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 many years the distribution of mutations was thought to be uniform, both in time and in the population (Luria and Delbrück 1943). However, starting at the end of the 1970's (Radman 1975), experiments with microorganisms gradually revealed that various stress responses induce a state of hypermutation 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 was referred to as "stress-induced hypermutation", "stress-induced mutagenesis", "adaptive mutation", "stationary-phase mutagenesis" or "condition-dependent mutation". Because mutations are a source of genetic variation in bacterial populations, it was suggested that stress-induced hypermutation 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).

This adaptive point-of-view was challenged by non-adaptive explanations. First, it was suggested that hypermutation is a by-product of stress and that mutations persist during stress due to lack of energy and resources which are needed to repair them (Tenaillon, Denamur, and Matic 2004). Second, because there is an inherent cost for high-fidelity DNA replication, it was suggested that an optimal mutation rate will balance between this cost of fidelity and the cost incurred by 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, recently Lynch proposed his "Drift-barrier" hypothesis (Lynch 2011). Although this hypothesis is much broader, it also suggests that enzymes that are only expressed during stress responses will be under weaker selection compared to enzymes 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 plausibility of the adaptive hypothesis is asexual populations (Ram and Hadany 2012). We studied the evolution of an allele which induces an increased mutation rate when maladapted to its environment using population genetics models with both constant and changing environments. We demonstrated that these stress-induced mutators can be expected to evolve in a wide range of parameters because they are favored by selection over mutators 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 virus carry off DNA segments with them when infecting new cells (Zinder and Lederberg 1952). Yeast perform sex when two cells in 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), and can lead to a "mutational meltdown" in a process named "Muller's ratchet" (Muller 1964; Haigh 1978; Lynch et al. 1993). These problems are mitigated by recombination. By combining co-occuring beneficial mutations recombination reduces "clonal interference" (Martens and Hallatschek 2011) and the waiting time for the production of an adapted genotype (Christiansen et al. 1998) – this has been called 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 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 any beneficial mutations it generated, it can pass those beneficial mutations to the competition – 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) hypermutation is favored by selection if environmental changes are frequent and recombination is weaker than mutation; (ii) when hypermutation is 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, hypermutation is still favored in rapidly changing environments, and stress-induced mutators are still favored over constitutive mutators.

# Model

We use stochastic individual-based simulations to model finite populations undergoing selection, mutation, recombination, and random genetic drift in changing environments. The simulations' source code is available at *http://proevolutionsimulation.googlecode.com*.

Our model is based on a Moran process (Otto and Day 2007, 581). Each simulation step is defined by five stages. At the first stage, which simulates genetic drift, a random individual is removed from the population. At the next stage, a random individual reproduces. The choice of an individual for reproduction is biased so that individuals with higher fitness have a higher probability to be chosen for reproduction. Thus, this stage simulates natural selection. At the next stage the new born individual mutates. The number of new mutations is Poisson distributed and mutations are uniformly distributed across a circular genome composed of 1,000 loci. At the recombination stage, which occurs after the mutation stage, the new born individual may transform its genome with a fragment of DNA from the genomes of recently removed individuals. The number of transformation events is drawn from a Poisson distribution and the length of the incorporated DNA fragment, as well as the position of the crossover, are uniformly distributed. At the final stage of each simulation step, environmental changes randomly occur with a constant probability, changing the identity of the favorable allele in four loci. Harmful alleles, as opposed to favorable alleles, reduce the fitness of individuals carrying them and decrease their chances to reproduce. The fitness of an individual is, where *x* is the number of harmful alleles the individual carries and *s* is the selection coefficient.

When a mutation occurs in a locus occupied with a favorable allele, it will change it to a harmful allele. However, when a mutation occurs in a locus occupied with a harmful allele, it has a probability β to change it to a favorable allele - β is the beneficial to deleterious mutation ratio.

The mutation rate and the recombination rate of an individual are each determined by three factors (see Table 1): First, there is a basal rate common for all individuals in the population. The basal mutation rate, following Drake (1991), is μ=0.003 mutations per individual per replication. The basal recombination rate *r* takes one of three different values: 0, 0.000075, 0.03 and 1.5 gene conversions per individuals per generation (matching to 0, 0.0000001, 0.00006, or 0.003 crossovers per individual per replication). The recombination to mutation ratio χ is therefore 0, 10 and 500. For comparison, the recombination to mutation ratios of *E. coli* and *H. pylori* are 1/60 (Milkman and Bridges 1990) and one (Falush et al. 2001), respectively. The simulations which use χ*=r*=0 are used for comparison with non-recombining (asexual) populations (Ram and Hadany 2012).

The second factor determining the mutation and recombination rates is the stress sensitivity thresholds π and φ, defined by the number of harmful alleles needed to induce a state of hypermutation or hyper-recombination, respectively. These thresholds are each determined by a separate modifier locus, and are therefore genetically inherited. They are not subject to mutation, however.

Third, each rate has a locus that determines the increase that is induced upon entering the hypermutation or hyper-recombination state. The mutation rate //fold-//increase is marked by *τ* and the recombination rate //fold-//increase is marked by *ρ*. These loci are also genetically inherited and are not affected by mutation.

Note that when an environmental change occurs most individuals will have four harmful alleles, so beneficial mutations could only occur in four of 1,000 loci. Therefore, even when β=1/2, the effective beneficial mutation rate will be (see Table 1 in Gordo, Perfeito, and Sousa 2011).

# Results

We simulated competitions between different mutators in populations of 100,000 bacteria experiencing different rates of recombination and 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* | *ρ* | Mutation rate | | Transformation rate | |
| *x<π* | *x≥π* | *x< π* | *x≥π* |
| Wild-type | WT | ∞ | 1 | 1 | *µ* | | *r* | |
| Constitutive mutator | CM | 0 | >1 | 1 | *τµ* | | *r* | |
| Constitutive recombinator | CR | 0 | 1 | >1 | *µ* | | *ρr* | |
| Constitutive mutator & recombinator | CMR | 0 | >1 | >1 | *τµ* | | *ρr* | |
| Stress-induced mutator | SIM | >0 | >1 | 1 | *µ* | *τµ* | *r* | |
| Stress-induced recombinator | SIR | >0 | 1 | >1 | *µ* | | *r* | *ρr* |
| Stress-induced mutator & recombinator | SIMR | >0 | >1 | >1 | *µ* | *τµ* | *r* | *ρr* |

**Legend:** *µ* - basal mutation rate; *r* – basal transformation rate; *x* – number of harmful alleles; *π* – stress sensitivity threshold; *τ* – mutation rate increase; *ρ* – transformation rate increase.