The evolution of stress-induced hypermutation 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 cost 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 long-term effect. Third, Lynch proposed the "drift barrier" hypothesis (Lynch 2011). Although this hypothesis is much broader, it also suggests that DNA repair proteins that are only expressed during stress will be under weaker selection compared to proteins that are constitutively expressed, and will therefore become more error prone due to genetic drift.

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

In a previous work we have addressed the likelihood of the adaptive hypothesis in asexual populations (Ram and Hadany 2012). We studied the evolution of mutator alleles that induce an increased mutation rate when maladapted to its environment in constant and changing environments. We demonstrated that these 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 gene transfer (HGT), using three mechanisms: transformation, in which cells recombine foreign DNA into their genome (Avery, Macleod, and McCarty 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, perform sex 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 three problems with 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" in a process named "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 [REF: Hartfield? Weissman?].

These problems are mitigated by recombination. Recombination reduces "clonal interference" by combining co-occurring beneficial mutations (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) [REF Hartfield? Weissman?].

The fate of a mutator – an allele that increases the mutation rate – depends on the mutations it generates. A mutator can "hitch-hike" to high frequencies with the beneficial mutations it generates in a process called "selective sweep" or "genetic hitch-hiking" (Maynard Smith and Haigh 1974; Taddei et al. 1997; Charlesworth 2007). However, a mutator may already have several deleterious mutations in its genetic background when a beneficial mutation is generated 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 the mutations it generates, 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 the beneficial mutations it generated, 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 is 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 the environment does not change frequently; (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 use stochastic Wright-Fisher simulations to model finite populations undergoing selection, mutation, recombination, and random genetic drift in changing environments.

In our model, the genome of every individual contains 1,000 fitness loci and four modifier loci. We keep track of how many individuals have each genome.

The fitness loci are bi-allelic - the alleles are marked by 0 and 1. The fitness of an individual is calculated by comparing the alleles at these fitness loci with the environmental optimal genome: if *x* is the number of loci in mismatch with the optimal genome, then the fitness of this individual is (1-*s*)*x*, with *s* as the selection coefficient. To model environmental changes, the optimal genome is changed by modifying the allele at several loci. The number of loci changed in each environmental change and probability that an environmental change will occur at a specific generation 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 in the number of mutations (random allele switching between 0 and 1) per individual per generation. If the 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 number of allele conversions per genome per generation; the threshold 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 description.

We start each simulation with a mutation-free population – all individuals have the optimal genome and continue with the simulation for a predefined number of generations. We model drift by sampling the number of individuals with each genome from a multinomial distribution determined by the fraction of individuals with each genome in this generation. Selection is model similarly, except that we multiply the fraction of individuals with each genome by the fitness of these individuals. Mutation and recombination are modeled by randomly drawing the number of events from a Poisson distribution with the mutation/recombination rate as the mean of the distribution, the randomly choosing the loci that are about to be changed from a uniform distribution (only fitness loci are subject to mutation and recombination), and finally choosing the new allele in the changed positions: mutation the current allele to the alternative one; 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, pandas, and Cython packages.

# Results

We simulated competitions between different strategies (see Table 1) in populations of bacteria experiencing changing environments. We estimated the fixation probability of such mutators from the fraction of competitions in which a mutator reached fixation in the population, that is, 100% of the individuals had the same mutator allele. If an allele is not affected by selection, it is neutral and will have a fixation probability that is equal to its frequency at the beginning of the simulation (50%). In contrast, if an allele is favored or disfavored by selection it will have a fixation probability that is significantly higher or lower than the initial frequency, respectively. Therefore, we tested whether the estimated fixation probabilities are significantly higher than 50% using a proportions test.

## Constitutive mutators

As shown before (Ram and Hadany 2012), constitutive mutators (CM) which increase the mutation rate τ-fold regardless of their condition, can fix in non-recombining )NR( populations undergoing rapid environmental changes, but not in populations which experience frequent recombination or slowly changing environments (Fig. 1). Selection, therefore, favors CM only if the environment changes very fast and mutation is stronger than recombination.

We hypothesized that the advantage of mutators decreases in recombining populations because recombination separates the mutators from the beneficial mutations they generate, that is, because recombination interferes with "genetic hitch-hiking" (Maynard Smith and Haigh 1974). To test this hypothesis, we used simulations with "recombination barriers", which prevent the transfer of DNA between individuals with different mutators, and thus prevent recombination from interfering with "hitch-hiking". The results agreed with our hypothesis: with "recombination barriers" the fixation probability of CM remained high even when the recombination rate of the population was high (Fig. 1b?).

## Stress-induced mutators

Next, we introduce stress-induced mutators (SIM). These mutators are alleles that induce increased mutation rates in response to stress. Our results show that if the recombination to mutation ratio is lower than one, SIM is favored over WT by selection (Fig. 2). Moreover, it is also favored over CM (Fig. 3), even when the environment is rapidly changing and CM is advantageous over WT. However, if recombination and mutation rates are equal, WT, and in some cases even CM, is favored over SIM. Again, we tested if the significant effect recombination is due to the interference with the "hitch-hiking" effect. With "recombination barriers" SIM is favored over WT and CM even when the recombination is as strong as mutation (Figs. 2b, 3b).

These results show that a regulated mutation rate is favored over a constant mutation rate by selection even in populations with recombination, as long as recombination is not too strong or does not occur between different mutators.

## Stress-induced recombination

# 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 this has an important implication 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 hypermutation in cancer cells, thereby promoting the acquisition of drug-resistance, cancer progression and metastasis (Podlaha et al. 2012). //to discussion?//

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, Macleod, and McCarty 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.