective size—a circumstance under which selection is greatly reduced. Several populations went extinct during this experiment, and these populations tended to evolve elevated mutation rates. In contrast, populations that survived to the end of the experiment tended to evolve decreased mutation rates. We discuss the relevance of our results to current ideas about the evolution, maintenance and consequences of high mutation rates.

1. Introduction

Because the genomes of all organisms harbour loci that affect the genome-wide mutation rate, mutation rates can evolve through the effects of natural selection and other evolutionary forces. If mutation-rate-modifier alleles have negligible direct effects on individual fitness, then natural selection can only alter mutation rates indirectly, via linkage disequilibrium between modifiers and fitness-affecting mutations [1–3]. Indirect selection to increase mutation rate is driven by hitchhiking of up-modifiers of mutation (mutators) with sweeping beneficial alleles and has been documented numerous times in experimental and natural microbial populations (reviewed in [4]). Indirect selection to decrease mutation rate depends on avoidance of mutational load, is expected to be relatively slow and weak, and has been observed less frequently (reviewed in [4]). Existing theory and observations thus suggest that where selection is minimized, rapid systematic evolution of mutation rate in either direction (up or down) is unlikely.

Selection is minimized intentionally in mutation accumulation (MA) experiments, in which replicate populations founded from a single ancestral genome are propagated at very small effective size (N_e) for many generations [5,6]. Because genetic drift governs the fate of mutations when their selective effect is less than approximately $1/N_e$ [7], numerous deleterious mutations that would otherwise be suppressed by purifying selection are free to accumulate along with truly neutral mutations in MA experiments, allowing estimation of their rate of occurrence [5,6]. It is important to note, however, that many MA experiments do not eliminate selection on beneficial and deleterious mutations of strong effect (for example, see [8]).

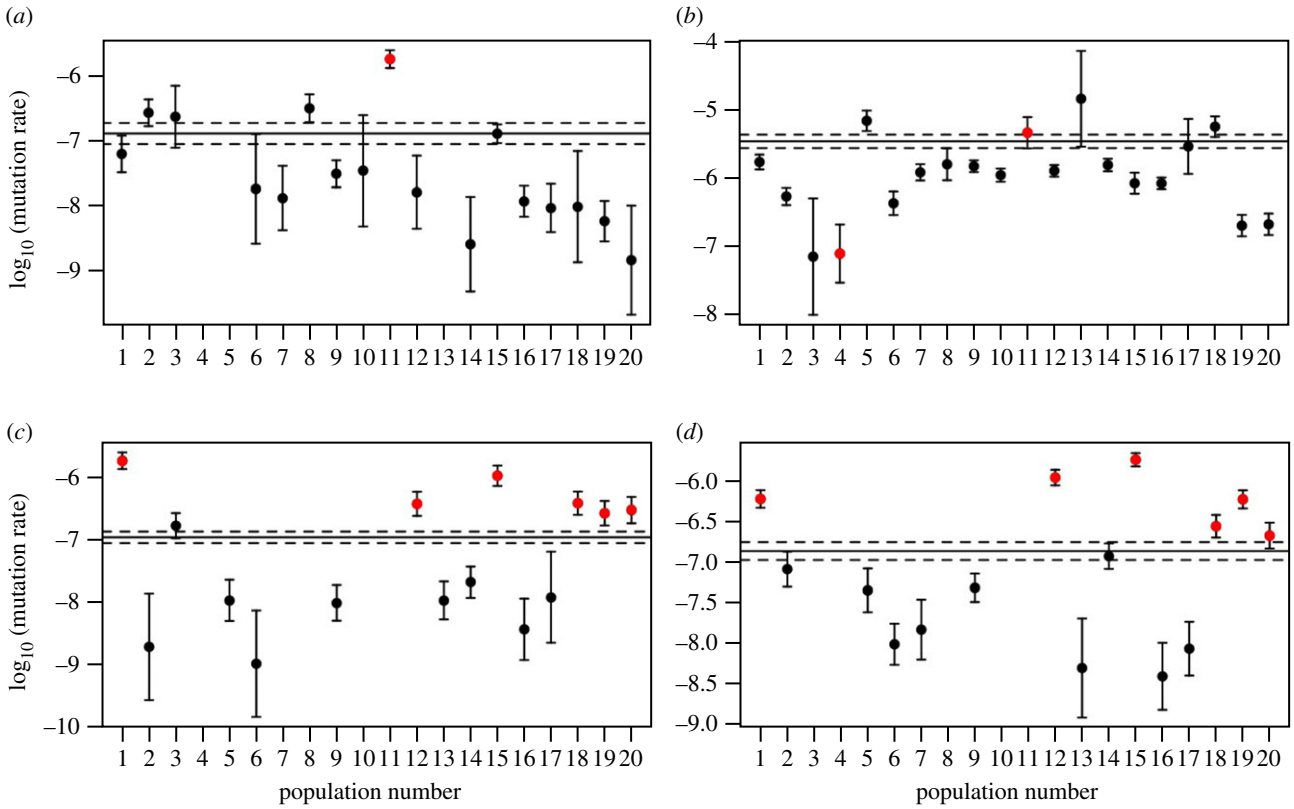

Figure 1. Maximum-likelihood estimates of mutation rates to streptomycin resistance and nalidixic acid resistance and their 95% confidence intervals for the LB (*a*,*b*) and MG (*c*,*d*) populations. Black markers denote populations that survived to the end of the experiment; red markers denote the populations that went extinct. (Some populations could not be assayed for mutation rate and are not represented: see §2.) Solid horizontal lines give the estimated mutation rate in the ancestral strain; upper and lower dotted lines represent its 95% CI.

We have carried out an MA experiment with replicate populations derived from a hypermutable *Escherichia coli* strain. Here, we report systematic evolution of mutation rates in this experiment. Populations that went extinct during the experiment tended to evolve elevated mutation rates, whereas those that survived tended to evolve decreased mutation rates; among the latter, a measure of fitness was negatively correlated with mutation rate in populations propagated on rich medium. We discuss the relevance of our results to current ideas about the evolution, maintenance and consequences of high mutation rates.

2. Material and methods

Full methods are provided in the electronic supplementary material.

(a) Mutation accumulation experiment

Forty independent MA populations were established using random isolates from *E. coli* strain PS2534, which is resistant to the antibiotic tetracycline, harbours defects in mismatch repair and proofreading (a non-synonymous substitution in *mutL (mutL13);* one non-synonymous substitution and four synonymous substitutions in *dnaQ (dnaQ905)*; see electronic supplementary material, table S6), and exhibits a genomic mutation rate that is as much as 4500-fold higher than that of wild-type *E. coli* [9]. Twenty populations were propagated on minimal glucose (MG) agar plates [10] and 20 populations were propagated on lysogeny broth (LB) agar plates [11]. All plates were supplemented with tetracycline (15 $\mu g\ ml^{-1}$). Populations were incubated at 37°C. Every 24 h, each population was bottlenecked to a size of one by streaking a random, isolated colony derived from a single cell to a fresh agar plate in order to isolate a new such colony. Populations were propagated for 50 transfers, corresponding to approximately 1250 generations (i.e. approximately 25 generations between transfers); a sample of each population was archived every fifth transfer.

(b) Fitness measurements

We estimated population fitnesses in liquid media every ten transfers by measuring growth rates and maximal (24 h) absorbance values at 600 nm. Frozen intermediate time points were inoculated into and grown in flat-bottomed 96-well microplates containing 15 $\mu g\ ml^{-1}$ of tetracycline in 250 µl of either Davis minimal medium (DM) supplemented with 1 $g\ l^{-1}$ of glucose [12], or LB. Because the ability of a line to produce colonies within 24 h should be positively related to its probability of being transferred in our experimental protocol, viable colony forming unit (CFU) counts were obtained as an additional measure of fitness by dilution and plating of 24 h liquid cultures to appropriate media.

(c) Estimation of mutation rates

Mutation rates to nalidixic acid resistance and streptomycin resistance (which arise at different genetic loci) were estimated in the ancestral strain and the evolved populations using a modified Luria–Delbrück fluctuation assay; statistical significance of mutation rate differences was inferred from non-overlap of 95% confidence limits of the estimated rates [13,14]. Mutation rates in surviving populations were measured in isolates stored at the end of the experiment; mutation rates in extinct populations were measured in isolates obtained from the last viable archived time point of each population. We were unable to obtain mutation rates from some populations that had evolved severe growth defects. All datasets from this study are accessible via the Dryad repository [15].

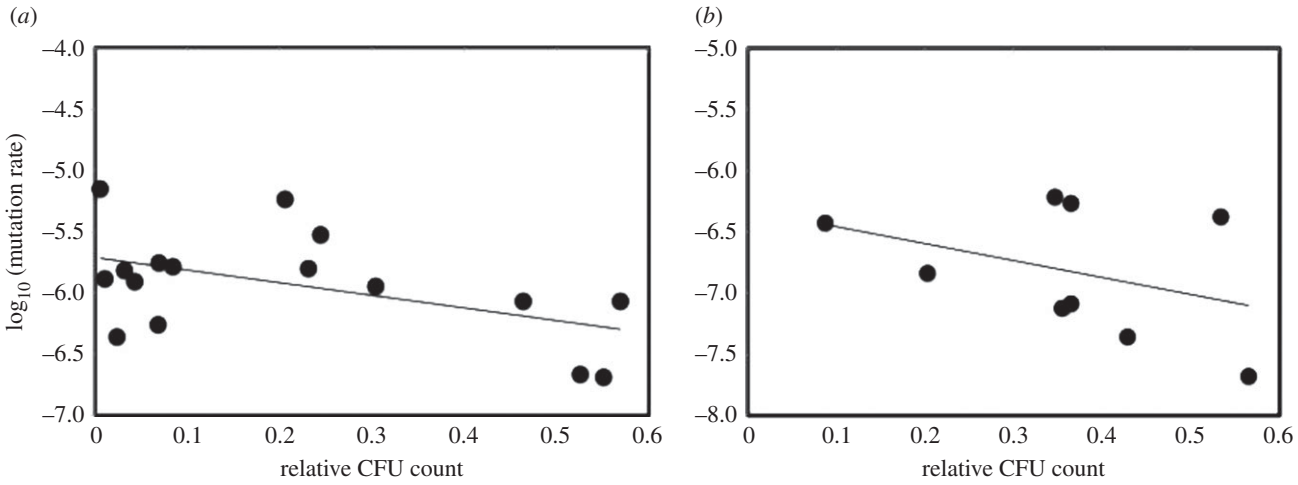

Figure 2. Endpoint fitness versus endpoint mutation rate to nalidixic acid resistance in the MG (*a*) and LB (*b*) populations that survived to the end of the experiment. Fitness is represented by the relative number of colonies obtained when plating identical dilutions of an evolved population and the ancestor (relative CFU count). The correlation between endpoint fitness and endpoint mutation rate is non-significant in the MG populations (p-value = 0.298) and significant in the LB populations (p-value = 0.045). The grey lines represent the least-squares fit regression lines for both datasets.

3. Results

Measures of fitness declined significantly in the populations during their propagation (electronic supplementary material, figure S1); moreover, two of the LB populations and nine of the MG populations went extinct by our experimental criteria (see electronic supplementary material). Among-population variance in fitness showed little evidence of increase over the entire course of propagation (for maximum growth rate; R^2 for LB = 0.00073. p-value = 0.960; R^2 for MG = 0.09889, p-value = 0.544, see electronic supplementary material, table S10); indeed, among-population variance in fitness was higher in the earlier time points assayed in both the MG and LB populations (see electronic supplementary material, figure S2).

Figure 1 illustrates the maximum-likelihood mutation rates in the ancestor and the MA populations. Ancestral mutation rates to nalidixic acid resistance and streptomycin resistance were higher in LB medium than in MG, consistent with previous studies [16]. Mutation rates to nalidixic acid resistance were significantly lower than that of the ancestor in seven of the nine surviving MG populations for which we could estimate mutation rates and in 12 of the 18 surviving LB populations. Mutation rates to streptomycin resistance were significantly lower than that of the ancestor in most populations for which nalidixic acid mutation rate had decreased. By contrast, mutation rates to streptomycin resistance were significantly higher than that of the ancestor in all of the populations that went extinct, and this pattern was generally corroborated by mutation rates to nalidixic acid resistance. Although there was no significant correlation between mutation rates to nalidixic acid and streptomycin resistance among the surviving populations ($R^2 = 0.346$, p-value = 0.098, see electronic supplementary material, table S7), when we pooled the surviving and extinct populations across the MG and LB treatments, nalidixic acid and streptomycin mutation rates were significantly correlated ($R^2 = 0.419$, p-value = 0.022, see electronic supplementary material, table S7). Fitness in the surviving populations appeared to be negatively related overall to mutation rate (see electronic supplementary material, table S9). A significant negative correlation between mutation rate and fitness was observed in one instance (figure 2), but this result should be regarded with caution given the number of comparisons in electronic supplementary material, table S9. Finally, among the MG populations mutation rates to both streptomycin resistance and nalidixic acid resistance were significantly higher overall in those populations that went extinct than in those populations that survived (two-tailed unpaired t-tests: nalidixic acid p-value = 5.852×10^{-5}; streptomycin p-value = 3.144×10^{-5}).

4. Discussion

A set of MA populations is expected to decline in average fitness over time because most mutations that affect the phenotype decrease fitness; because mutations accumulate in a stochastic manner, fitness variance among MA populations is expected to increase over time [5]. Based on the number of generations between bottlenecks, the effective size (using the harmonic mean of population size across generations) of our hypermutable MA populations was approximately 12 individuals [17]. Thus, mutations of absolute selective value substantially less than approximately 8% were free to accumulate in these populations. Indeed, these populations generally showed substantial and significant fitness declines and some went extinct. There was little evidence, however, for increase in fitness variance among our populations across the entire experiment. Because the rate of mutations affecting fitness in our *E. coli* ancestor strain was potentially as high as 0.9 per generation per genome [9], it may well be that substantial among-population variance in fitness was to be expected immediately in our MA experiment and that further increases in variance would be negligible. Other possible explanations for the lack of increased variance across the whole experiment may include selection against deleterious mutations of large effect and accumulation of deleterious mutations of mostly small, similar effects.

In general, indirect selection based on avoidance of mutational load is predicted to act only slowly and weakly to decrease mutation rates [2]. Systematic evolution of decreased mutation rates in our surviving MA populations was thus somewhat unexpected, although we note that Perfeito *et al.* [8] previously observed reductions in mutation rate in an MA experiment (see also [18]). Two factors could favour rapid evolution of reduced mutation rates in hypermutable MA

populations. First, selective pressure to avoid mutational load could become quite strong if fitness approaches a minimum viable value as deleterious mutations accumulate—especially, perhaps, if there is strong synergistic epistasis among deleterious mutations. Under such circumstances, a modifier that reduces genomic mutation rate substantially (an 'antimutator') could spread because it increases the relative fitness of an individual's descendants by more than the selective threshold imposed by the daily bottleneck regime. This possibility is qualitatively consistent with our findings that mutation rates were significantly higher in most populations that went extinct than in the populations that survived (figure 1). Second, the small effective population size of MA populations should greatly diminish any advantage that a high-mutation-rate lineage obtains through faster acquisition of beneficial mutations [19], thus further facilitating evolution of a reduced mutation rate. Indeed, a recent theoretical study [18] predicts that hypermutable populations can substitute antimutators via indirect selection when the deleterious mutation rate is high—a finding we have also obtained in individual-based computer simulations (see electronic supplementary material).

Our study is the first to document the evolution of higher mutation rates in mutation-accumulation lines and to show that higher mutation rates were significantly associated with extinction. Because very small effective population size should minimize opportunities for mutator hitchhiking by greatly reducing the frequency of selective sweeps, the evolution of higher mutation rates in our experimental system was unanticipated. While we cannot at present rule out mutator hitchhiking as an explanation for the evolution of higher mutation rates, an alternative possibility is that higher mutation rates evolved in these populations as a consequence of stochastic accumulation of mutator alleles under mutation pressure [20]. Discriminating between these two possibilities is the subject of ongoing work.

The results we have presented here have some implications for current ideas concerning mutation rate evolution and the fate of asexual populations. Recent studies [9,20,21] predict that recurrent mutator hitchhiking can cause mutation rate evolution to be upwardly biased in adapting asexual populations, perhaps even culminating in mutation rates that cause extinction [20]. Observations of such extremely high mutation rates have not been forthcoming so far in natural populations, with the exception of some viruses, perhaps because most populations exhibit some degree of horizontal genetic exchange that would tend to inhibit mutator hitchhiking [4]—or, perhaps, because asexual populations in which very high mutation rates do evolve quickly go extinct. Our study is most relevant to the latter possibility: the extremely small size and high mutation rate of our MA populations plausibly represent the terminal consequences predicted by models of recurrent mutator hitchhiking. Our results suggest that, under these circumstances, some populations may pull back from the brink of extinction—if only temporarily—by evolving reduced mutation rates, while others may be forced over the brink earlier by mutator hitchhiking or mutation pressure.

Data accessibility. Datasets available at Dryad: http://dx.doi.org/10.5061/dryad.dt115 [15].

Authors' contributions. M.H. and T.S. carried out all experimental procedures and participated in writing the manuscript. T.S. analysed the data and wrote computer simulations. P.S. participated in experimental design and helped write the paper. All authors agree to be accountable for all aspects of the work and give approval for publication of the manuscript.

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