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REVIEW

# Experimental evolution and the dynamics of genomic mutation rate modifiers

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Because genes that affect mutation rates are themselves subject to mutation, mutation rates can be influenced by natural selection and other evolutionary forces. The population genetics of mutation rate modifier alleles has been a subject of theoretical interest for many decades. Here, we review experimental contributions to our understanding of mutation rate modifier dynamics. Numerous evolution experiments have shown that mutator alleles (modifiers that elevate the genomic mutation rate) can readily rise to high frequencies via genetic hitchhiking in non-recombining microbial populations. Whereas these results certainly provide an explanatory framework for observations of sporadically high mutation rates in pathogenic microbes and in cancer lineages, it is nonetheless true that most natural populations have very low mutation rates. This raises the interesting question of how mutator hitchhiking is suppressed or its phenotypic effect reversed in natural populations. Very little experimental work has addressed this question; with this in mind, we identify some promising areas for future experimental investigation.
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## INTRODUCTION

More than 75 years ago, AH Sturtevant noted that alleles at some gene loci may modify the mutation rate and reasoned that such alleles could be subject to natural selection solely through their genetic associations with fitness-affecting mutations at other loci (Sturtevant, 1937). Theoretical studies began exploring the population genetics of mutation rate modifier alleles in the 1960s, and significant theory papers on mutation rate evolution have appeared in every decade since (Kimura, 1967; Leigh, 1970, 1973; Ishii *et al.*, 1989; Taddei *et al.*, 1997; Johnson, 1999; Andre and Godelle, 2006; Gerrish *et al.*, 2007; Lynch, 2008; Wylie *et al.*, 2009; Desai and Fisher, 2011). Direct experimental work on the population genetics of mutation rate modifiers began in the 1970s (Gibson *et al.*, 1970; Cox and Gibson, 1974) and has grown rapidly in recent years (Giraud *et al.*, 2001; Notley-McRobb *et al.*, 2002b; Thompson *et al.*, 2006; Pal *et al.*, 2007; Gentile *et al.*, 2011; Raynes *et al.*, 2011, 2012, 2014; McDonald *et al.*, 2012; Maharjan *et al.*, 2013; Wielgoss *et al.*, 2013). Here, we review advances in our understanding of mutation rate evolution that have been contributed by experimental studies. Most such studies have been dedicated to investigating the conditions under which modifiers that increase mutation rates (mutators) will spread within populations. We note that there remains a need for studies investigating the conditions under which the spread of mutators is inhibited or its phenotypic effect reversed, and we suggest some avenues for further research that can address this problem.

## SEMINAL EXPERIMENTS

In a pioneering series of experiments in the 1970s and 1980s, EC Cox and collaborators propagated mixed populations of isogenic mutator (bearing a defect at the *mutT* locus) and wild-type strains of the bacterium *Escherichia coli* in continuous chemostat cultures (Gibson *et al.*, 1970; Cox and Gibson, 1974; Chao and Cox, 1983). Their work showed that the *mutT* mutator strain would ultimately spread in such populations if initially present above a critical threshold frequency and decline if present below the threshold frequency. Notably, there was an initial lag period before the mutator began to spread during which its frequency remained relatively stable or even declined (Chao and Cox, 1983). Taken together, these results indicated that the mutator strain was able to supplant the wild type not because of some intrinsic fitness advantage of the defective *mutT* allele, but as a consequence of selection for beneficial mutations that arose at a higher rate in the *mutT* genetic background. The results of Cox and collaborators provided the first explicit experimental demonstration that mutation rate modifiers can be subject to indirect natural selection via their linkage to fitness-affecting alleles. The spread of mutators observed in these and subsequent similar experiments (discussed below) can be seen as a particular example of the general population genetic phenomenon in which neutral or even deleterious alleles rise in frequency—hitchhike—in linkage with alleles under positive selection (Maynard Smith and Haigh, 1974).

Although the early experiments of Cox and others provided compelling demonstrations of indirect selection on mutators, they raised many questions: If mutator alleles require a substantial initial frequency in a population in order to spread by hitchhiking, then how would a higher mutation rate ever evolve in a population in which mutators must themselves arise as rare mutations? How do the supply rates and distributions of fitness effects of beneficial and deleterious mutations affect the fates of mutators? What is the quantitative effect of recombination on mutation rate evolution: for example, how much recombination is required to inhibit mutator hitchhiking? How would

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the phenotypic effect of mutator hitchhiking be reversed, that is, how would alleles conferring 'lower' mutation rates invade mutator populations? Are mutator alleles intrinsically neutral, or do they themselves sometimes have direct effects on fitness? As we discuss in detail below, some of these questions and related questions have been addressed in experimental work since the early 1980s—in some cases, with surprising answers. However, we note that the question of how mutator hitchhiking is impeded or its phenotypic effect reversed remains little studied by experimentalists.

## MUTATOR HITCHHIKING AS A FUNDAMENTAL ASPECT OF ASEXUAL POPULATIONS

Since the original work by Cox and collaborators, numerous microbial evolution experiments have confirmed that mutator alleles will hitchhike when introduced into experimental asexual populations at substantial frequencies. Such experiments have primarily been performed *in vitro* in *E. coli* (Trobner and Piechocki, 1984a; Labat *et al.*, 2005; de Visser and Rozen, 2006; Loh *et al.*, 2010; Gentile *et al.*, 2011) and the yeast *Saccharomyces cerevisiae* (Thompson *et al.*, 2006; Raynes *et al.*, 2011, 2012, 2014), but some work has also been carried out *in vivo* by propagating *E. coli* populations in the mouse gut (Giraud *et al.*, 2001). The focus of these experiments has not necessarily been to demonstrate mutator hitchhiking anew, but rather to explore the influence of various population genetic factors on the likelihood and dynamics of mutator hitchhiking, as discussed below.

The question of whether rare, spontaneously originated mutator mutants can hitchhike to high frequency in asexual populations was first addressed experimentally in the 1990s. Studies of 12 long-term experimental evolution populations of *E. coli* founded by Lenski *et al.* (1991) showed that three of these populations evolved 100-fold elevated mutation rates within the first 10 000 generations of their propagation through hitchhiking of spontaneously originated mutator alleles (Sniegowski *et al.*, 1997; Shaver *et al.*, 2002). These results and related theoretical work (Taddei *et al.*, 1997; Tenaillon *et al.*, 1999; Andre and Godelle, 2006; Gerrish *et al.*, 2007) have revealed that although mutator hitchhiking is unlikely in the short run when mutators arise spontaneously and are rare, it can become highly probable over longer time spans due to chance associations between mutators and beneficial mutations. Work by Mao *et al.* (1997) showed that repeated rounds of lethal selection on large experimental populations of *E. coli* would also greatly increase the frequencies of spontaneous mutator mutants.

The original findings of Sniegowski *et al.* (1997) and Mao *et al.* (1997) have been corroborated by multiple independent studies demonstrating the spread of spontaneous mutator mutants in microbial experimental populations (Notley-McRobb *et al.*, 2002a,b; Pal *et al.*, 2007), including three more instances of spontaneous mutator hitchhiking in the 12 experimental populations of Lenski *et al.* (Barrick *et al.*, 2009; Blount *et al.*, 2012; Wiser *et al.*, 2013). In the study by Giraud *et al.* (2001), mutators were also detected at significant frequencies in two of the initially wild-type *E. coli* populations several weeks after colonization of the mouse gut. Moreover, observations of high mutator frequencies in natural populations of viruses and of pathogenic and commensal bacteria (Suarez *et al.*, 1992; LeClerc *et al.*, 1996; Matic *et al.*, 1997; Bucci *et al.*, 1999; Oliver *et al.*, 2000; Björkholm *et al.*, 2001; Gould *et al.*, 2007) and the widespread genomic instability observed in cancer cell populations (Negrini *et al.*, 2010; Loeb, 2011; Burrell *et al.*, 2013) are consistent with mutator hitchhiking occurring in natural populations as well as in experimental populations.

The apparent ease with which mutator alleles can hitchhike to high frequency in the absence of recombination has inspired theoretical work suggesting that asexual populations should have a net tendency to evolve ever-higher genomic mutation rates as long as they are substituting beneficial alleles of substantial effect (Andre and Godelle, 2006; Gerrish *et al.*, 2007). To date, there has been only one experimental test related to this idea: Gentile *et al.* (2011) used Chao and Cox-style competition experiments to show that a strain of *E. coli* bearing two mutator alleles (mismatch repair and proofreading deficiencies) can prevail over an isogenic strain bearing a single mutator allele and having a correspondingly lower mutation rate. These results are consistent with the theory that mutation rate evolution is upwardly biased in asexual populations undergoing adaptive evolution; whether spontaneously originated multiple mutator genotypes can evolve by hitchhiking, however, has yet to be investigated experimentally.

## DYNAMICS OF MUTATOR HITCHHIKING: EXPERIMENTS AFTER CHAO AND COX

In the simplest possible analysis, a subpopulation bearing a mutator allele in an asexual population is more likely to spread than to dwindle if its supply rate of beneficial mutations (that is, the product of its population size and its beneficial mutation rate) exceeds that of the wild type (Figure 1). This threshold criterion corresponds qualitatively to the frequency threshold for mutator success first inferred experimentally by Chao and Cox (1983). In reality, though,

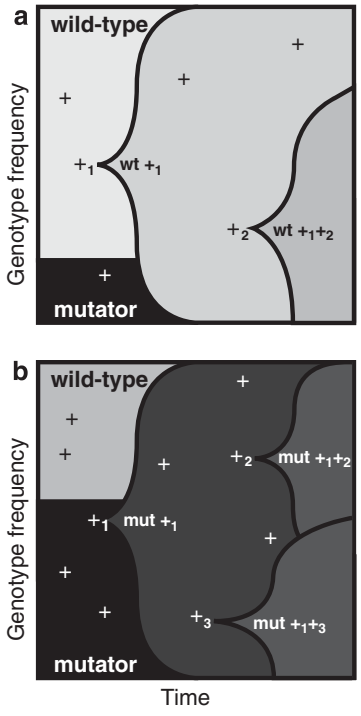


**Figure 1** Mutator hitchhiking depends on the relative mutation supply rates to mutator and wild-type subpopulations. (**a**) When a mutator allele is rare, the subpopulation with the wild-type mutation rate can have a higher mutation supply rate than the mutator subpopulation and therefore will tend to acquire new beneficial mutations more quickly. This keeps the mutator from rising in frequency. (**b**) If sufficiently common, a mutator subpopulation can have a higher mutation supply rate than the wild type. Each new beneficial mutation then has a higher tendency to arise in association with the mutator, causing the mutator to hitchhike toward higher frequency.

the situation is more complicated and a number of population genetic factors can influence the success of a mutator in an asexual population even if it is initially quite common. For example, population size bottlenecks may inhibit mutator hitchhiking (Raynes *et al.*, 2014) by reducing the probability of fixation of new beneficial mutations (Wahl and Gerrish, 2001). Alternatively, if beneficial mutations are so frequent that they co-occur in a population, then competition between mutator and wild-type lineages carrying beneficial mutations (Gerrish and Lenski, 1998; Wilke, 2004; Sniegowski and Gerrish, 2010) may also interfere with mutator hitchhiking. Mutator dynamics characterized by non-monotonic and slower-than-expected changes in frequency are consistent with the effect of such 'clonal interference' from the wild-type subpopulation and have been observed in multiple experimental studies in both bacteria and yeast (Shaver *et al.*, 2002; de Visser and Rozen, 2006; Gentile *et al.*, 2011; Raynes *et al.*, 2012); clonal interference effects on mutator hitchhiking have also been shown to strengthen with increasing population size as a result of higher overall beneficial mutation supply rate (Raynes *et al.*, 2012).

Mutator hitchhiking dynamics are also expected to depend on the distribution of fitness effects of new mutations. In particular, indirect selection in favor of mutators can be stronger when large-effect beneficial mutations facilitate rapid hitchhiking while reducing the time during which contending beneficial mutations can arise in the wild-type population. Consistent with this expectation, mutators in experimental yeast populations were markedly more successful in a stressful growth medium—in which selection is presumably stronger—than in a rich growth medium (Thompson *et al.*, 2006). By extension, this line of reasoning suggests that mutators should have a particularly strong advantage in populations experiencing lethal (hard) selection under which only mutants survive. Mutator enrichment has indeed been observed in experimental bacterial populations subjected to selection for survival on a novel carbon source (lactose: Mao *et al.*, 1997), resistance to lethal doses of antibiotics (Mao *et al.*, 1997; Gentile *et al.*, 2011) and resistance to bacteriophage infection (Pal *et al.*, 2007). Correspondingly, mutators have also been detected at substantial frequencies in pathogenic microbial populations that are presumably under continual and potentially lethal attack from host immune systems (Suarez *et al.*, 1992; LeClerc *et al.*, 1996; Matic *et al.*, 1997; Oliver *et al.*, 2000) and in experimental *E. coli* populations during the course of mouse gut colonization (Giraud *et al.*, 2001).

Assuming that many beneficial mutations are dominant, mutators should have a greater advantage in diploid than in equivalently sized haploid populations because the mutation supply rate is doubled. Furthermore, assuming that most deleterious mutations are recessive, higher ploidy should also decrease the strength of indirect selection against mutators that occurs because of their associations with deleterious mutations. Consistent with these predictions, mutators appear to be more successful in diploid than haploid populations of yeast (Thompson *et al.*, 2006; Raynes *et al.*, 2011). A recent study also found that haploid yeast mutator populations consistently switched to the diploid state during experimental evolution, potentially reducing the effects of deleterious mutations (McDonald *et al.*, 2012). It is important to note that despite the apparent advantage of homozygous mutators in experimental diploid populations, the evolutionary fate of spontaneously arising heterozygous mutators may be influenced considerably by the dominance of their mutation rate effects. In fact, assuming that many loss-of-function mutator mutations are likely to be recessive, higher ploidy may frequently hinder mutator evolution.

## WHY AREN'T ALL ASEXUALLY REPRODUCING POPULATIONS FIXED FOR MUTATOR ALLELES?

Despite the robustness of mutator hitchhiking that has been revealed in experimental populations and the circumstantial evidence for mutator hitchhiking in natural populations that we have described above, most natural populations of both sexual and asexual organisms have very low mutation rates—that is, they are clearly not fixed for mutator alleles (Sniegowski *et al.*, 2000). Indeed, the very presence of broadly conserved DNA error avoidance and repair mechanisms suggests pervasive selection for low mutation rates across most living things. This discrepancy between mutation rates predicted in experimental studies and those observed in natural populations was noted by Chao and Cox in 1983 (page 133). Experimental work on how mutator hitchhiking might be inhibited or its phenotypic effect reversed, however, has been surprisingly sparse.

An obvious potential explanation for the maintenance of low mutation rates in most natural populations is genetic recombination. Mutator hitchhiking depends on linkage disequilibrium (nonrandom genetic association) between mutators and alleles that increase fitness in a population, and recombination erodes linkage disequilibrium. Theoretical studies certainly support the intuitive conclusion that recombination can prevent mutator hitchhiking (Kimura, 1967; Leigh, 1970; Johnson, 1999; Sniegowski *et al.*, 2000; Tenaillon *et al.*, 2000). Because many natural populations that reproduce asexually can engage in recombination via horizontal gene exchange (Ochman *et al.*, 2000; Koonin *et al.*, 2001; Gogarten and Townsend, 2005), recombination potentially plays a critical role in disengaging mutators from beneficial mutations across a broad phylogenetic range of organisms (Sniegowski *et al.*, 2000; Gerrish *et al.*, 2007). We are aware, however, of only one experiment that has directly investigated the influence of recombination on mutation rate modifiers to date: Raynes *et al.* (2011) observed that a mutator allele of the mismatch repair locus *MSH2* declined rapidly in frequency in sexual but not asexual diploid experimental populations of yeast, seemingly in agreement with theory. However, the rate of *msh2* decline in the sexual populations in this experiment suggested a fitness cost of the mutator that did not manifest in parallel asexual populations; this fitness cost was possibly due to strongly deleterious and lethal recessive mutations that were unmasked in haploid spores during sexual reproduction. Thus, we still lack a convincing experimental demonstration that recombination inhibits mutator hitchhiking.

It is a well-accepted tenet of evolutionary genetics that most mutations with phenotypic effects are deleterious (Fisher, 1930; Eyre-Walker and Keightley, 2007). Mutator hitchhiking, then, is bought at the future cost of an increased load of deleterious mutations—an effect that can eventually favor the fixation of modifiers that decrease the mutation rate, even in a population that has no recombination. Numerous theoretical studies have examined how populations—whether asexual or sexual—can evolve optimum mutation rates that reflect a tradeoff between mutator hitchhiking and the cost of increased mutational load (Leigh, 1970; Tenaillon *et al.*, 1999; Orr, 2000; Clune *et al.*, 2008). However, the timescale over which such theories are applicable to the low mutation rates observed in natural populations appears to exceed what has been studied experimentally so far. The experimental studies discussed above have shown that mutator hitchhiking can take place rapidly (over a few tens or hundreds of generations) in a population that is adapting to a novel environment; in contrast, the accumulation of increased genetic load and subsequent replacement of a mutator allele by an allele conferring a lower mutation rate is likely to be a much slower process (Gerrish *et al.*, 2007). Thus, it is perhaps unsurprising that the

reestablishment of the wild type—that is, very low mutation rate—has, up to this point, not been observed in an experimental population fixed for a mutator allele. Experimental work has provided evidence for selection against very high mutation rates, but no evidence for selection in favor of very low mutation rates.

For example, Tröbner and Piechocki in an early study (1984b) showed that a mutator (*mutT*) strain of *E. coli* evolved a lower—although still higher-than-wild-type—mutation rate after propagation in a constant environment for 2200 generations. Tröbner and Piechocki were unable to characterize either the genetic basis or the population dynamics of the modifier(s) responsible for decreasing the mutation rate but showed that the *mutT* allele itself had not changed. Clones with lower mutation rates have also been isolated from *mutS* mutator populations of *E. coli* after experimental colonization of the mouse gut (Giraud *et al.*, 2001) and *in vitro* propagation by serial passaging (Turrientes *et al.*, 2013). Another recent example of such short-term selection against strong mutator phenotypes is provided by the work of Loh *et al.* (2010), who co-propagated 66 *E. coli* strains with different mutation rates for 350 generations: moderate-strength mutators were most favored in all of their experimental populations. Evolution of reduced—but not wild-type—mutation rates has also been observed in yeast populations by McDonald *et al.* (2012), who showed that *msh2* mutator strains in haploid populations were selected against because of the associated deleterious load. These results are consistent with earlier findings suggesting a high deleterious and lethal mutation rate in yeast (Wloch *et al.*, 2001) and showing that carriage of the *msh2* mutator allele sharply decreases fitness (Grimberg and Zeyl, 2005; Thompson *et al.*, 2006; Raynes *et al.*, 2011).

Some very recent evidence for indirect selection favoring reduction of high mutation rates comes from the work of Wielgoss *et al.* (2013), who showed that one of Lenski's long-term *E. coli* populations that had previously evolved a 150-fold elevated genomic mutation rate by fixing a defective *mutT* allele between 20 000 and 30 000 generations of propagation (Barrick *et al.*, 2009) was subsequently invaded (by 40 000 generations) by two separate *mutY* mutations that reduced the mutation rate by 40–60%. Wielgoss *et al.* estimated that the *mutY* mutations reduced the mutational load of the *mutT* background from 0.013 to 0.0073 and 0.0093, implying positive selection coefficients of $\sim$0.57% and $\sim$0.37% in favor of these mutations, respectively. In an asexual population, fixation of mutations with such small positive selective effects is likely only if other alleles with stronger beneficial effects are not present. Although the long-term evolution experimental populations studied by Wielgoss *et al.* still are substituting beneficial mutations after tens of thousands of generations of propagation in their constant environment (Barrick *et al.*, 2009; Wiser *et al.*, 2013), these mutations are apparently of small enough effect to allow scope for selection to favor the relatively small reduction in mutational load conferred by the *mutY* mutations.

In our discussion so far, we have implicitly assumed that mutation rate modifiers are themselves neutral and thus only affected by indirect selection. Whether mutation rate modifiers are neutral in real genetic systems is, however, an empirical matter. Given the potential for pleiotropy among diverse gene products that has been revealed by recent genomic approaches (Paaby and Rockman, 2013; Solovieff *et al.*, 2013), there remains a clear need for experiments testing whether mutation rate modifiers themselves have direct selective effects. Limited studies, for example, have shown that enhanced replication fidelity (lower mutation rate) may decrease replication rate in some viruses and thus be directly costly (Furio *et al.*, 2005; Furio *et al.*, 2007). A recent study in *Pseudomonas aeruginosa* also showed that a *mutS* mutator allele can provide inherent resistance to oxidative stress and so could have a direct fitness benefit in certain environments (Torres-Barceló *et al.*, 2013). On the other hand, a different study in *mutS E. coli* populations has documented the appearance of apparently directly beneficial variants with lower mutation rates (Turrientes *et al.*, 2013). In general, it is conceivable that mutation rate modifiers can have pleiotropic effects on fitness that affect their propensity to spread in natural populations, but at present it is unclear whether such effects would contribute to stabilizing the mutation rate at the low wild-type levels that prevail in most natural populations.

## FUTURE DIRECTIONS

Table 1 summarizes the forms of selection on mutation rate modifiers that we have discussed in this review. As we have pointed out, the overwhelming majority of experimental studies have provided support for the notion that mutators are likely to hitchhike to high frequency in asexual populations (first entry in Table 1). Yet, as we have noted, most natural populations of both sexual and asexual organisms have wild-type mutation rates that are quite low in comparison to mutation rates caused by mutator alleles. We have considered a number of factors that might explain why natural populations are seldom fixed for mutators. These include recombination, efficient selection against the increased deleterious load of mutations incurred by mutators in well-adapted populations and potential direct fitness effects of mutator alleles. All of these explanations are, in principle, amenable to future experimental investigation.

Many, if not most, natural populations engage in some form of genetic exchange, whether during reproduction or as part of horizontal gene exchange. Only one experiment to date has attempted to directly examine the effect of recombination on mutator hitchhiking (Raynes *et al.*, 2011) and further studies in recombining populations are clearly necessary to assess the role of various types and rates of recombination on the dynamics of mutation rate modifiers. Importantly, although recombination has been generally theorized to prevent mutator hitchhiking, the actual amount of recombination necessary to do so should depend critically on the strength of indirect selection favoring the mutator. Simulation studies suggest that even very rare recombination may effectively inhibit mutator hitchhiking in well-adapted populations in which beneficial

**Table 1 Potential forms of selection on mutation rate modifiers discussed in this review**

| *Source of selection* | *Type of selection* | *Strength* | *Outcome* |
|---|---|---|---|
| Hitchhiking with beneficial mutations | Indirect | Strong, immediate | Favors higher rate |
| Increased load of deleterious mutations | Indirect | Weak, delayed | Favors lower rate |
| Cost of genomic replication fidelity | Direct | Weak | Favors higher rate |
| Pleiotropic fitness effects of modifiers | Direct | Largely unknown | Unknown |

mutations are comparatively rare and generally of small effect (Tenaillon *et al.*, 2000). Correspondingly, recombination during horizontal gene transfer (Cohan, 1994; Koonin *et al.*, 2001; Gogarten and Townsend, 2005) may be sufficient to inhibit mutator hitchhiking in many asexually reproducing microbial populations and account for the scarcity of mutators in natural microbial populations. A phylogenetic analysis of the mismatch repair (MMR) genes from natural *E. coli* isolates has revealed extensive sequence mosaicism, consistent with recurrent recombination events within MMR genes (Denamur *et al.*, 2000). Given the high rates of homeologous recombination in MMR-deficient bacteria, the authors proposed that these recombination events occurred mostly in MMR-deficient lineages and could have acted as an effective mechanism for the reacquisition of the wild-type modifier alleles in well-adapted mutator populations. The widespread evidence of horizontal gene transfer presented in the study is particularly intriguing in light of the potential role of recombination in the maintenance of low wild-type mutation rates in natural *E. coli* populations.

Whereas rare recombination may effectively inhibit mutator hitchhiking in well-adapted populations, low rates of recombination may no longer be sufficient to prevent mutator hitchhiking in poorly adapted populations in which beneficial mutations are common. Correspondingly, mutators are frequently observed in pathogenic microbial populations, generally expected to be under strong selection, despite the well-documented horizontal gene transfer in these populations (Juhas, 2013). Further studies in *E. coli* populations with plasmid-mediated horizontal gene transfer (for example, Cooper, 2007) and in populations of facultatively sexual organisms (such as *S. cerevisiae*) in which the rate of recombination can be adjusted by the experimenter (Raynes *et al.*, 2011) are likely to help elucidate the role of recombination in mutation rate evolution.

Very long-term evolution experiments such as that carried out by Lenski and collaborators for more than 50 000 generations in a constant abiotic environment present the best opportunity to study mutation rate modifier dynamics under conditions in which the deleterious load may outweigh the advantage of increased beneficial mutation supply rate (Wielgoss *et al.*, 2013). Further studies in these experimental populations seem likely to provide additional important insights into the evolution of lower mutation rates and, potentially, the reversal of the phenotypic effects of mutators back to wild-type mutation rates in well-adapted populations. A possible direct approach to investigating the dynamics of re-evolution of wild-type mutation rates in these populations would be to assay whether evolved mutator clones that have been genetically restored to their wild-type modifier status are capable of displacing isogenic mutator competitors. That said, we caution that the extent to which selective effects in these populations—which have become extremely well adapted to their constant environment (Wiser *et al.*, 2013)—are representative of those in natural populations is unclear.

Because very little is known about possible pleiotropic effects of mutation rate modifiers on fitness, this is an area that should see some attention in future studies. Such studies should seek to address how common such pleiotropic effects are and whether their qualitative and quantitative effects on fitness can contribute to the maintenance of low wild-type mutation rates in natural populations. High-throughput growth rate screens (Blomberg, 2011; Hall *et al.*, 2014) of mutant libraries could be conducted as a first approximation of fitness effects of mutation rate modifiers in model organisms such as yeast and *E. coli*; such growth rate measurements, however, may not capture all aspects of competitive fitness and may differ significantly in different propagation conditions (for example, MutS may be directly beneficial to bacteria in oxidizing environments: Torres-Barceló *et al.*, 2013). Any comprehensive investigation of potential direct mutator fitness effects in asexual populations, moreover, will be complicated by the difficulty of separating direct fitness effects from indirect selective effects due to associated mutations.

## CONCLUSION

In discussing the pioneering mutator hitchhiking experiments of Cox and colleagues, John Maynard Smith wrote, 'It may be that the decisive step towards an understanding of the evolution of mutation rates was an experiment rather than a theory' (Maynard Smith, 1978; page 172). In the intervening decades, studies of mutator dynamics in microbial populations have indeed contributed greatly to our understanding of mutation rate evolution and have helped explain sporadic observations of mutators in asexual populations of infectious microbes and cancer cells. Yet, despite the observed tendency of experimental microbial populations to evolve higher mutation rates, it is clear that most populations in nature have very low genomic mutation rates. There remains considerable scope for further experimental investigation into how lower mutation rates evolve and are maintained. Important new insights are likely to emerge from studies of the effect of recombination on mutation rate evolution and studies of mutation rate modifier dynamics in well-adapted populations.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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