The role of mutational spectrum in the selection against mutator alleles

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Abstract (250)

Rapidly adapting microbe and cancer cell populations often evolve high mutation rates. Yet, once adaptive opportunity declines, antimutator alleles are expected to take over as a result of indirect selection against deleterious mutations. Theory indicates that the most important determinant of antimutator invasions is the extent of mutation rate reduction. However, inconsistent results from evolution experiments suggest that additional factors may also play a major role in antimutator dynamics. Here we show that the idiosyncratic mutation bias exhibited by different mutators—a previously unrecognized factor—can greatly alter mutator susceptibility to antimutator invasions. Using a simulation model calibrated to mimic a well-known long-term evolution experiment with bacteria, we show that differences in average deleterious load can account for order-of-magnitude changes in antimutator fitness for a realistic range of parameters. Since these parameters are known to vary with the environment, our results reveal an unanticipated source of variability in antimutator dynamics. Finally, we estimated the genome-wide average disruptive effect on proteins of mutations caused by different mutators, and found marked and systematic differences emerging across mutators and species with different genomic GC compositions. Taken together, our results suggest that antimutator dynamics may be highly dependent on the specific genetic, ecological and evolutionary history of a given population. Such dependence reveals a more complex picture than anticipated, being relevant for understanding mutators in clinical settings, as well as how hypermutability shapes the evolution of bacterial genome size and composition.
**Introduction**

The idea that the mutation rate is evolvable has captivated the interest of evolutionary biologists for decades (Sniegowski *et al.*, 2000). It was early recognized that, since the vast majority of mutations with phenotypic effects are deleterious, selection should primarily act to reduce the deleterious load, pushing mutation rates to be as low as physiologically affordable (Eshel, 1973; Kimura, 1960; Leigh, 1973; Liberman and Feldman, 1986; Sturtevant, 1937). However, strains with highly-elevated mutation rates (i.e. mutators) are readily selected in clinical and laboratory populations of bacteria (LeClerc *et al.*, 1996; Sniegowski *et al.*, 1997) and yeast (Healey *et al.*, 2016; Voordekers *et al.*, 2015), as well as in certain cancers (Loeb, 2011). Theory and experiments have explained this phenomenon in terms of selection pressures operating at different timescales: linkage with strong beneficial mutations enables mutators to rapidly reach fixation before their increased deleterious load becomes fully manifest, which requires the accumulation of multiple secondary deleterious mutations (Good and Desai, 2016). Due to this reliance on rapid hitchhiking, mutators are most likely to thrive whenever populations face strong selection pressures (Tenaillon *et al.*, 1999) and under conditions in which both recombination (Tenaillon *et al.*, 2000) and genetic drift (Raynes *et al.*, 2018) are unimportant.

But, eventually, populations fixed for a mutator phenotype are expected to re-evolve low mutation rates – provided that selective pressure subsides and restorative alleles are available. Given the longer timescales involved, the evolution of reduced mutation rates has proven much more difficult to observe than its reverse process, the selection for mutator alleles. Indirect evidence comes from the fact that DNA repair genes seem to undergo frequent horizontal transfer (Brown *et al.*, 2001; Denamur *et al.*, 2000; Elena *et al.*, 2005), and the observation of marked mutation rate polymorphisms within single-patient bacterial populations (Couce *et al.*, 2016). Direct, empirical evidence of the evolution of reduced mutation rates is limited to a handful of experimental evolution studies (McDonald *et al.*, 2012; Singh *et al.*, 2017; Tröbner and Piechocki, 1984; Turrientes *et al.*, 2013; Wielgoss *et al.*, 2013). The provisional picture that emerges from these studies is rather heterogeneous, with different experiments reporting contrasting findings in terms of timescales, mechanisms and magnitude of mutation rate reduction. Recent theoretical work has begun to provide a framework to account for these contrasting patterns, emphasising the role of several factors in determining the fixation probability of antimutator alleles. These factors include differences in population size, beneficial and deleterious mutation rates, mutator strength, and the
availability of secondary mutations compensating the cost of deleterious mutations (Good and Desai, 2016; Jain and James, 2017; James and Jain, 2016).

An additional, yet unexplored factor is the well-known mutational idiosyncrasy exhibited by different mutators (Miller, 1996). This idiosyncrasy arises from the particular molecular details of the mutation-avoidance mechanism that are impaired in each mutator genotype. In *Escherichia coli*, for instance, impairment of any of the enzymes removing oxidized guanine from the DNA (e.g. MutM, MutY) results into substantial elevations of G:C→T:A mutations, while disruption of the enzyme preventing its incorporation from the free nucleotide pool (e.g., MutT) leads to a marked increase in A:T→C:G mutations (Michaels and Miller, 1992). These sort of mutational biases shape the tendency of different mutators to generate mutations with different fitness effects, which can have dramatic consequences on mutator success when adaptation involves just a few strongly beneficial mutations (Couce et al., 2013). In analogy to this phenomenon, an intriguing hypothesis is that mutators that tend to generate stronger deleterious mutations may be more easily out-competed by an invading, low-mutation rate genotype. Similarly, mutators producing on average milder deleterious mutations than the wild-type may resist the invasion of antimutator alleles for longer. Whether these possibilities are plausible or not under realistic scenarios remains largely unknown.

In a first approach, at least two considerations argue against the idea that mutational spectrum differences can play any significant role in the evolution of reduced mutation rates. The first one comes from the classic Haldane-Muller principle (Haldane, 1937; Muller, 1950), which states that the reduction in fitness caused by recurring deleterious mutations is roughly on the order of the deleterious mutation rate ($u_d$), irrespective of the actual fitness cost of each individual mutation ($s_d$). Such independence from $s_d$ should preclude any spectrum-driven differences in mutational load among mutators. It is well-known, however, that this principle only holds as long as $s_d > u_d$ (Crow, 1970), a condition that may readily be violated in well-adapted, mutator populations of microbes. Second, different biases in the production of mutations are likely to translate into substantial fitness differences when just a few number of sites have a huge impact on fitness, as in the case of strongly beneficial antibiotic resistance mutations (Couce et al., 2013). It is unclear, however, to what extent these kind of spectrum-driven differences may balance out when considering a larger number of sites. Relevant to this issue is the observation that some amino acid substitutions tend to be much more disruptive to proteins than others, a well-established fact that forms the basis of many protein alignment tools (Yampolsky and Stoltzfus, 2005). This fact affords speculation that systematic
patterns may emerge at the genome-wide scale, so that different mutational spectra may produce, on average, deleterious mutations with characteristically different fitness effects.

Here, we used computer simulation to explore the extent to which the advantage of an antimutator allele deviates from the Haldane-Muller expectations under the relevant range of parameters. In addition, we estimated the genome-wide average disruptive effect on proteins of mutations caused by different mutational spectra. Importantly, since different codon usage patterns might alter the probability that a particular spectrum generates strong-effect amino acid changes, we also tested whether systematic differences are to be expected among mutators in species with widely-divergent genomic GC compositions. Overall, our results suggest that mutational spectrum differences may play an unsuspectedly important role in the selection against high mutation rates in bacteria.

Results and Discussion

Broad conditions allow mutational spectrum to impact antimutator evolution

To test whether mutational spectrum differences can alter the evolution of reduced mutation rates, we built a computer model that simulates the evolutionary dynamics of antimutator alleles invading a mutator population. The model was designed to capture the basic properties of the influential Lenski’s Long-Term Evolution Experiment (LTEE), in which 12 *Escherichia coli* populations have been serially propagated in the same glucose-limited medium for more than 60,000 generations (Good *et al.*, 2017). Crucially, one of these bacterial populations was observed to re-evolve reduced mutation rates after being dominated by a mutator phenotype for more than 10,000 generations (Wielgoss *et al.*, 2013). Inspired by this experiment, we considered the simple scenario of an asexual mutator population being serially propagated in a constant environment to which is already well-adapted (see Material and Methods). At the start of each simulation, a single antimutator allele, restoring the mutation rate to wild-type levels, is introduced. The trajectory of this allele is tracked until its either lost by drift or increases in frequency by a 1,000-fold factor. Multiple frequency trajectories are then used to estimate the average effective selection coefficient ($s_{eq}$) of the antimutator allele, computed empirically as the log change of the antimutator-to-mutator ratio per generation (see Material and Methods).

Our first aim was to test whether the Haldane-Muller principle can be violated over the range of parameters typically reported in experiments with mutator bacteria. In particular, the two most
important parameters for this matter are the mutation rate of mutators ($m$) and the average selection coefficient of deleterious mutations ($s_d$). Most estimates of $m$ are based on a few reporter genes, and so caution should be exercised when using them as a proxy for genome-wide rates (Foster, 2006).

However, while more than a dozen genes are known to increase bacterial mutation rates when inactivated (Miller, 1996; Oliver and Mena, 2010), only those causing order-of-magnitude elevations of $m$ are the ones typically observed in clinical and experimental evolution studies (Jolivet-Gougeon et al., 2011; Oliver and Mena, 2010). Therefore, the relevant range spans from slightly over a 10-fold increase (e.g. mutY, Notley-McRobb et al., 2002) to a 1,000-fold increase of $m$ (e.g. dnaQ, Ragheb et al., 2019), although all mutators observed in the LTEE fall in the several 100-fold range (e.g., mutT, Wielgoss et al., 2013; mutL, Sniegowski et al., 1997).

The estimates of $s_d$ also display a certain degree of uncertainty. Attempts to estimate $s_d$ classically relied on mutation accumulation experiments, in which populations are serially passaged through single-cell bottlenecks to restrain selection from purging deleterious mutations (Eyre-Walker and Keightley, 2007). However, since populations need to recover sufficiently after the single-cell bottleneck for the experiment to continue, there exists an upper limit on how deleterious a mutation can be to get detected, which may lead to an underestimation of $s_d$ according to the growth conditions employed (Elena and Lenski, 1997). Despite this limitation, different experiments have provided similar values for both the upper and the lower bounds of $s_d$. Using an early isolate from the LTEE, (Kibota and Lynch, 1996) estimated an upper bound for $s_d$ of 0.012. Two later studies, also using E. coli, reported slightly higher values ($s_d \sim 0.03$) (Elena and Lenski, 1997; Trindade et al., 2010). Of note, both studies pointed to differences in mutational spectrum as a possible explanation for their higher estimates (they examined a transposon-based insertion library, and a mutS mutator strain, respectively). More recently, a few studies have leveraged the resolution afforded by next-generation sequencing to provide a lower bound for $s_d$. These studies reported remarkably close values for this lower bound ($s_d \sim 0.0015 \text{ to } 0.0017$), even though they involved three different bacterial species (Salmonella typhimurium, Lind and Andersson, 2008; Pseudomonas aeruginosa, Heilbron et al., 2014 and Burkholderia cenocepacia, Dillon and Cooper, 2016). In addition, one of these studies found that $s_d$ can vary noticeably across environments (Dillon and Cooper, 2016).

Figure 1 provides a general overview of the invasion dynamics observed in the computer simulation model. In line with the Haldane-Muller expectations, we observed that the mutation rate of the resident mutator ($m$) strongly determines the speed of the antimutator invasion (Figure 1a).
However, in contrast with the Haldane-Muller principle, we found that the fitness cost of deleterious mutations ($s_d$) can also exert a substantial, albeit less dramatic effect on invasion speed (Figure 1b). While this dependence on $s_d$ is most pronounced when mutation rates are the highest and fitness costs the smallest, our results show that invasion dynamics can indeed be affected by $s_d$ over a large fraction of the relevant range of parameters (Supplementary Figure S1). Therefore, there are grounds to speculate whether spectrum-driven differences in $s_d$ may alter the propensity of different mutators to evolve reduced mutation rates. To examine this possibility, we expanded the computer simulation model to allow consideration of general biases in the production of deleterious mutations. We modelled these biases as a multiplicative factor ($\kappa$) that modifies the selection coefficient of deleterious mutations in the mutator background, such that when $\kappa=1$ there are no differences between mutators and antimutators (see Material and Methods).

Figure 2 captures how the interplay between $s_d$ and $m$ controls the degree to which mutational spectra differences ($\kappa$) impact on the success of antimutator alleles. Two patterns can readily be appreciated by observing the overall shape of the curves in Figure 2. First, the slopes become steeper with mutation rate ($m$) (which increases from panel a to d). In turn, the slopes become flatter with fitness cost ($s_d$) (which increases within each panel from bottom to top). In line with the discussion in the previous paragraph, these general patterns can be interpreted in terms of deviations from the Haldane-Muller principle. Thus, the impact of $\kappa$ is the greatest when mutation rate is maximal and fitness cost is minimal (Figure 2d, lowest line) – exactly the same conditions under which the dependence of invasion speed on $s_d$ is most pronounced (Figure 1b and Supplementary Figure S1). Conversely, when populations approach the regime in which the Haldane-Muller principle holds ($s_d > u_d$), the impact of $\kappa$ becomes rather modest, which visually translates into comparatively flatter slopes (Figure 2a, upper lines).

The previous analysis shows that the importance of mutational spectrum ultimately depends on how large the mutation rate is compared with the fitness cost of deleterious mutations. Therefore, a further natural parameter to consider is the basal deleterious mutation rate ($u_d$), that is, the absolute rate at which deleterious mutations are produced in the non-mutator background. Estimates of this quantity have classically been obtained through mutation accumulation experiments, and consequently suffer from the same uncertainties discussed for $s_d$. Throughout the previous simulations we set $u_d = 2 \times 10^{-4}$, as originally estimated by Kibota & Lynch (Kibota and Lynch, 1996) in *E. coli*. However, while reports in other bacteria have provided similar or slightly lower values ($u_d = 1.8 - 0.7 \times 10^{-4}$; Dillon and Cooper, 2016; Trindade *et al.*, 2010), estimates in yeast...
differ by more than an order of magnitude, depending on whether the strain is haploid ($u_d = 1.1 \times 10^{-3}$; Wloch et al., 2001) or diploid ($u_d = 0.6 – 0.5 \times 10^{-4}$; Joseph and Hall, 2004; Zeyl and DeVisser, 2001). On top of this, an additional layer of variability comes from the fact that the overall mutation rate can vary across growth conditions (Degnen and Cox, 1974; Krašovec et al., 2014; Chu et al., 2018; Shewaramani et al., 2017). In Figure 3 we explored how changes in $u_d$ within the empirically relevant range can alter the previously discussed results from Figure 2. A prominent pattern emerging from Figure 3 is that the slopes become steeper with larger values of $u_d$ (Figure 3c). This result mimics the pattern found for increasing $m$ in Figure 2, and can be understood in terms of populations moving gradually away from the Haldane-Muller regime. A more remarkable observation is that even for the lowest values tested, despite the relatively flatter slopes, the mutational spectrum is still capable of exerting a moderate but sizeable impact on the performance of invading antimutator alleles.

Overall, the results presented here support the notion that the impact of mutational spectrum on antimutator evolution can be substantial under a wide and relevant range of conditions. Finally, it is worth noting that the variation in the slopes in Figures 2 and 3 results in a large area of overlap among the curves obtained for different mutators under various combinations of parameters, especially for the smaller values of $\kappa$. This overlap represents the range of conditions under which a stronger mutator will actually be more robust to antimutator invasions than a weaker one. Since both $s_d$ and $U_d$ can vary appreciably across species and environments, such a counterintuitive outcome illustrates the importance of considering the mutational spectrum when investigating the evolution of reduced mutation rates.

**Different mutational spectra cause distinct genome-wide protein-disrupting patterns**

The previous results show that spectrum-driven differences in $s_d$ can greatly influence the evolution of reduced mutation rates in bacteria. It remains to be explored, nonetheless, whether spectrum-driven differences in $s_d$ are actually likely to occur among bacterial mutators. While differences in fitness have indeed been observed in the case of beneficial mutations involving a few genomic sites (Couce et al., 2013), the very large mutational target size for deleterious mutations may cause local, spectrum-driven differences to balance out at the genome-wide scale. However, a first look at the properties of the genetic code affords reasonable grounds for expecting the emergence of some general trends. Certainly, it has long been known that transversions are overall more detrimental than transitions, due to the fact that transversions underlie a larger fraction of non-synonymous
substitutions and, among these, tend to produce changes that are less conservative of the physicochemical properties of amino acids (Lyons and Lauring, 2017; Wakeley, 1996; Zhang, 2000). Besides these trends, a closer examination reveals that the 6 types of point mutations display fairly broad distributions of disruptive effects (see Supplementary Figure S2). Such breadth raises the possibility that, ultimately, the average disruptive effect of a given mutational spectrum may actually be determined by the highly-diverse codon usage preferences observed among bacterial species (Wan et al., 2004).

To explore these possibilities, we set out to quantify the average protein-disrupting effect of the specific point mutations elevated in 3 prominent types of mutators: mutY (G:C → T:A), mutT (A:T → C:G) and Mistmatch Repair (G:C → A:T, A:T → G:C) mutators (Miller, 1996; Oliver and Mena, 2010) (see Material and Methods). Briefly, we systematically computed all of the possible substitutions per codon associated with each mutational spectrum across a panel of bacterial genomes spanning a wide range of GC compositions. We then estimated the protein-disrupting effects of all these spectrum-specific substitutions by applying the well-known Grantham’s matrix of physicochemical distance (Grantham, 1974). This amino-acid substitution matrix was previously shown to provide the best predictions of empirical fitness effects among standard distance-based matrices (Jacquier et al., 2013). As validation, we also conducted the analyses applying an alignment-based substitution matrix (BLOSUM100, Henikoff and Henikoff, 1992), which provided comparable results. Moreover, using the specific case of the LTEE experiment, we have shown that the use of Grantham’s matrix provides an efficient alternative to more sophisticated and computationally intensive methods, such as Direct Coupling Analysis (Couce et al., 2017) (see Supplementary Figure 3). Finally, seeking to increase the likelihood of non-synonymous mutations being predominantly harmful, we initially conducted these analyses for genes belonging to the COG categories most commonly enriched in essential genes (H: Coenzyme metabolism, J: Translation and M: Cell wall/membrane/envelop biogenesis; Mandal et al., 2017) – although the overall patterns remained similar when considering whole genomes (see Supplementary Figure 4).

Figure 4 shows that there are indeed marked differences in the protein-disrupting effects of mutations caused by the different mutational spectra. The Mistmatch Repair spectrum displays the weakest disruptive effects in all tested backgrounds (Figure 4, green), which makes sense since this spectrum comprises the two transitions, well-known to be the most conservative among all possible point mutation types (Lyons and Lauring, 2017; Wakeley, 1996; Zhang, 2000). While interesting, we shall note that this result is probably an underestimation since Mistmatch Repair mutators, apart
from point mutations, also exhibit an elevated occurrence of indels and large recombination events (Elez et al., 2007). More remarkable is the fact that the disruptive effects associated with the mutY− and mutT− spectra exhibit a strong and opposite dependence on the GC content of the genetic background. In particular, we observe that the mutY− spectrum is highly detrimental in AT-rich backgrounds (Figure 4, red), while the mutT− spectrum inflicts its greatest disruption in GT-rich backgrounds (Figure 4, blue). This contrasting behavior is amenable to a straightforward explanation: whatever the processes causing the base composition bias may be, the last codons to be changed to conform to this bias should be the ones for which the change will produce the most harmful effects. These last codons are exactly the ones being predominantly altered by mutY−-specific mutations (G:C → T:A) and mutT−-specific mutations (A:T → C:G) in AT-rich and GT-rich backgrounds, respectively.

In addition, we should expect the fitness cost of altering these last, non-conforming codons to be the greatest in conditions where selection is weak compared to other evolutionary forces, since under such conditions selection can only prevent the most essential amino-acid sites from changing. This phenomenon would help explain why the most disruptive effects are found for the mutY− spectrum in the most AT-biased genomes – generally seen as reflective of highly-relaxed selective conditions (Hershberg and Petrov, 2010; Hildebrand et al., 2010; McCutcheon and Moran, 2011). This effect is better appreciated in the analyses with the distance-based instead of the aligned-based matrix (Figure 4b versus Figure 4c), perhaps because physicochemical distance is a more pure proxy for protein-disrupting effects than evolutionary conservation, which integrates the effects of several other factors (e.g. epistasis, basal mutational bias) (Yampolsky and Stoltzfus, 2005).

**Concluding Remarks**

Our analyses reveal that different mutators can be expected to produce deleterious mutations with distinctive fitness effects, and that such idiosyncrasy can greatly impact antimutator invasion dynamics. At least two points regarding these findings merit brief consideration. First, the simulations purposely focused on the effects of mutational spectra on deleterious mutations, leaving aside the complications of considering either compensatory or generally-beneficial mutations. While previous research has already studied the importance of these types of mutations on antimutator dynamics (Good and Desai, 2016; Jain and James, 2017; James and Jain, 2016), a full treatment of this problem should take into account the fact that spectrum-driven differences can also bias mutator access to both compensatory and generally-beneficial mutations – a non-trivial degree
of complexity that will be reserved for future research. Second, it is worth noting the breadth of conditions under which spectrum effects are noticeable, as well as the magnitude that these effects can reach – including the paradoxical situation of weak mutators exhibiting larger deleterious load that strong mutators. The breadth and magnitude of these effects lead us to conclude that, even if taken only as a first approximation, our analyses strongly support the notion that mutational spectrum differences can drive antimutator evolution in many biologically-relevant scenarios.

Finally, it is worth pointing out that the general finding of our study is that antimutator success depends not only on the extent of mutation rate elevation, but also on the mutational spectrum, the genetic background and the environmental conditions. Since the exact contribution of these factors is essentially an empirical question, it is possible that the likelihood of antimutator invasions in real-world scenarios may have to be evaluated on a case-by-case basis. Such dependence on the particulars of each case has at least two important consequences. In clinical settings, it can complicate predictions about the long-term persistence and transmissibility of mutators, thus being relevant to interventions aimed at curbing the contribution of mutators to antibiotic resistance evolution (Oliver and Mena, 2010). More broadly, it has implications for our views on how mutators shape the evolution of bacterial genomes. Episodes of hypermutability can be common along the evolutionary history of bacterial lineages, inflicting rapid changes in genome size and composition that can blur the signature of selection (Couce et al., 2017). Our results suggest that the length of these pulses of hypermutability, and therefore their potential impact, may be highly dependent on the specific genetic, ecological and evolutionary history of a given lineage – a possibility further complicating the interpretation of present-day patterns of bacterial genome diversity.

Materials and Methods

Computer simulation

The computer model simulates the serial passage of a bacterial population in a laboratory environment to which is already well-adapted. Mimicking serial passage, the algorithm recreates two events: population growth and bottleneck. In the first one, cells reproduce and accumulate mutations for ~ 6.7 generations (population sizes oscillate daily between $10^7$ and $10^9$) (Lenski et al., 1991). Reproduction is formulated in terms of discrete, non-overlapping generations (Chevin, 2011). Every generation, individuals reproduce according to their multiplicative growth rate,
defined as \( r = 2 + ns_d \), where \( n \) represents the number of accumulated deleterious mutations and \( s_d \) is the average deleterious selection coefficient. Mutation is implemented by using a Poisson-distributed pseudorandom number generator (the function \texttt{rpois} in R). Every generation, individuals acquire deleterious mutations with a probability depending on the basal deleterious mutation rate \( (u_d) \) and the mutator strength \( (m) \) (note that for antimutator alleles \( m=1 \)). Lethal mutations are implemented similarly, with a fixed value of \( u_l = 1 \times 10^{-5} \) (Robert et al., 2018; Tenaillon et al., 1999). The second part of the algorithm is executed when population size exceeds the limit of \( 10^9 \) individuals, and consist of taking a random sample of \( 10^7 \) individuals, after which growth is resumed.

Simulations start with a single antimutator allele entering a population of \( 10^7 \) mutator individuals, and terminate when the frequency this allele reaches either zero or increases by a 1,000-fold factor. The average effective selection coefficient of the antimutator allele is calculated empirically as \( s_{\text{eff}} = \log((p_g/q_g)/(p_0/q_0))/g \) where \( p \) and \( q \) represent the frequency of the antimutator and mutator allele, respectively, and \( g \) is the number of generations (Chevin, 2011). To implement the differential access of mutators to deleterious mutations with different fitness costs, we introduced a multiplicative factor \( (\kappa) \) that modifies \( s_d \) in the mutator background as \( r = 2 + \kappa ns_d \). Note that when \( \kappa=1 \) there are no differences between mutators and antimutators. For all tested parameter combinations, reported values of \( s_{\text{eff}} \) were computed from 200 replicates. All programming was performed in R version 3.2.3 (R Core Team, 2015), and basic codes are freely available upon request.

**Genome analyses**

To conduct the bioinformatic analyses we developed a series of scripts in Python (version 2.7.12) (www.python.org), freely available upon request. These codes were applied to a panel of 25 bacterial genomes, including relevant pathogens, and chosen to span a wide range of GC compositions. A summary of the main features of these genomes is presented in Supplementary Table S1. For all strains, the predicted coding sequences (CDSs) and their functional classification (COG) were retrieved from the Microscope platform from Genoscope (www.genoscope.cns.fr) (Vallenet et al., 2006). After formatting and parsing, we estimated the average protein-disrupting effect of different mutations for all CDSs across the panel of genomes. We achieved this by computing the Grantham and BLOSUM100 scores for all of the possible substitutions per codon associated with each mutational spectrum. The Grantham and BLOSUM100 matrices were obtained.
from the AAindex database (www.genome.jp/aaindex) (Kawashima and Kanehisa, 2000) and the NCBI FTP server (ftp.ncbi.nih.gov/blast/matrices), respectively. Codons harbouring incompletely specified bases (e.g. N, R, Y) were excluded from the analyses.

This article contains Supplementary Material available online

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Author contributions

Conceived the study: AC. Designed and conducted the simulations: AC. Analysed data: AC, OT. Wrote the paper: AC, OT.
Figures

Fig. 1.- Frequency trajectories of antimutator alleles invading well-adapted, mutator populations. Lines represent 100 independent simulations for each condition. (a) Invasion dynamics under various values of the mutation rate of the resident mutator (grey, $m = 1000$; magenta, $m = 300$; blue, $m = 100$; green, $m = 30$), and a fixed fitness cost of deleterious mutations ($s_d = 0.01$). (b) Invasion dynamics under various values of the fitness cost of deleterious mutations (from left to right, $s_d$ equals: 0.064, 0.032, 0.016, 0.008, 0.004, 0.002, 0.001), and a fixed mutation rate of the resident mutator ($m = 1000$). Other parameters as described in Material and Methods.
Mutational spectrum effects on the invasion speed of antimutator alleles. Points represent the effective selection coefficient ($s_{\text{eff}}$) of the invading antimutator alleles, averaged from 200 independent simulations. Panels correspond to different values of the mutation rate of the resident mutator ($a, m = 30$; $b, m = 100$; $c, m = 300$; $d, m = 1000$). Within each panel, lines depict different values for the fitness cost of deleterious mutations (from top to bottom, $s_d$ equals: 0.064, 0.032, 0.016, 0.008, 0.004, 0.002, 0.001). Mutational spectrum effects refers to the differential propensity of mutators to produce deleterious mutations with different fitness cost. We modelled this effect as a multiplicative factor ($\kappa$) that modifies $s_d$ in the mutator background, such that when $\kappa=1$ mutators and antimutators generate deleterious mutations of similar effect.
Fig. 3.- Impact of the basal deleterious mutation rate on antimutator dynamics. Panels (a) and (b) show antimutator fitness under two values of the basal deleterious mutation rate ($u_d = 8 \times 10^{-4}$ and $u_d = 0.5 \times 10^{-4}$, respectively). Points represent the effective selection coefficient ($s_{eff}$) of the invading antimutator alleles, averaged from 200 independent simulations. Lines depict different values of $s_{da}$ following the same convention as in Figure 2. Panel (c) shows the change in the antimutator’s effective selection coefficient ($s_{eff}$) for various values of the basal deleterious mutation rate (from top to bottom, $u_d$ equals: $1.6 \times 10^{-3}$, $8 \times 10^{-4}$, $4 \times 10^{-4}$, $2 \times 10^{-4}$, $1 \times 10^{-4}$, $0.5 \times 10^{-4}$). Fold change refers to the change in $s_{eff}$ from $\kappa = 0.25$ to $\kappa = 4$. In all cases, the mutation rate of the resident mutator was fixed to a single value ($m=300$). Other parameters as described in Material and Methods.
Fig. 4.- Protein-disrupting effects of mutations caused by different mutators in different genomes. Colours correspond to predictions for $\text{mutY}^-$ (red), $\text{mutT}^-$ (blue) and Mistmatch Repair$^-$ (green) mutators. For comparison, the effects of a unbiased spectrum are highlighted with a grey background. (a) Grantham scores in five different bacterial species for which hypermutability is of particular interest. These species are arranged, from left to right, according to increasing GC content. (b) Average Grantham scores across a panel of species with genomes spanning a wide range of GC compositions. (c) Average BLOSUM100 scores across the same panel. Details about these genomes are shown in Supplementary Table S1.
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