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PhD thesis

­­­­­The Evolution of Stress-Induced Mutagenesis: Causes and Consequences

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I dedicate this thesis to my grandfather, Eng. Herbert Zvi Littman.

# Abstract

Empirical studies show that in bacteria and eukaryotes, stress can induce a state of mutagenesis – a temporary increase in mutation rates. However, theoretical treatment of this phenomenon is lacking.

Using mathematical models and computer simulations, I have developed a theoretical basis to explain the evolution of stress-induced mutagenesis. My results show that (i) stress-induced mutagenesis is favored by selection under both changing and constant environments due to the beneficial mutations it generates; (ii) stress-induced mutagenesis increases the ability of populations to adapt to new conditions without jeopardizing their ability to remain adapted to stable environments.

In addition, I developed a new probabilistic approach to analyze the probability that a random mutation leads to an improved phenotype in Fisher's geometric model, a widely used model of adaptive evolution.

Because mutation is a fundamental evolutionary force, my PhD research has important significance to various aspects of biology. Most importantly, my research makes an important theoretical contribution to our understanding that mutation is more likely to occur in individuals who are mal-adapted to their environments and therefore are more likely to benefit from it.

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Access this thesis online at <https://github.com/yoavram/thesis>.

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Nothing in Evolution Makes Sense Except in Light of Population Genetics.

– Lynch, PNAS 2007

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# Introduction

## The evolution of the mutation rate

The evolution of the mutation rate is a long-standing problem in evolutionary biology, dating back to an article by Sturtevant [1] that suggested there are *“genes that affect general mutation rate”* (mutator alleles) and that *“in wild populations, such genes must be subject to selection”*. But how does selection affect these mutator alleles? And how do other evolutionary forces – mutation, recombination, and genetic drift – affect the evolutionary fate of mutator alleles? The answers to these questions depend on a number of evolutionary and ecological factors.

An optimal mutation rate balances between *adaptedness*, the ability to retain existing adaptations, and *adaptability*, the ability to produce new adaptations [2]. The distribution of mutational effects [3] is therefore an important factor for the evolution of the mutation rate. Briefly, in well-adapted populations beneficial mutations might be very rare and therefore selection will favor the reduction of the mutation rate [4]. In mal-adapted populations, on the other hand, beneficial mutations could be much more common. Mutator alleles can then “hitch-hike” [5] with the beneficial mutations they generate and reach high frequencies [6–8].

Kimura [9] suggested two hypotheses for the evolutionary adjustment of the mutation rate: (i) the mutation rate is optimized by intra-group selection to minimize the genetic and substitutional loads (respectively, the fitness costs due to the accumulation of deleterious mutations, and due to the elimination of mal-adapted individuals during the fixation of a well-adapted genotype), and (ii) most mutations are deleterious, and therefore the mutation rate is at the lowest level allowed by physical and physiological constraints. Leigh [2, 10] used an analytical model to demonstrate that in asexual populations evolving in a periodically changing environment, inter-group selection may favor mutator alleles due to the increased standing variation they generate, which allows rapid adaptation to environmental changes. Also, Leigh found that the optimal mutation rate in asexual populations is equal to the frequency of environmental change [see also 11, 12]. In sexual populations, however, selection will act against alleles that increase the mutation rate [10].

More recently, Lynch [13, 14] framed the *drift barrier hypothesis*. This hypothesis suggests that the reduction of the mutation rate is limited by the effective population size rather than by physical constraints: when the mutation rate is low enough, selection towards further decreases is too weak to overcome random genetic drift in small populations. Other authors explored a model in which any reduction of the mutation rate entails a reduction in fitness owing to a *cost of DNA replication fidelity* [15, 16]. This cost may be due to the resources and energy required to proof and repair replication errors, or the lower replication rate of high fidelity DNA polymerases. Under this model, the mutation rate has an optimal value which, in asexual populations, depends only on the relationship between the mutation rate and the *cost of fidelity*. In sexual populations this optimal value also depends on the magnitude of selection [15].

To summarize, the evolution of the mutation rate depends on a variety of population and environmental factors (reviewed by [17–19]; Figure 1). First, mutator alleles are subject to indirect negative and positive selection due to the deleterious and beneficial mutations they generate. The overall direction and magnitude of selection varies for different populations, environmental conditions, and molecular constraints. Second, mutator alleles are also under direct selection due to the *cost of fidelity*. Third, genetic drift affects the fate of mutator alleles by limiting the influence of direct and indirect selection and by driving the accumulation of deleterious mutations in small populations, which can lead to the extinction of mutator alleles [20]. Fourth, *clonal interference* reduces the adaptation rate of asexual populations and reduces the indirect selection for mutator alleles due to the beneficial mutations they generate [21]. Lastly, complex fitness landscape [22, 23] and co-evolution of different species [24] can have an important role in determining the evolutionary fate of mutator alleles.



Figure 1. Evolutionary forces involved in the evolution of mutator alleles. If we consider the optimal mutation rate rather than *adaptability*, drift is not considered.

## Stress-induced mutagenesis

During the beginning of the 21st century it became increasingly clear that *"various types of stresses induce responses that have mutagenic consequences"* [25] and that *"the same cellular stress responses long appreciated to shore-up damaged cellular hardware (other than DNA) can, surprisingly and importantly, also remodel genomic software (DNA) by increasing rates of random mutagenesis"* [26]. Research with laboratory strains of *Escherichia coli* has uncovered some of the mechanisms which underlie stress-induced mutagenesis [27–30]. This phenomenon is common in many bacterial species [26, 31–34], and evidence suggests that mutations are more common in stressed yeast [35], algae [36], flies [37], and even human cancer cells experiencing hypoxia stress [38, 39].

Several explanations for the evolutionary origin of stress-induced mutagenesis were proposed, and they can be roughly divided to adaptive and non-adaptive hypotheses [14, 17, 19, 40–42]: (i) the *adaptive hypothesis*, or *second-order selection hypothesis*, suggests that mutagenesis in times of stress is favored by natural selection because it increases variation and allows faster adaptation; (ii) non-adaptive hypotheses suggest that mutagenesis is an inevitable by-product of stress that is caused by lack of energy and resources needed to maintain replication fidelity or by some other causes, such as random genetic drift [43]. Non-adaptive hypotheses have received theoretical treatment [14, 44, 45] but, until now, the adaptive hypothesis has not been studied using population genetics models. Such models can determine if a mutator allele that induces elevated mutation rates in response to mal-adaptation can increase in frequency by indirect selection on the mutations it generates.

The consequences of stress-induced mutagenesis are largely unknown. Agrawal [46] studied a model of fitness-dependent mutation rates (FDMR) in an infinite population and showed that FDMR increases the *two-fold cost of sex* [47]. His model was later extended for finite populations [48], in which the correlation between the mutation rate and fitness was shown to slow or stop *Muller's Ratchet* [49], thus helping to explain how asexual populations cope with deleterious mutations to avoid mutational meltdown. Other outcomes of a plastic mutation rate could span a variety of evolutionary, epidemiological, and ecological scenarios: the evolution of drug-resistance in bacteria [50, 51], the evolution of cancer cells and the emergence of chemotherapeutic-resistance [38, 39, 52], the evolution of pesticide resistance in commercial crops [53], industrial applications using bacteria in stressful environments [54–56], host-parasite co-evolution [24, 57, 58], and the evolution of pathogen virulence [59–61]. More importantly, because the mutation rate is a fundamental element of population genetics models and theories [62], the basic notion that it is plastic rather than constant has a huge importance to our understanding of evolution and biology.

## Research objectives

Since the famous work of Luria and Delbrück (1943), evolutionary and ecological models assume that the mutation rate is constant and uniform. However, an alternative assumption, that the mutation rate is plastic and that stress induces elevated mutation rates, can lead to remarkably different results and conclusions. Furthermore, the lack of theory and applicable models contributes to the slow adoption of these ideas by evolutionary biologists and geneticists, as well as researchers in the medical sciences.

In light of this, the objectives of my PhD thesis are:

1. Explain the evolution of stress-induced mutagenesis in asexual populations:
   1. In constant and changing environments
   2. On smooth and rugged fitness landscapes
2. Explore the effect of stress-induced mutagenesis:
   1. On adaptation
   2. On the evolution of complex traits

## Thesis overview

During my PhD I have authored three peer-reviewed manuscripts on stress-induced mutagenesis and the evolution of the mutation rate.

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

In the first peer-reviewed manuscript [64] I have provided theoretical support for the *adaptive hypothesis*: I have shown that stress-induced mutagenesis is favored by natural selection due to the beneficial mutations it generates specifically in individuals that need them the most. Using a deterministic model, I analysed the evolution of asexual populations in a constant environment in which mutations are either deleterious or compensatory (*e.g.* compensating for existing deleterious mutations). I have shown that increasing the mutation rate in individuals with below average fitness increases the mean fitness of the population, but only if compensatory mutations are possible. This is a new and surprising result: selection is usually predicted to reduce the mutation rate rather than increases it [4]. Using stochastic computer simulations, I analysed evolution of asexual populations in a changing environment and considered models in which alleles for different mutational strategies compete against each other. Stress-induced mutator alleles, which induce increased mutation rates in mal-adapted individuals, were highly successful in competitions with non-mutator alleles and constitutive mutator alleles that induce a constant low and high mutation rate, respectively. Populations with stress-induced mutator alleles also had a higher mean fitness than populations with non-mutator or constitutive mutator alleles. Therefore, I concluded that stress-induced mutagenesis is likely to evolve in asexual populations because it is favored by natural selection, both in constant and changing environments. This is in contrast to constitutive mutagenesis, which can only be favored in rapidly changing environments, and is always selected against in a constant environment [65].

### Stress-induced mutagenesis and complex adaptation

In my second peer-reviewed manuscript [66] I studied the consequences of stress-induced mutagenesis on the evolution of complex traits. Complex traits require two or more mutations that are beneficial together but separately deleterious. The evolution of complex traits is an open question in evolutionary biology for over 80 years [67, 68], as it is not clear how individuals can accumulate the required mutations if each of them is deleterious on its own.

I have used mathematical analysis and computer simulations to estimate how stress-induced mutagenesis increases the rate of complex adaptation in comparison to normal mutagenesis and constitutive mutagenesis. Combining these estimates with estimates of population mean fitness in a constant environment, I have demonstrated that stress-induced mutagenesis is the most efficient mutational strategy, as it breaks the classical trade-off between *adaptability*, the ability to adapt to new conditions, and *adaptedness*, the ability to remain adapted to current conditions [10]. These results provide the first estimates of the effect of stress-induced mutagenesis on adaptation.

### The probability of improvement in Fisher's geometric model

Fisher's geometric model is a widely used model of adaptive evolution [69]. In my third published manuscript [70], I developed a new probabilistic approach for determining the probability that a random mutation leads to an improved phenotype in Fisher's geometric model. This new approach opens new opportunities for understanding and analysing Fisher's model because it provides an alternative interpretation of the relationship between the probability for a beneficial mutation and the model parameters: the effect size of the mutation, the number of traits affected by the mutation, and the distance from the current phenotype to the optimal one.

My previous research suggests that stress-induced mutagenesis is very sensitive to the probability that a mutation is beneficial; indeed, the advantage of stress-induced mutator alleles in a constant environment disappears if compensatory (beneficial) mutations are not possible [64], and the rate of adaptive evolution is an increasing function of the probability that a mutation is beneficial [66]. My new approach for determining the probability that a mutation is beneficial is therefore a step towards understanding the evolutionary origin of stress-induced mutagenesis.

## Methods overview

Next, I will describe theory-oriented methods which I used during my PhD research.

### Individual-based simulations

Individual-based simulations (also called *agent-based simulations*) consist of populations of individuals and a set of rules determining their life-cycle and interactions. These rules often include a random element. Emerging population dynamics are then studied using repeated runs of the simulations for different sets of parameters and by applying statistical analysis to determine the significance of the results.

To study competitions between different mutator alleles [64], I developed a Java open source framework for individual-based simulations [71] ([https://github.com/yoavram/proevolutionsimulation](https://github.com/yoavram/proevolutionsimulation/tree/charles)). This software was later used in a separate manuscript that I co-authored [72]. The software allows the definition of life-cycle rules necessary to model natural and sexual selection, mutation, recombination, sexual reproduction, migration, and random genetic drift. I performed over 100,000 simulation runs on the Hadany computer cluster.

### Wright-Fisher models

The *Wright-Fisher model* is a standard population genetic model [73]. It is used to describe the change in allele frequency from generation to generation (with non-overlapping generations) and can include the effects of natural selection, mutation, random genetic drift, recombination, and migration. In its simplest form, the Wright-Fisher model follows the frequency *p* of an allele of interest that has an adaptive advantage *s* over the rest of the alleles:

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where *p'* is the frequency of the allele of interest in the next generation. This example only includes natural selection. If the model includes more genotypes of interest, then it is described by a system of similar equations.

Wright-Fisher models can be studied using mathematical or computational analysis. With mathematical analysis, one attempts to find or approximate a solution to the set of equations defined by the model. In computational analysis, one attempts to approximate or estimate a solution by calculating the set of equations for specific parameter values. It is common to include random fluctuations to model the effect of random genetic drift; in this case, the analysis produces random samples from the solution distribution, and further statistical analysis is required.

The mutation-selection balance is the equilibrium of a model that only includes natural selection and mutation. At the mutation-selection balance, the expected allele frequencies do not change from generation to generation. I studied the mean fitness of populations with different mutational strategies at the mutation selection balance [64, 66] using eigenvalue analysis and found an analytic expression for the relation between the population mean fitness and the rate of mutation of individuals with *x* deleterious mutations [64]. I have also written numerical procedures for calculating the expected mean fitness of a population with a specific mutational strategy, given the basal mutation rate, the selection coefficient against deleterious mutations, and the rate of compensatory mutations (see supplementary at <https://github.com/yoavram/ruggedsim/blob/master/manuscript/supplementry.ipynb>).

Focusing on adaptive evolution, I have used a Wright-Fisher model in combination with a Branching process (see below) for estimating the adaptation rate with different mutational strategies. The model was used to estimate the waiting time for the appearance of an adaptive genotype that goes to fixation [66].

To validate the results of this mathematical analysis, I developed a software implementation of a Wright-Fisher model with selection, mutation, and random genetic drift (<https://github.com/yoavram/ruggedsim/tree/master/stochastic>). Over 100,000 simulation runs were performed on the Hadany computer cluster and all data relevant to the publication [66] was deposited on dryad [74].

### Branching processes

Branching processes are stochastic models that study the random extinction and fixation of different types in a population [75]. I used branching processes to calculate the fixation probability of beneficial mutations [76, 77], a crucial step in calculating the rate of adaptation.

### Probability theory

I developed a probabilistic approach to study the probability of improvement in Fisher's geometric model [70]. A geometric approach has been previously published by several authors [78, 79], albeit not by Fisher himself [69]. However, using probabilistic arguments I reached the same solution without applying geometric arguments. The analysis was implemented in Python and can be viewed at <https://mybinder.org/repo/yoavram/FGMProb> by clicking on the *Expected improvement.ipynb*.

# Discussion

I studied the evolution of stress-induced mutagenesis – the increase of mutation rate in response to stress and mal-adaptation. First, I have used population genetics models to show that stress-induced mutagenesis can be favored by natural selection in asexual populations due to the beneficial mutations it generates [64, 66]. Second, I have shown that stress-induced mutagenesis increases the rate of adaptation and the mean fitness of adapting populations without jeopardizing the fitness of well-adapted populations in a stable environment [64, 66]. Third, I have developed a new method for determining the probability that a mutation is beneficial, a crucial constraint on the evolution of stress-induced mutagenesis [70].

Despite evidence that stress-induced mutagenesis is a common mechanism in many species of bacteria [25, 26, 80] as well as in yeast [35], many authors doubted (i) if stress-induced mutagenesis can have a significant adaptive advantage, (ii) if such an advantage can lead to its evolution and maintenance in natural populations, and (iii) if stress-induced mutagenesis has an important role in evolution [41, 42, 81–83].

My results show, for the first time, that stress-induced mutagenesis can have an evolutionary advantage over constant mutation rates, low or high. Moreover, my results show that this evolutionary advantage is due to stress-induced mutagenesis breaking the trade-off between *adaptability* (the ability to adapt) and *adaptedness* (the ability to remain adapted), allowing rapid adaptation to environmental challenges without compromising the population mean fitness in a stable environment.

Classical theory predicts that in asexual populations with constant mutation rates the mean fitness can be estimated by *e-U*, which is a decreasing function of the mutation rate *U*, and that selection acts to reduce the mutation rate [84]. This effect has been referred to as the *Reduction Principle* [4, 85]. My results show natural selection only acts to reduce the mutation rates of individuals with above average fitness. In contrast, selection acts to increase the mutation rate of individuals with below average fitness, even in a constant environment [64].

Several non-adaptive hypotheses exist for the evolution of stress-induced mutagenesis. It has been suggested that decreasing the mutation rate carries a cost, called *the cost of DNA replication fidelity*. Such a cost may arise from the extra energy, time, and resources required by the DNA replication and proofing systems [15, 17]. Increasing the mutation rate during stress can therefore directly increase the fitness of the individual, regardless of beneficial mutations and adaptation. My models do not include a direct cost or benefit for increasing or decreasing the mutation rate. Although the *cost of fidelity* might increase the advantage of stress-induced mutator alleles over non-mutator alleles, it might also increase the success of constitutive mutator alleles in competitions, as these alleles will constitutively benefit from not paying the *cost of fidelity*. However, recent empirical studies suggest that the *cost of fidelity* does not play an important role in the evolution of constitutive mutator alleles in bacteria [29, 60, 86, 87]. Future studies can integrate the *cost of fidelity* into my models to try and find how the *cost of fidelity* affects the evolutionary advantage of stress-induced mutagenesis.

Another cost might be associated with the regulation of the mutation rate: to determine their condition, cells must invest in costly sensory and signaling mechanisms. However, such mechanisms already exist for various unrelated purposes, such as the maintenance of cell cycle and homeostasis. Therefore, I consider these mechanisms as "free", in terms of fitness costs. Indeed, in *E. coli* mutagenesis is induced by several stress responses that serve other cellular functions [25, 88].

My models assume that individuals have perfect information regarding their condition or fitness, so that the mutagenesis response is only induced in mal-adapted or stressed individuals. However, it is more reasonable to assume that such information is only an estimate and that sometimes well-adapted individuals induce mutagenesis, and stressed individuals fail to do so, by mistake. In such cases, an error correction mechanism, based on the population mean fitness or the parent fitness, can be used to mitigate errors in fitness estimation [89].

The *drift barrier hypothesis* [43] offers a different explanation for the evolutionary origin of stress-induced mutagenesis. This hypothesis suggests that stress-induced DNA replication and proofing enzymes are seldom expressed, and therefore experience less selection on their normal function – maintaining high fidelity DNA replication. This reduced selection allows deleterious mutations to accumulate in the genes encoding these enzymes (*e.g.*, DNA polymerases), making them error-prone. Future research could test this hypothesis by estimating the effective population size and the frequency of stress that allow stress-induced mutagenesis to evolve by the accumulation of deleterious mutations in stress-induced DNA polymerases.

Pathogens experience stress during drug treatment and when interacting with host immune systems. Neglecting the effect of these stresses on pathogen mutation rates can lead to incorrect conclusions on the ability of the pathogens to evolve virulence [59, 60] and drug resistance: Obolski and Hadany [51] demonstrated that stress-induced mutagenesis changes the recommended drug administration policy in hospital departments, whereas Cirz an co-workers [34] showed that inhibiting mutagenesis reduces the ability of *Staphylococcus aureus* to evolve drug resistance.

Similar effects of stress, caused by pesticides, are expected to occur in agricultural settings, allowing plant and livestock pathogens to develop resistance and virulence more rapidly than expected [53]. Likewise, microbes are commonly used in industrial applications, from cheese, yogurt, beer, and wine to antibiotics and biofuel production. However, the genetic integrity of industrial strains may be at a larger risk than previously thought, as in most cases microbes are kept in stressful conditions in order to produce the desired products [54].

An important corollary to microbial evolution is the development of cancer by the clonal proliferation of cancer cells [90]. Cancer cells are exposed to different stresses, due to therapy – chemotherapeutic drugs and radiation – as well as the fast growth of the tumor. It has been shown that mutagenesis is induced in cancer cells under hypoxia stress [91], which can lead to mutations that cause durg resistance, tumor progression, and metastasis [92, 93].

## Conclusions

Mutation is a fundamental evolutionary force and therefore affects diverse areas in biology. My research contributes and supports the ongoing shift in our understanding of mutation as a regulated response to mal-adaptation and stress, rather than an inevitable result of biophysical and biochemical processes. My results provide theoretical support to the observation that mutations are more likely to occur in mal-adapted individuals and in stressful environments.

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אוניברסיטת תל-אביב

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עבודת דוקטור

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מאת יואב רם

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