INVESTIGATION OF THE EFFECTS OF THE UNUSUAL GENOME ARCHITECTURE ON THE EVOLUTION OF THE CILIATE *Tetrahymena* USING COMPUTATIONAL MODELING

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Dedication

To the best advisors I have ever had, Dr. Ricardo Azevedo and Dr. Rebecca Zufall!

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Abstract

Tetraymena is a model organism in molecular biology and has a very unusual genome architecture, including nuclear dimorphism, amitotic division of the somatic nucleus during asexual reproduction, capability to control the copy number of chromosomes during amitosis, and the ability to have facultative sex when a germline nucleus is present. Recent studies also revealed that *Tetrahymena* has very special evolutionary characteristics, such as the prevalence of successful asexuality within the genus and the extremely low mutation rate found in the germline nucleus. How *Tetrahymena* evolves such unusual characteristics is still unclear, and whether and how the unusual genome architecture contributes to the unusual evolutionary characteristics remain largely untested or uninvestigated.

In this study, I investigated the effect of these unusual genome features on the evolution of *Tetrahymena* using computational modeling, particularly on their contributions to their successful asexuality and the extremely low mutation rate. I found that compared to mitosis, amitosis of somatic nucleus, together with copy number control, can both decelerate the operation of Muller's ratchet to a sexual-comparable extent and speed up the adaptation to changing environments. I also found that although not expressed during asexual generations, the mutations arising in the germline nucleus can also respond to selection acting on the somatic nucleus following sexual reproduction, which rejects the previous explanation for the low germline nucleus mutation rate in *Tetrahymena*. Instead, my results showed that the reproduction strategies adopted by *Tetrahymena*, including amitosis and facultative sex, can naturally promote the reduction of mutation rate under selection. This study highlighted the contribution of unusual genome architecture to the achievement of unusual evolutionary characteristics found in *Tetrahymena*, which both lead to a better understanding of the evolution of this organism and elucidate new mechanisms for eukaryotes to survive asexually and promote mutation rate reduction.

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Chapter 1. Introduction

1.1. What is sex?

Sex has different definitions in different scientific fields. Here in my dissertation, sex is defined as a process that generates genetically novel individuals by combining genetic material from two parents (Margulis and Sagan, 1990). For eukaryotes, sex generally involves two major processes, meiosis, during which recombination occurs and the gametes are generated, and syngamy, in which the chromosomes from each of the gametes get mixed and form the new individual (zygote). Although today when talking about sex, we usually associate sex with reproduction ('sexual reproduction'), sex does not necessarily link with the reproducing process and lead to individual number increase (Margulis and Sagan, 1990). A good example is provided by ciliates, in which the sex is just for nuclear exchange and reorganization but not for reproduction. The individual number does not increase during sexual conjugation (Corliss, 2016).

Sex is widespread within eukaryotes, with only a few exceptions (Rice, 2002). The most ancient sexual-like event can be tracked back to around two billion years ago, in which some bacteria started to exchange their genes through processes like transformation, conjugation, and transduction (Otto, 2008). Though quite different from modern sex within eukaryotes, these processes are still ongoing within prokaryotes today and contribute greatly to their evolution (Redfield, 2001). Sex within eukaryotes is proposed to have arisen when the Last Eukaryotic Common Ancestor (LECA) transitioned from a prokaryotic state. This process is assumed to have involved several evolutionary innovations, including cell ploidy alternation and mating-type regulation, and finally caused the transition of LECA to be sexual (Goodenough and Heitman, 2014).

Setting aside the origin of sex, the maintenance and persistence of sex is another focus and among the most puzzling questions in evolutionary biology. This is because that although sex is beneficial, it is also inherently costly compared to asexual reproduction (Otto and Lenormand, 2002). Several theories have been proposed to explain the prevalence of sex and are supported by empirical evidence (reviewed in Rice, 2002). However, none of these theories are universally accepted and leaves the maintenance of sex within eukaryotes an open question.

1.2. The costs and benefits of sex

1.2.1. Costs of sex

The costs of sex include cost of males, potential of breaking well-adapted genotypes, and extra time and energy costs to conduct a successful mating (Lehtonen, Jennions, and Kokko, 2012; Roze, 2012).

Unlike asexual reproduction, in which one individual can reproduce by generating a copy of itself, sexual reproduction within animals and some plants usually requires the participation of males. Only engaging in parental care within some species, the males do not directly generate offspring. However, the population needs to invest almost half of the total resources to generate males. Under the same condition, since every asexual individual can generate offspring directly, it will enjoy a two-fold advantage compared to sex (reviewed in Lehtonen et al., 2012 and Roze, 2012). Another cost of sex is that the recombination of chromosomes from parents may break up the well-adapted genetic combinations and generate less-adapted genotypes, thus lowering the overall fitness of the population (reviewed in Lehtonen et al., 2012 and Roze, 2012). Additionally, finding a mate is the prerequisite of a successful sexual reproduction, which may need extra investment of both time and energy (reviewed in Lehtonen et al., 2012 and Roze, 2012). Moreover, this process can also result in a higher risk of predation and parasite transmission within the population (reviewed in Lehtonen et al., 2012 and Roze, 2012).

1.2.2. Benefits of sex

Given the great costs suffered by sexual populations, sex is also beneficial. Generally the benefits of sex can be classified into two major categories: the direct benefits which can immediately increase the fitness of individuals generated by sex, and the indirect benefits that owe the evolutionary advantage of sex to the much greater genetic variation generated compared to asexual reproduction and thus providing more materials for selection to operate. Accordingly, two major classes of hypotheses based on direct and indirect advantages, respectively, have been proposed to explain the prevalence of sex within eukaryotes (Hartfield and Keightley, 2012; Kondrashov, 1993).

For the direct benefit hypotheses, one hypothesis suggested that meiotic recombination during sex can facilitate the repairing of DNA damage and help to maintain the genome intact (Bernstein, Hopf, and Michod, 1988; Hartfield and Keightley, 2012). However, little evidence has been found to support the argument that recombination is indispensable to the DNA repair process (Engelmoer and Rozen, 2011; Hartfield and Keightley, 2012; Redfield, 1993). Indeed, diploid asexuals can also get their DNA repaired by using the second copy of the alleles as template (Hartfield and Keightley, 2012; Otto and Lenormand, 2002). Moreover, for sexual reproduction, DNA double-strand breaks (DSBs) events occur frequently during meiosis, which greatly harms the intactness of the genome and contradicts the hypothesis (Hartfield and Keightley, 2012; Kleckner, 1996; Longhese et al., 2009). Another direct hypothesis states that sex can accelerate the spread of "selfish" genes (i.e., DNA sequences whose only function is rapidly self-replicated but having no host function) within the population, and thus sex should be favored to ensure the rapid spread of these "selfish" genetic elements even though these elements tend to reduce the fitness of their hosts (Goddard, Greig, and Burt, 2001; Hartfield and Keightley, 2012; Hickey, 1982). Nonetheless, this hypothesis may be reasonable to explain how sex initially emerged, but fail to explain the maintenance of sex. After a successful invasion and once reaching a relatively high frequency, these selfish elements will be propagated with the same rate in both sexual and asexual populations. Considering the extra costs of sex, the asexual population will eventually win the competition with sexual ones (Hartfield and Keightley, 2012; Otto and Lenormand, 2002). Besides those, sex can also confer a direct benefit to ciliate. Most ciliate lineages undergo an unusual amitotic division during their vegetative growth. One common consequence of such process is the uneven segregation of homologous chromosomes, which may lead to variation in copy number of chromosomes among daughter cells. The varied chromosome copy number may lead to the complete loss of some chromosomes as well as all the essential genes sitting on them, and finally cause the population extinction (except for one species, *Tetrahymena thermophila*, who has an unknown copy number control mechanism during amitosis). The sexual conjugation, resulting in the recreation of the new macronucleus, may be essential to reset the copy number of each chromosome to the normal ploidy, thus conferring the direct benefit to ciliate (Morgens and Cavalcanti, 2015; Prescott, 1994).

Due to the weakness of direct benefit hypotheses in explaining the evolution of sex, evolutionary biologists have turned to focus on the hypotheses based on indirect benefits conferred by sex. Initiated by the idea first proposed by August Weismann, the indirect benefit hypotheses attribute the benefits of sex to the altered variation and selection caused by sex (Weismann, 1887). By recombining alleles from different genetic backgrounds, sex can generate an enhanced genetic variation among offspring compared to asexual reproduction and improve the response to selection, which can be advantageous for the long-term persistence of the population. Generally, there are three major categories of indirect population-genetics hypotheses elucidating the evolutionary advantages of sex (Hartfield and Keightley, 2012; Kondrashov, 1993).

(1) Break up the Hill-Robertson interference among loci:

Within a population of finite-size, selection operating on one locus may interfere with selection on another linked locus and thus lead to a reduction in the response to selection, which is known as Hill-Robertson interference (Hill and Robertson, 1966). Sex can break up such interferences among loci through recombination and enhance the efficiency of selection (Lively and Morran, 2014). Two hypotheses explaining the widespread distribution of sex are based on this broad idea. One is Muller's ratchet, which states that asexual reproduction can result in an irreversible accumulation of deleterious mutations in finite populations, and sexual reproduction can avoid the operation of the ratchet by allowing selection to act independently on each arising mutation and thereby slowing down or preventing the accumulation of deleterious mutations (Hartfield and Keightley, 2012; Muller, 1964). Another one is the Fisher-Muller hypothesis, suggesting that sex can speed up adaption by combining beneficial mutations arising in different lineages into the same individual (Hartfield and Keightley, 2012).

These two hypotheses do not only differ in the distinct mutation types they focus on, but also have some inherent differences. For example, Muller's ratchet is triggered by genetic drift, particularly the loss of the mutation-free class, thus smaller populations may suffer a faster operation of the ratchet (Haigh, 1978). On

the contrary, implied by Fisher-Muller hypothesis, the advantage of sex will be more obvious in large populations (Green and Mason, 2013).

The idea of sex breaking up Hill-Robertson interference and thus facilitating its maintenance is well supported by experimental evolution research, as there are several studies showing that asexual lineages accumulate deleterious mutations much faster than their sexual relatives and sexual lineages can achieve accelerated adaptation compared to asexual ones (e.g., Rignot et al., 2006; Neiman et al., 2010; Kaltz and Bell, 2002).

(2) Enhance resistance to parasite infection (Red Queen hypothesis):

The Red Queen hypothesis suggests that the maintenance of sex is driven by coevolution between two or more species through negative frequency dependent selection (Hartfield and Keightley, 2012; Rabajante et al., 2016).

Almost every organism on the earth suffers pressure from its parasites, and coevolving parasites provide a continually changing environment for the hosts (Hamilton, Axelrod, and Tanese, 1990). Given a certain number of genes involved in parasite resistance, the asexual populations can only develop new resistance through the arising of *de novo* mutations. On the contrary, sexual reproduction can continually generate novel parasite resistant genotypes by recombining these existing resistance alleles, which is far more efficient than asexual reproduction to resist the parasite infection and thus facilitate the maintenance of sex (Auld, Tinkler, and Tinsley, 2016; Hartfield and Keightley, 2012).

The Red Queen Hypothesis is suggested to be the theory with the most empirical support to explain the prevalence of sex within eukaryotes, as there are field surveys and laboratory experimental studies illustrating that sexual populations can be stably maintained while asexual ones go extinct in the face of increased parasite infection and a higher level of sex would be selected in the presence of parasite infection (eg. Jokela, Dybdahl, and Lively, 2009; Morran et al., 2011; Morran, Parmenter, and Phillips, 2009).

(3) Mutational deterministic hypothesis:

This theory was proposed and advocated by Kondrashov, based on his research on an infinite-sized deterministic model (Kondrashov, 1982). This hypothesis assumed that most mutations arising in the population are only mildly deleterious but tend to act synergistically (i.e., once they are present together, their overall deleterious effect is greater than directly adding their individual effects). Thus the majority of individuals in the population will tend to carry a small number of mutations, and selection will favor the generation of individuals with fewer mutations (Kimura and Maruyama, 1966; Kondrashov, 1982). Once achieving equilibrium, the asexual population always reach an equilibrium fitness of e^{-U}, in which U is the genomic deleterious mutation rate. However, the recombination during sex allows the population to generate some offspring carrying fewer deleterious mutations, and some carrying more. Under the circumstance of synergistic epistasis mentioned above, the individuals carrying more deleterious mutations will tend to die out and get excluded from the population. Consequently, sexual populations can achieve a higher population equilibrium fitness, suggesting a deterministic benefit of sexual reproduction. Furthermore, a greater benefit of sex over asexual reproduction can be reached under a higher mutation rate. Thus, under a high enough deleterious mutation rate, this advantage conferred by sex may overcome the inherent two-fold cost of sex and lead to the evolution of higher levels of recombination (Hartfield and Keightley, 2012; Kondrashov, 1982, 1988, 1993).

This theory relies on two strict assumptions (i.e., high enough genomic mutation rate, typically greater than or equal to 1 per genome per generation, and prevalence of synergistic epistasis among deleterious mutation), which have received many criticisms since proposed. Although some studies indicate that the genomic mutation rate of some eukaryotes may exceed 1(Kondrashov, 1988), many sexual species also have genomic mutation rates much lower than 1 (Drake et al., 1998; Halligan and Keightley, 2009). Regarding epistasis, there is limited evidence demonstrating the widespread existence of synergistic epistasis within the genome (Sohail et al., 2017; but see Elena and Lenski, 1997). Also, it has been found that strength of synergistic epistasis has to be within a particular range to favor the evolution of increased recombination, and the range can be even reduced if the epistatic interactions vary among loci (Otto and

Feldman, 1997). As a consequence, the mutational deterministic hypothesis is not a currently favored explanation for the evolution of sex.

Besides the three main categories of hypotheses listed above, there are also several other theories, such as the idea of fitness-associated recombination/sex (FAR/FAS) and the argument that heterogeneous environments would favor sex and recombination, explaining the prevalence of sex (Hartfield and Keightley, 2012). However, they are not as well-accepted and prominent as the three hypotheses mentioned above. In this dissertation, I will focus on the indirect benefits provided by sex, particularly in the aspects of slowed down Muller's ratchet and accelerated adaptation.

1.3. Asexuality: evolutionary "dead-end"?

Without the benefits typically provided by sex, asexual reproduction has long been regarded as an evolutionary dead-end (Smith and Maynard-Smith, 1978). Consistent with this prediction, most existing asexual lineages are evolutionarily young and estimated to derive from their sexual ancestors only recently (Judson and Normark, 1996; Normark, Judson, and Moran, 2003; Rice, 2002).

1.3.1. Asexual reproduction usually accelerates mutation accumulation

Due to the lower efficiency in purging deleterious mutations compared to sexual reproduction, asexual reproduction is predicted to result in an accelerated deleterious mutation accumulation within the population, which prevents the long-term persistence of asexual populations.

Several empirical studies have demonstrated this prediction. For example, Paland and Lynch (2006) investigated the amino acid substitutions in the mitochondrial protein-coding genes of asexual and sexual *Daphnia pulex* lineages. They found that 4.4% of these substitutions are mildly deleterious and persist in both asexual and sexual linages, while an extra 13.3% moderately deleterious substitutions are only found in asexual lineages. Thus a 4-times faster deleterious amino acid substitution rate was found in the mitochondrial protein-coding genes of asexual *Daphnia pulex* lineages compared to that of sexual ones

(Paland and Lynch, 2006). Another study on *Timema* stick insects has revealed a 3.6 - 13.4 fold elevated mutation rate for three protein-coding genes analyzed in asexual lineages compared to the sexual ones. Moreover, they found that the mutations occurred in asexual lineages often lead to much greater changes of hydrophobicity for the encoded amino acids, implying coding mutations also seem to be much more severely deleterious in asexuals. These effects may work together to result in the short longevity of asexual *Timema* lineages (Henry, Schwander, and Crespi, 2012).

Similar results were also identified in asexual plants. Some plants can reproduce asexually through a process called apomixis, which generates clonal progeny via ploidy-unreduced and unfertilized seed. In a study comparing mutation accumulation in asexual and sexual relatives of *Arabidopsis*, many more mutations were found in asexual lineages. Moreover, more mutations tended to accumulate and persist in highly-conserved genomic sites in asexual lineages in comparison with sexual ones. These results suggest that mutations can dramatically influence the evolution of asexual plants, and the accumulation of mutations do enhance the extinction risk of asexual lineages (Lovell et al., 2017).

A number of studies have confirmed accelerated mutation accumulation in asexual lineages but few of them have directly revealed the link between mutation accumulation and asexual reproduction. This gap was filled by a study on the freshwater snail *Potamopyrgus antipodarum*, which demonstrated that the accelerated mutation accumulation in asexual lineages is definitely a consequence of asexuality, and furthermore, can occur rapidly enough to favor the maintenance of sexual reproduction (Neiman et al., 2010).

1.3.2. Asexual reproduction slows down adaption

Besides the accelerated accumulation of deleterious mutations, according to Weismann's theory, asexual reproduction also leads to a slowed adaptation compared to sex, due to it generating less genetic variation and thus resulting in a weaker response to selection.

Although not many studies focus on the adaptation of asexual populations, they usually served as controls for evaluating the idea that sex can accelerate adaptation. These investigations clearly showed that asexual reproduction results in much slower adaptation compared to sex, especially when facing a changing or novel environment.

For example, using the unicellular chlorophyte *Chlamydomonas reinhardtii* as the study system, Kaltz and Bell (2002) observed that the permanently asexual lineage exhibited slower adaptation compared to lines that ever underwent sexual events when facing a complex novel environment (Kaltz and Bell, 2002). Another study working on the facultative sexual rotifer *Brachionus calyciflorus* revealed a similar pattern (Becks and Agrawal, 2010). Once exposed to novel environments, a higher frequency of sex has been observed, indicating that a higher propensity of sex has evolved. Moreover, although sexual-derived populations resulted in a lower population fitness initially, the researchers showed that sex generates a variety of genotypes, some of which were well-adapted to the new environment. This finding directly demonstrated Weismann's theory and showed asexual reproduction is not favored during adaptation (Becks and Agrawal, 2010).

Although many experimental evolution studies investigated the adaptation of sexual and asexual populations to a changing or novel environment, it should be noticed that the changing environment is not a prerequisite for the sexual populations to overwhelm the asexual ones.

1.4. Successful asexual eukaryotes

As mentioned above, many asexual lineages are evolutionarily short-lived due to Muller's ratchet and less capacity for rapid adaptation. However, there are also some lineages which have survived completely asexually for quite a long time, suggesting that they can overcome such disadvantages of asexuality. This phenomenon further raised an important scientific question on how they achieve such a success. Although the answer to this question still remains unclear, recent studies on some long-lived asexual lineages implied

that their success may rely on some unique non-sexual genetic mechanisms which can confer sexual-like benefits in the absence of sex.

1.4.1. Bdelloid rotifers

The best known example of successful asexuals is the bdelloid rotifer, which has persisted in the absence of sex for at least 35 - 40 millions of years (Waggoner and Poinar, 1993; Welch and Meselson, 2000). The genome analyses of a clonal bdelloid rotifer, *Adineta vaga*, revealed that the tetraploid genome contains many rearranged allelic regions, with some even sitting on the same chromosome (Flot et al., 2014). Conventional meiosis cannot occur within such a genome structure, as it is impossible for the allelic regions that sit on the same chromosome to pair and segregate during meiosis. Thus, the obligate asexuality of bdelloid rotifers has been confirmed. The genome of *A. vaga* exhibits a low average inter-allelic divergence (ASD) of 4.4% at nucleotide level, which is comparable to that found in sexual species and suggests the frequent occurrence of gene conversion events within the genome. Furthermore, more than 8% of the genes within *A. vaga* are not originated from metazoan and supposed to be acquired through horizontal gene transfer (HGT) (Flot et al., 2014). These processes may contribute to the successful asexuality of *A. vaga* by slowing down the operation of Muller's ratchet. For instance, during the gene conversion process, an allele can replace its homologous allele in the same locus and consequently make this locus homozygous. This process is of great value in eliminating deleterious mutations: if the wild-type allele erases the mutation, then this locus will be free of mutation after the conversion; what's more, with a recessive mutation, even if the mutated allele overwrites the wild-type one, mutation will be homozygous in this locus and subjected to natural selection directly. The HGT process makes it possible for bdelloid rotifers to acquire external genes, not only from rotifer to rotifer, but also from other non-metazoan species to rotifer, which would contribute to the genome diversification of bdelloids. Furthermore, there is also evidence showing that this HGT process is still ongoing. Hence, although obligately asexual, the bdelloid rotifers can avoid Muller's ratchet through gene-conversion and HGT, which confer sexual-like benefits of homogenizing and diversifying, respectively (Flot et al., 2014).

1.4.2. Non-marine Darwinuloidea ostracods

Ostracods of the superfamily Darwinuloidea is another famous ancient asexual "scandal" besides bdelloid rotifers (Judson and Normark, 1996), and similar strategies as bdelloid rotifers were found in several species of non-marine Darwinuloidea ostracods to achieve successful asexuality. Indicated by extremely low genetic diversity as well as the absence of males in all the existing fossil records which can date back to 25 million years ago, one species of Darwinuloidea ostracods, *Darwinula stevensoni*, is believed to be a true long-persisted asexual (Schön, Rossetti, and Martens, 2009). Non-sexual genetic homogenizing mechanisms, such as gene conversion, somatic recombination, and DNA repair, are likely to contribute to reducing the mutation load during asexual reproduction and thus allowing the long-term persistence of *D. stevensoni* (Schön and Martens, 2003). Moreover, there are evidences implying *D. stevensoni* has a General Purpose Genotype (GPG), which makes it possible for *D. stevensoni* to tolerate a wide range of niches and alleviate the need to adapt to environmental change. Thus, *D. stevensoni* can 'arrest' evolution. The presence of GPG also confirmed the obligate asexuality of *D. stevensoni* (as sexual reproduction will break apart the combinations of alleles making up GPG) and the occurrence of non-sexual homogenizing mechanisms listed above (Schön et al., 2009).

1.4.3. *Diploscapter*

Being a close relative of the sexual model organism *Caenorhabditis elegans*, *Diploscapter* has recently been identified and confirmed to form a successful asexual clade within the Protorhabditis group of nematodes (Fradin et al., 2017). This asexual clade has been estimated to be derived from a sexual ancestor around 18 million years ago as indicated by phylogenic analyses, which is among the oldest asexual lineages identified so far. Like other successful asexuals, the asexual *Diploscapter* clade also exhibits unusual genome architecture. Different from the sexually reproducing species within Protorhabditis groups, which usually maintains six or seven pairs of chromosomes, all the lineages within the asexual clade only have one chromosome pair and there are no intermediate karyotypes detected. Furthermore, the reduction of chromosome number occurred approximately simultaneously with the origination of the asexual clade,

suggesting a potential correlation between these two events (Fradin et al., 2017). The genome sequencing of one lineage within the clade, *D. pachys*, revealed that the single chromosome pair was formed by the fusion of six chromosomes within the sexual ancestor according to a certain order. The fusion event may result from the loss of telomeres, as no telomere sequence and maintenance proteins were identified. Moreover, *D. pachys* may conduct a modified partial meiosis that only involves Meiosis II Phase during the maturation of oocyte, and many essential genes for Meiosis I got lost during evolution. Since the Meiosis I Phase which results in the segregation of homologous chromosomes is skipped, the mother's entire genetic materials are transferred to the offspring and its entire heterozygosity has also been maintained. As a result, the individuals within the population exhibit no variation and maintained the same level of sexual-comparable heterozygosity (an average of ~4% difference between alleles) in the genome, which may facilitate the long term persistence of this asexual clade (Fradin et al., 2017).

1.5. Evolution of mutation rate

Mutation is the cause of all genetic variation and is the crucial factor for initiating evolution. The mutation rate, defined as the number of new mutations generated within a single gene or an organism per unit time, is one of the most important parameters in genetics and evolutionary biology (Crow, 1997). Like most other genetic traits, the mutation rate is also modified by both evolutionary forces and the environment. As most mutations are deleterious, generally a lower mutation rate is favored by selection (Eyre-Walker and Keightley, 2007; Sniegowski and Raynes, 2013). However, the forces that prevent the mutation rate evolving to zero and the lower bound that mutation rate can evolve are still puzzling (Sniegowski and Raynes, 2013; Sturtevant, 1937).

1.5.1. Hypothesis based on the needs of beneficial mutations

As beneficial mutations are essential for the long-term adaptation of populations, it may seem intuitive that the need for beneficial mutations is one of the main driving forces that limit the reduction of mutation rate

and determine the genomic mutation rate (Sniegowski et al., 2000). Although beneficial mutations are rare, a higher mutation rate leads to a higher probability of generating more beneficial mutations, which may facilitate adaptation. Thus, the achieved mutation rate is supposed to be a reconciling consequence of generating sufficient beneficial mutations while keeping the whole genome from getting excessive numbers of deleterious mutations.

However, the circumstances that can achieve an effective selection for increasing mutation rate seem to be quite constrained (Johnson, 1999; Sniegowski et al., 2000; Sniegowski and Raynes, 2013). As most mutations are deleterious, the more deleterious mutations that arise along with elevated mutation rate will generate a large mutation load and lead to a persistent selection pressure for the population, thus lower genomic mutations are favored most of the time. Moreover, under most evolutionary circumstances, the selection for increasing beneficial mutation rate tends to be much weaker than the selection for decreasing deleterious mutation rate. Consequently, this hypothesis is implausible to solve the puzzling question on mutation rate evolution (Johnson, 1999; Sniegowski et al., 2000; Sniegowski and Raynes, 2013).

1.5.2. "Cost of fidelity" hypothesis

Another famous hypothesis regarding the evolution of mutation rate is known as "cost of fidelity" hypothesis. First proposed by Kimura in 1967, it highlights the effects of the costs for improving DNA replication fidelity on reducing mutation rate (Kimura, 1967). Maintaining a low mutation rate requires a high level of fidelity during DNA replication, which may be costly in both time and energy and thus reduce the individual fitness. Additionally, as deleterious mutations are constantly generated within the population, it is impossible to correct all the DNA replication errors. Accordingly, Kimura suggested that it is the fitness cost of improving DNA replication fidelity or the physiological accuracy limits of the DNA replicating and repairing mechanisms that set the lower bound of mutation rate evolution (Kimura, 1967).

Being generally accepted and further developed by many evolutionary biologists since it was proposed, however, the "cost of fidelity" hypothesis is far from perfect as it contradicts with several existing scientific facts. For example, the enhanced DNA replication fidelity may result in a slowed replication speed and

hence should be more costly in unicellular microbes. However, it was found that unicellular microbes usually maintain a much lower genomic mutation rate compared to multicellular organisms (Lynch, 2010; Sniegowski and Raynes, 2013). Moreover, many organisms use a similar mechanism to replicate and repair their genome. Therefore if the physiological accuracy of such a mechanism does set the limits for mutation rate evolution, we would expect to see a similar mutation rate among organisms having similar physiologies. Nonetheless, the per-site mutation rate varies greatly among those organisms (Sniegowski and Raynes, 2013). Thus, the "cost-of-fidelity" hypothesis may be insufficient to explain the mutation rate evolution either.

1.5.3. Drift-barrier hypothesis

To solve the obvious discrepancy between the "cost of fidelity" hypothesis and experimental findings, Lynch (2010) instead proposed that the reduction of mutation rate may be limited by the intrinsic incapability of selection but not the physiological constraints (Lynch, 2010). Based on this idea, Sung et al. (2012) promoted the drift-barrier hypothesis, which argues that the powers of natural selection on mutation rate evolution are limited by the stochastic effects of random genetic drift and there is a drift barrier impeding the further reduction of mutation rate (Sung et al., 2012a). Due to the fact that the majority of mutations are deleterious, a lower mutation rate would be favored, and natural selection should minimize mutation rate to the lower bound ("the drift barrier") which the benefits of further mutation rate reduction are equivalent to the effects of genetic drift.

As both more efficient selection and weaker drift effect can be achieved under a larger effective population size, according to the drift-barrier hypothesis, the resulting mutation rate is expected to be negatively correlated with the effective population size (Lynch, 2008, 2010). Consistent with this prediction, a significant negative correlation between mutation rate and effective population size has been identified in prokaryotes and eukaryotes, respectively (Sniegowski and Raynes, 2013; Sung et al., 2012a). The prokaryotes exhibited a greatly elevated regression (i.e., a much higher regression slope) between mutation rate and effective population size (Figure 1.1(a)), which may result from the fewer protein-coding genes

carried within the prokaryote genomes and thus lead to a less efficient selection to reduce the mutation rate. As shown in Figure 1.1(b), by calibrating the mutation rate as the effective mutated rate of the protein-coding sites that are subject to selection, the regression difference between prokaryotes and eukaryotes is eliminated and a uniform correlation between mutation rate and effective population size has been established (Sniegowski and Raynes, 2013; Sung et al., 2012a).

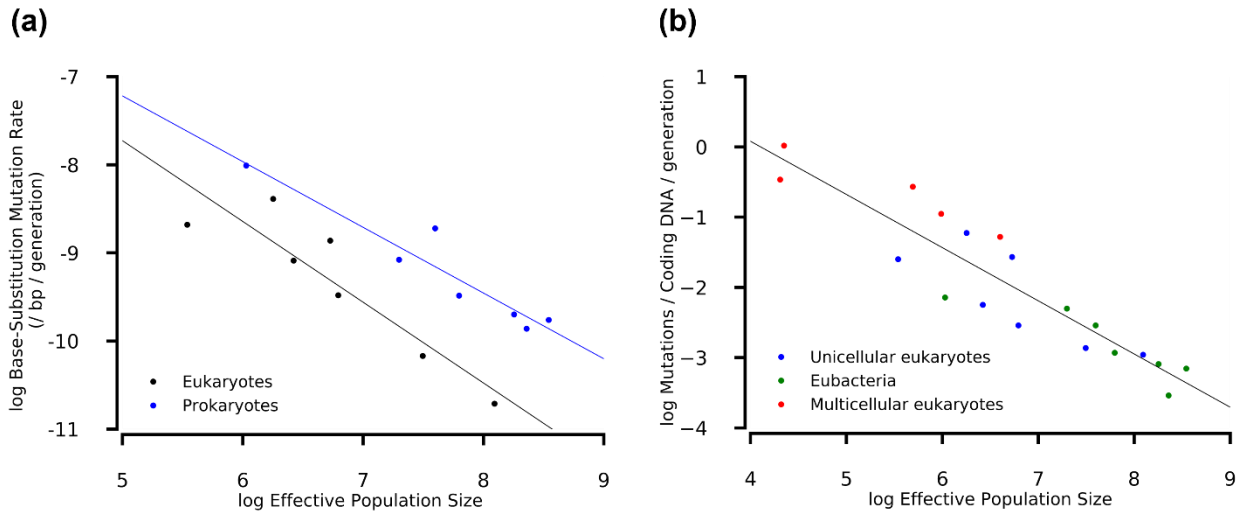

Figure 1.1: A significant negative correlation exists between mutation rate and effective population size. The prokaryotes exhibited a greatly elevated regression between mutation rate and effective population size than eukaryotes (a), and the difference is eliminated after calibrating the mutation rate as the effective rate of the protein-coding sites that are subject to selection (b). Curves show the regression (a) between mutation rate (indicated as base-substitutional mutation rate per base pair per generation) and effective population size within eukaryotes and prokaryotes and (b) between the effective mutation rate in protein-coding sites and effective population size. These figures were remade based on the data provided for Figure 1(B) and (C) in Sung et al. (2012) (Sung et al., 2012a). Note that both x and y axes are shown in log scale.

1.5.4. Impact of sex on mutation rate evolution

Selection acting on a mutation rate modifier (i.e., a locus that can modify the mutation rate) can be either direct or indirect (Sniegowski et al., 2000). The direct selection relies on the direct fitness effect of the modifier allele instead of its effect on mutation rate, and the indirect selection depends on the linkage disequilibrium between the modifier alleles and other alleles affecting fitness. As currently there is no experimental evidence showing that the modifier alleles can directly affect the carriers' fitness, the

dynamics of modifiers are most likely influenced by the indirect selection caused by linkage disequilibrium (Sniegowski et al., 2000).

By "hitchhiking" with the arising beneficial mutations, the mutator alleles can be favored by selection and lead to an increase in the mutation rate within the population (Sniegowski et al., 2000). Such a mutator hitchhiking process has been suggested to account for the selection and fixation of mutator alleles within asexual populations (Sniegowski et al., 2000). However, for a sexual population, the recombination process occurring during sex can efficiently remove the linkage disequilibrium between the modifier allele and fitness-affecting allele. As most mutations are deleterious, the recombination has a much greater destructive impact on the indirect selection of increasing the mutation rate (i.e., favoring mutators) than the indirect selection of lowering the mutation rate (Sniegowski et al., 2000). Indeed, several theoretical studies on modifier models have demonstrated that the mutator hitchhiking was unlikely to be crucial for shaping the genomic mutation rate within the sexual population (Kimura, 1967; Sniegowski et al., 2000). Instead, based on the available evidence at that time, Sniegowski et al. (2000) suggested that the mutation rate within a sexual population is more likely to be set by the tradeoff between the direct selective cost caused by increasing DNA replication fidelity and the indirect selection generated by the selection against deleterious mutations (Sniegowski et al., 2000).

1.6. Biology of *Tetrahymena*

Tetrahymena is a free-living ciliate protist and also a model organism in molecular biology. It has very unusual genomic features and also has been suggested to be a long lived asexual lineage (Doerder, 2014). Previous research proposed that the unusual genomic features may facilitate the achievement of successful asexuality within *Tetrahymena* lineage (Doerder, 2014; Zufall, 2016). However, this idea has not been fully evaluated.

1.6.1. Unusual genomic features of *Tetrahymena*

Tetrahymena has several unusual genomic features, including nuclear dimorphism, amitotic division of the macronucleus, chromosome copy number control during amitosis, the ability to have facultative sex, and also the extremely low mutation rate within the micronucleus (see below) (Orias, Cervantes, and Hamilton, 2011).

(1) Nuclear dimorphism within a single cell:

Like other ciliates, normally *Tetrahymena* maintains two nuclei within a single cell: the soma-like polyploid macronucleus (MAC) and the germ-like diploid micronucleus (MIC) (Orias et al., 2011). The MAC is transcriptionally active and responsible for the normal growth and asexual reproduction, while the MIC remains transcriptionally silent during these processes and is only involved in sexual conjugation. The MIC is necessary but not sufficient for *Tetrahymena* to have sex (Orias et al., 2011). Without a MIC, *Tetrahymena* can still survive and reproduce asexually but lose the capability of having sex.

(2) Amitotic division of MAC:

During asexual reproduction, the MIC divides by normal mitosis while the MAC applies an unusual amitotic division strategy (Orias et al., 2011). Lacking kinetochores and mitotic spindle, amitotic division of the MAC allows random distribution of parental alleles among daughter cells (see Figure 2.1 in Chapter 2). This process is similar to the recombination process that occurs during sex and can generate a much greater genetic variation than the normal mitotic division (Orias et al., 2011). This process further leads to "phenotypic assortment", in which the MAC can become completely homozygous in around 200 generations (Doerder, Deak, and Lief, 1992). I predict this key feature allows the purging of deleterious mutations as well as accumulate beneficial mutations without sex.

(3) Copy number control mechanism during amitosis:

For amitosis, one common consequence is the uneven segregation of homologous chromosomes, which may lead to chromosome copy number variations among daughter cells. Such variation can be amplified in

the following divisions, eventually leaving the cells with no copies of some chromosomes (Morgens and Cavalcanti, 2015).

Since all genes within the missing chromosomes also get lost, the complete loss of a chromosome would greatly reduce fitness and even be lethal, and may be a cause of population extinction observed in many asexual lineages of ciliates (Bell, 1988). To solve this problem, some ciliates without a copy number control mechanism need to have sex frequently to reset the copy number of each chromosome to the normal ploidy, which means they cannot conduct asexual reproduction infinitely (Morgens et al., 2013; Prescott, 1994). However, unlike other ciliates, at least one lineage within *Tetrahymena* genus, *Tetrahymena thermophila* can maintain MAC ploidy roughly unchanged (i.e., around the normal ploidy 45) during amitotic division, thus allowing it to avoid extinction caused by ploidy changes during asexual reproduction (Orias et al., 2011). However, the mechanism behind this phenomenon and whether other *Tetrahymena* lineages have such a mechanism still remain unknown.

(4) Ability to have facultative sex in the presence of MIC:

With the MIC present in the cell, *Tetrahymena* can also undergo sexual conjugation. This process is typically induced by starvation, and involves the fusion of meiotic gametes, degradation of the old MAC, and generation of the new MIC and MAC (Orias et al., 2011). Programmed genome rearrangement occurs during the development of new MAC, and generates a highly fragmented MAC genome. In *T. thermophila,* this process will result in a MAC composed of around 200 chromosomes (fragments of the 5 chromosomes in the MIC), with each of them containing several hundred of genes and present in 45 copies (Orias et al., 2011). During this process, approximately one third of the DNA sequences, mostly repetitive sequences, within the MIC get eliminated (Hamilton et al., 2016). After one round of conjugation, due to the inherent adolescence period, the progenies cannot reproduce sexually again for around 60~100 generations (Rogers and Karrer, 1985).

(5) Extremely low mutation rate within MIC:

Revealed by mutation accumulation experiment, one species within *Tetrahymena* lineage, *T. thermophila*, was found to maintain an extremely low mutation rate (~7.61×10^{-12} per site per generation) within the MIC (Long et al., 2016b). Such low mutation rate is consistent with the value identified in another ciliate species, *Paramecium tetraurelia*, and is the lowest mutation rate found within eukaryotes. How *T. thermophila* achieves this low mutation rate is still a mystery, and it has been suggested that the facultative sexual strategy and large effective population size may account for it. Given that *T. thermophila* cannot conduct sex with a frequency higher than once every 60 - 100 generations, and the mutations within MIC are not expressed and supposed not subject to selection until following sex, natural selection should favor a low mutation rate (Long et al., 2016b). Considering an estimated effective population size of 1.12×10^8 for this species, the power of natural selection should be very strong to promote the reduction of mutation rate (Long et al., 2016b). However, this explanation has yet to be evaluated.

1.6.2. *Tetrahymena*: a successful asexual lineage?

Tetrahymena has been suggested to be a successful asexual lineage, as evidenced by both experiment and phylogenetic analyses. Although rare in other ciliates, obligate asexuality is quite widespread within the *Tetrahymena* genus as ~25% of the natural isolates were found to be amicronucleates (and thus asexual) (Doerder, 2014). Further phylogenetic analyses showed that these amicronucleates have multiple origins, and some of them may be as old as millions of years (Doerder, 2014). Indeed, some asexual cultures of *T. thermophila* have been maintained continuously in lab condition for more than 80 years, which is around 10^5 generations (Doerder and Brunk, 2012).

The successful survival of amicronucleate *Tetrahymena* is supposedly a consequence of its unusual architectural features, especially the amitotic division of MAC (Doerder, 2014; Zufall, 2016). During asexual reproduction, the amitotic process within the MAC can generate a much greater genetic variation compared to mitosis, and selection can thus work more efficiently to purge the deleterious mutations and accumulate the beneficial ones. As a consequence of amitosis, the resulting "phenotypic assortment" allows *Tetrahymena* to get new mutations purged or fixed in the absence of sex within a relatively short period of

time. Moreover, the unknown copy number control mechanism makes the *Tetrahymena* free of extinction caused by copy number variation during amitosis. These unusual genome features may work together to confer sexual-like benefits in the absence of sex and make it possible for *Tetrahymena* to achieve successful asexuality. Nonetheless, this prediction has not been demonstrated, and whether the benefits provided by these unusual genome architectures in preventing "evolutionary dead-end" are comparable to that of sex remains unknown.

1.7. Outline of dissertation

This dissertation is aimed at elucidating the effects of unusual genome architectures on the evolution of the ciliate *Tetrahymena* using computational modeling. More specifically, I focused on the contributions of these unusual genome architectures to achieve the successful asexuality and the extremely low mutation rate found in *Tetrahymena*.

In Chapter 2, I evaluated the roles the unusual genome features, particularly amitosis of the MAC and the copy number control, play on the successful asexuality of *Tetrahymena*. Using a stochastic Wright-Fisher model involving different reproduction strategies (i.e., amitosis, mitosis, obligate sex, and facultative sex), I found that amitosis with copy number control can confer sexual-like benefits by slowing down the operation of Muller's ratchet and speeding up adaptation. Moreover, as demonstrated by mathematical analyses (done by my advisor, Dr. Ricardo Azevedo), amitosis was found to be able to reduce the mutation load carried in a large population compared to mitosis.

In Chapter 3, I further investigated whether amitosis with copy number control can accelerate the adaptation of *Tetrahymena* to changing environments. Adopting a polyploid Fisher's Geometric Model, I compared the adaptation trajectories under different reproduction strategies once facing a constant and a changing environment. My results showed that because of the overdominance among alleles, the amitotic population may suffer a genetic load and achieve a lower fitness level than the mitotic one within a constant

environment. However, the greater variation generated by amitosis can accelerate the adaptation process in changing environments. No matter whether the environment changes or not, the obligate sexual population can only achieve a high fitness level under low MAC ploidies but not high ones, due to the fact that the allele amplification process may drive the phenotype to overshoot the optimum allele copy number under high MAC ploidies.

In Chapter 4, I explored the possible mechanisms that account for the evolution of extremely low mutation rate found in *Tetrahymena*. I first tested the hypothesis proposed by Long et al. (2016), that the low MIC mutation rate evolves as a consequence of selection only operating on the "invisible" MIC mutations accumulated during the many rounds of asexual generations between two rounds of sex (Long et al., 2016b). By investigating the mutation load carried within the MIC, I found that this explanation is invalid as the MIC mutations also respond to selection during asexual cycles as they are present in the MAC after having sex. Instead, by introducing mutation rate modifiers and investigating the mutation evolution trajectories under different reproduction strategies, I found that certain reproduction strategies, especially amitosis and facultative sex, can naturally promote the reduction of mutation rate under selection force.

Chapter 2. Amitosis confers benefits of sex in the absence of sex to *Tetrahymena*[1]

[1] This chapter is based on our manuscript that is going to be submitted for publication. A preprinted version can be found at *bioRxiv* doi: https://www.biorxiv.org/content/10.1101/794735v1. I am responsible for the stochastic simulations in this manuscript. The contents have been reorganized and the figures have been adjusted to be consistent with other chapters.

2.1. Introduction

Sex appears to be the most successful reproductive strategy in eukaryotes despite its many costs (Bell, 1982; Smith and Maynard-Smith, 1978; Weismann, 1887). While a complete explanation for sex's success remains elusive, several evolutionary benefits of sex have been identified (Burt, 2000; Kondrashov, 1993), such as, the purging of deleterious mutations (Kondrashov, 1988; Muller, 1964), the accumulation of beneficial mutations(Fisher, 1930; Muller, 1932), and an advantage in biotic interactions (Bell, 1982; Hamilton et al., 1990; Otto and Nuismer, 2004). It is predicted that, by forgoing these benefits, asexual lineages are evolutionary dead-ends (Smith and Maynard-Smith, 1978; Stebbins, 1957) due to genetic deterioration and/or an inability to adapt to environmental changes. Consistent with this prediction, many asexual lineages show signs of accelerated accumulation of deleterious mutations compared to their sexual relatives (Henry et al., 2012; Hollister et al., 2015; Johnson and Howard, 2007; Neiman et al., 2010; Paland and Lynch, 2006; Tucker et al., 2013). Despite these low expectations, some asexual eukaryotic lineages appear to be successful, including the ciliate *Tetrahymena* (Doerder, 2014). Here, we show that the mechanism of somatic nuclear division in *Tetrahymena*, termed amitosis, provides benefits similar to sex, allowing for the long-term success of asexual lineages. We found that, when compared to mitosis, amitosis with chromosome copy number control reduces mutation load deterministically, slows the accumulation of deleterious mutations under genetic drift, and accelerates adaptation. These benefits arise because, like sex, amitosis can generate substantial genetic variation in fitness among (asexual) progeny. Our results indicate that the ability of *Tetrahymena* to persist in the absence of sex may depend on non-sexual genetic mechanisms conferring benefits typically provided by sex, as has been found in other asexual lineages (Flot et al., 2014; Gladyshev, Meselson, and Arkhipova, 2008; Maciver, 2016; Seidl and Thomma, 2014).

Although rare throughout ciliates, obligately asexual lineages are abundant, and possibly ancient, in the genus *Tetrahymena* (Doerder, 2014). The reason for this abundance is unknown. One possibility is that the peculiar genomic architecture of *Tetrahymena* allows it to avoid some of the negative consequences of asexuality (Doerder, 2014; Zufall, 2016). Ciliates are microbial eukaryotes characterized by the separation of germline and somatic functions into two distinct types of nuclei within a single cell. The somatic macronucleus (MAC) is the site of all transcription during growth and asexual reproduction, and the germline micronucleus (MIC) is responsible for the transmission of genetic material during sexual conjugation (Figure 2.1). Following conjugation, a zygotic nucleus divides and differentiates into the two types of nuclei (Figure 2.1(a), (b)). During this differentiation, the macronuclear genome undergoes massive rearrangements resulting in a genome with many small, highly polyploid, acentromeric chromosomes (Chalker, 2008). This genome structure results in amitotic macronuclear division (Figure 2.1(c), (d)). Amitosis generates variation among individuals in the number of each allele at a locus. In most ciliates, amitosis results in differing numbers of chromosomes among progeny, which eventually leads to senescence and death (Bell, 1988). However, *Tetrahymena* have an unknown mechanism to control chromosome copy number during amitosis that results in roughly constant ploidy (Orias et al., 2011). 25% of 2,609 *Tetrahymena*-like wild isolates lacked a MIC and were, therefore, asexual (Doerder, 2014). To test whether amitosis with chromosome copy number control can account for the relative success of asexual *Tetrahymena*, we examined the evolutionary consequences of various forms of reproduction, nuclear division, and ploidy.

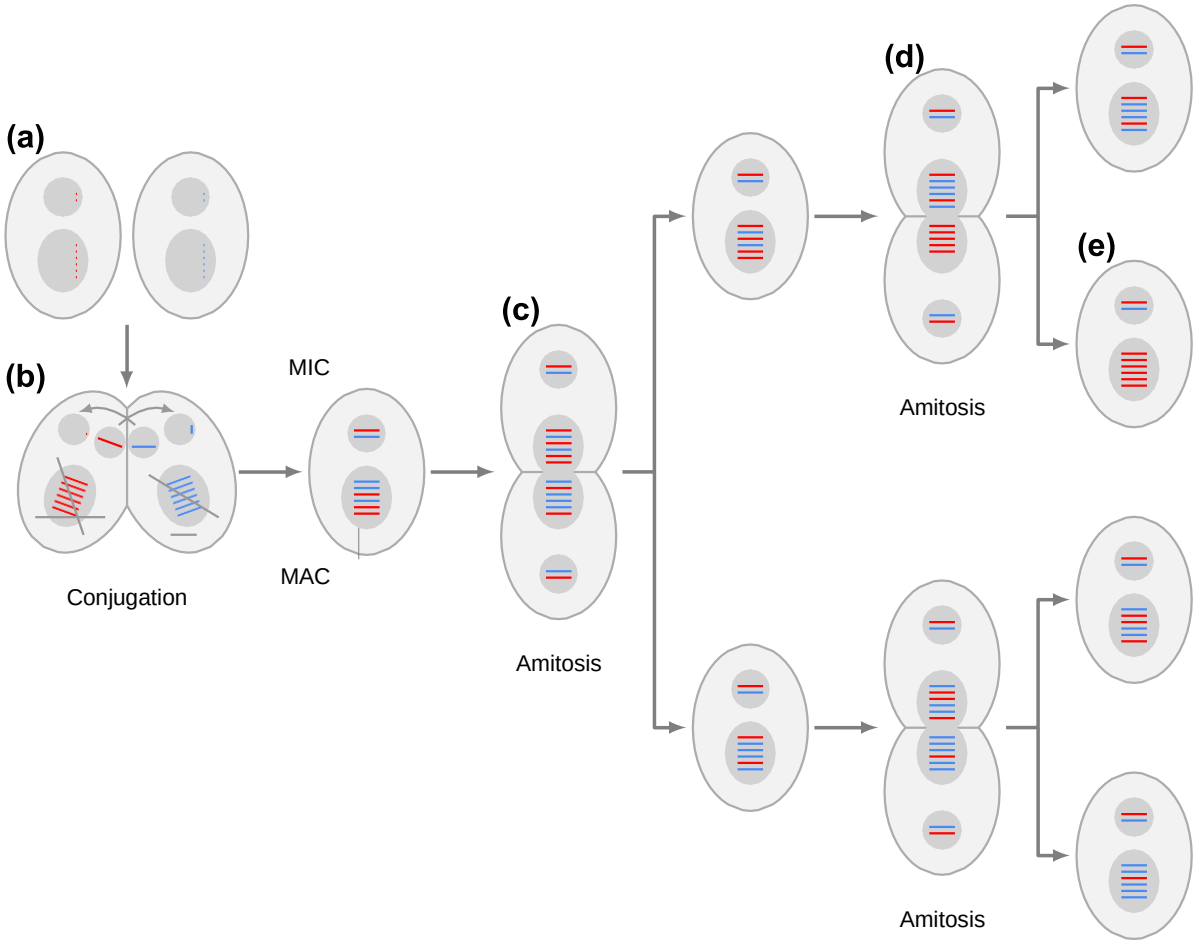

Figure 2.1: Amitosis with chromosome copy number control generates variation among individuals. Schematic of sexual conjugation followed by two rounds of asexual division. For simplicity, only one chromosome is shown: it occurs in two copies in the micronucleus (MIC) and six copies in the macronucleus (MAC) (in reality, each chromosome occurs in 45 copies in the *Tetrahymena thermophila* MAC). a, During sexual reproduction (conjugation), the diploid MIC undergoes meiosis (Jahn and Klobutcher, 2002; Orias et al., 2011). b, Two cells can fuse transiently and exchange haploid meiotic products. A resident meiotic product then fuses with the transferred meiotic product to produce a new diploid zygotic nucleus, which divides to generate the new MIC and MAC (the old MAC is destroyed). During asexual reproduction (c, d), the MIC divides by mitosis while the MAC divides by amitosis. Amitosis allows the random segregation of parental chromosomes among daughter cells generating variation among individuals. Ultimately, this results in phenotypic assortment, in which individual chromosomes in the MAC become completely homozygous within several generations (Doerder et al., 1992). (e). *T. thermophila*, has an unknown copy number control mechanism that results in an approximately equal number of homologous chromosomes in each daughter cell (Orias et al., 2011).

2.2. Methods

2.2.1. Model

We model an infinite-sized population of asexual organisms of ploidy *n* reproducing in discrete generations. We begin by considering a single locus. The state of the population is given by a vector of frequencies $\vec{x} = (x_0, x_1, \ldots, x_n)$, where x_i is the frequency of the genotype with *i* deleterious mutations and *n* – *i* wildtype alleles. Every generation, the population undergoes reproduction (mitosis or amitosis), mutation, and selection.

An individual with *j* deleterious mutations reproducing by amitosis has offspring with *i* mutations with probability

$$\mathrm{P}_{i,j} = \begin{cases} \dfrac{\binom{2j}{i}\binom{2n-2j}{n-i}}{\binom{2n}{n}} & \text{if} \quad 0 \le i \le \min(2j, n) \\ 0 & \text{otherwise} \end{cases} \tag{1}$$

Thus, amitosis changes x_i by $\Delta^{\mathrm{amit}} x_i = (\sum_{j=0}^{n} x_j P_{i,j}) - x_i$. Mitosis has no effect on genotype frequencies: $\Delta^{\mathrm{mit}} x_i = 0$.

An individual with $i < n$ deleterious mutations will mutate into an individual with $i + 1$ mutations with probability $\mu_d(n - i)$ where μ_d is the deleterious mutation rate per wildtype allele per generation. We assume that all mutations are deleterious and irreversible. Thus, mutation will change x_i by

$$\Delta^{\mathrm{mut}} x_i = x_{i-1}(n - i + 1)\mu_d - x_i(n - i)\mu_d \tag{2}$$

Natural selection causes the frequency of individuals with *i* deleterious mutations to change by $\Delta^{\mathrm{sel}} x_i = x_i\, W_i/\overline{W} - x_i$, where $W_i = 1 + i s_d/n$, $s_d < 0$ is the effect of a deleterious mutation in a homozygous state, and $\overline{W} = \sum_i x_i W_i$ is the mean fitness.

We assume that these population genetic processes operate independently every generation. So, for example, evolution under reproductive strategy *X* (mitosis or amitosis) is described by

$$\vec{x}' = \vec{x} + \Delta^X \vec{x} + \Delta^{\text{mut}} \vec{x} + \Delta^{\text{sel}} \vec{x} \quad (3)$$

where $\vec{x}'$ are the genotype frequencies in the next generation. A population of individuals with $x_0 > 0$ evolving according to Equation 3 evolves towards a stable equilibrium where the mean fitness is $\widehat{W}_{X,1}$. If there are *L* fitness loci with the same μ_d and s_d, and there is linkage equilibrium between these loci, the mean fitness at equilibrium will be $\widehat{W}_{X,L} = (\widehat{W}_{X,1})^L$. When ploidy was $n > 3$, $\widehat{W}_{\text{amit}}$ was calculated numerically by iterating Equation 3. Equilibrium was inferred when the Euclidean distance between consecutive $\vec{x}$ was smaller than 10^{-6}. See Equations 4 and 5 and Appendix for analytical expressions for diploids ($n = 2$).

2.2.2. Stochastic simulations

Stochastic, individual-based simulations were conducted within a Wright-Fisher framework (Ewens, 2004). Individuals have *L* fitness loci and undergo a mutation–selection–reproduction life cycle. Population have constant size *N*. Initially, all individuals are mutation-free and have a fitness of $W = 1$.

Every generation, each individual may acquire a new mutation at a fitness locus with the probabilities shown in Equation 2. An individual can acquire multiple mutations, but only one per locus. Under asexual reproduction (mitosis or amitosis), *N* individuals are chosen at random to reproduce, with replacement, with probability proportional to their fitness. Each individual chosen to reproduce is allowed to generate one offspring. Under mitosis, the offspring is an exact copy of the parent; under amitosis, the number of mutant alleles inherited by the offspring at each locus is drawn at random with probability given by Equation 1. The parents are discarded after reproduction. Under sexual reproduction, 2*N* individuals are chosen randomly with replacement, with probability proportional to their fitness. We then create *N* pairs of individuals from this set at random without replacement. Each pair is allowed to generate one offspring with free recombination among the *L* loci. The parents are discarded after reproduction.

The rate of accumulation of drift load was measured as the slope of a linear regression of population $\ln \overline{W}$ against generation. Equilibrium was evaluated using the slope of a linear regression of population $\overline{W}$ against generation. Equilibrium was inferred when the average slope was not statistically significantly different from zero over a large number of replicate populations. The slopes were evaluated between generations 300 and 600 in diploids and between generations 9×10^3 and 10^4 in 45-ploids.

2.3. Results and Discussion

2.3.1. Amitosis can confer deterministic benefit by reducing the mutation load within the population

Most mutations with effects on fitness are deleterious but natural selection cannot remove all of them from populations. As a result, many individuals carry deleterious mutations that reduce their fitness, which leads to a reduction in the mean fitness of populations, or mutation load. We begin by investigating the extent to which amitosis with chromosome copy number control affects mutation load. A population of asexual diploids that reproduces by mitosis is expected to show the following mean fitness at equilibrium (Crow, 1970; Haldane, 1927; Kimura and Maruyama, 1966; Kondrashov and Crow, 1988):

$$\widehat{W}_{\mathrm{mit}} = \exp\left(-U_d\right) \tag{4}$$

where $U_d = 2L\mu_d$ is the deleterious mutation rate per diploid genome per generation, L is the number of loci influencing fitness, and μ_d is the deleterious mutation rate per locus per generation (see Appendix). In contrast, if an asexual diploid population reproduces by amitosis, its mean fitness at equilibrium is given by

$$\widehat{W}_{\mathrm{amit}} = \exp\left[-U_d\left(\frac{1-3s_d}{2-3s_d}\right)\right] \tag{5}$$

where $s_d < 0$ is the effect on fitness of a deleterious mutation in a homozygous state (see Appendix). This scenario is purely theoretical because no diploid nucleus is known to reproduce amitotically. Equations 4 and 5 rely on several assumptions: (i) population size is very large, so we can ignore genetic drift; (ii) mutations are irreversible; μ_d is (iii) low and (iv) equal across loci; (v) there is linkage equilibrium

among fitness loci; all mutations (vi) have the same deleterious effect s_d, and contribute to fitness (vii) additively within loci (i.e., are codominant) and (viii) multiplicatively among loci (i.e., do not interact epistatically). Equations 4 and 5 show that amitosis can reduce mutation load compared to mitosis in diploid populations. For example, if $U_d = 0.1$ and $s_d = -0.1$, the mean fitness at equilibrium is $\widehat{W}_{mit} = 0.905$ under mitosis and $\widehat{W}_{amit} = 0.945$ under amitosis. Thus, amitosis has a selective advantage over mitosis of $\widehat{W}_{amit}/\widehat{W}_{mit} - 1 = 4.4\%$. The deleterious mutation rate, U_d, has a large effect on the benefit of amitosis: doubling the value of U_d more than doubles the advantage of amitosis to 9.1% (Figure 2.2(a)). The selection coefficient of a deleterious mutation, s_d, however, has a comparatively small effect on the benefit of amitosis: making mutations one tenth as deleterious ($s_d = -0.01$) causes the advantage of amitosis to increase to only 5.0% (Figure 2.2(b)).

Amitosis with copy number control is observed in the genus *Tetrahymena*, which have high ploidy in their macronuclear genome (e.g., *T. thermophila* are 45-ploid). Interestingly, the benefit of amitosis relative to a mitotically reproducing organism with the same ploidy increases with ploidy (Figure 2.2). For example, if $U_d = 0.1$ and $s_d = -0.1$, the benefit of amitosis increases to 6.7% in tetraploids, 7.9% in octoploids, 8.7% in 16-ploids, and so on. Further increases in ploidy cause diminishing returns in the benefit of amitosis. These expected benefits are conservative because they assume that the deleterious mutation rate, U_d, is constant across ploidies. If, for example, doubling ploidy causes an increase of 10% in U_d, a substantially greater benefit of amitosis would be achieved at high ploidies (Figure 2.2(a), dashed line). A mutation accumulation study estimated that *T. thermophila* has a deleterious mutation rate in the MIC of $U_d^{(MIC)} = 0.0094$ per genome per generation and that mutations have an expected deleterious effect of $s_d^{(MIC)} = -0.11$ in a homozygous state (Long et al., 2016a). If we assume that the MAC genome has $U_d^{(MAC)} = (45/2) \times U_d^{(MIC)} = 0.2115$ and $s_d^{(MAC)} = s_d^{(MIC)}$, we estimate that amitosis has a benefit of 21.0% relative to mitosis in this species.

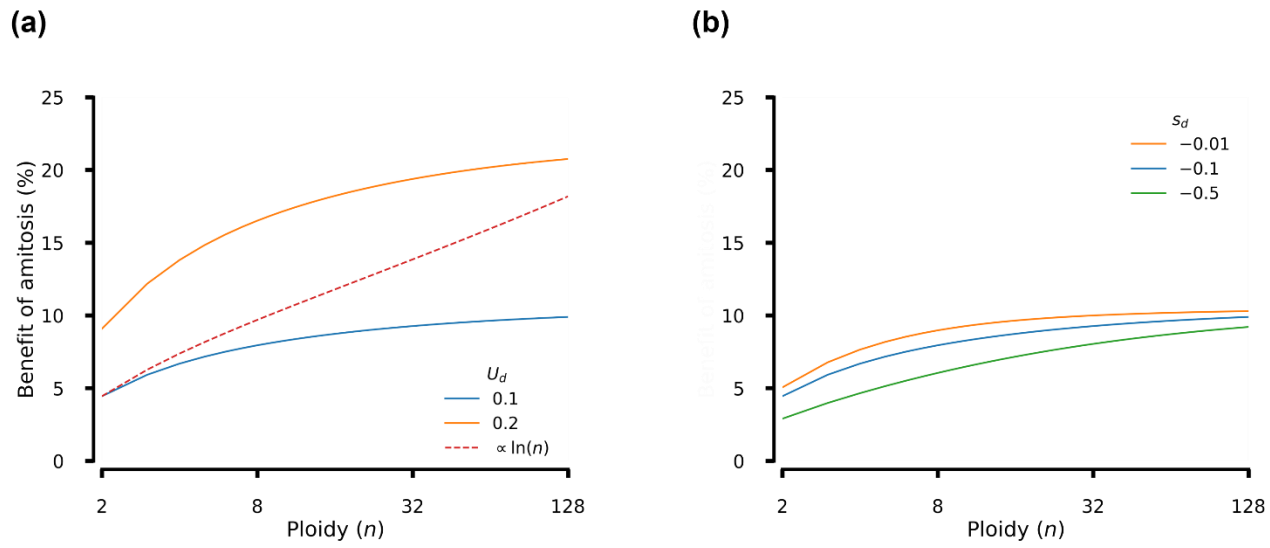

Figure 2.2: Amitosis with chromosome copy number control reduces mutation load relative to mitosis in large populations. Values show the selective advantage of amitosis over mitosis, $\widehat{W}_{amit}/\widehat{W}_{mit} - 1$, at different ploidies ($\widehat{W}_X$ is the mean fitness at equilibrium of a population of individuals following reproductive strategy X for a certain ploidy). a, Effect of the genomic deleterious mutation rate, U_d. Solid lines show selective benefits corresponding to constant values of U_d at all ploidies. The dashed line assumes that a doubling of the ploidy results in a 10% increase in U_d. Mutations have a deleterious effect of $s_d = -0.1$ at all ploidies. b, Effect of the selection coefficient of a deleterious mutation, s_d. We set $U_d = 0.1$ at all ploidies. In both a and b we assumed that there were $L = 100$ fitness loci. Note that ploidy is shown in a log scale.

2.3.2. Amitosis can confer stochastic benefit by slowing down the operation of Muller's ratchet

The analyses so far have ignored the effect of genetic drift. Drift can cause a population to accumulate deleterious mutations stochastically, further increasing genetic load, or drift load (Crow, 1970; Kimura and Maruyama, 1963; Poon and Otto, 2000). In asexuals this phenomenon is known as Muller's ratchet (Felsenstein, 1974; Haigh, 1978; Muller, 1964). We now evaluate the extent to which amitosis with copy number control can slow down the accumulation of drift load. Populations of $N = 10$ or 100 diploid mitotic individuals experience strong Muller's ratchet when $U_d = 0.1$ and $s_d = -0.1$ (Figure 2.3(a)). Increasing population size to $N = 10^3$ individuals causes the ratchet to slow down considerably, allowing populations to achieve mutation-selection equilibrium (Figure 2.3(a)). Reproduction through amitosis makes populations less susceptible to Muller's ratchet. The accumulation of drift load slows down by 39% (95%

confidence interval, CI: 31%, 46%) in diploid populations of N = 10 individuals, and effectively halts in populations of N = 100 individuals (Figure 2.3(c)).

The benefit of amitosis in slowing down the accumulation of drift load, like the deterministic benefit, increases with ploidy. Muller's ratchet operates in populations as large as $N = 10^4$ mitotic 45-ploid individuals (Figure 2.3(b)). Amitosis is able to halt the accumulation of drift load in populations with as few as N = 100 45-ploid individuals (Figure 2.3(d)). Even when amitotic populations are small enough to accumulate drift load, they do so more slowly than mitotic ones. For example, populations of N = 10 amitotic 45-ploid individuals accumulate drift load 64% (95% CI: 59%, 68%) more slowly than mitotic populations of the same size (Figure 2.3(b), (d)).

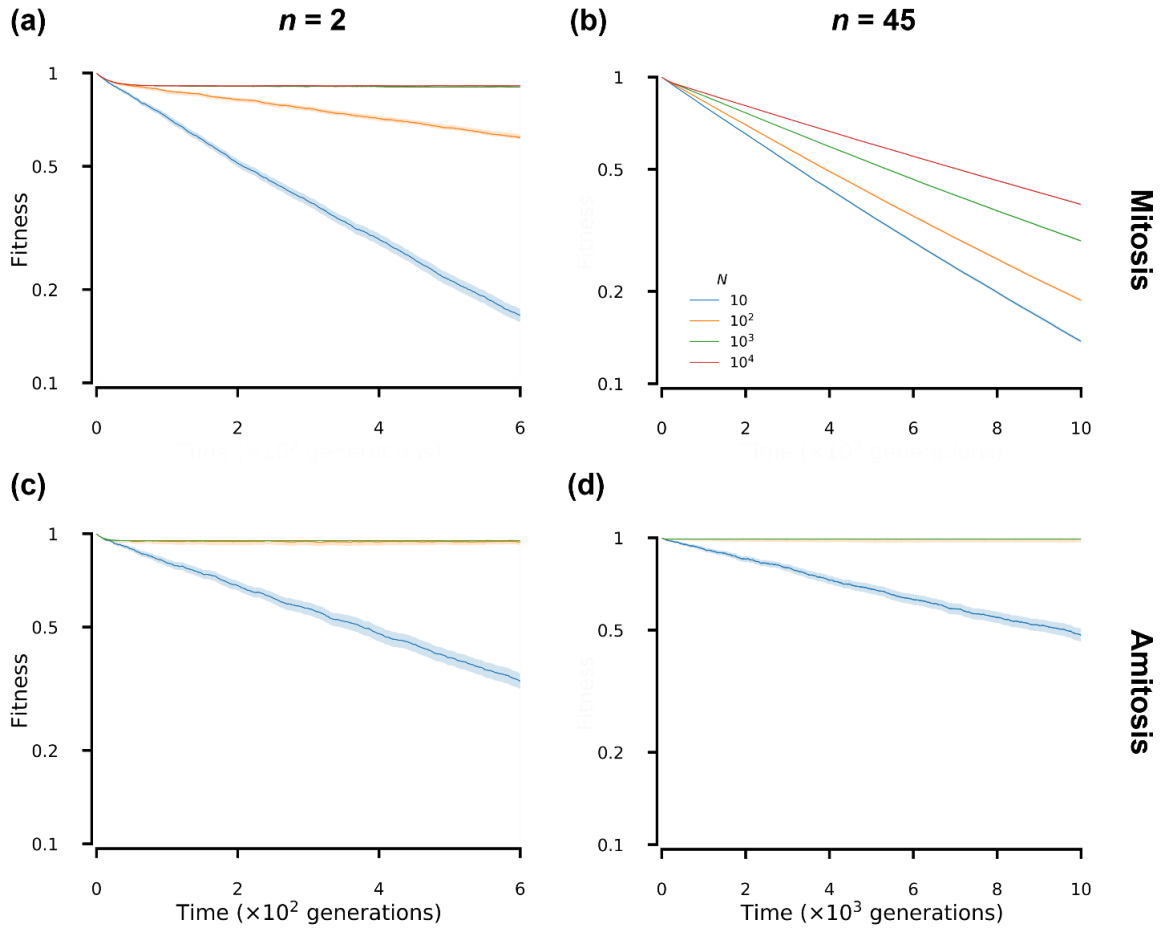

Figure 2.3: Amitosis with chromosome copy number control slows down the accumulation of drift load relative to mitosis. Evolutionary responses of mean fitness in populations of different sizes (N) and

plodies (n), following different reproductive strategies. Lines show the means of stochastic simulations of 100 populations; shaded regions represent 95% CIs. a, Mitosis in diploids ($n = 2$). b, Mitosis with a ploidy of $n = 45$. c, Amitosis in diploids ($n = 2$). d, Amitosis with a ploidy of $n = 45$. We assumed $L = 100$ fitness loci, a genomic deleterious mutation rate of $U_d = 0.1$ per generation, that mutations have a deleterious effect of $s_d = -0.1$ in a homozygous state, and that, initially, all individuals are unmutated. Note that fitness is shown in a log scale.

2.3.3. The stochastic benefit provided by amitosis is comparable to that of sex

The benefits of amitosis over mitosis identified so far are analogous to benefits of sexual over asexual reproduction. In diploids, sexual reproduction by selfing confers a deterministic advantage over mitosis almost identical to that of asexual amitosis shown in Equations 4 and 5 (see Appendix). Unlike amitosis, sex with random mating in diploids only confers a deterministic advantage over asexual reproduction if there is negative epistasis between deleterious mutations (Kondrashov, 1988; Otto and Feldman, 1997), or if deleterious mutations are partially recessive (Chasnov, 2000; Otto, 2003). Sex can also counteract Muller's ratchet (Felsenstein, 1974; Muller, 1964), much like amitosis (Figure 2.3(a), (c)). Are the benefits of asexual amitosis also similar to those of sexual reproduction when ploidy is high? We investigated this question in populations of $N = 20$ individuals of a 45-ploid organism like *T. thermophila* experiencing $U_d = 0.1$ and $s_d = -0.1$. Amitosis slows down the accumulation of drift load relative to mitosis by 90% (95% CI: 88%, 92%; Figure 2.4(a)). An organism like *T. thermophila* but reproducing sexually, with outcrossing, every generation (i.e., obligate sex with no amitosis) and then generating a 45-ploid macronucleus from the recombinant diploid micronucleus (see Figure 2.1(a), (b)) would slow down the accumulation of drift load by 92% (95% CI: 90%, 94%; $\tau = 1$, Figure 2.4(a)). However, *T. thermophila* cannot reproduce sexually every generation; rather, it requires $\sim$ 100 asexual cell divisions to reach sexual maturity (Doerder et al., 1995; Nanney, Caughey, and Tefankjian, 1955). Facultative sex every $\tau = 100$ generations slows down the ratchet by only 68% (95% CI: 64%, 72%; measured based on fitness in the generation immediately before the population reproduces sexually), much less than amitosis (Figure 2.4(a)). The benefit of amitosis is also comparable to that of sex in larger populations in the presence of beneficial mutations. In an evolutionary scenario under which asexual populations are not able to adapt, both amitosis and obligate sex every

generation (τ = 1) allow populations to adapt, and more rapidly than facultative sex every τ = 100 generations (Figure 2.4(b)).

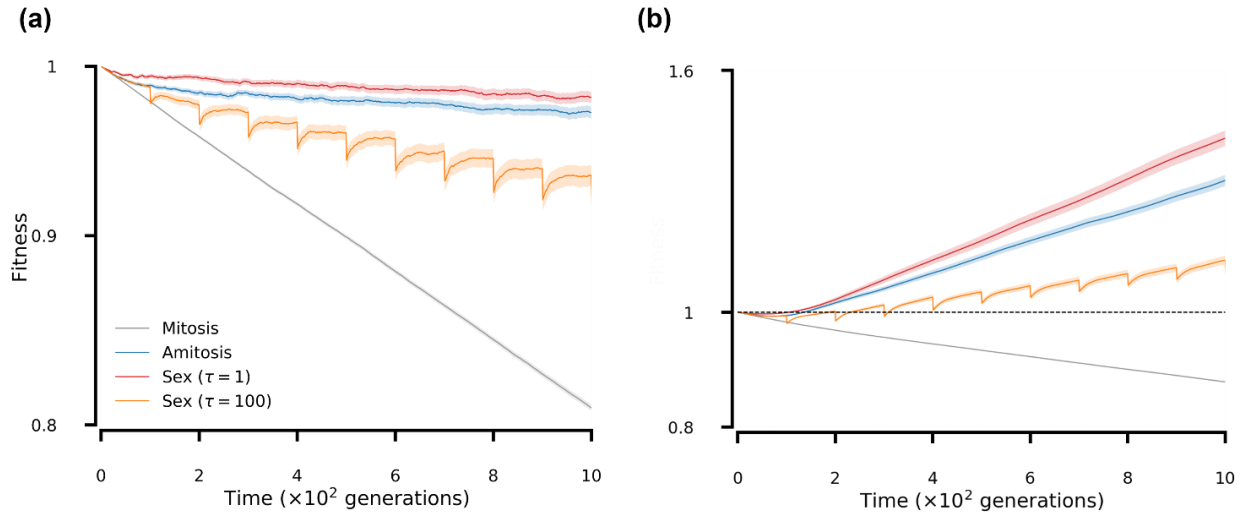

Figure 2.4: The benefit of amitosis with chromosome copy number control is similar to that of sex. Evolutionary responses of population mean fitness under different reproductive strategies. Lines show the means of stochastic simulations of 500 populations; shaded regions represent 95% CIs. a, Populations of N = 20 individuals with a deleterious mutation rate of U_d = 0.1 per genome per generation. All mutations are deleterious and have a selection coefficient of $s_d = -0.1$ in a homozygous state. b, Populations of $N = 10^3$ individuals with a genomic mutation rate of U = 0.1 per generation; 99% of mutations are deleterious and 1% are beneficial with selection coefficients of $s_d = -0.1$ and s_b = 0.1, respectively. We assumed that individuals have a MAC ploidy of n = 45 with L = 100 fitness loci, and that, initially, they carry no mutations. Sexual reproduction takes place with random mating and free recombination every τ generations. Note that fitness is shown in a log scale.

The results shown in Figure 2.4 raise the intriguing possibility that amitosis is actually evolutionarily superior to facultative sex in *T. thermophila* and its relatives, which have $\tau \approx 100$. If true, this would lead to the prediction that asexual lineages should outcompete sexual ones in *Tetrahymena*. This could explain why obligately asexual lineages are abundant in *Tetrahymena* (Doerder, 2014). If this explanation is correct, we would expect that asexual lineages of *Tetrahymena* do not show the typical signs of accelerated accumulation of deleterious mutations compared to their sexual relatives (Henry et al., 2012; Hollister et al., 2015; Johnson and Howard, 2007; Neiman et al., 2010; Paland and Lynch, 2006; Tucker et al., 2013).

The hypothesis outlined in the previous paragraph may be invalid for two reasons. First, our analysis may overestimate the benefit of amitosis relative to facultative sex. Our hypothesis assumes that chromosome copy number control during amitosis is perfect, or at least, highly precise on an evolutionary time-scale. However, the precision of copy number control is unknown even in *T. thermophila*. Control of chromosome copy number could be less precise than we have assumed and, therefore, confer a smaller benefit to *Tetrahymena*. Second, our analysis may underestimate the benefit of facultative sex relative to amitosis. We have considered only two possible benefits of sex, both "mutational" in nature (Kondrashov, 1993). Other benefits of sex are not guaranteed to show the same pattern. For example, we have not considered the potential benefits of sex in the face of biotic interactions (Bell, 1982; Hamilton et al., 1990; Otto and Nuismer, 2004). Even if our hypothesis is correct, it is also conceivable that there are additional factors contributing to the relative success of asexual *Tetrahymena*. For example, it has been proposed that high ploidy alone may inhibit the accumulation of deleterious mutations through gene conversion (Maciver, 2016). However, this proposed advantage has not been modelled, and therefore it is difficult to evaluate.

What is the mechanistic basis of the benefits of amitosis identified here? The main difference between the two types of nuclear division is that amitosis, like sex, can generate more genetic variation in fitness than mitosis. For example, an n-ploid individual (we assume n is even for simplicity) with $n/2$ wild-type alleles and $n/2$ deleterious alleles will have a fitness of $W = 1 - s_d/2$. Mutation will generate a variance in fitness of

$$V_{\mathrm{mut}} = \frac{\left(u_d - u_d^2\right) s_d^2}{n^2} \tag{6}$$

every generation, where $u_d = n\mu_d$ is the deleterious mutation rate at the locus per generation. Mitosis is not expected to generate any variance in fitness in addition to mutation (i.e., $V_{\mathrm{mit}} = V_{\mathrm{mut}}$). Amitosis will, however, increase the variance in fitness further

$$V_{\mathrm{amit}} = V_{\mathrm{mut}} + \frac{s_d^2}{8n - 4} \tag{7}$$

every generation (Schensted, 1958). Since u_d is likely to be low, amitosis is expected to increase the variance in fitness to a much greater extent than mutation, and therefore mitosis ($V_{\mathrm{amit}} \gg V_{\mathrm{mit}}$).

We propose that amitosis causes an increase in the additive genetic variance in fitness, therefore making natural selection more efficient—an analog of Weismann's hypothesis for the advantage of sex (Burt, 2000; Kondrashov, 1993; Weismann, 1887). Consistent with this idea, the variance in fitness generated by amitosis relative to mitosis increases approximately linearly with ploidy ($V_{\mathrm{amit}}/V_{\mathrm{mit}} \approx n/(8u_d)$; Equations 6 and 7), which explains why the benefit of amitosis relative to mitosis increases with ploidy. We conclude that amitosis with chromosome copy number control confers benefits of sex in the absence of sex and can account for the high incidence of obligately asexual lineages in *Tetrahymena* (Doerder, 2014).

2.4. Appendix[2]

2.4.1. Mitosis

Model

A population of diploid individuals reproducing by mitosis evolves at one fitness locus according to the following system of recursion equations

$$\begin{aligned} x_0' &= \frac{x_0}{\overline{W}} - 2\mu_d x_0 \\ x_1' &= \frac{(2+s_d)\,x_1}{2\overline{W}} + 2\mu_d x_0 - \mu_d x_1 \\ x_2' &= \frac{(1+s_d)\,x_2}{\overline{W}} + \mu_d x_1 \end{aligned} \tag{S1}$$

where the mean fitness of the population is

[2] All the mathematic analyses shown in the Appendix were done by Dr. Ricardo Azevedo. This work is presented here for illustration.

$$\overline{W} = x_0 + \left(1 + \frac{s_d}{2}\right) x_1 + (1 + s_d) x_2 \tag{S2}$$

x_i is the frequency of the genotype with i deleterious mutations and $n - i$ wildtype alleles, μ_d is the deleterious mutation rate per wildtype allele per generation, and $s_d < 0$ is the effect of a deleterious mutation in a homozygous state. Note that $\sum_i x_i = 1$.

Equilibrium

The population is in equilibrium when $\vec{x}' = \vec{x}$, where $\vec{x} = (x_0, x_1, x_2)$. There is one equilibrium where unmutated individuals are present in the population ($x_0 > 0$)

$$\begin{aligned} \hat{x}_0 &= \frac{(s_d + 2\mu_d (1 + s_d))^2}{s_d^2 (1 + 2\mu_d)^2} \\ \hat{x}_1 &= -\frac{4\mu_d (s_d + 2\mu_d (1 + s_d))}{s_d^2 (1 + 2\mu_d)^2} \\ \hat{x}_2 &= \frac{4\mu_d^2}{s_d^2 (1 + 2\mu_d)^2} \end{aligned} \tag{S3}$$

Stability of the equilibrium

The Jacobian matrix of the system is

$$\mathbf{J} = \begin{pmatrix} \frac{1}{\overline{W}} + \frac{s_d x_0}{\overline{W}^2} - 2\mu_d & \frac{s_d x_0}{2\overline{W}^2} \\ \frac{s_d(2 + s_d) x_1}{2\overline{W}^2} + 2\mu_d & \frac{2 + s_d}{2\overline{W}} + \frac{s_d(2 + s_d) x_1}{4\overline{W}^2} - \mu_d \end{pmatrix} \tag{S4}$$

(We only need to consider x_0 and x_1 because $x_2 = 1 - x_0 - x_1$.) The eigenvalues of **J** (Equation S4) evaluated at the equilibrium in Equation S3 are

$$\begin{aligned} \lambda_1 &= (1 + s_d)(1 + 2\mu_d) \\ \lambda_2 &= 1 + \mu_d + 2\mu_d^2 + \frac{1}{2} s_d (1 + 2\mu_d)^2 \end{aligned} \tag{S5}$$

The equilibrium in Equation S3 is stable (Otto and Day, 2007) if $-1 < \lambda_i < 1$, $\forall_i$. If we assume that mutations occur at a low rate ($0 < \mu_d << 1$) and are deleterious ($-1 \leq s_d < 0$), the equilibrium is stable if

$$-1 \leq s_d < -\frac{2\mu_d}{1+2\mu_d} \tag{S6}$$

The equilibrium in Equation S3 is valid ($\forall_i : 0 \leq x_i \leq 1$) when the condition in Equation S6 is met.

Mean fitness at equilibrium

The mean fitness (Equation S2) at the equilibrium defined by Equation S3 is

$$\widehat{W}_{\mathrm{mit},1} = \frac{1}{1+2\mu_d} \tag{S7}$$

If there are L fitness loci with the same μ_d and s_d, and there is linkage equilibrium between these loci, the mean fitness at equilibrium will be $\widehat{W}_{\mathrm{mit},L} = (\widehat{W}_{\mathrm{mit},1})^L$. Taking a first-order Taylor expansion of ln $(\widehat{W}_{\mathrm{mit},L})$ around $\mu_d = 0$ we get Equation 4

$$\widehat{W}_{\mathrm{mit}} = \exp(-U_d) \tag{S8}$$

where $U_d = 2L\mu_d$ is the genomic deleterious mutation rate.

2.4.2. Amitosis

Model

A diploid individual with $j = 0$ or 2 deleterious mutations reproducing by amitosis has offspring with j mutations with probabilities $P_{j,j} = 1$. If the individual has $j = 1$ mutations, its offspring will have $i = 0$, 1, or 2 mutations with probability (Schensted, 1958) $P_{i,1} = 1/6$, 2/3, and 1/6, respectively (see Methods, Equation 1). Thus, amitosis changes $\vec{x}$ relative to Equation S1 by

$$\Delta^{\text{amit}} x_0 = \frac{x_1}{6}$$
$$\Delta^{\text{amit}} x_1 = -\frac{x_1}{3}$$
$$\Delta^{\text{amit}} x_2 = \frac{x_1}{6}$$

A population of diploid individuals reproducing by amitosis evolves at one fitness locus according to the following system of recursion equations

$$\begin{aligned} x_0' &= \frac{x_0}{\overline{W}} - 2\mu_d x_0 + \frac{x_1}{6} \\ x_1' &= \frac{(2+s_d)x_1}{2\overline{W}} + 2\mu_d x_0 - \mu_d x_1 - \frac{x_1}{3} \\ x_2' &= \frac{(1+s_d)x_2}{\overline{W}} + \mu_d x_1 + \frac{x_1}{6} \end{aligned} \tag{S9}$$

See Mitosis for details on the notation.

Equilibrium

There is one equilibrium where unmutated individuals are present in the population ($x_0 > 0$)

$$\begin{aligned} \hat{x}_0 =& \beta \left(4 + s_d\left(12 + 37 s_d\right) + 40\mu_d + 2 s_d \mu_d \left(76 + 71 s_d\right) + 36\mu_d^2 \left(1 + s_d\right)\left(3 + 5 s_d\right) + \right. \\ &\left. + 72\mu_d^3 \left(1 + s_d\right)^2 - \alpha\left(2\left(1 + \mu_d\right)\left(1 + 6\mu_d\right) + s_d\left(9 + 4\mu_d\left(5 + 3\mu_d\right)\right)\right)\right) \\ \hat{x}_1 =& 2\beta \left(3 s_d^2 \left(1 + 2\mu_d\right)\left(5 + 2\mu_d\left(7 + 6\mu_d\right)\right) + 2\left(1 + \mu_d\right)\left(1 + 6\mu_d\right)\left(-2 + \alpha - 6\mu_d\right) + \right. \\ &\left. + s_d\left(-4 + 5\alpha + 2\mu_d\left(-26 + 7\alpha + 6\mu_d(-5 + \alpha)\right)\right)\right) \\ \hat{x}_2 =& -\beta\left(1 + 6\mu_d\right)\left(3\left(s_d + 2 s_d \mu_d\right)^2 + 2\left(1 + \mu_d\right)\left(-2 + \alpha - 6\mu_d\right) + \right. \\ &\left. + s_d\left(4 + \alpha + 2\mu_d\left(12 + \alpha + 12\mu_d\right)\right)\right) \end{aligned} \tag{S10}$$

where

$$\alpha = \sqrt{(2 - 3s_d)^2 + 12\mu_d(2 + 3s_d(1 + s_d)) + 36\mu_d^2(1 + s_d)^2}$$
$$\beta = \frac{1}{16 s_d^2 (1 + \mu_d)(2 + 3\mu_d)^2}$$

Stability of the equilibrium

The Jacobian matrix of the system is

$$\mathbf{J} = \begin{pmatrix} \frac{1}{\overline{W}} + \frac{s_d x_0}{\overline{W}^2} - 2\mu_d & \frac{1}{6} + \frac{s_d x_0}{2\overline{W}^2} \\ \frac{s_d(2+s_d)x_1}{2\overline{W}^2} + 2\mu_d & -\frac{1}{3} + \frac{2+s_d}{2\overline{W}} + \frac{s_d(2+s_d)x_1}{4\overline{W}^2} - \mu_d \end{pmatrix} \tag{S11}$$

(We only need to consider x_0 and x_1 because $x_2 = 1 - x_0 - x_1$.)

The characteristic equation of **J** evaluated at the equilibrium in Equation S10 is

$$a_1 + a_2\lambda + \lambda^2 = 0 \tag{S12}$$

with coefficients

$$\begin{aligned} a_1 =& \frac{1}{108(2+s_d)^2}(4(134 - 31\alpha + 3\mu_d(82 - 16\alpha + 3\mu_d(38 - 5\alpha + 18\mu_d))) + \\ &+ 4s_d(197 - 46\alpha + 3\mu_d(186 - 32\alpha + 3\mu_d(97 - 9\alpha + 60\mu_d))) + \\ &+ 3s_d^2(122 - 21\alpha + 2\mu_d(313 - 32\alpha + 6\mu_d(103 - 5\alpha + 72\mu_d))) + \\ &+ 9s_d^3(1 + 2\mu_d)(19 - \alpha + 2\mu_d(35 - \alpha + 36\mu)) + 27s_d^4(1 + 2\mu_d)^3) \end{aligned}$$

$$a_2 = -\frac{1}{12}\left(26 - 3\alpha + 3s_d(3 + 4\mu_d(2 + \mu_d)) + \frac{2\mu_d}{2+s_d}(20 - \alpha + 18\mu_d + s_d(14 - \alpha + 12\mu_d))\right)$$

The equilibrium in Equation S10 is stable if the following Routh-Hurwitz conditions are met (Otto and Day, 2007)

$$\begin{aligned} 1 + a_1 + a_2 &> 0 \\ 2 + a_2 &> 0 \end{aligned} \tag{S13}$$

If we assume that mutations occur at a low rate ($0 < \mu_d << 1$) and are deleterious ($-1 \leq s_d < 0$), the conditions in Equation S13 are met when

$$\begin{aligned} &\left(-1 \leq s_d \leq \frac{1}{31}\left(-21 + \sqrt{7}\right)\right) \vee \\ &\left(\frac{1}{31}\left(-21 + \sqrt{7}\right) < s_d < 0 \ \wedge \ 0 < u < \frac{1}{12}\sqrt{\frac{1 - 11s_d}{1 + s_d}} - \frac{1 + 7s}{12(1 + s_d)}\right) \end{aligned} \tag{S14}$$

The equilibrium in Equation S10 is valid ($\forall_i : 0 \leq x_i \leq 1$) when the condition in Equation S14 is met. The

condition in Equation S14 is broader than that in Equation S6.

Mean fitness at equilibrium

The mean fitness (Equation S2) at the equilibrium defined by Equation S10 is

$$\widehat{W}_{\mathrm{amit},1} = \frac{14 + 3s_d + 6\mu_d(3 + s_d) + \alpha}{8(1 + \mu_d)(2 + 3\mu_d)} \tag{S15}$$

If there are L fitness loci with the same μ_d and s_d, and there is linkage equilibrium between these loci, the mean fitness at equilibrium will be $\widehat{W}_{\mathrm{amit},L} = (\widehat{W}_{\mathrm{amit},1})^L$. Taking a first-order Taylor expansion of ln $(\widehat{W}_{\mathrm{amit},L})$ around $\mu_d = 0$ we get Equation 5

$$\widehat{W}_{\mathrm{amit}} = \exp\left[-U_d\left(\frac{1 - 3s_d}{2 - 3s_d}\right)\right] \tag{S16}$$

where $U_d = 2L\mu_d$ is the genomic deleterious mutation rate.

2.4.3. Selfing

Model

A diploid individual with j = 0 or 2 deleterious mutations reproducing by selfing has offspring with j mutations with probabilities $P_{j,j} = 1$. If the individual has $j = 1$ mutations, its offspring will have i = 0, 1, or 2 mutations with probability $P_{i,1}$ = 1/4, 1/2, and 1/4, respectively. Thus, selfing changes $\vec{x}$ relative to Equation S1 by

$$\Delta^{\mathrm{self}} x_0 = \frac{x_1}{4}$$
$$\Delta^{\mathrm{self}} x_1 = -\frac{x_1}{2}$$
$$\Delta^{\mathrm{self}} x_2 = \frac{x_1}{4}$$

A population of diploid individuals reproducing by selfing evolves at one fitness locus according to the following system of recursion equations

$$
\begin{aligned}
x_0' &= \frac{x_0}{\overline{W}} - 2\mu_d x_0 + \frac{x_1}{4} \\
x_1' &= \frac{(2+s_d)x_1}{2\overline{W}} + 2\mu_d x_0 - \mu_d x_1 - \frac{x_1}{2} \\
x_2' &= \frac{(1+s_d)x_2}{\overline{W}} + \mu_d x_1 + \frac{x_1}{4}
\end{aligned}
\tag{S17}
$$

See Mitosis for details on the notation.

Equilibrium

There is one equilibrium where unmutated individuals are present in the population ($x_0 > 0$)

$$
\begin{aligned}
\hat{x}_0 =& \frac{\varepsilon}{2}\left(2 + 5s_d + 11s_d^2 + 2\mu_d\left(7 + s_d\left(22 + 19s_d\right)\right) + 4\mu_d^2\left(1 + s_d\right)\left(7 + 11s_d\right) + \right. \\
&\left. + 16\mu_d^3\left(1 + s_d\right)^2 - \gamma\left(2 + 7s_d + 2\mu_d\left(5 + 7s_d\right) + 8\mu_d^2\left(1 + s_d\right)\right)\right. \\
\hat{x}_1 =& 2\varepsilon\left(s_d\left(-1 + 2\gamma + \mu_d\left(-8 + 5\gamma + 4\mu_d(-2 + \gamma)\right)\right) + \right. \\
&\left. + s_d^2\left(1 + 2\mu_d\right)\left(2 + \mu_d\left(5 + 4\mu_d\right)\right) + \left(1 + \mu_d\right)\left(1 + 4\mu_d\right)\left(-1 + \gamma - 2\mu_d\right)\right. \\
\hat{x}_2 =& -\frac{\varepsilon}{2}\left(1 + 4\mu_d\right)\left(\left(s_d + 2s_d\mu_d\right)^2 + 2\left(1 + \mu_d\right)\left(-1 + \gamma - 2\mu_d\right) + \right. \\
&\left. + s_d\left(1 + \gamma + 2\mu_d\left(4 + \gamma + 4\mu_d\right)\right)\right)
\end{aligned}
\tag{S18}
$$

where

$$
\begin{aligned}
\gamma &= \sqrt{(1-s)^2 + 4u\left(1 + s + s^2\right) + 4u^2(1+s)^2} \\
\epsilon &= \frac{1}{s_d^2(1+\mu_d)(3+4\mu_d)^2}
\end{aligned}
$$

Stability of the equilibrium

The Jacobian matrix of the system is

$$
\mathbf{J} = \begin{pmatrix}
\frac{1}{\overline{W}} + \frac{s_d x_0}{\overline{W}^2} - 2\mu_d & \frac{1}{4} + \frac{s_d x_0}{2\overline{W}^2} \\
\frac{s_d(2+s_d)x_1}{2\overline{W}^2} + 2\mu_d & -\frac{1}{2} + \frac{2+s_d}{2\overline{W}} + \frac{s_d(2+s_d)x_1}{4\overline{W}^2} - \mu_d
\end{pmatrix}
\tag{S19}
$$

(We only need to consider x_0 and x_1 because $x_2 = 1 - x_0 - x_1$.)

The characteristic equation of **J** evaluated at the equilibrium in Equation S18 is given by Equation S12 with coefficients

$$\begin{aligned}a_1 =& \frac{1}{4(2+s_d)^2}(23-15\gamma+2\mu_d(23-12\gamma+2\mu_d(15-5\gamma+6\mu_d))+\\ &+s_d(31-22\gamma+2\mu_d(47-23\gamma+2\mu_d(36-9\gamma+20\mu_d)))+\\ &+s_d^2(11-7\gamma+2\mu_d(35-11\gamma+2\mu_d(36-5\gamma+24\mu_d)))+\\ &+s_d^3(1+2\mu_d)^2(6-\gamma+12\mu_d)+s_d^4(1+2\mu_d)^3)\end{aligned}$$

$$a_2 = -\frac{1}{4}\left(9-3\gamma+s_d(3+4\mu_d(2+\mu_d))+\frac{2\mu_d}{2+s_d}(7-\gamma+6\mu_d+s_d(5-\gamma+4\mu_d))\right)$$

The equilibrium in Equation S18 is stable if the following Routh-Hurwitz conditions in Equation S13 are met (Otto and Day, 2007). If we assume that mutations occur at a low rate ($0 < \mu_d << 1$) and are deleterious ($-1 \leq s_d < 0$), the conditions in Equation S13 are met when

$$\begin{aligned}&\left(-1 \leq s_d \leq \frac{1}{22}(-15+\sqrt{5})\right)\\ \vee&\left(\frac{1}{22}(-15+\sqrt{5}) < s_d < 0 \wedge 0 < u < \frac{1}{8}\sqrt{\frac{1-7s_d}{1+s_d}} - \frac{1+5s}{8\,(1+s_d)}\right)\end{aligned} \tag{S20}$$

The equilibrium in Equation S18 is valid ($\forall_i : 0 \leq x_i \leq 1$) when the condition in Equation S14 is met. The condition in Equation S20 is broader than that in Equation S6 and approximately equal to that in Equation S14.

Mean fitness at equilibrium

The mean fitness (Equation S2) at the equilibrium defined by Equation S18 is

$$\widehat{W}_{\text{self},1} = \frac{5+s_d+2\mu_d(3+s_d)+\gamma}{2(1+\mu_d)(3+4\mu_d)} \tag{S21}$$

If there are L fitness loci with the same μ_d and s_d, and there is linkage equilibrium between these loci, the mean fitness at equilibrium will be $\widehat{W}_{\text{self},L} = (\widehat{W}_{\text{self},1})^L$. Taking a first-order Taylor expansion of ln $(\widehat{W}_{\text{self},L})$ around $\mu_d = 0$ we get

$$\widehat{W}_{\text{self}} = \exp\left[-U_d\left(\frac{1-2s_d}{2-2s_d}\right)\right] \tag{S22}$$

where $U_d = 2L\mu_d$ is the genomic deleterious mutation rate. Note that $\widehat{W}_{\text{self}} \approx \widehat{W}_{\text{amit}}$ (Equation S16).

Chapter 3. Investigation of the effect of amitosis with copy number control on adaptation to a changing environment

3.1. Introduction

The fact that sex is widespread within eukaryotes has puzzled evolutionary biologists for a long time. Weismann proposed that sex is advantageous and prevalent as it can generate more genetic variation compared to asexual reproduction, thus making selection more efficient (Weismann, 1887). This idea has been broadly accepted and promoted after proposing, and has been suggested as the foundation of several modern hypotheses explaining the widespread nature of sex (Hartfield and Keightley, 2012; Kondrashov, 1993). According to this idea, sex is expected to be able to facilitate the adaptation process, especially under a changing or novel environment. This prediction has been confirmed by many experimental evolution studies. Once exposed to a novel environment, sexual populations can exhibit an accelerated adaptation compared to their asexual relatives (Colegrave, 2002; Goddard et al., 2005), and higher frequencies of sex, especially out-crossing, were found to be favored during adaptation (Becks and Agrawal, 2012; Luijckx et al., 2017). Furthermore, it is even suggested that a frequently changing environment is a crucial factor in maintaining sex within the population (Lively and Morran, 2014).

In Chapter 2, I have shown that the unusual asexual reproduction strategy adopted by *Tetrahymena*, amitosis, can confer sexual-like benefits by slowing down the operation of Muller's ratchet and speeding up adaptation in a constant environment. However, it is unlikely that *Tetrahymena* always live in a constant environment. Living in freshwater ponds and streams, *Tetrahymena* may have to experience many changing variables in their habitats, such as the daily temperature fluctuations. In this chapter, I investigated whether and to what extent that amitosis can help *Tetrahymena* to get adapted to environmental changes using Fisher's geometric model with a moving optimum.

Fisher's geometric model (FGM) was first proposed by R. A. Fisher in 1930. In this model, the phenotype of each individual was represented by a single point in an *L*-dimension landscape, with each dimension corresponding to a phenotypic trait under stabilizing selection and having a single optimum. The fitness in FGM is calculated based on the Euclidian distance to the optimum, as a shorter distance indicates a higher fitness. The mutations are characterized by random vectors which can be either beneficial or deleterious,

depending on whether they can drive the mutated genotype closer to the optimum. For simplicity, the mutations are usually assumed to be universally pleiotropic, which means each mutation can affect all the dimensions simultaneously (Tenaillon, 2014).

FGM is a widely used model in evolutionary biology, especially in the field of studying adaptation. Despite making several simplifying assumptions, FGM has been shown to capture well certain features of adaptation. For example, one prediction made by FGM is that the population adaption should be proceeded by the substitution of small effect beneficial mutations as small effect beneficial mutations are more numerous. Such prediction has been demonstrated by empirical studies working on bacteriophage $\phi 6$ (Burch and Chao, 1999; Tenaillon, 2014). By allowing the optimum to move during evolution, FGM is also useful to study adaptation to a changing environment (Matuszewski, Hermisson, and Kopp, 2014). However, the classic FGM only considers haploid mitotic populations, with only a few working on diploid systems and considering sexual process (Manna, Martin, and Lenormand, 2011; Peck, Barreaut, and Heath, 1997; Peck, Yearsley, and Barreau, 1999; Tenaillon, 2014).

In this study, following the method introduced for diploid populations (Manna et al., 2011), I further extended FGM to be polyploid. To compare the performances of different reproduction strategies on adaptation, the processes of amitosis and sex were simulated and incorporated into FGM. Different pleiotropy levels were manipulated in my revised FGM. As the first step, the behaviors of the revised FGM were studied under a fixed optimum, and it was found that amitosis can cause a genetic load due to the prevalent overdominance within the model and resulted in a lower population fitness increase compared to mitosis. Further allowing the movement of optimum has elucidated that amitosis can accelerate the adaptation process in changing environments as it can generate a much greater variation and thus make selection more efficient. However, no matter whether the optimum moves, sex was found to only speed up adaptation to a similar extent as amitosis under low ploidies but not high ones, because the phenotype may be driven to overshoot the optimum after the mutation copy number amplification during sex.

3.2. Methods

3.2.1. Model

An individual-based FGM is used to simulate the evolution of *Tetrahymena* under different reproduction strategies with a finite population size $N = 500$. Key genomic features of *Tetrahymena*, including separate micronucleus (MIC) and macronucleus (MAC), polyploidy and amitotic division of MAC, and capability of having facultative sex, are incorporated into the model.

General process: Similar to the model used in Chapter 2, generally this model adopts a Wright-Fisher strategy, which assumes a constant population size N in each discrete generation undergoing mutation, selection, and reproduction subsequently. Each individual within the population maintains two genomes, i.e., a diploid MIC and an n-ploid MAC, both carrying L loci. Different values of n, including 1, 2, 4, 8, 16, 32, 45, 64, and 128 are simulated. The phenotype of each individual is only determined by the genotype of the MAC and represented as a single point in an L-dimension FGM landscape. Pleiotropy levels are manipulated, and the number of loci within the genome is always equal to the number of dimensions in the FGM landscape. In this study, I set the value of L to be 10. The fitness is calculated based on the distance of the phenotype to the optimum:

$$W(d) = e^{-\frac{d^2}{2}} \quad (1)$$

in which d is the Euclidian distance between the phenotype and the optimum. Within a MAC, each locus can accumulate as many as n mutations, with their phenotypic effects in the FGM landscape being additive in their corresponding dimensions. Within a MIC, mutations can also occur but cause no phenotypic effect in the landscape until following sex. The fitness dynamics during evolution are monitored and compared among different reproduction strategies.

Generation of ancestor population: For initial fitness set to be 1, the ancestor is sitting at the optimum. For initial fitness lower than 1, a point in L-dimensional space is randomly generated, with the position at each dimension sampled from a normal distribution with mean of 0 and standard deviation (SD) of 0.01.

The fitness of this point is calculated and its position is adjusted by multiplying (if higher than the pre-set initial fitness) or dividing (if lower than the initial fitness) a gradually increasing scale factor starting from 1 until reaching the pre-set initial fitness. The ancestor population is monomorphic, composed of N mutation-free individuals with the same initial phenotype in the landscape.

Manipulation of pleiotropy level: The mapping between loci and dimensions is established before starting the evolutionary process and kept constant during evolution. Different pleiotropy levels, including universal pleiotropy, restricted pleiotropy, and no pleiotropy, are tested. Under universal pleiotropy, each locus within the genome will control all the dimensions of the landscape, i.e., a mutation at one locus can cause a phenotypic change along all the dimensions. For another extreme, no pleiotropy, each locus will only correspond to one pre-selected dimension within the landscape, and the mutations can only move the phenotype along its corresponding dimension. Similarly, for restricted pleiotropy, the mutations generated within each locus will direct the phenotypic movement along a certain number of pre-selected dimensions in the landscape. For example, with a pleiotropy level of 2, the mutations at locus 1 may influence the movement of phenotype along two pre-selected dimensions (e.g., dimension 1 and 3).

Mutation: The population is mutation-free and thus completely homozygous initially. Mutations are allowed to occur at each locus in both the MAC and MIC with the same rate per site per generation. Here I set the MAC genomic mutation rate to be $U = 0.01$ per generation. Mutation is simulated as a binomial process, with $U/(nL)$ being the probability of success and the ploidy being the number of trials, and the mutated allele is allowed to mutate again with the mutation rate unchanged. The phenotypic effect of each newly arising mutation is generated from a normal distribution (for no pleiotropy case) or a multivariate normal distribution (for universal and restricted pleiotropy cases) with mean of 0 and a pre-set SD. For universal and restricted pleiotropy, the SD is adjusted by dividing the square root of the pleiotropy level to keep the total SD constant across different pleiotropy levels. Only the mutations arising within the MAC can drive the phenotype to move within the landscape. For mutations accumulated in the MIC, their

phenotypic effects are generated and stored but have no effect on moving the phenotype until following sex.

Selection: After mutation, selection proceeds by randomly selecting *N* individuals with replacement from the previous generation, with each individual's chance to be selected being proportional to its somatic fitness. Those selected individuals are 'parents' for the new generation, and they are allowed to reproduce by mitosis, amitosis, obligate sex, or facultative sex to construct the population of the next generation. Only one progeny will be generated and kept for each parent during reproduction.

Asexual reproduction: During asexual reproduction, the MIC is divided by mitosis, which is achieved by directly getting a copy of the MIC for each selected parent. For the division of MAC, two asexual reproduction strategies, mitosis and amitosis, are simulated in the model.

(i) Mitosis: Same as the MIC, the mitotic division of MAC is modeled as generating an identical MAC for each of the selected parents.

(ii) Amitosis: For each of the selected parents, the *n* alleles within each locus are first duplicated, and then *n* alleles are picked randomly with equal probability and kept as the alleles inherited from the previous generation after amitosis. The total phenotypic effects of these inherited alleles are then summed together and may move the individual to new phenotype in the landscape.

Sex: Here sex is simulated as an outcrossing process, which is achieved through random mating in the model. For this process, as described in subsection "Selection", two sets of parents are selected randomly with replacement based on their relative fitness. Each set of parents produce haploid nuclei once from their MICs. Then the alleles from these two sets of haploid nuclei get fused, and generate new MICs and MACs.

For the generation of haploid nuclei, one allele is picked randomly from each MIC locus and maintained in the haploid nuclei. Then two haploid nuclei are fused into a diploid zygotic nucleus and develop into the new MIC and MAC. The new MIC is identical to the zygotic nucleus, by directly getting a copy of the diploid nucleus. For the new MAC, generally it is a process of amplifying the copy number of alleles in the

diploid nucleus. If the ploidy of MAC, *n*, is an even number, each allele within the diploid nucleus will be directly amplified by *n*/2 times and formed the new MAC. If *n* is an odd number, the copy number of each allele will first get magnified for (*n* - 1)/2 times, and then the remaining allele will be randomly picked from the two alleles within each locus and create the new MAC. Their total phenotypic effects are then summed together and move the individual to new phenotype in the landscape.

3.2.2. Quantification of the genetic load generated during adaptation by amitosis

Under no pleiotropy, the amitotic population with a pre-set initial fitness is first allowed to adapt for 10,000 generations and is checked for equilibrium by the method introduced in Chapter 2. Here the linear regression slopes of the fitness in the last 1,000 generations are used for equilibrium checking. If the population reached equilibrium, the mean variance for the phenotypic effects within each dimension are obtained at the final generation. To quantify the genetic load generated during adaptation by amitosis (I term it as "amitosis load"), another population with initial fitness of 1 is allowed to evolve under amitosis. Instead of being mutation-free initially, the new population used to measure amitosis load carries *n*/2 copies of positive and *n*/2 copies of negative alleles in each locus which can generate equal mean variance as that in the adapting population. Due to the segregation of alleles within each locus during amitosis, the population cannot stably stay in the optimum and will suffer fitness loss. Once this population achieves equilibrium, the fitness loss caused by the segregation of alleles is what I mean by "amitosis load".

3.2.3. Quantification of the mutation load generated during adaptation by amitosis

Following 3.2.2, to quantify the mutation load generated during adaptation by amitosis, a new population with initial fitness of 1 and subject to the same mutating process as the adapting amitotic population (i.e. having the same mutation generating rate and regime as that in the adapting population) is allowed to evolve under mitosis. Once this mitotic population reaches equilibrium, the fitness loss is the mutation load suffered by adapting amitotic population.

3.2.4. Check of the best copy number for beneficial mutations

Under the assumption of no pleiotropy and fixed optimum, an ancestral population with a pre-set initial fitness is generated based on the method introduced in Section 3.2.1. Instead of having the normal mutation-selection-reproduction lifecycle, here I only focused on the mutation process. Up to 10,000 beneficial mutations are generated in the ancestor population. For each beneficial mutation, its fitness effects are checked once present between 1 to *n* copies within the ancestor population, in which *n* is the MAC ploidy and the maximum possible copy number for the mutation, and the best copy number is the one which can lead to the highest fitness increase.

3.2.5. Movement of optimum

To simulate the changing environment, here I further allow the optimum to move during evolution. Within each generation, the optimum first moves, followed by the normal mutation-selection-reproduction lifecycle. Three optimum movement scenarios are tried here, and the parameters of movement are selected to achieve moderate optimum moving rate, which would lead to a fitness loss of ~0.4 after 1,000 generations solely caused by movement of the optimum (see subsection 3.3.5 for details).

(i) Moving along all dimensions: every generation the optimum moves along all the dimensions of the landscape, with the movement in each dimension sampled from a normal distribution with mean of 0 and a given SD of 0.01.

(ii) Moving along a certain dimension: one dimension is pre-selected and the optimum is only allowed to move along this certain dimension. Similar to the first strategy, the movement in this dimension is sampled from a normal distribution with mean of 0 and a constant SD of 0.05.

(iii) Moving along a straight line across the landscape: every generation the optimum moves along a straight line that connects the origin and the point (1, 1, 1,…, 1) across all the *L* dimensions of the landscape, with a constant moving rate of 0.003 along each dimension per generation.

3.3. Results

3.3.1. Mitosis allows population to achieve a higher population mean fitness than amitosis under a fixed optimum in FGM

To set a baseline for the performance of amitosis and mitosis in a changing environment, I first used the polyploid FGM to investigate the adaptation of amitotic and mitotic populations in a constant environment. Also, as we assumed no epistasis or overdominance existed in the model used in Chapter 2, the inherent epistasis and overdominance within FGM allowed us to study whether and how they can affect the adaptation process.

Using a 10-dimension and 45-ploid FGM, the adaptation of a 45-ploid population with size N = 500 and initial fitness w_0 = 0.5 was simulated for 2,000 generations undergoing mitosis and amitosis, respectively. Three different pleiotropy levels, including universal pleiotropy, no pleiotropy, and a restricted pleiotropy level of 2 (i.e., each locus corresponds to 2 randomly selected dimensions within the landscape) were tested. Assuming a MAC genomic rate U_{MAC} = 0.01, and generating the phenotypic effect of each arising mutation from a normal distribution with mean of 0 and a total SD of 0.05, it was found that pleiotropy level has little effect on the adaptation process (Figure 3.1). After evolving for 2,000 generations, mitosis under all three pleiotropy levels led to a population mean fitness of ~0.85 (universal pleiotropy: mean: 0.836, 95% confidence intervals, CI: 0.830 - 0.843; restricted pleiotropy level of 2: mean: 0.829, 95% CI: 0.820 - 0.838; no pleiotropy level: 0.855, 95% CI: 0.849 - 0.861), and amitotic population reached a population fitness level of around 0.80 (universal pleiotropy: mean: 0.796, 95% CI: 0.789 - 0.802; restricted pleiotropy level of 2: mean: 0.773, 95% CI: 0.764 - 0.783; no pleiotropy level: 0.757, 95% CI: 0.746 - 0.767). Since the treatment of no pleiotropy led to the highest fitness increase in mitotic populations but lowest increase in amitotic ones, in the following parts of this chapter, unless specified, I focused on the no pleiotropy assumption, and the evolution parameters (e.g., population size, SD of generating phenotypic effect of mutations, etc.) were kept constant through the whole chapter.

Comparing adaptation under mitosis and amitosis, however, showed that mitotic and amitotic populations exhibited different fitness dynamics patterns. For the mitotic population, although slowly, the population mean fitness increases continuously during the evolution of 2,000 generations, while for the amitotic one, the population mean fitness increased dramatically in the first hundreds of generations but then the rate of increase slowed down and finally reached a plateau. Furthermore, amitosis has led to a lower population mean fitness than mitosis, as the population mean fitness achieved by amitosis after evolving 2,000 generations is ~0.1 less than that got by mitosis. This result suggested that mitosis is better than amitosis when adapting towards a fixed optimum in FGM, which was not consistent with my expectation and also different from the conclusion I got in Chapter 2. Then I turned to investigate the reasons that caused the weaker adaptation of amitosis in FGM.

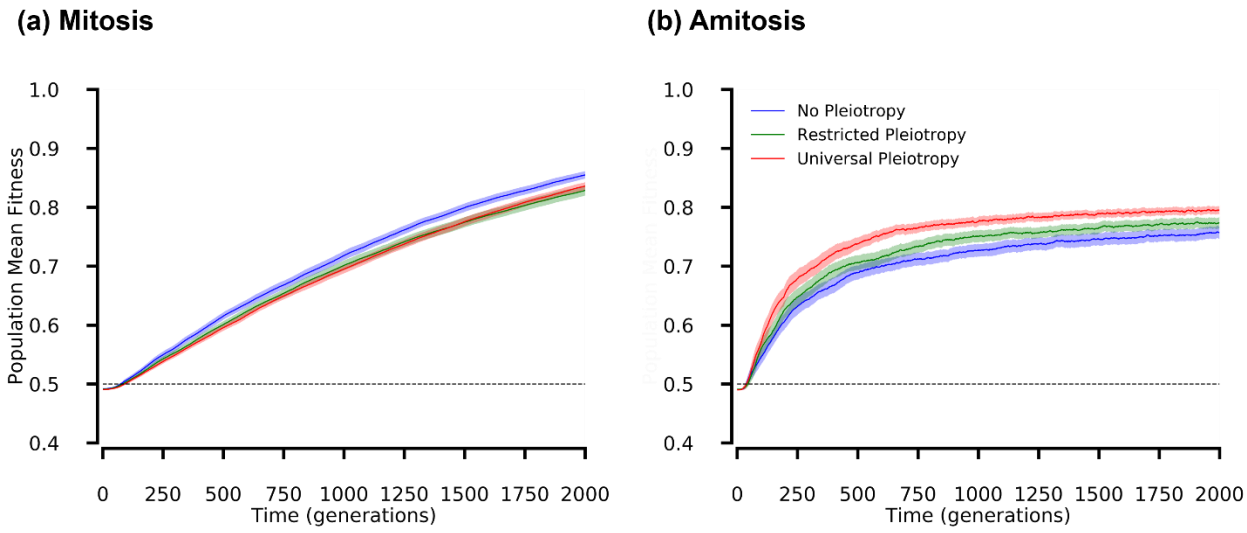

Figure 3.1: Amitosis results in a lower population mean fitness compared to mitosis in a constant environment. Values show the population mean fitness during adaptation to a fixed optimum with different levels of pleiotropy in a 10-dimension FGM, under mitosis (a) and amitosis (b), respectively. Solid lines show the means calculated from the simulation results of 100 replicated populations; shaded regions represent 95% confidence intervals. The black dash line shows the initial fitness level w_0. Here I assumed a population size N = 500 with ploidy n = 45 and initial fitness w_0 = 0.5. There were 10 loci within the genome, with a genomic mutation rate of 0.01 per generation. Mutations were generated from a normal (for no pleiotropy case) or multivariate normal (for restricted and universal pleiotropy) with mean of 0 and a total SD of 0.05.

3.3.2. Amitosis can cause a genetic load during adaptation due to the overdominance of mutations

The slow adaptation of amitosis may be caused by the overdominance of alleles in FGM, which is missing in the model I used in Chapter 2. Within FGM, the overdominance arises from the stabilizing selection on each phenotypic trait. Under overdominance, there is an optimal copy number between 0 and the ploidy *n* which can produce the highest fitness for a certain allele. For a mitotic population, the only source of difference between parents and offspring is the new mutations generated, which leads to slow and small movements within the landscape, and selection would generally move the population towards to the optimum. During adaptation, every time a beneficial mutation arises, it is preserved within the population unless it mutates again, thus allowing the subsequent mutations to build and be selected upon the new genetic background. Consequently, once the population is reaching the optimum, the phenotype can stay there stably. On the contrary, for an amitotic population, setting aside the new mutations generated, the segregation of alleles within each locus during amitosis could cause dramatic movement in the landscape. The greatly increased variation can lead to an accelerated adaptation initially. However, once approaching the optimum, the segregation and reassortment of the alleles within each locus may drive the allele composition away from the optimal state and lead to the departure of the phenotype from the optimum. As a result, amitosis would cause a genetic load (here I termed it as "amitosis load"), especially under high ploidies, during adaptation, which may result in a weaker adaptation level compared to mitosis. The widespread prevalence of overdominance within FGM has been demonstrated and will be illustrated in Subsection 3.3.4.

To quantify the amitosis load, I first simulated the adaptation of 128-ploid amitotic populations with size N = 500 and initial fitness w_0 = 0.5 for 10,000 generations. Here I increased the ploidy to be 128 as the population used to measure amitosis load did not reach equilibrium under 45-ploid and the amitosis load may be more obvious under higher ploidies. The adapting population achieved an equilibrium fitness of 0.794 (95% CI: 0.786 - 0.802) and a mean phenotypic effect variance of ~0.0000270 (95% CI: 0.0000249 - 0.0000292) in the final generation. Thus, the population used to quantify the amitosis load was set to carry

64 copies of +0.005199 alleles and 64 copies of -0.005199 alleles within each locus (here ± 0.005199 represents the phenotypic movement caused by an allele along its corresponding dimension), which would cause a total effect of 0 along each dimension but generate the same phenotypic effect variance as the adapting population. The amitosis load quantifying population sits at the optimum initially and was allowed to evolve for 10,000 generations under amitosis. If the fitness "plateau" of the adapting population does result from the amitosis load, then I would expect to see that the fitness levels of the adapting population and the amitosis load quantifying population are approximately the same. As shown in Figure 3.2, the amitosis load quantifying population finally achieved an equilibrium population mean fitness of 0.803 (95% CI: 0.800 - 0.805), suggesting that the amitosis load has caused a ~0.2 fitness loss. As I expected, the equilibrium fitness reached under amitosis load was approximately equal to that achieved by an adapting population, which indicated that the weaker adaptation level of the amitotic population is mainly caused by the genetic load generated during amitosis. The discrepancy between the final equilibrium fitness of the adapting population and amitosis load quantifying population may result from the additional mutation load suffered by the adapting population. Given a genomic mutation rate of 0.01 and a SD of 0.05 to generate new mutations, the 128-amitotic population is expected to suffer a mutation load of 0.0095 (95% CI: 0.0090 ~ 0.0101), which matched the fitness difference between these two populations and explained the discrepancy.

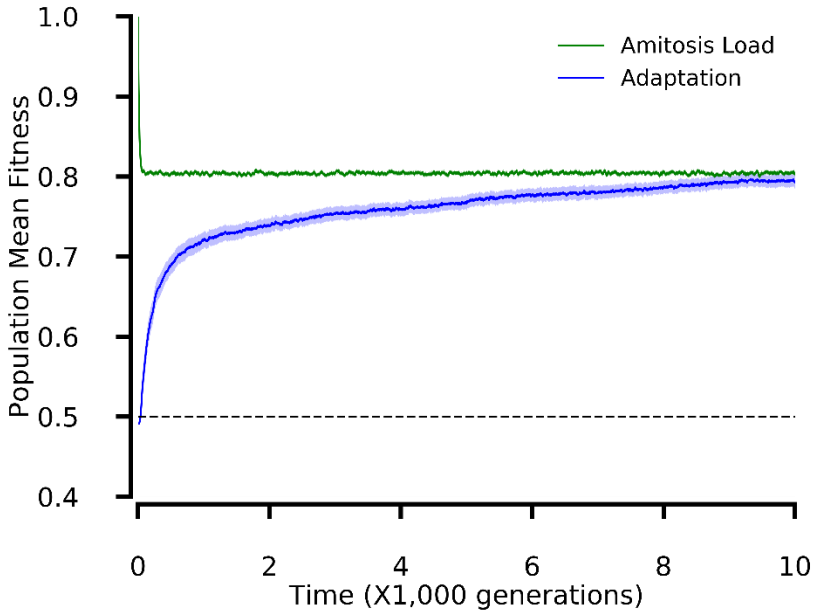

Figure 3.2: Amitosis causes a genetic load during adaptation in a constant environment. The blue line shows the population mean fitness of 128-ploid population with size N = 500 and initial fitness w_0 = 0.5 undergoing amitosis during adaptation to a fixed optimum. The green line shows the fitness dynamics of 128-ploid amitotic population with size N = 500 and initial fitness w_0 = 1 but carrying 64 copies of +0.005199 alleles and 64 copies of -0.005199 alleles within each locus. The black dash line shows the initial fitness level of the adapting population. Here I assumed no pleiotropy within the model. Means and 95% confidence intervals are calculated from the simulation results of 100 replicate populations for each setting.

3.3.3. Sex can only lead to a higher population mean fitness than asexual reproduction when ploidy is low and under a fixed optimum

After comparing the adaptation dynamics under amitosis and mitosis in FGM, I further incorporated sex into the model and investigated its effect on adaptation. Both obligate sex every generation (τ = 1) and facultative sex every τ = 100 generations were simulated for a variety of MAC ploidies, including 1, 2, 4, 8, 16, 32, 45, 64, and 128.

Interestingly, my results showed that obligate sex every generation (τ = 1) can only lead to a slightly higher equilibrium mean fitness than amitosis in low ploidies (up to 16 in the ploidies I considered). For example, with a MAC ploidy of 16, the obligate sexual population achieved a population mean fitness of 0.893 (95% CI: 0.887 - 0.900) after evolving 2,000 generations, which is marginally higher than that reached by amitosis

(mean: 0.858; 95% CI: 0.849 - 0.868) (Figure 3.3). However, for MAC ploidy higher than 16, obligate sex every generation ($\tau = 1$) resulted in a lower population mean fitness than amitosis. For instance, with a MAC ploidy of 128, the mean fitness within obligate sexual population was only 0.558 (95% CI: 0.544 - 0.571), which was almost the same as the initial fitness level of 0.5 and much weaker than that achieved by amitosis (mean: 0.741; 95% CI: 0.732 - 0.750) and mitosis (mean: 0.845; 95% CI: 0.839 - 0.852) (Figure 3.3). The reason for this unexpected behavior of sex is investigated in the following subsection.

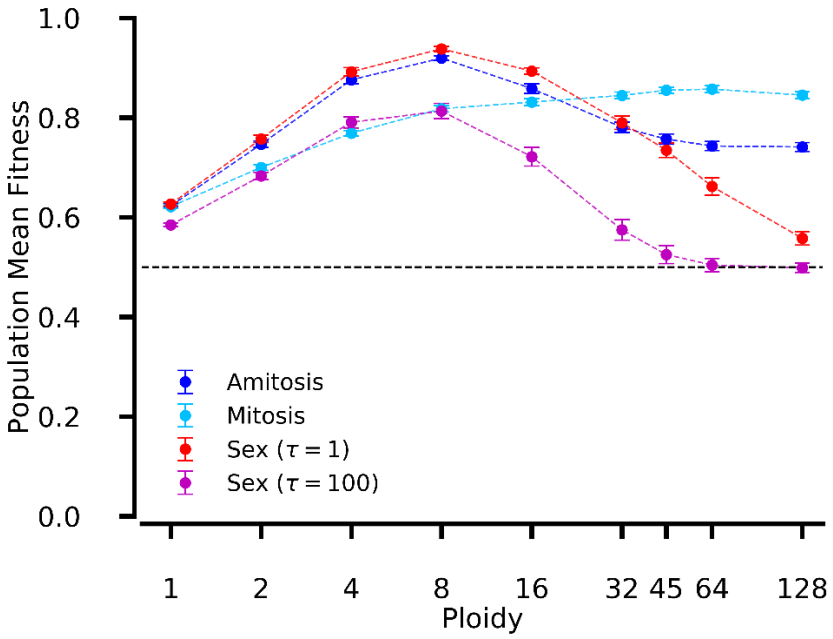

Figure 3.3: Sex only speeds up adaptation under low ploidies but not high ploidies in constant environment. Values show the means and 95% CIs of population mean fitness with different MAC ploidies under amitosis, mitosis, obligate sex every generation ($\tau = 1$), and facultative sex every $\tau = 100$ generations after adapting to a constant environment for 2,000 generations. The MAC ploidy levels of 1, 2, 4, 8, 16, 32, 45, 64, and 128 were investigated. Sex was simulated as an outcrossing process. Other parameters were the same as that in Figure 3.1. The black dash line shows the initial fitness level of the adapting population. Means and 95% confidence intervals are calculated from the simulation results of 100 replicate populations for each setting. Note that the ploidy is shown in a log scale.

Facultative sex every $\tau = 100$ generations, similar to what I found in Chapter 2, generated stair-like fitness dynamics, due to the recreation of a new MAC following sex, and the population fitness achieved was always lower than that reached under amitosis with no sex for all ploidies tested (Figure 3.3).

3.3.4. Most beneficial mutations generated in FGM exhibit overdominance

The remarkable adaptation pattern under sexual reproduction is probably caused by the allele multiplication process that occurs following sex. For *Tetrahymena*, once having sex, two haploid nuclei generated from the MICs of parental cells fuse into a diploid nucleus and then develop into new MIC and MAC. The development of a new MAC involves chromosomal amplification, in which each allele within the diploid nucleus will amplify its copy number from 1 to $n/2$. As illustrated in Figure 3.4, even for beneficial mutations, there may exist an intermediate best copy number which can lead to the greatest fitness increase. Exceeding the best copy number may drive the phenotype overshoot the optimum, which will result in little fitness increase and can even change the beneficial effect to be deleterious.

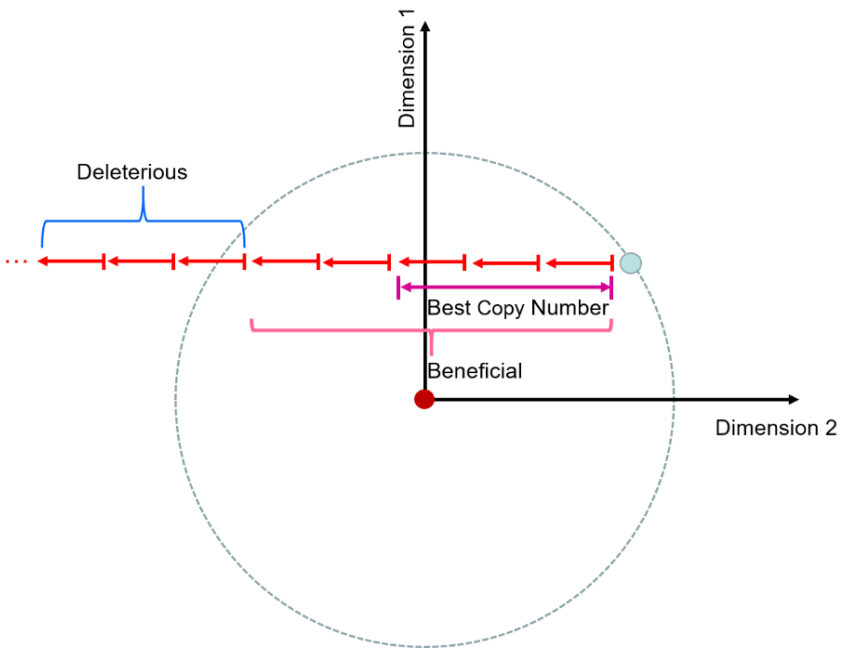

Figure 3.4: Example of best copy number for beneficial mutations in FGM. As shown in the 2-dimension FGM, the circle represents all the possible phenotypes which can lead to the same fitness. Suppose a phenotype is sitting on the cyan point initially. For a beneficial mutation which can drive the phenotype closer to the optimum, there is a best copy number which makes the phenotype closest to the optimum and thus lead to the highest fitness increase (in this example, the best copy number is 3). Increasing the copy number to be 4 or 5, although the overall effect is still beneficial, the fitness increase is lower than that having 3 copies. Further increasing the copy number to be 6 or more, the phenotype will move outside of the circle and result in a lower fitness than initial value, meaning that the copy number increase process turn to be deleterious.

To test this hypothesis, on the background of ancestor populations with size N = 500 and initial fitness w_0 = 0.5, I generated 10,000 beneficial mutations for all the MAC ploidies tested in Subsection 3.3.3. The mean best copy numbers of the beneficial mutations under each MAC ploidy were then obtained and compared with the value of $n/2$.

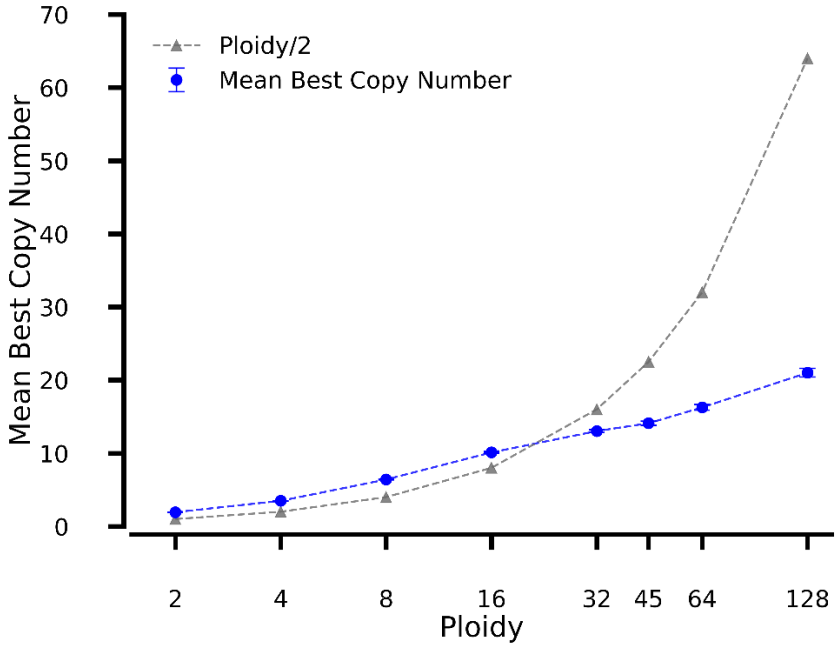

Figure 3.5: Most beneficial mutations generated in FGM exhibit overdominance. Graph shows the comparison of the best copy number of beneficial mutations with 0.5 MAC ploidies. The blue dots represent the mean and 95% CIs of best copy numbers calculated from 10,000 beneficial mutations generated on the background of ancestor populations with size N = 500 and initial fitness w_0 = 0.5. The error bars represent the 95% CIs. The grey triangles indicate the value of 0.5 MAC ploidy. Here the MAC ploidy ranges from 2~ 128. Note that the ploidy is shown in a log scale.

As shown in Figure 3.5, supporting my argument in Subsection 3.3.2, overdominance is prevalent within FGM as the mean best copy numbers for beneficial mutations were lower than the MAC ploidy n, especially under high ploidies. Consistent with what I found in Subsection 3.3.3, up to MAC ploidy of 16, the mean best copy number of beneficial mutations was slightly higher but close to $n/2$. For example, with n = 16, the mean best copy number calculated from the 10,000 beneficial mutations was 10.110 (95% CI: 10.005 - 10.215), thus the copy number amplification from 1 to 8 during sex won't cause the mutated phenotype to overshoot the optimum and can result in a great fitness increase (Figure 3.3). However, for MAC ploidy

greater than 16, the mean best copy number of beneficial mutations is much lower than $n/2$. For instance, under the highest MAC ploidy I tested here, $n = 128$, the value of $n/2$, 64, greatly exceeded the best copy number of beneficial mutations (mean: 21.004, 95% CI: 20.411 - 21.597). Consequently, having obligate sex every generation ($\tau = 1$) under $n = 128$ drove the mutated phenotype to overshoot the optimum and led to a weak adaptation (Figure 3.3).

3.3.5. Amitosis can accelerate the adaptation to changing environments compared to mitosis

After studying the adaptation process under a fixed optimum in FGM, as my research aim in this chapter is to investigate whether amitosis can speed up the adaptation to a changing environment, I then simulated changing environments by allowing the optimum to move during evolution. Here serving as an indicator of the optimum movement magnitude, I explored the rate of environmental deterioration (showing as the achieved fitness of the moving optimum relative to its initial position) under the three movement scenarios tested. Shown in Figure 3.6, all the three movement regimes suffered approximately the same fitness loss of ~0.4 after 1,000 generations. Given the SD of moving along each dimension set to be 0.01, the moving optimum achieved a fitness of 0.397 (95% CI: 0.369 - 0.425) after moving for 2,000 generations, and the second moving scenario with a SD of 0.05 along a certain dimension reached a fitness level of 0.515 (95% CI: 0.442 - 0.587). The third moving scenario, moving along the diagonal with a rate of 0.003 per generation, got a fitness of 0.165.

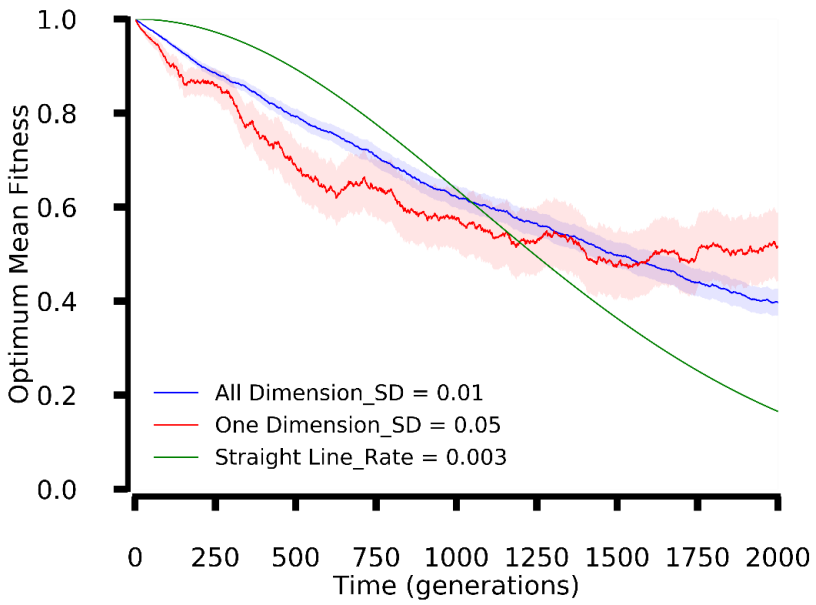

Figure 3.6: Changing environment under 3 movement scenarios. The lines show the rates of environmental deterioration under three movement scenarios: A: optimum moving along all the 10 dimensions with a SD of 0.01; B: optimum moving along a certain dimension with a SD of 0.05; C: optimum moving along a straight line across the landscape with a constant moving rate of 0.003 along each dimension per generation. Means and 95% confidence intervals (shown as shade regions) are calculated from the simulation results of 100 replicate populations for each setting.

The adaptive trajectories of amitotic and mitotic populations under all the three optimum movement scenarios were investigated. Given the parameters for moving the optimum, amitosis was found to achieve a much better adaptation than mitosis for all the optimum moving scenarios. Under the first movement scenario, the amitotic population reached a population mean fitness of 0.653 (95% CI: 0.638 - 0.668) after evolving for 2,000 generations, while the mitotic one only achieved little increase in fitness and ended in a fitness level of 0.543 (95% CI: 0.513 - 0.573). Similarly, for the second moving scenario, compared to the fitness loss suffered by the mitotic population (mean fitness: 0.419; 95% CI: 0.363 - 0.474), the amitotic population gained large fitness increase to 0.689 (95% CI: 0.669 - 0.710). Further demonstrating my hypothesis that amitosis can facilitate the adaptation to changing environments, the third optimum moving scenario has resulted in a population mean fitness of 0.596 (95% CI: 0.581 - 0.610) in amitotic populations while only 0.341(95% CI: 0.320 - 0.362) in mitotic ones after evolving for 2,000 generations (Figure 3.7). The better adaptation of amitosis results from the greater variation generated, which makes the selection

more efficient and allows the population to better chase the moving optimum. On the contrary, the only source of variation in mitotic population is the new arising mutations, which cannot generate enough variation for the population to purse the optimum when it is moving. Based on these results, I drew the conclusion that compared to mitosis, amitosis can accelerate adaptation to changing environments.

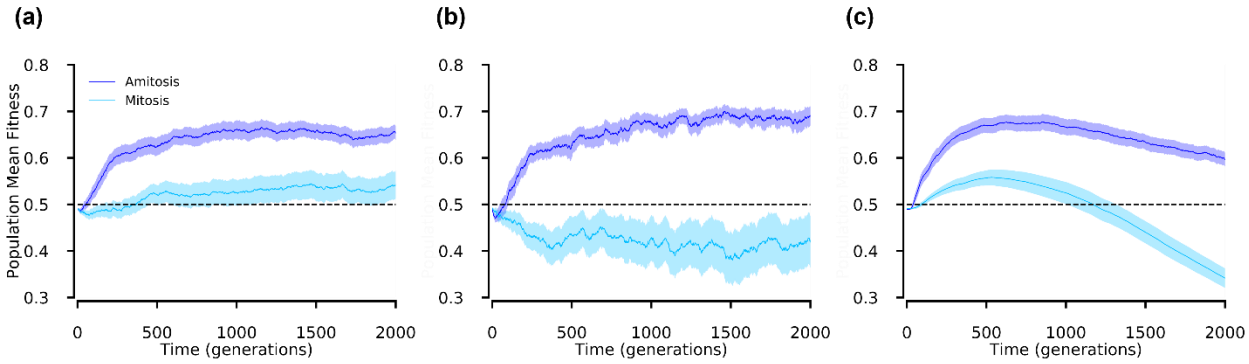

Figure 3.7: **Amitosis accelerates the adaptation to a changing environment compared to mitosis.** Figures show the fitness dynamics of mitotic and amitotic populations during adaptation to three continuous-changing environments as mentioned in Figure 3.6 (a: moving along all 10 dimensions with a SD of 0.01; b: moving along a certain dimension with a SD of 0.05; c: moving along the diagonal with rate of 0.003). Other evolutionary parameters were the same as that in Figure 3.1. Means and 95% CIs (shown as shade regions) are calculated from the simulation results of 100 replicate populations for each setting.

However, it should be notified that amitosis cannot always lead to a better adaptation to changing environments. For instance, under a slowly moving optimum, amitosis and mitosis would behave analogously to that under a fixed optimum, which the amitotic population would suffer the "amitosis load" and result in lower fitness than the mitotic one (Figure 3.8, SD of optimum movement = 0.002). On the other hand, if the magnitude of optimum movement is too high, then even the amitotic population cannot adapt and will suffer fitness loss (Figure 3.8, SD of optimum movement = 0.05). Thus, the result that amitosis can accelerate adaptation to a changing environment is only valid within a certain range of optimum moving magnitude. Based on my exploration, for the first optimum moving strategy, such magnitude is to move along each dimension within the SD range of 0.006 - 0.021 (Figure 3.8).

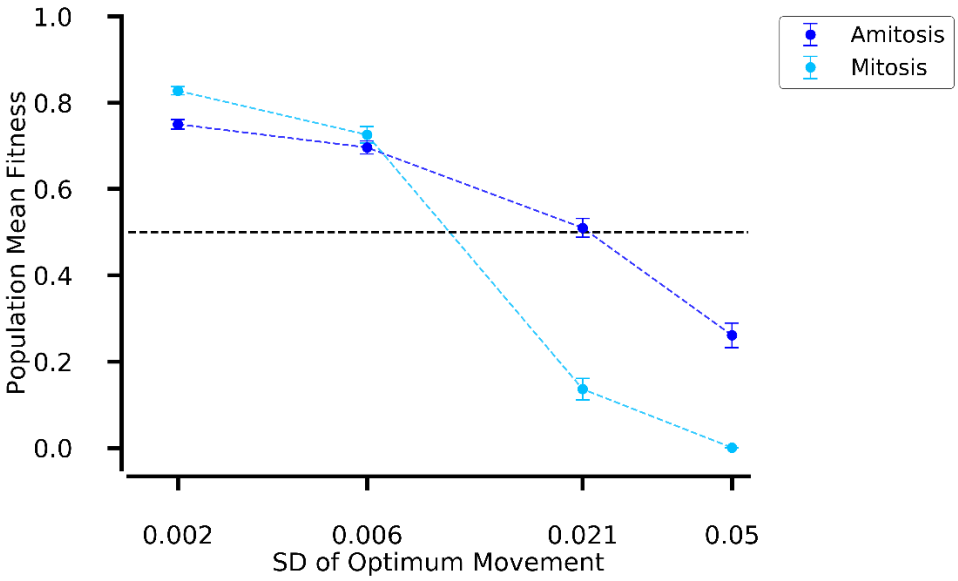

Figure 3.8: **The result that amitosis can accelerate the adaptation to changing environments is not universal.** Values show the population mean fitness of mitotic and amitotic populations after adapting to a continuous-changing environment for 2,000 generations. Here the optimum moved according to the scenario (A) but with several SD 0.002, 0.006, 0.021, and 0.05, respectively. Other evolutionary parameters were the same as that in Figure 3.1. The error bar shows the 95% CIs of population mean fitness. Means and 95% confidence intervals are calculated from the simulation results of 100 replicate populations for each setting. Note the x axis is shown in log scale.

3.3.6. Sex can only accelerate adaptation to changing environments under low ploidies

As it is known that sex can facilitate the adaptation to changing environments, I further compared the adaptation achieved by amitosis with that reached by sex under a moving optimum in FGM. The evolution under different reproduction strategies, including mitosis, amitosis, obligate sex every generation ($\tau = 1$), and facultative sex every $\tau = 100$ generations, were simulated and compared under MAC ploidy levels of 1, 2, 4, 8, 16, 32, 45, 64, and 128. Since a similar pattern was found for all the three optimum movement scenarios, here I illustrated my findings using the results from the first moving scenario.

In agreement with my expectation, obligate sex every generation ($\tau = 1$) does speed up the adaptation when facing a moving optimum, and the population mean fitness reached is slightly better than that achieved by amitosis. However, same as what I found under a fixed optimum, this is only true under low MAC ploidies up to 16. As shown in Figure 3.9, with a MAC ploidy $n = 16$, the obligate sexual population can reach a

population mean fitness of 0.765 (95% CI: 0.750 - 0.780) after evolving 2,000 generations, and the amitotic population can achieve a similar level of adaptation by getting a fitness level of 0.709 (95% CI: 0.691 - 0.726). Neither mitosis nor facultative sex can lead to adaptation due to their inability to generate enough variation for selection to operate (under mitosis: mean final fitness = 0.514, 95% CI: 0.483 - 0.545; under facultative sex: mean final fitness = 0.454, 95% CI: 0.421 - 0.487).

Further increasing the MAC ploidy caused obligate sexual populations to fail to adapt to the changing environment. For example, with a MAC ploidy of 128, both obligate sexual and facultative sexual populations suffer great fitness loss after evolving for 2,000 generations (obligate sexual populations: mean fitness = 0.311, 95% CI: 0.283 - 0.340; facultative sexual populations: mean fitness = 0.231, 95% CI: 0.207 - 0.256). However, the mitotic population can roughly maintain its population mean fitness unchanged (mean fitness = 0.537, 95% CI: 0.507 - 0.567), and the amitotic one can still gain fitness increase and result in a final population mean fitness of 0.619 (95% CI: 0.600 - 0.638) (Figure 3.9). The reason for the behavior of sex is similar to that under fixed optimum, for which the amplification of mutations from 1 to $n/2$ greatly exceeds the optimal value and turns to be deleterious.

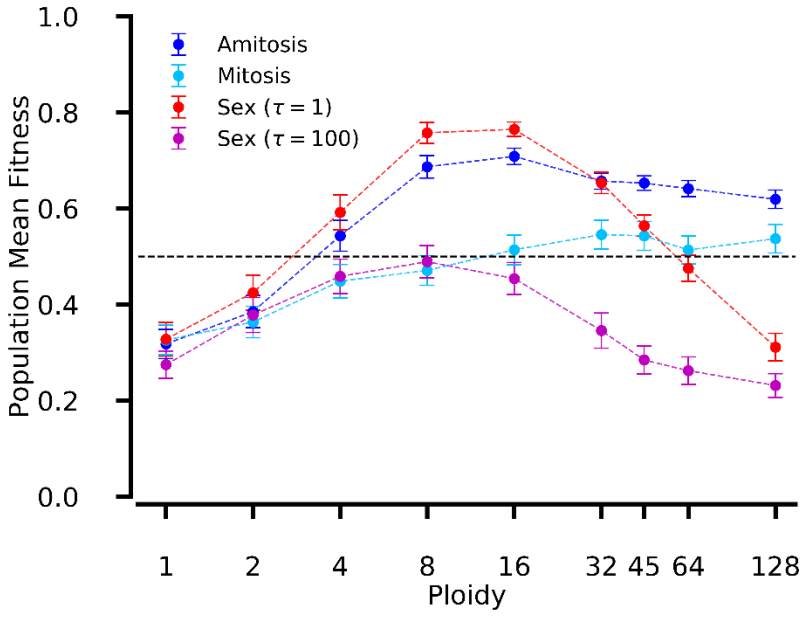

Figure 3.9: **Sex can only accelerate the adaptation to changing environments under low ploidies but not high ones.** Values show the means and 95% CIs of population mean fitness with different MAC ploidies

under amitosis, mitosis, obligate sex every generation ($\tau = 1$), and facultative sex every $\tau = 100$ generations after adapting to a changing environment for 2,000 generations. Optimum movement was modeled according to the movement scenario (A) in Figure 3.6, with SD setting to be 0.01. Sex was simulated as an outcrossing process. Other parameters were the same as that in Figure 3.3. The black dash line shows the initial fitness level of the adapting population. Means and 95% confidence intervals are calculated from the simulation results of 100 replicate populations for each setting. Note that the ploidy is shown in a log scale.

3.4. Discussion

In this chapter, to investigate whether the unusual amitotic division adopted by *Tetrahymena* can confer sexual-like benefit by speeding up adaptation to a changing environment, I built a new model based on classic FGM. To capture the genome architecture of *Tetrahymena*, I extended the classic FGM to be polyploid and also incorporated sexual and amitotic processes for comparison. The optimum was allowed to move during evolution to simulate the changing environment.

Using the polyploid FGM, I found that pleiotropy level has little effect on evolution trajectories under both mitosis and amitosis. When adapting to a constant environment, amitosis was found to cause a genetic load and achieve a weaker adaptation level compared to mitosis, due to the segregation of alleles within each locus. However, when facing a changing environment, compared to mitosis, the greater variation generated by amitosis leads to a more efficient selection and thus allows the population to better chase the moving optimum. Counterintuitively, under both constant and changing environments, sex can only achieve a slightly better adaptation compared to amitosis under low ploidies but not high ploidies. Further investigation has revealed that overdominance is prevalent for the mutations generated in FGM, which may cause the mutated phenotype to overshoot the optimum once sex occurs.

As an important parameter of FGM, the pleiotropy level defines how many phenotypic traits are affected by a single locus (Stearns, 2010; Tenaillon, 2014). Mainly for simplicity, classic FGM assumed a universal pleiotropy (i.e., one locus can affect all the dimensions within the landscape simultaneously) (Tenaillon, 2014). This assumption has been rejected by recent quantitative genomic studies on model systems such as *Escherichia coli* and mouse, in which most mutations were found to only affect a limited number of

phenotypic traits (Nichols et al., 2011; Tenaillon, 2014; Wagner et al., 2008). Although the actual pleiotrophy level within *Tetrahymena* remains unknown, the results I got here showed that different pleiotropy levels seem to have little effect on the adaptation process. However, here I only assumed a 10-dimension FGM landscape, a more complex FGM with a higher number of dimensions should be adopted to check the robustness of my findings.

Caused by overdominance of mutations in FGM, under amitosis, the segregation and reassortment of alleles within each locus can lead to the departure from the optimal allele composition and drive the phenotype to move away from the optimum. Such process generated a genetic load similar to the segregation load identified before (Haag and Roze, 2007) and impede adaptation. This is a novel finding as no one has used FGM to study the amitotic process under polyploidy, and for the first time, I have found a potential evolutionary disadvantage of amitosis, that it may break up well-adapted genotypes and result in fitness loss.

My model revealed that the overdominance is prevalent for beneficial mutations generated, which is consistent with the previous finding in diploid FGM (Sellis et al., 2011). By investigating and comparing the adaptation process in haploid and diploid populations using FGM, Sellis et al. (2011) found that the overdominance evolves naturally and is quite common within the diploid population, given that the phenotypic effects of some mutations are large enough to drive the mutated phenotype to overshoot the optimum. They argued that the prevalence of overdominance is very robust regarding the parameters they used in the model, such as population size, mutation rate, and the number of dimensions in the FGM. Here my study further extended the finding of widespread overdominance to polyploid populations. Sellis et al. (2011)'s work has got some experimental supports. For example, as revealed in Sellis et al. (2016), during the adaptation to glucose-limited environment, all the three beneficial mutations arising in diploid *Saccharomyces cerevisiae* populations exhibit overdominance (Sellis et al., 2016). However, whether overdominance is widespread within the *Tetrahymena* genome has yet to be evaluated.

Once facing a changing environment, the greater variation generated by amitosis allows the population to better chase the optimum when it is moving. Here I tried three different optimum movement scenarios to simulate three distinct types of environmental changes that the populations may experience in reality. The first optimum movement scenario is based on the "shaking optimum" regime introduced in Gordo and Campos's (2013) study and describes a possible environmental change regime which can affect all the phenotypic traits simultaneously with some uncertainties on the changing direction and magnitude (Gordo and Campos, 2013), and the second one focuses on a similar regime that can only cause an effect on a certain phenotypic trait. These two optimum movement scenarios represent two extremes on how environmental changes may impact the phenotypes: the first scenario simulates an environmental change than can lead to the maladaptation of all the phenotypes (e.g., the temperature change which may affect the metabolic enzyme activity); and the second one models another extreme, that such environment change can only result in the maladaptation of a certain phenotype, such as the supply change of a specific nutrient. The third movement scenario, differing from the first one, represents an environmental change regime that is deterministic in both the changing direction and magnitude. Global warming is a good example of this environmental change scenario, which has a certain changing direction and approximately constant changing magnitude. Under all of the three optimum movement scenarios, my simulation results showed that amitosis can speed up adaptation compared to mitosis. However, the better adaptation of amitosis can only be achieved within a certain range of optimum moving magnitude. The amitotic populations cannot overcome the "amitosis load" and will achieve a lower fitness than the mitotic ones if the magnitude of environmental change is below the lower bounder of that range. On the other hand, once facing an environmental change that exceeds the upper bounder of that magnitude range, even the amitotic population cannot adapt and will suffer fitness loss.

Due to the widespread overdominance within FGM, the amplification of allele copy number following sex may exceed the best copy number and drive the mutated phenotype to overshoot the optimum, which leads to a weak adaptation. This finding suggests that sex can only accelerate adaptation process under low

ploidies but not high ones. Consistent with my result, current experimental evolution studies have revealed that sex does speed up adaptation under low ploidy (Colegrave, 2002; Goddard et al., 2005). However, the performance of sex during adaptation under high ploidies largely remains unknown and needs to be investigated. My results also indicate the good adaptation of sex requires a match between the allele amplification during sex and the best copy number of beneficial mutations, which means the evolutionary matching between MAC ploidy and the mutation effects. In the future, to test my findings, genome sequencing and mutation accumulation experiment may be performed to check the overdominance of alleles and measure the effect of new mutations.

In this study, I investigated the adaptation process of *Tetrahymena* in changing environments using an extended polyploid FGM. My results suggested that amitosis can help *Tetrahymena* to get adapted to the environmental changes and highlighted the significance of amitosis in achieving the successful asexuality of *Tetrahymena*. Furthermore, this is the first research that extended FGM to be ploypoid and incorporated amitotic process, which also leads to a better understanding about the behavior of FGM in complex systems.

Chapter 4. Investigation of the evolutionary causes of the extremely low mutation rate in *Tetrahymena*

4.1. Introduction

Mutation accumulation (MA) experiments have revealed that *Tetrahymena thermophila* maintains an extremely low MIC mutation rate. Estimated from 10 *T. thermophila* MA lines, the MIC base-substitution mutation rate was found to be 7.61×10^{-12} per site per generation (95% CI: 0.691×10^{-12} - 14.53×10^{-12}). This value is consistent with that obtained from another ciliate species *Paramecium tetraurelia* (mean = 1.94×10^{-11}, 95% CI: 1.22×10^{-11} - 2.66×10^{-11}), and is the lowest among studied eukaryotes and even lower than many prokaryotes (Long et al., 2016b; Sung et al., 2012b). Such a low mutation rate raises the question of how they achieve this, given the fact that most eukaryotes studied so far have mutation rates that are at least one order of magnitude higher (Sung et al., 2012b).

Sung et al. (2012) proposed that the low MIC mutation rate of *P. tetraurelia* can be explained by the drift-barrier hypothesis, in which natural selection should minimize mutation rate to the lower bound which the benefits of further mutation rate reduction are equivalent to the effects of genetic drift (Sung et al., 2012b). Based on Sung et al. (2012)'s idea, Long et al. (2016) argued that the exceptionally low MIC mutation rate may be a consequence of the unusual nuclear dimorphism and facultative sexual strategy adopted by *T. thermophila*. During asexual generations between two cycles of sex, the MIC remains transcriptionally silent. Since the mutations accumulated within the MIC are not expressed, they are supposedly not exposed to selection until following sex. Given that the maximum sexual frequency of *T. thermophila* is approximately once every 100 generations, the number of mutations expressed following sex will be at least 100 times the average number of MIC mutation generated per generation, thus natural selection should favor the reduction of mutation rate (Long et al., 2016b). Furthermore, based on the measured mutation rate, Long et al. (2016) estimated the effective population size of *T. thermophila* may be as large as 1.12×10^{8}. Such a large effective population size should lead to a very strong selection to promote the mutation rate reduction (Long et al., 2016b). However, this idea has not been experimentally or theoretically evaluated.

According to Long et al. (2016)'s argument, the mutations within the MIC will keep accumulating but not be subject to selection during the asexual generations. Thus, the naïve expected total MIC mutations

between two rounds of sex would be the MIC mutation rate per generation times the number of asexual generations between two rounds of sex, from which the naïve expected mutation load carried within the MIC can be calculated. In this study, I first investigated the mutation load of the MIC using computational modeling, and compared the obtained values with the naïve expectation. I found that the MIC carried a much lower mutation load than expected. Further study elucidated that although not expressed, the mutations that occurred in the MIC can also respond to selection during asexual generations because they are now present in the MAC in the same cell following the occurrence of sex, resulting in a low mutation load within the MIC.

Since the preliminary hypothesis cannot explain the low MIC mutation rate, I introduced mutation rate modifiers into the stochastic model used in Chapter 2, and investigated mutation rate evolution under different reproduction strategies. I found certain reproduction strategies are capable of lowering the mutation rate, as amitosis is able to lower the MAC mutation rate and facultative sex can lower both MAC and MIC mutation rates. Furthermore, under facultative sexual reproduction, a greater reduction of mutation rate can be achieved in the 45-ploid MAC population compared to that of the diploid system.

4.2. Methods

4.2.1 Quantification of mutation load carried in MIC

Using the stochastic model described in Chapter 2, I simulate the evolution of *Tetrahymena* population with size N = 500 undergoing different frequencies of sexual reproduction for 20,000 generations. Other evolution parameters are identical to that used in Chapter 2, i.e., MIC and MAC have the same mutation rate per site, with the genomic mutation rate for 45-ploid MAC U_{MAC} set to be 0.1 per generation, and the mutations have a deleterious effect of -10% once present in homozygous state. Different sexual frequencies, including obligate sex every generation ($\tau = 1$), and facultative sex every τ = 2, 5, 10, 20, 50, 100, 200, and 500 generations, are tested. Here the value of τ describes the sexual period, which is the number of asexual

generations between two rounds of sex, and the reciprocal of τ is the sexual frequency. In this study, sex is also simulated as an outcrossing process with random mating strategy. The population mean fitness immediately after sexual reproduction is obtained and checked for equilibrium based on the method introduced in Chapter 2. Here I used the population mean fitness of the last 2,000 generations thus there are at least 5 data points for conducting linear regression and following equilibrium checking. If the populations are found to be at equilibrium, the population mean fitness immediately after the sexual cycles at the last 2,000, 1,000, and 0 generations will be used as an indicator of the MIC mutation load, and compared with the expected value calculated based on mutation load theory.

4.2.2 Testing of the response of MIC mutation to selection during asexual generations

(A) Testing of the hypothesis that MIC mutations also respond to selection during asexual generations

To test the hypothesis that MIC mutations may also respond to selection during asexual generations, on the basis of the original model, instead of allowing mutation every generation in both MAC and MIC, mutations are turned off in the MAC and only generated once within MIC just before having sex. With other evolution parameters keeping unchanged, the MIC mutation rate has been raised to be 1 prior to sex. The mean number of mutations within MIC, as well as the population mean fitness just before and after sex under different sexual frequencies, are obtained.

(B) Checking the robustness of the MIC mutations responding to selection

To check the robustness of MIC mutation responding to selection, I further allow the generation of MAC mutations in the model from section 4.2.2A and check whether these mutations can affect the efficiency of selection operating on MIC mutations. As the mutations originally within MAC will get lost immediately after having sex, here MAC mutations are generated with a total mutation rate of 45 that evenly scaled between two rounds of sex (e.g., a constant MAC genomic mutation rate of 0.45 per generation with a sexual frequency of once every 100 generations, i.e., $\tau = 100$), which will then lead to an average twice

mutation rate per site per generation in MAC compared to that in MIC. The population mean fitness and mean number of MIC mutations are then compared with those where there are no MAC mutations.

4.2.3 Investigation of mutation rate evolution under different reproduction strategies

(A) Investigation of mutation rate evolution in the 45-ploid system

To investigate the mutation rate evolution in a 45-ploid system like *Tetrahymena*, the mutation rate modifier loci are incorporated into the original stochastic model. These modifiers do not directly contribute to the fitness but control the mutation rate. The mutation rate within the MAC and MIC are controlled by two different sets of modifiers within the MAC, each composed of 10 mutator loci in which the mutated alleles can increase the mutation rate and 10 antimutators that can get mutated alleles lowering the mutation rate. Since the MIC remains silent, the MIC mutation rate represents the "genotypic" mutation rate, i.e., the mutation rate that is determined by the allele composition in the modifier loci within the MAC, but not the "phenotypic" mutation rate that can get expressed and exhibit phenotypic changes.

Mutations can occur within both fitness and modifier loci, with their rate being determined by the allele composition of modifier loci. For the fitness loci, the MAC and MIC have the same initial mutation rate per site per generation, with the initial MAC genomic mutation rate being 0.1. For the modifier loci, the wild type alleles within the mutators and antimutators have an initial total mutation rate set to be 0.01 and 0.002, respectively, thus there is always a mutational bias to increase the mutation rate within the genome (Gerrish et al., 2006). When a mutated allele gets fixed within a modifier locus, it can change the mutation rate by 90% (for mutators: +90%; for antimutators: -90%) within the genome (including both fitness and modifier loci), and, as with the fitness loci, they also act additively within the locus and multiplicatively among loci.

To investigate and compare the effects of different reproduction strategies on mutation rate evolution, the mutation rate evolution under mitosis, amitosis, obligate sex every generation ($\tau = 1$), and facultative sex every $\tau = 100$ generations are simulated. To achieve an efficient selection on mutation rate, I assume a

population size of 2,000. All individuals are mutation-free initially. The alleles in both fitness loci and modifier loci will be distributed among offspring according to a certain reproduction strategy. Here I allow two mutation generation contexts at fitness loci: either all mutations are deleterious, or a certain amount of mutations are beneficial. For the second context, it is simulated differently from that mentioned in Chapter 2. Starting with the model where all mutations are deleterious, another set of 100 loci which can only accumulate beneficial mutations are integrated, with the beneficial mutation rate setting to be a certain proportion of that for deleterious mutations. The mutations can alter the fitness by 10% (for beneficial ones: +10%; for deleterious ones: -10%) once getting fixed within the fitness loci. Population mean fitness and mutation rate in both MAC and MIC are monitored for 10,000 generations.

(B) Investigation of mutation rate evolution in diploid system

On the basis of 4.2.3 (A), I further investigated the mutation rate evolution under facultative sex in the diploid system. The ploidy of MAC is set to be 2. The initial mutation rate per site per generation and the effects of mutations in both modifier loci and fitness loci are kept the same as that in the 45-ploid MAC system. Population mean fitness and mutation rate in both MAC and MIC are monitored for 10,000 generations and compared with that obtained in the 45-ploid system.

4.3. Results

4.3.1. MIC carries a much lower mutation load than expected

(A) Facultative sexual population can achieve mutation-selection balance

To evaluate Long et al. (2016)'s hypothesis that the low MIC mutation rate is a consequence of the many rounds of asexual generations between two rounds of sex, I first investigated the mutation load carried within the MIC.

Mutation load is the fitness loss due to the presence of deleterious mutations at mutation-selection balance (Agrawal and Whitlock, 2012). As the first step to quantify the mutation load carried in the MIC, I

determined the population mean fitness immediately after having sex, in which the MIC mutations accumulated during the asexual cycles get expressed, and checked whether they have reached equilibrium. All of the simulated facultative sexual populations have reached equilibrium and achieved an equilibrium fitness between 0.875 and 0.996. As shown in Figure 4.1(a), generally a lower equilibrium fitness was achieved under a lower facultative sexual frequency (i.e., a longer sexual period τ).

(B) Simulation gets a much higher equilibrium fitness than expected value

As mentioned in Section 4.1, according to Long et al. (2016)'s argument, the mutations accumulate neutrally and are not subject to selection between two rounds of sex. Thus the expected total MIC mutations accumulated between two rounds of sex would be the MIC mutation rate per generation, U_{MIC}, times the sexual period, τ. Since the diploid MIC also divides mitotically during the asexual cycles, according to mutation load theory (Agrawal and Whitlock, 2012; Haldane, 1927), the equilibrium fitness immediately after sex, when the MIC mutations get expressed, would be:

$$\widehat{w} = e^{-U_{MIC} \times \tau} \quad (1)$$

As here I assumed identical mutation rate per site per generations within MIC and MAC, U_{MIC} is $0.1 \times 2/45 \approx 0.0044$, and τ ranges from 1 to 500.

I then compared the expected values calculated based on Equation 1 with the simulation results. However, as shown in Figure 4.1 (b), the equilibrium fitness from the simulations was found to be much higher than the expected value, except for that from having sex every generation. Assuming that the simulation is a better representation of "reality", these results indicated that the MIC carried a much lower mutation load than expected. The great discrepancy between simulation and prediction was not caused by the approximation made in mutation load theory but implied that selection also acts against the MIC mutations during asexual generations.

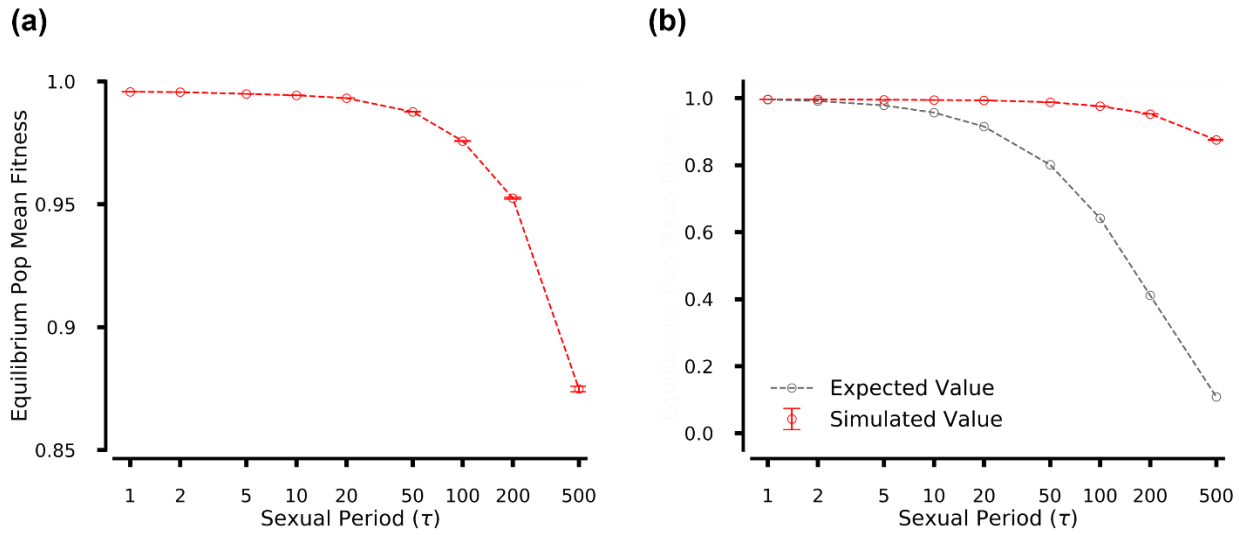

Figure 4.1: The MIC carried a much lower mutation load than expected. Values shows (a) the equilibrium fitness just after sex under different sexual frequencies (shown as sexual periods) got from simulation, and (b) comparison with the naïve expectation calculated based on mutation load theory. For the values got from simulation, the mean and 95% CIs are calculated using the population fitness just after sexual cycles at the last 2,000, 1,000, and 0 generations. For the simulation, I assumed a population size of N = 500 with a MAC ploidy n = 45. Each individual carries 100 initially mutation-free loci in both the MAC and MIC. All mutations are assumed to be deleterious and generated with a MAC genomic mutation rate U_{MAC} = 0.1 per generation. Mutations have a selection coefficient s_d = -10% once present in homozygous state. Sex reproduction is simulated as an outcrossing process with random mating. 100 replicated populations were run for each value of sexual period τ. Note that the x axis is shown in a log scale.

4.3.2. MIC mutations respond to selection during asexual generations

During sex in *Tetrahymena*, a haploid pronucleus will be produced from the MIC, and then two haploid pronuclei exchanged between paired cells will get fused into a diploid zygotic nucleus and further develop into the new MIC and MAC. The new MIC is generally a descendant of the diploid nucleus and still maintains a condensed and diploid genome, while the differentiation of new MAC from the diploid zygotic nucleus involves chromosomal amplification and programmed genome rearrangement (Orias et al., 2011). Since both new MAC and MIC are differentiated from the same diploid nucleus, they are correlated with each other, and inherit the same set of alleles originally in the zygotic nucleus. During the following asexual generations, selection directly acts on the MAC mutations, which may lead to correlated changes in allele

frequencies within the MIC. Thus, it is possible that there is indirect selection operating on the MIC via the selection on the MAC during asexual generations.

If the idea that there is indirect selection operating on the MIC is correct, then a lower facultative sexual frequency (i.e., a greater sexual period) is expected to achieve a higher selective efficiency on MIC mutations, as there are more rounds of asexual generations that selection can act on. To test this idea, I then allowed the populations conducting different frequencies of facultative sex to have the same mutation generation process within the MIC and investigated the fitness achieved after having sex. Here I only allow the mutation to generate once within the MIC just prior to sex. Since there is no mutation generation within the MAC, the only source for the MAC to get mutations is from the MIC during sexual process, when the old MAC is degraded and new MAC is created. Also, since there is only one round of mutation just before having sex, under different sexual frequencies, the population will get roughly the same number of mutations but have different numbers of asexual generations during which selection may operate. As a result, if MIC mutations respond to selection following sex, as sex becomes less and less frequent, a higher and higher equilibrium fitness immediately after sex would be achieved, as there are more asexual generations for selection to operate on the MIC mutations generated prior to sex.

(A) Higher equilibrium fitness is achieved once sex is less frequent

With the revised model, I then allowed the sexual frequencies to vary from having sex every generation to having sex once every 500 generations (i.e., τ = 1, 2, 5, 10, 20, 50, 100, 200, and 500). To get a clearer pattern on the impact of sexual frequency on the equilibrium fitness after sex, I raised the MIC genomic mutation rate to be 1 prior to sex. All tested sexual frequencies can lead to mutation-selection balance within a population composed of 500 individuals. A shown in Figure 4.2, the obligate sexual population reached an equilibrium fitness of 0.3636 (95% CI: 0.3635 - 0.3637), which matched that predicted by mutation load theory (i.e., $e^{-1} \approx 0.3679$). As expected, a higher and higher equilibrium fitness can be achieved as sex becomes less and less frequent, which indicated that the MIC mutations also respond to selection operating on the MAC during asexual generations (Figure 4.2).

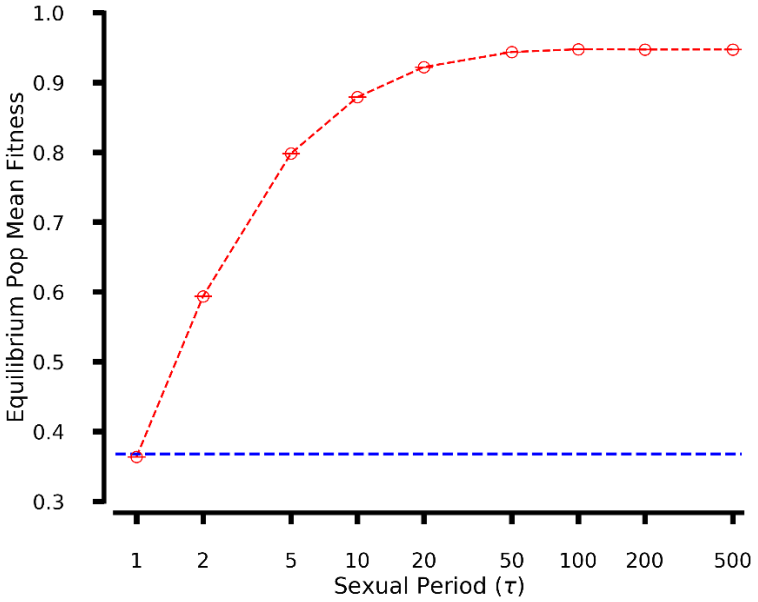

Figure 4.2: A higher equilibrium population mean fitness can be achieved as sex occurs less frequently. Values shows the equilibrium fitness just after sex under different sexual frequencies (shown as sexual periods). The mean and 95% CIs are calculated using the population fitness just after sexual cycles at the last 2,000, 1,000, and 0 generations. The blue dash line is the expected equilibrium fitness with a MIC mutation rate of 1 under obligate sex every generation ($\tau = 1$), which is $e^{-1} \approx 0.3679$. Here I revised the original model by only allowing the mutation to generate in the MIC just before having sex, with a MIC genomic mutation rate $U_{MIC} = 1$. Other parameters are the same as that in Figure 4.1. Note that the x axis is shown in a log scale.

With a sexual frequency of once every 100 generations ($\tau = 100$), the equilibrium fitness achieved the maximum equilibrium fitness of ~0.95, and further lowering the sexual frequencies (i.e., increasing the value of τ) did not result in an obvious equilibrium fitness increase. This is due to that once the frequency of sexual process is low enough, the many rounds of selection occurred during asexual generations are sufficient to remove all the MIC mutations generated previously. Since here I monitored the population mean fitness immediately after sex, the ~0.05 fitness loss was caused by the new mutations generated during the following new round of sex. Given a genomic mutation rate of 1, MIC will approximately get 1 mutation just before having sex. Then after having sex, the MAC will get 22 or 23 mutant alleles from the MIC, and result in a ~0.05 fitness loss under a mutation selection coefficient of -0.1.

(B) The response of MIC mutations to selection is robust in the presence of MAC mutation

To check the robustness of my findings on the selection of MIC mutations during asexual divisions as well as to make the model more realistic, I turned on the mutation generating process in the MAC again and investigated whether the selection acting on the mutations originally from the MIC was interfered by the new mutations generated within the MAC. Since the MAC will be recreated after sex and lose all previous mutations, I allowed MAC mutation to be generated every generation, with a total genomic mutation rate of 45 evenly scaled between two sexual cycles (e.g., a constant MAC genomic mutation rate of 0.9 per generation with a sexual frequency of once every 50 generations). As a result, the MAC has an average mutation rate per site per generation twice that of the MIC.

In the presence of new mutations generated in the MAC, the equilibrium fitness immediately after sex was obtained and compared with that assuming no MAC mutations. As shown in Figure 4.3, the equilibrium fitnesses under these two settings were almost the same, suggesting that the response of MIC mutations to selection during asexual divisions is robust and unaffected by the new mutations generated within the MAC.

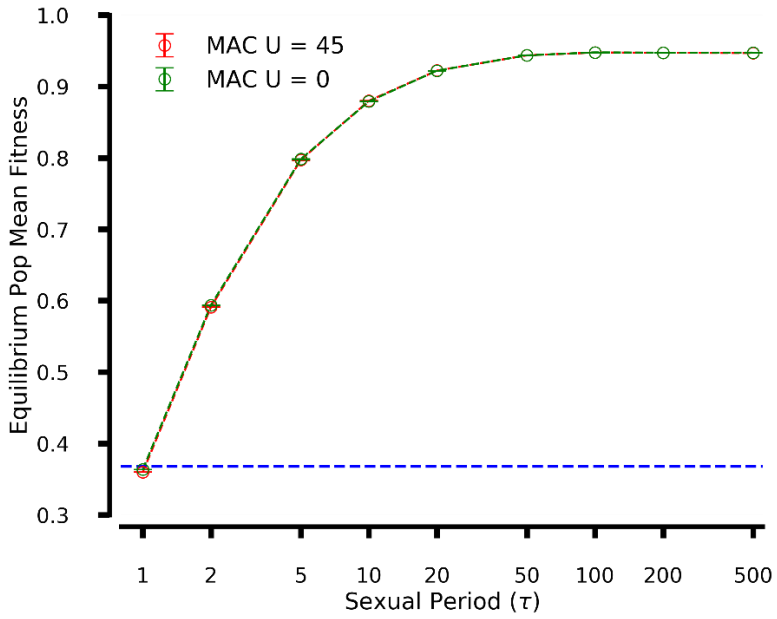

Figure 4.3: Similar equilibrium fitness can be achieved in the presence of MAC mutation generation. Values show the equilibrium population mean fitness just after sex in the absence and presence of MAC mutation generation under different sexual frequencies (shown as sexual periods). The mean and 95% CIs are calculated using the population fitness just after sexual cycles at the last 2,000, 1,000, and 0 generations. For the presence of MAC mutation generation, MAC mutations are generated every generation, with a total

genomic mutation rate of 45 evenly scaled between two rounds of sex (thus MAC has an average twice mutation rate per site per generation compared to MIC). The blue dash line is the expected equilibrium fitness with a MIC mutation rate of 1 under obligate sex every generation ($\tau = 1$), $e^{-1} \approx 0.3679$. Note that the x axis is shown in a log scale.

As demonstrated above, although the MIC mutations are not expressed during asexual generations, there is indirect selection acting on the MIC through the selection operating on the MAC, as both MAC and MIC inherit the same set of mutations from the diploid zygotic nucleus during sex. Moreover, the indirect selection acting on the MIC is very robust and remains approximately unaffected even under a doubling of mutation rate within the MAC. Thus, one of the theoretical bases of Long et al. (2016)'s explanation for the low MIC mutation rate within *T. thermophila*, that MIC mutations are accumulated neutrally and not under selection during asexual cycles, is incorrect, and renders their explanation invalid.

4.3.3. Certain reproduction strategies can lower mutation rate in *Tetrahymena*

(A) Amitosis and facultative sex can reduce mutation rate

I further hypothesized that unusual reproduction strategies adopted by *Tetrahymena*, i.e., amitosis and facultative sex, may be able to reduce the mutation rate naturally under selection. To test this hypothesis, I introduced mutation rate modifiers and investigated how mutation rate evolves under different reproduction strategies.

I first simulated mutation rate evolution assuming all mutations are deleterious. Due to the deleterious effect of mutations, a lower mutation rate would be favored by natural selection. Allowing the mutation rate to evolve and simulating for 10,000 generations, even with a population size of 2,000, mitotic populations still suffered great fitness loss caused by Muller's ratchet (mean and 95% CI of final population mean fitness: 0.1141, 0.1063 ~ 0.1218), while the other 3 reproduction strategies, amitosis, obligate sex every generation ($\tau = 1$), and facultative sex every $\tau = 100$ generations, allowed the population to achieve a fitness level close to 1 (Figure 4.4; mean and 95% CI of final population mean fitness: amitosis: 0.9992 ± 0.0001; obligate sex every generation: 0.9950 ± 0.0012; facultative sex every $\tau = 100$ generations: 0.9960 ± 0.0010).

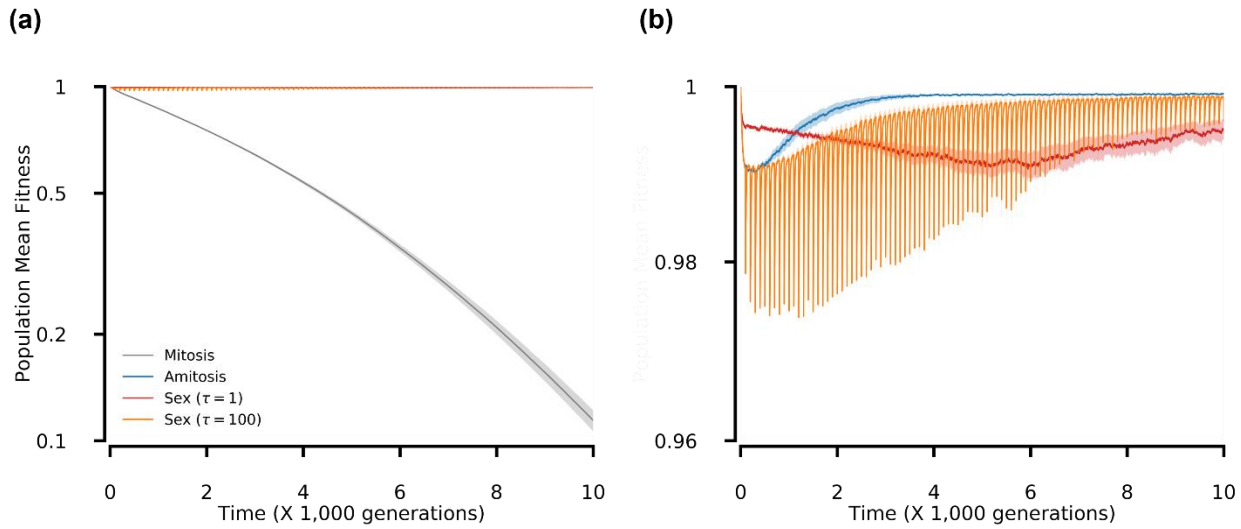

Figure 4.4: Assuming all mutations are deleterious, amitosis and sex can slow down the operation of Muller's ratchet relative to mitosis under an evolvable mutation rate. Evolutionary response of population mean fitness achieved by different reproduction strategies under an evolvable mutation rate. Lines and shade regions show the means and 95% CIs of stochastic simulations of 100 replicate populations. Subfigure (a) shows the results under all four reproduction strategies: mitosis, amitosis, obligate sex every generation ($\tau = 1$), and facultative sex every $\tau = 100$ generations, and subfigure (b) shows 3 reproductions excluding mitosis. Here I assumed a population size N = 2,000 with a MAC ploidy n = 45. Each individual carries 100 fitness-affecting loci, 10 mutators and 10 antimutators in both the MAC and MIC. All loci are mutation-free initially. For fitness-affecting loci, all mutations are deleterious and generated with an initial MAC genomic mutation rate U_{MAC} = 0.1 per generation. The deleterious mutations have a selection coefficient s_d = -10% once present in homozygous state. For mutator and antimutators, they accumulate mutation with an initial total rate of 0.01 and 0.002, respectively. The mutations present in homozygous state can change the mutation rate by +90% (in mutators) and -90% (in antimutators), respectively. Sexual reproduction is simulated as an outcrossing process with random mating. Note that the population mean fitness is shown in a log scale.

Further looking into the mutation rate evolutionary trajectories in the MAC and MIC, revealed that certain reproduction strategies can promote the reduction of mutation rate. To see the results more clearly, I divided the obtained mutation rate by the initial rate to show the relative mutation rate during evolution. Serving as a control, I also simulated mutation rate evolution under mitosis with a population size of 1, in which selection cannot operate on the mutation rate and finally led to a dramatic mutation rate increase (Figure 4.5; mean and 95% CI of mutation rate increase in MAC: 13.536, 13.370 - 13.702; in MIC: 13.536, 13.370 - 13.702). For the mutation rate within the MAC, with a larger population size of N = 2,000, due to the effect of selection for lower mutation rate, the mitotic population increased mutation rate less (mean of

relative mutation rate after 10,000 generations: 6.833; 95% CI: 6.763 - 6.902). However, selection within the mitotic population is not sufficient to reduce the mutation rate. Obligate sex every generation ($\tau = 1$) does not result in a reduction in the mutation rate either (Figure 4.5(a); mean of relative MAC mutation rate: 1.341; 95% CI: 1.297 - 1.385), but caused less of a mutation rate increase compared to mitosis. The failure of obligate sex in reducing mutation rate is caused by a different reason than that for mitosis, which I will address in the Discussion part. Both amitosis and facultative sex every $\tau = 100$ generations can greatly reduce the MAC mutation rate: after evolving for 10,000 generations, the amitotic population achieved a MAC mutation rate of 0.092 (95% CI: 0.090 - 0.093), and facultative sex every $\tau = 100$ generations led to a similar mutation rate reduction (mean relative MAC mutation rate: 0.128; 95% CI: 0.120 - 0.136).

However, within the MIC, except for facultative sex, none of the other three reproduction strategies can reduce the mutation rate. Mitosis resulted in an even greater mutation rate increase in the MIC compared to the MAC (mean of relative mutation rate: 6.833; 95% CI: 6.763 - 6.902), and amitosis caused a slight mutation rate increase (mean of relative mutation rate: 1.221; 95% CI: 1.197 - 1.246). This is likely due to the fact that the MIC does not get expressed under asexual reproduction strategies, thus selection cannot act on it.

Interestingly, obligate sex every generation ($\tau = 1$) cannot lower the MIC mutation rate and it remains roughly unchanged after 10,000 generations (mean of relative mutation rate: 1.131; 95% CI: 1.076 - 1.186), although the MIC mutations got expressed and subject to selection every generation. Similar to what happened in the MAC, facultative sex every $\tau = 100$ generations can also reduce the mutation rate within the MIC (Figure 4.5(b); mean of relative mutation rate: 0.165; 95% CI: 0.158 - 0.173).

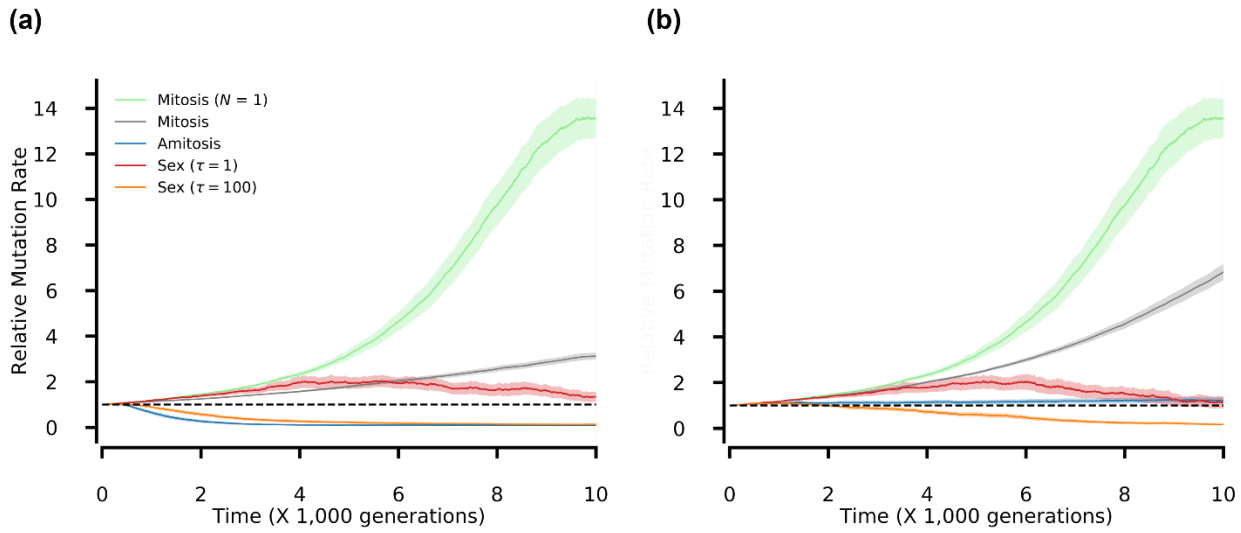

Figure 4.5: Amitosis and facultative sex can reduce the mutation rate in the absence of beneficial mutations. Evolutionary response of mean mutation rate per site per generation in the (a) MAC and (b) MIC under different reproduction strategies. Lines and shade regions show the means and 95% CIs of stochastic simulations of 100 replicate populations. The green line shows the mutation rate evolution dynamics within a mitotic population composed of 1 individual. The black dash line shows the relative mutation rate of 1, which is the initial mutation rate level. Note that the y axis is shown as mutation rate changes relative to the initial value.

I then investigated the evolution of mutation rate in the presence of 1% beneficial mutations. For mitosis, the fitness dynamics and mutation rate evolving trajectories were almost identical to that under no beneficial mutation, as mitotic populations cannot accumulate the small amount of beneficial mutations efficiently (Figure 4.6 and Figure 4.7). However, for the other three reproduction strategies, the presence of only 1% beneficial mutations made a great difference in both fitness dynamics and mutation rate evolution trajectories. After evolving for 10,000 generations, the amitotic, obligate sexual, and facultative sexual populations reached a population mean fitness greater than 1 (Figure 4.6), indicating adaptation happened. Similar to the pattern we found in Chapter 2, obligate sex every generation ($\tau = 1$) led to the greatest fitness increase (mean of population mean fitness after 10,000 generations: 1246.975; 95% CI: 882.495 - 1611.456), followed by amitosis (mean of population mean fitness: 35.449; 95% CI: 26.193 - 44.705) and facultative sex every $\tau = 100$ generations (mean of population mean fitness: 9.817; 95% CI: 8.059 - 11.575).

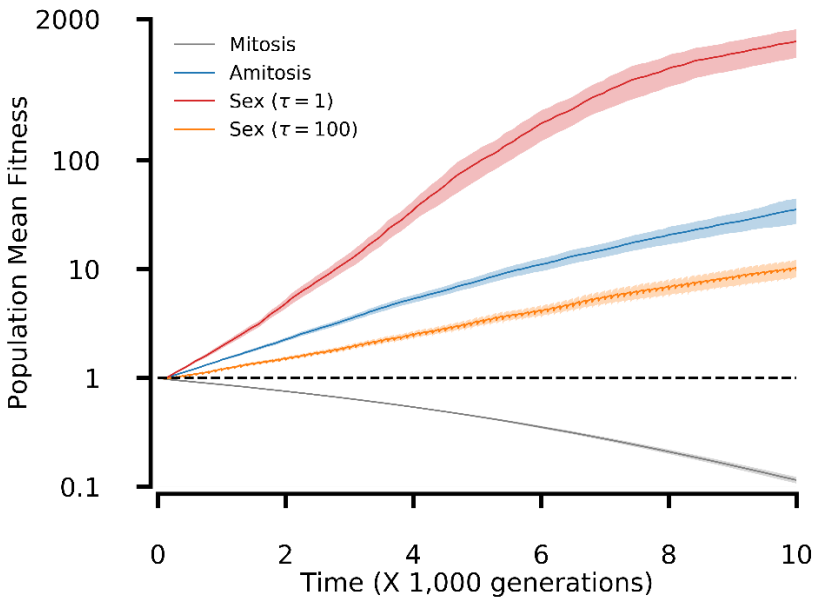

Figure 4.6: Under an evolvable mutation rate, amitosis and sex can lead to adaptation in the presence of 1% beneficial mutations. Evolutionary response of population mean fitness achieved by different reproduction strategies under an evolvable mutation rate. Lines and shade regions show the means and 95% CIs of stochastic simulations of 100 replicate populations. Here I introduce another 100 fitness-affecting loci that can only get beneficial mutations. The beneficial mutations have a selection coefficient s_b = 0.1 once present in homozygous state, and are generated with a total rate of 1% of that for deleterious mutations (i.e., 0.001 per generation initially). The grey dash line shows the population mean fitness level of 1. Other evolutionary parameters are the same as that in Figure 4.4. Note that the population mean fitness is shown in a log scale.

For the evolution of mutation rate, however, the obligate sexual population evolved to almost the same mutation rate as that under no beneficial mutations within both the MAC and MIC (Figure 4.7; for MAC: mean of relative mutation rate: 1.074, 95% CI: 1.033 - 1.116; for MIC: mean of relative mutation rate: 1.150, 95% CI: 1.090 - 1.210). Amitosis can still reduce the MAC mutation rate with a lower magnitude (mean of relative mutation rate: 0.501, 95% CI: 0.475 - 0.527), but caused a much greater mutation rate increases within MIC (mean of relative mutation rate: 3.441, 95% CI: 3.220 - 3.662). Similarly, having facultative sex every τ = 100 generations can also decrease the MAC mutation rate, although the extent of reduction was less than that under no beneficial mutations (mean of relative mutation rate: 0.255, 95% CI: 0.243 - 0.267), and maintained the MIC mutation rate roughly unchanged (mean of relative mutation rate: 1.016, 95% CI: 0.939 - 1.092).

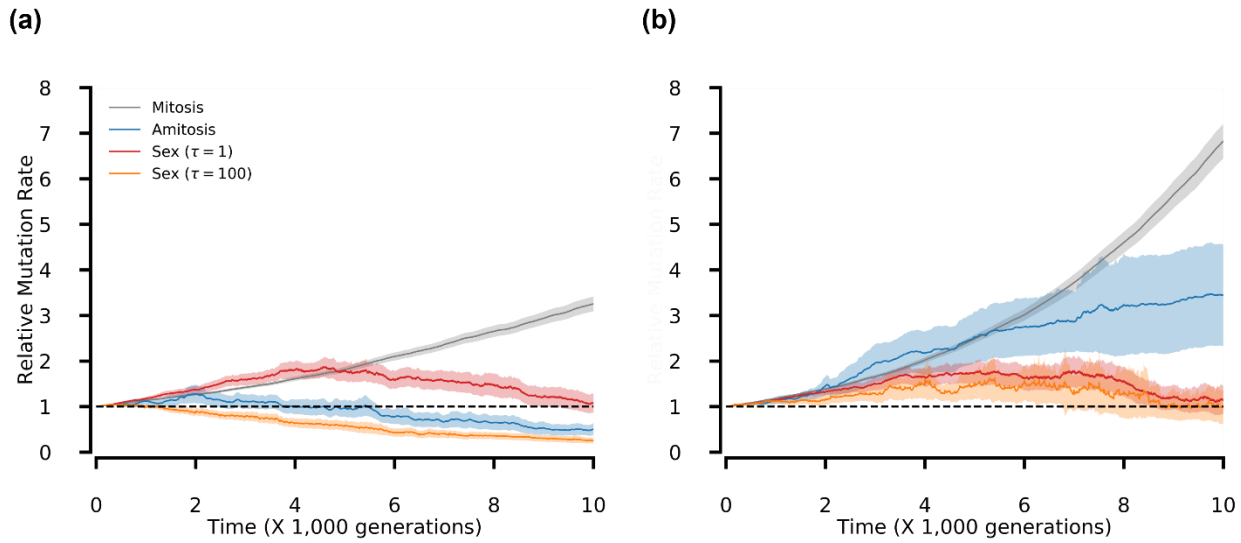

Figure 4.7: Amitosis and facultative sex can reduce the mutation rate in the presence of beneficial mutations. Evolutionary response of mean deleterious mutation rate per site per generation under different reproduction strategies in the presence of 1% beneficial mutations. Subfigure (a) and (b) are for the deleterious mutation within the MAC and MIC, respectively. Lines and shade regions show the means and 95% CIs of stochastic simulations of 100 replicate populations. Note that the y axis is shown as mutation rate changes relative to the initial value.

(B) Amitosis of modifier loci accounts for lowering mutation rate during amitosis

As amitosis has been shown to be able to reduce the MAC mutation rate, I then investigated whether it is the amitotic division of fitness loci or modifier loci that accounts for this. By allowing fitness and modifier loci to divide in different ways, I ran another two sets of simulations: set fitness loci to divide by amitosis while modifier loci divided by mitosis, and vice versa. Such simulations can be regarded as "thought experiments" and do not represent any real organisms.

Compared with the previous results where both fitness loci and modifier loci were divided by amitosis, I found it was the amitotic division of the modifier loci that is responsible for reducing the MAC mutation rate. Assuming all mutations are deleterious, having only modifier loci divided by amitosis led to a similar mutation rate evolution both the MAC and MIC (Figure 4.8; final MAC relative mutation rate: mean: 0.070, 95% CI: 0.061 - 0.079; final MIC relative mutation rate: mean: 1.285, 95% CI: 1.146 - 1.424). On the other hand, having only fitness loci divided by amitosis generated approximately the same mutation rate evolution

as that having both fitness loci and modifier loci divided by mitosis (Figure 4.8; final MAC relative mutation rate: mean: 3.056, 95% CI: 2.925 - 3.187; final MIC relative mutation rate: mean: 6.611, 95% CI: 6.306 - 6.916). The same patterns were also found in the presence of beneficial mutations. With the presence of 1% beneficial mutations, the populations having only modifier loci divided by amitosis reached a relative deleterious mutation rate of 0.066 (95% CI: 0.058 - 0.075) in the MAC and 1.135 (95% CI: 1.036 - 1.234) in the MIC, while the ones having only fitness loci divided by amitosis achieved a relative deleterious mutation rate of 10.406 (95% CI: 9.881 - 10.931) in the MAC and 13.629 (95% CI: 12.926 - 14.332) in the MIC (Figure 4.8). Taking these results together, I concluded that it is the division mode of the modifier loci that actually determines the mutation rate evolution trajectories under both mitosis and amitosis.

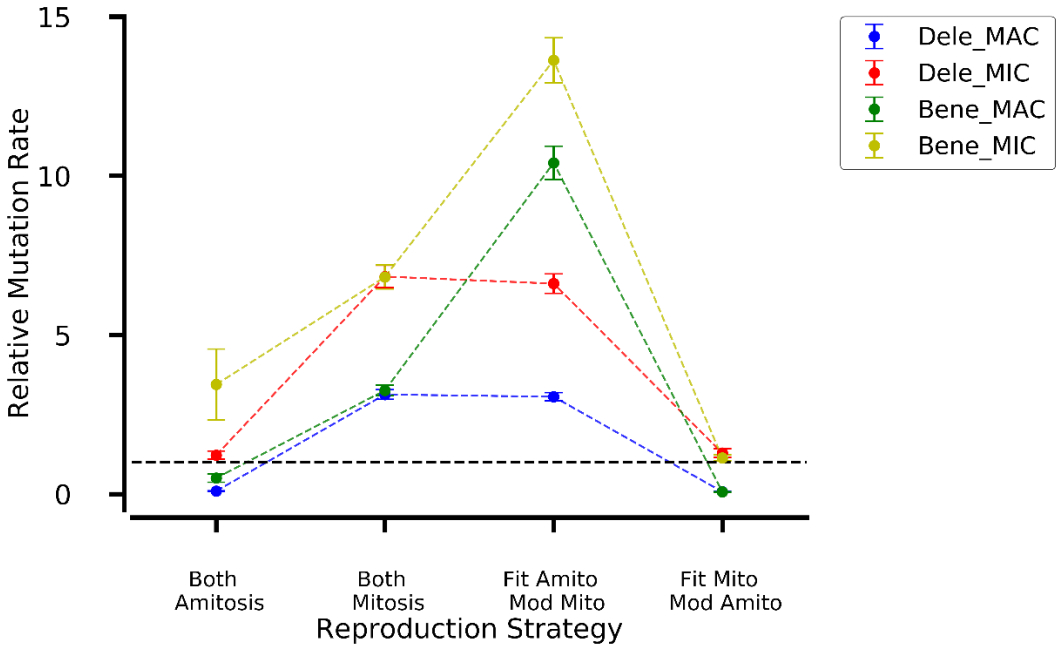

Figure 4.8: The division mode of the modifier loci determines the mutation rate trajectories under both amitosis and mitosis. Values show the relative mutation rate per site per generation within the MAC and MIC after evolving for 10,000 generations under 4 different division modes of fitness and modifier loci: a. both types of loci are divided by amitosis; b. both types of loci are divided by mitosis; c. fitness loci divided by amitosis while modifier loci divided by mitosis; d. fitness loci divided by mitosis while modifier loci divided by amitosis. The error bar shows the 95% confidence intervals. Means and 95% CIs are calculated from the simulation results of 100 replicate populations for each setting. The black dash line shows the relative mutation rate of 1, which is the initial mutation rate level. The evolutionary parameters are the same as that in Figure 4.5 (for assuming all mutations are deleterious) and 4.7 (for assuming 1% mutations are beneficial).

(C) The sexual process but not the amitotic process during facultative sex reduces the mutation rate

As introduced in subsection 4.3.3(A), I found that facultative sex every $\tau = 100$ generations has an effect on mutation rate evolution in both the MAC and MIC. Since facultative sex involved two distinct processes (i.e., sexual and asexual cycles), I then asked which process is responsible for promoting mutation rate evolution. To answer this question, I simulated another form of facultative sex, in which there were mitotic cycles between two rounds of sex.

Compared to the mutation rate evolution under facultative sex with amitosis, the new form of facultative sex with mitosis generated similar results in both the MAC and MIC (Figure 4.9). Under the assumption that all mutations are deleterious, facultative sex with mitosis led to a MAC relative mutation rate of 0.047 (95% CI: 0.038 - 0.056) and MIC relative mutation rate of 0.213 (95% CI: 0.152 - 0.275) after evolving 10,000 generations, which is close to that evolved under facultative sex with amitosis (mutation rate in the MAC: mean: 0.128, 95% CI: 0.086 - 0.170; in the MIC: mean: 0.165, 95% CI: 0.126 - 0.205). An even closer MAC and MIC mutation rates were identified in the presence of 1% beneficial mutations (within the MAC: facultative sex with mitosis: 0.163, 95 % CI: 0.120 - 0.206 vs. facultative sex with amitosis: 0.255, 95 % CI: 0.192 - 0.317; within the MIC: facultative sex with mitosis: 0.754, 95 % CI: 0.576 - 0.933 vs. facultative sex with amitosis: 1.016, 95 % CI: 0.630 - 1.402). Thus, it is the sexual process but not the amitotic process during facultative sex that is responsible for the reduction of mutation rate within both MAC and MIC.

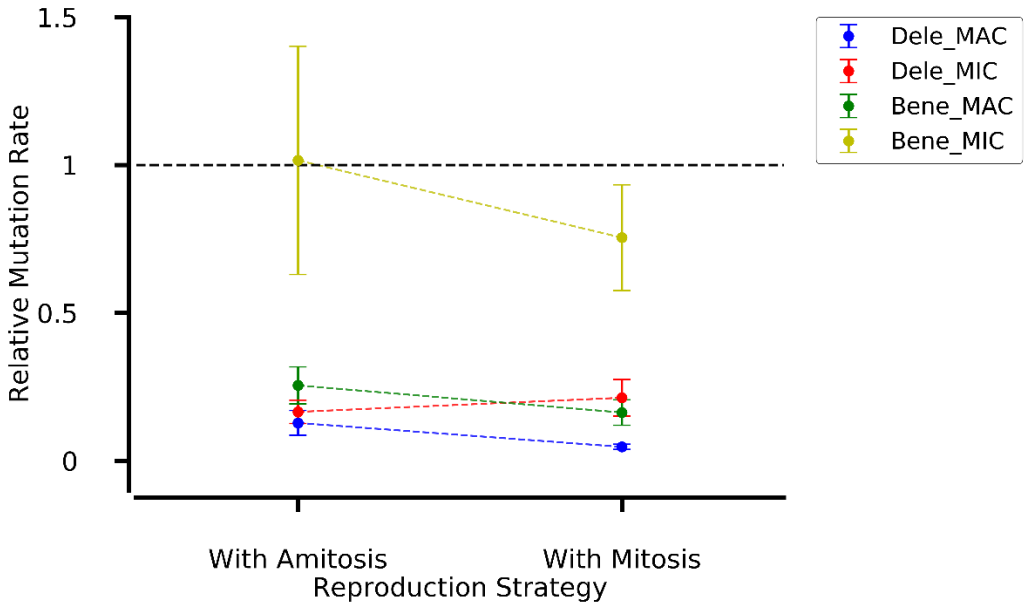

Figure 4.9: The sexual process of facultative sex contributes to lowering the mutation rate. Values show the relative mutation rate per site per generation within the MAC and MIC after evolving for 10,000 generations under facultative sex with amitosis and mitosis, respectively. The error bar shows the 95% confidence intervals. Means and 95% CIs are calculated from the simulation results of 100 replicate populations for each setting. The black dash line shows the relative mutation rate of 1, which is the initial mutation rate level. The evolutionary parameters are the same as that in Figure 4.5 (for assuming all mutations are deleterious) and 4.7 (for assuming 1% mutations are beneficial).

4.3.4. Greater reduction of mutation rate can be achieved in polyploid system

As mentioned above, within a 45-ploid system, facultative sex can efficiently promote the reduction of mutation rate in both the MAC and MIC. I then asked how facultative sex affects mutation rate evolution in a normal diploid system.

Assuming all mutations are deleterious, the facultative sexual population with a diploid MAC generated similar fitness and mutation rate evolution trajectories as that with a 45-ploid MAC, although the population with a diploid MAC suffered greater fitness loss once having sex. After evolving for 10,000 generations, within a population size of 2,000, facultative sex with a diploid MAC allowed the populations to reach a population mean fitness of 0.9906 (95% CI: 0.9884 - 0.9929), which was almost the same as that in 45-ploid system (Figure 4.10(a)). For the evolution of mutation rate, the facultative sexual population with a diploid MAC achieved a relative mutation rate of 0.2464 (95% CI: 0.1815 - 0.3112) in the MAC and 0.2445

(95% CI: 0.1797 - 0.3093) in the MIC, both of which were higher than that got within the 45-ploid MAC system (Figure 4.11).

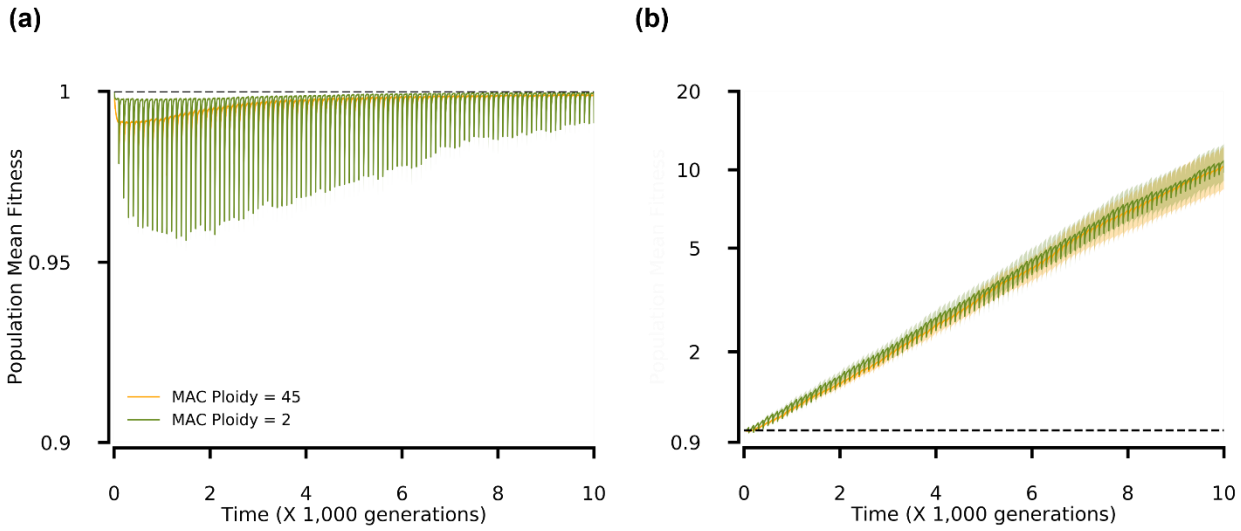

Figure 4.10: Facultative sex with diploid MAC achieves similar population mean fitness compared to that with 45-ploid MAC. Evolutionary response of population mean fitness achieved by facultative sex within diploid and 45-ploid MAC under (a) all mutations are deleterious and (b) 1% mutations are beneficial. Lines and shade regions show the means and 95% CIs of stochastic simulations of 100 replicate populations. I assumed the same initial mutation rate per site per generation as well as same homozygous mutation effect within diploid and 45-ploid MAC. The grey dash line shows the population mean fitness level of 1. Other evolutionary parameters are the same as that in Figure 4.4 (for assuming all mutations are deleterious) and 4.6 (for assuming 1% mutations are beneficial). Note that the population mean fitness is shown in a log scale.

The pattern I found for population mean fitness was robust in the presence of beneficial mutation. Introducing 1% beneficial mutations, the facultative sexual population with a diploid MAC achieved almost identical population mean fitness compared to that got in 45-ploid system after evolving 10,000 generations (Figure 4.10 (b); population mean fitness with a diploid MAC: mean: 9.8007, 95% CI: 8.2298 - 11.3716). However, for mutation rate evolution, they exhibited different patterns. Within the MAC, facultative sex with a diploid MAC did not reduce but led to an increase for the MAC mutation rate (Figure 4.11; mean mutation rate: 1.7236, 95% CI: 1.2524 - 2.1947), and achieved a much higher MIC mutation rate than that got within the 45-ploid MAC system (Figure 4.11; mean and 95% CI of the mutation rate with a diploid MAC: 1.3912, 0.9970 - 1.7854).

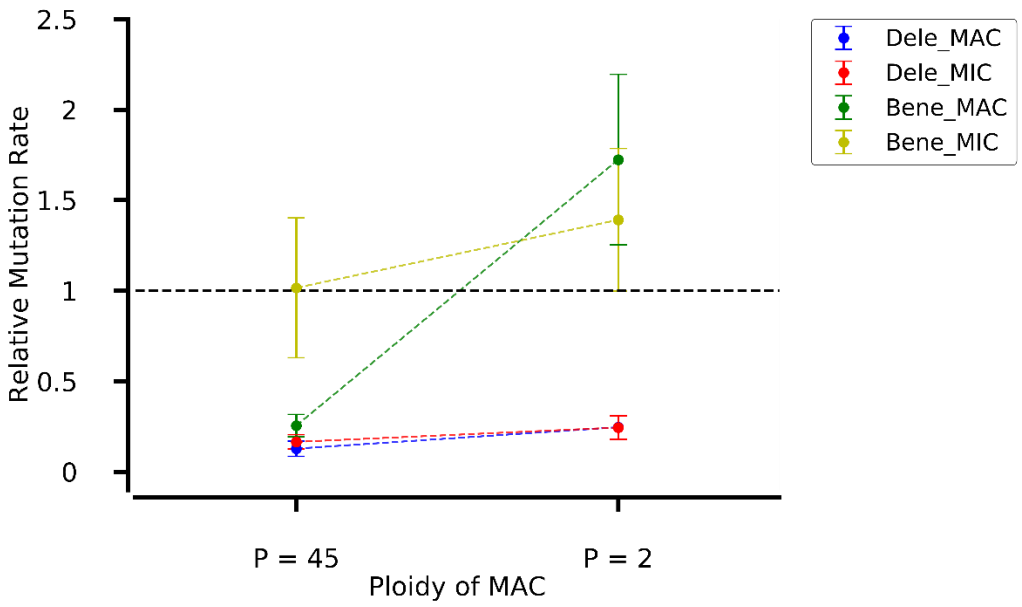

Figure 4.11: Facultative sex in the diploid MAC system achieves a less reduction of mutation rate compared to that in the 45-ploid MAC system. Values show the relative mutation rate per site per generation in the MAC and MIC under facultative sex with a diploid and 45-ploid MAC, respectively. The error bar shows the 95% confidence intervals. Means and 95% CIs are calculated from the simulation results of 100 replicate populations for each setting. The black dash line shows the relative mutation rate of 1, which is the initial mutation rate level. The evolutionary parameters are the same as that in Figure 4.5 (for assuming all mutations are deleterious) and 4.7 (for assuming 1% mutations are beneficial).

The comparison of results got in the diploid and 45-ploid MAC systems suggested that a greater reduction of mutation rate can be achieved in polyploid system, regardless of the presence of beneficial mutations.

4.4. Discussion

4.4.1. MIC mutations respond to selection during asexual generations as they are present in the MAC following sex

In this chapter, I investigated the evolutionary causes of the extremely low mutation rate found in the MIC of *T. thermophila*. Long et al. (2016) argued that the low MIC mutation rate evolved as a consequence of long periods of asexuality between two sexual cycles. They claimed that the MIC mutations are not expressed and supposed that they are not "visible" to selection until having sex, thus a larger mutation load

will be generated and natural selection should favor the reduction of mutation rate (Long et al., 2016b). However, by investigating the equilibrium population mean fitness immediately after sex, I found that the MIC carried a much lower mutation load than the expected value assuming there is no selection acting on MIC mutations until having sex. Further study revealed that although not expressed until having sex, the MIC mutations are also subject to selection during asexual generations as they are present in the newly created MAC and can respond to selection via the selection operating on the MAC. Under the evolution parameters I used for simulation, with a sexual frequency of once every 100 generations ($\tau = 100$), selection during asexual generations is sufficient to remove approximately all deleterious mutations generated before having sex again. Furthermore, the response of MIC mutations to selection is robust in the presence of MAC mutations. Even with an average MAC mutation rate two times that of the MIC, the response of MIC mutations to selection remains approximately unaffected. Thus the previous hypothesis proposed by Long et al. (2016) was theoretically invalid to explain the low MIC mutation rate found in *T. thermophila*. Long et al. (2016)'s argument on mutation load carried within the MIC also contradicts with the experimental data. Suggested by Long et al. (2016), if their hypothesis is correct, then the MAC would exhibit a higher mutation rate which is more similar to other eukaryotes, in contrast to the MIC, as the mutations in the MAC are subject to selection every generation. However, recent experimental measurement has shown that the MAC maintains a similar mutation rate to the MIC (personal communication with Dr. Rebecca Zufall).

4.4.2. Amitosis and facultative sex can reduce mutation rate under selection

In 1937, A.H. Sturtevant suggested that there may be some loci which can modify the mutation rate and thus are subject to selection via their genetic associations with the loci affecting the fitness of their carriers (Sturtevant, 1937). These loci are termed mutation rate modifiers, and generally can be classified into two major categories: the mutators whose alleles can cause an increase of mutation rate and the antimutators in which the alleles can lower the mutation rate (Gerrish et al., 2006; Schaaper, 1998). Since the introduction of mutation rate modifiers, many theoretical papers on mutation rate evolution have been published. For example, by introducing mutators into the population and examining the factors that may affect the mutation

rate evolution, Kimura (1967) proposed the famous "cost of fidelity" hypothesis, that the reduction of mutation rate is constrained by the extra fitness cost of further improving DNA replication fidelity and the physiological accuracy limits of the DNA replication and repair mechanisms (Kimura, 1967). Liberman and Feldman (1986) studied a diploid deterministic population genetic model undergoing random mating and involving two loci (i.e., one fitness locus subject to selection and one modifier loci controlling the mutation rate), and suggested that natural selection should generally select a minimal mutation rate within a stable environment ('reduction principle') (Liberman and Feldman, 1986). These findings greatly increase our understanding on mutation rate evolution.

Here by incorporating mutation rate modifiers, I investigated mutation rate evolution under different reproduction strategies. Those modifiers will not contribute to the fitness directly but affect the mutation rate of the fitness loci. Since the mutation rate determines how many mutations are generated in the fitness loci, the mutation rate and fitness are correlated. Thus selection on the fitness loci can also impact the mutation rate.

The effects of fitness-based selection on mutation rate can be described using the Price equation. Derived by George R. Price in 1970s, the Price equation quantifies how the magnitude of a certain quantitative trait changes over time under selection (Price, 1970, 1972). This equation can be expressed as:

$$\Delta\bar{z} = \frac{1}{\overline{w}} Cov(w, z) + \frac{1}{\overline{w}} E[w\Delta z] \quad (2)$$

in which w is the fitness, $\overline{w}$ is the population mean fitness, z is a certain quantitative trait, $\Delta\bar{z}$ is the changing amount of the mean quantitative trait from one generation to the next generation, and $E[w\Delta z]/\overline{w}$ is the expected changing amount of the trait caused by reproduction. The Price equation shows that $\Delta\bar{z}$ is determined by the covariance between this trait and fitness, together with the expected quantity change of the trait due to fitness. Under the same level of $E[w\Delta z]/\overline{w}$, a stronger correlation (indicated by a higher covariance) between the studied trait and fitness will lead to a greater amount of change in this trait under selection.

In my study, the quantitative trait under study is mutation rate, and the term $E[w\Delta z]/\overline{w}$ captures the impact of reproduction on the mutation rate. The Price equation can help to explain my findings, that certain reproduction strategies, especially amitosis and facultative sex, can promote the reduction of mutation rate, while mitosis and obligate sex every generation ($\tau = 1$) cannot. Since there is the same mutation bias for different reproduction strategies, approximately the same level of $E[w\Delta z]/\overline{w}$ will be achieved. Thus, the different mutation rate evolution patterns are caused by the difference in covariance between fitness and mutation rate under different reproduction strategies.

Under the assumption that all mutations are deleterious, a lower mutation rate would be favored by selection as fewer mutations can be generated. The mutation rate is not directly under selection, but via the selection based on individual fitness. For asexual reproduction strategies, since the mutations within the MIC cannot contribute to the individual fitness, meaning that there is no correlation between MIC mutation rate and fitness, the selection can only act on the mutation rate within the MAC. As demonstrated in Chapter 2, the mitotic population cannot generate enough variation for selection to operate, thus the selective force acting on mutation rate is also too weak to lower the mutation rate. Consequently, mitosis cannot reduce the mutation rate in either the MAC or MIC. On the other hand, for amitosis, although the "recombination-like" effect weakens the correlation between mutation rate and fitness, the much enhanced variation generated also leads to much more efficient selection compared to mitosis, which would then efficiently promote the reduction of the mutation rate within the MAC.

Surprisingly, obligate sex every generation ($\tau = 1$) resulted in an increase of mutation rate in both the MAC and MIC, while maintaining a high population mean fitness level of ~0.995 simultaneously. This is likely to be caused by the breakup of the linkage disequilibrium (and, therefore, the covariance) between fitness and modifier loci in every generation under obligate sex. Thus according to Price equation, the selection will have a weak effect on mutation rate evolution and result in mutation rate increase. This idea can be tested by running another two simulations: once having sex, only let part of the genome, either the fitness or the modifier loci, recombine, while other divide by mitosis. If my explanation is correct, then the new

simulation that only allows the modifier loci to recombine will generate approximately the same results as that setting both fitness and modifier loci to recombine on mutation rate evolution. However, even facing an increased mutation rate and more arising mutations, obligate sex every generation ($\tau = 1$) can still generate sufficient variation for selection to remove the deleterious mutations, thereby avoiding Muller's ratchet and maintaining a high fitness level. Nonetheless, lower the sexual frequency to once every 100 generations can achieve a mutation rate reduction in both MAC and MIC. This is probably because the MAC are regenerated from the diploid zygotic nucleus once having sex, which would thus create a correlation between the MAC and MIC. Thus, the selection can also operate on the mutation rate within the MIC. Furthermore, as there are many rounds of asexual cycles between two rounds of sex, same as what happened under amitotic division, selection can efficiently promote the reduction of MAC mutation rate. As a result, facultative sex every $\tau = 100$ generations can lead to the mutation rate reduction within both nuclei. In the future, the frequency of facultative sex can be manipulated to test the explanation here. If my explanation is valid, I would expect that the facultative sex with a lower frequency would result in a lower selection efficiency on the MIC mutations, and with a low enough sexual frequency, the mutation rate evolution within both MAC and MIC would be approximately identical to that under solely amitosis.

Indeed, my preliminary analyses have shown that different reproduction strategies generate different patterns for covariance between population mean fitness $\overline{w}$ and mean mutation rate within the MAC and MIC ($\overline{u}_{MAC}$and $\overline{u}_{MIC}$). I found that mitosis has led to a weak negative covariance between $\overline{w}$ and $\overline{u}_{MAC}$. Compared to mitosis, amitosis generates a negative covariance between $\overline{w}$ and $\overline{u}_{MAC}$ with a much greater magnitude initially, which is in step with the great MAC mutation rate reduction in the first ~2,000 generations. Obligate sex every generation ($\tau = 1$) results in a fluctuated covariance around 0 between $\overline{w}$ and both $\overline{u}_{MAC}$ and $\overline{u}_{MIC}$, while facultative sex every $\tau = 100$ generations leads to a very strong negative covariance between $\overline{w}$ and both $\overline{u}_{MAC}$ and $\overline{u}_{MIC}$. Such patterns of covariance are consistent with my explanations for the mutation rate evolution. In the future, new simulations can be run to measure the covariance between the population mean fitness and mean mutation rate within both MAC and MIC, as

well as the expected mutation rate changes caused by reproduction, under different reproduction strategies. With these measured parameters and also the population mean fitness, the theoretical changes of mutation rate can be calculated using Price equation and compared with the simulation results. I would expect the results obtained from simulation and theoretical calculation match with each other.

The presence of 1% beneficial mutations have a great effect on the fitness dynamics and mutation rate evolution trajectories for amitosis and sex. Consistent with the findings in Chapter 2, the amitotic and sexual (both obligate and facultative) populations can efficiently accumulate the rarely arising beneficial mutations and achieve adaptation, while the mitotic population changed little compared to that in the absence of beneficial mutations and still suffered severe Muller's ratchet. For the mutation rate evolution, both amitotic and facultative sexual populations achieved a higher mutation rate in the MAC and MIC compared to that assuming all mutations are deleterious, as a higher mutation rate can generate more beneficial mutations and may be favored by selection under these circumstances. However, the mutation rate evolution trajectories remained roughly unchanged in the obligate sexual and mitotic populations. For mitotic one, it is due to the less efficiency to accumulate the beneficial mutations thus the presence of 1% beneficial mutation would be "invisible" to selection. For the sexual population, as the correlation between the fitness and modifier loci breaks up every generation, the incorporation of beneficial mutations would have little effect on mutation rate evolution according to Price equation.

By decoupling the division strategy of fitness and modifier loci under amitosis, I found it is the amitotic division of modifier loci that accounts for the reduction of MAC mutation rate. Interestingly, an even greater mutation rate reduction can be reached under one of the decoupled strategies, that only modifier loci were divided by amitosis. This phenomenon results from the enhanced correlation between fitness and mutation rate under this reproduction strategy, thus based on Price equation, selection based on fitness can operate more efficiently on mutation rate evolution. Same explanation also applies to the pattern I found for facultative sex with amitosis and with mitosis, that the facultative sexual population adopting mitotic asexual reproduction strategy achieves an even greater mutation rate reduction than the one adopting

amitotic asexual strategy. Compared to the diploid population, the polyploid population can achieve a more prominent mutation rate reduction under facultative sexual reproduction strategy as a much greater variation can be generated for selection to operate.

4.4.3. Summary and future directions

How *T. thermophila* achieves such a low mutation rate is still a mystery, and it has been suggested that the low mutation rate is a universal genetic feature of ciliates (Long et al., 2016b; Long, Doak, and Lynch, 2018). Here I showed that the previous hypothesis proposed by Long et al. (2016), that the transcription-silent MIC did not get exposed to selection until having sex thus should favor a low mutation rate, is invalid. The mutations accumulated within the MIC do respond to selection via the selection acting on the MAC as they turn to be present in the new created MAC after sexual conjugation. Instead, my study suggested that the unusual reproduction strategy adopted by *T. thermophila* may account for its extremely low mutation rate. As demonstrated in the model, the reproduction strategy adopted by *T. thermophila*, amitosis and facultative sex, can naturally promote the reduction of mutation rate under selection. For future direction, as mentioned before, the covariance between fitness and mutation rate in both MAC and MIC, as well as the expected mutation rate changes due to reproduction, can be collected in the simulation, and thus the expected changes of mutation rate can be calculated using Price equation and compared to that got from simulation. If my explanation is correct, then these two sets of data would match with each other. Also, the model used here can be extended to study the mutation rate evolution within *P. tetraurelia*, who adopted a different facultative sexual strategy that involves autogamy (Sung et al., 2012b). In addition, more mutation accumulation experiments should be conducted on other ciliate lineages to check the generality of the low mutation rate within ciliate, as current knowledge is limited to several species (such as *T. thermophila*, *P. tetraurelia*, and *P. biaurelia*) (Long et al., 2016b; Long et al., 2018; Sung et al., 2012b). Moreover, there is evidence showing that the DNA polymerases within ciliates have different amino acid sequences in the highly conserved catalytic sites compared to other eukaryotes. However, whether these sequence changes would improve the DNA replication fidelity remains unknown (Sung et al., 2012b). Further study should

also focus on elucidating the molecular basis for achieving and maintaining such a low mutation rate within ciliates.

Chapter 5. Conclusion and future directions

5.1. Overall conclusion

Tetrahymena is a model organism in molecular biology and has very unusual genome features, including nuclear dimorphism, amitotic division of the MAC, chromosome copy number control mechanism, and ability to have facultative sex (Orias et al., 2011). Recent studies also revealed that *Tetrahymena* has very special evolutionary characteristics, such as the prevalence of successful asexuality within the lineage and the extremely low mutation rate found in the MIC (Doerder, 2014; Long et al., 2016b). However, the relationship between the unusual genome architectures and evolutionary characteristics largely remain untested or uninvestigated. In this dissertation, I investigated the effects of these unusual genome architectures on the evolution of the ciliate *Tetrahymena*, particularly the achievement of successful asexuality and extremely low mutation rate, using computational modeling.

The unusual amitotic division of the MAC within *Tetrahymena* has been suggested to allow it to avoid some common consequences of asexuality and confer sexual-like benefits in the absence of sex (Doerder, 2014; Zufall, 2016). One consequence resulting from asexuality is the large fitness loss within the population caused by Muller's ratchet (Muller, 1964). In Chapter 2, I investigated whether amitosis of the MAC, together with copy number control, can slow down the operation of Muller's ratchet. I found that within a relatively small population, amitosis can decelerate the operation of Muller's ratchet to an extent comparable to that observed in obligate sexuals. Moreover, even with a very small proportion of beneficial mutations present, the amitotic population can efficiently accumulate them and achieve an adaptation level similar to the sexual ones. Furthermore, the mathematical analyses done by my advisor, Dr. Ricardo Azevedo, revealed that compared to mitosis, amitosis can reduce the mutation load carried in large populations. Therefore, amitosis can confer both a deterministic benefit by reducing mutation load and a sexual-like stochastic benefits by slowing down the operation of Muller's ratchet and accelerating adaptation.

Due to the inability to generate sufficient variation for selection to operate, asexual reproduction generally leads to a slowed adaptation compared to sex, especially when facing a novel or changing environment

(Colegrave, 2002). In Chapter 3, I explored whether amitosis can also facilitate the adaptation process in changing environments. Using a polyploid Fisher's Geometric Model, I compared the adaptation of populations adopting different reproduction strategies in constant and changing environments. I found that within a constant environment, the overdominance of alleles can generate a genetic load in amitotic populations and thereby result in a low population mean fitness compared to the mitotic ones. However, when facing a changing environment, owing to the enhanced variation generated, the amitotic populations do achieve an accelerated rate of adaptation compared to the mitotic ones. Regardless of environmental changes, sex can only speed up adaptation under low MAC ploidies. For high MAC ploidy levels, sexual populations gain only little fitness increase. This is due to the fact that the optimal copy number is much lower than half of the MAC ploidy, which is the copy number reached following sex. My results from this chapter suggest that amitosis can also accelerate adaptation to changing environments.

Besides the prevalence of successful asexuality within *Tetrahymena* lineage, another unusual evolutionary trait is the extremely low mutation rate found within the MIC (Long et al., 2016b). Long et al. (2016) proposed that since mutations in the MIC are not expressed during asexual reproduction, they are only exposed to selection when individuals have sex. Thus natural selection should favor and promote the reduction of mutation rate within the MIC. However, by evaluating the equilibrium fitness just after having sex, I found that the MIC carries a much lower mutation load than the naïve expectation (i.e., assuming mutations keep accumulating in the MIC and are not exposed to selection). Further study revealed that the MIC mutations also respond to selection during asexual generations as they are present in the MAC after sex occurred. Thus the hypothesis proposed by Long et al. (2016) is invalid to explain the evolution of exceptionally low mutation rate in *Tetrahymena*. Instead, by exploring mutation rate evolution in the presence of mutation rate modifiers, I found that the two reproduction strategies adopted by *Tetrahymena*, amitosis and facultative sex, can naturally promote the reduction of mutation rate under selection. Based on my findings in this chapter, I proposed that the extremely low mutation rate within *Tetrahymena* is a joint consequence of its unusual nuclear-dimorphic genome architecture and reproduction strategy.

To sum up, my study highlights the contribution of the unusual genome architecture carried by *Tetrahymena* to its extraordinary evolutionary characteristics. I found that the unusual amitotic division of MAC, together with copy number control, can contribute to the successful asexuality of *Tetrahymena* by slowing down the operation of Muller's ratchet and accelerating adaptation to changing environments. Furthermore, the amitotic and facultative sexual reproduction strategies were found to be able to promote the reduction of mutation rate. This study not only leads to a better understanding on the evolution of this model organism, but also elucidates new mechanisms for eukaryotes to survive asexually and control their mutation rate, which will definitely shed new light on understanding the evolutionary costs and benefits of sex as well as mutation rate evolution.

5.2. Future directions

5.2.1. Investigate the contribution of other genome features on achieving successful asexuality

In this dissertation, I mainly focused on the roles that amitosis with copy number control played on the successful asexuality of *Tetrahymena*. Assuming perfect chromosome copy number control, my results showed that amitosis can generate a much greater genetic variation and enhance the efficiency of response to selection, thereby slowing down the operation of Muller's ratchet and accelerating adaptation to a sexual-comparable extent. However, other unusual genome features besides amitosis, such as copy number control mechanism itself and chromosome fragmentation, are also likely to contribute to the successful asexuality of *Tetrahymena* and have yet to be investigated.

In the models I used throughout the dissertation, I assumed perfect chromosome copy number control during amitosis. Currently the copy number control mechanism is only known in one species of *Tetrahymena*, *T. thermophila*, and how precise it really is needs to be investigated by experiment (Orias et al., 2011). Also, whether other species within *Tetrahymena* lineage have a similar mechanism and how this mechanism works have not been studied. A recent study on another ciliate species *Chilodonella uncinata*

suggested stabilizing selection may act to control the copy number during asexual reproduction, although currently no evidence indicates this species has a copy number control mechanism (Spring, Pham, and Zufall, 2013). Assuming such selection also works in *Tetrahymena*, one possible direction would be to explore the strength of stabilizing selection which is sufficient for maintaining a roughly constant copy number. Furthermore, the effect of this copy number control mechanism can be then investigated by comparing evolution under amitosis with and without such mechanism.

In my models, for simplification, I assumed single locus-sized chromosomes in both the MAC and MIC, which ignored the Hill-Robertson Interference among loci sitting on the same chromosome. Indeed, all ciliates investigated so far are found to have highly fragmented genomes, and some species even contain around 20,000 gene-sized chromosomes with each being present for thousands of copies (Orias et al., 2011; Prescott 1994; Riley and Katz, 2001). *Tetrahymena* maintains a moderate fragmentation level, with several hundreds of loci in a MAC chromosome. This fragmentation process occurs during the generation of new MAC in sexual conjugation, and the evolutionary significance of this process is still unclear (Orias et al., 2011). I hypothesize that a more fragmented chromosome can achieve a higher efficiency to purge deleterious mutations and accumulate the beneficial ones, and this hypothesis can be tested by comparing the evolution trajectories under different sizes of chromosomes. Additionally, having cross-over has been suggested to be very effective to enhance the selection efficiency (Burt, 2000). Thus, cross-over events can also be introduced into the model used in this dissertation to explore how much recombination is needed to overcome the Hill-Robertson interference among loci.

By evaluating the contributions of other genome features besides amitosis, we will have a much better understanding about roles of different genome architectures playing on successful asexuality within *Tetrahymena* lineage.

5.2.2. Explore the benefits of unusual genome architecture on enhancing the resistance to parasite infection

In this dissertation, I mainly investigated whether the unusual amitotic division of MAC can confer sexual-like benefits regarding generating more genetic variation and enhancing the response to selection, i.e., the benefits suggested by Weismann. As mentioned in Chapter 1, there are several other theories explaining the benefits of sex besides the ones based on Weismann's idea. Among them, currently the best experimentally supported theory is the Red Queen Hypothesis (Lively and Morran, 2014). However, whether and how the successful asexual lineages can escape parasite infection has yet to be elucidated (Judson and Normark, 1996).

One of the future directions for this study would thus be exploring whether the unusual genome architecture of *Tetrahymena*, especially amitosis with copy number control, can provide sexual-like benefits regarding enhancing resistance to parasite infection. To study this question, new models can be established to simulate and compare the parasite resistance evolution within *Tetrahymena* populations under different reproduction strategies.

Through exploring whether the unusual amitotic division can confer sexual-like benefits regarding improving parasite resistance, a more robust conclusion can be made for the contributions of unusual genome architectures to the achievement of successful asexuality within *Tetrahymena*.

5.2.3. Compare the efficiency of different mechanisms in facilitating successful asexuality

In my study, I focused on the contribution of unusual genome architectures maintained by *Tetrahymena* to achieve successful asexuality within the lineage. As reviewed in Chapter 1, the persistence of ancient asexual scandals may rely on a variety of genetic mechanisms conferring sexual-like benefits in the absence of sex. For example, gene conversion events were found to be prevalent within both bdelloid rotifers and Darwinuloidea ostracods, which can generate homozygous loci in the absence of sex. This process can either remove the deleterious mutations or make them homozygous and subject to selection directly, hence slowing down the operation of Muller's ratchet and reducing the mutation load (Flot et al., 2014; Schön

and Martens, 2003). Also, the ongoing horizontal gene transfer events within bdelloid rotifers makes it possible to get external genes and contribute to the genome diversification (Flot et al., 2014). Thus, it will be interesting to compare the efficiency of different genetic mechanisms in facilitating successful asexuality within a system like *Tetrahymena*.

This question can be investigated by incorporating gene conversion and horizontal gene transfer processes to the general model used in Chapter 2, and comparing their efficiencies to purge deleterious mutations and accumulate beneficial ones with that of amitosis. The answer to this question will illustrate the capability of several most common genetic mechanisms adopted by successful asexual lineages on avoiding some common consequences of asexuality and conferring sexual-like benefits within a system like *Tetrahymena*.

5.2.4. Investigate how sex can be maintained within an amitotic population

Throughout the dissertation, the performance of amitosis and sexual reproduction has been compared separately, and then it is natural to ask how they will behave if these two reproduction modes coexist and compete within the same population. This question is of great significance as the prevalence of sexual reproduction is one of the most puzzling questions in evolutionary biology due to the severe costs associated with sex (Roze, 2012). However, sex is still the only possible reproduction mode for most eukaryotes, which is confusing and needs an explanation.

As mentioned in the Chapter 1, although normally *Tetrahymena* reproduces by amitotic division, it also maintains the ability to reproduce sexually. Thus one possible future direction is to study how sex can persist within the amitotic *Tetrahymena* population. Given the fact that amitosis can confer sexual-like benefits without suffering the costs associated with sex, it should be more difficult to maintain sex within *Tetrahymena* than other eukaryotes which adopt mitotic strategy. The answer to this question of how sex can be maintained in an amitotic population will serve as an upper bound for the maintenance of sex within asexual populations, and will help us to gain insight into the persistence and maintenance of sex among eukaryotes.

5.2.5. Investigate the effect of facultative sex on mutation rate evolution within a normal single nucleus system

In Chapter 4, I found that facultative sex is distinctive in promoting the reduction of mutation rate compared to other reproduction strategies, as it can lower the mutation rate in both the MIC and MAC. Since mutation rate evolution was investigated within an unusual two-nuclear system like *Tetrahymena*, one future direction would be to evaluate how facultative sex affects the mutation rate evolution within a normal single nucleus system.

This question can be addressed by revising the mutation rate evolving model used in Chapter 4 to only involve a single haploid or diploid nucleus and simulating the mutation rate evolution under different facultative sexual frequencies. Since no haploid and diploid organisms have been found to conduct amitotic division, the facultative sexual reproduction will be modeled as sexual process together with mitotic division. The mutation rate evolution under facultative sex will then be compared with that under mitosis and obligate sex. To the best of my knowledge, all existing studies on mutation rate evolution only focus on the evolution trajectories under either mitosis or obligate sex, and this result will greatly increase our knowledge on mutation rate evolution.

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