The evolution of stress-induced mutagenesis in the presence of recombination

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# Introduction

## The controversy of the evolution of stress-induced hypermutation

For years the mutation rate was considered constant and uniform, both in time and in the population (Luria and Delbrück 1943). However, since the 1970s (Radman 1975), experiments with microorganisms revealed that various stress responses induce a state of mutagenesis in which the mutation rate of an individual is increased by several orders of magnitude (Galhardo, Hastings, and Rosenberg 2007; Foster 2007; Heidenreich 2007). This process is referred to as *stress-induced mutagenesis* (SIM). Because mutations are the ultimate source of genetic variation, it was suggested that stress-induced mutagenesis evolved to promote the adaptation of cells to new environmental conditions and stresses (Tenaillon, Denamur, and Matic 2004; Saint-Ruf and Matic 2006; Rosenberg et al. 2012; Ram and Hadany 2012).

This adaptive explanation is challenged by several non-adaptive explanations. First, mutagenesis can be a by-product of stress - mutations can be generated more often during stress because there is not enough energy and resources to repair them (Tenaillon, Denamur, and Matic 2004). Second, because there is a cost for high-fidelity DNA replication, it was suggested that an optimal mutation rate will balance between this "cost of fidelity" and the mutational load due to the accumulation of deleterious mutations (Dawson 1998). This balance may change during stressful times because, for example, the "cost of fidelity" is a short-term effect whereas the mutational load is a medium-term effect. Third, Lynch proposed the "drift barrier hypothesis" (Lynch 2011). Although this hypothesis has broader implications, it also suggests that DNA repair proteins that are primarily expressed during stress will be under weaker selection compared to proteins that are constitutively expressed, and will therefore become error prone due to genetic drift.

## The evolution of stress-induced hypermutation in asexual populations

In a previous work we have addressed the adaptive hypothesis in asexual populations (Ram and Hadany 2012). We studied the evolution of mutator alleles that induce an increased mutation rate in maladapted individuals in constant and changing environments. We demonstrated that these stress-induced mutator alleles can evolve in a wide range of conditions because they are favored by selection over mutator alleles that induce a constant rate of mutation.

## Recombination in microbial populations

Nevertheless, most microorganisms are not entirely asexual. Bacteria perform recombination, or horizontal/lateral gene transfer (HGT/LGT), using three mechanisms: transformation, in which cells recombine foreign DNA into their genome (Avery 1944); conjugation, in which plasmids are transferred between cells (Lederberg and Tatum 1946); and transduction, in which viruses carry off DNA segments with them when infecting new cells (Zinder and Lederberg 1952). Other microbes, such as yeast, sometimes reproduce sexually when two cells in a haploid state (spores) with opposite mating types mate to produce a diploid cell [REF].

## The effect of recombination on the evolution of mutators

The rate of adaptation of asexual (non-recombining) populations is largely dependent on the mutation rate, because mutation is the sole provider of novel adaptations in clonal populations.

However, there are several factors that can lower the overall adaptation rate of this *adaptation-by-mutation* strategy. First, competition between beneficial mutations can reduce their fixation probability, thus limiting the rate of adaptation in asexual populations in a process called "clonal interference" (Gerrish and Lenski 1998). Second, if two or more beneficial mutations are needed for adaptation to environmental conditions then they must occur in sequence, rather than in parallel (Muller 1932)//Fisher?//. Third, because most mutations are deleterious, increasing the mutation rate causes a mutational load which disrupts selection for beneficial mutations (Orr 2000), can lead to a "mutational meltdown" and loss of adaptation in a process called "Muller's ratchet" (Muller 1964; Haigh 1978; Lynch et al. 1993), and can lead to the hitch-hiking of deleterious mutations with fixating beneficial mutations (Hartfield and Glémin 2014).

These issues are mitigated by recombination. Recombination reduces "clonal interference" by combining co-occurring beneficial mutations (Cooper 2007; Martens and Hallatschek 2011). Combining co-occurring beneficial mutations also reduces the waiting time for the production of an adapted genotype (Christiansen et al. 1998) – this is sometimes referred to as the "Fisher-Muller effect" (Felsenstein 1974). In addition, recombination generates less-loaded individuals that were lost due to drift, halting "Mullter's ratchet" (Lynch and Conery 1995), and helps to purge deleterious mutations more effectively, reducing the mutational load (Keightley and Otto 2006).

The fate of a mutator – an allele that increases the mutation rate – depends on the mutations associated with it. A mutator can "hitch-hike" to high frequencies with the beneficial mutations it generates in a process called "selective sweep" or "genetic hitch-hiking" (Smith and Haigh 2009; Taddei et al. 1997; Charlesworth 2007), even if those mutations were originally deleterious when generated (Leigh 1970). However, a mutator may have several deleterious mutations in its genetic background together with a beneficial mutation and this deleterious background can prevent selection from effectively acting on the beneficial mutation (Orr 2000). This is called the "Hill-Robertson effect" (Hill and Robertson 1966; note that the "Hill-Robertson effect" is sometimes used in a broader sense that includes both the "Fisher-Muller effect" and "clonal interference" - see Comeron, Williford, and Kliman 2008). Recombination will have a contrasting effect on the fate of such a mutator: because recombination tends to break the linkage between the mutator and its associated mutations, it will counter both the "hitch-hiking effect" and the "Hill-Robertson effect". However, it is important to note that when recombination breaks the linkage between a mutator and its beneficial mutations, it can pass those beneficial mutations to competitor alleles – other mutator alleles or the wild-type allele. Although recombination will also pass deleterious alleles, we expect that the gain of beneficial mutations by other mutator alleles will be more significant and that the net effect of recombination on the fate of mutators will be negative (but see Orr 2000).

Due to this contrasting effect of recombination on the evolution of mutators, both at the population and the individual levels, it is important to examine if stress-induced mutators are limited to asexual populations or can readily evolve in the presence of recombination.

## Stress-induced hypermutation in the presence of recombination (and sir? )

We used simulations to study the evolution of stress-induced mutators in the presence of recombination in finite populations evolving in changing environments.

We show that (i) mutators are favored by selection if environmental changes are frequent and recombination is weaker than mutation; (ii) when mutators are advantageous, stress-induced mutators are favored over constitutive mutators; (iii) stress-induced mutators can be favored even when constitutive mutators are not; (iv) recombination reduces the selection for mutators primarily by disrupting the "hitch-hiking effect" rather than by the "Fisher-Muller effect"; and (v) if recombination is also stress-induced, mutators are still favored in rapidly changing environments, and stress-induced mutators are still favored over constitutive mutators.

# Model

We used stochastic Wright-Fisher simulations to model finite populations undergoing selection, mutation, recombination, and random genetic drift in changing environments.

The genome of every individual contains 1,000 fitness loci and four modifier loci. Individuals are grouped by their genotype and we keep track of the number of individuals in each group.

The fitness loci are bi-allelic and the alleles are marked by 0 and 1. The fitness of an individual is calculated by comparing the alleles at the fitness loci with the environmental optimal genome: the fitness of an individual with *x* loci in mismatch with the optimal genome is (1-*s*)*x*, where *s* is the selection coefficient. To model environmental changes, the optimal genome is altered by modifying alleles at several loci. The number of loci changed in each environmental change and the probability that an environmental change occurs are parameters of the model.

The modifier loci affect the rates of mutation and recombination. These loci are denoted by the letters π, *τ*, *ϕ*, and *ρ* and have numerical allele values. The basal mutation rate *µ* is measured by the number of mutations per individual per generation. If an individual has π or more mismatches with the environmental optimal genome, it increases its mutation rate *τ*-fold. The basal recombination rate is denoted by r and measured by the number of gene conversions per genome per generation; the threshold for recombination rate increase is ϕ and the rate increase is *ρ*-fold. These modifiers allow us to model different mutation and recombination strategies. See Table 1 for a full list of these strategies.

Each simulation starts with a mutation-free population (all individuals start with the optimal genome) and continues for a predefined number of generations. We model drift by sampling the number of individuals in each group in the next generation from a multinomial distribution, where the probability assigned to each group is equal to its relative size (*i.e.*, its frequency). Selection is modeled similarly, except we multiply the group relative size by the fitness of the individuals in the group (individuals in the same group have the same genotype and therefore the same fitness). Mutation and recombination are modeled by randomly drawing the number of events from a Poisson distribution with the mutation and recombination rates as the means of the distribution, then randomly choosing the loci that are about to be altered from a uniform distribution (only fitness loci are subject to mutation and recombination), and finally choosing the new allele in the altered loci: mutation changes the current allele to the beneficial allele with probability β and to the deleterious one with probability 1-β (β is the probability for a beneficial mutation); recombination replaces the current allele with a randomly chosen one based on the frequency of each allele in the population.

The simulations were written in Python 2.7 using the NumPy, SciPy, and pandas packages.

# Results

Previously, we showed that stress-induced mutators can invade asexual populations (Ram and Hadany 2012). However, many microbe populations are not entirely asexual. The rates of horizontal gene transfer in bacteria greatly differ between different species and mechanisms, as does the rate of sex and recombination in yeast [REF].

We simulated competitions between different mutation and recombination modifier alleles (see Table 1) in populations of bacteria experiencing changing environments. We estimated the fixation probability of modifier alleles from the fraction of competitions in which they reached fixation in the population. If an allele is neutral its fixation probability is equal to its initial frequency (50%). In contrast, if an allele is favored or disfavored by selection its fixation probability is significantly higher or lower than the initial frequency, respectively. Therefore, we tested whether the estimated fixation probabilities are significantly different from 50% using a 2-tail proportions test. In addition, we also measured the time for adaptation in populations homogenous at the modifier loci and the dynamics of mean, max, and min fitness during the adaptation process.

## Evolution of SIM in the presence of recombination

Figure ‎1 shows the fixation probability of constitutive (CM; dashed red) and stress-induced mutators (SIM; solid blue) under a range of recombination rates, starting with the low rates measured in *E. coli* and up to the high rates measured in *H. pylori* and *S. cerevisiae* [REF]. The figure shows that recombination reduces the fixation of both mutators, as shown before for CM (Tenaillon et al. 2000). However, SIM is always more successful than CM and it can be favored by selection in the presence of mild recombination, even when CM cannot.



Figure - invasion\_SIMvsCM\_pop\_1e6\_2014-02-23

Why is SIM advantageous when CM is not? Figure 2 shows that when 9 out of 10 mutations are deleterious (β=0.1; left panel) SIM fixation is more likely than CM fixation. However, when all mutations are beneficial (β=1; right panel), there is no difference between SIM and CM fixation. This suggests that SIM is advantageous over CM because it generates less deleterious mutations due to lower mutation rates in adapted individuals. Alternatively, the reason could be that SIM purges deleterious mutations more successfully than CM, but we have shown before (Ram and Hadany 2012) that purging of deleterious mutations doesn't affect SIM in a constant environment.



Figure - invasion\_SIMvsCMvsNM\_pop\_1e5\_1e6\_2014-03-09

More information on the advantage of SIM is given by the introduction of "recombination barriers". These barriers cause individuals to reject recombination with DNA from individuals with different modifier alleles. This prevents individuals with non-mutator alleles from "stealing" beneficial mutators and allows mutator alleles to "hitch-hike" with their beneficial mutations (Smith and Haigh 2009). Our results (Figure S2) show that "recombination barriers" do not have a significant effect on the dynamics, suggesting the effect of recombination on "hitch-hiking" and the transfer of beneficial mutations between sub-populations is not an important factor in this system.

The effect of population size on the dynamics is explored in Figure 3 (also see Figure S1). As the population size increases (from left to right) the effect of recombination on the fixation probability is stronger: with 105 individuals (left column), recombination only mildly affects the fixation probabilities with τ=10 (bottom row); with 107 individuals (right column) recombination has a very significant effect. For example, the fixation probability of SIM (solid blue) with τ=5 (middle row) and N=107 (right column) decreases from ≈0.75 to ≈0.31 when the recombination rate increases from 0 to 0.3, whereas there is no effect at all with N=105 (left column). This effect is similar for SIM and CM and is consistent with results for CM in (Tenaillon et al. 2000).



Figure - invasion\_SIMvsCMvsNM\_pop\_sizes\_2014-02-23

## Co-evolution of mutators and recombinators

Figure 4 shows results of competitions between alleles that modify the recombination rate – "recombinator" alleles. Both constitutive recombinators (CR; dashed purple) and stress-induced recombinators (SIR; solid orange) win competitions against wildtype, and their advantage increases with the general recombination rate and the population size.

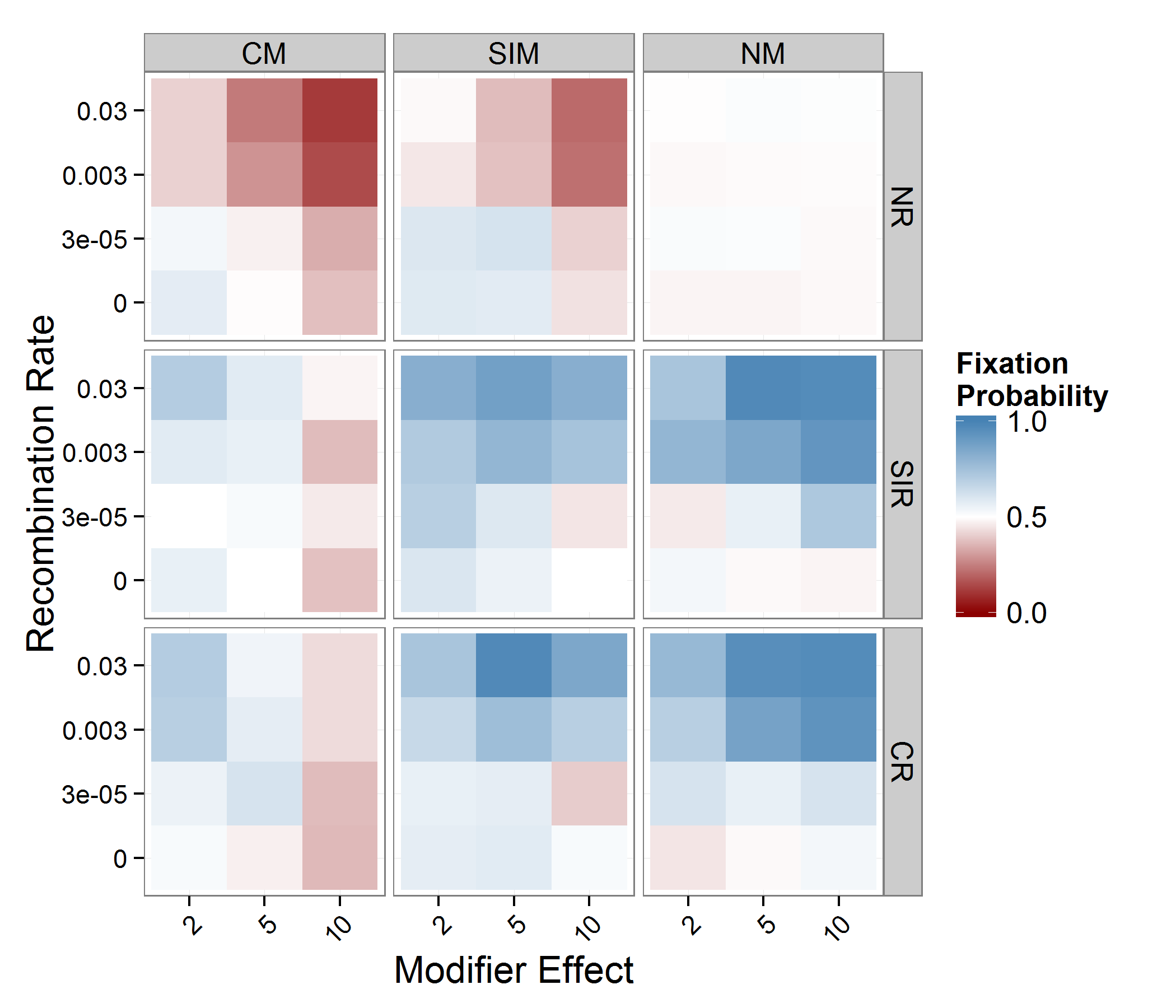


Figure - invasion\_SIRvsCR\_pop\_sizes\_2014-02-26

When a modifier allele affects both the mutation and the recombination rate, the dynamics are more complex (Figure 5). For a smaller population size (105), SIM (with NR, CR or SIR) is the most successful, and if it's combined with a recombinator (CR or SIR) its success increases with the baseline recombination rate. For a larger population size (106), this advantage of SIM over NM diminishes, and it is as good as NM with a recombinator and worse without one (reminiscent of Figure 1). However, CM is always less successful then both NM and SIM.



Figure - invasion\_combined\_tau\_5\_pop\_sizes\_2014-02-26



**Figure 5 alternative for pop size 1e6 - invasion\_invasion\_combined\_heatmap\_N\_1e6\_2014-03-04**

We also simulated competitions where the mutator allele also induces recombination in populations that were otherwise effectively asexual, with recombination rate *r*=1e-16. In this case, SIM was always most advantageous, but as the modifier increased the rate of recombination, the fixation probability of all modifiers increased and the advantage of SIM over NM disappeared – see Figure 6.

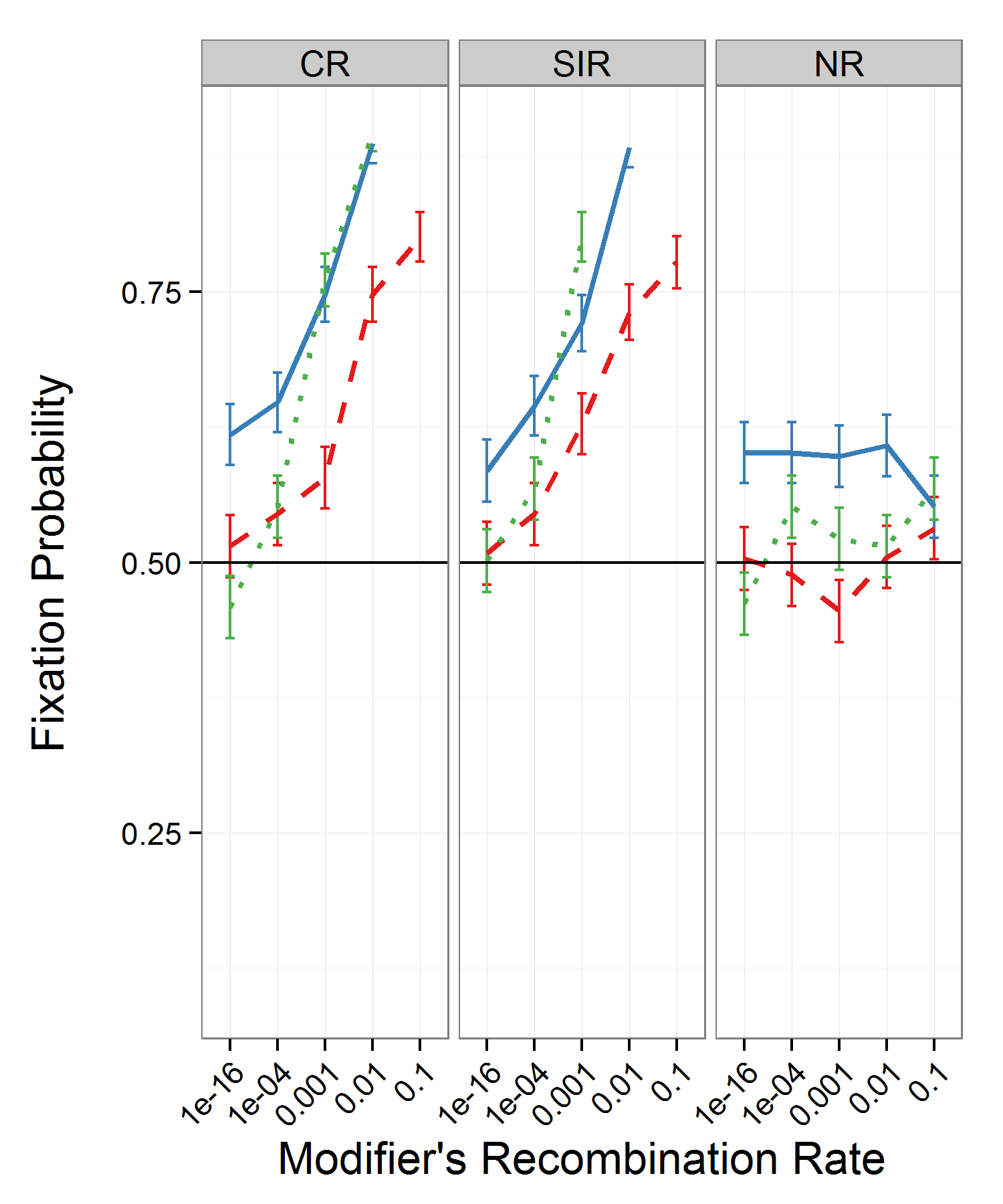


Figure - invasion\_SIMvsCMvsNM\_asexuals\_2014-03-10

## Adaptation time

Next, we investigate how recombination affects a population-level trait – the average time until the population is adapted after an environmental change.

Figure 6 shows the adaptation time of populations homogenous at the modifier locus (NM in dotted green; CM in dashed red; SIM in solid blue) with different levels of mutation rate increase τ (from left to right), populations size *N* (bottom to top), and number of beneficial mutations required for adaptation (one in circles; four in triangles; fitness effects of all mutations are equal and independent, *i.e.* there is no epistasis).

First, increasing rates of recombination (on the x-axis) reduces the time for adaptation with four beneficial mutations (triangles) in agreement with (Tenaillon et al. 2000). However, increasing the rate of recombination has not effect on adaptation with a single beneficial mutation (circles). The effect of recombination is stronger in large populations, where the mutation supply is larger and therefore recombination is more likely to recombine beneficial mutations from different backgrounds (Tenaillon et al. 2000).

Second, both mutator populations (SIM and CM) adapt much faster than non-mutator populations, and increasing the mutation rate decreases the adaptation time. Remarkably, in the time it takes a non-mutator population to fix a single beneficial mutation, a mutator population can fix a combination of four beneficial mutations, even without recombination (see panels where triangles are below circles). The difference between CM and SIM is only meaningful when adaptations require a single beneficial mutation (circles) and is very small even then.

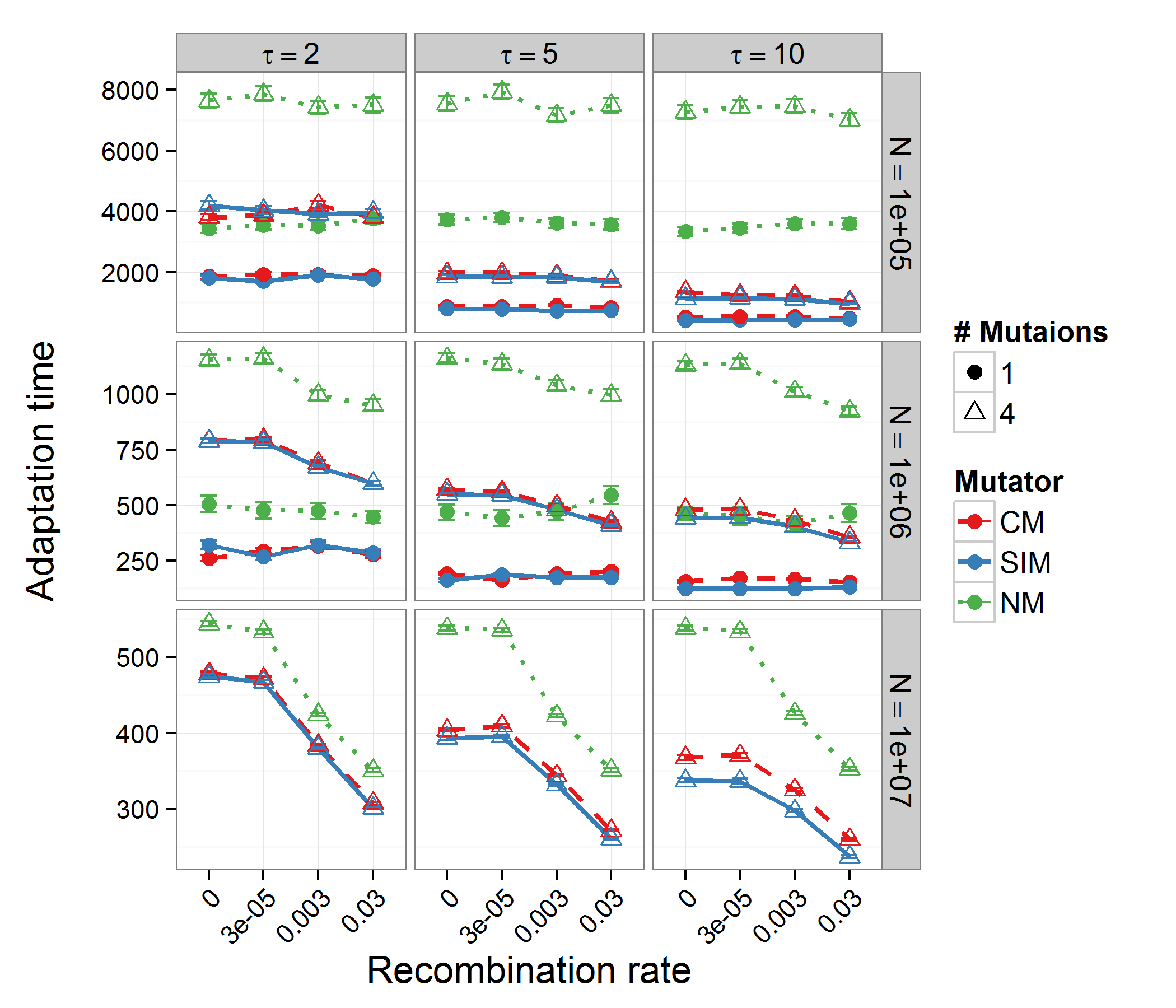
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Figure - adaptation\_NR\_2014-03-10

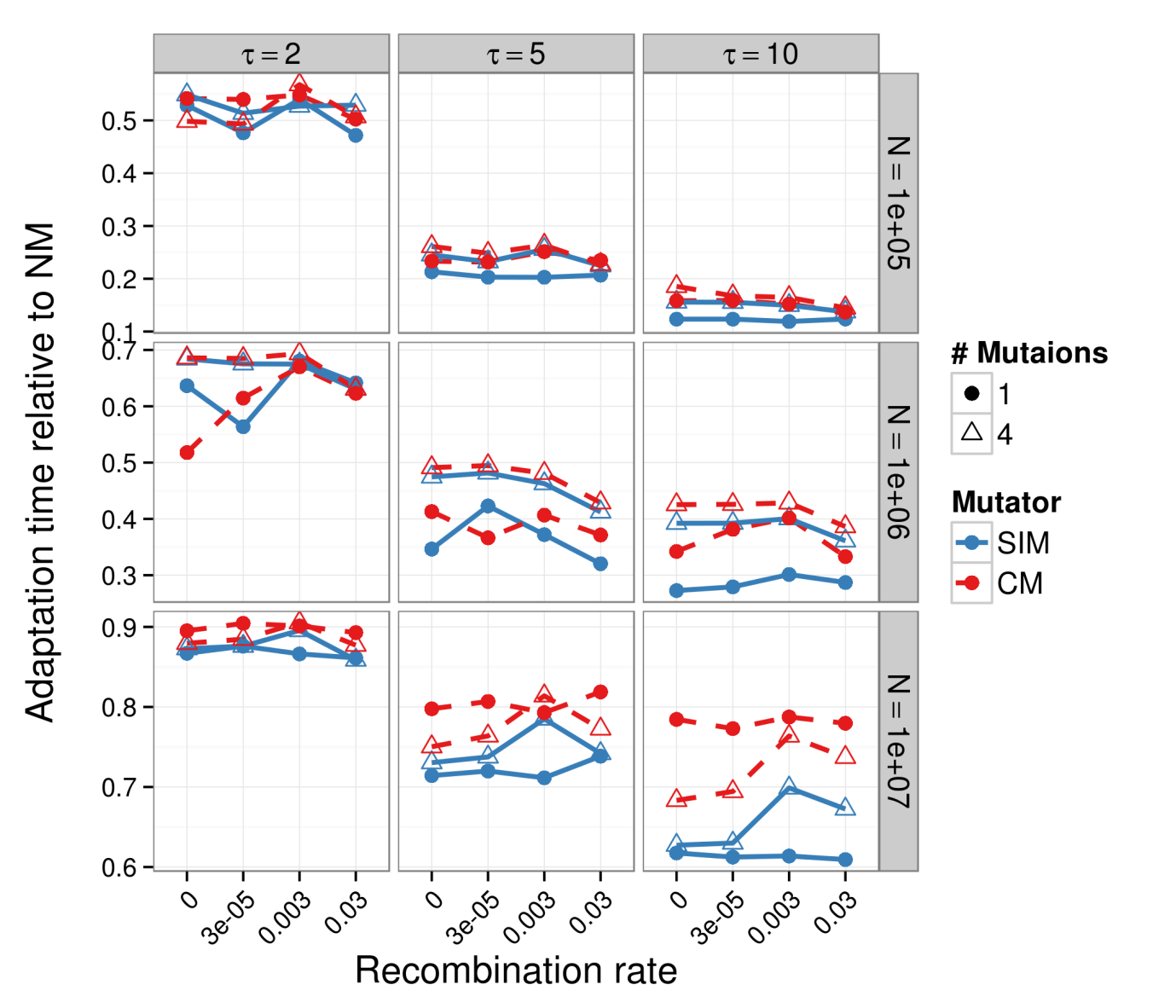


Figure ’ - adaptation\_NR\_2014-05-11

# Discussion

The consequences of stress-induced mutation and recombination go well beyond discussions of evolutionary theory and population genetics. Pathogens have been shown to acquire drug-resistance via stress-induced mechanisms (Cirz and Romesberg 2007; Cirz et al. 2005), and both stress-induced mutation and recombination have important implications for the design of antibiotic treatment strategies (Obolski and Hadany 2012) and pesticide application (Gressel 2011). Virulence (Ubeda et al. 2005)? Similarly, chemotherapy and radiation are predicted to induce mutagenesis in cancer cells, thereby promoting the acquisition of drug-resistance, cancer progression and metastasis (Podlaha et al. 2012).

The co-evolution of the rates of mutation and recombination has received some remarkable theoretical treatment. Using computer simulations, Tenaillon *et al.* (2000) studied the evolution of mutator alleles in a population adapting to a new environment which requires a combination of several beneficial mutations for optimal adaptation. They have shown that "*even rare genetic exchanges can lead to a large decrease in the probability of mutator fixation*". They suggest that the "Hill-Robertson effect" doesn't play an important role in this decrease and that the acceleration of adaptation rate by the "Fisher-Muller effect" plays a more significant role than the prevention of the "hitch-hiking effect".

A different approach was taken by Levin and Cornejo (2009). They used computer simulations to show that recombination increases the rate of adaptation in bacterial populations and that recombining populations have a selective advantage over non-recombining populations. However, they found that this advantage is reduced if non-recombining individuals cannot serve as donors and their frequency is greater than that of recombining individuals. The same occurs when the population density is too low, because in order to recombine an individual must make physical contact with a donor or a viable DNA strand from a donor. Furthermore, they demonstrated that the advantage of recombination depends on the amount of genetic variation available in the population, because the effect of recombination on the adaptation rate depends on the availability of genetic variants that it can shuffle - the "Fisher-Muller effect".

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Cost of fidelity – in competitions between CM and SIM this could have changed the picture, giving the CM a better fitness in the constant environment regime

//Nevertheless, in a previous study we have shown that if mutation rates are increased in individuals with below average fitness and reduced in individuals with above average fitness, then the mutation load actually decreases (Ram and Hadany 2012). //??to discussion??

//Barton 2007 summarizes from Maynard Smith & High – hitch-hiking effect - that "the effect of a sweep on variability is dependent on the ratio r/s; this must be substantially less than 1 for there to be much of an effect".//

Recombination occurs by bacterial transformation (Avery 1944; Redfield 1988). This mechanism allows individual cells to recombine foreign DNA into their genome. Of the three known mechanisms for bacterial horizontal gene transfer (the other two being conjugation and transduction), transformation is the only one actively regulated by the cells and the most likely to have evolved due to its adaptive properties (Redfield 1993; Redfield, Schrag, and Dean 1997; Tenaillon et al. 2001; Matic, Taddei, and Radman 1996).

Stress-induced recombination has been documented in bacteria (Beaber, Hochhut, and Waldor 2004; Prudhomme et al. 2006; Maiques et al. 2006), yeast (Abdullah and Borts 2001), plants (Lucht et al. 2002; Yao and Kovalchuk 2011), and flies (Parsons 1988; Zhong and Priest 2010; Tedman-Aucoin and Agrawal 2012).

stress-induced combined modifiers, which modify both the mutation and the recombination rate, provide the optimal strategy in comparison with other evolutionary strategies studied here (see Table 1 for a summary of strategies used in this study). This last finding supports empirical evidence that recombination is induced by stress (see references above), and that mutation is correlated with recombination and regulated by the same mechanisms (Torkelson et al. 1997; Velkov 1999; Bull et al. 2000).

# References

# Figures

# Tables

Table 1. Evolutionary strategies summary

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Strategy | Abbr. | *π* | *τ* | *ϕ* | *ρ* | Mutation rate | | | Recombination rate | | |
| x<π | | x≥π | x<*ϕ* | | x≥*ϕ* |
| Wild-type | WT | 1 | 1 | 1 | 1 | *µ* | *µ* | | *r* | *r* | |
| Constitutive mutator | CM | 0 | >1 | >1 | 1 | *τµ* | *τµ* | | *r* | *r* | |
| Constitutive recombinator | CR | 0 | 1 | 1 | >1 | *µ* | *µ* | | *ρr* | *ρr* | |
| Constitutive mutator & recombinator | CMR | 0 | >1 | >1 | >1 | *τµ* | *τµ* | | *ρr* | *ρr* | |
| Stress-induced mutator | SIM | >0 | >1 | >1 | 1 | *µ* | | *τµ* | *r* | *r* | |
| Stress-induced recombinator | SIR | >0 | 1 | 1 | >1 | *µ* | *µ* | | *r* | | *ρr* |
| Stress-induced mutator & recombinator | SIMR | >0 | >1 | >1 | >1 | *µ* | | *τµ* | *r* | | *ρr* |

**Legend:** *µ* - basal mutation rate; *r* – basal recombination rate; *x* – number of mismatches; *π* – mutation rate threshold; *τ* – mutation rate increase; *ϕ* – recombination rate threshold*; ρ* – recombination rate increase.

# Supporting material



Figure S - invasion\_SIMvsCM\_r\_02014-02-26



Figure - invasion\_RB\_SIMvsCM\_pop\_1e6\_2014-03-09