Sign of selection on mutation rate modifiers depends on population size

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The influence of population size (*N*) on natural selection acting on alleles that affect fitness has been understood for almost a century. As *N* declines, genetic drift overwhelms selection and alleles with direct fitness effects are rendered neutral. Often, however, alleles experience so-called indirect selection, meaning they affect not the fitness of an individual but the fitness distribution of its offspring. Some of the best-studied examples of indirect selection include alleles that modify aspects of the genetic system such as recombination and mutation rates. Here, we use analytics, simulations, and experimental populations of *Saccharomyces cerevisiae* to examine the influence of *N* on indirect selection acting on alleles that increase the genomic mutation rate (mutators). Mutators experience indirect selection via genomic associations with beneficial and deleterious mutations they generate. We show that, as *N* declines, indirect selection driven by linked beneficial mutations is overpowered by drift before drift can neutralize the cost of the deleterious load. As a result, mutators transition from being favored by indirect selection in large populations to being disfavored as *N* declines. This surprising phenomenon of sign inversion in selective effect demonstrates that indirect selection on mutators exhibits a profound and qualitatively distinct dependence on *N*.

mutation rate | mutators | population size | indirect selection | experimental evolution

Genetic variation—the raw material for evolution—is ultimately generated by mutation. The genomic rate of mutation is affected by DNA replication and repair enzymes that act to reduce mutation rate at the expense of energy and replication time. Genes encoding DNA replication and repair enzymes can themselves be altered by mutation. Allelic variants of these enzymes that raise the genomic mutation rate, for example, loss-of-function mutants of the mismatch repair pathway, are known as mutators (1). Mutators emerge in a variety of settings including cancer cells (2, 3); viral, bacterial, and fungal infections (4–8); and laboratory microbial populations (9–15). Even when they have no intrinsic effect on fitness, mutators can experience indirect selection via genomic associations with fitness-affecting mutations that they rapidly generate elsewhere in the genome (1). In nonrecombining (asexual) populations, indirect selection is particularly strong because the association between mutators and new fitness-affecting mutations is permanent.

Most fitness-affecting mutations are expected to be deleterious (16), resulting in continuous indirect selection against mutator alleles. Nevertheless, theoretical models generally predict that mutators should be favored by indirect selection in nonrecombining populations because they can hitchhike (17) with occasional, beneficial mutations (18–20) [an important exception is the "drift barrier" theory, which, however, focuses almost exclusively on deleterious mutations (21)]. Consistent with these predictions, mutators have been repeatedly observed to spontaneously emerge and achieve fixation via hitchhiking with beneficial mutations in microbial evolution experiments (9–11, 13).

Importantly, most theoretical and empirical studies of mutators have focused on large, unstructured populations (refs. 22 and 23

are exceptions). Here, we develop an analytic approximation for the probability of mutator fixation, which leads us to predict that while mutators are favored in large populations, they become disfavored as population size declines. We refer to this phenomenon as "sign inversion." We confirm our analytic approximation in stochastic computer simulations and then demonstrate sign inversion in experimental yeast populations. We conclude by addressing the importance of sign inversion for mutation rate evolution in nature and how it may help understand the relatively sporadic occurrence of mutators despite their frequent emergence in laboratory populations. More generally, we speculate that the proposed mechanism of sign inversion may be broadly applicable to other instances of indirect selection; intriguingly, sign inversion has already been documented in a few other studies (24–26). Sign inversion may thus represent a previously unappreciated but critical role of population size in evolution.

Results and Discussion

Heuristic Approximation for the Fixation Probability of a Mutator Allele. Building on earlier studies (20, 27–30), we hypothesized that, in sufficiently small nonrecombining populations (i.e., not large enough to contain multiple, competing lineages), mutator alleles can fix by only one of two classically understood processes. Namely, a mutator may produce a beneficial mutation that escapes drift and sweeps to fixation, taking the mutator with

Significance

It has been known since the early days of population genetics that population size plays a critical role in natural selection. In small populations, selection on alleles that intrinsically affect fitness can be overwhelmed by genetic drift, rendering both beneficial and deleterious alleles selectively neutral. In contrast, alleles that increase the genomic mutation rate (mutators) experience indirect selection, which acts not on their fitness effects but on their genomic associations with fitness-affecting alleles. Here, we show that the sign of indirect selection on mutators can change depending on population size. This phenomenon of sign inversion in selective effect reflects a qualitatively distinct role of population size in evolution and may be broadly important in other instances of indirect selection.

it. Alternatively, the mutator can fix if random genetic drift happens to overcome its increased load of deleterious mutations.

To fix by hitchhiking in this simple population-genetic model, a mutator must first generate a beneficial mutation, which occurs with the probability equal to the ratio of the beneficial to total mutation rate, $U_{ben}/(U_{del} + U_{ben})$. Here, U_{ben} and U_{del} are the beneficial and deleterious mutation rates, respectively. As typically $U_{del} \gg U_{ben}$, we can approximate $U_{ben}/(U_{del} + U_{ben}) \approx U_{ben}/U_{del}$ (Fig. S2). Then the beneficial mutation has to sweep to fixation, which for a mutation with selection coefficient s occurs with probability $P_{\text{fix}}(N, s) = (1 - e^{-2s})/(1 - e^{-2Ns})$ given by Kimura in ref. 31. Thus, we assume that mutator fixation is determined by the first beneficial mutation acquired, and not by subsequent beneficial or deleterious mutations (27). In particular, we assume that subsequent deleterious mutations cannot spoil the original beneficial mutation (Fig. S2).

In addition to hitchhiking, a mutator allele can also fix by genetic drift, if drift can overpower indirect selection against the mutator driven by linked deleterious mutations. Assuming that the population is at a mutation selection equilibrium (in which case deleterious mutations have zero fixation probability; Fig. S2; ref. 32), the selective disadvantage of a mutator due to the deleterious load is simply the increase in the rate of deleterious mutations over the population mean (33, 34).

Algebraically, we can therefore approximate the fixation probability of a mutator as

$$P_{\text{fix}}^{\text{mutator}} = P_{\text{fix}}^{\text{drift}} + P_{\text{fix}}^{\text{hitchhiking}}, \qquad [\mathbf{1a}]$$

where the fixation probability by drift is

$$P_{\text{fix}}^{\text{drift}} = P_{\text{fix}}(N, -\Delta U_{del}) \qquad [\mathbf{1b}]$$

and the fixation probability by hitchhiking is

$$P_{\text{fix}}^{\text{hitchhiking}} = \frac{U_{ben}}{U_{del}} \cdot P_{\text{fix}}(N, s_{ben}). \qquad [\mathbf{1c}]$$

Here, ΔU_{del} is the difference in the deleterious mutation rate between mutator and nonmutator, and s_{ben} is the selection coefficient of a beneficial mutation.

$NP_{\text{fix}}^{\text{mutator}}$ (i.e., $P_{\text{fix}}^{\text{mutator}}$ normalized by the fixation probability of a neutral allele, $P_{\text{fix}}^{\text{neutral}} = 1/N$) for a 100-fold mutator is plotted in Fig. 1 as a function of N, and its constituents $NP_{\text{fix}}^{\text{drift}}$ and $NP_{\text{fix}}^{\text{hitchhiking}}$ are plotted in Fig. S1. As Fig. 1 shows, the approximation for $NP_{\text{fix}}^{\text{mutator}}$ (Eq. **1a**) is closely borne out in stochastic Wright–Fisher (18) simulations of asexual populations (see Fig. S2 for simulations testing the assumptions made by our analytic approximation).

Fig. 1 also illustrates the most surprising behavior of $NP_{\text{fix}}^{\text{mutator}}$: as population size declines, it crosses the neutral expectation ($NP_{\text{fix}}^{\text{neutral}} = 1$), meaning that mutators transition from being favored by selection to being disfavored. Hereafter, we will refer to this N-dependent inversion in the sign of selection as sign inversion. Setting $NP_{\text{fix}}^{\text{mutator}}$ to 1 (i.e., equal to $NP_{\text{fix}}^{\text{neutral}}$), we find that selection is predicted to favor the mutators above $N_{crit} \approx U_{del}/(P_{\text{fix}}^{\text{direct}}(N, s_{ben}) \cdot U_{ben})$ and disfavor them below. This transition occurs because, on one hand, selection against the deleterious mutational load makes mutator fixation by drift (Eq. **1b**) very unlikely (generally $NP_{\text{fix}}^{\text{drift}} \ll 1$). Meanwhile, mutator hitchhiking probability (Eq. **1c**) is everywhere depressed compared with a directly favored mutation by the requirement to first generate such a mutation. Consequently, unlike NP_{fix} of a directly favored mutation, which asymptotically approaches the neutral expectation as N declines (Fig. 1), $NP_{\text{fix}}^{\text{hitchhiking}}$ of a mutator drops below it at N_{crit}. At this point, the
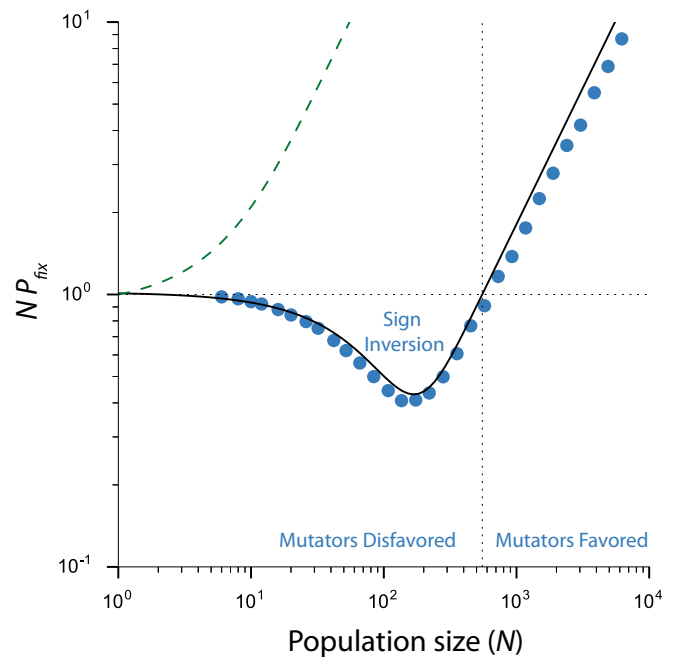

Fig. 1. Population size (N) determines the sign of indirect selection on mutators. Sign inversion for mutators occurs when their normalized fixation probability ($NP_{\text{fix}}^{\text{mutator}}$) crosses the neutral expectation (horizontal dotted line). Mutators fare better than neutral alleles above $N_{crit} \approx U_{del}/(P_{\text{fix}}(N, s_{ben}) \cdot U_{ben})$ (vertical line), but worse below. Solid black line: analytic approximation (see text). Blue dots: stochastic simulations, 10^6 replicates. In contrast, the normalized fixation probability of a directly beneficial mutation [$P_{\text{fix}}(N, s_{ben})$; green dashed line] asymptotically approaches but never crosses the neutral threshold. Parameter values: $U_{del} = 10^{-4}$, $U_{ben} = 10^{-6}$, $s_{ben} = 0.1$, and $s_{del} = -0.1$. Mutators mutate 100 times faster than nonmutators.

likelihood of mutator hitchhiking to fixation falls below that of a neutral mutation fixing solely by genetic drift. As N continues to decline, indirect selection against the deleterious mutational load results in sign inversion and keeps $NP_{\text{fix}}^{\text{mutator}}$ below the neutral expectation until the cost of the load is overpowered by drift (i.e., $NP_{\text{fix}}^{\text{drift}}$ approaches 1).

Sign Inversion in Experimental Yeast Populations. To empirically test the prediction of sign inversion, we conducted competitions between mutator and nonmutator strains of *S. cerevisiae*. The mutator strain carried a deletion of a mismatch repair gene MSH2, which in this genetic background resulted in approximately a 20-fold increase in the mutation rate over the nonmutator. [Simulations show that sign inversion occurs for a 20-fold mutator as well (Figs. S3 and S5).] We initiated competitions at approximately equal frequencies (thereby changing the neutral fixation probability, $P_{\text{fix}}^{\text{neutral}}$, from $1/N$ to ~0.5) and propagated them at three different population sizes by regular dilutions into fresh medium. Following other experimental studies and population genetics theory (28, 35, 36), we manipulated population size by varying the size of the dilution bottleneck. Based on simulations of 20-fold mutator dynamics in bottlenecked populations, we predicted that sign inversion would occur at a bottleneck somewhere between ~50 and ~1,000 cells (Fig. S3).

We acknowledge that population size bottlenecks have also been predicted to reduce survival and fixation probabilities of beneficial mutations (36, 37). Note, however, that while deeper population bottlenecks may weaken indirect selection in favor of mutators, they cannot strengthen indirect selection against mutators. They thus cannot lead to sign inversion. Indeed, simulations confirmed that

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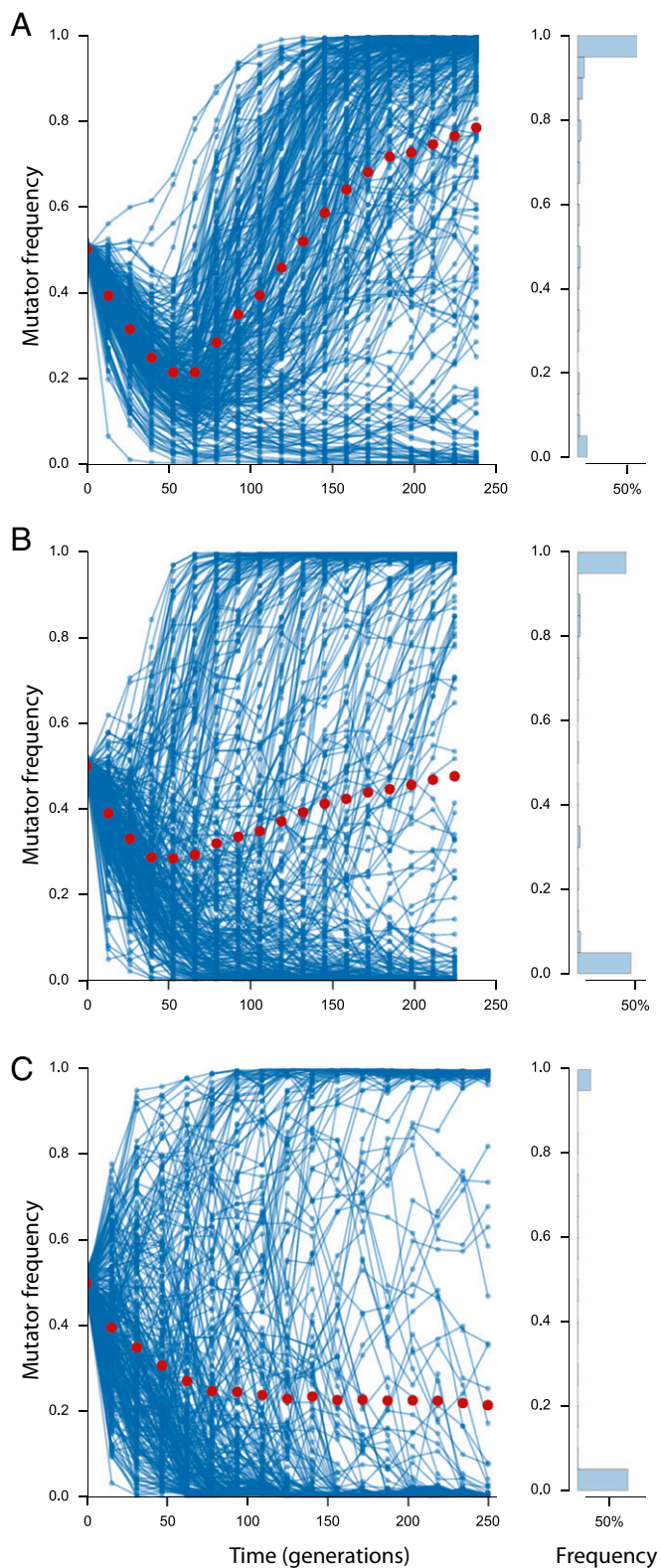
Fig. 2. Sign inversion in experimental yeast populations: Mutators are favored in large populations but disfavored in small populations. (*Left*) Mutator dynamics in populations propagated through bottlenecks of differing size: (*A*) large (~8,000 cells), (*B*) medium (~80 cells), and (*C*) small (~20 cells). Blue curves: individual population frequencies; red dots: all population averages. (*Right*) Frequency histograms on the last day of propagation.

while small bottlenecks weaken selection on mutators, sufficiently deep bottlenecks still cause sign inversion (Fig. S3).

Fig. 2 presents the results of competitions propagated through large bottlenecks of ~8,000 cells (Fig. 2*A*), intermediate bottlenecks of ~80 cells (Fig. 2*B*), and small bottlenecks of ~20 cells (Fig. 2*C*) (Dataset S1). As predicted, mutators were strongly favored in large populations but strongly disfavored in small populations. In large populations mutators won in 162 of the 276 competitions (*Methods*) in the first ~250 generations (~58.7%, significantly above the neutral expectation; two-sided, binomial test, $P < 0.01$) and lost in only 25 (Fig. 2*A*). In addition, the mean mutator frequency in the 89 unresolved competitions (~0.63 ± 0.03 SEM) was significantly higher than the starting frequency (two-sided, paired t test, $t_{df = 88} = 4.4870$, $P << 0.01$), consistent with selection favoring mutators. In intermediate populations, mutators won in 112 of 275 competitions (~40.7%) and lost in 124 (~45.1%) in the first ~225 generations (Fig. 2*B*). The mean mutator frequency in the remaining 39 competitions (~0.49 ± 0.05 SEM) was not significantly different from the mean starting frequency in these populations (two-sided, paired t test, $t_{df = 38} = 0.1747$, $P = 0.8622$). Thus, it appears that fixation probability of the mutator was roughly equal to that of a neutral allele, suggesting that the size of these intermediate populations is close to N_{crit}. Finally, in small populations (Fig. 2*C*), mutators won in only 53 of 273 competitions (~19.4%; significantly below the neutral expectation; two-sided, binomial test, $P << 0.01$) and lost in 208 (~76.2%) in ~250 generations. The mean mutator frequency in the remaining 12 competitions (~0.43 ± 0.07 SEM) was not significantly different from their starting frequency (two-sided, paired t test $t_{df = 11} = 0.8173$, $P = 0.4311$).

We note that, while all populations reached the same cell density by the end of each dilution cycle, smaller populations experienced lower densities than large populations early in the cycle, raising the possibility of density-dependent (38) differences in selection. Critically, while population density could influence the rates or effects of fitness-affecting mutations, and, therefore, affect indirect selection on mutators, it cannot account for the observed sign inversion. Trivially, an increase in either U_{ben} or s_{ben} or a decrease in U_{del} with lower population density would put mutators at an advantage in our small population treatment. Conversely, a decline in either U_{ben} or s_{ben} with population density would only weaken selection in favor of the mutator, but it would not cause selection against the mutator, as required to produce sign inversion. In addition, while an increase in U_{del} with lower population density could strengthen selection against the mutator, no such response was observed in our data. (See SI Text for estimates of U_{ben}, U_{del}, and s_{ben} in this experiment.)

The Mechanism of Sign Inversion in Experimental Populations. To confirm that the mechanism driving sign inversion in our competitions was consistent with the predictions of our analytic model, we measured mean fitness values of winning mutator and nonmutator populations. In addition, we simulated competitions initiated at equal frequencies of mutators and nonmutators and propagated through small bottlenecks of 20 cells and large bottlenecks of 8,000 cells. Consistent with our expectation that at population sizes above N_{crit} mutator fixation is primarily driven by hitchhiking with beneficial mutations, all mutator winners in simulated (Fig. 3*A*) and experimental (Fig. 3*B* and Fig. S4) large populations were significantly fitter than their ancestors (in experiments: two-sided t tests, all $P < 0.01$; Dataset S2).

In contrast, our theory predicts that below N_{crit} mutators may fix by either hitchhiking or genetic drift. Correspondingly, in both simulated (Fig. 3*A*) and experimental (Fig. 3*B* and Fig. S4) small populations, some of the mutator winners were considerably fitter than their ancestors (i.e., fixed by hitchhiking), while some were about as fit or even less fit than their ancestors (i.e., fixed by drift). However, nonmutators are expected to fix not by hitchhiking but
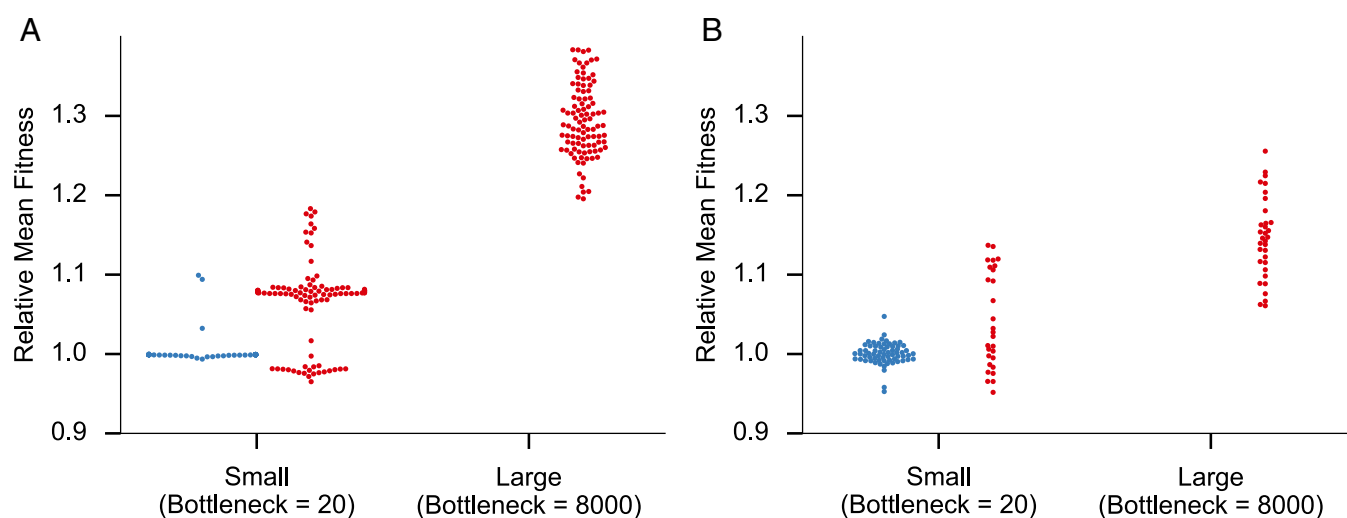
Fig. 3. Mutators win large competitions by hitchhiking; nonmutators win small competitions by outlasting the mutators. (*A*) Mean fitness of simulated competition winners. In simulation, mutators (red dots) are favored at large *N* ($P_{fix}^{mutator} \sim 99\%$) and disfavored at small *N* ($P_{fix}^{mutator} \sim 20\%$). Large *N* mutator winners are always fitter than their ancestors. In contrast, some small *N* mutator winners are fitter than their ancestors, while some are not. Nonmutator winners (blue dots) of small *N* simulations are almost never fitter than their ancestors. $P_{fix}^{mutator}$ averaged over 10^6 runs. Each point represents population fitness at the end of simulation (a random 100 replicates shown for clarity; 99/100 nonmutator winners have a relative fitness of 1). Parameter values: $U_{del} = 10^{-3}$, $U_{ben} = 10^{-5}$, $s_{ben} = 0.1$, $s_{del} = -0.1$. Mutators mutate 20 times faster than nonmutators. (*B*) Mean fitness of experimental competition winners. Mean fitness of large populations in which mutators had won (red dots) is always considerably higher than their ancestors, consistent with hitchhiking. In contrast, only some small populations in which mutators had won show evidence of hitchhiking. Mean fitness of small populations in which nonmutators had won (blue dots) is very close to their ancestors, ruling out hitchhiking. Each point represents an average of five replicate fitness assays. See Fig. S4 for 95% CI.

by outlasting the mutators lost to selection against the deleterious load. Indeed, nonmutator winners of simulated small populations (Fig. 3*A*) were almost never fitter than their ancestors. Likewise, most nonmutator winners of experimental small populations (Fig. 3*B*) were not significantly fitter than the ancestor while the rest were not fit enough (~1% more fit on average) to have fixed by hitchhiking so quickly (Dataset S2). Thus, we reason that their fixation was most likely still driven by outlasting the mutators lost to the deleterious load.

Conclusion

Taken together, our results demonstrate that indirect selection favors mutators in large populations but suppresses them in small populations. Understanding the role of *N* in mutation rate evolution helps elucidate where and why mutators are likely to prevail. In particular, it may help explain why mutators often emerge in experimental microbial populations but have been rarely found in nature, except sporadically in cancer cells (2, 3) and pathogenic infections (5, 7). Importantly, whereas natural populations are likely to be large, they are also frequently structured in both time and space [e.g., transmission bottlenecks (39), biofilms (40)]. Our results suggest that population structure may help inhibit mutators in nature by dividing large populations into smaller demes, in which mutators may be more vulnerable to selection against the deleterious load.

More generally, our results dramatically extend our understanding of the influence of *N* on natural selection. It has been long known that small *N* can weaken selection on alleles that directly affect fitness, rendering both beneficial and deleterious alleles neutral. However, beneficial alleles never become deleterious and deleterious alleles never become beneficial. We have shown that *N* can actually change the sign of indirect selection on mutators. Intriguingly, the mechanistic basis of sign inversion may not be unique to mutators. Any mechanism that introduces random genetic or phenotypic variation is more likely to negatively affect fitness than to improve it. Consequently, other indirectly selected modifiers of variation may also experience sign inversion. As in the case of mutators, other modifier alleles may

be generally less likely to achieve fixation by drift than neutral alleles, due to selection against the predominantly deleterious variants they produce. Meanwhile, their probability of fixation via hitchhiking with beneficial variants may be tempered by the low probability of producing such variants and, thus, bound to decline below the expected fixation probability of a neutral allele as *N* declines. Indeed, sign inversion has apparently already been observed in models of evolution of bet-hedging (24), cooperation (26), and recombination rate (25). Whether these phenomena can be united in a single theoretical framework remains an open question that we are actively exploring. In any case, our results add to a growing appreciation of nonclassical population size dependence in evolution by natural selection (41, 42).

Methods

Stochastic Simulations. We consider an asexual population evolving in discrete, nonoverlapping generations. The population is composed of genetic lineages, that is, all individuals that have the same genotype. A genotype is modeled as a vector of 100 loci, including 99 fitness-affecting loci and 1 mutation rate modifier. The mutation rate locus acts solely on the mutation rate (i.e., it has no intrinsic effect on fitness) and cannot be mutated during simulation. The fitness loci can generate both deleterious and beneficial mutations. We assume fixed fitness effects for all mutations and that they are additive in nature. Fitness is thus calculated as the sum of fitness contributions of all mutated fitness-affecting loci: given *x* deleterious mutations and *y* beneficial mutations (with selection coefficients of s_{del} and s_{ben}, respectively), fitness is $w = 1 - xs_{del} + ys_{ben}$.

Simulations start with *N* individuals divided into two genetic lineages—mutators and nonmutators, all free of fitness-affecting mutations and only differing in their mutation rate. Simulations end when mutators either reach fixation (frequency of 100%) or go extinct (frequency of 0%). Fixation probability is then calculated as the fraction of replicate simulation runs in which mutators fixed. Every generation, the population reproduces according to the Wright–Fisher (43) model, in which the representation of each genetic lineage in the next generation is drawn from a multinomial distribution with expectation determined by its frequency multiplied by its relative fitness in the previous generation. Unless otherwise stated, all simulations were conducted with populations of constant size (*N*). In simulations of bottlenecked populations, populations double every generation

until they reach the maximum population size N_{max} (8×10^5 cells in all simulations; this is the approximate carrying capacity in our experiments). Populations are then reduced to the bottleneck size by sampling every lineage from the population with probability proportional to its frequency.

Upon reproduction, each lineage acquires a Poisson distributed number of mutations, X with mean determined by the size of the lineage multiplied by the total per-individual mutation rate ($U_{del} + U_{ben}$). The counts of beneficial and deleterious mutations are then drawn from a binomial distribution with $n = X$ and $P = U_{ben}/(U_{ben} + U_{del})$. Loci to be mutated are randomly chosen from the nonmutated ones.

Simulations were conducted with empirically supported parameter values from experimental yeast populations (44–48): $U_{ben} \sim 10^{-6}$ to 10^{-5}, $s_{ben} \sim 0.02$ to 0.1, and $U_{del} \sim 10^{-3}$ to 10^{-5}. Simulation code was written in Julia 0.5 and is available at https://github.com/yraynes/Sign-Inversion.

Strains, Media, and Propagation Conditions. Isogenic strains yJHK111and yJHK112 labeled with ymCitrine and ymCherry, respectively, were generously provided by the laboratory of Andrew Murray, Harvard University, Cambridge, MA, and have been previously described (49). To engineer mutator strains, we replaced the coding sequence of *MSH2*, a component of the yeast mismatch repair pathway, in both yJHK111 and yJHK112 with a kanamycin resistance knockout cassette as previously described (50, 51). The cassette provides resistance to the antibiotic geneticin (G418) and has been previously shown to have a minimal effect on fitness (52). Successful replacement of *MSH2* was confirmed with PCR. Fluctuation tests conducted as previously described (50) indicated an ~20-fold increase in the mutator mutation rate toward nystatin (at 4 μM) resistance as well as 5-fluoro-orotic acid (5FOA) (at 1 mg/mL) resistance associated with MSH2 deletion. Low-glucose minimal medium (6.7 g of YNB+nitrogen, 0.2 g of glucose per 1 L) was used in all experiments to reduce the carrying capacity (to ~4×10^6 cells per mL) and lower population size. Medium was supplemented with ampicillin (100 μg/mL) and tetracycline (20 μg/mL) to prevent bacterial contamination. Populations were propagated in 200 μL of medium in wells of standard flat-bottom 96-well plates. Plates were sealed in plastic Ziploc bags to prevent evaporation and incubated at 30 °C with shaking at 1,250 rpm in a microplate shaker (Multi-Microplate Genie; 1-mm orbit; Scientific Industries). All populations were periodically frozen in 15% glycerol at −80 °C.

Experimental Propagation. Preliminary fitness tests as described below indicated a slight but significant advantage of ymCherry over ymCitrine in both the nonmutator background (ymCherry relative fitness = 1.006, $t_{df = 4}$ = 2.9714, $P = 0.04$) and the mutator background (ymCherry relative fitness = 1.013, $t_{df = 4}$ = 6.4172, $P = 0.003$). To control for this fitness difference, we employed a blocking design in which one-half of the competitions were conducted with mutators labeled with ymCherry and one-half with mutators labeled with ymCitrine. To start, ymCherry (mutator and nonmutator)- and ymCitrine (mutator and nonmutator)-labeled strains were streaked onto YPD agar plates from frozen stocks. After 2 d of growth, 288 individual colonies of each of these four genotypes were picked into 200 μL of medium and allowed to reach saturation. All populations were then diluted 10,000-fold and allowed to regrow for 2 more days to physiologically acclimate to the new conditions before competitions started. Once saturated, appropriate mutator and nonmutator cultures were combined at equal volumes into 288 independently founded competitions between ymCherry-labeled mutators and ymCitrine-labeled nonmutators and 288 independently founded competitions between ymCitrine-labeled mutators and ymCherry-labeled nonmutators. Mutator frequencies in all populations were then estimated by analyzing about 20,000 cells on the Attune NxT Flow Cytometer (Invitrogen). The 138 populations of each labeling scheme (276 total) closest to 50% mutator frequency were chosen for the experiment. Populations bottlenecked at 8,000 and 80 cells were initiated from the same set of 276 populations. Populations bottlenecked at 20 cells were initiated from a new set of 276 populations started in the same way as described above. In the populations used to start the two sets of larger populations, ymCherry-labeled mutators were initially at an average frequency of 49.7% ± SD 1.17% and ymCitrine-labeled mutators were at an average frequency of 50.8% ± SD 1.12%. In the populations used to start the 20 cell competitions, ymCherry-labeled mutators were initially at an average frequency of 49.9% ± SD 1.92% and ymCitrine-labeled mutators were at an average frequency of 49.9% ± SD 1.87%. Experiments were performed in 96-well plates with four wells per plate filled only with medium to control for cross-contamination.

Populations were propagated by dilution into fresh medium once the maximum population size was reached (~800,000 cells at the density of ~4×10^6 cells per mL). Large populations were transferred daily through 1:100 dilutions into fresh medium, resulting in a bottleneck of about 8,000

cells and about $\ln_2(100) = 6.6$ generations between transfers. To allow sufficient time to recoup the dilution, intermediate populations bottlenecked at 80 cells were transferred every 2 d through 1:10,000 dilutions into fresh medium, resulting in about $\ln_2(10,000) = 13.4$ generations between transfers. Small populations bottlenecked at 20 cells were transferred every 2.5 d through 1:40,000 dilutions into fresh medium, resulting in $\ln_2(40,000) = 15.3$ generations between transfers. One of the intermediate populations and three small populations were lost to accidental extinction during dilution and excluded from the data set. Mutator frequencies (Dataset S1) were assessed by periodically analyzing about 20,000 cells from each population on the Attune NxT Flow Cytometer.

Competitions were propagated until the sign of indirect selection became clear as assessed by comparing the average frequency and the realized probability of mutator fixation to our neutral expectation (the starting frequency of mutators in our competitions). Note that because the timing of mutator fixation and mutator loss are unknown we did not directly compare realized fixation and loss probabilities after stopping the experiment.

In simulations of bottlenecked populations (Fig. S3), we observed that the probability of fixation (reaching 100% frequency) for a mutator was almost indistinguishable from the probability of reaching 95% (over 10^6 replicates): mutators failed to fix after reaching 95% in ~0.1% of simulated small populations and considerably fewer of intermediate (~0.001%) and large (none) populations. Likewise, the probability of extinction in simulations was practically the same as the probability of dropping below 5%: mutators went on to fix after having dropped to below 5% in ~0.08% of small populations, ~0.2% of intermediate populations, and ~0.001% of large populations. In our analysis, we thus considered a mutator to have won when it reached a frequency of at least 95% and lost when its frequency dropped to at most 5%.

Fitness Assays. Relative fitness (Dataset S2) was assayed in short-term competition experiments. Competitors were first inoculated into 200-μL cultures in our low-glucose medium and grown to saturation overnight. They were then diluted 1,000-fold into the same medium and allowed to regrow for another day to acclimate to the growth environment. After 24 h (~10 generations), competitors were combined 50:50 in wells of a 96-well plate and propagated through two 1:100 bottlenecks (2 d, 13.4 generations). The frequency of competitors before and after 13 generations of growth was determined by flow cytometry using the Attune NxT Flow Cytometer. Relative fitnesses were calculated from the change in competitors' frequencies over the 13.4 generations of competition using standard population genetics (32). Each competition was replicated five times.

Winning mutator and nonmutator populations were isolated by sampling experimental (whole) populations in which they had reached a frequency >99%. Frozen stocks were sampled from the first available time point immediately after winners had reached >99% to minimize the effect of further adaptation after fixation. Winning nonmutator lineages from small populations bottlenecked at 20 cells were isolated from populations frozen after about 60 generations (four transfers) or 120 generations (eight transfers) of propagation. Since mutators were able to fix in only a single population after 60 generations (Fig. 2), all mutator winners from our smallest populations were isolated after 120 generations of propagation. Mutator winners of our large populations were isolated from populations frozen after 192 generations of propagation.

To control for the disadvantage of the higher deleterious load in mutators, nonmutators were always competed against nonmutators carrying the opposite fluorescent marker while mutators were always competed against mutators. Fitness values of evolved populations reported in Fig. 3 and Fig. S4 were normalized by the relative fitness of their ancestral strain.

Statistical Analysis. A two-sided binomial test (53) was used to assess whether realized fixation probability in our experiments was significantly different from the neutral expectation (given by the starting frequency). A two-sided paired *t* test (53) was used to compare mean mutator frequency at the end of the experiment to mean mutator frequency at the beginning. A two-sided unpaired *t* test (53) was used to compare mean population fitness of evolved and ancestral populations.

1. Sniegowski PD, Gerrish PJ, Johnson T, Shaver A (2000) The evolution of mutation rates: separating causes from consequences. *BioEssays* 22:1057–1066.
2. Loeb LA (2011) Human cancers express mutator phenotypes: origin, consequences and targeting. *Nat Rev Cancer* 11:450–457.
3. Lengauer C, Kinzler KW, Vogelstein B (1997) Genetic instability in colorectal cancers. *Nature* 386:623–627.
4. Matic I, et al. (1997) Highly variable mutation rates in commensal and pathogenic *Escherichia coli*. *Science* 277:1833–1834.
5. LeClerc JE, Li B, Payne WL, Cebula TA (1996) High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Science* 274:1208–1211.
6. Suárez P, Valcárcel J, Ortín J (1992) Heterogeneity of the mutation rates of influenza A viruses: isolation of mutator mutants. *J Virol* 66:2491–2494.
7. Oliver A, Cantón R, Campo P, Baquero F, Blázquez J (2000) High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* 288:1251–1254.
8. Healey KR, et al. (2016) Prevalent mutator genotype identified in fungal pathogen *Candida glabrata* promotes multi-drug resistance. *Nat Commun* 7:11128.
9. Sniegowski PD, Gerrish PJ, Lenski RE (1997) Evolution of high mutation rates in experimental populations of *E. coli*. *Nature* 387:703–705.
10. Shaver AC, et al. (2002) Fitness evolution and the rise of mutator alleles in experimental *Escherichia coli* populations. *Genetics* 162:557–566.
11. Barrick JE, et al. (2009) Genome evolution and adaptation in a long-term experiment with *Escherichia coli*. *Nature* 461:1243–1247.
12. Notley-McRobb L, Seeto S, Ferenci T (2002) Enrichment and elimination of mutY mutators in *Escherichia coli* populations. *Genetics* 162:1055–1062.
13. Pal C, Maciá MD, Oliver A, Schachar I, Buckling A (2007) Coevolution with viruses drives the evolution of bacterial mutation rates. *Nature* 450:1079–1081.
14. Mao EF, Lane L, Lee J, Miller JH (1997) Proliferation of mutators in A cell population. *J Bacteriol* 179:417–422.
15. Raynes Y, Sniegowski PD (2014) Experimental evolution and the dynamics of genomic mutation rate modifiers. *Heredity (Edinb)* 113:375–380.
16. Eyre-Walker A, Keightley PD (2007) The distribution of fitness effects of new mutations. *Nat Rev Genet* 8:610–618.
17. Smith JM, Haigh J (1974) The hitch-hiking effect of a favourable gene. *Genet Res* 23:23–35.
18. Gerrish PJ, Colato A, Perelson AS, Sniegowski PD (2007) Complete genetic linkage can subvert natural selection. *Proc Natl Acad Sci USA* 104:6266–6271.
19. Taddei F, et al. (1997) Role of mutator alleles in adaptive evolution. *Nature* 387:700–702.
20. André JB, Godelle B (2006) The evolution of mutation rate in finite asexual populations. *Genetics* 172:611–626.
21. Sung W, Ackerman MS, Miller SF, Doak TG, Lynch M (2012) Drift-barrier hypothesis and mutation-rate evolution. *Proc Natl Acad Sci USA* 109:18488–18492.
22. Perfeito L, Pereira MI, Campos PRA, Gordo I (2008) The effect of spatial structure on adaptation in *Escherichia coli*. *Biol Lett* 4:57–59.
23. Travis ER, Travis JMJ (2004) Mutators in space: the dynamics of high-mutability clones in a two-patch model. *Genetics* 167:513–522.
24. Gillespie JH (1974) Nautural selection for within-generation variance in offspring number. *Genetics* 76:601–606.
25. Whitlock AOB, Peck KM, Azevedo RBR, Burch CL (2016) An evolving genetic architecture interacts with Hill-Robertson interference to determine the benefit of sex. *Genetics* 203:923–936.
26. Nowak MA, Sasaki A, Taylor C, Fudenberg D (2004) Emergence of cooperation and evolutionary stability in finite populations. *Nature* 428:646–650.
27. Wylie CS, Ghim C-M, Kessler D, Levine H (2009) The fixation probability of rare mutators in finite asexual populations. *Genetics* 181:1595–1612.
28. Raynes Y, Halstead AL, Sniegowski PD (2014) The effect of population bottlenecks on mutation rate evolution in asexual populations. *J Evol Biol* 27:161–169.
29. Tenaillon O, Toupance B, Le Nagard H, Taddei F, Godelle B (1999) Mutators, population size, adaptive landscape and the adaptation of asexual populations of bacteria. *Genetics* 152:485–493.
30. Good BH, Desai MM (2016) Evolution of mutation rates in rapidly adapting asexual populations. *Genetics* 204:1249–1266.
31. Kimura M (1962) On the probability of fixation of mutant genes in a population. *Genetics* 47:713–719.
32. Crow JF, Kimura M (1970) *An Introduction to Population Genetics Theory* (Harper and Row, New York).
33. Kimura M (1967) On the evolutionary adjustment of spontaneous mutation rates. *Genet Res* 9:23–34.
34. Johnson T (1999) The approach to mutation-selection balance in an infinite asexual population, and the evolution of mutation rates. *Proc Biol Sci* 266:2389–2397.
35. Lenski RE, Rose MR, Simpson SC, Tadler SC (1991) Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. *Am Nat* 138:1315–1341.
36. Wahl LM, Gerrish PJ, Saika-Voivod I (2002) Evaluating the impact of population bottlenecks in experimental evolution. *Genetics* 162:961–971.
37. Wahl LM, Gerrish PJ (2001) The probability that beneficial mutations are lost in populations with periodic bottlenecks. *Evolution* 55:2606–2610.
38. Clarke B (1972) Density-dependent selection. *Am Nat* 106:1–13.
39. Bergstrom CT, McElhany P, Real LA (1999) Transmission bottlenecks as determinants of virulence in rapidly evolving pathogens. *Proc Natl Acad Sci USA* 96:5095–5100.
40. Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol* 2:95–108.
41. Cvijović I, Good BH, Jerison ER, Desai MM (2015) Fate of a mutation in a fluctuating environment. *Proc Natl Acad Sci USA* 112:E5021–E5028.
42. Graves CJ, Weinreich DM (2017) Variability in fitness effects can preclude selection of the fittest. *Annu Rev Ecol Evol Syst* 48:399–417.
43. Ewens W (2004) *Mathematical Population Genetics* (Springer, New York).
44. Joseph SB, Hall DW (2004) Spontaneous mutations in diploid *Saccharomyces cerevisiae*: more beneficial than expected. *Genetics* 168:1817–1825.
45. Levy SF, et al. (2015) Quantitative evolutionary dynamics using high-resolution lineage tracking. *Nature* 519:181–186.
46. Zeyl C, DeVisser JA (2001) Estimates of the rate and distribution of fitness effects of spontaneous mutation in *Saccharomyces cerevisiae*. *Genetics* 157:53–61.
47. Wloch DM, Szafraniec K, Borts RH, Korona R (2001) Direct estimate of the mutation rate and the distribution of fitness effects in the yeast *Saccharomyces cerevisiae*. *Genetics* 159:441–452.
48. Frenkel EM, Good BH, Desai MM (2014) The fates of mutant lineages and the distribution of fitness effects of beneficial mutations in laboratory budding yeast populations. *Genetics* 196:1217–1226.
49. Koschwanez JH, Foster KR, Murray AW (2013) Improved use of a public good selects for the evolution of undifferentiated multicellularity. *eLife* 2:e00367.
50. Raynes Y, Gazzara MR, Sniegowski PD (2011) Mutator dynamics in sexual and asexual experimental populations of yeast. *BMC Evol Biol* 11:158.
51. Grimberg B, Zeyl C (2005) The effects of sex and mutation rate on adaptation in test tubes and to mouse hosts by *Saccharomyces cerevisiae*. *Evolution* 59:431–438.
52. Goldstein AL, McCusker JH (1999) Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast* 15:1541–1553.
53. Sokal RR, Rohlf FJ (1995) *Biometry: The Principles and Practice of Statistics in Biological Research* (Freeman, New York), 3rd Ed.

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