GENE MIXABILITY IN AN EVOLVING GENETIC NETWORK

THE EFFECT OF SEX AND MIXABILITY ON THE EVOLUTION OF AN IDEALIZED GENETIC NETWORK

BY

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Abstract

Why sexual reproduction and recombination are prevalent among living organisms is one of the most intriguing questions in biology. It has been studied extensively from a multitude of perspectives ranging from multi-locus population genetics models, in-vivo and more recently in-silico systems. The analysis of complex metabolic networks in living organisms reveals that they can be decomposed into several functionally distinct sub-groups, called modules. This property of modular organization has been accepted as a general organizational feature of biological networks, and has important consequences for the evolution of biologically complex features through different combinations of simpler functions. In this light it has been shown that sexual populations can develop a form of modularity on the genetic level, called mixability, where alleles are selected for their ability to function under a wide variety of genetic contexts, much like a module.

However the functional implications of mixability still remain to be seen. We wish to assess whether mixability can develop in a simplified model of populations undergoing evolution for increased biological complexity through the construction of their genomes into simple metabolic chains. We modelled the fitness and growth of complexity in sexual and asexual populations in the presence of recurrent mutations which increase the ability of genes to interact with one another. Our results show

that mixability is selected for in sexual populations when genetic diversity is high and under certain conditions gives sexual populations a competitive edge over asexual populations through increased genetic complexity. This provides a starting point for examining the effect of mixability upon growing genetic networks and its role in influencing larger scale modularity, which thus far has not been significantly explored.

Acknowledgments

I would like to thank my supervisor Dr.Jonathan Dushoff for his guidance and mentorship during the course of my Masters work. I would also like to thank Dr.Paul Higgs, my co-supervisor, for insightful discussion as this thesis was taking shape. To the members of the Theoretical Biology Lab in LSB 216, I am grateful for all your encouragement. And lastly, a thank you to my parents, my girlfriend, and my best friend.

This thesis is dedicated to my mother and my father.

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Chapter 1

Introduction

The majority of modern eukaryotes reproduce sexually, in that two parental organisms contribute half of their genetic material through the fusion of meotically derived gametes to produce a single offspring. During the process of meiosis the recombination and segregation of homologous chromosomes further ensures that gametes will receive different combinations of genetic material from each parent. Bacteria on the other hand do not undergo meiosis and produce offspring asexually through binary fission, but they also can acquire genetic material from different sources via the mechanisms of conjugation, transduction and transformation [33].

It is difficult to explain why sex has persisted in eukayrotes when it seems that asexual reproduction is more efficient at producing offspring; two parents are required to produce a single offspring sexually, whereas only one parent is necessary for asexual reproduction. Therefore a sexual couple must produce at least twice as many surviving offspring to equal the reproductive capacity of a single asexually reproducing organism. This is famously referred to as the "two-fold cost of sex" [29]. The underlying idea is that females must spend half of their reproductive energy producing males,

which is expected often, but not always to be the case [53], and that males contribute little or nothing to producing and raising offspring. The two-fold cost of sex can be mitigated if males contribute to providing for or protecting offspring. But there might be other costs associated with mating that decrease the fitness of males and females, including finding, choosing and competing for mates [8]. Why should males persist? In some cases multicellular sexual species can become asexual through female self-fertilization (parthenogensis). These "unisexual" species are distributed over a diverse array of eukaryotic phyla, such as fish, molluscs and insects [46], but they are rare and of recent divergence. In mammals the loss of males through parthenogenesis has not been observed, perhaps due to several factors such as maintenance of diploidy and egg activation by sperm [10]. However symmetrical sex through meiosis arose about 850 Mya in the earliest ancestors of the eukaryotes [47, 43], and despite a few exceptions it has been persisted among the majority of unicellular and multicellular eukaryotic species to date. Why sex is so ubiquitous despite its cost, known as the "paradox of sex", is one of the most puzzling questions in biology to date.

The main explanation for the maintenance of sex is that the exchange of genetic information is beneficial in increasing environmental adaptation. Nearly a century of research studying the benefit of sexual recombination has generated many hypotheses, which can be divided into two major categories; the first are mutational-based hypotheses that aim to show that in an unchanging environment recombination can incorporate beneficial mutations and/or decrease the accumulation of deleterious mutations better than asexual populations, and the second are ecological-based hypotheses show that recombination is beneficial in producing better adaption to fluctuating changes in selection caused by biotic or abiotic factors over time[23]. Although in the

past models have typically explored mutational and environmental factors separately, it is becoming more evident that each may work together as evolutionary forces in shaping the evolution of sex [52, 30].

In the following section we will primarily review the general findings of mutational-based models, as their study aims to address general conditions under which sex and recombination are favoured. We will then review some recent findings suggesting that sex may increase the modular organization of genes and genomes that may lead to long-term evolutionary advances. We will explore this possibility further by examining a model where sex can influence the construction of a simplistic genetic network.

1.1 Mutation-based hypotheses of the benefit of sex

1.1.1 Modeling approaches

Mutational-based modeling approaches study the advantage of sex in two ways; by comparing the equilibrium mean fitnesses of sexual and asexual populations that have evolved in isolation [6, 29], or by studying how the introduction of a neutral modifier allele which increases recombination will spread through a population at equilibrium [34]. The first method address the long-term advantage of sex in preventing invasion by asexuals, while the second studies if increased genetic recombination confers an immediate advantage to a population [38].

These studies assess the advantage of sex by utilizing population genetics models, where a population containing a fixed number of individuals undergoes repeated selection and reproduction of offspring for a set number of generations. Individuals

possess a genotype consisting of a set of haploid or diploid loci undergoing selection. Mutational events introduce beneficial or deleterious mutations and produce new alleles at each locus. The reproductive fitness of an individual, which is defined as the relative proportion of offspring that survive to reproduce in the next generation, is determined by the combinations of alleles that make up its genotype. Each mutation introduced changes the ability of an individual to reproduce by a multiplicative amount if they act independently. Otherwise, the effect of a given mutation can be either reinforced or counteracted by the presence of other mutations at other loci, called epistasis. The effect of epistasis is accounted for by introducing an additional term to account for the degree of deviation from multiplicative fitness effects between loci. In asexual populations offspring are direct copies of their parents, with the exception of genetic variation introduced by mutation. In sexual populations individuals mate randomly according to their fitnesses; a pair of individuals is chosen which undergo recombination between their respective loci to produce a single recombinant offspring genotype. Whether populations are of infinite or finite size has important consequences to the final distribution of alleles in the population. Under the former, mutation and fitness are events that occur at fixed rates, while under the latter they are probabilistic events where random fluctuations play an important role.

In models addressing the long-term advantage of sex recombination between loci occurs at a fixed rate, while models studying the immediate advantage of sex separate "recombination-modifier" loci control recombination. In the latter, the effect of alleles which increase recombination can be assessed either when the population is undergoing selection or after it has evolved to equilibrium. In the following sections findings from both kinds of models shall be presented.

1.1.2 Increased accumulation of beneficial alleles

Scientists have explored a variety of hypotheses to explain the apparent paradox of sexual reproduction [23, 38, 52]. Early models of the evolution of sex were inspired by an idea engendered by both Fisher[14] and Muller[15], called the Fisher-Muller hypothesis, which states that recombination is advantageous because it allows useful, but rare, mutations occurring in different individuals to be united into a single lineage, thus accelerating the rate of evolution.

This advantage of recombination comes from the observation that asexual populations can only incorporate beneficial mutations through repeated mutation of the same genotype through successive generations. But in sexual populations the incorporation of mutations can occur much faster through recombination [32, 6, 29]. The advantage of sex in this context depends on how long it takes for a new mutation to appear in a population (T_e) and the number of generations it takes to become fixed through selection (T_s) [29]. If $T_s >> T_e$, a new beneficial mutation may arise before another one has finished spreading through the population, thus we can expect beneficial mutations to arise in different lineages and recombination will be advantageous. If $T_e >> T_s$ each mutation will spread throughout the population before the next one arises, thus mutations to become successively incorporated in asexual populations and there will be no advantage to recombination. Therefore we expect conditions that favour recombination to be those that increase T_s, such as increased population size and low selective advantage of mutations, and those that decrease T_e, such as decreased mutation rates [6, 7].

Generally, recombination can only be beneficial if mutations act independently and breaking up existing combinations of mutations does not have a negative impact on

population fitness as a whole. When mutations act independently they have the same effect in isolation as they would in combination. But if this is not the case, certain combinations of mutations could be better adapted than others, and breaking them up through recombination would be detrimental to the fitness of an sexual population in the long term [5, 12]. Thus by preserving beneficial combinations of mutations, asexual populations are able to attain a greater fitness than sexual populations [6, 34, 1]. Given that complex biological processes involved in cell regulation and metabolism are based on sets of co-evolved genes [41], the effect of interaction among mutations is an important factor to consider in the evolution of sex.

Sex can be beneficial if it restores beneficial allelic combinations that are lost randomly in small populations through genetic drift [13, 23]. In small populations reproductive processes are probabilistic events that introduce random fluctuations in allele proportions over time. The effect of drift diminishes for larger population sizes as selection dominates in maintaining beneficial alleles, decreasing the advantage of recombination [36]. However, genetic drift-like effects can occur in populations of large size when the number of evolving loci is large. In this situation it is unlikely that the most fit allelic combination will be represented in the population, thus a scenario results where recombination among genotypes is able to create them [18].

1.1.3 Decreased accumulation of deleterious mutations

Recombination can provide another advantage to sexual populations by producing offspring with fewer deleterious mutations than asexual populations, improving population fitness overall [23]. The overall benefit of sex in the presence of deleterious

mutation depends on population size as well as interactions between deleterious mutations at different loci. In populations of infinite size sex is particularly beneficial when deleterious mutations are worse in combination than if their effects are independent. This results in a scenario where individuals with many deleterious mutations are strongly selected against, leaving behind genotypes with intermediate levels of mutation from which recombination can restore a greater frequency of unmutated individuals [11, 4, 37].

Although these findings are plausible from a theoretical standpoint, they are unlikely to apply in real populations where deleterious alleles are not always worse in combination [9]. However, the presence of genetic interactions becomes less dominant at smaller population sizes where the evolution of alleles are subject to genetic drift. In this case asexual populations of small size face a major problem in maintaining mutation-free genomes because they can be randomly lost. Over time this results in the steady decline of asexual population fitness through the irreversible accumulation of deleterious mutations, known as Muller's Ratchet [32, 13]. Sexual populations can escape Muller's Ratchet through recombination between individuals possessing unmutated alleles, restoring genotypes of increased fitness. Muller's Ratchet can also effectively prevent the spatial spread of an asexual invader through sexual populations of large size, allowing them to persist over time [45]. Additionally, the presence of Muller's Ratchet has been verified in different biological systems where population sizes are small and when recombination is absent, such as recently diverged asexual lineages of Potamopyrgus antipodarum freshwater snails [35], the non-recombining neo-Y chromosome of Drosophila miranda [3], and human mitochondrial DNA [27].

A major limitation to the Muller's Ratchet hypothesis is that is can only explain

the benefit and maintenance of sex in the presence of genetic drift, ie., when populations are small. But if mutation rates are large (1 per genome per generation), mutations act in combination to reinforce negative effects, and only individuals possessing few mutations are viable, then Muller's Ratchet could also occur at larger finite population sizes [22]. However the lack of experimental evidence for large genomic mutation rates [20, 21] and negative interactions between deleterious effects [9] precludes Muller's Ratchet as a general mechanism of maintaining sex in real populations.

Without the presence of interactions between mutations or large mutation rates sex can still be beneficial in populations of larger finite size. This is because the individual effects of mutated and unmutated loci interfere with each others selection, causing random fluctuations in individual viability equivalent to the effect of genetic drift. Because loci remain associated with one another in asexual populations, selection against mutated loci becomes inefficient and the existence of unmutated genotypes becomes rarer over time. Recombination is then an advantage because it can restore unmutated genotypes that would rarely exist through selection alone [19].

1.2 Modularity and Mixability

Modularity is considered to be an organizing principle of biological systems, producing the wide range of functional and structural diversity among the taxa of life [17, 42]. A module is a collection of interacting cellular components that perform a certain task distinguishable from other such collections; the components can be RNA structural motifs, proteins, or DNA [51]. These components create an extensive network of molecular interactions, which can be represented in graph structure by a set of nodes

(molecules) with edges (interactions) connecting them. Studies of molecular networks in this way reveal that cellular organization can be broken down into small clusters of densely connected nodes, representing modules, which are sparsely connected with each other [17]. Studies of small-scale eukaryotic and bacterial metabolic and protein-interaction networks reveals that molecules are organized into modules related to their specific function in the cell and are highly conserved across taxa [49, 44, 2].

An important consequence of modularity is that it can allow biological complexity to develop by creating novel and complex functions from sets of simpler ones. The functional independence of modules from their genetic surroundings makes them particularly amenable to genetic exchange, which organisms can utilize to develop novel metabolisms and inhabit new environments [25, 42, 39, 24]. In experiments using the digital life platform Avida, it was found that sexually reproducing organisms are able to develop modular genetic architectures, while asexually reproducing organisms are not [31]. This is because organisms in the Avida system, called "Avidans", contain a genome comprised of computer instructions, analogous to genes, which allow them to extract energy from the environment. The more modular a genome, the more likely a set of genes involved in a given function will be found in proximity to one another. Increasing the physical "linkage" of coadapted genes makes it more likely that they will be inherited as a whole and remain functional in future offspring. Although the work of Avida provides useful insights into the processes of evolution and a illustration of the benefits of modularity, its general applicability to biological systems still remains contested.

Recently, it has been shown that sexual populations can develop modularity on the level of individual genes, called mixability [26]. The authors of this work defined

mixablity as the ability for alleles to do well over many different genetic contexts. To study this the authors employed a multi-locus model of allele frequencies evolving under selection alone. Asexual and sexual populations begin with a random set of alleles assigned to each individual. The fitness of conferred by each allele depends on the combination of alleles at other loci, possessing varying degrees of epistasis. While asexual populations select for highly coadapted combinations of alleles, sexual populations select for alleles which do better on average in many different genetic combinations. These results show that mixability does not necessarily have an immediate fitness advantage, so why might it be beneficial for sexual populations to be mixable? The authors suggest that the ultimate benefit of gene mixability may arise through the future evolution of complex gene networks, but the limitations of their model prevent them from providing a further analysis. To our knowledge this is the first hypothesis examining the benefit of sexual recombination by increasing genetic complexity through the concept of genetic modularity, and we wish to explore it further in a simple model of an evolving network of genetic interactions.

We wish to assess whether mixability can develop in a simplified model of populations undergoing evolution for increased biological complexity by connecting genes into simple metabolic chains. In this model, called the "chain model", genes accumulate "characters" through mutation, allowing them to interact with neighbouring loci. Biological complexity is represented by the maximum number of genes that are connected through character interactions, called a "character chain". In sexual populations genes are recombined into new genetic contexts, which may be potentially disruptive to the construction of character chains. To circumvent this problem we expect sexual populations to select for genes with mixability, i.e. those that carry

many characters.

Our results reveal three different behaviours in the chain-model depending on the probability of mutational events and the functional benefit and cost of carrying characters. The first behaviour results when mutation is frequent and the benefit and cost of carrying characters are nearly equal, creating a Muller's Ratchet scenario where sexual populations accumulate fewer excess characters than asexual populations, resulting in increased population fitness. The second behaviour results when sexual populations are able to build character-chains faster than asexual populations, which occurs when characters are rare and beneficial and the cost of carrying characters is low, called Sexual Efficiency behaviour. The third behaviour occurs when asexual populations are more efficient at constructing character chains and attain a greater fitness than sexual populations, which results when mutations are frequent and the cost of carrying characters is reduced, called Asexual Efficiency behaviour.

We performed mixability assays of sexual and asexual populations by measuring the population fitness of alleles that have evolved at different times under the three behaviours described above. The population fitness of an allele is the relative change in average population fitness when every individual possesses it, which measures how well a given allele functions in the population as a whole. We found that mixability is increased in sexual populations and correlates with the number of characters an allele possesses. This occurs regardless if they develop greater chain length than asexual populations or not, but requires that the cost of carrying characters is low. That mixability can be beneficial by increasing the biological complexity of sexual populations is a novel finding, which was not considered by previous work [26]. Allele mixability is also selected during evolutionary transient events that occur early during

our model simulations when population genetic diversity peaks. Thus mixability may serve to aid the discovering novel evolutionary invention through recombination.

Chapter 2

Model

Individual organisms are composed of genetic information in the form of DNA that encodes for a variety of traits, such as eye colour in humans, or the ability to use sulfur as an energy source in bacteria. These traits correspond to segments of the genetic material, called genes, which are transmitted from parental organisms to their progeny from one generation to another. A population of individuals can possess different versions of genes, called alleles. Some of these alleles are more beneficial for an organism to possess, because they encode for traits which increase survival in a given environment, thus they are more likely to be passed on to future generations. This process of environmental adaptation of organisms over time is known as natural selection.

Population genetics studies the evolution of genetic diversity of real populations over time through the use of idealized models. A typical population genetic model begins with a population of a discrete fixed number of individuals, each possessing a collection of characteristics, called "alleles", which constitute a "genome". Individuals are randomly chosen to reproduce in proportion to their "fitness", which is a

function of their genome, and possibly of other factors (others' genomes, an external environment). During reproduction individuals pass on their characteristics to offspring with some probability of each gene being modified through mutation. In asexual populations an offspring receives all of its genes from a single parent, while in sexual populations an offspring receives each gene with equal probability from two chosen parents. After reproduction is complete, a randomly chosen individual is killed and subsequently replaced by the newly generated offspring.

Population-genetics models in finite populations are complex, stochastic models, which are difficult to solve mathematically. Even approximations are difficult, since alleles are frequently lost randomly, and it is difficult to predict what new alleles will arise. Fortunately computational simulation has made it easier to explore the behaviours of stochastic population genetics models, such as the one we propose below.

Our goal is to examine under which conditions gene mixability develops in asexual and sexual populations of finite size and how it is influenced by an evolving set of genetic interactions. To do this we employ a cartoon depiction of an interacting gene network, which we call the "chain model", which represents a simplistic model of a biological network. Despite this shortcoming, its simplicity allows us to avoid the complications inherent in highly mechanistic models [31] and draw general conclusions about how mixability can influence the evolution of genetic networks that are lacking in the results of previous work [26].

We model populations of N individuals, each possessing a circular genome, g, of ℓ loci. Each gene is denoted by g_i, where $i = 1, 2, \ldots, \ell$, and possesses a set of "characters", $\{c_k^i\}$, where k goes from 1 to C_i, the number of characters at locus i.

Characters have a cost to the organism, but allow genes to interact with genes at neighbouring loci, to create a "chain" of interactions.

Mutations occur with probability μ per gene in each replication. These probability that a mutation is a gain, rather than a loss, of character, decreases with the number of characters present at a locus, and is given by: $1 - \frac{C_i}{2\varepsilon}$. Here, ε is the equilibrium number of characters reached by the mutation process in the absence of selection (when $C_i = \varepsilon$, the probability of loss and gain are both 1/2). New characters are assigned a random value in the range 0-1. If character loss occurs, a randomly chosen character from $\{c_k^i\}$ is removed.

We make a simple, mechanistic model of fitness by assuming that organism function is based on chains of characters, and that these characters have costs. "Chains" of characters are defined as sequences of increasing character values at consecutive loci in a single direction along the circular genome. Chains can begin at any locus position. The longest such character chain in a genome is called its chain length, m, and can begin at any locus position. We also set the maximum chain length attainable as a multiple of genome size, n, where m stops elongating once it has reached a size of $n\ell$.

Individual fitness(w) is given by: $w = 1 + fm - \beta C_{\text{total}}$, where f is the functional benefit of elongating character chains, β is the cost of carrying a character and C_{total} is the total number of characters an individual possesses, calculated by $C_{\text{total}} = \sum_{i=1}^{l} C_i$. m is the maximum chain length as described above. From the equation for individual fitness, w, we can see that excess characters that are not involved in genetic interactions are costly for an organism to possess, however they can have potential benefits, such as increasing gene mixability. In sexual populations

recombination places genes into different genetic contexts and their functionality depends on the assortment of characters they possess. Therefore we expect sexual populations to develop alleles with increased mixability, aided by the development of excess characters.

In the following section we present results from simulating the model described above for asexual and sexual populations for a set number of generations under the same sets of parameters(μ, f, ε and N) for many runs. Individuals in both sexual and asexual populations are chosen to reproduce in proportion to their fitness, passing on their genes to a single offspring with the probability μ that each loci is mutated through the gain or loss of a character. In asexual populations the genotype of the offspring comes from a single chosen parent, while in the sexual population two parents are chosen and the offspring has an equal probability of inheriting a gene from either.

In both versions of the model the resulting offspring replaces a randomly chosen individual in the population. This procedure continues until N replications have occurred and constitutes a single generation. For each generation the average chain length, total characters and fitnesses of asexual and sexual populations are calculated and compared. Mixability behaviour results when sexual populations develop greater chain-lengths and fitness than asexual populations under the same set of parameters. For these simulations we assess the mixability of each allele in sexual and asexual populations by calculating the relative change in population fitness if every individual were to possess that allele. This demonstrates the overall ability of an allele to function in different genetic contexts, and the most mixable alleles are those which produce the highest relative fitness values.

Chapter 3

Results

In this section, we explore the chain model under different combinations of parameters. The goal is to investigate what kinds of qualitative behaviours the model shows, particularly behaviours which given an advantage to either sexual or asexual populations, relative to each other. We have identified three different qualitative behaviours: Asexual Efficiency, Muller's Ratchet, and Sexual Efficiency. Asexual Efficiency behaviour results when asexual populations develop a greater fitness due to their ability find and preserve beneficial gene combinations more efficiently than sexual populations. Muller's Ratchet behaviour results when sexual populations develop a greater relative fitness because asexual populations accumulate deleterious excess characters which sexual populations are able to purge through recombination. Sexual Efficiency behaviour results when sexual populations to create beneficial allele combinations faster than asexual populations. The occurrence of a given behaviour depends on the interaction between the total number of new alleles arising per generation as a result of μ, and the spread of newly arising alleles in successive generations as determined by the selection parameters f and β.

The approximate number of new alleles arising per generation is given by $\mu N\ell$, where μ is the probability that a mutation event occurs at a given locus, N is the population size and ℓ is the number of loci per individual. This value declines as alleles approach ε characters on average and character losses become more frequent. Thus when ε is large we expect the majority of mutational events to result in producing new alleles. Once a new allele appears, its spread through the population depends on the fitness of the individual possessing it, given by $W = 1 + fm - \beta C_{total}$. This can be rewritten as $W = 1 + f(m - \frac{\beta}{f} C_{total})$. It is assumed that the benefit of increasing a character chain outweighs the cost of carrying a character, thus $\frac{\beta}{f} < 1$. When the fitness benefit of interacting characters, f, is increased, beneficial alleles that increase chain length will spread faster through the population.

3.1 Model Behaviours

The following parameter explorations will begin from a set of baseline parameters; character mutations are rare, $\mu = \frac{1}{Nl}$ such that 1 mutation occurs per generation, $\varepsilon = 5$, gene interactions have a small effect such that $f = \frac{1}{N} = 0.001$ and $\beta = 0.0001$. Genome size is set to $\ell = 10$, $n = 2$ so the maximum chain length attainable is $\ell n = 20$, and the population size is $N = 1000$.

When the fitness benefit of chains is relatively small, then character and fitness levels are subject to a large amount of random variation (Figure 4.1). Increasing μ allows character chains to be built faster, and the number of excess characters quickly accumulate. The fitness and character levels still fluctuate in both populations due to inefficiency of selection and random loss due to genetic drift (Figure 4.2). Larger values of f increase the strength of selection for beneficial alleles, increasing the length of

character chains and reducing fluctuations in character and fitness levels (Figure 4.3). The number of excess characters is still great due to the low cost of carrying characters, β. Overall, sexual and asexual populations under these parameters are not distinguishable in character or fitness values.

Increasing the cost of carrying characters, β, and decreasing proportion of back-mutation by increasing the average number of characters genes can accumulate, $\varepsilon = 10$, results in Muller's Ratchet behaviour (Figure 4.4). In this scenario, asexual populations accumulate costly excess characters that cause average population fitness to decrease. Sexual populations on the other hand are able to maintain greater population fitness by purging excess characters through recombination between genomes with fewer accumulated characters. Increasing β also affects the rate at which character chains are constructed in both asexual and sexual populations (compare with Figure 4.3). A possible explanation might be that alleles that increase chain length also possess many costly excess characters, which prevents them from spreading through populations. By increasing the proportion of back-mutations by decreasing the number of characters genes can accumulate, $\varepsilon = 5$, the number of harmful excess characters becomes be reduced in both sexual and asexual populations (Figure 4.5). Although this reduces the separation between the character and fitness levels of sexual and asexual populations, but does not entirely eliminate Muller's Ratchet behaviour.

When f is increased Asexual Efficiency behaviour results (Figure 4.6). Increasing f allows for stronger selection for increasing chain-length, allowing sexual and asexual populations to counteract the accumulation of costly excess characters. Beneficial

alleles spread faster through recurrent mutation in asexual populations than in sexual populations, which is subject to losing beneficial allele combinations by chance through recombination.

When ε is increased and μ is decreased, a new outcome emerges where sexual populations construct longer charager chains than asexual populations (Figure 4.7). This results in Sexual Efficiency behaviour where sexual populations develop more beneficial characters and higher fitness than asexual populations. Making mutations less costly and increasing f further improves the separation of chain length between sexual and asexual populations (Figure 4.8).

Asexual efficiency behaviour can also be seen at larger population sizes, N=5000 (Figure 4.9). Interestingly, this behaviour changes after 1000 generations as character-chains are built at an increased rate in sexual populations, eliminating the increased fitness of asexual populations. Asexual populations have an initial advantage because of rapid clonal spread of beneficial allele combinations. However, once excess character levels increase substantially in sexual populations recombination is able to quickly catch up.

When f is increased and μ and β are decreased at the larger population size, we again see Sexual Efficiency behaviour (Figure 4.8). Again, however, this is a transient effect: if left to run for 5000 generations, then the fitness and character values of sexual and asexual populations converge (results not shown).

3.2 Mixability assays

Allele mixability assays are performed to investigate the functional ability of alleles that have evolved in sexual and asexual populations when recombined in new contexts. Under Sexual Efficiency behaviour (Figure 4.10), at 250 generations sexual populations develop alleles with greater mixability than asexual populations (Figure 4.11). These mixable alleles yield relative fitness values of 1 or greater after being recombined into a new genetic contexts, which shows a positive correlation with the number of characters possessed. Over time the proportion of alleles with high mixability increases in sexual populations, which is accompanied by a significant increase in sexual population chain length seen around 500 generations (Figure 4.12). At this point the relative fitness of mixable alleles declines to approximately 1 and growth rate of character chains in sexual populations also decreases.

The decline of gene mixability in sexual populations occurs due to the decrease of population genetic diversity over time. The variance-to-mean ratio of the total number of characters possessed by individuals (population VMR) reveals that genetic diversity peaks at 250 generations in sexual populations when mixability is greatest and declines thereafter (Figure 4.13). Thus as new alleles are introduced to the population recombination is able to select for those with increased mixability, which eventually assemble into favourable combinations that increase chain length and cause a rapid increase in population diversity as they spread. Over time these alleles become prevalent in the population and genetic diversity declines, at which point their role is preventing the disruption of character chains and maintaining population fitness. Asexual populations undergo greater variation in genetic diversity over time as beneficial allele combinations arise randomly through mutation and rapidly fix.

Evidently this method of increasing chain length is also effective but occurs over a longer period of time.

Pop VMR plots of other model behaviours also follow the same shape, with the only difference being the magnitude of the peak height and width, and when it occurs (results not shown). Performing allele mixability assays for other behavioural regimes when pop VMR is at its peak reveals that allele mixability is also increased in sexual populations evolving under asexual efficiency parameters (Figure 4.14, but not those of muller's ratchet where the cost of excess characters is increased (Figure 4.15)

Chapter 4

Figures

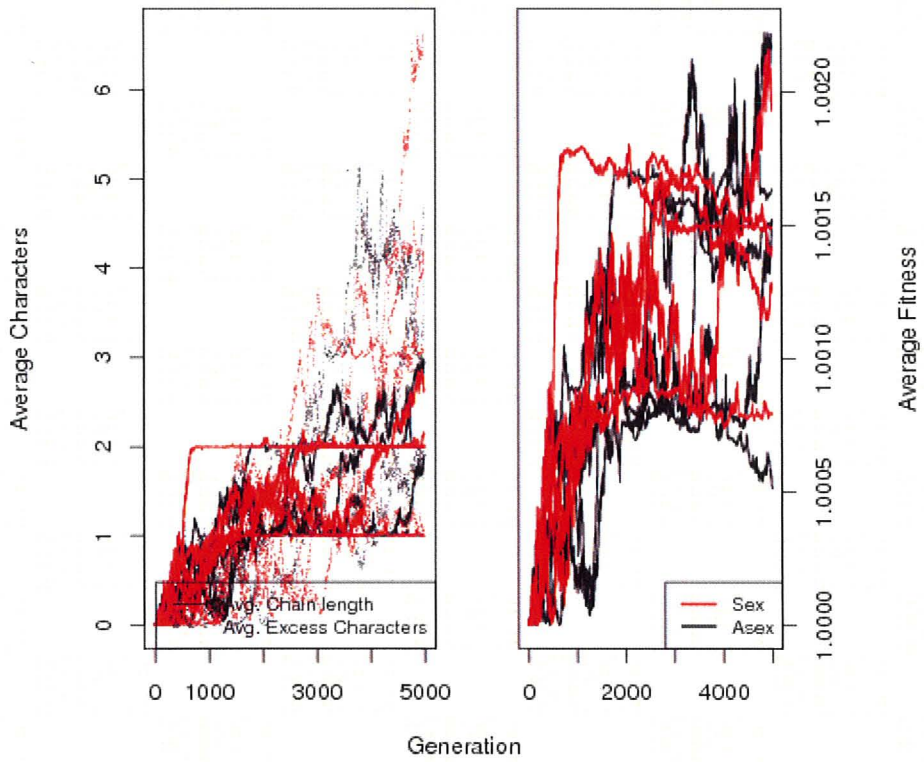

Figure 4.1: Plot of average population fitness and character values per generation when mutational events, μ, are rare and the benefit of functional characters, f is low. Asexual and sexual populations are represented by black and red lines, respectively. The left panel shows the average number of functional characters incorporated into character chains (solid lines) and the average number of excess characters not incorporated into character chains (dashed lines). The right panel shows average population fitness (solid lines). Results for five sexual and asexual simulations are shown; each line represents a single simulation generated using the baseline parameters: $\beta = 0.0001$ $\varepsilon = 5$ $\mu = 0.0001$ $f = 0.001$ $N = 1000$ $n = 2$.

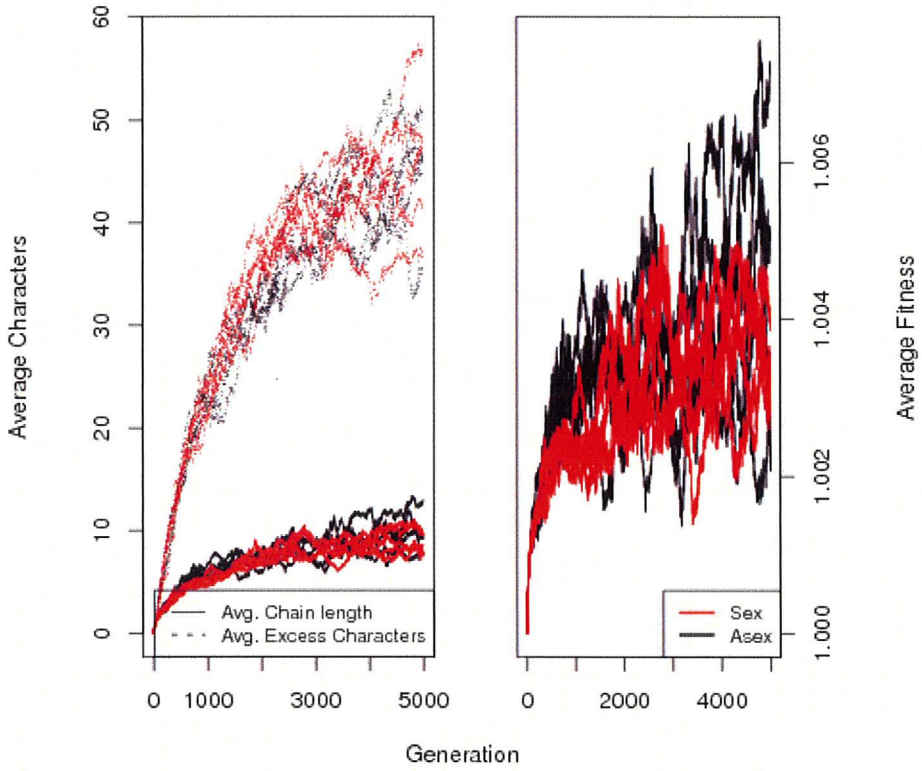

Figure 4.2: Plot of average population fitness and character values per generation when mutational events, μ, are more frequent and benefit of functional characters, f is low. Asexual and sexual populations are represented by black and red lines, respectively. The left panel shows the average number of functional characters incorporated into character chains (solid lines) and the average number of excess characters not incorporated into character chains (dashed lines). The right panel shows average population fitness (solid lines). Results for five sexual and asexual simulations are shown; each line represents a single simulation generated using the baseline parameters (see Figure 4.1) with the frequency of mutational events, μ increased to 0.005.

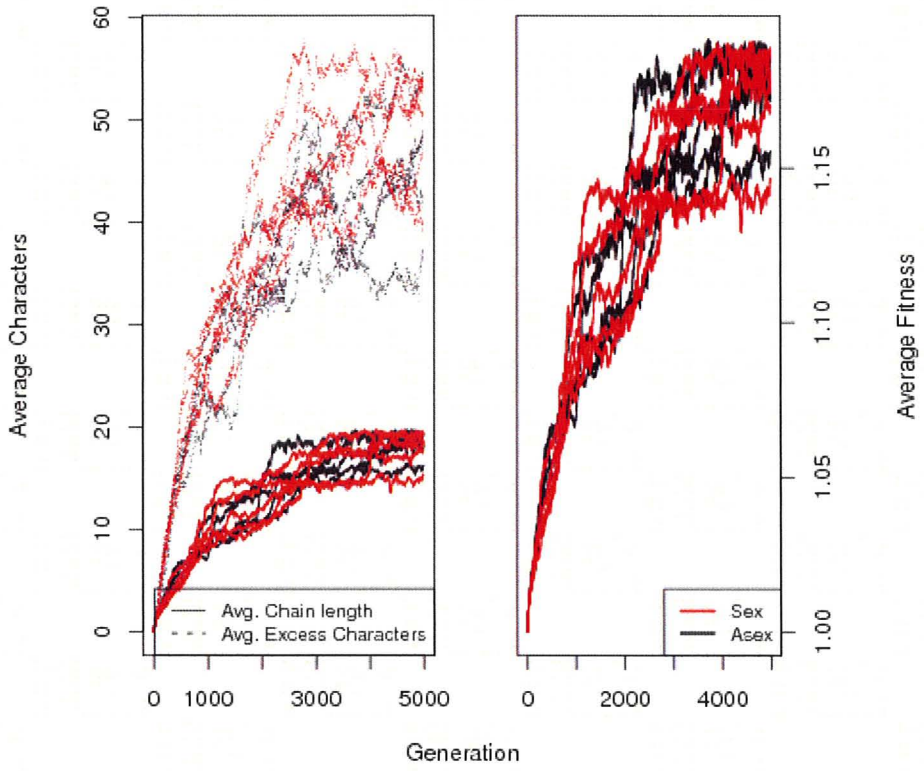

Figure 4.3: Plot of average population fitness and character values per generation when mutational events, μ are more frequent and the benefit of functional characters, f is increased. Asexual and sexual populations are represented by black and red lines, respectively. The left panel shows the average number of functional characters incorporated into character chains (solid lines) and the average number of excess characters not incorporated into character chains (dashed lines). The right panel shows average population fitness (solid lines). Results for five sexual and asexual simulations are shown; each line represents a single simulation generated using the baseline parameters (see Figure 4.1) with $\mu = 0.005$ and $f = 0.01$

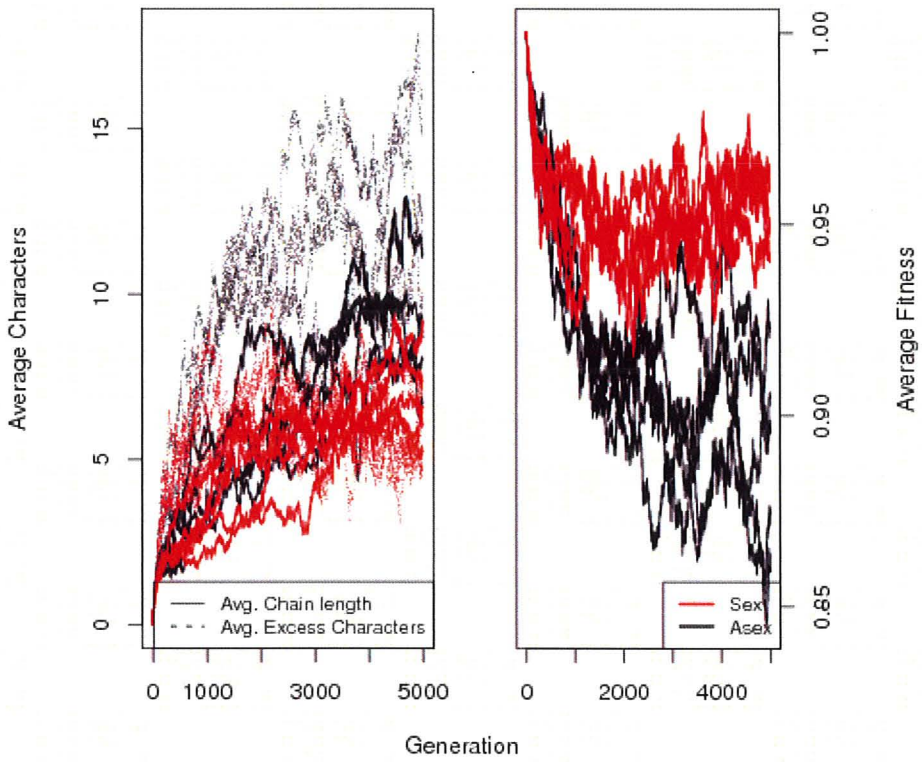

Figure 4.4: Plot demonstrating Muller's Rachet behaviour using baseline parameters when mutational events, μ are frequent, the functional benefit of characters, f and cost of carrying characters, β is increased, and the average number of characters a gene can possess, ε is increased. Asexual and sexual populations are represented by black and red lines, respectively. The left panel shows the average number of functional characters incorporated into character chains (solid lines) and the average number of excess characters not incorporated into character chains (dashed lines). The right panel shows average population fitness (solid lines). Results for five sexual and asexual simulations are shown; each line represents a single simulation generated using the baseline parameters (see Figure 4.1) with $\mu = 0.005$, $f = 0.01$, $\beta = 0.009$ and $\varepsilon = 10$. The average fitness of asexual (black lines) populations is decreased relative to sexual (red lines) fitness due to increased levels of excess characters.

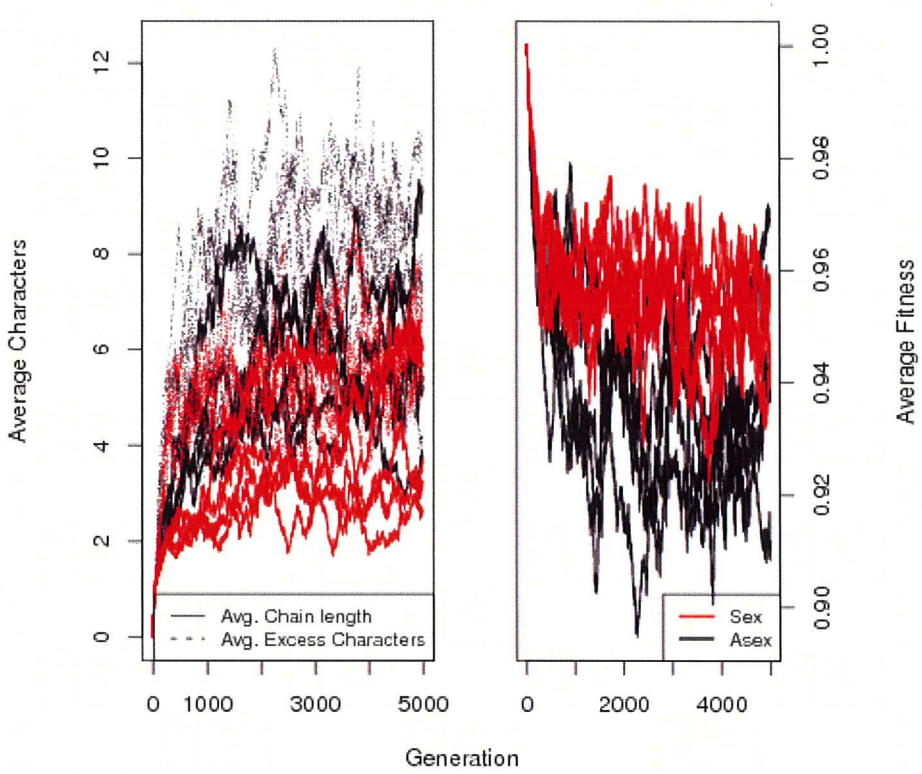

Figure 4.5: Plot of Muller's Ratchet parameters when the average number of characters a gene can possess, ε, is decreased. Asexual and sexual populations are represented by black and red lines, respectively. The left panel shows the average number of functional characters incorporated into character chains (solid lines) and the average number of excess characters not incorporated into character chains (dashed lines). The right panel shows average population fitness (solid lines). Results for five sexual and asexual simulations are shown; each line represents a single simulation using Muller's Ratchet parameters (see Figure 4.4) with $\varepsilon = 5$.

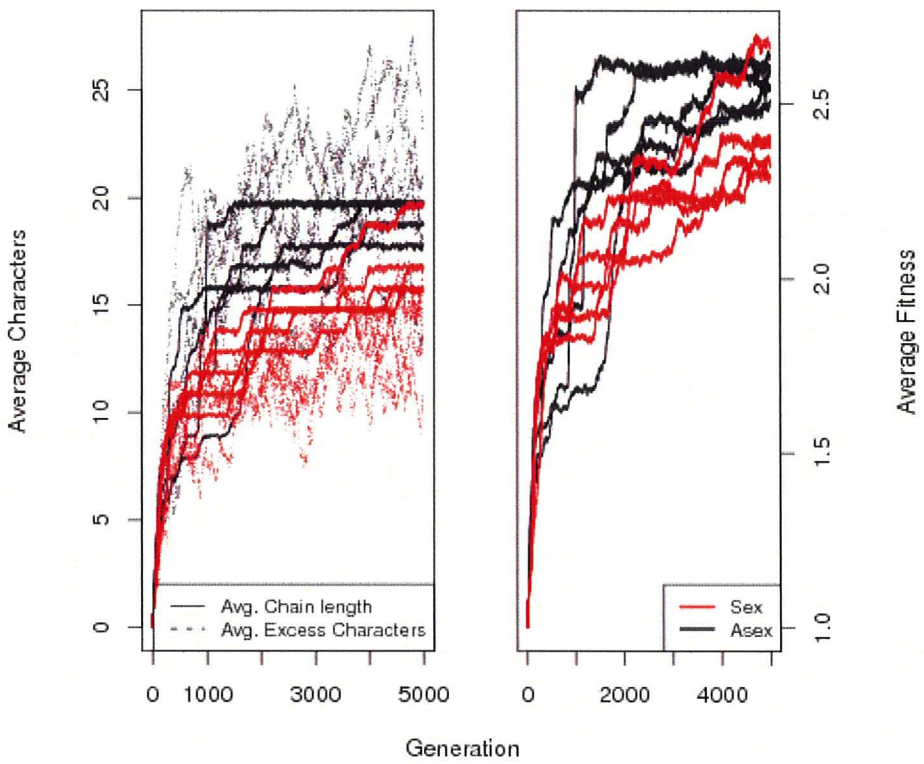

Figure 4.6: Plot showing transition to asexual efficiency behaviour from Muller's Ratchet behaviour when the functional benefit of characters, f, is increased. Asexual and sexual populations are represented by black and red lines, respectively. The left panel shows the average number of functional characters incorporated into character chains (solid lines) and the average number of excess characters not incorporated into character chains (dashed lines). The right panel shows average population fitness (solid lines). Results for five sexual and asexual simulations are shown; each line represents a single simulation using parameters from Muller's Ratchet parameters (see Figure 4.5) with $f = 0.1$. Asexual populations (black lines) develop greater average fitness by building longer character chains than sexual populations (red lines).

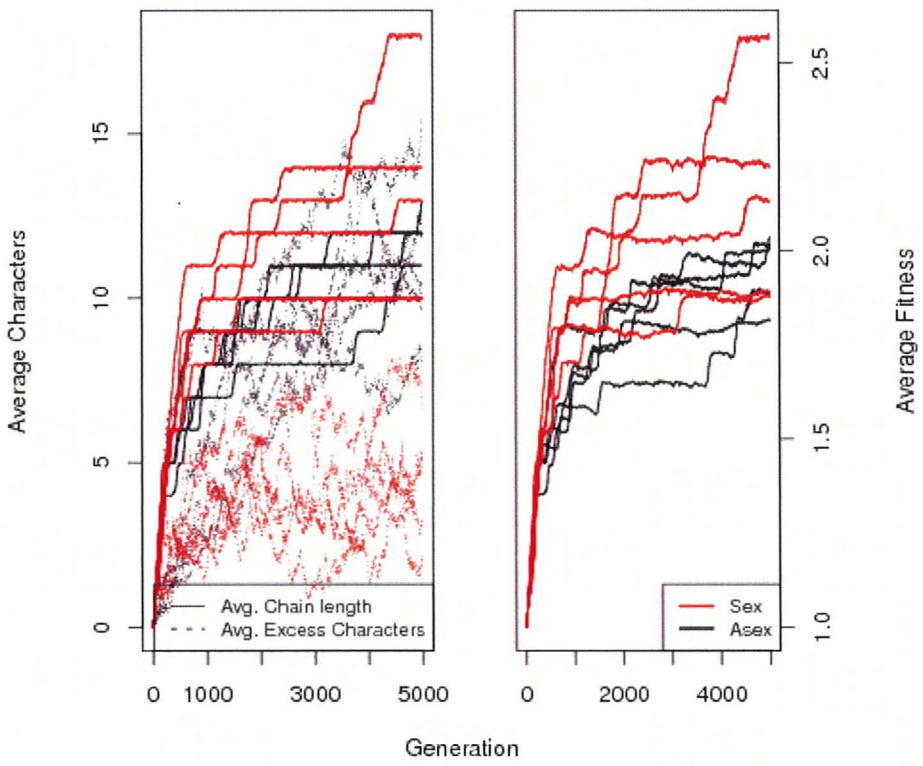

Figure 4.7: Plot showing the transition to Sexual Efficiency behaviour from Asexual Efficiency behaviour when mutational events, μ, are less frequent. Asexual and sexual populations are represented by black and red lines, respectively. The left panel shows the average number of functional characters incorporated into character chains (solid lines) and the average number of excess characters not incorporated into character chains (dashed lines). The right panel shows average population fitness (solid lines). Results for five sexual and asexual simulations are shown; each line represents a single simulation using Asexual Efficiency parameters (see Figure 4.6) with $\mu = 0.0005$. Sexual populations (red) accumulate longer chain lengths than asexual populations.

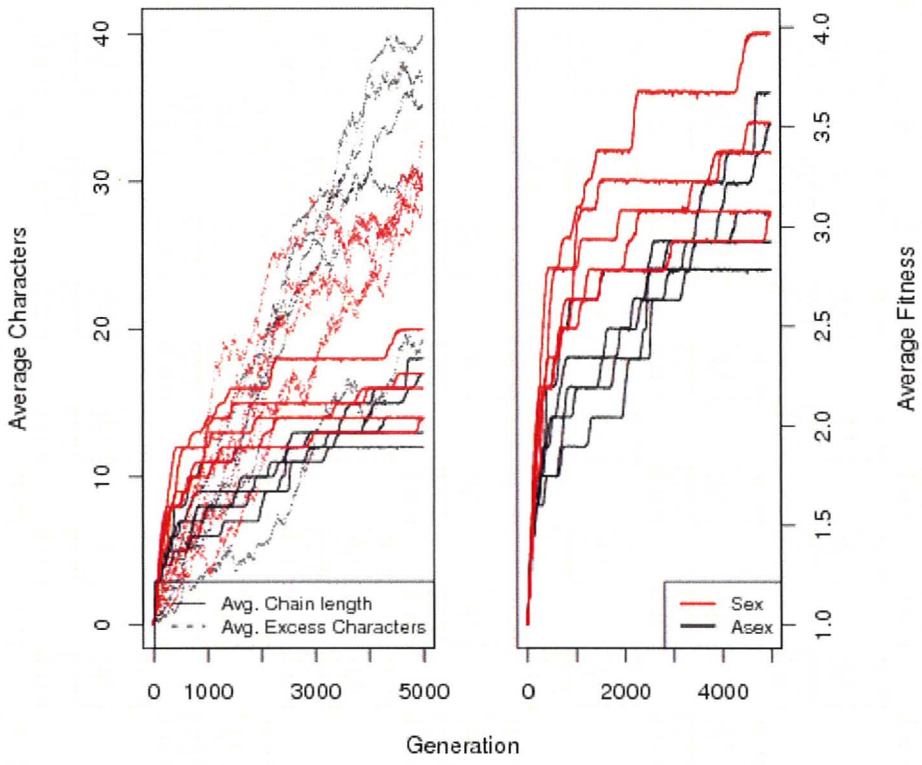

Figure 4.8: Plot showing Sexual Efficiency behaviour when the functional benefit of characters, f, is increased. Asexual and sexual populations are represented by black and red lines, respectively. The left panel shows the average number of functional characters incorporated into character chains (solid lines) and the average number of excess characters not incorporated into character chains (dashed lines). The right panel shows average population fitness (solid lines). Results for five sexual and asexual simulations are shown; each line represents a single simulation using Sexual Efficiency parameters (see Figure 4.7) with $f = 0.15$. Better separation between sexual and asexual character chain and population average fitness values results.

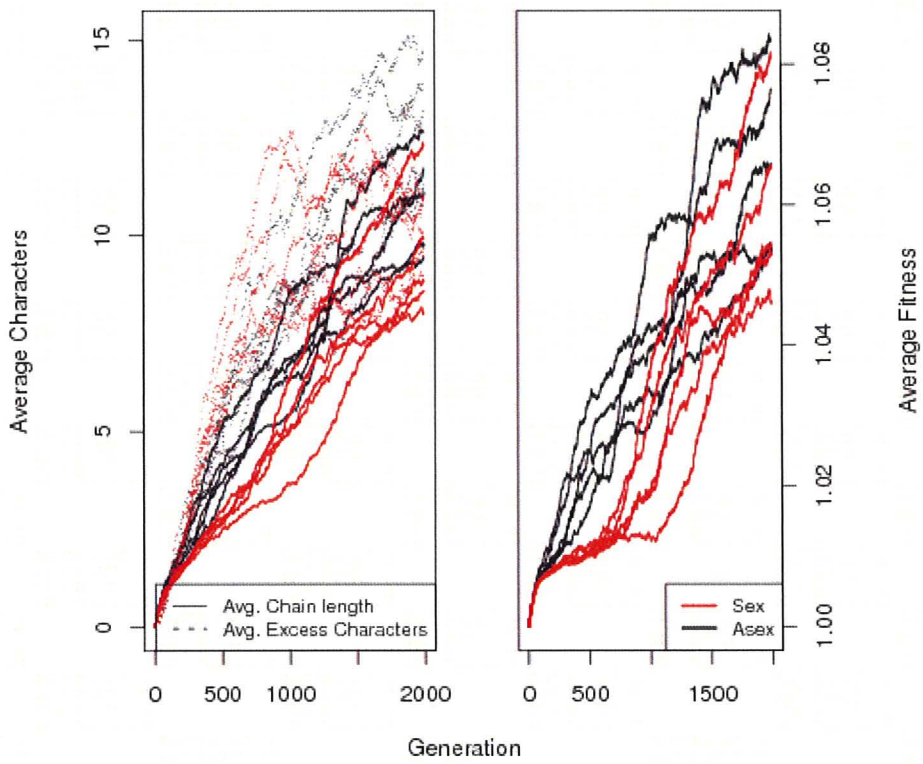

Figure 4.9: Plot showing asexual efficiency behaviour at increased population size. Asexual and sexual populations are represented by black and red lines, respectively. The left panel shows the average number of functional characters incorporated into character chains (solid lines) and the average number of excess characters not incorporated into character chains (dashed lines). The right panel shows average population fitness (solid lines). Results for five sexual and asexual simulations are shown; each line represents a single simulation using the following parameters: $N = 5000$ $\beta = 0.0018$ $\varepsilon = 5$ $\mu = 0.002$ $f = 0.01$ $n = 2$. Asexual Efficiency behaviour appears during the first 1000 generations of the simulations and disappears after sexual populations rapidly increase average chain length and fitness values.

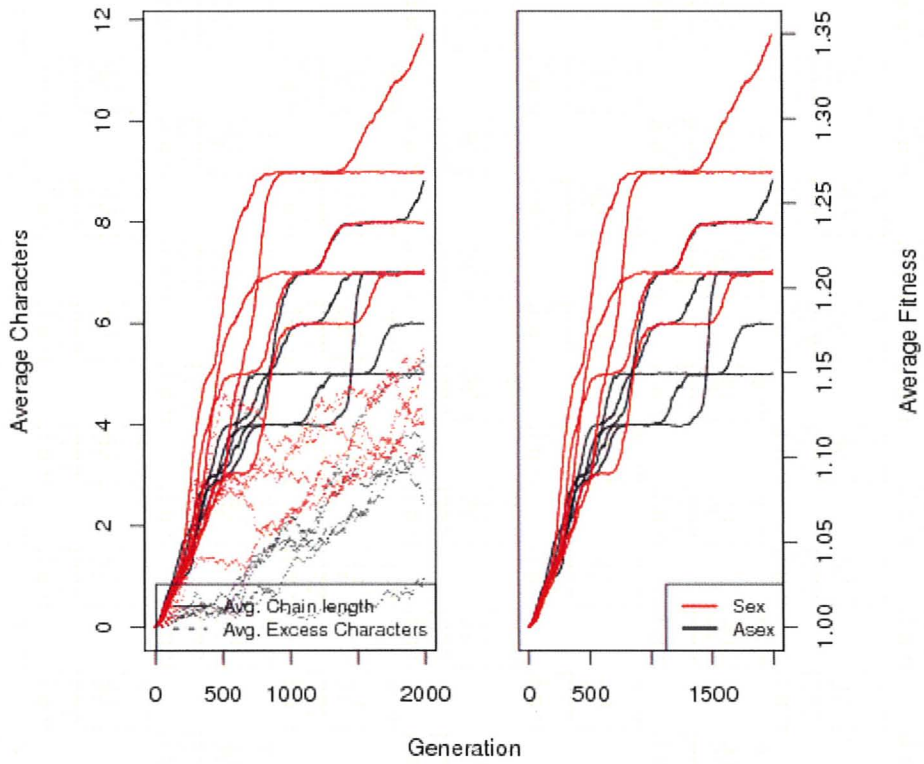

Figure 4.10: Plot showing Sexual Efficiency behaviour at increased population size. Asexual and sexual populations are represented by black and red lines, respectively. The left panel shows the average number of functional characters incorporated into character chains (solid lines) and the average number of excess characters not incorporated into character chains (dashed lines). The right panel shows average population fitness (solid lines). Results for five sexual and asexual simulations are shown; each line represents a single simulation using the following parameters: $N = 5000$, $\beta = 0.0001$ $\varepsilon = 5$ $\mu = 0.0002$ $f = 0.03$ $n = 2$. Sexual Efficiency behaviour appears during the first 1000 generations of the simulation, with sexual populations also developing more excess characters on average than asexual populations.

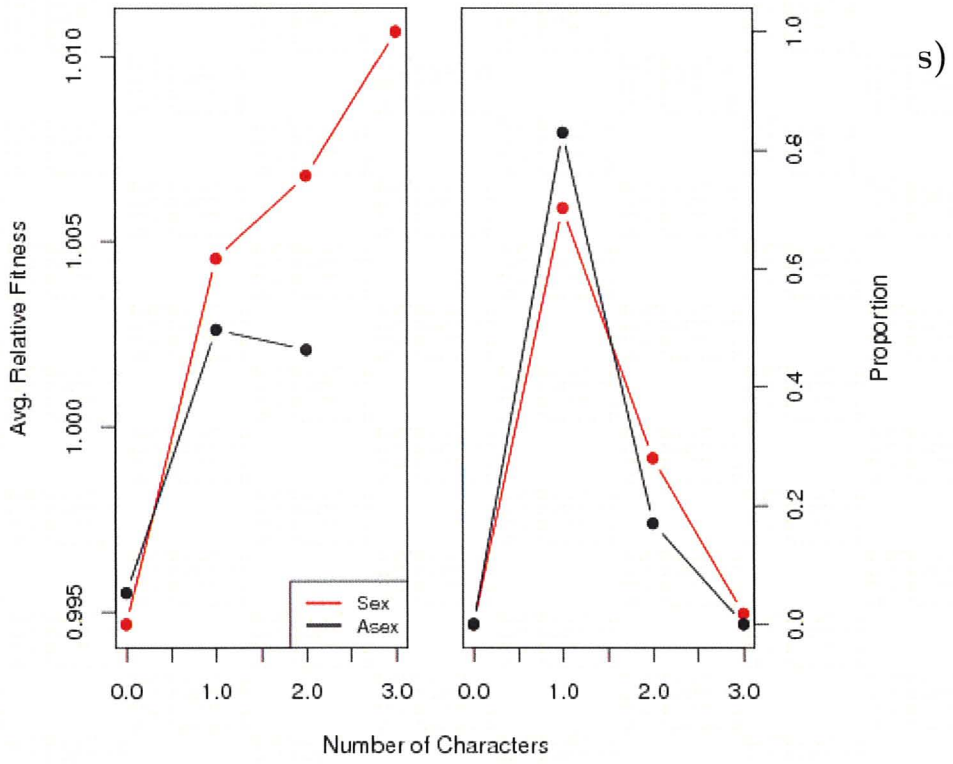

Figure 4.11: Assay of allele mixability of asexual (black points and lines) and sexual (red points and lines) populations from the simulations generated using Sexual Efficiency parameters (Figure 4.10) at 250 generations. Average allele mixability (left panel) and allele proportion (right panel) is plotted over the number of characters an allele possesses. The mixability of any given allele is represented by the relative change in average population fitness when it is possesed by every individual in the population.

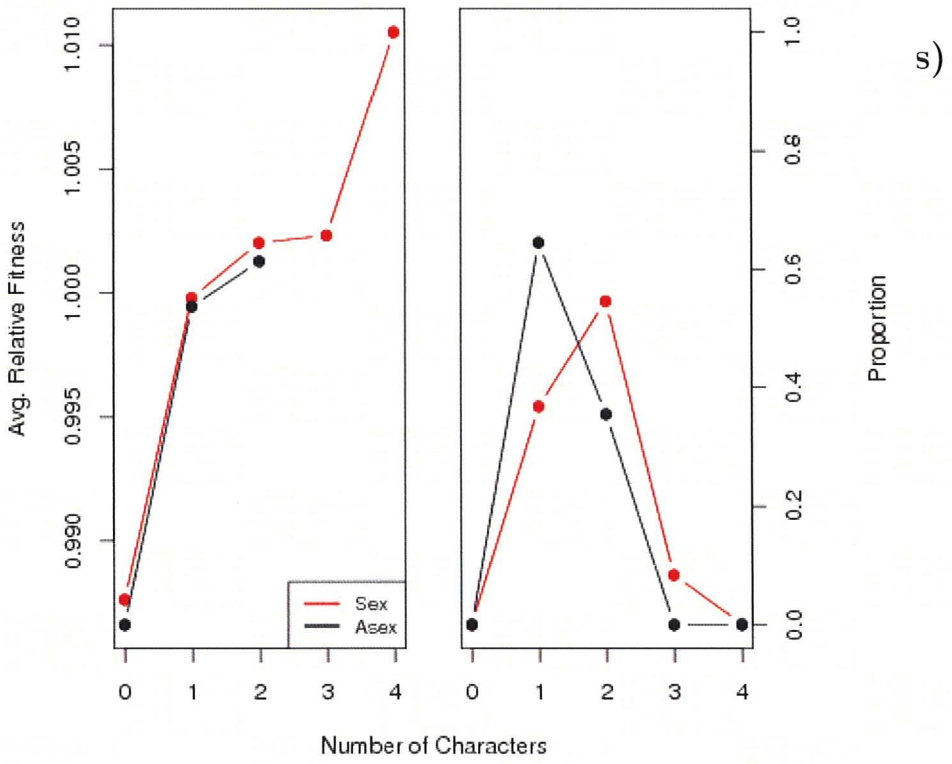

Figure 4.12: Assay of allele mixability of asexual (black points and lines) and sexual (red points and lines) populations from the simulations generated using Sexual Efficiency parameters (Figure 4.10) at 500 generations. Average allele mixability (left panel) and allele proportion (right panel) is plotted over the number of characters an allele possesses. The mixability of any given allele is represented by the relative change in average population fitness when it is possesed by every individual in the population.

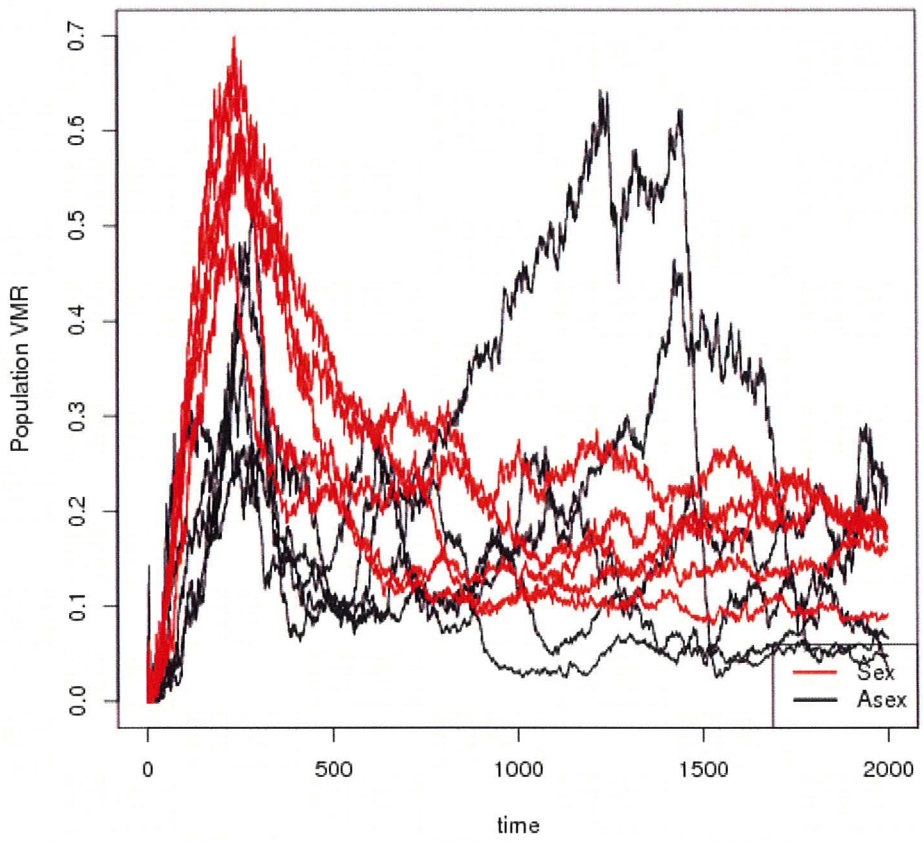

Figure 4.13: Population variance-to-mean ratio (Population VMR) of asexual (black lines) and sexual (red lines) populations for Sexual Efficiency behaviour (see Figure 4.10). Each line represents a single simulation; five sexual and asexual simulations are shown. Population VMR measures population genetic diversity for each generation by dividing the variance of total characters possesed by individuals by the average number of total characters in the population.

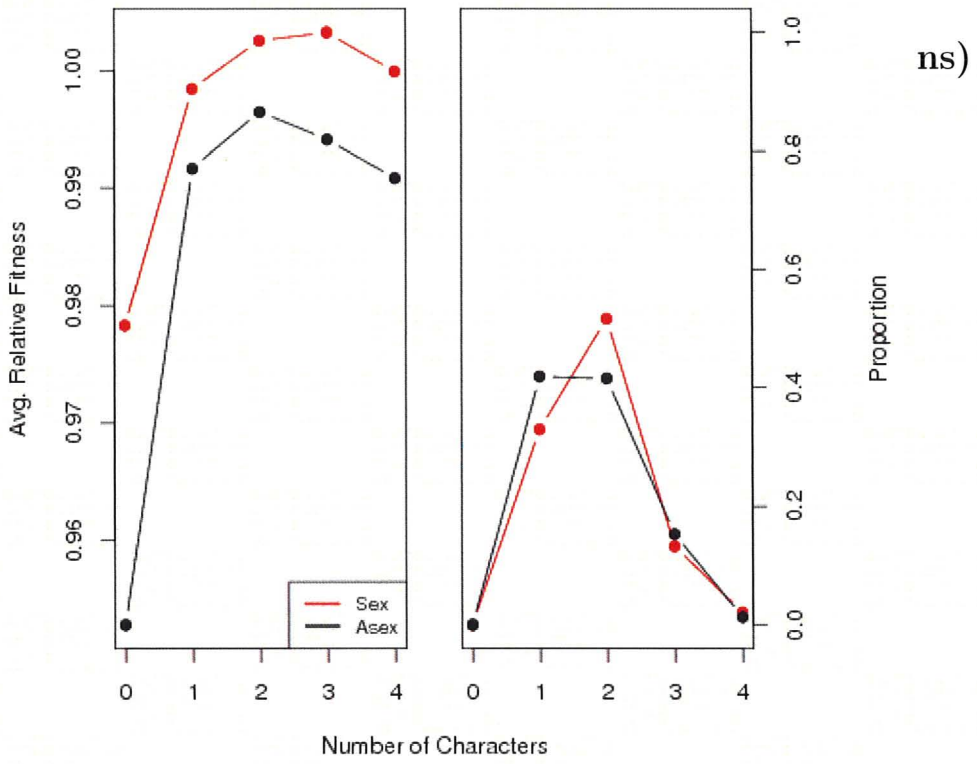

Figure 4.14: Assay of allele mixability of asexual (black points and lines) and sexual (red points and lines) populations from the simulations generated using Asexual Efficiency parameters (see Figure 4.6) at 100 generations. Average allele mixability (left panel) and allele proportion (right panel) is plotted over the number of characters an allele possesses. The mixability of any given allele is represented by the relative change in average population fitness when it is possesed by every individual in the population.

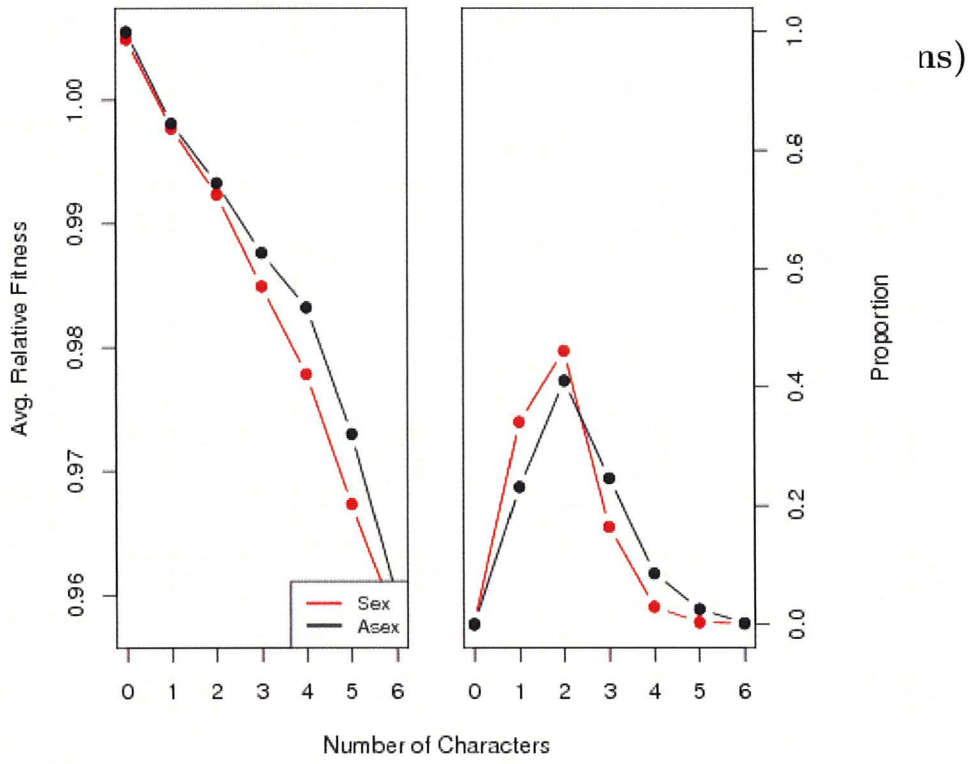

Figure 4.15: Assay of allele mixability of asexual (black points and lines) and sexual (red points and lines) populations from the simulations generated using Muller's Ratchet parameters (see Figure 4.4) at 100 generations. Average allele mixability (left panel) and allele proportion (right panel) is plotted over the number of characters an allele possesses. The mixability of any given allele is represented by the relative change in average population fitness when it is possesed by every individual in the population.

Chapter 5

Discussion

We have investigated the development of mixability in sexual and asexual populations using an explicit model of an evolving genetic network, named the chain model. The results presented here demonstrate three different model behaviours; Muller's Ratchet, Asexual Efficiency and Sexual Efficiency. Muller's ratchet occurs when the mutation rate is elevated and characters are costly to an organism, causing asexual populations to be overwhelmed by irreversible accumulation of mutations. Sexual populations are able to maintain greater fitness by producing genotypes with lower levels of mutation through recombination. By increasing the functional advantage of characters the effect of muller's ratchet is mitigated and model behaviour transitions into asexual efficiency, where beneficial alleles spread quickly enough through clonal replication to allow character chains to be built more efficiently than in sexual populations. The construction of character chains in sexual populations is slowed by random loss of alleles due to recombination. When mutations become less frequent and the cost of carrying characters is insignificant Sexual Efficiency behaviour occurs, where recombination becomes advantageous in the manner proposed by Sexual Efficiency by

bringing together rarely occurring alleles in new functional configurations faster than they would occur in asexual populations. Asexual efficiency and Sexual Efficiency behaviours also occur at larger population sizes and follow the general trends in parameter differences as described above. However, they do not result from the direct translation of parameters at smaller population sizes, ie., by reducing μ, f and β in proportion to the increase in N. Attempts to replicate Muller's Ratchet behaviour at large population size in this fashion have thus far been unsuccessful, which requires further study. This may indicate that the effect of genetic drift has an effect in the simulations where $N = 1000$.

We find that sexual populations promote increased allele mixability under Asexual Efficiency and Sexual Efficiency behaviours. The selection for mixable alleles occurs when genetic diversity reaches its peak, shortly after characters are introduced into a homogeneous population intially possessing no characters. Furthermore mixability is positively correlated with the number of characters an allele possesses, because characters allow alleles to function in a greater number of genetic contexts. Under conditions producing Muller's Ratchet behaviour, where the cost of carrying excess characters is nearly equal to their functional benefit, selection favours alleles possessing one or zero characters and allele mixability does not develop.

Our finding that mixability is acts over the evolutionary transient agrees with previous work by Livnat et al. [26]. The evolutionary transients in the chain model have biological significance, representing the period of time where populations undergo adaptation to a new environment. These periods of adaptation occur when the population has not yet reached a state of evolutionary equilibrium, which is the point where the total character levels in the population and population fitness do

not change over time. The number of characters attained at equilibrium depends on the cost of carrying characters, β. When β is low, we expect equilibrium populations to develop $\varepsilon\ell$ characters on average, while as β increases we expect selection to act against the accumulation of excess characters and less than $\varepsilon\ell$ characters to be reached. How soon a population reaches this equilibrium is determined by the probability of mutational events, μ, and the functional benefit of characters, f.

In the Chain Model there is another evolutionary equilibrium determined by the maximum chain length that populations can attain. It is implausible to expect that real populations, which demonstrate extraordinary potential to evolve and face varied selective pressures from changing environments, ever truly reach any such end point in their evolution. Therefore, by exploring evolutionary transient events we can assess how mixability can realistically influence the rate of evolution in evolving sexual and asexual populations. This is only relevant under Asexual and Sexual Efficiency behaviours where conditions favour increased biological complexity. In Muller's Ratchet behaviour biological complexity is hindered by negative mutational processes. Under such conditions the evolutionarily transient events are of less importance than the ability of populations to survive over the long term.

We show that it is be possible for sexual populations to have a competitive advantage over asexual organisms through Sexual Efficiency behaviour. There are two related possible explanations for this behaviour. The first is "mixability". This is the idea that sexual reproduction selects for alleles adapted to interact in many different genetic contexts, and therefore favors alleles with more characters, which can accelerate evolution of long chains under favorable circumstances. The mixability assays provide evidence that multi-character alleles do function well in different genetic

contexts, particularly in populations that evolved with sex (Figure 4.11). The second reason is the Fisher-Muller mechanism of bringing together favorable alleles that evolved in different lineages. This mechanism can work even without the assistance of mixability.

Future work could attempt to evaluate more clearly the contributions of the Fisher-Muller mechanism and mixability in Sexual Efficiency behaviour. We could ask for example how much advantage a sexual population would retain in a model where alleles can only develop a single character. If mixability does not play an important role in Sexual Efficiency behaviour, we should expect little difference between the rate of construction of character chains if alleles possessed only one, or many characters.

Although our character-chain based biological network is an over-simplified cartoon, it may have lessons for real biological networks. There is extensive evidence of interactive protein domains that play important roles in cellular regulation and signalling [40]. These may be analogous with our "characters" in that they may be accumulated, and their fitness contribution may depend strongly on how well they mix with other characters. The question still remains how mixable genes can influence the large-scale evolution of biological systems. Theoretical work shows that modular systems of genetic interactions can evolve solely through the process of network growth, eg. through gene duplication and random loss of genetic functional associations over time [48, 16]. In many cases it is difficult to explain the evolutionary significance of certain structural features of genetic systems, particularly those of gene regulation [50, 28]. Regardless, selection of mixable alleles in sexual populations could possibly play a role as intermediaries between existing modules, or reconnect new modules in novel ways. This may have important consequences in the area of

cell regulation where different developmental pathways could be generated leading to increased complexity.

Chapter 6

Conclusion

Understanding why recombination is predominant among living organisms is an outstanding question in biology that has not been entirely exhausted. Recent work has revealed that biological systems are constructed from smaller functionally isolated subsystems in a modular fashion. This organizational property has important influence the evolution of organismal complexity and genetic recombination. So far it has been shown that sexual populations can select for modularity on the level of individual genes, called mixability, where genes can function well in any genetic context but at the cost of breaking up highly coadapted combinations of alleles.

By using a simplified mechanistic model of an evolving genetic network, which we have called the "Chain Model", we were able to address the functional implications of allele mixability. In the Chain Model neighbouring genes on a circular genome develop interactions with one another by acquiring "characters" through mutation, which evolves into a simple biological network with a chain structure improving individual fitness. The complexity of the network is determined by the number of genes that are incorporated into the chain, and is called the chain length. In sexual populations

evolving character chains are under a risk of being broken apart by the recombination of loci from incompatible genetic backgrounds. This can be overcome by alleles that possess many characters and can function in different genetic contexts, i.e. mixability.

We wished to know what conditions favoured mixability in the Chain Model. We tracked the average sexual and asexual population fitness and character levels over time using different combinations of parameters; the probability of mutational events, μ, the functional benefit of characters, f, and the cost of characters, β. Three distinct model behaviours emerged, which we have called Muller's Ratchet, Asexual Efficiency and Sexual Efficiency. Under muller's Ratchet sexual populations attain a greater fitness than asexual populations by eliminating excess costly characters, resulting when mutations are frequent and f and β are nearly equal. Under Asexual Efficiency asexual populations construct character chains faster than sexual populations and attain a greater fitness, resulting when mutations are frequent and f is high while β is low. Under Sexual Efficiency the reverse occurs, and is the result when mutations are rare, f is high and β is low.

Under the different model behaviours we assayed the mixability of alleles evolved from sexual and asexual populations. Allele mixability was assessed by measuring the relative fitness of the population if every individual possessed it, which gives an estimate of the ability of an allele to function under different genetic contexts. We discovered that allele mixability was increased in sexual populations under Asexual Efficiency and Sexual Efficiency behaviours and was positively correlated with the number of characters possessed. Furthermore, we have also shown that alleles undergo selection for mixability during evolutionarily transient events when population genetic diversity is maximized, which increases the likelihood that recombined alleles will be

placed in different genetic contexts. Under Muller's Ratchet behaviour mixability fails to develop as a result of selection acting against the accumulation of excess characters. Therefore from our results we can conclude that mixability is favoured in sexual populations when the cost of carrying characters is low relative to their benefit, and when the appearance of characters is rare mixability could also be advantageous by allowing sexual populations to develop biological complexity faster than sexual populations. How large scale modularity develops from genetic mixability remains to be elucidated, but it is evident that future work on more refined network-based models will shed more light onto this question as well as the advantage of sex and recombination.

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