CAUSES AND CONSEQUENCES OF THE EVOLUTION OF MUTATION RATE

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Doctor of Philosophy

By

Bingjun Zhang

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Bingjun Zhang

APPROVED:

Ricardo B. R. Azevedo, Chairman
Department of Biology and Biochemistry

Tim F. Cooper
Department of Biology and Biochemistry

Blaine J. Cole
Department of Biology and Biochemistry

Krešimir Josić
Department of Mathematics

Gábor Balázsi
Laufer Center for Physical
Quantitative Biology at Stony Brook University

Dean, College of Natural Sciences and Mathematics

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Abstract

As the ultimate source of all genetic variation, mutation is required for evolution. The mutation rate measures the rate at which mutations occur over time. How the mutation rate evolves and how it interacts with other evolutionary processes are still far from clearly understood. In this thesis, I employ individual-based simulations and aim to understand how the evolution of mutation rate interacts with other evolutionary forces such as mutation biases, selection for evolvability, genetic drift and the evolution of recombination rate.

In Chapter 3, I studied the role of mutation bias (bias towards high mutation rate) in the evolution of mutation rate, which has been long ignored in the literature. I found that the effect of mutation bias on the evolution of mutation rate is significant when compared to that of other broadly promoted evolutionary forces such as natural selection, mutator hitchhiking, and genetic drift. Even in sexual populations, I found that mutation bias can still operate and drive the evolution of mutation rate. In Chapter 4, when the mutation rate (U) and recombination rate (R) are not allowed to evolve, I found that there exists an optimal mutation rate (U_{opt}), at which a population can achieve their maximal evolvability (E). Populations displayed negative evolvability if U was above a critical value (U_{crit}). Asexual and sexual populations showed similar relationships between E and U. Moreover, increasing R also increased E, U_{opt}, and U_{crit}. In Chapter 5, I found that selection for evolvability cannot optimize U when it is allowed to evolve because U increased without bound — a phenomenon known as mutation rate catastrophe. In addition, the effect of mutation bias is much stronger than the selection for evolvability in sexual populations, indicating the selection for evolvability is not the only force that

can affect the evolution of U and is a weak selection. However, U cannot be optimized when both forces are operating. Lastly, I found that selection can optimize the recombination rate for high evolvability. High R can prevent populations from experiencing the mutation rate catastrophe, although populations do not always evolve high R.

Contents

List of Figures

List of Tables

Chapter 1

General introduction

1.1 The evolutionary importance of mutation rate

Charles Darwin was the first to effectively present that inheritable variation is the ultimate fuel for the process of evolution (Darwin, 1859). Evolution cannot occur without variation. Mutations are changes in the DNA in an organism and introduce variation into populations. As the ultimate source of all genetic variation, mutation is the first step of evolution by creating new alleles and genotypes. It is critical to the evolution of all populations on the planet.

The mutation rate, which is defined as the rate of generation of mutations, plays a central role in several evolutionary processes. Wright (1931) was one of the first to propose that mutation rate is critical for the maintenance of genetic variation. Kimura and Ohta (1971) proposed that the random fixation of neutral

mutations accounts for the polymorphism of proteins and drives their sequence evolution. Charlesworth (1994) had identified three effects of "background selection" on molecular evolution. First, Genetic variation can be reduced significantly due to selection against strong deleterious mutations. Second, meanwhile, the fixation rate of slightly deleterious mutations can be increased through background selection. Third, as for the beneficial mutations, they may not be able to fix in the population due to the linkage disequilibrium with deleterious alleles (the "a ruby-in-the-rubbish" effect) (Peck, 1994). This increased probability of fixation of deleterious mutations and reduced probability of fixation of beneficial mutations, reduces the rate of adaptation (Barton, 1995).

Overall, mutation rate affects several evolutionary processes mentioned above. However, how mutation rate interacts with these evolutionary processes is still far from clearly understood. The evolution of mutation rate plays a critical role in these interactions. Understanding the forces and factors that influence the evolution of mutation rate remains a central challenge for evolutionary biology.

1.2 The molecular mechanisms of the evolution of mutation rate

DNA-based organisms have evolved sophisticated mechanisms that reduce mutation rate by ensuring DNA replication fidelity and repairing DNA damage. Mutations in the genes involved in these mechanisms can result in either increases or decreases in

mutation rate (Miller, 1996; Kunz *et al.*, 1998; Friedberg *et al.*, 1995; Fijalkowska *et al.*, 2012; Herr *et al.*, 2011). Genotypes with elevated or reduced mutation rates are known as *mutators* and *antimutators*, respectively. Take, for example, the gene *mutD*, which encodes the ϵ subunit of DNA polymerase III in *Escherichia coli*. The ϵ subunit is necessary for the exonuclease-associated proofreading activity during DNA replication. Loss-of-function mutations occur in the *mutD* gene disrupt the proofreading activity (Wu and Marinus, 1994; Schaaper, 1993). The defective proofreading function leads to significantly increased mutation rate in *E. coli* and causes a mutator phenotype (Horst *et al.*, 1999). Similarly, mutations (*dam*, *mutH*, *mutS*, and *mutL*) that occur in genes involved in the mismatch repair system, which is the system for recognizing and repairing errors during DNA replication, have also been found to create mutator phenotypes in *E. coli* (Horst *et al.*, 1999).

Mutator phenotypes have been reported in natural populations of many species such as *E. coli* (LeClerc *et al.*, 1996; Matic *et al.*, 1997; Björkholm *et al.*, 2001), *Salmonella enterica* (LeClerc *et al.*, 1996), *Neisseria meningitidis* (Bucci *et al.*, 1999), *Pseudomonas aeruginosa* (Oliver *et al.*, 2000), *Staphylococcus aureus* (Prunier *et al.*, 2003), *Haemophilus influenzae* (Watson *et al.*, 2004), *Streptococcus pneumoniae* (del Campo *et al.*, 2005), *Saccharomyces cerevisiae* (Magni and Borstel, 1962; Esposito and Bruschi, 1993; Strathern *et al.*, 1995; Heidenreich and Wintersberger, 1997), and *Ascobolus immersus* (Paszewski and Surzycki, 1964).

For example, strains of *Streptococcus pneumoniae* with a mutator phenotype have been isolated from patients with Cystic Fibrosis (CF) (del Campo *et al.*, 2005). They found that the proportion of stains with elevated mutation rates to antibiotic

resistance in these isolates (60%) were significantly higher ($P = 0.02$) than the strains isolated from non-CF patients in the same institute (37%).

The evolution of mutation rate has also been observed directly in experimental populations (e.g., Chao and Cox, 1983; Mao *et al.*, 1997; Giraud *et al.*, 2001; Barrick *et al.*, 2009; Loh *et al.*, 2010; Wielgoss *et al.*, 2012). For example, a population of *Escherichia coli* adapting to a constant environment evolved a frameshift mutation in the *mutT* gene at $\sim 2.5 \times 10^4$ generations (Barrick *et al.*, 2009). This mutator mutation increased mutation rate by $\sim$ 150-fold (Wielgoss *et al.*, 2012). Subsequently, the population evolved two antimutator alleles in the *mutY* gene that approximately halved the mutation rate (Wielgoss *et al.*, 2012).

1.3 The evolutionary forces that determines the fate of alleles modifying mutation rate

The fact that mutation rates vary by many orders of magnitude among and within different forms of life, from 10^{-4} to 10^{-11} per base or base pair per generation (Drake *et al.*, 1998; Drake, 2006; Conrad *et al.*, 2011; Hodgkinson and Eyre-Walker, 2011), leads to the question of, what evolutionary forces may influence and shape the fate of alleles modifying mutation rates in different populations?

Sturtevant (1937) was perhaps the first to recognize that the fate of mutator and antimutator alleles is likely to be determined by an interaction between multiple evolutionary forces. He discussed the mutations that affect the general mutation rate

of the organism in 1937, long before the discovery of the structure of DNA and the molecular mechanisms of mutations. Four main forces have since been proposed to drive the evolution of mutation rate.

The first two evolutionary forces involve selection at the individual level. First, direct individual selection. The vast majority of mutations with fitness effects are deleterious mutations (Halligan and Keightley, 2009; Lynch *et al.*, 1999). High-mutation rates are selected against due to the high-mutational load, which is the fitness cost that caused by the accumulation of deleterious mutations. However, Sturtevant was the first asking the question that, why mutation rate does not evolve to zero if low-mutation rates may be selected for (Sturtevant, 1937)? A possible answer to his question was proposed by Kimura (1967) — the so-called "cost of fidelity" hypothesis. The hypothesis suggests that maintaining a low-mutation rate might be physiologically costly in terms of time and energy during DNA replication. The fact that deleterious mutations are occurring constantly in the population makes it impossible to repair all the errors during DNA replication (Drake, 1991). As mutation rate decreases, the cost of fidelity during DNA replication and repair could increase. This results in a fitness cost for individuals maintaining low-mutation rates. Thus, the cost of fidelity may lead to the selection for mutators that increase the general-mutation rate (Kimura, 1967; Dawson, 1998; Sniegowski *et al.*, 2000). Furió *et al.* (2005) showed that vesicular stomatitis virus clones with low-mutation rate had reduced fitness. The general idea of cost of fidelity has been promoted broadly (Kimura, 1967; Kondrashov, 1995; Dawson, 1998; Drake *et al.*, 1998; Sniegowski *et al.*, 2000; André and Godelle, 2006; Baer *et al.*, 2007).

Second, indirect individual selection caused by linkage disequilibrium between a mutation-rate modifier and subsequent mutations with effects on fitness (Sturtevant, 1937; Kimura, 1967; Leigh, 1970; Dawson, 1998; Johnson, 1999a,b; Tenaillon *et al.*, 1999). Selection for beneficial mutations is expected to favor mutators, causing *mutator hitchhiking*. It has been widely believed that the selection and fixation of mutator alleles in asexual populations is the consequences of mutator hitchhiking (Sniegowski *et al.*, 2000). Conversely, selection against deleterious mutations is expected to indirectly favor antimutators.

The hitchhiking hypothesis implies that the beneficial mutations generated by mutators can fix in the population regardless of the excess deleterious mutations carried by the individual in which they arise. However, excessive deleterious mutations may create a large mutational load. As mutation rate increases, mutator alleles may not be fixed in the population due to the mutational load. The disadvantages of excess deleterious mutations are not trivial and should not be ignored. The theory of background selection (see Section 1.1 above), which emphasizes the effects of deleterious mutations, describes the scenarios where new mutations are less likely to be fixed due to selection against linked-deleterious mutations (Charlesworth *et al.*, 1993; Charlesworth, 1994).

Similarly, Peck (1994) studied a mathematical model and found an effect he called "a ruby-in-the-rubbish". In asexual populations, the probability that beneficial mutations are lost is greatly enhanced by deleterious mutations in the background. These studies suggest that the deleterious mutations have negative effects on the fate of mutator alleles and antimutators are expected to be favored by selection.

Other than the indirect selection regarding beneficial or deleterious mutations, recombination is also expected to interfere with this process by breaking up linkage disequilibrium between mutator alleles and the background they arise from (Tenaillon *et al.*, 2000). Another interesting result from Peck (1994) is that the "ruby" may not be affected significantly by the "rubbish" in sexual populations. The deleterious mutations in the background have little effect on the ultimate fate of beneficial mutations. Based on previous investigations, the effects of both types of indirect individual selection on the evolution of mutation rate are still not clearly understood and it is not easy to predict how the dynamics are influenced by linkage disequilibrium.

The third evolutionary force is group selection. For example, Kimura (1967) proposed that populations with high mutation rate may have higher rates of adaptation than populations with low mutation rate because of the increased supply of beneficial mutations. For example, in a long-term evolution experiment with *E. coli*, 3 out of 12 replicate populations evolved a ∼100-fold higher mutation rate within 8.5×10^3 generations. As a result, these 3 populations adapted more quickly than the 6 populations that retained the ancestral mutation rate over the following $\sim 4 \times 10^4$ generations (Wiser *et al.*, 2013). A study on pathogenic *S. enterica* and *E. coli* isolates found a nonsignificant but positive association between mutators and pathogenicity, suggesting a slight advantage of mutators adapting to a rapidly changing environment (LeClerc *et al.*, 1996). However, a later study failed to confirm such an association (Matic *et al.*, 1997). Thus, the existing knowledge does not provide a clear and coherent theory to explain how this type of group selection affects the evolution of mutation rate.

Alternatively, populations with high mutation rate may be more likely to go extinct (Gabriel *et al.*, 1993; Gerrish *et al.*, 2007; Bull *et al.*, 2007; Gerrish *et al.*, 2013). For example, Gerrish *et al.* (2007) reported a phenomenon they called "mutation-rate catastrophe" whereby asexual populations evolve intolerably high mutation rate and eventually go extinct. I will investigate the causes of this phenomenon in Chapter 3.

The fourth and final force is genetic drift (Sturtevant, 1937; Palmer and Lipsitch, 2006; Lynch, 2008, 2011). Lynch (2008, 2011) has shown that, even if natural selection favors antimutators to reduce mutational load, mutation rate cannot be minimized indefinitely in a finite population. As a result, larger populations are expected to evolve lower mutation rates when compared to smaller populations. This prediction is supported by comparative data (Lynch, 2010). Among multiple phylogenetic lineages, the base substitutional mutation rate (u) was found to decrease as the effective population size (N_e) increases (Lynch, 2006; Lynch and Walsh, 2007). A similar pattern has been found for mammalian mitochondrial genomes (Piganeau and Eyre-Walker, 2009). The significant negative correlation between u and N_e is in agreement with the proposed drift hypothesis.

The studies of these four main evolutionary forces provide most of the insights of the evolution of mutation rates. In this dissertation, I systematically study these evolutionary forces with individual-based simulations. Besides the four main evolutionary forces, I studied a new force that has been long neglected in the field, mutational biases toward increased mutation rates. I aim to analyze how multiple evolutionary forces interact and shape the evolution of mutation rate in both asexual and sexual populations. I try to understand the extent to which populations are

able to adjust their mutation rate and recombination rate to achieve a higher rate of adaptation.

Chapter 2

Model and general methods

2.1 Overview

In this study, I employ an individual-based Wright-Fisher model of adaptive evolution. The Wright-Fisher model was developed independently by Sewall Wright (1931) and R. A. Fisher (1930a). Among the several models developed by geneticists, the Wright-Fisher model has become the most widely used stochastic model in population genetics. It was originally proposed as an idealized model for the process of genetic drift. It examines how the frequencies of alleles vary stochastically over time to understand the loss or fixation of alleles of interest, thus allow us to investigate the genes transmission from generation to generation.

While the Wright-Fisher model allows an elegant approach to investigate the underlying dynamics of the evolution of an idealized population, it makes a number

of crucial assumptions :

- Populations have a finite population size (N), which is kept constant during the course of evolution;
- Discrete, non-overlapping generations. This assumes all individuals in the population have the same life expectancy. The reproduction and death of all individuals occur simultaneously and are synchronized among the population.
- Random mating at reproduction;
- Populations have no social or geographical structure.

Many variations of the Wright-Fisher model were developed since it was introduced in the 1930s. The one I developed in this dissertation is an infinite-alleles model with mutation and recombination. In this model, any new mutation at an existing locus creates a new allelic type not observed before. With the assumptions above, I model haploid organisms undergoing a selection-reproduction-mutation life cycle (see Section 2.5 and 2.6 below). Populations evolve in a constant environment and beneficial mutations are never exhausted.

Unless otherwise stated, I used the default parameter values shown in Table 2.1. The default deleterious mutation parameters are comparable to those of the RNA virus $\phi 6$ (Burch *et al.*, 2007).

2.2 Population

I begin by generating a haploid founder individual with multiple loci (see next section). The founder individual is considered free of mutations, and is assigned a genomic mutation rate of U_0, and a recombination rate of R_0 (see Table 2.1). The founder individual is cloned to create a population of N identical individuals.

2.3 Genome

The genome of an individual consists of a linear chromosome containing three different types of loci: L fitness loci, one mutation rate modifier locus, and one recombination rate modifier locus. Loci occupy $L + 2$ evenly spaced positions along the chromosome. The position of each locus in the founder individual is picked at random. The resulting genome organization remains constant throughout the simulation. In asexual populations, the position of loci does not affect the reproduction. In sexual populations, crossovers occur at random positions of the genome (see Section 2.5 below).

The fitness loci determine individual fitness, W (Equation 2.1). The mutation and recombination rate modifier loci determine the mutation and recombination rate, respectively (Equation 2.3); neither modifier locus directly affects individual fitness (Equation 2.1).

2.4 Fitness

The fitness of an individual carrying no mutations is $W = 1$. Mutations act independently and multiplicatively on fitness:

$$W = \prod_{i=1}^{L} \left[\prod_{j=1}^{k_i} (1 + s_{ij}) \right] \quad , \tag{2.1}$$

where L is the number of fitness loci, k_i is the number of mutations at fitness locus i, and s_{ij} is the effect of the jth mutation at the ith locus on fitness. Our model does not incorporate epistasis for fitness.

2.5 Selection and reproduction

To produce the following generation, N haploid individuals are picked at random, with replacement, with probability proportional to their fitness. The N individuals are assigned randomly to $N/2$ pairs, and each pair forms a transient diploid stage. Two haploid offspring are produced by recombining the two parental genomes, such that the number of crossovers between them, x, is assumed to be Poisson distributed with mean R. If $x = 0$, then each parental genome is copied to produce offspring in the following generation. If $x > 0$, then the genomic position of each crossover is chosen randomly, and both recombinant offspring are transferred to the following generation. For simplicity, I refer to $R = 0$ as asexual reproduction, and to $R > 0$ as sexual reproduction.

Table 2.1: Model parameters

Parameter	Description [default value, if applicable]
W	Individual fitness (see Equation 2.1)
$\overline{W}$	Mean fitness of a population
U	Individual mutation rate (see Equation 2.3)
U_0	Mutation rate in the founder [0.1]
$\overline{U}$	Mean mutation rate of a population
R	Individual recombination rate
R_0	Recombination rate in the founder [0.1]
$\overline{R}$	Mean recombination rate of a population
N	Population size [10^4]
L	Number of fitness loci [100]
p_b	Proportion of beneficial mutations [10^{-3}]
p_d	Proportion of deleterious mutations [0.5]
p_m	Proportion of mutator mutations [0.01]
p_a	Proportion of antimutator mutations [10^{-4}]
p_{r+}	Proportion of mutations that increase R [0.01]
p_{r-}	Proportion of mutations that decrease R [0.01]
$U_{m/a/r+/r-}$	Mutation rate of certain allele [$U_0 \times p_{m/a/r+/r-}$]
$\overline{s}_b$	Mean effect of a beneficial mutation [0.03]
$\overline{s}_d$	Mean effect of a deleterious mutation [-0.03]
$\overline{s}_m$	Mean effect of a mutator mutation [0.05]
$\overline{s}_a$	Mean effect of a antimutator mutation [-0.05]
$\overline{s}_{r+}$	Mean effect of a mutation that increases R [0.05]
$\overline{s}_{r-}$	Mean effect of a mutation that decreases R [-0.05]

2.6 Mutation

After an individual offspring is generated, it experiences random mutation. There are six types of mutations, denoted by different subscripts: beneficial (subscript b) and deleterious mutations (d) at the fitness loci, which increase and decrease fitness, respectively; mutator (m) and antimutator mutations (a) at the mutation-rate modifier locus, which increase and decrease mutation rate, respectively; mutations at recombination modifier locus that increase ($r+$) and decrease ($r-$) recombination rate.

The number of mutations, k_i, of type i acquired by an individual is assumed to be Poisson distributed with mean $U_i = Up_i$, where U is the current genomic mutation rate and p_i is the proportion of mutations of type i, such that $0 < \sum_i p_i \leq 1$ (note that $\sum_i p_i$ may be lower than 1 because I ignore mutations that do not affect fitness, mutation rate, or recombination rate). Thus, mutator and antimutator mutations change the rates of all types of mutations. The values of p_i are constant for all types of mutations and are not allowed to evolve in the simulations presented here.

The effect of a mutation of type i, s_i, is chosen randomly from an exponential distribution with mean $|\overline{s}_i| = 1/\lambda$ truncated at 1 with probability density function

$$f(x;\lambda) = \lambda e^{-\lambda x} \tag{2.2}$$

Mutations that increase fitness, mutation rate or recombination rate have positive s_i values; mutations that decrease these traits have negative s_i values. The values of $\overline{s}_i$ remain constant for all types of mutations during the course of a simulation.

2.7 Mutation rate and recombination rate

The mutation rate, U, of an individual is calculated by:

$$U = U_0 \prod_{i=1}^{k} e^{s_i} \quad , \tag{2.3}$$

where U_0 is the mutation rate of the individual that founded the population, k is the number of mutations at the modifier locus, and s_i is the effect of the ith mutation on U. The recombination rate, R, of an individual is calculated in the same way as U (Equation 2.3, but replacing U by R, and U_0 by R_0). I assume that mutations at the modifier loci act independently on U or R (no epistasis).

2.8 Implementation

I developed a software framework in Java™ for implementing the Wright-Fisher model described above and simulating the evolutionary processes in all experiments in this dissertation. I use Java Development Kit (JDK) version 1.6 to build the framework. For scientific computing and statistic analysis, I use the Apache Common Math library for Java™.

Chapter 3

The role of mutation bias in the evolution of mutation rate

3.1 Introduction

Other than the four main evolutionary forces (see Section 1.3 in Chapter 1) that influence the fate of mutator and antimutator alleles, I consider another evolutionary force: mutation bias towards increased-mutation rate. This bias can take one of two forms: mutator mutations may occur more often and/or have larger effects on average than antimutator mutations. I will refer to these two types of biases as rate and effect biases, respectively (Figure 3.1). There is no evidence that either kind of mutation bias exists but they seem plausible for two reasons (Ninio, 1991).

First, mutator mutations are typically loss-of-function mutations (Miller, 1996;

Kunz *et al.*, 1998; Friedberg *et al.*, 1995; Fijalkowska *et al.*, 2012) and loss-of-function mutations are thought to be more common than other kinds of mutations because there are so many different ways of disrupting the function of a gene (e.g., insertions, deletions, frameshifts, nonsense mutations, missense mutations). Second, certain kinds of loss-of-function mutations, such as whole gene deletions, are expected to be impossible to revert in a single mutation. Despite these arguments, many antimutator mutations have been isolated even though antimutators are believed to be more difficult to detect than mutators (Schaaper, 1998; Kunz *et al.*, 1998; Herr *et al.*, 2011).

Interestingly, despite the lack of evidence for their existence several theoretical analyses of the evolution of mutation rate have assumed the operation of mutation biases towards increased mutation rate (e.g., Taddei *et al.*, 1997; Tenaillon *et al.*, 1999, 2000; Gerrish *et al.*, 2007; Sloan and Panjeti, 2010; Desai and Fisher, 2011; Lynch, 2011; Jain and Nagar, 2012). However, the evolutionary consequences of those mutation biases have only rarely been examined, and never in depth. For example, Lynch (2011) assumed "an upward mutational bias toward the production of mutator versus antimutator alleles" in one model and showed that it could drive an evolutionary increase in mutation rate. However, that study focused on the effect of genetic drift, not mutation bias.

Here I investigate the ability of an upward mutation bias to drive the evolution of mutation rate. I consider the roles of mutation biases, different types of natural selection, genetic drift, and mode of reproduction, and the way they interact, to influence the evolution of mutation rate. I conclude that mutation bias can play a major

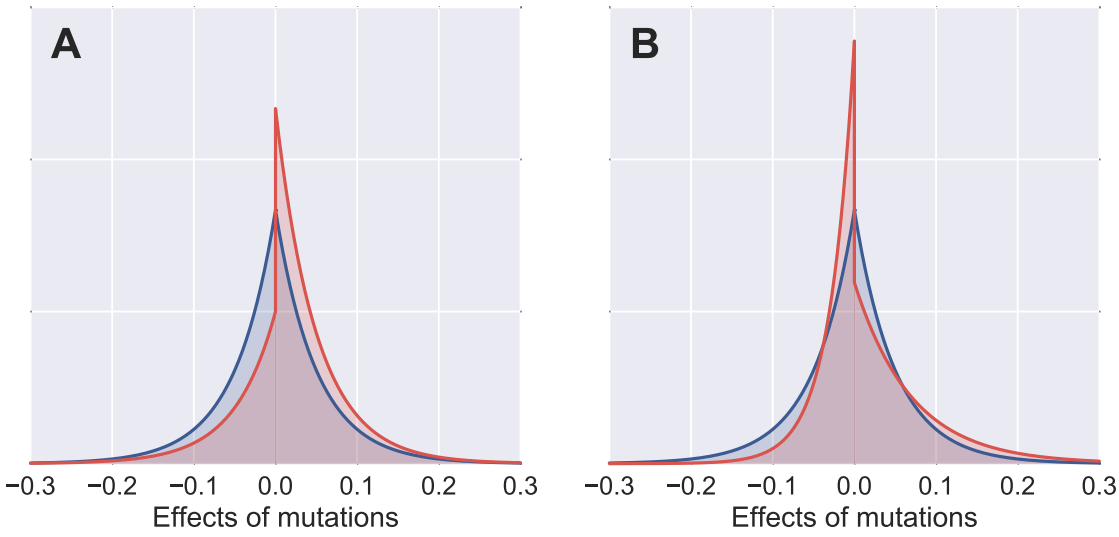

Figure 3.1: Examples of biased and unbiased distributions of mutational effects on mutation rate. Following the notation in Equation 3.4, mutational effects are defined as $x' - x$. The blue distribution in both (A) and (B) is unbiased ($\bar{s}_m = \bar{s}_a = 0.05\,,\ p_m/p_a = 1$). The red distribution in (A) shows *rate* bias towards increasing mutation rate: mutator mutations occur more often than antimutator mutations ($p_m/p_a = 2.33$), but their effects are identically distributed ($\bar{s}_m = \bar{s}_a = 0.05$). The red distribution in (B) shows *effect* bias towards increasing mutation rate: mutator and antimutator mutations occur at the same rate ($p_m/p_a = 1$), but mutator mutations have larger effects than antimutator mutations on average ($\bar{s}_m = 0.07\,,\ \bar{s}_a = 0.03$).

causal role in the evolution of mutation rate under a broad range of evolutionary scenarios.

3.2 Models

I used individual-based simulations to investigate the way mutation bias interacts with natural selection to determine the evolution of mutation rate, U. I considered either direct or indirect selection on U. Populations evolve according to a Wright-Fisher model with a selection-reproduction-mutation life cycle, constant population size, N, and discrete, non-overlapping generations.

3.2.1 Direct selection

I assumed (i) that mutations at a mutation rate modifier locus affect mutation rate directly and (ii) have pleiotropic effects on fitness, and (iii) that no other loci influence fitness. Because there is heritable variation for mutation rates, they are subject to direct selection (Sturtevant, 1937).

Genome. The genome of an individual consists of a single mutation-rate modifier locus determining both the mutation rate, and fitness.

Population. A population consists of N haploid individuals, each with mutation rate U and fitness W determined by the mutation-rate modifier locus (see Table 2.1). At the beginning of the simulation, individual values of log mutation rate $x = \ln U$ (i.e., genotypic values at the mutation-rate modifier locus) are either constant, or drawn randomly from a normal distribution or gamma distribution (See equation 3.1).

Gamma distribution. Log mutation rate is gamma distributed with probability density function

$$g(x) = \frac{(x-\mu)^{\alpha-1}}{\theta^{\alpha}\,\Gamma(\alpha)}\,\exp\left(-\frac{x-\mu}{\theta}\right) \quad \text{if } \; x > \mu\;, \tag{3.1}$$

and $g(x) = 0$ otherwise, where μ is a location parameter, $\theta > 0$ is a scale parameter, $\alpha > 0$ is a shape parameter, and Γ is the gamma function. The mean, variance, third, and fourth central moment of x are

$$\bar{x} = \mu + \alpha\theta\;, \;\; V(x) = \alpha\theta^2\;,$$
$$S(x) = 2\alpha\theta^3\;, \;\; K(x) = 3\alpha(2+\alpha)\theta^4\;.$$

The gamma distribution can only show positive skewness, $S(x) > 0$.

Mutation rate has the following mean, variance, and third central moment:

$$\begin{aligned} \overline{U} &= e^{\mu}(1-\theta)^{-\alpha}\;, \\ V(U) &= e^{2\mu}(1-2\theta)^{-\alpha} - \overline{U}^2\;, \\ S(U) &= e^{3\mu}(1-3\theta)^{-\alpha} - \overline{U}\left(\overline{U}^2 + 3\,V(U)\right)\;. \end{aligned} \tag{3.2}$$

Selection. I assume that log mutation rate, x, is under direct directional selection. The fitness of an individual is

$$W = 1 + \beta(x - \bar{x})\;, \tag{3.3}$$

where β is the linear selection gradient on x. This formulation ensures that mean fitness is $\overline{W} = 1$. Typically, I assume selection for a low mutation rate, $\beta < 0$.

Reproduction. To produce the next generation, N haploid individuals from parent generation were picked at random, with replacement, with probability proportional to their fitness. Once N offspring were generated, the parents were discarded. A selected individual was allowed to produce a single offspring with the same value of x.

Mutation. After an individual offspring was generated, it experienced random mutation at the mutation rate modifier locus. I assumed that mutator and antimutator mutations act additively on log mutation rate, x. The log mutation rate, x', of offspring of an individual with log mutation rate x has distribution

$$x' = x + I_m X_m - I_a X_a \ , \tag{3.4}$$

where I_m and I_a are indicator random variables following Bernoulli distributions with parameters Up_m and Up_a, respectively; $U = e^x$ is the mutation rate of the parent; p_m and p_a are the proportions of mutator and antimutator mutations, respectively; X_m and X_a are random variables following exponential distributions (see equation 2.2) with means $\bar{s}_m$ and $\bar{s}_a$, respectively. The values of p_m, p_a, $\bar{s}_m$ and $\bar{s}_a$ are constant and are not allowed to evolve during the simulations.

The distribution in Equation 3.4 implies that an individual can acquire at most one mutator and one antimutator mutation. Thus, this model is only meaningful when both of the following conditions are met: $Up_m \ll 1$ and $Up_a \ll 1$.

Mutation bias. Mutation bias can take two forms. *Rate* bias occurs when the proportions of mutator and antimutator mutations differ: $p_m \neq p_a$ (Figure 3.1A, red). *Effect* bias occurs when the mean effects of mutator and antimutator mutations differ: $\bar{s}_m \neq \bar{s}_a$ (Figure 3.1B, red). Typically, I assume that there is a mutation bias towards increased mutation rate (i.e., $p_m > p_a$ or $\bar{s}_m > \bar{s}_a$).

3.2.2 Indirect selection

I assumed (i) that mutations at a mutation-rate modifier locus directly affect mutation rate and (ii) have no pleiotropic effects on fitness, and (iii) that other loci determine fitness. Mutation-rate modifier alleles experience indirect selection due to the linkage disequilibrium with beneficial and deleterious alleles at fitness loci (Johnson, 1999b).

Indirect selection was implemented as described in Chapter 2. A few modifications to the main model are:

- The recombination rate was not allowed to evolve in sexual populatoins;
- Population size $N = 10^3$ for experiments in Figure 3.7 to 3.9.

Effective population size. I also implemented a neutral locus to estimate the effective population size of each population (Keightley and Otto, 2006). The neutral locus encodes a genotypic value z. Initially, $z = 0$. The locus mutates once in every individual, every generation, regardless of the value of U. Mutations act independently and additively on z. The effects of mutations are normally distributed

with mean zero and unit variance. I estimate the effective population size, N_e, of a population as the within-population variance of z at equilibrium.

Selection gradient. I estimated the strength of indirect selection on log mutation rate using the relationship that is given by the Price (1970) equation (first equation in system of Equations 7)

$$\begin{aligned}\Delta_s \bar{x} &= \mathrm{cov}(x, W)/\overline{W} \\ &= \mathrm{cov}(x, 1 + \beta(x - \bar{x})) \\ &= \beta \left[\overline{(x - \bar{x})^2}\right] \\ &= \beta \mathcal{V} \,, \end{aligned} \tag{3.5}$$

where $\mathrm{cov}(x, W)$ is the covariance between log mutation rate and fitness (see Equation 1), β is the linear selection gradient on x, and $\mathcal{V}$ is the variance of x.

Using the equation 3.5, I get the selection gradient

$$\beta = \frac{\mathrm{cov}(x, W)}{\overline{W}\,\mathcal{V}} \,, \tag{3.6}$$

For a particular population, I calculated the average values of $\mathrm{cov}(x, W)/\overline{W}$ and $\mathcal{V}$ over the entire time series. I then estimated β for the population as the ratio of the two averages.

3.3 Results

3.3.1 Natural selection and mutation bias can both drive the evolution of mutation rate

I investigated how opposing natural selection and mutation bias interact to drive the evolution of mutation rate. I began by allowing populations to evolve under different strengths of direct selection (β) for low mutation rate in the presence of a 10-fold rate mutation bias towards the generation of mutator mutations (Figure 3.2). In the absence of selection ($\beta = 0$), the mutation bias caused the mutation rate to increase. Weak selection ($\beta = -0.01$) slowed the increase in mutation rate, but was not sufficient to prevent it. Stronger selection ($\beta = -0.02$) halted change in mutation rate (Figure 3.2, red). Stronger selection still ($\beta \lesssim -0.03$) overcame the mutation bias and caused the mutation rate to decline.

Next, I took the evolutionary scenario shown in red in Figure 3.2 ($\beta = -0.02$), and manipulated the strength of rate mutation bias (p_m/p_a, Figure 3.3). A stronger mutation bias ($p_m/p_a = 100$) overwhelmed natural selection and caused the mutation rate to increase. Under weaker mutation biases ($p_m/p_a < 10$), selection overcame the mutation bias and caused the mutation rate to decline (Figure 3.3). Effect mutation bias had similar effects to rate mutation bias (Figure 3.4).

The direction of change in mean log mutation rate in Figures 3.2, 3.3, and 3.4 was determined by the relative strengths of mutation bias and selection (Equations 3.7–3.9): mutation rate increased when the mutation bias was stronger than selection,

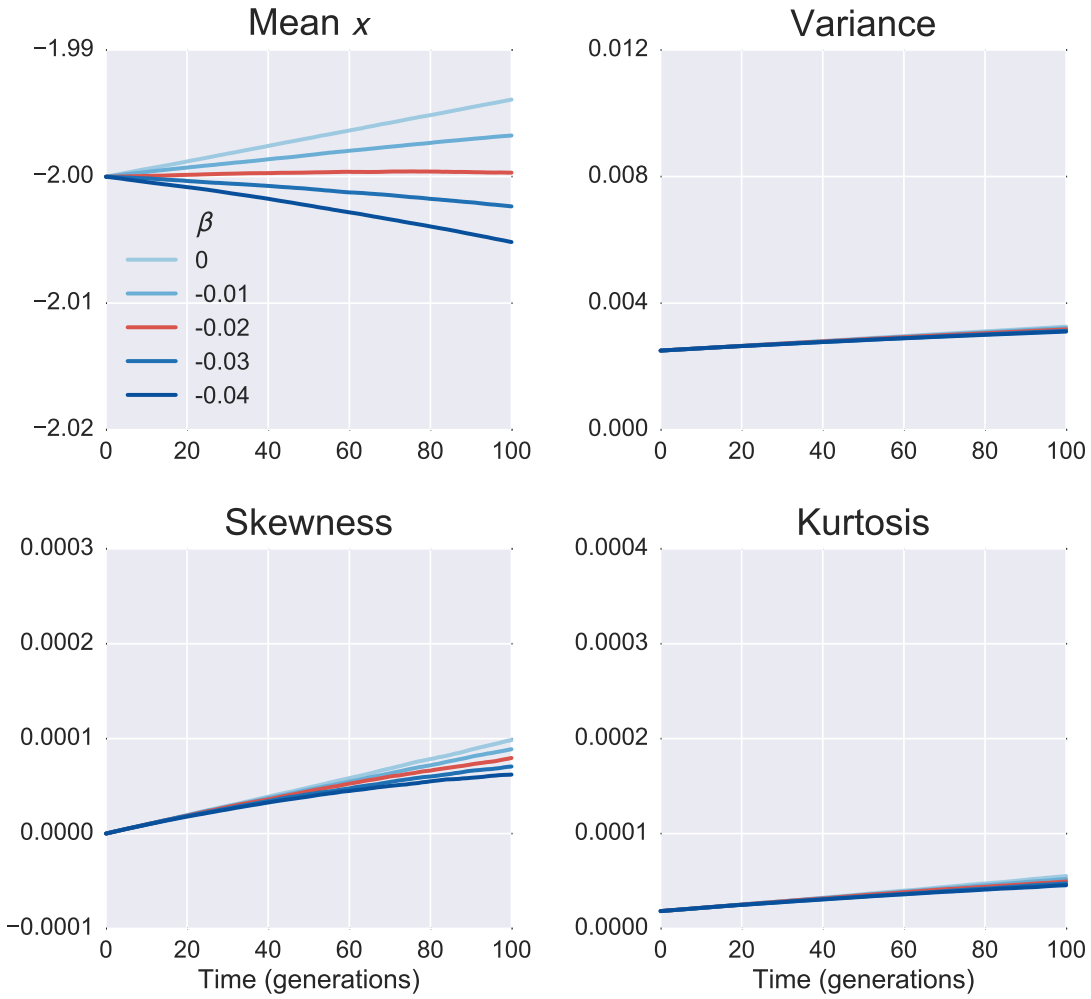

Figure 3.2: Natural selection can overcome a mutation bias. Evolutionary responses in the mean, variance, skewness, and kurtosis of log mutation rate in populations experiencing different strengths of selection for low mutation rate (β) and a 10-fold proportion mutation bias towards the generation of mutator mutations ($p_m = 0.01, p_a = 0.001$). Lines show means of stochastic simulations of 10^4 replicate populations. The lines encompass 95% confidence intervals, CIs. Note that there are five sets of lines in all plots. All populations consisted of $N = 10^4$ individuals. The same founder population was used in all simulations. Individual log mutation rates in the founder population were drawn at random from a normal distribution with mean $\bar{x} = -2$ and variance $\mathcal{V} = 0.0025$. There was no effect mutation bias ($\bar{s}_m = \bar{s}_a = 0.05$) in these simulations. Simulations in red are also shown in Figures 3.3 and 3.5.

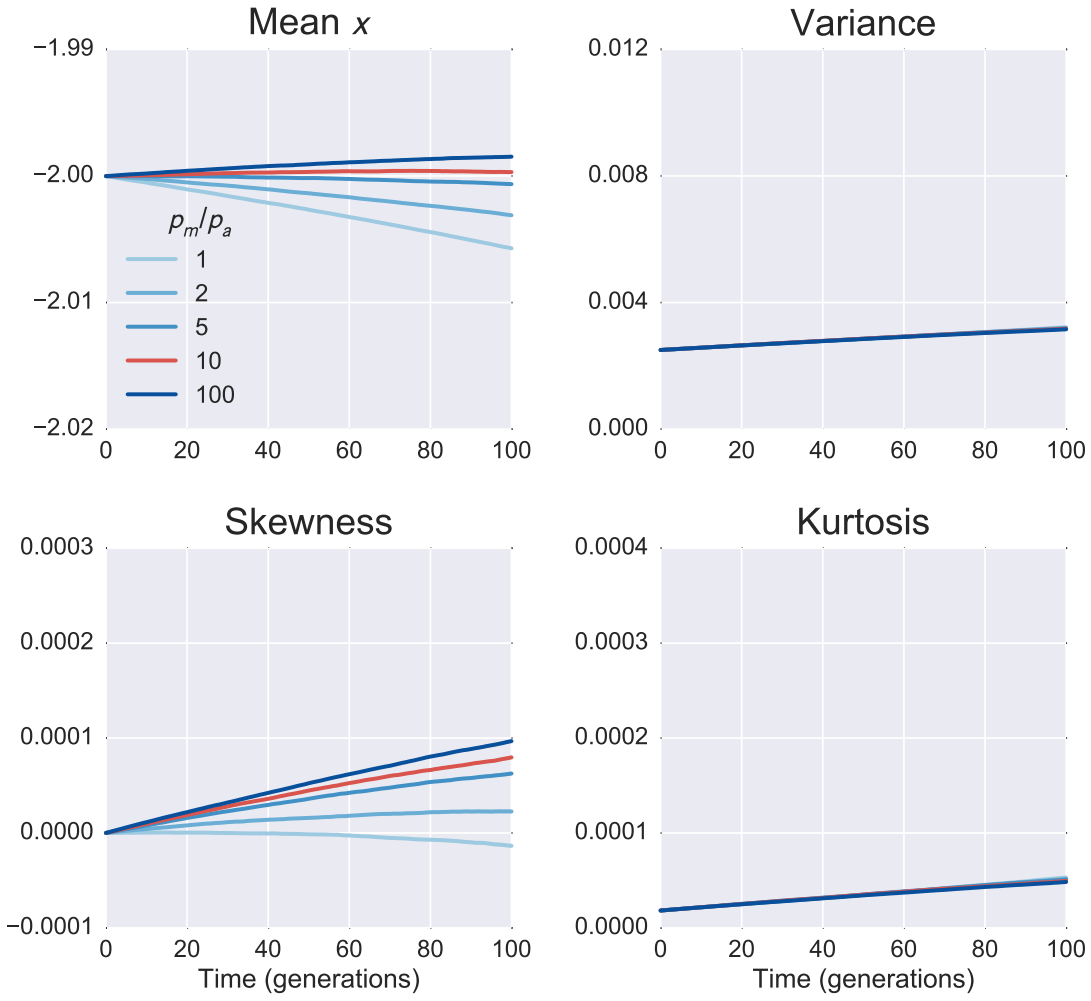

Figure 3.3: A mutation bias can overcome natural selection. Evolutionary responses in the mean, variance, skewness, and kurtosis of log mutation rate in populations experiencing different strengths of rate mutation bias towards mutator mutations (p_m/p_a) and selection for low mutation rate ($\beta = -0.02$). Lines show means of stochastic simulations of 10^4 replicate populations. The lines encompass 95% CIs. Note that there are five sets of lines in all plots. All populations consisted of $N = 10^4$ individuals. The overall proportion of mutations affecting mutation rate was constant in all simulations ($p_m + p_a = 0.011$). The same founder population as in Figure 3.2 was used in all simulations. There was no effect mutation bias ($\bar{s}_m = \bar{s}_a = 0.05$) in these simulations. Simulations in red are also shown in Figures 3.2 and 3.5.

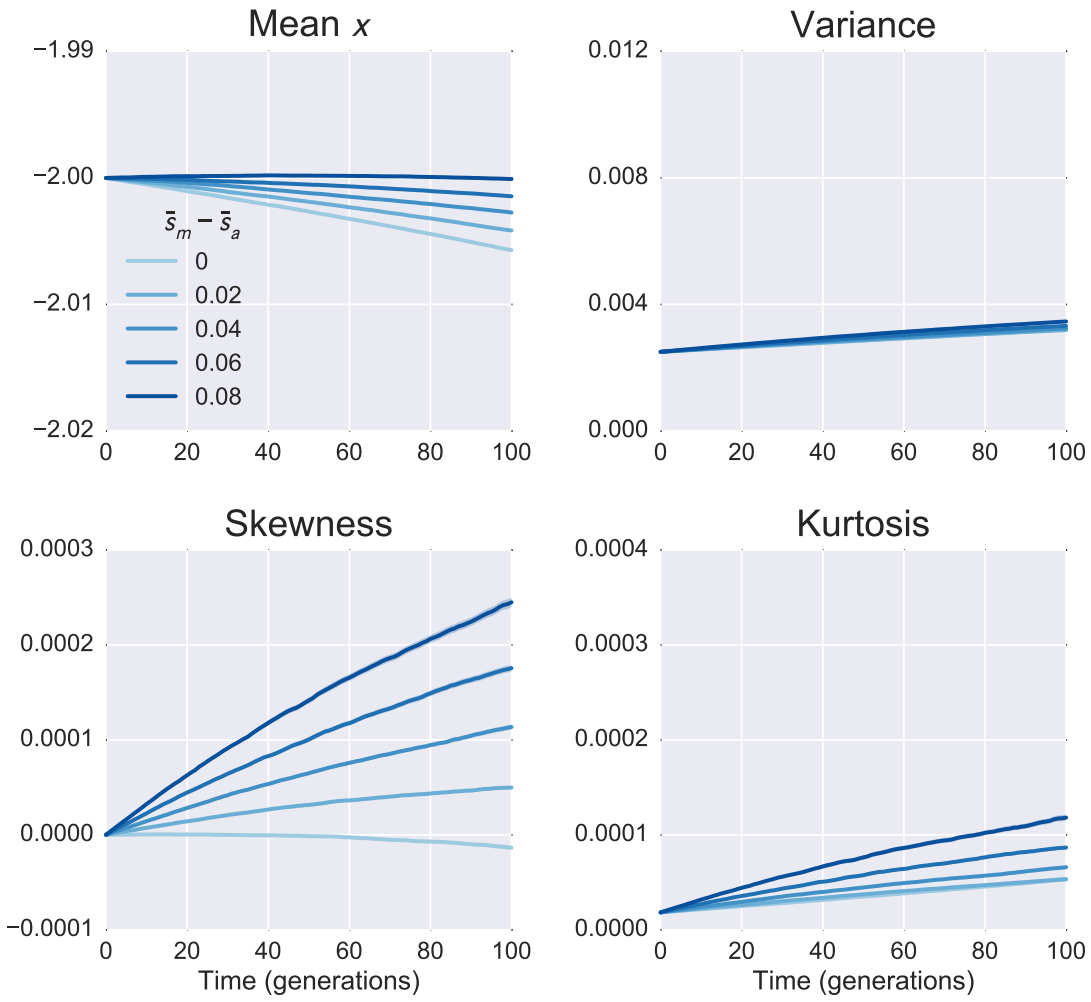

Figure 3.4: A mutation bias can overcome natural selection. Evolutionary responses in the mean, variance, skewness, and kurtosis of log mutation rate in populations experiencing different strengths of effect mutation bias towards mutator mutations ($\bar{s}_m - \bar{s}_a$) and selection for low mutation rate ($\beta = -0.02$). Lines show means of stochastic simulations of 10^4 replicate populations ($N = 10^4$). Shaded regions show 95% CIs (most lines encompass these regions). The overall proportion of mutations affecting mutation rate was constant in all simulations ($p_m + p_a = 0.011$). The same founder population as in Figure 3.2 was used in all simulations. There was no rate mutation bias ($p_m = p_a = 0.0055$) in these simulations. The simulations with no effect bias ($\bar{s}_m - \bar{s}_a = 0$) are the same as the simulations with no rate bias in Figure 3.3 ($p_m/p_a = 1$).

$\phi_1 > -\beta\mathcal{V}$, and decreased when the mutation bias was weaker than selection, $\phi_1 < -\beta\mathcal{V}$, where ϕ_1 is a measure of the strength of mutation bias (Equation 3.10).

Genetic drift did not play a significant role in these results. For example, the failure of selection to overcome the evolutionary effects of a mutation bias in the results summarized in Figure 3.2 ($\beta = -0.01, p_m/p_a = 10$), was not caused by genetic drift. Populations were started with a variance in log mutation rate of $\mathcal{V} = 0.0025$. This means that the 20% fittest individuals experienced an average selective benefit of 0.14% relative to the 20% least fit individuals, calculated from equation 3.5 with x normally distributed. This selection coefficient is over an order of magnitude greater than $1/N = 0.01\%$, indicating that selection operated efficiently in these populations.

3.3.2 The variance in mutation rate determines the effectiveness of selection for low mutation rate

One salient feature of the results summarized in Figures 3.2 and 3.3 is that the variance in log mutation rate increased at a rate determined by mutation, but largely independent of either selection or rate mutation bias: $\Delta\mathcal{V} \approx 2\phi_2$ (Equations 3.7–3.9). I investigated the long-term evolutionary consequence of this increase in variance by evolving populations with different initial variances in log mutation rate under direct selection for low mutation rate ($\beta = -0.02$) and a 10-fold rate mutation bias towards the generation of mutator mutations ($p_m/p_a = 10$). I found that higher variance increased the effectiveness of selection for low mutation rate (Figure 3.5). In an initially monomorphic population ($\mathcal{V} = 0$), the mutation bias overcame selection,

causing the mutation rate to increase. When the variance was higher ($\mathcal{V} \gtrsim 0.005$), selection overcame the mutation bias, causing the mutation rate to decrease. These results indicate that mutation bias is likely to play a more important role in the evolution of mutation rate in smaller populations because genetic drift will more rapidly erode variation in mutation rate (Equation 3.11).

3.3.3 The skewness in log mutation rate has little effect on the evolution of mutation rate

Unlike the variance, the evolution of the skewness in log mutation rate, $\mathcal{S}$, depended on both selection and mutation bias (Figures 3.2, 3.3 and 3.4). Selection for low mutation rate tended to reduce $\mathcal{S}$ (Figure 3.2), whereas mutation bias towards the generation of mutator mutations tended to increase $\mathcal{S}$ (Figure 3.3). Within the range of parameters explored, mutation bias was a stronger force than natural selection, causing $\mathcal{S}$ to increase.

I investigated the long-term evolutionary consequence of this increase in skewness in log mutation rate by evolving populations with the same initial variance but different initial values of $\mathcal{S}$ under direct selection for low mutation rate ($\beta = -0.02$) and a 10-fold rate mutation bias towards the generation of mutator mutations. I found that higher initial values of $\mathcal{S}$ slightly slowed down the increase in the variance in log mutation rate, but had only a slight effect on the response in mean log mutation rate (Figure 3.6).

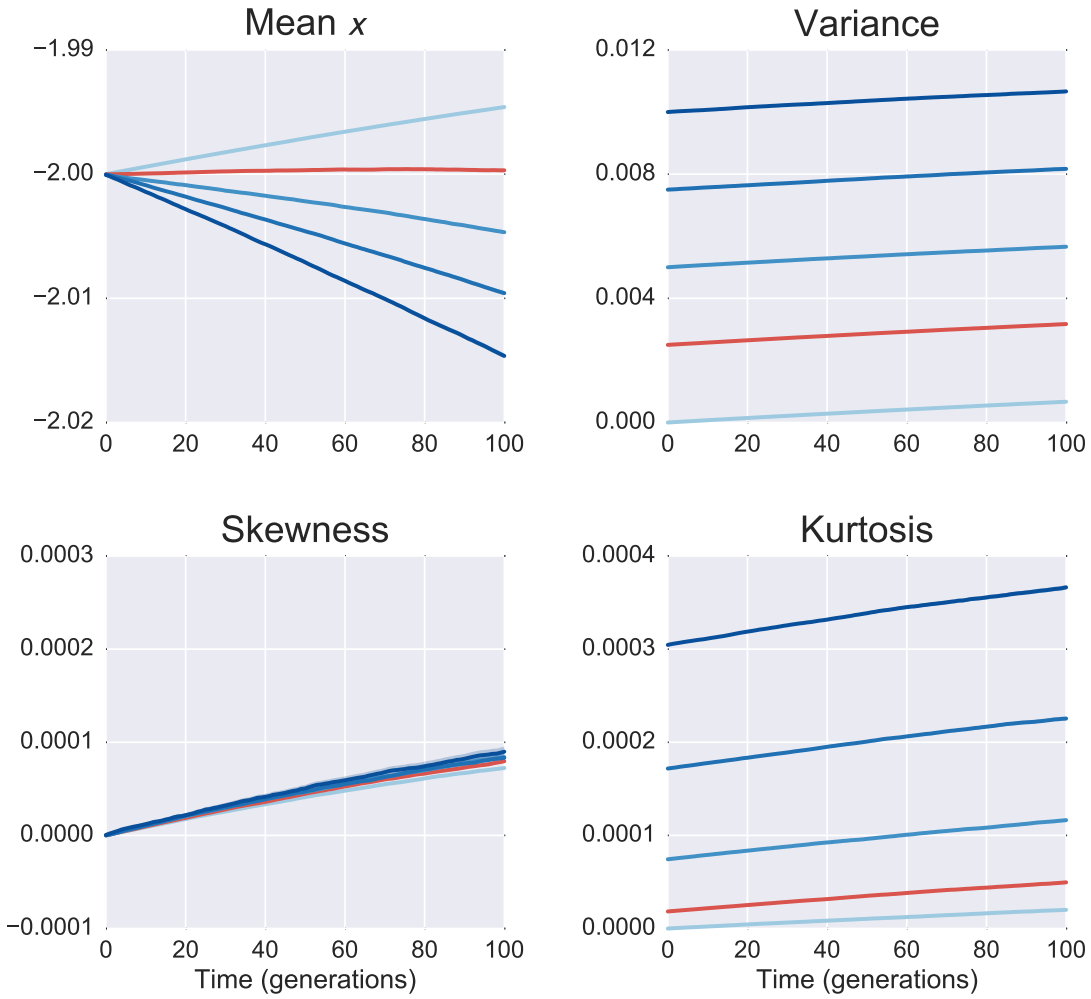

Figure 3.5: The variance in mutation rate determines the effectiveness of selection for low mutation rate. Evolutionary responses in the mean, variance, skewness, and kurtosis of log mutation rate in populations with different initial variance in log mutation rate experiencing selection for low mutation rate ($\beta = -0.02$) and a 10-fold proportion mutation bias towards the generation of mutator mutations ($p_m = 0.01, p_a = 0.001$). Lines show means of stochastic simulations of 10^4 replicate populations ($N = 10^4$). Shaded regions show 95% CIs (most lines encompass these regions). The same founder population was used in all simulations for a particular variance $\mathcal{V}$. Individual log mutation rates in the founder population were drawn at random from a normal distribution with mean $\bar{x} = -2$ and variance $\mathcal{V}$. There was no effect mutation bias ($\bar{s}_m = \bar{s}_a = 0.05$) in these simulations. Simulations in red are also shown in Figures 3.2 and 3.3.

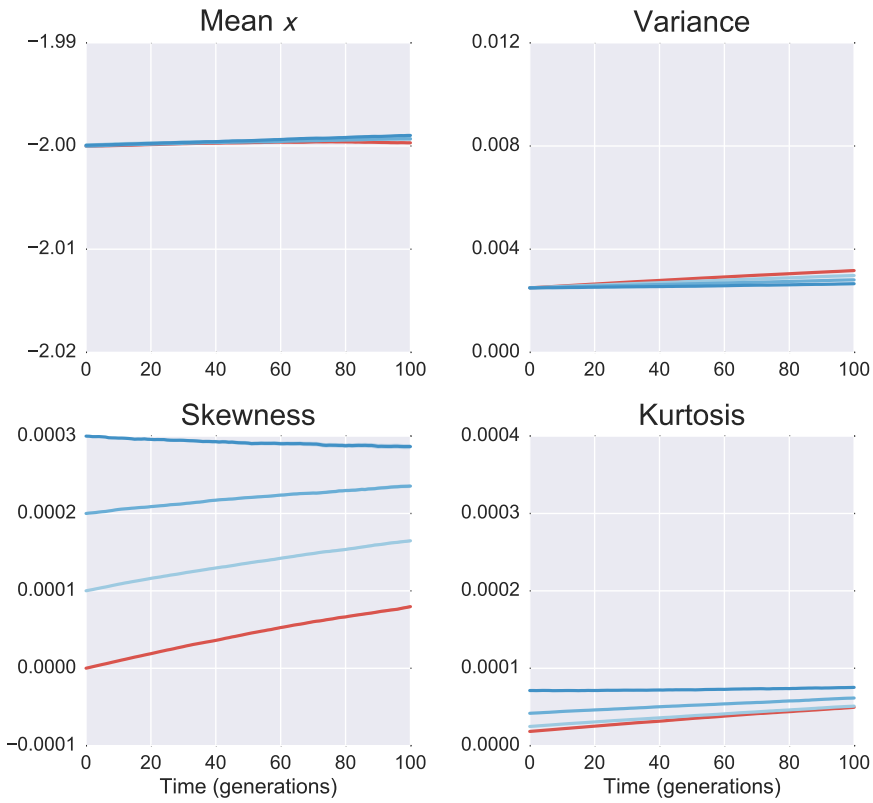

Figure 3.6: The skewness in log mutation rate has little effect on the evolution of mutation rate. Evolutionary responses in the mean, variance, skewness, and kurtosis of log mutation rate in populations with different initial skewness in log mutation rate experiencing selection for low mutation rate ($\beta = -0.02$) and a 10-fold rate mutation bias towards the generation of mutator mutations ($p_m = 0.01, p_a = 0.001$). Lines show means of stochastic simulations of 10^4 replicate populations. Shaded regions show 95% CIs (most lines encompass these regions). Note that there are five sets of lines in the mean plot. All populations consisted of $N = 10^4$ individuals. The same founder population was used in all simulations for a particular skewness $\mathcal{S}$. Individual log mutation rates in the founder population of the simulations shown in red were drawn at random from a normal distribution with mean $\bar{x} = -2$ and variance $\mathcal{V} = 0.0025$. Individual log mutation rates in the founder population of the simulations shown in blue were drawn at random from a gamma distribution with mean $\bar{x} = -2$, variance $\mathcal{V} = 0.0025$, and skewness $\mathcal{S}$ (See Section 3.5 above). For comparison, $\mathcal{S} = 0.00025$ corresponds to an exponential distribution. There was no effect mutation bias ($\bar{s}_m = \bar{s}_a = 0.05$) in these simulations. Simulations in red are also shown in Figures 3.2, 3.3 and 3.5.

3.3.4 Mutation bias can cause mutation rate to increase even if all mutations with an effect on fitness are deleterious

So far I have assumed that mutation rate is under *direct* selection. Natural selection can also *indirectly* act on mutation rate. For example, since mutator genotypes acquire more deleterious mutations than antimutator genotypes, there is indirect selection for low mutation rate even if mutation rate has no direct effect on fitness (Sturtevant, 1937; Kimura, 1967; Leigh, 1970; Dawson, 1998; Johnson, 1999a).

I used individual-based simulations to investigate the extent to which the theoretical framework of direct selection introduced above can provide insight into indirect selection on mutation rate. I began by simulating asexual populations experiencing a 10-fold rate mutation bias towards the generation of mutator mutations where all mutations with effects on fitness were deleterious ($p_d = 0.5, p_b = 0$). Mutation rate had no direct effect on fitness, but the accumulation of deleterious mutations generated indirect selection for low mutation rate of an average strength of $\beta \approx -0.036$ (Equation 3.6; Figure 3.7B, blue). This selection gradient is consistent with theoretical expectations (File S3; Lynch, 2011). Populations had a constant census size of $N = 10^3$, but the operation of natural selection reduced effective-population size to $N_e = 231$ (Equation 3.6; Figure 3.7B, blue).

Despite this indirect selection for low mutation rate, mutation rate increased (Figure 3.7A, solid blue line). The response was not caused by mutator hitchhiking because there were no beneficial mutations. To test whether mutation bias can account for the increase in mutation rate, I ran direct-selection simulations under the

parameters estimated from the indirect-selection simulations: $\beta = -0.036$ and $N = N_e = 231$ (I ignored the small decreases in N_e that take place in the direct selection simulations). The results indicate that direct-linear selection on log mutation rate (Figure 3.7A, dashed blue line) provides a good approximation to indirect selection on mutation rate. Thus, the increase in mutation rate was driven by mutation bias ($\phi_1 > -\beta\mathcal{V}$).

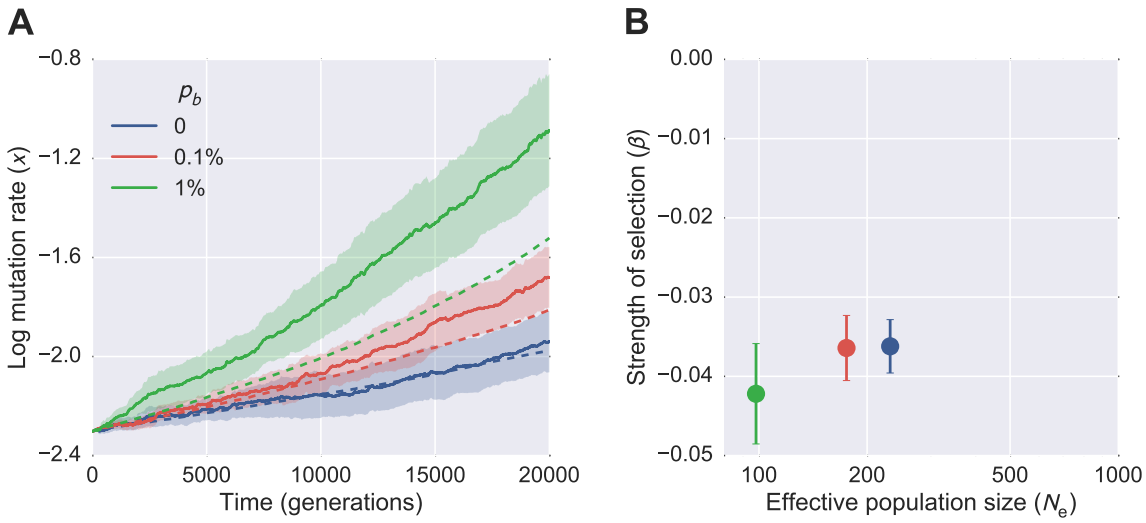

Figure 3.7: Mutation bias can cause mutation rate to increase when selection acts indirectly on mutation rate. (A) Solid lines show evolutionary responses in the mean of log mutation rate in 30 populations experiencing a 10-fold rate mutation bias towards the generation of mutator mutations ($p_m = 0.01, p_a = 0.001$) and different proportions of beneficial mutations (p_b). Mutation rate had no direct effect on fitness. In all populations, half of all mutations were deleterious ($p_d = 0.5$) and there was no effect mutation bias ($\bar{s}_m = \bar{s}_a = 0.05$). All populations consisted of $N = 10^3$ individuals and reproduced asexually. Shaded regions show 95% CIs. Dashed lines show evolutionary responses in the mean of log mutation rate in 2,500 populations experiencing the same distribution of mutational effects on mutation rate and direct selection on mutation rate. The strength of selection on mutation rate (β) and the population size in the direct selection simulations were set to the average β and effective population size (N_e) observed in the the populations experiencing indirect selection on mutation rate, shown in (B). Error bars in (B) are 95% CIs. Data in red is also shown in Figure 3.8.

3.3.5 Mutation bias can drive the evolution of mutation rate in the presence of mutator hitchhiking

Mutator alleles can fix because of mutator hitchhiking with the beneficial alleles they help generate (Taddei *et al.*, 1997; Tenaillon *et al.*, 1999; Gerrish *et al.*, 2007), causing mutation rate to increase. To investigate how mutator hitchhiking and mutation bias interact, I ran indirect selection simulations like those shown in blue in Figure 3.7, but allowing beneficial mutations ($p_b > 0$). When 0.1% of mutations were beneficial, the overall strength of selection for low mutation rate did not change because deleterious mutations were 500-fold more common than beneficial mutations. I found that mutation rate increased more rapidly when there were beneficial mutations (Figure 3.7A, solid blue and red lines). However, this higher rate of evolution of x was not completely explained by mutator hitchhiking; it was caused in part by a reduction in N_e (Figure 3.7B) which made selection for low mutation rate less effective (Figure 3.7A, dashed blue and red lines). Note that the dashed red line is approximately half way between the solid blue and red lines in Figure 3.7A, indicating that approximately half of the increase in the rate of evolution of x in the presence of beneficial mutations was caused by mutation bias and genetic drift.

Increasing the proportion of beneficial mutations to $p_b = 1\%$ caused the rate of evolution of x to increase further (Figure 3.7, green). Again, the increase was explained in part by an increased opportunity for mutator hitchhiking, and in part by a further reduction in N_e. However, the results show that the direct selection approximation breaks down when mutator hitchhiking becomes too frequent.

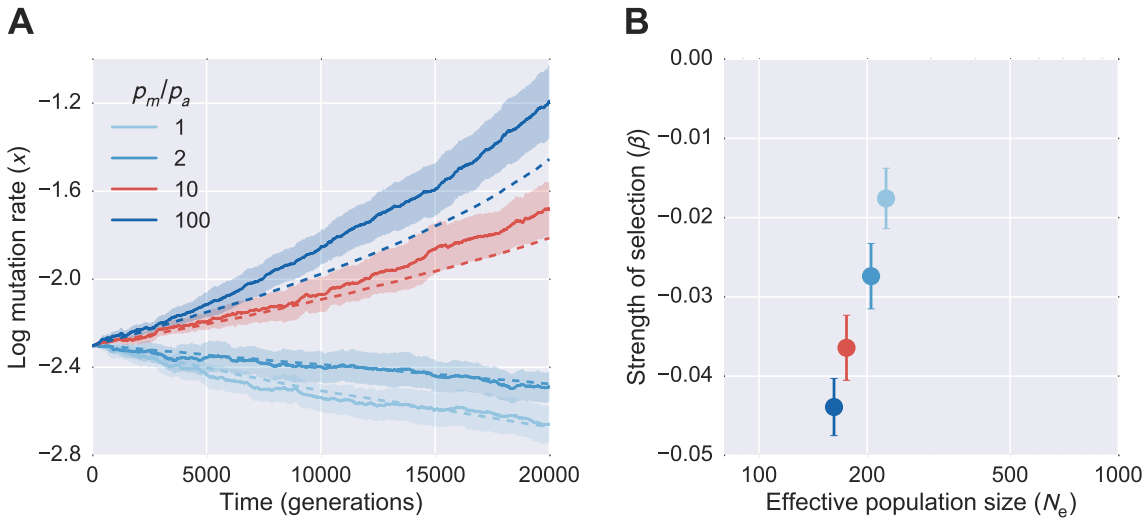

Figure 3.8: A strong mutation bias is required for mutator hitchhiking to cause mutation rate to increase. (A) Solid lines show evolutionary responses in the mean of log mutation rate in 30 populations experiencing a different strengths of rate mutation bias towards the generation of mutator mutations (p_m/p_a). The overall proportion of mutations affecting mutation rate was constant in all simulations ($p_m + p_a = 0.011$). Mutation rate had no direct effect on fitness. In all populations, half of all mutations were deleterious and 0.1% were beneficial ($p_d = 0.5, p_b = 0.001$), and there was no effect mutation bias ($\bar{s}_m = \bar{s}_a = 0.05$). All populations consisted of $N = 10^3$ individuals and reproduced asexually. Shaded regions show 95% CIs. Part (B) and the dashed lines in (A) were obtained as explained in the legend of Figure 3.7. Data in red is also shown in Figure 3.7.

To further evaluate the contribution of mutation bias to the evolution of mutation rate by mutator hitchhiking, I manipulated the strength of rate mutation bias in the presence of beneficial mutations. I found that a strong mutation bias was necessary for mutator hitchhiking to be capable of increasing mutation rate. A 2-fold mutation bias was not sufficient (Figure 3.8A). Surprisingly, increasing the strength of rate-mutation bias resulted in stronger selection for low mutation rate and lower effective population size.

3.3.6 Mutation bias can drive the evolution of mutation rate in the presence of recombination

Even low levels of genetic exchange and recombination can prevent the spread of mutator alleles by breaking up the associations between mutator alleles and the beneficial alleles they bring forth (Tenaillon *et al.*, 2000). To investigate how mutation bias operates in the presence of recombination, I ran indirect selection simulations like those shown in blue and red in Figure 3.7, but with recombination. In the absence of beneficial mutations, and therefore of mutator hitchhiking, recombination caused a 75% reduction in the average strength of selection for low mutation rate and a 4-fold increase in the effective population size (Figure 3.9B). Both effects are consistent with the expectation that recombination reduces Hill-Robertson interference (Hill and Robertson, 1966; Felsenstein, 1974; Comeron *et al.*, 2008). Mutation rate increased as expected under the influence of mutation bias (Figure 3.9A). In the presence of beneficial mutations, mutation rate increased slightly faster than in their

absence. However, this increase is not caused by mutator hitchhiking but by a slight reduction in effective population size (Figure 3.9). I conclude that mutation biases can operate in the presence of recombination.

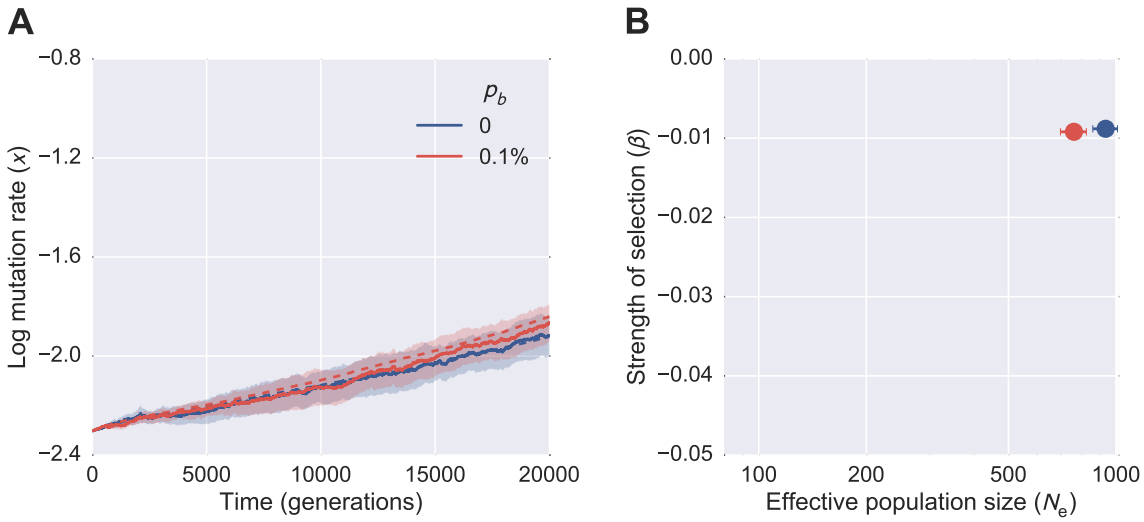

Figure 3.9: Mutation bias can cause mutation rate to increase even in the presence of recombination. Data in blue and red were obtained from simulations identical to those use to obtain the data of the same color in Figure 3.7, except that populations reproduced sexually with $R = 1$.

3.4 Discussion and conclusion

Here, I considered the roles of four processes on the evolution of mutation rate: mutation bias, natural selection, genetic drift, and recombination. My results show that biases in the relative rates of generation or in the relative effects of mutators and antimutators can play a role in the evolution of mutation rate comparable to that of any of the other three processes.

I showed that a mutation bias can cause the mutation rate to increase even when high mutation rate is deleterious, directly or indirectly. When selection acted only indirectly through deleterious mutations (assuming deleterious-mutation parameters similar to those of the RNA virus ϕ6, Burch *et al.*, 2007), even a 10-fold mutation bias could counteract selection and cause mutation rate to increase. When beneficial mutations were added to the model, mutator hitchhiking also caused mutation rate to increase, but the effect of mutation bias was still detected. Mutation biases were also able to operate in the presence of recombination. Mutation biases can have effects in populations of all sizes, but are likely to be specially important in small populations because natural selection will tend to be less efficient. These results indicate that mutation biases should be considered a possible cause for the spread of mutators in natural (e.g., Gross and Siegel, 1981; LeClerc *et al.*, 1996; Matic *et al.*, 1997; Oliver *et al.*, 2000; Björkholm *et al.*, 2001; Denamur *et al.*, 2002; Giraud *et al.*, 2002; Richardson *et al.*, 2002; Prunier *et al.*, 2003) and experimental populations (Cox and Gibson, 1974; Chao and Cox, 1983; Chao *et al.*, 1983; Mao *et al.*, 1997; Sniegowski *et al.*, 1997; Giraud *et al.*, 2001; Notley-McRobb *et al.*, 2002; Thompson

et al., 2006; Pal *et al.*, 2007) alongside mutator hitchhiking.

I believe that my results also prompt the reevaluation of some earlier theoretical results. For example, Gerrish *et al.* (2007) showed that if there is recurrent mutation at a mutation rate modifier locus generating both mutators and antimutators, the spread of mutator alleles as populations adapt can raise mutation rates to intolerably high levels, eventually driving populations to extinction. I continue investigating this "mutation-rate catastrophe" phenomenon in Chapter 5.

Whether mutation biases on mutation rate actually exist in nature remains an open question. There is general agreement that they do, although that belief appears to be largely built on plausibility arguments (Ninio, 1991). Direct evidence is anecdotal at best and is difficult to evaluate because screening for antimutators is more difficult than screening for mutators (Schaaper, 1998). My results indicate that knowing the precise nature (rate and/or effect) and magnitude of the mutation biases on mutation rate will be essential to elucidate the extent to which they contribute to the evolution of mutation rate. Another relevant empirical question is the extent to which the biases themselves can evolve. For example, a mutator genotype may have a weaker upward mutation bias than an antimutator genotype.

My analysis begs the question of why mutation biases on mutation rate have been neglected for so long? One possibility is that theoretical population geneticists have simply felt that the effects of mutation bias on the evolution of mutation rate are too straightforward to study in detail. Another possibility is that this is part of a broader pattern of neglect of the effect of mutation biases in population genetics (Yampolsky and Stoltzfus, 2005; Stoltzfus, 2006)—a bias against mutation biases, as it were.

This is ironic given that another mutation bias (towards deleterious mutations) has been recognized as central to the problem of the evolution of mutation rates since Sturtevant (1937). Whatever the reason, I hope that my work will stimulate further interest in the role of mutation biases in the evolution of mutation rate.

3.5 Appendix

3.5.1 Analysis

[1]

Price equations. Under the models described above the mean x, variance ($\mathcal{V}$) and third central moment (skewness, $\mathcal{S}$) of log mutation rate are expected to evolve according to a coupled system of Price (1970) equations

$$\begin{cases} \Delta\bar{x} = \Delta_s\bar{x} + \Delta_m\bar{x} \\ \Delta\mathcal{V} = \Delta_s\mathcal{V} + \Delta_m\mathcal{V} \\ \Delta\mathcal{S} = \Delta_s\mathcal{S} + \Delta_m\mathcal{S} \end{cases} \tag{3.7}$$

Each Price Equation 3.7 partitions the total change in the statistic from one generation to the next (Δ) into two components: change caused by selection (Δ_s), and change caused by mutation (Δ_m). Mutation takes place after, and independently from, selection.

Below I derive the coupled system of Price Equations 3.7 for the **Direct selection** model.

[1] All the analytical work was done by Dr. Ricardo Azevedo. This work is presented here for illustration purposes.

Selection. The selection components of the coupled system of Equations 3.7 are

$$\begin{cases} \Delta_s \bar{x} = \bar{x}' - \bar{x} = \beta \mathcal{V} \\ \Delta_s \mathcal{V} = \mathcal{V}' - \mathcal{V} = \beta \mathcal{S} - (\beta \mathcal{V})^2 \\ \Delta_s \mathcal{S} = \mathcal{S}' - \mathcal{S} = \beta \mathcal{K} - 3\beta \mathcal{V} \mathcal{V}' - (\beta \mathcal{V})^3 \end{cases} \tag{3.8}$$

where $\bar{x}$, $\mathcal{V}$, $\mathcal{S}$ and $\mathcal{K}$ are the mean, variance, third central moment (skewness), and fourth central moment (kurtosis) of log mutation rate *before* selection, respectively; $\bar{x}'$, $\mathcal{V}'$ and $\mathcal{S}'$ are the mean, variance and skewness of log mutation rate *after* selection (but before mutation), respectively.

The coupled system of selection Equations 3.8 does not rely on any assumptions about the distribution of x. However, it is not closed because the evolution of $\mathcal{K}$ is not predicted by the system.

Mutation. To derive the mutation components of the coupled system of Equations 3.7 I assume that log mutation rate after selection, x', is normally distributed with mean $\bar{x}'$, and variance $\mathcal{V}'$ and calculate the moments of a parameter mixture distribution of the form of Equation 3.4

$$\begin{cases} \Delta_m \bar{x} = \bar{x}'' - \bar{x}' = \phi_1 \\ \Delta_m \mathcal{V} = \mathcal{V}'' - \mathcal{V}' = 2\left(\phi_2 + \mathcal{V}'\phi_1 - \psi\right) - \phi_1^2 \\ \Delta_m \mathcal{S} = \mathcal{S}'' - \mathcal{S}' = 6\phi_3 - 2\phi_1\left(3\phi_2 - \phi_1^2\right) + 3\mathcal{V}'\left(2\phi_2 - 2\phi_1^2 + \mathcal{V}'\phi_1\right) - \\ \qquad\qquad -6\psi\left(2\mathcal{V}' - \phi_1 + \bar{s}_m - \bar{s}_a\right) \end{cases} \tag{3.9}$$

where $\bar{x}''$, $\mathcal{V}''$ and $\mathcal{S}''$ are the mean, variance and skewness of log mutation rate after mutation, respectively;

$$\phi_n = \left[p_m(\bar{s}_m)^n + p_a(-\bar{s}_a)^n\right] e^{\bar{x}' + \frac{\mathcal{V}'}{2}} \ , \tag{3.10}$$

and

$$\psi = p_m \bar{s}_m p_a \bar{s}_a e^{2(\bar{x}' + \mathcal{V}')} \ .$$

If x is normally distributed, the mean mutation rate is $\overline{U} = e^{\bar{x} + \frac{\mathcal{V}}{2}}$. Thus, ϕ_1 is a measure of strength of mutation bias of a genotype. The coupled system of mutation Equations 3.9 indicates that mutation will cause the mutation rate to evolve if there is a mutation bias.

The coupled system of mutation Equations 3.9 is closed (unlike the system of selection Equations 3.8).

Genetic drift. The system of Price Equations 3.7 is deterministic. In a finite population, genetic drift is not expected to have an effect on $\bar{x}$. I model the effect of genetic drift on $\mathcal{V}$ and $\mathcal{S}$ by reducing the values obtained from Equations 3.7 every generation

$$\begin{cases} \mathcal{V}^* = \mathcal{V}\left(1 - \dfrac{1}{N}\right) \\ \mathcal{S}^* = \mathcal{S}\left(1 - \dfrac{1}{N}\right)\left(1 - \dfrac{2}{N}\right) \end{cases} \tag{3.11}$$

where N is the population size.

Chapter 4

Static analysis of the effects of mutation and recombination rate on evolvability

4.1 Introduction

The ultimate goal of a population evolving under natural selection is to attain the best possible fit to the environment in which it finds itself. Most often this is achieved by the spread of alleles that increase the fitness of the genotypes that carry them. The rise of high-fitness genotypes, in turn, causes the mean fitness of the population to increase. Natural selection can also favor alleles that increase the rate of adaptation, even if they do not contribute to fitness directly (Wagner and Altenberg, 1996; Partridge and Barton, 2000; Sniegowski and Murphy, 2006).

The evolvability of a population is determined by its ability to generate high-fitness genotypes. Here I estimate evolvability as the rate of adaptation of the population under study. Two genetic mechanisms are known to contribute to the evolvability: mutation is the ultimate source of all beneficial alleles, and recombination can create new, high-fitness combinations of existing alleles. Thus, the rates of both mutation and recombination are expected to be important determinants of evolvability (Pigliucci, 2008; Colegrave and Collins, 2008).

The effects of mutation rate on evolvability are expected to be complex. On the one hand, population cannot adapt without beneficial mutations. High mutation rate may promote the evolvability of a population because it increases the supply of beneficial mutations. On the other hand, it is well known that the vast majority of spontaneous mutations with detectable effects on fitness are deleterious. High mutation rate introduces excessive deleterious mutations, which may be selected against due to the mutational load it creates. The elimination of recurrent deleterious mutations lowers evolvability through background selection (see section 1.1). Thus, it is reasonable to predict that optimal mutation rate exists, at which populations will display the highest evolvability.

Recombination rate, as another evolvability trait, affects genetic variation by mixing genetic variants from different lineages, thus affects the population's ability to respond to natural selection. Specifically, recombination may affect the rate of adaptation in at least two ways. First, recombination reduces the effects of background selection (see section 1.1) by breaking the linkage disequilibrium between beneficial mutations and their deleterious background. It may facilitate the fixation

of beneficial mutations. That may not only prevent an overly high mutational load, but may also lead to a high rate of adaptation. Second, the fixation of multiple beneficial mutations is not efficient in asexual populations due to clonal interference — a phenomenon that beneficial mutations in different genomes have to compete for fixation and cannot fix simultaneously (Hill and Robertson, 1966; Barton, 1995; Gerrish and Lenski, 1998; Orr, 2000). However, in sexual populations, multiple beneficial mutations that are generated in different genomes may be recombined into single genome — known as the Fisher–Muller effect (Fisher, 1930b; Muller, 1932). Meanwhile, This process reduces the effect of clonal interference and may lead to the acceleration of adaptation. Here I predict that optimal recombination rate may exist, at which populations show highest evolvability.

Gerrish *et al.* (2013) has studied the effects of various static mutation rates on evolvability in asexual populations in the presence of both beneficial and deleterious mutations. The joint effects of mutation rate and recombination rate on evolvability are complicated and have not been clearly understood. Here I use individual-based simulations to investigate what are the effects of mutation rate and recombination rate on evolvability when neither rate is allowed to evolve. My results show that populations show maximum evolvability at intermediate mutation rates and high recombination rates. I also found that there are critical mutation rates, at which populations show negative evolvability.

4.2 Model

I utilize the Wright-Fisher model described in Chapter 2. The parameters for simulations in this chapter follow the default parameters in Table 2.1 except that the mutation and recombination rates are kept constant during evolution. I evolve populations under mutation rates ranging from $U = 2 \times 10^{-3}$ to 10 and recombination rates ranging from $R = 0$ (completely asexual populations) to 1.

Evolvability. I define the evolvability, E, of a population as its adaptation rate. I measure E by the slope of the linear regression of $\ln \overline{W}$ against time in generations, where $\overline{W}$ is the mean fitness of the population.

The relationship between E and U was modeled using the modified skew-normal distribution introduced by Urban *et al.* (2013):

$$E(u) = e^{-\frac{2\psi\phi u + u^2}{2\phi^2}} \left(\frac{\psi u}{\phi} + 1 \right) E_{\max} \tag{4.1}$$

where $u = \ln(U/U_{\text{opt}})/\sigma$ is a transformed mutation rate; U_{opt} is the mutation rate at which $E = E_{\max}$, the maximum evolvability; σ is a scale parameter; $\phi = \sqrt{\psi^2 + 1}$; ψ is a skew parameter. If $\psi > 0$ then $E(u)$ shows a long right tail and steep left shoulder; if $\psi < 0$ then $E(u)$ shows a long left tail and steep right shoulder; if $\psi = 0$ then $E(u)$ is symmetric (Gaussian). For a relationship between E and U obtained for a specific combination of N and R, I estimated the parameters $E_{\max}$, U_{opt}, σ, and ψ using nonlinear least squares (we considered only estimates where $E > 0$). The critical mutation rate at which $E = 0$ is estimated as $U_{\text{crit}} = U_{\text{opt}} - \sigma\phi/\psi$.

4.3 Results

In this chapter, I ask how the mutation rate (U) and recombination rate (R) influence the evolvability (E) of both asexual ($R = 0$) and sexual ($0 < R \leq 1$) populations when U and R are not allowed to evolve.

4.3.1 There is an optimal mutation rate that maximizes the evolvability of an asexual population

My results (Figure 4.1) confirm earlier theoretical and simulation results on asexual populations (Orr, 2000; Bachtrog and Gordo, 2004; Gerrish *et al.*, 2013).

When the mutation rate is low ($NU_b \ll 1$), populations adapt by fixing beneficial mutations sequentially, a regime known as strong selection, weak mutation (Gillespie, 1984). Under this regime, evolvability is determined almost entirely by the supply of beneficial mutations; increases in U cause corresponding increases in E (Figure 4.1, $U \ll 0.1$).

As U continues to rise, however, the increase in E starts to slow down for four reasons (Figure 4.1, $U \approx 0.1$). First, as beneficial mutations become more abundant in the population they start to compete with each other, which slows down their spread—a process known as clonal interference (Fisher, 1930a; Muller, 1932; Gerrish and Lenski, 1998). Second, selection for beneficial mutations causes the fixation of linked deleterious mutations as a side effect—a process known as hitchhiking (Maynard Smith and Haigh, 1974). Third, as deleterious mutations become

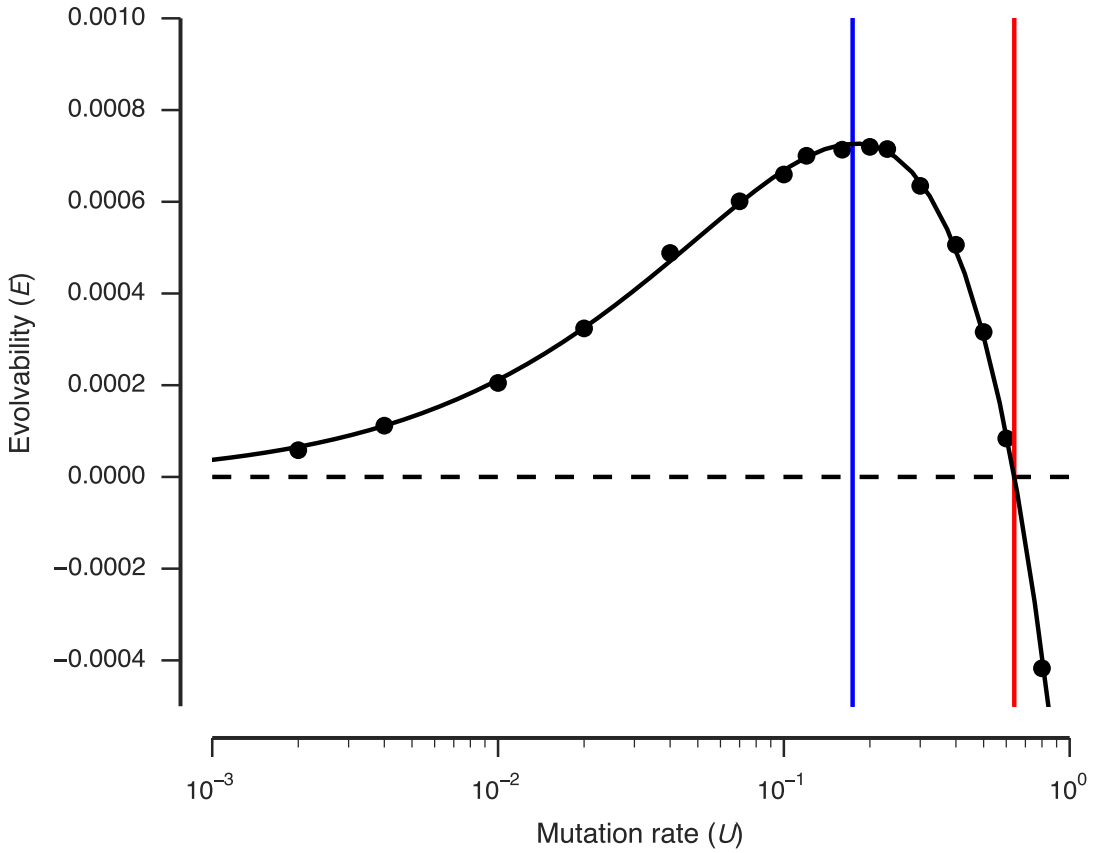

Figure 4.1: **Evolvability** (E) **is maximized at an intermediate mutation rate** (U) **in asexual populations.** Values are average E of 18 ± 9 (mean $\pm$ standard deviation) populations evolving under constant U and R. The evolvability of a population is measured as the slope of $\ln \overline{W}$ on time over 10^4 generations. Error bars show 95% confidence intervals (c.i.). Lines were obtained by fitting Equation 4.1 to values of E and U for a given value of R using nonlinear least squares. All coefficients of determination were $\geq 99.7\%$. The parameter values for the simulations are shown in Table 2.1 except that U_0 was set to the values of U shown and were not allowed to evolve (i.e., $p_m = p_a = 0$). $R = 0$ and was not allowed to evolve ($p_{r+} = p_{r-} = 0$). The vertical blue and red lines show U_{opt} and U_{crit}, respectively.

more abundant they begin to accumulate stochastically—a process known as Muller's ratchet (Muller, 1964; Gordo and Charlesworth, 2000a,b). Fourth, selection against deleterious mutations removes some linked-beneficial mutations from the population as a side effect—a process known as background selection (Charlesworth, 1994; Peck, 1994). The four processes are known collectively as Hill-Robertson interference (Hill and Robertson, 1966; Felsenstein, 1974; Comeron *et al.*, 2008).

A further increase in U causes E to reach a maximum value ($E_{\max}$), beyond which the gain in E caused by an increased supply of beneficial mutations is outweighed by the loss in E caused by an intensification of Hill-Robertson interference; we refer to the mutation rate at which $E = E_{\max}$ as *optimal* (Figure 4.1, $U_{\text{opt}} = 0.174 \pm 0.007$, estimate and 95% confidence interval, c.i.; see **Section 4.2 Evolvability** for more details).

Increasing U beyond the optimal value causes E to decrease. Eventually, a *critical* mutation rate, U_{crit}, is reached at which $E = 0$. On average, populations evolving at $U = U_{\text{crit}}$ do not adapt (Figure 4.1, $U_{\text{crit}} = 0.64$). Increasing U beyond U_{crit} causes the population to go extinct because $E < 0$. Next, I investigate how recombination influences evolvability.

4.3.2 Recombination promotes evolvability

Weismann's hypothesis (Weismann, 1887; Kondrashov, 1993; Burt, 2000) posits that sex increases evolvability because sex reshuffles genes to create individual variation upon which natural selection acts. To test this hypothesis, I investigated how the

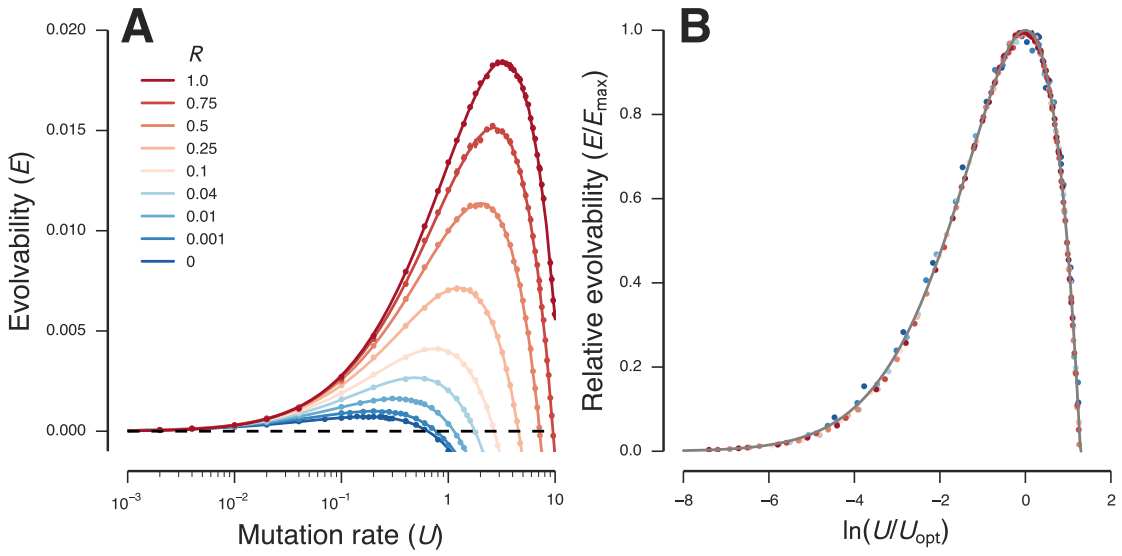

Figure 4.2: **Both the mutation rate and the recombination rate influence evolvability.** (A) Evolvability (E) increases with the recombination rate (R) and is maximized at an intermediate mutation rate (U). Values are average E of 18 ± 9 (mean $\pm$ standard deviation) populations evolving under constant U and R. The evolvability of a population is measured as the slope of $\ln \overline{W}$ on time over 10^4 generations. Error bars show 95% c.i.. Lines were obtained by fitting Equation 4.1 to values of E and U for a given value of R using nonlinear least squares. All coefficients of determination were $\geq 99.7\%$. The parameter values for the simulations are shown in Table 2.1 except that U_0 and R_0 were set to the values of U and R shown and were not allowed to evolve (i.e., $p_m = p_a = p_{r+} = p_{r-} = 0$). (B) The recombination rate does not change the relationship between relative evolvability ($E/E_{\max}$) and mutation rate ($\ln U - \ln U_{\text{opt}} = u\sigma$, see Equation 4.1). Both $E_{\max}$ and U_{opt} were estimated from the fits described in (A). The skew and scale parameters are approximately invariant with R: $\psi = -2.90 \pm 0.23$ and $\sigma = 1.218 \pm 0.021$ (mean $\pm$ s.d. of estimates for different values of R); the gray line shows $E/E_{\max}$ from Equation 4.1 with these values of ψ and σ.

recombination rate (R) influences the relationship between E and U described above if neither U nor R are allowed to evolve.

Increasing the recombination rate in the range $0 \leq R \lesssim 1$ has two effects on the relationship between E and U (Figure 4.2A). First, for any value of U, E increases with increasing R, in agreement with Weismann's hypothesis. Second, raising R increases both the optimal and the critical values of U. Both effects arise, presumably, because recombination reduces Hill-Robertson interference (Barton and Otto, 2005; Keightley and Otto, 2006; Hartfield *et al.*, 2010; Iles *et al.*, 2003).

Increasing the recombination rate beyond $R \approx 1$ has no effect on the relationship between U and E. Thus, the optimal recombination rate is $R_{\text{opt}} \gtrsim 1$. The exact pattern of saturation of R_{opt} is a function of the number of fitness loci, L, used in my simulations: increasing L causes E_{max} to increase (Figure 4.3); I predict that the lower bound of R_{opt} will also increase with L.

The results suggest that recombination promotes evolvability. Recombination disrupts the linkage disequilibrium between the mutator alleles and novel beneficial mutations. Thus, recombination may allow populations to evolve high evolvability without the cost of high mutational load.

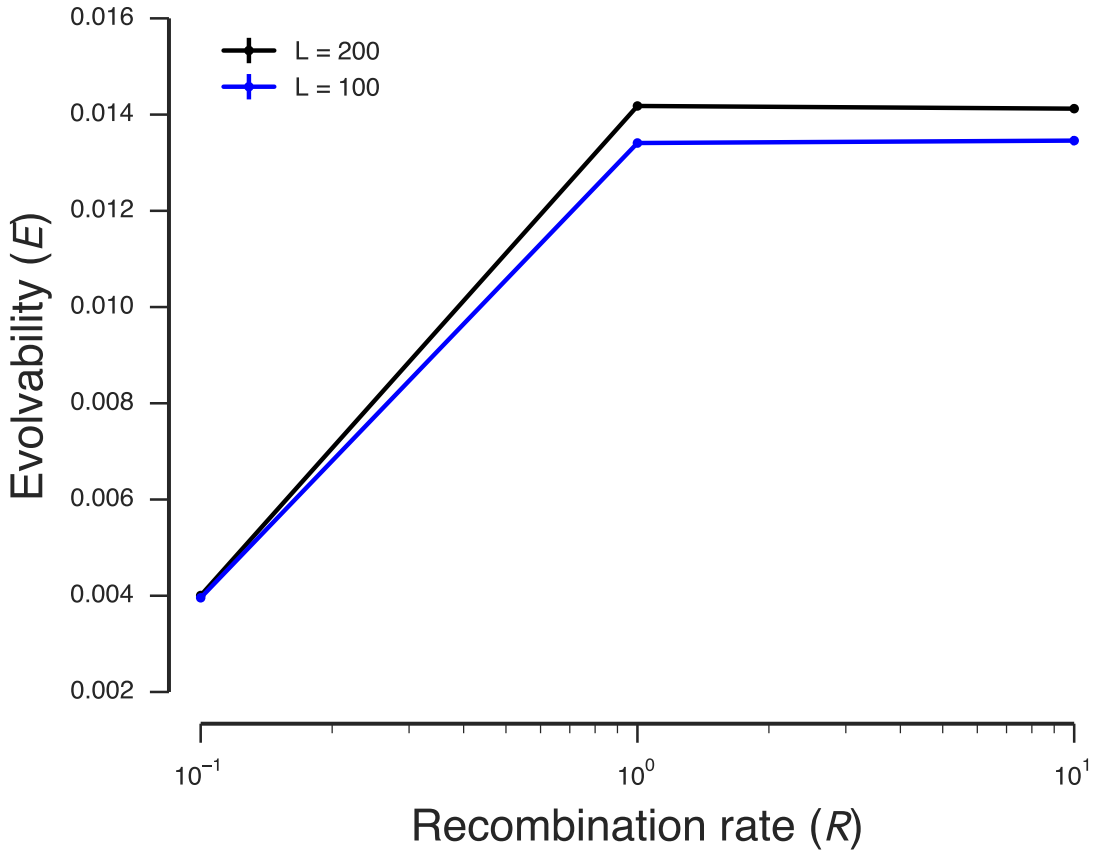

Figure 4.3: **Populations with larger number of loci evolve higher evolvability.** The parameter values are shown in Table 2.1 except R are set to the values shown and were not allowed to evolve. The number of loci L is set to 200 in black line.

4.4 Discussion and conclusion

In this chapter, I study the effects of mutation rate and recombination rate on evolvability when both rates are kept constant during evoluton. I first explored a relatively large range of mutation rate in asexual populations (Figure 4.1). I found that evolvability can reach its maximum at intermediate mutation rate. I referred that mutation rate as optimal mutation rate (U_{opt}). Also, at very high mutation rate, populations show negative evolvability. This mutation rate was termed as critical mutation rate (U_{crit}). The results confirm earlier theoretical and simulation results on asexual populations (Orr, 2000; Bachtrog and Gordo, 2004; Gerrish *et al.*, 2013). The results reveal patterns of the relationship between the mutation rate and evolvability, which can be used as a reference model for further investigations.

The discovery of critical mutation rate leads to a question – can mutation rate evolve higher than the critical mutation rate even if the population evolves negative evolvability? As introduced in Section 3.4 in Chapter 3, populations that experience the "mutation-rate catastrophe" evolve intolerably high mutation rate. In this scenario, populations that go extinct evolve negative evolvability. Here, I propose a hypothesis that populations go extinct if their mutation rates evolve higher than the critical mutation rate. I will continue investigating this hypothesis in Chapter 5.

I also studied the effects of both mutation and recombination rates on evolvability when both rates are not allowed to evolve (Figure 4.2). When I introduced recombination into the populations, I found similar patters as shown in asexual populations. No matter what recombination rate it is, populations all reach their highest

evolvability at an intermediate mutation rate. Meanwhile, the results suggest that recombination promotes evolvability by increasing both optimal (U_{opt}) and critical (U_{crit}) mutation rates.

My results are consistent with Weismann's hypothesis that sex increases evolvability Weismann (1887). Briefly, Weismann's hypothesis posits that sex makes natural selection more efficient because it causes an increase in the additive genetic variance in fitness by breaking up deleterious combinations of alleles within or among loci (Weismann, 1887; Kondrashov, 1993; Burt, 2000). In agreement with Weismann's hypothesis, Colegrave *et al.* (2002) found that a single round of sexual reproduction in *Chlamydomonas reinhardtii* increased the short-term evolvability of a population in novel environments.

These results imply the advantage of sex since sexual populations display higher evolvability than their asexual counterparts under the same constant mutation rates. It leads to the question that, if the recombination rate is allowed to evolve, will populations evolve higher recombination rate for higher evolvability? This problem will be further investigated in Chapter 5.

Another surprising pattern from this results is that the recombination rate—and, therefore, the strength of Hill-Robertson interference—has no effect on the relationship between *relative* evolvability ($E/E_{\max}$) and U (Fig. 4.2B). The causes of this pattern has not been understood and will be continue investigating in future study. This result indicates that selection for adaptation rate should act similarly on U regardless of the value of R.

Overall, I demonstrated the relationships among mutation rate, recombination rate and evolvability systematically. The results are consistent with earlier theories on the effects of mutation and recombination rates on evolvability (Fisher, 1930b; Muller, 1932; Hill and Robertson, 1966; Gerrish *et al.*, 2013) and provide quantitative references for various combinations of mutation and recombination rates. I will use these findings to further study the effects of mutation and recombination rates on evolvability when I allow one or both rates to evolve in next chapter.

Chapter 5

Selection for evolvability can optimize the recombination rate but not the mutation rate

5.1 Introduction

In Chapter 4, I studied the effects of mutation and recombination rates on evolvability when both rates are kept constant during evolution. However, both rates evolve in nature.

Genotypes with high mutation rate (i.e., "mutators") have been found in both natural (Gross and Siegel, 1981; LeClerc *et al.*, 1996, 1998; Matic *et al.*, 1997; Oliver

et al., 2000; Björkholm *et al.*, 2001; Denamur *et al.*, 2002; Giraud *et al.*, 2002; Richardson *et al.*, 2002; Prunier *et al.*, 2003) and experimental populations (Cox and Gibson, 1974; Chao and Cox, 1983; Chao *et al.*, 1983; Mao *et al.*, 1997; Sniegowski *et al.*, 1997; Giraud *et al.*, 2001; Notley-McRobb *et al.*, 2002; Thompson *et al.*, 2006; Pal *et al.*, 2007). Mutator phenotypes are often caused by mutations in genes involved in DNA repair systems (see Chapter 1 for more details on mutator alleles).

Recombination rates have also evolved extensively. For example, several lineages of *Daphnia pulex* consist of obligately asexual females that have lost the ability to undergo meiosis but retain the ability to produce males, some of which can interbreed with sexual females and produce obligately asexual female offspring (Lynch *et al.*, 2008; Eads *et al.*, 2012). Therefore, these asexual lineages can, in principle, "convert" sexual lineages to asexuality. Recent surveys of mammals (Dumont and Payseur, 2008) and angiosperms capable of sexual reproduction (Tiley and Burleigh, 2015) found 7- and 8-fold variation in genomic recombination rate, respectively.

As studied in Chapter 4, mutation and recombination rates are both important determinants of evolvability. Meanwhile, selection for evolvability has been proposed to contribute to the evolution of both the mutation rate and the recombination rate (Taddei *et al.*, 1997; Tenaillon *et al.*, 1999; Weismann, 1887; Colegrave *et al.*, 2002). Populations may optimize mutation and recombination rates for high evolvability.

However, the hypothesis that selection for evolvability drives the evolution of evolvability is controversial for several reasons:

- It is likely to be weak (Partridge and Barton, 2000).

- Natural selection lacks foresight (Dickinson and Seger, 1999; Sniegowski and Murphy, 2006).
- It requires group selection or clade selection (Kirschner and Gerhart, 1998; Dickinson and Seger, 1999; Brookfield, 2001; Sniegowski and Murphy, 2006; Lynch, 2007) but see (Wagner, 1981).
- Recombination will break the association between the high evolvability allele and the beneficial mutations or gene combinations it generates (Partridge and Barton, 2000; Sniegowski and Murphy, 2006; Lynch, 2007).
- Most mutations and novel combinations of genes are likely to be deleterious (Partridge and Barton, 2000; Sniegowski and Murphy, 2006).
- Lack of comparative evidence (Lynch, 2007).

Although mutation and recombination rates both evolve and influence evolvability, the extent to which the evolution of either rate is actually driven by selection for evolvability remains an open question.

Selection for evolvability can favor the spread of mutator alleles in large asexual populations, but mutator alleles can also fix because of genetic drift (Lynch, 2010) or hitchhiking with the beneficial alleles they help generate (Taddei *et al.*, 1997; Tenaillon *et al.*, 1999). One study using digital organisms found that natural selection fails to optimize mutation rates when populations evolve on rugged fitness landscapes (Clune *et al.*, 2008). Even when fitness landscapes are smooth, the spread

of mutator alleles can raise mutation rates to intolerably high levels, ultimately driving populations to extinction—a phenomenon dubbed "mutation-rate catastrophe" (Gerrish *et al.*, 2007).

The coevolution of the two rates has rarely been investigated. However, there is evidence that changes in one rate can influence the evolution of the other rate. For example, even low levels of genetic exchange and recombination can prevent the spread of mutator alleles by breaking up the associations between mutator alleles and the beneficial alleles they bring forth (Tenaillon *et al.*, 2000). Another study found that allowing the deleterious mutation rate to evolve can favor asexual reproduction (Sloan and Panjeti, 2010).

Here I use individual-based simulations on a smooth fitness landscape to investigate the extent to which selection for evolvability can optimize mutation and recombination rates. Figure 4.2 showed that intermediate mutation rate and high recombination rate can maximize evolvability. It leads to two predictions – (1) selection for evolvability will favor the intermediate mutation rates, at which evolvability is maximized; (2) selection for evolvability will maximize recombination rate for high evolvability. I then test these predictions by allowing mutation rate and/or recombination rate to evolve. I find that selection for evolvablity can optimize the recombination rate but not the mutation rate.

5.2 Model

I used the model described in Chapter 2 with evolvable mutation rate and/or recombination rate. The parameters for simulations in this chapter follow the default parameters in Table 2.1 except where noted.

Equilibrium mutation rate. The mutation rate at equilibrium, $\widehat{U}$, was estimated from multiple replicate time series of $\overline{U}$. I discarded the initial two thirds of all time series as a burn-in period. I then pooled the remaining one third of all time series and tested for a temporal trend in three population statistics of U using linear regression: mean, standard deviation (s.d.), and skewness of U within populations. If there was a statistically significant trend in any statistic ($P < 0.05$), I ran the same number of simulations for a longer evolutionary time and repeated the procedure until statistical significance disappeared. I averaged the values of $\overline{U}$ of the last one third of each replicate time series and estimated $\widehat{U}$ as the grand mean of the replicate means.

Extinction. Since my model employs soft selection, populations never actually go extinct. I define extinction as a population evolving a mean fitness of $\overline{W} < 10^{-8}$.

5.3 Results

5.3.1 Selection for evolvability fails to optimize the mutation rate of asexual populations

Intuitively, a population evolving under selection for evolvability might be expected to evolve an equilibrium mutation rate of $\widehat{U} = U_{\text{opt}}$ because that corresponds to maximum evolvability. However, this prediction may be incorrect for two reasons. First, because selection for adaptation rate is asymmetric (Figure 5.4). Urban *et al.* (2013) have recently shown that if the mean phenotype of a population is at the peak of an asymmetric fitness landscape then individuals on the flat side of the fitness peak will be fitter than individuals on the steep side of the fitness peak. In other words, there will be directional selection towards the flat side of the fitness peak. If the asymmetric selection is dominant, I predict that asymmetric selection will cause $\widehat{U} \lesssim U_{\text{opt}}$. Second, because there is a mutational bias towards high U in my simulations (Urban *et al.*, 2013): the rate of mutator mutations is 100-fold higher than the rate of antimutator mutations ($p_m/p_a = 100$; Table 2.1). If mutational bias is dominant, I predict that the mutational bias will cause $\widehat{U} \gtrsim U_{\text{opt}}$.

To predict the mutation rate at equilibrium under selection for evolvability alone, taking into account asymmetric selection and mutation bias, I used a simplified version of my evolutionary model where the mutation rate, U, is the sole determinant of individual fitness. I modeled evolvability, E, using Equation 4.1 (Figure 4.2) and assumed that fitness was given by: $W = E$ if $E > 0$ and $W = 0$ otherwise. I

allowed U to evolve under the control of a single modifier locus, as in the main model. Applying this approach to the asexual case ($R = 0$), I obtained a prediction of $\widehat{U} = 0.1843 \pm 0.0002$ (mean and 95% c.i., based on on 200 runs for 3×10^4 generations; see Section **5.2, Equilibrium mutation rate** for more details). This prediction is slightly higher than $U_{\text{opt}} = 0.174$.

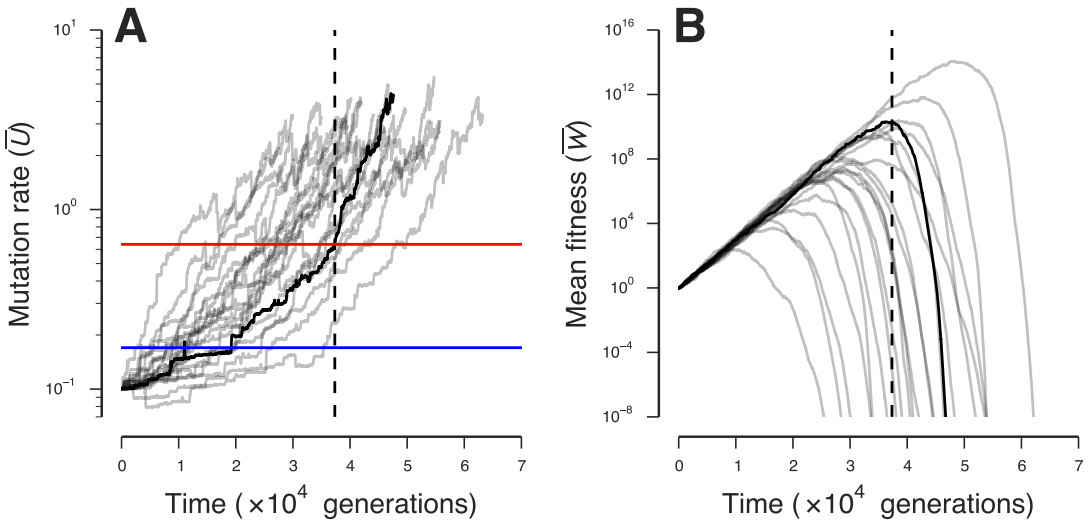

Figure 5.1: **Asexual populations experience a mutation-rate catastrophe when mutation rate evolves higher than critical mutation rate** (U_{crit})**.** Values show the (A) mean mutation rate, $\overline{U}$, and (B) mean fitness, $\overline{W}$, of 20 asexual populations with an evolvable mutation rate. The parameter values are shown in Table 2.1 except that $R_0 = 0$ and R was not allowed to evolve ($p_{r+} = p_{r-} = 0$). The horizontal blue and red lines in (A) show U_{opt} and U_{crit}, respectively (see Figure 4.1). The solid black lines highlight the mutation-rate catastrophe in one population. The vertical dashed lines show the time when this population achieves $\overline{U} = U_{\text{crit}}$ (A) and $\overline{W}$ reaches its maximum value (B). An additional 64 populations show a similar pattern to the 20 shown here. The evolutionary dynamics of $\overline{U}$ and $\overline{W}$ are similar to those shown in Figure 1B of Gerrish *et al.* (2007), even though they differ from the parameter values quoted in the legend of that figure. The discrepancy is attributable to a typo in Gerrish et al. (2007) (P. J. Gerrish, personal communication).

I tested this prediction by studying 84 asexual populations like those described in Figure 4.2 ($R = 0$) but with an evolvable mutation rate. All populations started with a suboptimal mutation rate of $U_0 = 0.1$ (Figure 5.1A). On average, populations reached the predicted $\widehat{U}$ within approximately 10^4 generations (Figure 5.1A, blue line). But populations did not remain at the predicted $\widehat{U}$. Rather, mutation rates continued to rise steadily and reached a critical mutation rate ($U_{\text{crit}} = 0.644$ calculated from data in Figure 4.1; Figure 5.1A, red line) within approximately 2.5×10^4 generations. From Figure 4.1, I learn that populations can evolve negative evolvability if the mutation rate is higher than the critical mutation rate (U_{crit}). As a result, the mean fitness of the populations began to decline (Figure 5.1B), causing populations to go extinct at $4.1 \pm 0.83 \times 10^4$ generations (mean $\pm$ s.d.). In Figure 5.1A, I randomly chose a population (solid black line) as an example and compared the expected U_{crit} (red line) with the mutation rate of the chosen population. I found out the generation at which mutation rate hit the U_{crit} and aligned it in Figure 5.1B (dashed vertical line). As shown, fitness started declining right after the generation at which mutation rate evolved to U_{crit}.

These results show that selection for evolvability cannot optimize the mutation rate of asexual populations in my model with the chosen parameter settings. The runaway increase in $\overline{U}$ followed by extinction has been termed "mutation-rate catastrophe" and is robust to changes in many of the parameters in Table 1 (Gerrish *et al.*, 2007). Next, I investigate whether the mutation-rate catastrophe is also robust to two mutational parameters — antimutator effects and mutation rates to antimutator.

5.3.2 Large antimutator effects and reduced mutation bias can arrest the "mutation-rate catastrophe"

What causes the mutation-rate catastrophe? One possibility is that although selection to lower the mutation rate is operating, populations lack sufficient genetic variation in mutation rate, and therefore cannot lower the mutation rate efficiently. To test this hypothesis, I introduced additional genetic variation in mutation rate in two ways. First, by increasing the effect of antimutator mutations ($\bar{s}_{\mathrm{a}}$). Second, by reducing the rate mutation bias towards mutator mutations through increasing the antimutator mutation rate (U_{a}).

I began by exploring only the antimutator effects within a range from $\bar{s}_{\mathrm{a}} = 0.03$ to 0.4 when kept other mutator parameters the same as in Table 2.1. I measured the proportion of populations that go extinct under different parameter combinations. The results reveal that, the smaller the antimutator effects, the higher the proportion of populations go extinct (Figure 5.2A). Populations evolving with small antimutator effects are not able to efficiently lower the mutation rate to a tolerable level. Populations go extinct due to high mutational load. As $\bar{s}_{\mathrm{a}}$ increased over 0.07, the proportion of extinction started delining, i.e., more and more populations are able to evolve mutation rates at tolerable levels. When $\bar{s}_{\mathrm{a}}$ is higher than 0.15, all the 100 populations were able to avoid catastrophe. All populations evolving with 100-fold rate mutational bias ($U_{\mathrm{m}} = 10^{-3}$, $U_{\mathrm{a}} = 10^{-5}$). I noticed that, even with $\bar{s}_{\mathrm{a}} = 0.1$ and $\bar{s}_{\mathrm{m}} = 0.05$, there were still 30% of the populations go extinct regardless the fact that $\bar{s}_{\mathrm{a}}$ is twice of $\bar{s}_{\mathrm{m}}$. The results show that, in my model, the antimutator

effect plays an important role in the mutation-rate catastrophe.

Other than antimutator effect, mutation rate to antimutator (U_{a}) also determines how efficient populations can purge the mutators. Next, I explored U_{a} within a range from 10^{-5} to 10^{-3} while kept other parameters the same as in Table 2.1 ($U_{\mathrm{m}} = 10^{-3}$, $\bar{s}_{\mathrm{a}} = \bar{s}_{\mathrm{m}} = 0.05$). Similarly, I also observed the transition in proportion of extinction. When U_{a} increased higher than 3×10^{-5}, some of the populations are able to avoid mutation-rate catastrophe. When $U_{\mathrm{a}} \geq 9 \times 10^{-5}$, none of the populations goes extinct. In terms of mutation bias as described in Chapter 3, changing in U_{a} is reducing the rate bias. The results show that, when $\bar{s}_{\mathrm{a}} = \bar{s}_{\mathrm{m}}$, slight reduction in rate bias can arrest the catastrophe.

Both results from $\bar{s}_{\mathrm{a}}$ and U_{a} suggest that, when populations are able to purge mutators faster, they are more likely to arrest mutation-rate catastrophe.

5.3.3 Selection for evolvability also fails to optimize the mutation rate of sexual populations

The mutation-rate catastrophe shows that selection for evolvability cannot optimize the mutation rate of asexual populations. Is the same true in sexual populations? Figure 4.2 indicates that sexual populations are expected to evolve even higher mutation rates than asexuals ones. Recombination might, however, prevent the mutation-rate catastrophe because it interferes with the spread of mutator alleles (Tenaillon *et al.*, 2000). To evaluate the extent to which selection for evolvability can optimize the $\overline{U}$ of sexual populations, I repeated the simulations described in the previous

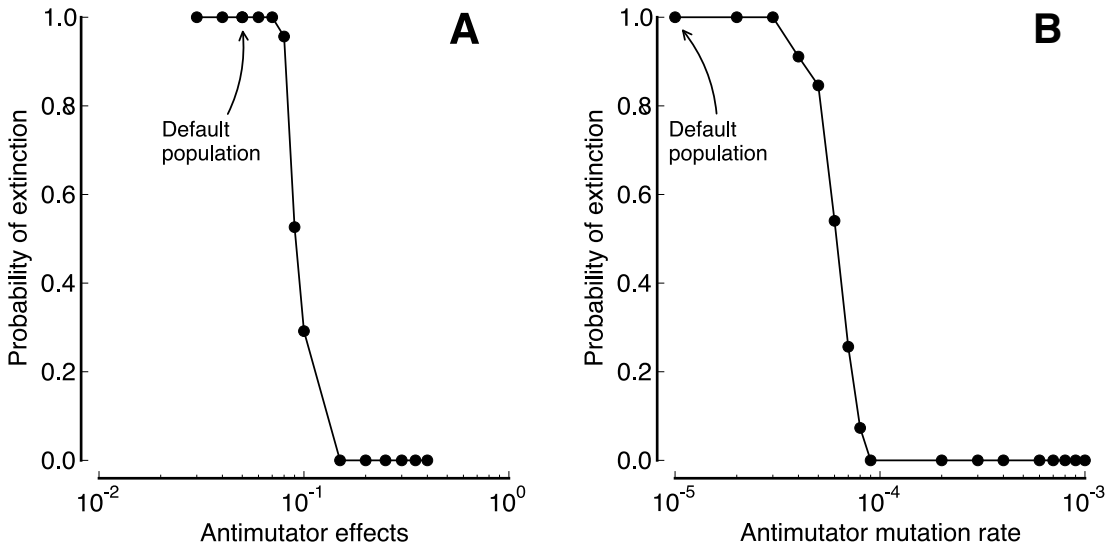

Figure 5.2: **Large antimutator effects and reduced rate mutation bias can prevent mutation-rate catastrophe.** (A) Populations with large antimutator effects extinct less often. The parameters are the same as in Table 1 except the antimutator effects (s_a) vary as shown. (B) Populations with high antimutator mutation rates extinct less often. The parameter are the same as in Table 1 except the mutation rates of antimutator (U_a) vary as shown. The default populations are the same populations as shown in Figure 5.1.

section but using populations with a broad range of fixed recombination rates, R (Figure 5.3).

When the recombination rate was low ($R \lesssim 0.01$) all populations evolved supercritical mutation rates and went extinct within 7×10^4 generations. Populations with higher R evolved higher $\overline{U}$ before going extinct (Figure 5.3A, gray points). Thus, the mutation-rate catastrophe persists in the presence of low levels of recombination. At higher recombination rates ($0.01 \lesssim R \lesssim 0.1$), some populations were still alive after 7×10^4 generations. However, within this range of R, recombination appears to have delayed, rather than prevented, the mutation-rate catastrophe; populations that did not go extinct also evolved supercritical $\overline{U}$ suggesting that they would have gone extinct eventually. When $R \gtrsim 0.2$, all populations were still alive after 7×10^4 generations. In addition, these populations evolved subcritical $\overline{U}$ indicating that, within this range of R, sex actually prevented the mutation-rate catastrophe (Figure 5.3).

These results indicate that recombination improves the ability of selection for evolvability to optimize the mutation rate to the extent that it prevents the mutation-rate catastrophe. But is the equilibrium mutation rate, $\widehat{U}$, of sexual populations consistent with optimization by selection for evolvability? Based on the relationship between E and U, I predict that a population with $R = 1$ (optimal recombination rate, Figure 4.2) evolving only under selection for evolvability will evolve an equilibrium mutation rate of $\widehat{U} = 4.017 \pm 0.004$ (mean and 95% c.i., based on 200 runs for 2.5×10^4 generations; Figure 5.4A, dashed line). To test this prediction, I evolved populations like those summarized in Figure 5.3 with $R = 1$ but starting

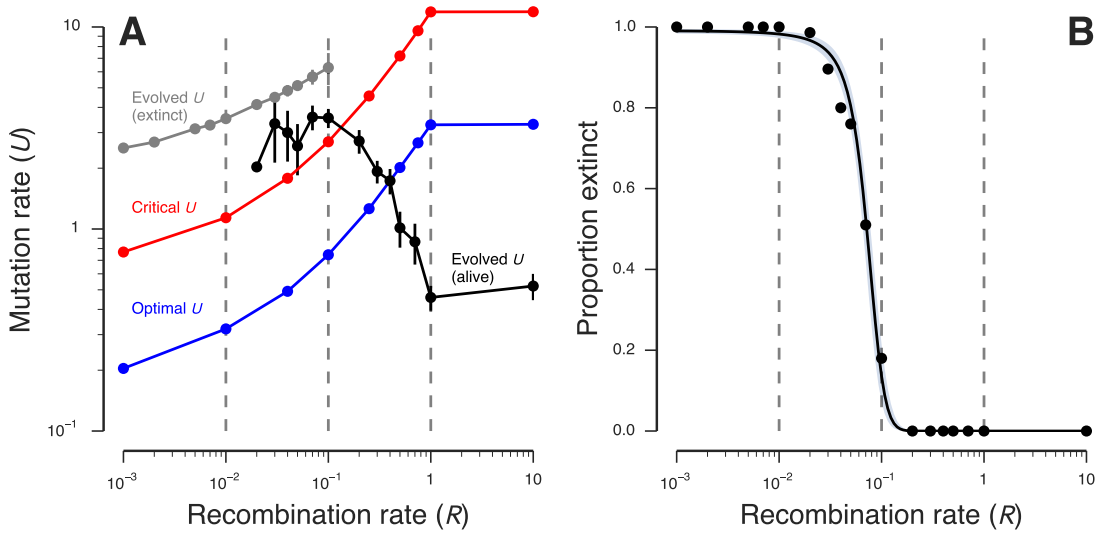

Figure 5.3: **High recombination rates prevent the mutation-rate catastrophe** (A) Blue and red circles show U_{opt} and U_{crit}, respectively, corresponding to different values of R. Both parameters were estimated from the data summarized in Figure 4.2A using nonlinear least-squares (see **Section 4.2, Evolvability**). Gray points indicate the mean mutation rates, $\overline{U}$, evolved by populations with different values of R that went extinct within 7×10^4 generations; black points show $\overline{U}$ of populations that were still alive after 7×10^4 generations. The mutation rate of each population was estimated as the mean value of $\overline{U}$ during the last 10^3 generations, either before extinction or before 7×10^4 generations. Error bars are 95% c.i. (B) Proportion of populations that go extinct within 7×10^4 generations. The line shows a logistic regression model. The shaded region indicates the 95% confidence region based on 10^4 bootstrap samples. The parameter values are shown in Table 2.1 except that R_0 was set to the value of R shown and was not allowed to evolve ($p_{r+} = p_{r-} = 0$). For a given value of R we simulated at least 50 populations; the gray and black points in (A) are based on subsets of these populations.

from a broad range of initial mutation rates, U_0. Figure 5.4B summarizes the results for three values of U_0 and shows that the actual equilibrium mutation rate is much lower than that predicted under the hypothesis that $\overline{U}$ is evolving solely under selection for adaptation rate: $\widehat{U} = 0.975 \pm 0.102$ (mean and 95% c.i., based on 10 runs for 4×10^4 generations, middle trend in Figure 5.4B; Figure 5.4A, solid line).

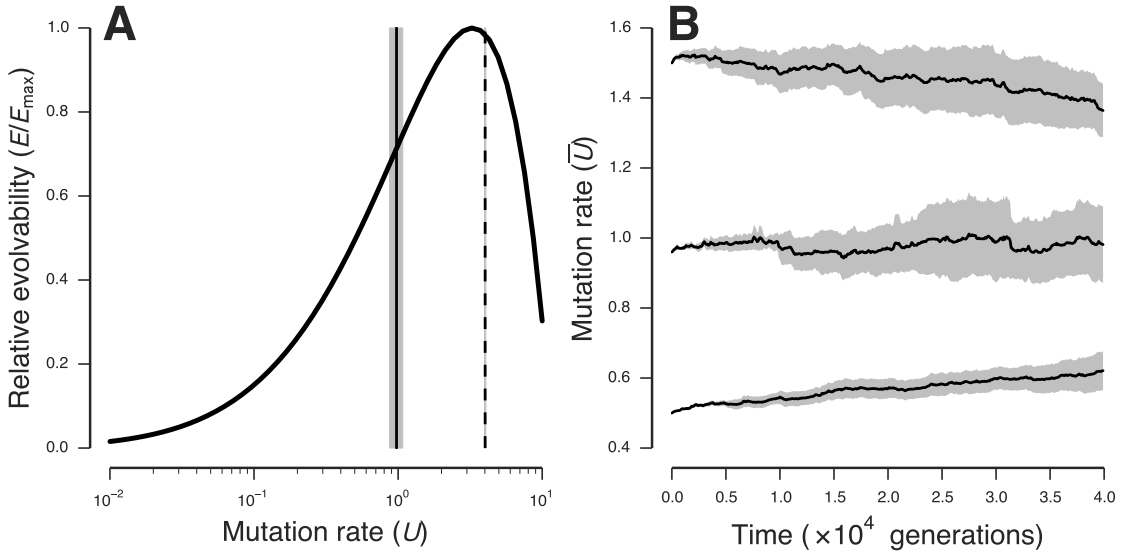

Figure 5.4: **Selection for evolvability fails to optimize the mutation rate of sexual populations** (A) Relationship between relative adaptation rate ($E/E_{\max}$) and mutation rate (U) for sexual populations with an optimal recombination rate $R = 1$ (modified from Figure 4.2). The dashed vertical line shows the equilibrium mutation rate, $\widehat{U}$, that would be expected to evolve if only selection for adaptation rate were to act on $\overline{U}$. The solid vertical line shows the actual $\widehat{U}$ evolved in our model based on the data in (B); shading indicates the 95% c.i. of the estimate. (B) Evolution of mutation rate, $\overline{U}$, in populations like those summarized in Figure 5.3 with $R = 1$ but starting from three different initial mutation rates, U_0. The equilibrium-mutation rate is shown by the solid vertical line in (A).

A possible explanation for this failure of optimization is that selection for evolvability is not the only type of selection acting on $\overline{U}$ and is a weak selection. To test this hypothesis, I began by measuring direct selection on U in populations at equilibrium for $\overline{U}$ and found that they experienced weak selection for lower U (standardized linear selection gradient: $\beta = -0.00213 \pm 0.0003$, mean and 95% c.i.). These results suggest that the equilibrium mutation rate, $\widehat{U}$, results from a balance between selection for evolvability acting to increase $\overline{U}$ and a cost of high mutation rate acting to decrease $\overline{U}$.

5.3.4 The mutation bias influences the equilibrium value of mutation rate in sexual populations

The results from Chapter 3 suggest that the previous section ignored another potential cause of the equilibrium value: mutation bias. Here I test whether mutation bias can influence the equilibrium value of mutation rate in sexual populations and how strong it is compared to the selection for evolvability.

I began by evaluating how beneficial mutations, which determine the strength of selection for evolvability, influence the relationship between mutation rate and evolvability (Figure 5.5). I did a similar analysis to that summarized in Figure 4.2 using smaller sexual populations ($N = 10^3$, $R = 1$) either with ($p_b = 10^{-3}$) or without ($p_b = 0$) beneficial mutations. Populations evolved with beneficial mutations (Figure 5.5A) showed a similar relationship between mutation rate and evolvability to that in Figure 4.2. Evolvability is maximized at an intermediate mutation rate. In contrast,

the populations evolved incapable of generating beneficial mutations showed more and more negative evolvability as mutation rate increased. This pattern was due to the accumulation of deleterious mutations (Figure 5.5B).

If the mutation rate is allowed to evolve in these two scenarios, what equilibrium mutation rate will it evolve? If selection for evolvability can optimize mutation rate, mutation rate is expected to evolve to U_{opt}. As I showed in Figure 5.4, selection for evolvability fails to optimize the mutation rate of sexual populations because the selection is weak and indirect. I predict that the mutation rate will also evolve to a suboptimal level here. In addition to selection, mutation bias is also expected to influence the evolved mutation rate. I predict that mutation rate will evolve to a higher equilibrium value in populations with a rate mutation bias than in populations without any mutation bias.

To test these predictions, I evolved sexual populations ($R = 1$) in four scenarios: (1) with 10-fold rate mutation bias ($p_{\text{m}}/p_{\text{a}} = 10$) and beneficial mutations ($p_b = 10^{-3}$) (Figure 5.6A); (2) with 10-fold rate mutation bias ($p_{\text{m}}/p_{\text{a}} = 10$) and without any beneficial mutations ($p_b = 0$) (Figure 5.6B); (3) without mutation bias ($p_{\text{m}} = p_{\text{a}}$) and beneficial mutations ($p_b = 10^{-3}$) (Figure 5.6C); (4) without mutation bias ($p_{\text{m}} = p_{\text{a}}$) and beneficial mutations ($p_b = 0$) (Figure 5.6D). In all scenarios, deleterious mutations are present in the populations ($p_d = 0.5$).

The results have four salient features. First, as expected, beneficial mutations do affect the equilibrium, however, they do so only slightly. Within each mutation bias treatment, populations that evolved with beneficial mutations (Figure 5.6A and C) all evolved slightly higher mutation rates at equilibria than populations without

beneficial mutations (Figure 5.6B and D).

Second, the mutation bias affects the evolution of mutation rate significantly. Populations that evolved with 10-fold rate mutation bias (Figure 5.6A and B) all evolved much higher mutation rates at equilibria than their counterparts without mutation bias (Figure 5.6A and B). These results indicate that mutation bias affects the evolution of mutation rates more than the natural selection.

Third, when I compare the equilibria of mutation rates with the evolvability patterns (vertical lines in Figure 5.5), all of them failed to evolve to the expected U_{opt} except when there is no mutation bias and no beneficial mutations (dark blue line in Figure 5.5B). First of all, in panel (A), the indirect selection for evolvability, together with other forces including hitchhiking, mutational load, and genetic drift, tend to optimize U at U_{opt}. The mutation bias tends to increase the evolved mutation rate. However, even when both are present (red line), the mutation rate still evolved to suboptimal level. This indicates that both natural selection and mutation bias failed to optimize mutation rates in sexual populations. When there was no mutation bias but only selection for evolvability (light blue line), mutation rate evolved much further from the U_{opt}. This suggests that mutation bias plays and important role in pushing mutation rate towards U_{opt}.

In panel (B), the U_{opt} is expected to be 0 when there is only deleterious but no beneficial mutations. Mutation bias itself is expected to increase the mutation rate. As expected, when mutation bias is present (orange line), mutation rate evolves to much higher level than U_{opt}. However, when mutation bias is absent, mutation rate evolves to much lower level, which is close to U_{opt}.

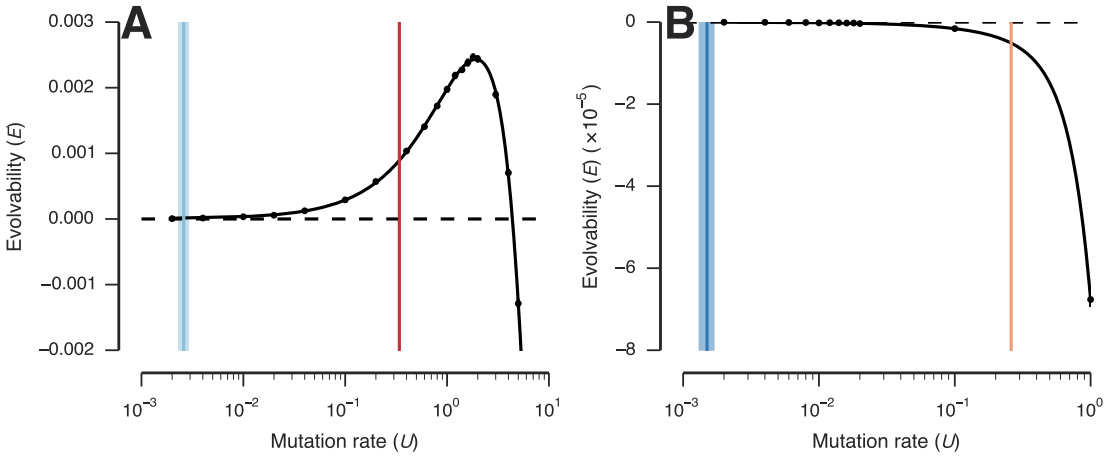

Figure 5.5: **The beneficial-mutation rate influences evolvability.** (A) shows evolvability is maximized at an intermediate-mutation rate when sexual populations ($R = 1$) evolving with 0.1% beneficial-mutation rate. Values are average E of 30 populations evolving under constant U and $R = 1$. The evolvability of a population is measured as the slope of $\ln \overline{W}$ on time over 10^4 generations. Error bars show 95% c.i.. Lines were obtained by fitting Equation 4.1 to values of E and U using nonlinear least-squares. All coefficients of determination were $\geq 99.7\%$. The parameter values for the simulations are shown in Table 2.1 except that (1) $N = 10^3$, (2) U_0 was set to the values of U shown and were not allowed to evolve (i.e., $p_m = p_a = 0$). $R = 1$ and was not allowed to evolve ($p_{r+} = p_{r-} = 0$). (B) shows evolvability is maximized at lowest mutation rate when sexual populations ($R = 1$) evolving with no beneficial mutations. Values are average E of 30 populations evolving under constant U and $R = 1$. The evolvability of a population is measured as the slope of $\ln \overline{W}$ on time over 10^4 generations. Error bars show 95% c.i.. The parameter values for the simulations are shown in Table 2.1 except that (1) $N = 10^3$, (2) U_0 was set to the values of U shown and were not allowed to evolve (i.e., $p_m = p_a = 0$), and (3) no beneficial mutations (i.e. $p_b = 0$). $R = 1$ and was not allowed to evolve ($p_{r+} = p_{r-} = 0$). Vertical lines show equilibria of mutation rates from Figure 5.6. Shadings shows 95% c.i.. Colors match the ones as in Figure 5.6.

Fourth, the mutation rates in Figure 5.6C and D were driven to very low levels, at which populations almost stop evolving due to the lack of variation. Populations in panel C and D evolve lowest mutation rate at 0.0026 ± 0.00029 and 0.0015 ± 0.0019 (mean $\pm$ 95% c.i.), separately. It means that these populations can each only gain $\sim$2 mutations at each generation. Populations are not able to reduce mutation rates further due to the "drift barrier" (Lynch, 2007).

The results above show that mutation bias instead of selection for evolvability drives the evolution of mutation rate, although mutation rate cannot be optimized when both forces are operating.

Next, I investigate how mutation influences the evolution of recombination rate.

5.3.5 Selection for evolvability can optimize the recombination rate of populations

The results summarized in Figure 4.2 indicate that selection for evolvability should lead to the evolution of an optimal recombination rate of $R_{\text{opt}} \gtrsim 1$ regardless of the mutation rate. I tested this prediction by studying populations like those described in Figure 4.2 with a constant mutation rate U but with an evolvable recombination rate. Figure 5.7A shows the results of simulations where populations started with a suboptimal recombination rate of $R_0 = 0.1$. The populations differed in their mutation rate at fitness loci but not at the recombination modifier locus. $\overline{R}$ increased in all populations, but the rate of change increased with U. Hartfield *et al.* (2010) found that both the selective advantage of a modifier of recombination and its probability

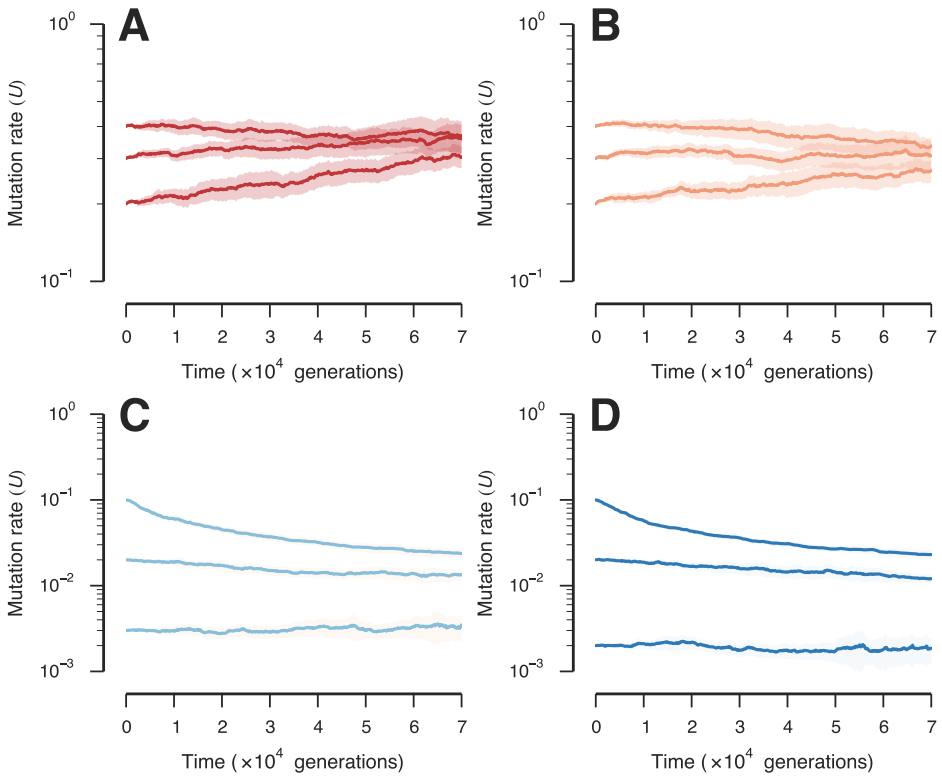

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Figure 5.6: **10-fold rate mutation bias drives populations to evolve higher equilibrium of mutation rate than populations without mutation bias.** (A) Evolution of mutation rate, $\overline{U}$, in sexual populations ($R = 1$) with 10-fold rate mutation bias ($p_{\mathrm{m}}/p_{\mathrm{a}} = 10$) and 0.1% beneficial mutation rate (p_b), starting from three different initial mutation rates, U_0. The equilibrium mutation rate is shown by the solid vertical line in 5.5 with the same color. $\widehat{U} = 0.34 \pm 0.004$ (mean ± 95% c.i.) was estimated from 30 populations (middle trend) for the last 5000 generations. (B) Evolution of mutation rate, $\overline{U}$, in sexual populations ($R = 1$) with 10-fold rate mutation bias ($p_{\mathrm{m}}/p_{\mathrm{a}} = 10$) but without beneficial mutation ($p_b = 0$), starting from three different initial mutation rates, U_0. The equilibrium mutation rate is shown by the solid vertical line in 5.5 with the same color. $\widehat{U} = 0.26 \pm 0.002$ (mean ± 95% c.i.) was estimated from 30 populations (middle trend) for the last 5000 generations. (C) Evolution of mutation rate, $\overline{U}$, in sexual populations ($R = 1$) without rate mutation bias ($p_{\mathrm{m}} = p_{\mathrm{a}}$) and 0.1% beneficial mutation rate (p_b), starting from nine different initial mutation rates, U_0. The equilibrium mutation rate is shown by the solid vertical line in 5.5 with the same color. $\widehat{U} = 0.0026 \pm 0.00029$ (mean ± 95% c.i.) was estimated from 30 populations (bottom trend) for the last 5000 generations. (D) Evolution of mutation rate, $\overline{U}$, in sexual populations ($R = 1$) without rate mutation bias ($p_{\mathrm{m}} = p_{\mathrm{a}}$) but without beneficial mutation ($p_b = 0$), starting from ten different initial mutation rates, U_0. The equilibrium-mutation rate is shown by the solid vertical line in 5.5 with the same color. $\widehat{U} = 0.0015 \pm 0.0019$ (mean ± 95% c.i.) was estimated from 30 populations (bottom trend) for the last 5000 generations.

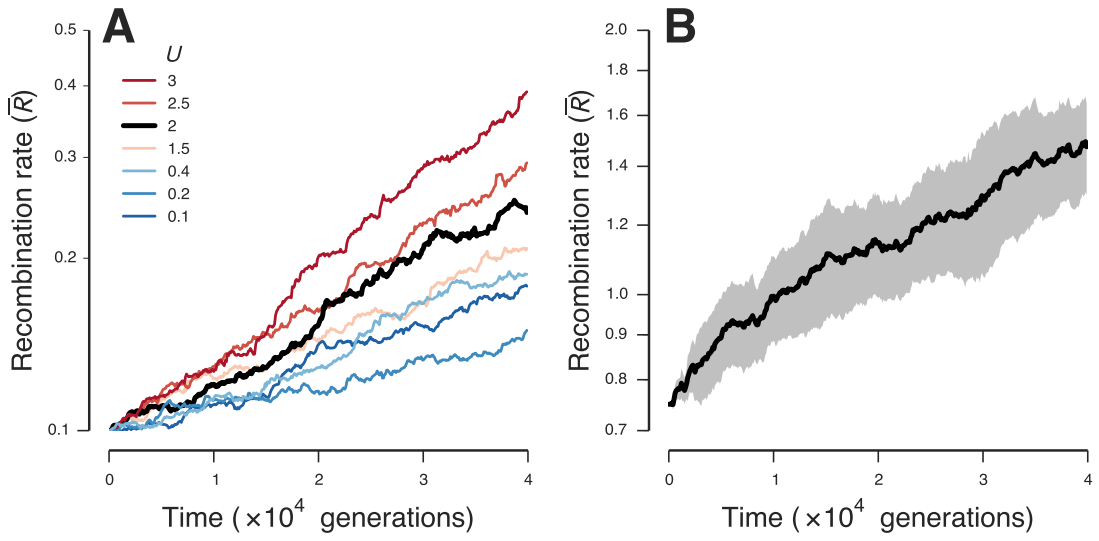

Figure 5.7: **The strength of selection for higher recombination rate increases with the mutation rate.** (A) Evolution of the recombination rate $\overline{R}$ in populations with a constant U. The parameter values are shown in Table 2.1 except that $U = U_0$ and was not allowed to evolve ($p_m = p_a = 0$). In addition, populations evolving under different values of U did not differ in the supply of mutations at the recombination modifier locus ($Up_{r+} = Up_{r-} = 10^{-3}$). Lines show mean responses of 30 populations. (B) Evolution of $\overline{R}$ with a constant $\overline{U} = 2$ but evolving from $R = 0.75$. Lines and shading show mean responses and 95% c.i. of 30 populations.

of fixation increased with the mutation rate in a similar model to mine.

Figure 5.7B shows that a population with $U = 2$ with a suboptimal recombination rate of $R_0 = 0.75$ evolves an optimal mutation rate. Thus, selection for evolvability can optimize the recombination rate in this model. Next I consider how $\overline{U}$ and $\overline{R}$ coevolve in response to selection for evolvability.

5.3.6 Selection for evolvability optimizes the recombination rate but not the mutation rate

The results so far indicate that selection for evolvability can optimize recombination rate but not mutation rate when only one of the rates is allowed to evolve. Here, I test the extent to which selection for evolvability can cause $\overline{U}$ and $\overline{R}$ to coevolve to an optimal state.

I allowed 40 populations to evolve for 7×10^4 generations with the parameters listed in Table 2.1, except with $R_0 = 10^{-2}$. The populations experienced one of two fates. First, 11 populations (27.5%) went extinct within 7×10^4 generations (Figure 5.8J). The recombination rate changed little and remained suboptimal in these populations ($\overline{R} \lesssim 0.1 \ll R_{\text{opt}}$; Figure 5.8K). In contrast, the mutation rate increased steadily to supercritical levels ($\overline{U} \gtrsim 1$; Figure 5.8L and gray solid point Figure in 5.9). In other words, these populations evolved exactly like asexual populations undergoing mutation-rate catastrophe. Second, the remaining 29 populations (72.5%) did not go extinct within 7×10^4 generations (Figure 5.8D, G). They evolved an optimal recombination rate ($\overline{R} \gtrsim 1$; Figure 5.8E, H) but a suboptimal mutation rate

($\overline{U} \approx 1$; Figure 5.8F, I and black circle/point in Figure 5.9). These populations did not go extinct since their mutation rates are lower than critical mutation rates (red line in 5.9)

The two fates are correlated with the evolution of recombination rate: populations that evolved a high $\overline{R}$ escaped extinction. The correlation appears to have a causal basis. Of the 29 populations that evolved high evolvability, 22 populations (76%) started to experience the mutation-rate catastrophe but were "rescued" by an increase in $\overline{R}$ (Figure 5.8G–I). To understand the causal relationship between R and U, I further analyzed these populations by aligning the transition point in adaptation rate together (Figure 5.10A). In 20 of these populations, the increase in $\overline{R}$ at least 5000 generations preceded the decline in $\overline{U}$ (Figure 5.10C and D). This pattern indicates that the increase in $\overline{R}$ prevents the populations evolving to supercritical mutation rates and going extinct.

5.4 Conclusion and discussion

In this chapter, I asked the question that if the selection for evolvability can optimize the mutation rate and recombination rate. The mutation-rate catastrophe phenomenon shows that mutation rate cannot be optimized at least in multiple scenarios studied here and previously (Gerrish *et al.*, 2007). Gerrish *et al.* (2007) showed that if there is recurrent mutation at a mutation rate modifier locus generating both mutators and antimutators, the spread of mutator alleles as populations adapt can

raise mutation rates to intolerably high levels, eventually driving populations to extinction. The authors interpreted this "mutation-rate catastrophe" as a result of the fact that natural selection is a "short-sighted process" that favors the short-term benefit of high mutation rate (generation of beneficial mutations) despite its long-term cost (accumulation of deleterious mutations). However, the study also assumed a strong mutation bias towards increased mutation rate (typically a 100-fold rate bias). Although the authors noted that the mutation bias was "sufficient but not necessary" for the mutation-rate catastrophe, they focused primarily on the causal role of natural selection (Gerrish *et al.*, 2007).

In chapter 4, I showed that critical mutation rate (U_{crit}) exists, beyond which populations evolve negative evolvability. I began by using the U_{crit} to investigate the fitness decline in populations that experience "mutation-rate catastrophe". As predicted, when mutation rate evolved higher than U_{crit}, fitness started declining. The results demonstrated that supercritical mutation rates directly cause the catastrophe.

Then, I tested if the mutation-rate catastrophe is robust to the change of two mutational parameters — antimutator effects on mutation rate ($\bar{s}_{\text{a}}$) and antimutator mutation rate (p_{a}). The results in Figure 5.2 showed that populations can escape from catastrophe if $\bar{s}_{\text{a}}$ or p_{a} is relatively large since they can lower the mutation rate more efficiently and drive mutation rate below U_{crit}. My results demonstrated that, rather than the causal role of natural selection as promoted by Gerrish *et al.* (2007), the biases in mutational parameters that result in supercritical mutation rate played important role in the catastrophe.

Next I investigated that if recombination can prevent catastrophe. The results in

Figure 5.4 showed that recombination improved the ability of populations to escape from catastrophe by evolving mutation rates lower than U_{crit}. This is consistent with earlier theoretical study that showed recombination can interference with the fixation of mutators (Tenaillon *et al.*, 2000).

The possible reasons that evolution for evolvability cannot optimize the mutation rate might because there are multiple forces shape the evolution of mutation rates, including evolution for evolvability, mutation bias, and genetic drift. I tested the influence of these forces and found that mutation bias affects the evolved mutation rate much more significantly than the selection for evolvability (Figure 5.5 and 5.6). This indicates that the selection for evolbability is likely weak, which supports earlier claims (Partridge and Barton, 2000). Earlier results suggested that mutation rate could be optimized in smooth fitness landscapes. However, the mutation bias was also operating in their model (Clune *et al.*, 2008). My results suggest that the mutation bias may explain their results.

Further more, when I allow both the mutation and recombination rates to evolve, my results show that selection for evolvability can optimize the recombination rate but not the mutation rate of a population (Figure 5.4). Populations with high $\overline{R}$ cannot evolve an optimal mutation rate, $\overline{U}$. This dependency of the evolution of U on the value of R is unlikely to result from selection on evolvability because the relationship between relative adaptation rate and U is approximately invariant with R (Figure 4.2B). Rather, it appears to result from a direct cost of high U.

Overall, I investigated the interactions among the evolution of evolvability, the evolution of mutation rate and the evolution of recombination rate in this chapter.

I hope my work can improve our understanding of these complex but intriguing evolutionary processes.

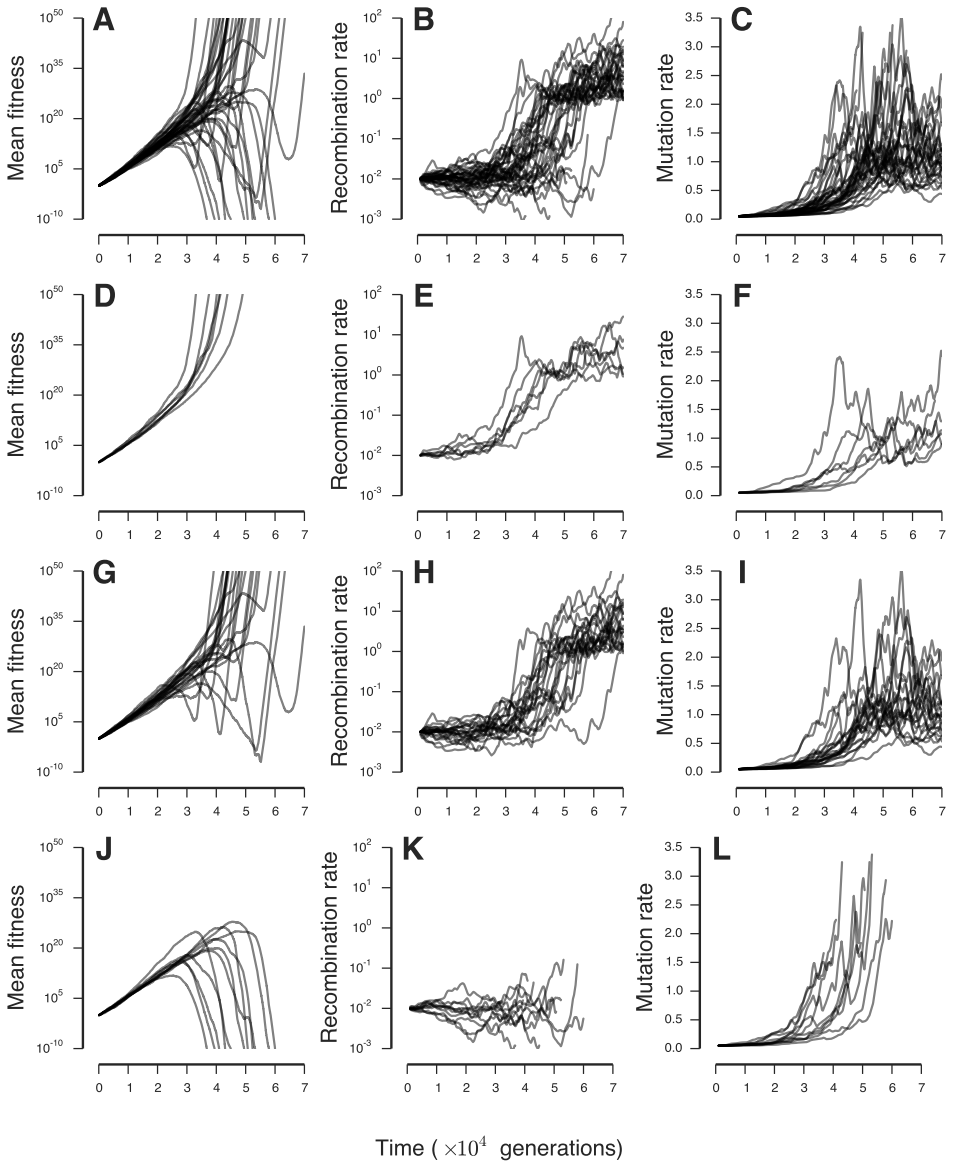

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Figure 5.8: **The evolution of recombination rate determines the fate of populations with evolvable mutation and recombination rates.** Populations were allowed to evolve for 7×10^4 generations with the parameters shown in Table 2.1, except with $R_0 = 10^{-2}$. Lines show moving averages of mean fitness ($\overline{W}$), recombination rate ($\overline{R}$), and mutation rate ($\overline{U}$) for each population with a window of 1000 generations. The first row (A–C) show the trajectories of all 40 populations. The remaining rows show subsets of these populations. (D–F) Show the trajectories of seven populations that evolve high adaptation rate without showing any signs of mutation-rate catastrophe. (G–I) Show the trajectories of 22 populations that start to undergo a mutation-rate catastrophe but are rescued by an increase in $\overline{R}$. (J–L) Show the trajectories of 11 populations that undergo a mutation-rate catastrophe and go extinct.

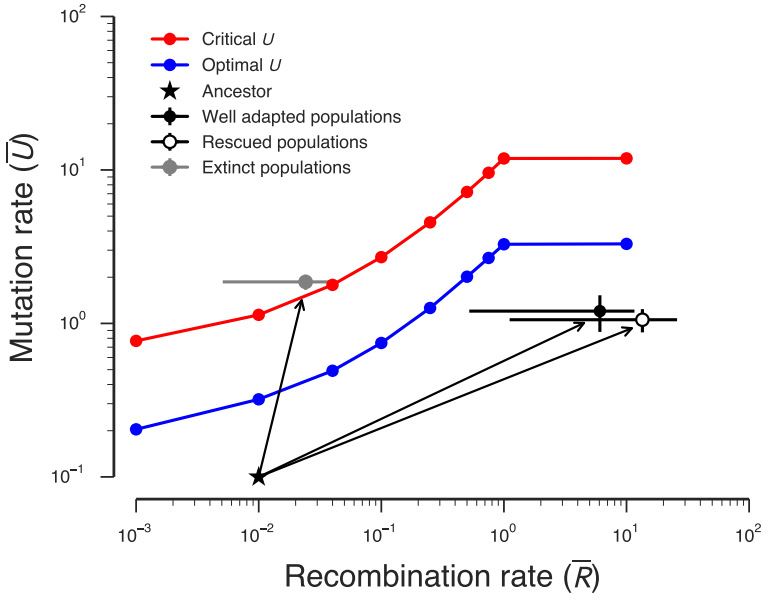

Figure 5.9: **Selection for evolvability optimizes the recombination rate but not the mutation rate.** Blue and red circles show U_{opt} and U_{crit}, respectively, corresponding to different values of R (see legend of Figure 5.3). Solid gray circles indicate the average values of $\overline{U}$ and $\overline{R}$ evolved by 11 populations that under go a mutation-rate catastrophe (Figure 5.8J–L). Open black circles indicate the average values of $\overline{U}$ and $\overline{R}$ evolved by 22 populations that start to undergo a mutation-rate catastrophe but are rescued by an increase in $\overline{R}$ (Figure 5.8G–I). Solid black circles indicate the average values of $\overline{U}$ and $\overline{R}$ evolved by 7 populations that evolve high-adaptation rate without showing any signs of mutation-rate catastrophe (Figure 5.8D–F). The $\overline{U}$ and $\overline{R}$ of each population were estimated as the mean values during the last 10^3 generations, either before extinction or before the cutoff of 7×10^4 generations. Error bars are 95% c.i.

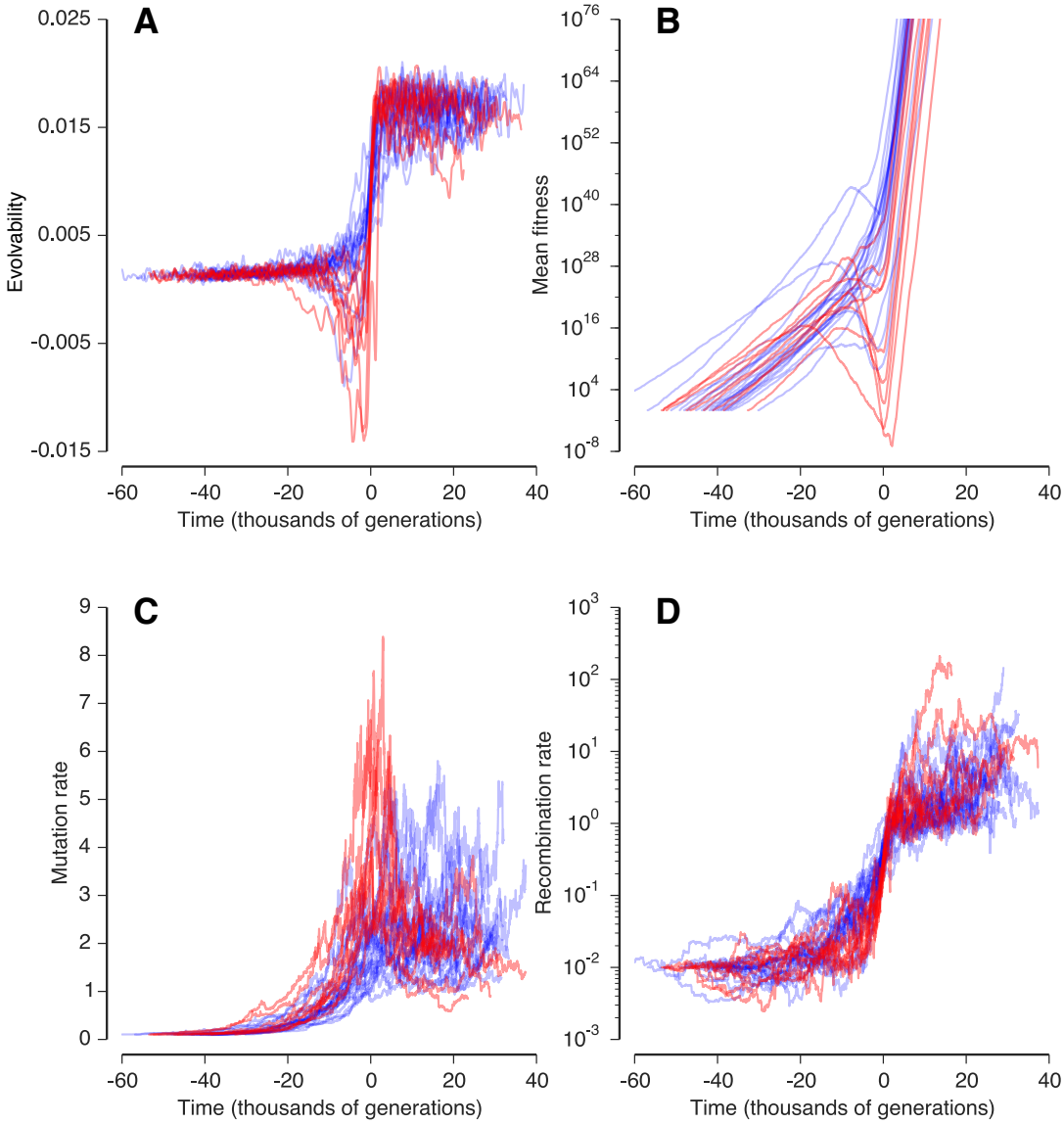

Figure 5.10: **Populations are aligned at transition point of evolvability show the increase in $\overline{R}$ rescues populations from the mutation-rate catastrophe.** The data are the same as in Figure 5.8D–I. All x axes show the time points relative to the transition point of fitness. Red lines show 20 populations that first evolve to extremely high mutation rates are able to evolve much lower mutation rates after the increase in $\overline{R}$ later during the evolution.

Chapter 6

Conclusions

The evolution of mutation rate is complex. First of all, mutations are required for evolution but populations cannot evolve high fitness with very high mutation rate due to the fact that most mutations that have fitness effects are deleterious. It is a trade-off for populations that are adapting. Second, the mechanisms that modifying mutation rate of individuals are complicated and indirect through mutations that create mutator and antimutator alleles. Third, there are several evolutionary processes that interact with the evolution of mutation rate (Wright, 1931; Kimura and Ohta, 1971; Charlesworth, 1994; Peck, 1994; Barton, 1995), which makes it hard to predict how mutation rate evolves under different circumstances.

In this dissertation, I aim to understand how mutation rate evolves in multiple scenarios and how the evolution of mutation rate interacts with other evolutionary forces, including mutation biases, selection for evolvability, genetic drift, and recombination.

6.1 The role of mutation bias in the evolution of mutation rate

In Chapter 3, I studied the role of mutation bias in the evolution of mutation rate. The effects of mutation bias on the evolution of mutation rate have been long ignored in the literature, although several theoretical studies had assumed the mutation bias in their models (e.g., Taddei *et al.*, 1997; Tenaillon *et al.*, 1999, 2000; Gerrish *et al.*, 2007; Sloan and Panjeti, 2010; Desai and Fisher, 2011; Lynch, 2011; Jain and Nagar, 2012). Here, I study the ability of an upward mutation bias to drive the mutation rate, which includes two types of mutation biases — the rate bias toward high mutation rate to mutators and the effect bias toward large mutator effects on the mutation rate. I implement models of both direct selection and indirect selection to study the role of mutation bias under different scenarios.

I began by evolving asexual populations under the direct selection model (see Section 3.2.1 for more details) and found that natural selection and mutation bias can both drive the evolution of mutation rate. The evolved mutation rate is determined by the relative strength of natural selection and mutation bias. Mutation rate increased when the mutation bias was stronger than selection, $\phi_1 > -\beta\mathcal{V}$, and decreased when the mutation bias was weaker than selection, $\phi_1 < -\beta\mathcal{V}$, where ϕ_1 is a measure of the strength of mutation bias, β is the linear selection gradient on log mutation rate, and $\mathcal{V}$ is the variance of log mutation rate (Equation 3.10) (Figures 3.2, 3.3 and 3.4). In addition, I found that higher variance increased the effectiveness of selection for low mutation rate (Figure 3.5).

Then I use the indirect selection model (see Section 3.2.2 for more details) to study how mutation bias affects the evolution of mutation rate when natural selection only act indirectly on mutation rate. It has been broadly promoted that mutator hitchhiking plays an important role in the fixation of mutator alleles (Taddei *et al.*, 1997; Tenaillon *et al.*, 1999; Gerrish *et al.*, 2007). Beneficial mutations are required in the process of hitchhiking. To compare the effect of mutator hitchhiking and mutation bias on the evolution of mutation rate, I first evolved asexual population with 10-fold rate mutation bias and only deleterious mutations but no beneficial mutations, i.e., no mutator hitchhiking during evolution. I found that mutation rate can cause mutation rate to increase even if all mutations with an effect on fitness are deleterious (Figure 3.7). This result demonstrated that the increase in mutation rate was driven by mutation bias.

Next, I evolved the same populations but added beneficial mutations (mutator hitchhiking is present). I found that mutation rate increased more rapidly when there were beneficial mutations (Figure 3.7A, solid blue and red lines). However, this higher rate of evolution of log mutation rate was not completely explained by mutator hitchhiking; it was caused in part by a reduction in effective population size (Figure 3.7B) which made selection for low mutation rate less effective (Figure 3.7A, dashed blue and red lines). In addition, when I manipulated the strength of rate mutation bias in the presence of beneficial mutations. I found that a strong mutation bias was necessary for mutator hitchhiking to be "capable of" increasing mutation rate. The results suggest that mutation bias drives the evolution of mutation rate in the presence of mutator hitchhiking.

Lastly, I studied the effects of mutation bias in sexual populations. In the presence of recombination, mutator hitchhiking is not efficient due to the disruption of the associations between mutator alleles and beneficial mutations. I evolved sexual populations with 10-fold mutation bias and with/without beneficial mutations. I found that, even without beneficial mutations, mutation rate was still increased as expected under the influence of mutation bias (Figure 3.9A). In the presence of beneficial mutations, mutation rate increased slightly faster than in their absence. However, this increase is not caused by mutator hitchhiking but by a slight reduction in effective population size (Figure 3.9). I conclude that mutation biases can operate in the presence of recombination.

My results suggest that, besides other evolutionary forces such as natural selection and genetic drift, the mutation bias can also affect the equilibrium mutation rate with a comparable strength. Genetic variation is required for populations to adapt in new environments. When other evolutionary forces are not strong enough to increase the mutation rate for more genetic variation, the mutation bias works as an effective force to do so. This point has not been carefully studied in the literature. Whether mutation bias exist in nature still remains an open question due to the lack of direct evidence, although it is generally assumed in earlier studies (Taddei *et al.*, 1997; Tenaillon *et al.*, 1999, 2000; Gerrish *et al.*, 2007; Sloan and Panjeti, 2010; Desai and Fisher, 2011; Lynch, 2011; Jain and Nagar, 2012). My results indicate that knowing the precise nature (rate and/or effect) and magnitude of the mutation biases on mutation rate will be essential to elucidate the extent to which they contribute to the evolution of mutation rate.

Overall, my results in Chapter 3 demonstrated the important role of mutation bias in the evolution of mutation rate. I hope this work can stimulate more interest on the study of mutation bias.

6.2 Static analysis of the effects of mutation and recombination rate on evolvability

In Chapter 4, I ask how the mutation rate (U) and recombination rate (R) influence the evolvability (E) of both asexual ($R = 0$) and sexual ($0 < R \leq 1$) populations when U and R are not allowed to evolve. The evolvability of a population is determined by its ability to generate high-fitness genotypes. The rates of both mutation and recombination are expected to be important determinants of evolvability (Pigliucci, 2008; Colegrave and Collins, 2008). I began by finding that there is an optimal intermediate mutation rate that maximizes the evolvability of an asexual population. Increasing U beyond the optimal value causes E to decrease. Eventually, a *critical* mutation rate, U_{crit}, is reached at which $E = 0$. On average, populations evolving at $U = U_{\text{crit}}$ do not adapt (Figure 4.1, $U_{\text{crit}} = 0.64$). Increasing U beyond U_{crit} causes the population to go extinct because $E < 0$.

Next, I investigated how the recombination rate (R) influences the relationship between E and U described above if neither U nor R are allowed to evolve. I found that increasing the recombination rate in the range $0 \leq R \lesssim 1$ increases E (Figure 4.2). In addition, raising R increases both the optimal and the critical values

of U. The results is in agreement with Weismann's hypothesis that sex increases evolvability (Weismann, 1887; Kondrashov, 1993; Burt, 2000).

Overall, these results imply an advantage of sex since sexual populations show higher evolvability than their asexual counterparts under the same constant mutation rates. It raises the question that, if the recombination rate is allowed to evolve, will populations evolve higher recombination rate for higher evolvability? This problem was be further investigated in Chapter 5.

6.3 Selection for evolvability can optimize the recombination rate but not the mutation rate

In Chapter 5, I investigated how selection for evolvability influences the evolution of mutation and recombination rates. The hypothesis is that populations may optimize mutation and recombination rates for high evolvability if selection for evolvability drives the evolution of both rates.

I began by allowing mutation rate to evolve in asexual populations. I found that selection for evolvability fails to optimize the mutation rate of asexual populations. Instead of optimizing mutation rate, asexual populations can evolve supercritical mutation rate that drives population to extinction — a phenomenon termed "mutation-rate catastrophe" (Figure 5.1).

To test what may cause the catastrophe, I investigated the effects of two mutational parameters on the mutation-rate catastrophe — antimutator effects ($\bar{s}_{\mathrm{a}}$) and

mutation rates to antimutator (U_{a}). Both large $\bar{s}_{\mathrm{a}}$ and high U_{a} (i.e., reduced rate mutation bias) allow populations to lower the mutation rate more efficiently. The results show that, as predicted, large $\bar{s}_{\mathrm{a}}$ and reduced rate mutation bias can prevent asexual populations from the mutation-rate catastrophe (Figure 5.2). Gerrish *et al.* (2007) interpreted this "mutation rate catastrophe" as a result of the fact that natural selection is a "short-sighted process" that favors the short-term benefit of high mutation rate (generation of beneficial mutations) despite its long-term cost (accumulation of deleterious mutations). However, my results showed that, besides natural selection, the mutation bias also plays a crucial role in the catastrophe.

Next I tested if mutation rate can be optimized in sexual populations since recombination can interfere with the spreading of mutator alleles. I repeated the simulations described in the previous paragraph but using populations with a broad range of fixed recombination rates, R (Figure 5.3). I found that high recombination rate can prevent the mutation-rate catastrophe (Figure 5.3B). The result indicates that recombination improves the ability of selection for evolvability to optimize the mutation rate to the extent that it prevents the mutation-rate catastrophe. However, the mutation rate evolved to a much lower level of mutation rate than the expected level (Figure 5.3A). It suggests that selection for evolvability cannot optimize mutation rate in sexual populations.

A possible explanation for this failure of optimization is that selection for evolvability is not be the only type of selection acting on $\overline{U}$ and is a weak selection. I tested this hypothesis by comparing the effects of selection for evolvability and mutation bias on the evolution of mutation rate. The results suggest that the selection

for evolvability is much weaker than the mutation bias. Mutation bias, instead of the selection for evolvability, drives the evolution of mutation rates in the examined scenarios (Figures 5.5) and 5.6). However, both forces cannot optimize the mutation rate to its expected equilibrium.

In contrast to the mutation rate, selection can optimize the recombination rate for high evolvability. In addition, I found that the strength of selection for higher recombination rate increases with the mutation rate (Figure 5.7). When I allowed recombination to evolve while keeping mutation rate constant, $\overline{R}$ increased in all populations, but the rate of change increased with U. Hartfield *et al.* (2010) found that both the selective advantage of a modifier of recombination and its probability of fixation increased with the mutation rate in a similar model to mine.

Lastly, I investigated the extent to which selection for evolvability can cause $\overline{U}$ and $\overline{R}$ to coevolve to an optimal state. I evolved sexual populations and allowed both rates to evolve. Again, I found that selection for evolvability can optimize the recombination rate but not the mutation rate (Figure 5.9). I also tested if the sexual populations can escape from the mutation-rate catastrophe. The results show that populations do not always escape the catastrophe (Figure 5.8). But in the cases they do, they all evolve high recombination rate. Some populations experienced the decline in fitness due to the intolerably high mutation rate at the beginning of the evolution but were able to be rescued from catastrophe. Further investigations showed that they evolved high recombination rate right before the mutation rate dropped back to normal level and they were rescued from catastrophe (Figure 5.10). These results indicate that sex could provide an advantage by rescuing asexual populations

from the mutation-rate catastrophe. Besides, although I did not model other genetic architectures such as modularity, they may also influence evolvability as promoted in earlier studies (Riedl, 1977; Wagner, 1996).

6.4 Future directions

In this thesis, I investigated several forces that affect the evolution of mutation rate including the mutation bias, background selection, mutator hitchhiking, genetic drift, and selection for evolvability. Mostly I focused on the effects of mutation bias and selection for evolvability on the evolution of mutation rate. My results emphasized the mutation bias as an important force that is comparable to other forces. In my work, the chosen deleterious mutation rate is comparable to those of the RNA virus $\phi 6$ (Burch *et al.*, 2007). In the future, it is important to test the predictions with broader range of mutational parameters and apply the predictions on other populations.

I hope my work can stimulate more studies on the mutation bias. For example, experimental work on directly estimating the mutation bias, both rate bias and effect bias, will be critical to further study their effects on the evolution of mutation rate. It has been found that both mutators and antimutators occurred in *Saccharomyces cerevisiae* populations (McDonald *et al.*, 2012), which make the estimation of mutation rate to mutators and antimutators possible.

Moreover, I studied the interplays among the evolution of mutation rate, selection for evolvability and recombination. My work improves our understanding of the

extent to which the selection for evolvability can optimize the mutation and recombination rate. In previous study, *E. coli* strains of moderate mutators have been found to out-compete the wildtypes and antimutator strains after evolving for 350 generations in stable environment (Loh *et al.*, 2010). The results are consistent with my predictions that intermediate mutation rates lead to high rate of adaptation. If all parameters can be estimated accurately, it will be interesting to compare the mutation rates of winner strains to the predictions from my model to examine if the optimal mutation rates in these populations fall into the predicted optimal range of mutation rates. In addition, in their experiments, the mutation rates did not change during the evolution. It will be interesting to evolve the mutator strains further and investigate that whether low mutation rate will be restored as reported by McDonald *et al.* (2012) or not. Moreover, will the restored low mutation rate be suboptimal as predicted by my model? If the mutation rates to mutator and antimutator are able to be estimated, my model will contribute to further investigating what forces shape the evolved mutation rates in these experimental strains.

Overall, understanding the relationship between mutagenesis and adaptability is of primary importance for further investigating complex processes that are mutation driven such as cancer progression, emergence of drug resistance, and speciation (Radman *et al.*, 1999; Salk *et al.*, 2010; Levin *et al.*, 2000; Johns and Joyce, 2005)

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