

Migration promotes mutator alleles in subdivided populations

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Mutator alleles that elevate the genomic mutation rate may invade nonrecombining populations by hitchhiking with beneficial mutations. Mutators have been repeatedly observed to take over adapting laboratory populations and have been found at high frequencies in both microbial pathogen and cancer populations in nature. Recently, we have shown that mutators are only favored by selection in sufficiently large populations and transition to being disfavored as population size decreases. This population size-dependent sign inversion in selective effect suggests that population structure may also be an important determinant of mutation rate evolution. Although large populations may favor mutators, subdividing such populations into sufficiently small subpopulations (demes) might effectively inhibit them. On the other hand, migration between small demes that otherwise inhibit hitchhiking may promote mutator fixation in the whole metapopulation. Here, we use stochastic, agent-based simulations and evolution experiments with the yeast *Saccharomyces cerevisiae* to show that mutators can, indeed, be favored by selection in subdivided metapopulations composed of small demes connected by sufficient migration. In fact, we show that population structure plays a previously unsuspected role in promoting mutator success in subdivided metapopulations when migration is rare.

KEY WORDS: Indirect selection, migration, mutation rate, mutator, sign inversion.

Allelic variants of genes that influence the genomic mutation rate, i.e. genes for DNA replication and repair enzymes, may experience natural selection even when they have no direct effect on an individual's fitness. Instead, these mutation rate modifiers experience indirect selection, which acts through statistical association between modifiers and fitness-affecting mutations elsewhere in the genome (Kimura 1967; Leigh 1973; Sniegowski et al. 2000; Lynch 2010). Thus, for example, the spread of modifier alleles that elevate the genomic mutation rate (mutators) is inhibited by selection against deleterious mutations they produce. On the other hand, selection for beneficial mutations may allow mutators associated with them to rise in frequency and even sweep to fixation in a population through a process called genetic hitchhiking (Maynard Smith and Haigh 1974). Mutator hitchhiking with beneficial mutations is particularly likely in the absence of recombination, which otherwise acts to erode statistical association between mutators and other mutations. Correspondingly,

mutator evolution has been repeatedly demonstrated in studies of experimental, asexual microbial populations adapting to laboratory conditions (reviewed in Raynes and Sniegowski 2014) and corroborated by simulations and analytical theory (Taddei et al. 1997; Sniegowski et al. 2000; Wylie et al. 2009; Desai and Fisher 2011; Good and Desai 2016). Outside the laboratory, mutators often reach high frequencies in asexual populations of viral and microbial pathogens (Suarez et al. 1992; LeClerc et al. 1996; Matic et al. 1997; Oliver et al. 2000; Healey et al. 2016). Mutation rates also frequently increase in nonrecombining somatic cells during cancer emergence and progression (Merlo et al. 2006).

Overall, these observations seem to imply that selection may generally favor mutators in nonrecombining populations undergoing adaptive evolution (in the absence of beneficial mutations, indirect selection always favors lower mutation rates; e.g., Lynch 2008). Several studies, however, have suggested that mutators may, in fact, be favored by selection only in sufficiently

large populations (Tenailon et al. 1999; Andre and Godelle 2006; Wylie et al. 2009; Raynes et al. 2014, 2018). Most recently, Raynes et al. (2018) have produced an analytic approximation for the fixation probability of a mutator allele, $P_{\text{fix}}^{\text{mut}}$, which predicts its transition from being favored by selection in large populations to disfavored in small populations at critical population size N_{crit} . Hereafter, following Raynes et al. we refer to this transition in selective effect as sign inversion.

In brief, Raynes et al. (2018) considered the combined probability of mutator fixation via hitchhiking with a sweeping beneficial mutation and random genetic drift. Because beneficial mutations are rare compared to deleterious ones (Eyre-Walker and Keightley 2007), the probability of a mutator hitchhiking to fixation is tempered by the low probability of a beneficial mutation appearing in its genetic background. In fact, Raynes et al. (2018) showed that in populations smaller than N_{crit} , the probability of hitchhiking is lower than the fixation probability of a neutral mutation— $1/N$. All the while, the probability of a mutator drifting to fixation is always lower than $1/N$ because of selection against the increased load of deleterious mutations. As a result, mutators fare worse than neutral mutations in populations below N_{crit} and better than neutral mutations in population above it. Raynes et al. (2018) confirmed sign inversion in agent-based computer simulations and in competitions between isogenic mutator and nonmutator strains of the budding yeast *Saccharomyces cerevisiae*. As predicted, mutators were favored by selection in large populations but disfavored by selection in small populations.

The phenomenon of population size-dependent sign inversion raises the question of how population structure may influence mutation rate evolution. Because mutators are disfavored in small populations, subdividing a metapopulation that otherwise would favor higher mutation rates into small, completely isolated subpopulations (demes) should effectively inhibit mutator hitchhiking. On the other hand, sufficient migration may be expected from population genetics theory to allow individual small demes in a metapopulation to evolve together as a single, large metapopulation (Maruyama 1970; Slatkin 1985; Kryazhimskiy et al. 2012), effectively increasing population size experienced by a mutator. As a result, mutators might switch from being disfavored in small, isolated demes to being favored in a sufficiently connected large metapopulation. Here, we explore the role of migration between demes in promoting mutator evolution in metapopulations. We employ the island model (Wright 1943) in which a metapopulation is subdivided into partially isolated demes of equal size, with each deme connected to all others via reciprocal migration. We show in simulation and experimental yeast populations that frequent migration allows metapopulations composed of small demes (individually below N_{crit}) to evolve as large, panmictic populations that favor mutators. More surprisingly, mutators fare even better in metapopulations with only moderate or rare mi-

gration. Indeed, we show that increased population structure can help mutators to persist in a metapopulation with rare migration despite the associated cost, and to eventually sweep to fixation in all of the connected demes.

Methods

COMPUTER SIMULATIONS

To investigate the role of migration in mutation rate evolution, we developed and simulated an individual-based, stochastic model of an evolving metapopulation, extending the earlier work of Raynes et al. (2018). As before, we consider strictly asexual populations of constant size, evolving in discrete, nonoverlapping generations according to the Wright-Fisher model (Ewens 2004). Populations are composed of genetic lineages, i.e. individuals with the same genotype. A genotype comprises 100 loci, 99 of which affect fitness and one that sets the mutation rate. The mutation rate modifying locus has two states: nonmutator and mutator and cannot itself be altered by mutation. The mutator state elevates the lineage mutation rate 100-fold, unless otherwise stated. The fitness loci can be affected by both beneficial and deleterious mutations. For computational efficiency, we assume constant fitness effects: $s_{\text{ben}} = 0.1$ for beneficial mutations and $s_{\text{del}} = -0.1$ for deleterious mutations. We also assume additive fitness effects and calculate fitness of a lineage with x beneficial and y deleterious mutations as $w_{xy} = 1 + xs_{\text{ben}} - ys_{\text{del}}$.

Unlike our earlier work (Raynes et al. 2018), we allow either $d = 3$, $d = 6$, or $d = 24$ demes, i.e. subpopulations, evolving in parallel. All demes in a metapopulation are equal in size, set at $N_{d=3} = 400$, $N_{d=6} = 200$, $N_{d=24} = 50$. The total size of a metapopulation is, thus, always $N_M = d \cdot N_d = 1200$. Simulations start with mutator and nonmutator lineages in every deme without any fitness-affecting mutations. Simulations end when mutators are either fixed (reach 100% in all demes) or extinct (0% in all demes) in the metapopulation.

Within a metapopulation, demes undergo reproduction and selection, mutation, and migration as follows.

Reproduction and selection

Every generation, the size of a lineage with x beneficial and y deleterious mutations is randomly sampled from a multinomial distribution with expectation $N f_{xy}(w_{xy}/\bar{w})$, where N is the size of the deme, f_{xy} is the frequency of the lineage in the previous generation, w_{xy} is as defined above, and \bar{w} is the average fitness of the deme (w_{xy}/\bar{w} is, thus, the relative fitness of the lineage).

Mutation

Upon reproduction, each surviving lineage acquires a Poisson distributed number of mutations, M , with mean determined by the size of the lineage multiplied by the total per-individual mutation

rate ($U_{\text{ben}} + U_{\text{del}}$) for nonmutators and $100 \times (U_{\text{ben}} + U_{\text{del}})$ for mutators, where U_{ben} and U_{del} are the deleterious and beneficial mutation rates, respectively. The number of beneficial mutations is then drawn from a binomial distribution with $n = M$ and $P = U_{\text{ben}}/(U_{\text{ben}} + U_{\text{del}})$, with the remainder representing the number of deleterious mutations. Each new beneficial and deleterious mutation is assigned to a randomly chosen not mutated locus producing a new genotype. For each new genotype, an individual is removed from the lineage and is assigned to a different existing lineage if the new genotype is already represented in the populations or to a new lineage of size 1.

Migration

To model migration, we implement the island model (Wright 1943) in which equal-sized demes exchange a constant number of migrants with all other demes. Every g generations, each deme contributes a Poisson distributed number of migrants with mean mN_d to a common migrant pool. Migrant individuals are randomly sampled without replacement from all the individuals in the deme. Next, each deme samples without replacement from the common migrant pool to return to size N_d . In our simulations, we manipulate both g and m to change migration rate.

EVOLUTION EXPERIMENTS

Strains, media, and propagation conditions

The haploid yeast strains used in this experiment have been previously described in Raynes et al. (2018). In brief, isogenic strains labeled with either ymCitrine (yJHK111) or ymCherry (yJHK112) were made into mutators by replacement of the mismatch repair gene, MSH2, with a kanamycin resistance knockout cassette. The deletion of MSH2 resulted in an approximate 20-fold increase in the mutation rate. As in Raynes et al. (2018), populations were propagated in 200 μL of low-glucose minimal medium (6.7 g YNB + ammonium sulfate, 0.2 g glucose per 1 L) supplemented with ampicillin (100 $\mu\text{g}/\text{mL}$) and tetracycline (20 $\mu\text{g}/\text{mL}$) to prevent bacterial contamination. Standard flat-bottom 96-well microtiter plates (Greiner Bio-One 655161) with lids were used. Plates were sealed in plastic Ziploc bags to prevent evaporation and incubated at 30°C with shaking at 1250 rpm in a microplate shaker (Multi-Microplate Genie; 1 mm orbit; Scientific Industries, Inc., Bohemia, New York).

Experimental populations

Mutator and nonmutator strains carrying both fluorescent labels were first streaked onto agar plates from frozen stocks. After two days of growth, 192 individual colonies of each of the four types were picked into 200 μL of low-glucose minimal medium and incubated as described above until saturation. All populations were then diluted 10,000-fold into 200 μL of fresh medium and incubated for two more days to acclimate to propagation condi-

tions. Populations were then combined to produce 192 populations of ymCherry-labeled mutators and ymCitrine-labeled nonmutators and 192 populations of ymCherry-labeled nonmutators and ymCitrine-labeled mutators. Note that $\sim 20,000$ cells from each population were analyzed on the Attune NxT Flow Cytometer (Invitrogen). Ninety-six populations (48 of each labeling scheme) closest to the target mutator frequency of 50% were chosen for the experiment and grouped together on a single 96-well microtiter plate. Competitions shown in Figure 3 were initiated from the same set of 96 populations. In these populations ymCherry-labeled mutators were at an average frequency of $48.03\% \pm \text{S.D. } 2.08\%$ and ymCitrine-labeled mutators were at an average frequency of $49.15\% \pm \text{SD } 2.75\%$. Competitions shown in Figure 4 were initiated several months later from a different set of 96 populations constructed as above. In these populations ymCherry-labeled mutators were at an average frequency of $50.02\% \pm \text{SD } 1.45\%$ and ymCitrine-labeled mutators were at an average frequency of $50.29\% \pm \text{SD } 1.71\%$.

Experimental propagation

As in Raynes et al. (2018) populations were propagated through 1:40,000 dilutions into fresh medium every 2.5 days resulting in regular bottlenecks of ~ 20 cells and $\log_2(40,000) \approx 15.3$ generations between transfers. As in the earlier work, transfers without migration were performed by sampling ~ 20 cells from each population into the corresponding well in the new plate. The effective population size of these populations could be estimated as $N_e = gN_0 = 15.3 \times 20 \approx 306$, where g is the number of generations between transfers and N_0 is the bottleneck size (Lenski et al. 1991). Transfers with migration were performed by first mixing samples of all demes in a metapopulation (see below) in a common reservoir – a single well in a standard deep-well microtiter plate. The mixture was then diluted and ~ 20 cells of the mixture were sampled into each well of the metapopulation in the new plate.

For experiments with frequent migration, metapopulations underwent migration at every transfer, that is, every ~ 15.3 generations (Fig. S3B). Ninety-six replicate populations were used to initiate three parallel treatments, in which populations were grouped into either 24 metapopulations of four demes, 16 metapopulations of six demes, or eight metapopulations of 12 demes.

For experiments with rare migration, 96 replicate populations were used to initiate three parallel treatments in which populations were either propagated completely in isolation (Fig. S3A) or were grouped into 16 metapopulations of six demes that went through either four or eight transfers between migration events (i.e., ~ 61.2 or ~ 122.4 generations respectively; Fig. S3C). (As a matter of convenience, these latter experiments were initialized from the fully isolated populations. After ~ 61.2 generations [four bottlenecks], samples were taken from the fully isolated populations to initiate 16 new replicate metapopulations of

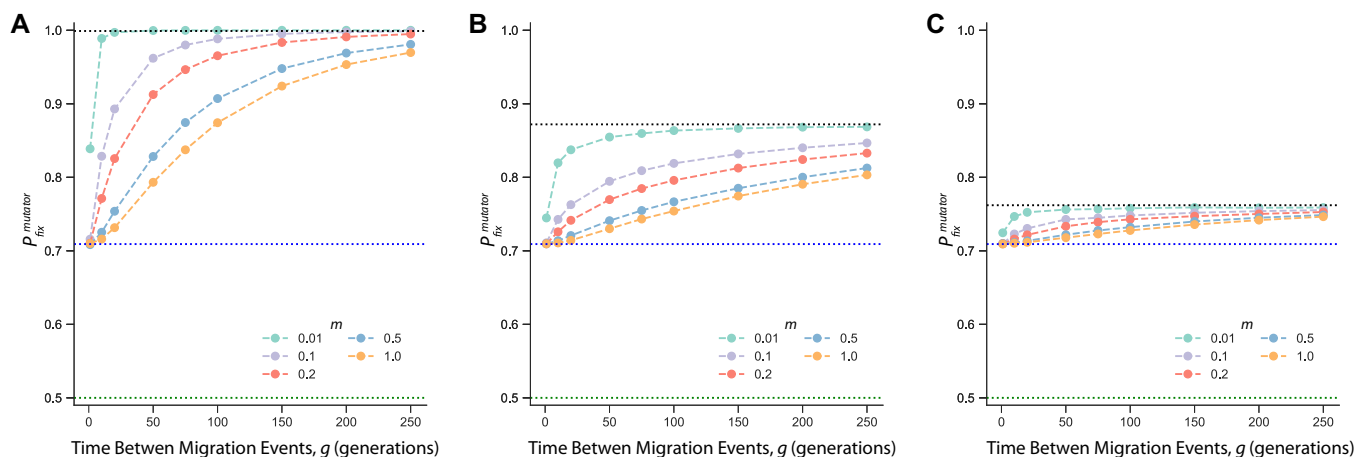


Figure 1. The effect of migration on indirect selection on mutators in simulated metapopulations. Mutator fixation probability was assessed in simulations of metapopulations comprising (A) 24 demes of size $N_d = 50$ individuals, (B) six demes of size $N_d = 200$ individuals, or (C) three demes of size $N_d = 400$ individuals. m represents the fraction of individuals each deme exchanges with the common migrant pool during a migration event. Horizontal dashed lines: green—neutral expectation given the starting frequency, blue—fixation probability expected in a panmictic population of size N_M , black—the binomial probability of mutator fixation in at least one deme of the metapopulation given by the number of demes and the expected P_{fix}^{mut} at the corresponding N_d . Parameter values: $U_{del} = 10^{-4}$, $U_{ben} = 10^{-6}$, $s_{ben} = 0.1$, $s_{del} = -0.1$. Mutators mutate 100 \times faster than nonmutators. P_{fix}^{mut} calculated over 10^6 runs of simulation.

six demes each, which then underwent migration and were then propagated with subsequent migration every ~ 61.2 generations. After ~ 122.4 generations [eight bottlenecks], the fully isolated populations were used to initiate another set of 16 replicate metapopulations that were then propagated with migration every ~ 122.4 generations.)

Mutator frequencies were periodically assayed by analyzing $\sim 20,000$ cells from each population on the Attune NxT Flow Cytometer. Following Raynes et al. (2018) we considered mutators fixed in a metapopulation when their frequency increased above 95% in all demes and lost when their frequency dropped below 5% in all demes. Propagation of each metapopulation was stopped when either the mutator or the nonmutator was fixed in all of its demes. Fixation time was assessed as the first time point at which the frequency of mutators was measured to be above 95% in all demes.

Results and Discussion

MIGRATION BETWEEN DEMES FAVORS MUTATORS IN SIMULATED METAPOPOPULATIONS

We used stochastic simulations to examine the influence of migration between demes on the fixation probability of a mutator allele, P_{fix}^{mut} , in a subdivided metapopulation. Simulations were initialized with a 100-fold mutator allele at a frequency of 50% in every deme of a metapopulation and continued until mutators reached fixation or went extinct in all of them (Fig. 1); additional simulations were performed starting with a single mutator in each deme (Fig. S2). To manipulate migration between demes, we var-

ied the number of generations, g , between migration events, and the fraction of migrants, m , sent out and received by each deme during each migration event. To ascertain whether mutators were favored or disfavored at different migration regimes, we compared P_{fix}^{mut} to the neutral expectation, i.e. the mutator starting frequency. The size of a metapopulation in our model was set at $N_M = 1200$ individuals, subdivided into either three large, six intermediate, or 24 small demes of size $N_d = 50$, $N_d = 200$, and $N_d = 400$, respectively. Population sizes were chosen such that, for our parameter values, mutators were expected to be favored in panmictic populations of size N_M (i.e., $N_M > N_{crit}$) but disfavored in panmictic populations of size N_d (i.e., $N_d < N_{crit}$). In preliminary simulations (Fig. S1), we estimated P_{fix}^{mut} to be ~ 0.71 for populations of size $N_M = 1200$, ~ 0.39 for $N_d = 50$, ~ 0.29 for $N_d = 200$, and ~ 0.38 for $N_d = 400$ (all when started at 50%).

As expected, simulated metapopulations with the most connected demes (i.e., migration events every generation, $g = 1$; $m = 1.0$) evolve as large, panmictic populations. Correspondingly, the realized P_{fix}^{mut} in these populations is as high as the expected P_{fix}^{mut} in panmictic populations of size N_M (Figs. 1, 2S) and clearly above the neutral expectation (0.5 for Fig. 1 and d/N_M for Fig. 2S). In other words, with frequent migration, indirect selection can favor mutators in metapopulations composed of demes that would individually disfavor them. There is, correspondingly, no discernable difference in P_{fix}^{mut} between metapopulations of the same size subdivided into different numbers of demes as they all evolve approximately as a panmictic population of size N_M (Fig. 1).

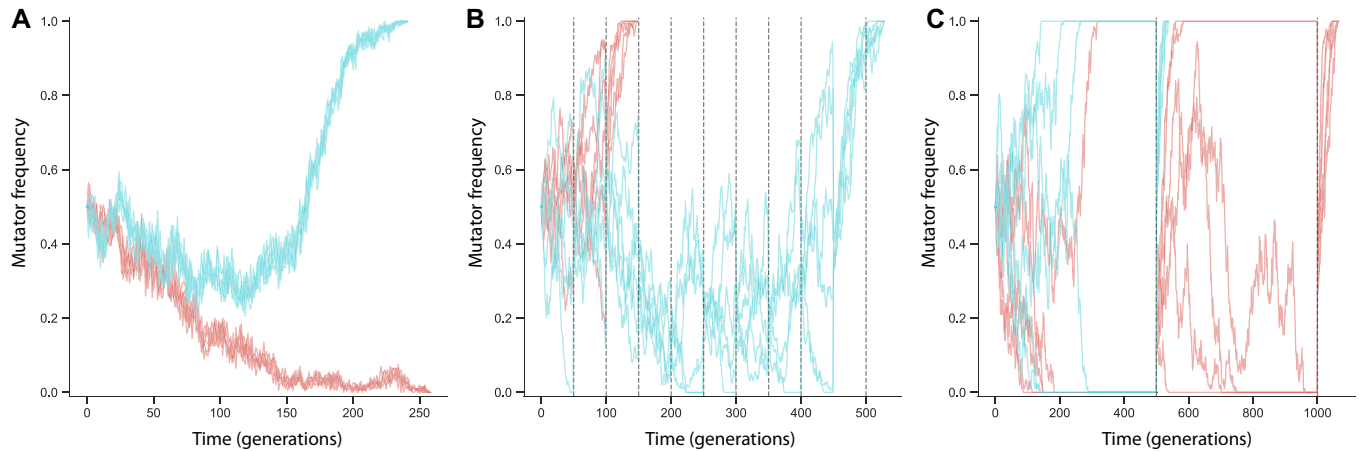


Figure 2. Mutator dynamics in representative simulated metapopulations. Red and teal lines show mutator frequencies in two randomly chosen metapopulations of six demes (each line shows mutator dynamics in an individual deme of the metapopulation) with migration events (A) every generation, (B) every 50 generations, and (C) every 500 generations. Demes are of size $N_d = 200$. Parameter values: $U_{del} = 10^{-4}$, $U_{ben} = 10^{-6}$, $s_{ben} = 0.1$, $s_{del} = -0.1$. Mutators mutate $100\times$ faster than nonmutators. Dashed vertical lines in panels (B) and (C) indicate the times of migration events.

More surprisingly, for any combination of nonzero values of g and m , P_{fix}^{mut} in a structured metapopulation of size N_M is at least as high as that expected in a panmictic population of the same size. In fact, as both the number of migrants and the frequency of migration events decrease, P_{fix}^{mut} in a structured metapopulation rises monotonically above that of a panmictic population. To see why P_{fix}^{mut} in a more structured metapopulation may be higher than expected for a panmictic population, it is informative to consider mutator dynamics in individual demes comprising the metapopulation. Such dynamics at different levels of migration are illustrated with representative simulation runs in Figure 2. As expected, demes in the most connected metapopulations (Fig. 2A) evolve almost as if they are a single panmictic population of size N_M —mutator frequencies within each deme follow closely the average metapopulation frequency. Correspondingly, mutators reach fixation in most connected metapopulations with probability given by P_{fix}^{mut} expected for population size N_M .

In less connected (i.e., more structured) metapopulations (Fig. 2B), mutator frequencies in individual demes begin to diverge, resulting in mutators experiencing selection more and more as if they were in small populations of size N_d . Note that demes with more common mutators can reintroduce mutators back into the metapopulation, raising the probability that they establish in other demes and eventually take over the metapopulation. Finally, in the least connected metapopulations (Fig. 2C), mutators evolve completely independently in individual demes. Competitions in most demes are resolved before migration can significantly affect mutator frequency and because mutators are disfavored in populations of size N_d , they generally lose in most of them. Population structure, however, allows mutators to persist in a metapopulation despite being disfavored by selection by fixing in at least

some of its mostly isolated demes. Moreover, because we model population undergoing adaptive evolution, mutators in these refugia demes continue substituting beneficial mutations at a faster pace than nonmutator populations in other demes. Eventually, migration spreads the more fit mutators into other less fit demes resulting in mutator fixation in the whole metapopulation.

Thus, we find that with sufficiently rare migration P_{fix}^{mut} can be approximated simply by the expected probability of mutators persisting in a metapopulation by reaching fixation in at least one of the demes. Indeed, P_{fix}^{mut} can be estimated simply as the binomial probability of $X > 0$ successes in d trials, where d is the number of demes in a metapopulation, and the probability of success in one deme is given by the expected P_{fix}^{mut} at population size N_d . Correspondingly, in each metapopulation in Figures 1, P_{fix}^{mut} approaches the binomial probability of mutator success in at least one deme (given by the number of demes: $d = 24, 6$, or 3 , and the expected P_{fix}^{mut} for a panmictic population of size $N_d = 50, N_d = 200$, and $N_d = 400$, respectively). This probability is, as expected, highest in metapopulations with the most demes (i.e., trials). Critically, the probability of mutator fixation in at least one of d demes of size N_d is also always higher than P_{fix}^{mut} in a panmictic population of size N_M (where $N_M = d \cdot N_d$). As a result, P_{fix}^{mut} in a metapopulation of size N_M with rare migration is also always higher than both that in a panmictic population of size N_M and that in a well-connected metapopulation of the same size (expected to evolve as a panmictic population). Figure S3 demonstrates that these results are also robust to the strength of the mutator (as tested in simulation over the range of 20-fold to 250-fold).

Note that while mutator fixation may be more likely with rare migration, the time needed for the mutator lineages that have

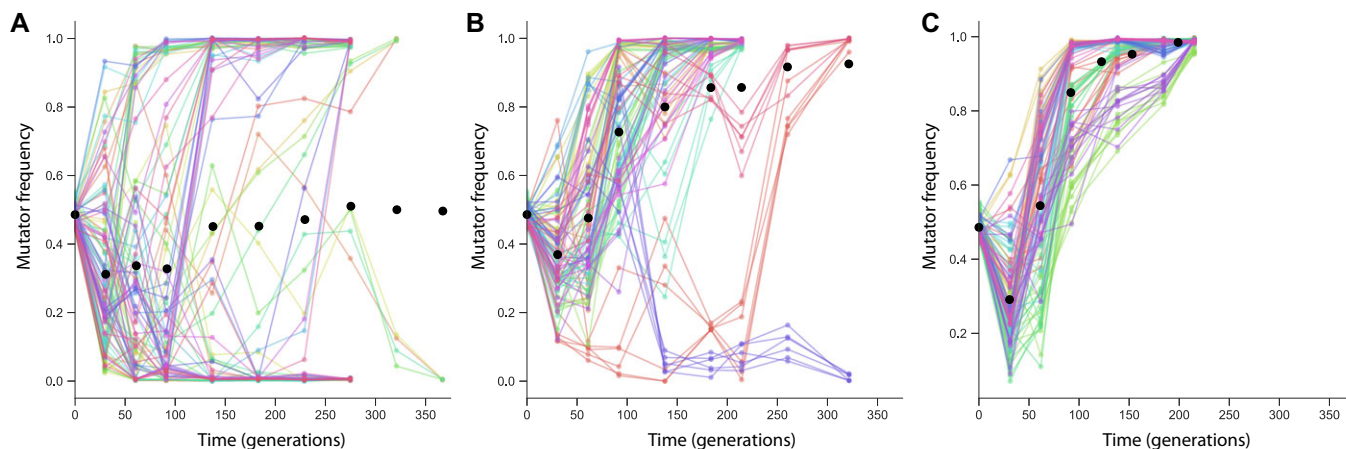


Figure 3. Mutator dynamics in experimental metapopulations of yeast. Metapopulations were composed of (A) four demes, (B) six demes, and (C) 12 demes. Each deme was propagated through a bottleneck of 20 cells sampled from a mixture of all demes in the metapopulation. Different color curves represent demes within each metapopulation. Black dots represent averages of all metapopulations (last measured values used for already stopped populations).

fixed in some of the demes to spread to all other demes via rare migration can make mutator fixation take considerably more time than in more well-connected metapopulations (Fig. S4).

MUTATOR DYNAMICS IN EXPERIMENTAL YEAST METAPOPOPULATIONS OF DIFFERENT SIZE

Our simulations show that with frequent migration between demes, $P_{\text{fix}}^{\text{mut}}$ in a metapopulation of size N_M is given by the expected fixation probability in a panmictic population of the same size. We tested this prediction empirically by conducting competitions between mutator and nonmutator strains of *S. cerevisiae* yeast in metapopulations of differing size (the experimental protocol is illustrated in Fig. S5). Because we have previously shown that larger panmictic populations could more effectively favor mutators than smaller populations (Raynes et al. 2018), we expected that, given sufficient migration between demes, mutators would also fare better in larger metapopulations.

We initiated 96 replicate populations at approximately equal frequencies of two strains and propagated them through regular dilutions into fresh medium. In parallel treatments, these populations were grouped into either 24 small metapopulations of four demes, 16 medium metapopulations of six demes, or eight large metapopulations of 12 demes. Previously, populations undergoing regular bottlenecks of ~ 20 cells (Fig. S5A) were shown to be small enough to disfavor mutators in the laboratory environment used in our experiments (Raynes et al. 2018). Here, we again subjected individual demes to bottlenecks of ~ 20 cells, but unlike in the earlier work, these 20 cells were now sampled from a mixture of all demes of the metapopulation, that is, metapopulation went through migration at every bottleneck (Fig. S5B).

Figure 3 presents mutator dynamics in metapopulations of different size composed of either four, six, or 12 demes (frequency data in Supporting Information Dataset 1). Mutators fixed in 12 out of 24 small metapopulations composed of four demes, meaning that they appeared to be close to neutral (Fig. 3A). Intriguingly, in the previous experiment Raynes et al. (2018) showed that in populations undergoing regular bottlenecks of 80 cells, mutators also fixed in approximately half of the competitions. Thus, as predicted by simulations, the effect of subjecting populations to bottlenecks of 80 cells was roughly recapitulated by metapopulations composed of four demes each of which was subjected to the 20-cell bottleneck undergoing frequent migration. (Note that populations bottlenecked to 80 cells are only approximately four times larger than population bottlenecked to 20 cells as they experience $\log_2(4) = 2$ fewer generations between transfers; for more on estimating population size in regularly bottlenecked populations, see Lenski et al. 1991.) In further agreement with our expectations, mutators were more successful in metapopulations composed of more demes (Figs. 3B, C). Mutators were able to fix in 15 out of 16 medium metapopulations composed of six demes and all eight of eight large metapopulations composed of 12 demes.

MUTATORS DYNAMICS IN EXPERIMENTAL METAPOPOPULATIONS OF YEAST WITH RARE MIGRATION

Our simulations also show that $P_{\text{fix}}^{\text{mut}}$ in a structured metapopulation with rare migration between demes is never lower, and may, in fact, be higher than that expected in a panmictic population of the same size. On the other hand, time to mutator fixation may be considerably longer than in metapopulations with more

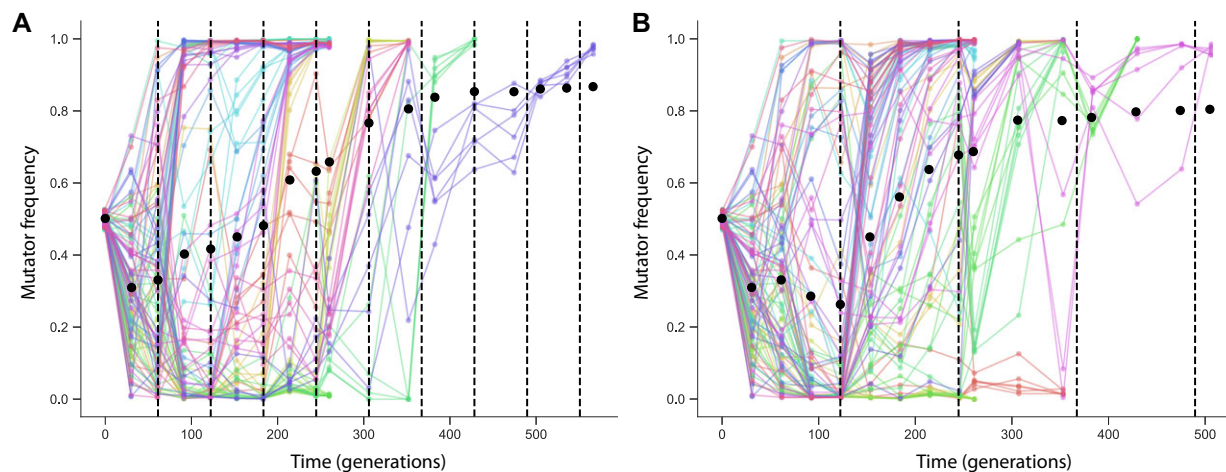


Figure 4. Mutator dynamics in experimental metapopulations of yeast with rare migration. (A) Sixteen metapopulations composed of six demes, undergoing migration every ~ 61.2 generations. (B) Sixteen metapopulations were composed of six demes, undergoing migration every ~ 122.4 generations. Different color curves represent demes within each metapopulation. Black dots represent averages of all metapopulations (last measured values used for already stopped populations). Dashed vertical lines indicate the times of migration events.

frequent migration. We tested these predictions empirically by conducting competitions between mutator and nonmutator yeast in metapopulations of the same size with differing migration rates (experimental protocol is illustrated in Fig. S5C; mutator frequency data available in Supporting Information Dataset 2). Once again, we initiated 96 replicate populations at approximately equal frequencies of the mutator and the nonmutator strains. In parallel treatments, these populations were propagated either without migration, with migration every ~ 61.2 generations, or with migration every ~ 122.4 generations. Populations without migration were propagated through regular bottlenecks of 20 cells every ~ 15.3 generations for a total of ~ 290 generations to confirm that mutators were disfavored at this population size (Fig. S6, which recapitulates results shown in Fig. 2C in Raynes et al. 2018). Populations with migration were propagated through four or eight cycles of growth in isolation (again experiencing 20 cell bottlenecks every ~ 15.3 generations, yielding ~ 61.2 or ~ 122.4 generations of growth, respectively) between migration events for up to ~ 566 generations until all competitions were resolved (Fig. 4).

Figure 3 shows that with sufficiently common migration, mutators were strongly favored in experimental metapopulations composed of six demes. In light of our simulation results in Figure 1, we expected that mutators would be just as favored in metapopulations of the same size (i.e., six demes) but connected by less frequent migration. Consistent with our expectation, mutators were favored by selection under both new migration regimes (Fig. 4). Mutators were able to fix in 14 out of 16 metapopulations propagated with migration every ~ 61.2 generations (Fig. 4A), and 13 out of 16 metapopulations propagated

with migration every ~ 122.4 generations (Fig. 4B). Mutator fixation probability in either migration regime was, as predicted, not significantly different than that observed for metapopulations with common migration (Fisher's exact test; for $g \sim 61.2$: two-tailed $P = 1.0$, for $g \sim 122.4$: two-tailed $P = 0.5996$). We note that unlike in simulations (Fig. 1), mutators did not fare better in metapopulations with rare migration than they did in populations with common migration. However, given the success of mutators in metapopulations in common migration, it seems likely that the number of metapopulations in our experiment was insufficient to detect any increase in the fixation probability.

Moreover, mutator fixation took significantly more time in metapopulations with rare migration than with common migration as predicted in our simulations. The average fixation time in metapopulations with migration every ~ 15.3 generations (Fig. 3B) was 172.38 ± 18.57 SEM. The average fixation times in metapopulations with migration every ~ 61.2 and ~ 122.4 generations (Fig. 4) were 263.37 ± 34.29 SEM and 280.12 ± 26.39 SEM, respectively. Both were significantly longer than in populations with migration every ~ 15.3 generations (for $g \sim 61.2$: two-sided t -test $t_{df=27} = 2.3070$, $P = 0.0290$; for $g \sim 122.4$: two-sided t -test $t_{df=26} = 3.3133$, $P = 0.0027$).

Conclusion

Here, we have used stochastic simulations and experimental populations of yeast to show that in a structured metapopulation, migration between demes can favor mutator fixation even when mutators are disfavored in individual demes. As expected from population genetics theory, we found that metapopulations with

sufficiently common migration evolved as large, panmictic populations (reviewed in Slatkin 1985). As a result, mutators were able to reach fixation in these metapopulations at a rate expected for the panmictic population of the same size. More surprisingly, our simulations showed that mutators might fare even better with only intermediate or even rare migration, as population structure may allow mutators to persist in a metapopulation at a higher than expected rate. The reason for this is that although the mutator fixation probability in individual demes is relatively low, the probability of fixation in at least one of the demes in a metapopulation can be considerably higher than that in a panmictic population of the same size. And any demes in which mutators do fix act as refugia for mutator populations, allowing mutators to remain in the metapopulation despite selection against the deleterious load. From these refugia, mutators can be reintroduced to other demes by migration, raising the overall fixation probability. Moreover, further adaptation by mutators in refugia makes it even more likely that these persistent populations eventually outcompete remaining nonmutators in other demes.

The evolutionary success of mutators with only minimal migration between demes is, intriguingly, reminiscent of the potential advantage of otherwise costly recombination (e.g., Peck et al. 1999; Whitlock et al. 2018) and cooperation (Nowak 2006; Taylor and Nowak 2007) in sufficiently structured populations. Similar to its effect on mutators, population subdivision allows costly recombiner alleles to persist in a metapopulation despite indirect selection against the recombination load—the cost of unfavorable combinations of alleles. Eventually, nonrecombining demes succumb to deleterious mutation and drift (i.e., Muller's ratchet), allowing recombining migrants to take over the metapopulation. Likewise, cooperators are generally exploited by cheaters in panmictic populations, but may overtake a sufficiently structured metapopulation if they can persist in clusters (demes) dominated by cooperators that outperform clusters dominated by cheaters.

Finally, we have previously suggested that population structure may inhibit mutators by subdividing otherwise large populations into small, isolated subpopulations (demes) (Raynes et al. 2018), which could contribute to their sporadic occurrence in nature (outside of cancers and pathogenic infections). The present work shows that even rare migration may be a powerful contributor to mutator success in structured environments, suggesting it too could be an important determinant of mutation rates in natural populations. We note, however, that we have examined only a simple model of population structure in which equal sized demes are equally connected to all others. Future work using more realistic models of population subdivision and migration will help shed more light on the role of migration in structured populations that, like real ecological habitats, contain demes of varying size and connectivity to one another.

AUTHOR CONTRIBUTIONS

All authors conceived of the study. YR designed the study, wrote and conducted simulations, and performed evolution experiments with yeast. YR and DMW analyzed the results. YR wrote the first draft of the manuscript. All authors reviewed, edited, and approved the final draft of the manuscript.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Fixation probability of mutators in panmictic populations.

Fig S2. Mutator fixation probability in simulated metapopulations initialized with a single mutator allele in each deme.

Fig S3. The effect of migration on mutators of different strengths.

Fig S4. Mutator fixation is delayed in metapopulations with rare migration.

Fig S5. Experimental propagation protocol.

Fig S6. Mutator dynamics in experimental metapopulations of yeast with no migration.

Dataset 1

Dataset 2