AN EMPIRICAL APPROACH TO UNDERSTANDING THE RELATIONSHIP BETWEEN RECOMBINATION AND FITNESS

By

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A thesis submitted in conformity with the requirements for the degree of Masters of Science
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Abstract

An empirical approach to understanding the relationship between recombination and fitness
M. Sc. 2008
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The persistence of sex is a recurrent conundrum in evolutionary biology because sex is costly. These costs may be accounted for by looking at the outcome of sex, namely that sex causes genetic mixing. Recombination is one of the processes by which sex causes genetic mixing; determining when recombination is advantageous may alleviate some of the costs of sex. The advantages of recombination are in the effects of recombination and the influences thereupon. The first experiment focuses on the effects of recombination on the mean fitness and variance in fitness. A second experiment examines the influences on recombination by addressing whether recombination is a general response to poor condition. Specifically, the impact on recombination rate of genotypes with variable fitness is investigated. Differing fitness effects are not correlated to recombination rates. Conversely, coincidence, a recombination related trait, is positively correlated with fitness.
Acknowledgement

A whole is never more than the sum of its parts. For this thesis, all of the parts would not have been possible if it weren’t for the people around me in the last two years. With any one of those people missing, this thesis would not be whole.

I am greatly indebted to my supervisor, Professor Aneil Agrawal, for the constant challenges he placed before me. I can not begin to describe how difficult it is to come from an undergraduate degree in Anthropology into this science and I can not imagine how hard it must be to have your first graduate student come into this science from Anthropology. Not only was my supervisor patient throughout but he never hesitated to ask me the hard questions and push me in my learning. He had this innate ability to know which of my “gloss over” explanations were truly hiding miscomprehension. He was incredibly understanding of the work that goes into experiments of this nature. He helped me with my experiment when I was especially stuck, including coming into the lab on Christmas day. In addition, he instilled in me a need to be meticulous in all of my experiments. I present these results with the confidence of knowing that I never took a short cut in any of the experiments.

I would also like to thank my committee member, Professor Locke Rowe, for encouraging me to look at the results from angles I had never imagined. A thank you also goes to the other members of my defense Professor John Stinchcombe and Professor Asher Cutter for advice, comments and encouragement.

A very special thank you goes to my lab mates, I think our lab was the scientific equivalent of Seinfeld - the only music we could all agree on was William Shatner. Without the guidance and advice of Chrissy Spencer this thesis would not be what it is. A very special thank you to her for providing thorough editing, advice and last minute help even during the peak of her own experiments. I would also like to thank my lab mates Nathaniel Sharp and Alethea Wang for providing feedback. All three of them were supportive and encouraging and took the time, a lot of time, to share their expertise about working with Drosophila. I would like to thank all of the Agrawal lab in the past two years especially Azadeh Laffafian, James King, Sean Clarke, for everything from advice to sharing laughs.

My second experiment encompassed scoring almost a total of 750 000 flies. This would not be possible without the help of an army of volunteers: Sanjana Sen, Bojana Bogelic, Alison Smeaton, Stephanie Chou, Yibo Yu, Royce Fan, Rhamak Kosravi, and Gang Wang. A ridiculously extra special thank you goes to Jadene Young who gave up her Christmas break, her spring break and basically all of her time off to provide endless hours of support and never stopped smiling the entire time. Jadene’s dedication can be summed by a single incident: I got to the lab at 7am on Christmas Eve and she had already been there for a while (did I mention that she lives two hours away?). I am still really sorry about Adv.

Anyone who has worked with Drosophila knows that experiments of this kind can keep you in the lab 80 days straight at a time and up to 14 hours a day. Flies don’t break for holidays, birthdays or anniversaries and this truly applied to my experiments. A big thank you to my friends and family who have tolerated my extreme periods of absenteeism and stressed out demeanor over the past two years. Especially to my wonderfully loving and extremely encouraging family: Jamie & Chris Aucoin, Amanda Aucoin, Craig Willchuk,
Emily Willchuk, Ian Willchuk, Rachel Aucoin, Abigail Aucoin, Meghan Aucoin, Jen & Jon Jones, Ann and Syd Duval, Maryse Menard & Michael Goyette, Pierre Lefebvre, Peter & Linda Tedman, and Dianne Munro for all understanding. A special thank you to my “mom” Karen Willchuk for always encouraging, supporting, and listening and always reminding me that our greatest weakness lies in giving up. Finally, to my biggest fan even on my worst day, my husband Daniel. He put up with me, supported me, encouraged me, fed me, loved me and even married me during this thesis. He selflessly did whatever he could to help me. He reminded me to keep things in perspective; just put one foot in front of the other and don’t worry about what comes after that. Thank you Dan for everything you are and all the things that you do.

Thank you to NSERC, the department of Ecology and Evolutionary Biology, the University of Toronto and to Professor Aneil Agrawal for the generous financial support.
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General Introduction

“No area of evolutionary biology offers the curious investigator a more fascinating mixture of strange phenomena and deep intellectual puzzles than the evolution of sex and its consequences” (Stearns 1987)

The evolution of the ‘evolution of sex’ quandary

Asexual organisms marked the start of life and the earliest regular sexual reproduction began among single-celled eukaryotes (Charlesworth 2006). Sexual reproduction evolved and was maintained by most ancient lineages of multicellular eukaryotes; asexual lineages are only recently re-established, with few exceptions (Charlesworth 2006). The maintenance of sex presents two problems: First, it breaks up any genetic combinations that are adaptively selected. Second, sex is expensive; the minimum cost is two-fold (Maynard Smith 1978). The two-fold cost of sex, also known as “the cost of males”, is incurred for the reason that an asexual counterpart has double the advantage of producing children as a sexual counterpart because only half of what the sexual counterpart produces can, themselves, produce offspring (Maynard Smith 1978). In addition to the two-fold cost of sex there are other costs to sex including the cost of finding a mate, costs associated with sexually transmitted diseases and costs for mating in which there is a risk of predation (Agrawal 2006). How are these costs overcome? Many attempts have been made to theorize how sex is maintained in spite of these costs, but to date the explanation remains elusive.

The molecular processes of sex, namely segregation and recombination, had not been identified in the early scientific community. As a result, the advantages of sex were poorly understood. The early leading theory of sex in the 19th century was the idea of
blending inheritance; sex results in an offspring being the average of the two parents (Darwin 1866). However, this explanation was insufficient to ease the inquisitive nature of many. Darwin (1861 in Charlesworth 2006) noted:

“We do not even in the least know the final cause of sexuality; why new beings should be produced by the union of the two sexual elements, instead of by a process of parthenogenesis”

Darwin later conceptualized the advantages of sex based on hybrid vigor; the characteristics of the hybrid are a combination of the advantageous traits of the parents and thus a sexually produced offspring is better suited to the environment than a clone of either parent (Darwin 1889). Other theories existed, such as the theory that sex creates variation, but offered little evidence on how sex was maintained (Weismann 1889). The processes of sex unfolded when the early works of Mendel (1865) were rediscovered by Correns (1900) and highly publicized; the concept of segregation was established (Bateson 1902, Corcos and Monaghan 1987). Finally, with the influence of Hugo de Vries in 1903, evidence by F. A. Janssen in 1909 and the work of Morgan et al. (1915) chromosomal crossing over and recombination was recognized and genetic research flourished (Richmond 2001).

Since the establishment of genetics, more intricate theories on the maintenance of sex have developed. Sex has long been considered a means of increasing variation to facilitate rapid adaptation in differing environments (Bell 1982). Moreover, that sex is advantageous because it brings together beneficial mutations has been posited (Fisher 1930, Muller 1932). Muller (1964), conversely, demonstrated that sex is beneficial because it facilitates purging deleterious mutations from a population, an idea that has received some empirical support (Zeyl and Bell 1997, Bachtrog 2003). One of the leading
theories for the evolution of sex is that it provides a means by which parasites can be resisted by changing the host genotype once a parasite becomes adapted for that genotype (Peters and Lively 1999). Computer simulations developed and expanded the conditions under which proposed theoretical hypotheses hold true (Felsenstein 1974, Keightley and Otto 2006, Salathe et al. 2007). Even though theories abound, the predicament of sex remains because the conditions under which the benefits can outweigh the costs are few and rarely observed in natural populations.

Recombination

One of the essential processes of sex is recombination. Recombination occurs during the gamete formation process of meiosis, specifically in the prophase of meiosis I. During this process non-sister homologous chromatids align, one from each parent, and portions of the chromatids crossover. Recombination is the exchange of genetic material between non-sister homologous chromatids, the outcome is variable gametes with differing combinations of their parental chromosomes.

Both recombination and segregation are the mechanisms by which sexual reproduction causes genetic mixing. Because there are two mechanisms for genetic mixing, recombination alone need not be advantageous enough to overcome the two-fold cost of sex. However, recombination must outweigh some of the costs. By finding the conditions under which recombination is advantageous some costs are alleviated and a basis established for understanding why sex persists.
Aim

This thesis set out to study important aspects of recombination. I asked two questions:

1. How does a single round of recombination alter mean fitness and variance in fitness?
2. How do genotypes of varying fitness affect the rate of recombination?

*Drosophila*

A professor of entomology and a breeder of *Drosophila*, Woodworth was the first to suggest using *Drosophila* for genetical experiments in 1909 (Sturtevant 2001). Since this time, *Drosophila* has become one of the most widely used lab species for genetic experiments. There are multiple reasons for this and these reasons are concurrent with why we selected *Drosophila* to perform the experiments in this thesis. First, *Drosophila melanogaster* has a short breeding time that allows for many generations to be constructed and tested in a single year of study; a generation can take from eight to 14 days. Second, *Drosophila melanogaster* is a diploid organism with only four chromosomes that have been mapped and well studied. The bulk of the genes available for study are on the second and third chromosomes. The first chromosome is the sex chromosome and was not used for the experiments in these chapters. The fourth chromosome is small and, while it was once thought not to recombine and lacked variation in genetic material, these assumptions are disproved (Bridges 1935, Wang et al. 2004). Regardless, the genomic content on the fourth chromosome is minimal in comparison to the second and third chromosomes. Finally, the genes selected for the
experiments of this thesis are phenotypically detectable, making for a quick and inexpensive means by which a genotype can be evaluated.

In addition to the large body of knowledge available on the genetics of *Drosophila melanogaster*, the genetic structural features of the organism lend themselves well to manipulation. Inversions are structural elements of the *Drosophila* genome that suppress recombination in different regions. Furthermore, *Drosophila melanogaster* males lack recombination so that changes in genotype can be fully attributable to recombination in the female when segregation is controlled for. These features are extremely important for both chapters of this thesis, as the goal of each is to measure some effect of recombination and it is necessary to know where that recombination event took place.

**Thesis Chapters**

**Chapter 1: The effect of recombination on mean fitness and variance in fitness**

Recombination acts on the linkage disequilibrium, or the association between alleles, in a population. Changes in linkage disequilibrium cause changes in the mean fitness and variance in fitness. Many theoretical works posit that recombination has a long-term advantage because it increases the variance in fitness in a population by breaking up negative associations (Bell 1982, Koella 1993, Kouyos et al. 2007, Maynard Smith 1971). Recombination, however, is often cited as unfavorable because it breaks up advantageous positive associations and reduces mean fitness in the short term (Bell 1982, Koella 1993, Kouyos et al. 2007). Alternately, recombination can break up detrimental allelic combinations and cause a short-term advantage by increasing mean fitness under certain circumstances (Peters and Lively 1999, Maynard Smith 1976). Theoretical
hypotheses for the evolution of sex then need to be categorized based on what they infer about changes in mean and variance in fitness. Historically, they have been categorized in a variety of ways but never by the proposed method (Korol et al. 1994, Koella 1993, Bell 1982). In this chapter I reclassify the theories and I measure and compare the mean fitness and variance in fitness in recombinant and non-recombinant lines of lab-adapted Drosophila melanogaster. Differences between the lines allow for the evaluation of the genetic associations in the population and can be used to narrow the many hypotheses for the evolution of sex.

The effects of recombination on the mean and the variance of a population are previously tested but the results are complex and contradictory (Charlesworth and Charlesworth 1975, Deng and Lynch 1996, Kelley et al. 1988). A caveat of existing literature is that selective pressures, under which a population evolved, are altered during the experiment. That is, the environment in which the associations were made is different from the environment in which the associations were measured. As a result, it is difficult to make conclusions about the associations of the populations in question. I set out to test the effects of recombination on a lab-adapted stock population of Drosophila melanogaster that is reared and tested in a stable and consistent environment. In addition, I control for the effects of segregation because they can confound the effects of recombination.

Chapter 2: Recombination rate and variable genotypes

Fisher (1930) quantified how variance can increase and decrease with sex. Moreover, he observed that recombination in certain situations is only selected for if the rate of recombination is suddenly reduced (Fisher 1930). If recombination is plastic,
meaning it has some flexibility in its rate in response to condition, the circumstances for
the advantages of recombination are much less restrictive. On the one hand, the plasticity
of recombination is well established experimentally (Zhuchenko et al. 1986, Hayman and
Parsons 1960, Graubard 1932, Plough 1917, Plough, 1921, Lawrence 1958, Belyaev and
2002, Priest et al. 2007). On the other hand, computer simulations established,
theoretically, that the spread of a plastic modifier in a diploid population is selectively
neutral, except under narrow circumstances such as maternal effects (Agrawal et al.
2005). So how has plasticity come to evolve in diploid organisms? Most current literature
supports the plasticity of recombination in response to an external influence, such as
environmental stress, that creates poor condition (Zhuchenko et al. 1986, Hayman and
Parsons 1960, Graubard 1932, Plough 1917, Plough, 1921, Lawrence 1958, Parsons
1987). However, these studies do not report fitness results thus the extent of the stress
cannot be evaluated. Fitness is key to understanding of the biological basis of condition.
When poor condition causes an increase in recombination it may help to establish the
advantages of recombination. As opposed to external stress, some studies using indirect
internal stress report a correlation between fitness and recombination (Tucic et al. 1981).
However, there are few studies in existence and the evidence is problematic because it is
confounded by uncontrolled sources of variation. Consequently, observed changes in
recombination cannot be attributable solely to changes in fitness (Tucic et al. 1981). I test
a range of genotypes with varying levels of fitness on the recombination rate in
Drosophila melanogaster. I controlled and homogenized the genetic background of all
genotypes being tested. As a result, any observed differences in recombination are
attributable to the only factor that differed between the lineages: poor condition created by mutations with deleterious fitness effects. These mutations have no known direct effects on recombination rate. I established the number and type of mutations, or the condition of the individual, to test whether recombination changes as a general response to poor condition.

**Results**

Overall, the goal of this thesis is to explore the extent of the relationship between recombination and fitness. What effect does recombination have on fitness? Does recombination respond to altered condition based on varying fitness? The impact of recombination on the mean fitness and variance in fitness is evaluated. The long-term and short-term advantages of recombination are explored as well as the implications for theories on the evolution of sex. While the results in Chapter 1 are statistically insignificant, this does not imply that there is no effect of recombination for one generation. Experimental limitations are considered but there are possible theoretical explanations as well. This chapter provides a firm starting point for future experiments.

In Chapter 2, as a proxy for condition, differing fitness levels are used to explore the influence on the rate of recombination. Recombination rate did change between the genotypes but not in a manner that is correlated with fitness. In addition, there is a statistically significant negative correlation between fitness and the degree of interference, or the incidence of recombination in adjacent chromosomal regions. This is in stark contrast to previous studies that repeatedly found reduced amounts of double recombinants under stressful conditions (Hayman and Parsons 1960, Graubard 1932,
Plough 1917, Plough, 1921, Lawrence 1958). The implications of these results are discussed for previous studies and the advantages of recombination.
References


Chapter 1

The effect of recombination on mean fitness and variance in fitness
Introduction

The ubiquity of sex can be investigated by examining what is genetically involved in sexual reproduction in contrast to asexual reproduction. One of the most obvious differences between sexual and asexual lineages is recombination, the shuffling of allelic combinations during meiosis. Selection works to maximize favorable allelic combinations so why break them up? In evolutionary biology, the advantages of this shuffling have been explored but a recurring issue arises in overcoming the two-fold cost of sex that makes the advantages of sexual reproduction beneficial enough to maintain sexual lineages. This two-fold cost occurs from females only passing on half of their genetic makeup to their offspring. When males contribute nothing to the upbringing of these offspring, a sexual female has to bare twice as many children to pass on as much genetic material as an asexual counterpart, whose offspring contains their full diploid compliment (Maynard Smith 1978). In addition to the two-fold cost of sex, there are other costs to sex such as the cost of finding a mate, the risk of contracting sexual diseases and the interference of the mating process with the ability to escape predators (Agrawal 2006). So how are these costs overcome? Recombination is the key. As recombination is one of the two ways in which sexual reproduction mixes genes, it has to be beneficial enough to help alleviate some of this burden. However, current research depicts the advantages of recombination occurring in only limited situations (Keightley and Otto 2006). In this chapter, I will investigate the effects of recombination, the theoretical hypotheses for why recombination persists, and the empirical evidence for these theories. Finally, I will discuss a new empirical undertaking along with the possible implications of its outcome.
I shall start by defining a hypothetical population in order to better demonstrate the effects of recombination. The population is defined by two loci at each of which there are two possibilities $B$ or $b$ and $F$ or $f$. The fitness of an individual ($W$) can be defined in terms of its genotype such that $W_{BF} = 1$; $W_{bF} = (1 - S_b)$, where $S_b$ is the selection costs of having allele $b$; $W_{BF} = (1 - S_f)$, where $S_f$ is the selection costs of having allele $f$; finally, $W_{bf} = (1 - S_b)(1 - S_f) + \epsilon$, in which $\epsilon$ is the additional fitness costs of having both $b$ and $f$, it is the deviation from multiplicative effects known as epistasis (Keightley and Otto 2006).

Whenever the average fitness of the extreme types outweighs the average fitness of the intermediates, $W_{BF} + W_{bf} > W_{BF} + W_{BF}$, epistasis must be positive, $\epsilon > 0$. Negative epistasis, on the other hand, occurs when an individual who has $b$ and $f$ is less fit than expected from having either just $b$ or $f$, in this case $W_{BF} + W_{bf} < W_{BF} + W_{BF}$ and $\epsilon < 0$.

Recombination directly impacts the linkage disequilibrium ($LD$) of a population. In terms of the defined population, when genetic associations are positive, $LD > 0$, there is an overrepresentation of the extreme types, $BF$ and $bf$, and recombination produces the intermediate types, $bF$ and $Bf$. Negative linkage disequilibrium, $LD < 0$, exists when there is an excess of intermediate types, $bF$ and $Bf$, in which case recombination would create $BF$ and $bf$. When there is no linkage disequilibrium in a population, $LD = 0$, recombination has no effect.

Linkage disequilibrium, as I have demonstrated, is the foundation upon which recombination operates. What causes these associations in the first place? Sources of negative linkage disequilibrium include mutation, drift, selection and migration. One particular source of negative linkage disequilibrium is the interaction between drift and

I can illustrate the Hill Robertson effect through elaboration of the previously defined population. Assume $B$ and $F$ are advantageous, $b$ and $f$ are deleterious and by chance drift creates $bF$ and $Bf$. Since selection aims to keep both $B$ and $F$ even though they are on deleterious backgrounds, $f$ and $b$ respectively, both $bF$ and $Bf$ will persist resulting in negative linkage disequilibrium. The Hill Robertson effect is this result of drift creating negative linkage disequilibrium, $LD<0$, where selection has little to act upon and so the combinations persist (Keightley and Otto 2006). Alternately, if drift creates positive disequilibrium, in this case $BF$ and $bf$, selection will eliminate $bf$ and all that will be left is $BF$. In such an extreme case, no genetic associations exist because only the one genotype remains. From these situations it can be deduced that genetic drift and selection, on average, create negative linkage disequilibrium.

Another important source of both positive and negative linkage disequilibrium is epistatic selection ($\varepsilon$) because selection acts to generate an excess of the most favorable allelic combinations. Epistatic interactions ($\varepsilon$) are the deviation of a genotype from multiplicative effects, as I have previously described. If a genotype is just as fit as expected, $W_{bf} = (1 - S_b)(1 - S_f)$, based on the allelic composition of the individual, there is no epistasis as $\varepsilon=0$ (figure 1.1c). Negative epistasis is the result of a genotype being less fit than expected and produces negative linkage disequilibrium, where $\varepsilon<0$ then $W_{bf} < (1 - S_b)(1 - S_f)$ resulting in $LD<0$ (figure 1.1a). Positive epistasis exists when a genotype is more fit than expected which generates positive linkage disequilibrium, where $\varepsilon>0$ then
$W_{bf} > (1 - S_b)(1 - S_f)$ and creates $LD > 0$ (figure 1.1b). Other forces that can result in positive linkage disequilibria include drift, migration and mutation.

What are the consequences of altering the genetic associations of a population? Recombination acts on linkage disequilibrium which causes changes in the mean fitness and variance in fitness of a population (see table 1.1). Changes in mean fitness have immediate implications for the population. Whether this impact is beneficial is governed by both the fitness of the allelic combinations and the sign of the genetic associations. To demonstrate, in this first scenario, assume the extreme types are more fit than the intermediates, such that $W_{BF} + W_{bf} > W_{BF} + W_{bf}$. If associations are positive, $LD > 0$, recombination causes a decrease in mean fitness by reducing the most fit genotypes to the least fit. Since there is a decrease in mean fitness then recombination is disadvantageous in the short-term. Conversely, if the associations in a population are negative, $LD < 0$, recombination will produce the fit extreme types and thus be beneficial in the short-term by increasing the mean fitness. The opposite can also be established, in a second scenario, where the intermediates are more fit than the extreme types, such that $W_{BF} + W_{bf} < W_{BF} + W_{bf}$. On the one hand, if the associations are positive, $LD > 0$, recombination will increase the mean fitness by making the most fit genotypes from the least fit and therefore recombination has a short-term advantage. If linkage disequilibrium is negative, on the other hand, $LD < 0$, then recombination suffers a short-term disadvantage from decreasing the mean fitness. In addition to these two scenarios, it is also possible that there are no associations between alleles in a population, $LD = 0$. In such a case, recombination has no effect on fitness since alleles are not found in shortage or in excess combinations with other alleles (figure 1.2c).
I have discussed the short-term effects of recombination on mean fitness. Recombination, however, can affect the variance in fitness and thus have long-term consequences. Consider a population with negative linkage disequilibrium, $LD < 0$, accordingly having an excess of intermediates, $hF$ and $Bf$. Recombination will cause a shift from the intermediate types to the extreme types and increase the variance in the population (figure 1.2b). Conversely, if the genetic associations of a population are positive, $LD > 0$, there is an excess of extreme types and recombination will cause a decrease in variance (figure 1.2a).

Recombination has both long-term effects on the variance of a population and short-term consequences for the mean fitness of a population. A genetic system then has to be in a careful state between maximizing fitness in the current environment while maintaining flexibility for the future (Korol et al. 1994). Because a constant environment is when sex should give way to parthenogenesis, it is essential to determine which of these effects predominate in a constant environment (Bell 1982); however, this has yet to be determined. Many theories exist that demonstrate either the long-term or the short-term advantage of sex is plausible (Bell 1982, Maynard Smith 1976, Kouyos et al. 2007, Peters and Lively 1999, Koella 1993, Salathe et al. 2007, Otto and Barton 2001, Felsenstein 1974, Williams 1966, Muller 1964, Fisher 1930). These theories are classified in many different ways including by beneficial versus deleterious mutations, or whether recombination works to conserve the genome or enhance it (Bell 1982, Korol et al. 1994, Koella 1993). Nevertheless, theories must be classified on the basis of what their predictions are about linkage disequilibrium and, where applicable, epistasis as these are the fundamentals upon which recombination acts.
Theoretical considerations

I have reclassified many of the theories by their effects on mean and variance (see table 1.2). Major theories, including the Fisher-Muller Hypothesis, Lottery models, the Hitchhiker and Tangled Bank, are based on recombination increasing variance so that in times of environmental shifts, temporal or spatial, a species that has the most variation will adapt the fastest and outcompete any clonal lineages (Bell 1982, Koella 1993, Maynard Smith 1971, Fisher 1930, Muller 1932). As I have discussed, this is an increase in variance from recombination breaking up negative linkage disequilibrium, $LD < 0$. In temporal environmental heterogeneity theories this is a means to avoid extinction (Bell 1982, Kondrashov and Yampolsky 1996, Waxman and Peck 1999). In spatial environmental heterogeneity models recombination allows individuals to use different niches and thus slow the depletion of resources (Bell 1982, Koella 1993, Lenormand and Otto 2000). For these theories then the existence of sex is to maintain variability for a long-term advantage. However, few theories make full assertions about the short-term consequences for breaking up negative linkage disequilibrium. The Best Man Hypothesis and the Tangled Bank entertain the notion that, because some unfit genotypes result from the process of recombination, it is possible that mean fitness decreases (Bell 1982).

As opposed to solely focusing on long-term advantages, a small number of theories postulate that sex persists because it increases the mean fitness and thus has a short-term advantage (Maynard Smith 1976, Peters and Lively 1999, Maynard Smith 1971). Consider a case where epistasis is negative and linkage disequilibrium is of opposite sign, $LD > 0$. Recall that recombination causes reduction in variance but an immediate short-term advantage by increasing the mean fitness. The Red Queen
hypothesis proposes that there are fluctuating selective pressures that result in changes in the sign of epistasis once a rare genotype has become common; this is also known as negative frequency dependent selection (Peters and Lively 1999, Salathe et al. 2007). Since linkage disequilibrium results from epistatic selection, a change in the sign of epistasis results in a time lagged change in the sign of linkage disequilibrium because it takes time for selection and recombination to build up the most fit genotype. If the time lag is on the order of two to five generations, the sign of linkage disequilibrium is often of opposite sign to epistasis and recombination consistently aids in responding to selection by increasing the presence of the most fit genotype (Peters and Lively 1999). Thus, recombination incurs a short-term advantage by increasing the frequency of the most fit genotype and increasing the mean fitness.

Some researchers are turning to a more pluralistic theoretical approach by combining aspects of certain theories (West et al. 1999). Indeed, what may cause recombination to persist at one time may become a byproduct of it another and thus the explanation for the maintenance of recombination may be constantly changing itself (Bell 1982, Korol et al. 1994, West et al. 1999). This is theoretically sound, but the necessary empirical evidence is lacking to qualify which aspects of the theories are true.

It must be noted that this is not meant to be an exhaustive discussion of all of the theories and they are not mutually exclusive. While theoretical hypotheses are continually put forth, negated and re-invented, the question of the ubiquity of sex remains. What is firm is that recombination affects the mean and variance in fitness of a population and most theories are based on either a long-term effect of recombination causing an increase in variance or, recombination gaining an immediate benefit from its effect on mean
fitness. To date, however, little empirical evidence exists to substantiate any of the theories. More studies are needed in which the mean and variance of fitness of sexually produced offspring are compared to asexually produced offspring.

In experiments of this type, three outcomes are possible. The three possible outcomes include: that there are no differences detected between the non-recombinant and the recombinant lineage, second, that the recombinant lineage has a different variance in fitness than the non-recombinant and finally, that the recombinant lineage has a different mean fitness than the non-recombinant lineage. I explore the consequences of such findings.

**Possible outcomes**

If the recombinant and non-recombinant lineage have no significant differences in mean and variance several explanations are possible. These explanations include: that recombination had nothing to act upon, $LD=0$, the differences between the lineages were lost by chance, or that other forces are important for the effects of recombination. Such forces may include mutation and selection shifts, brought about by the heterogeneity of environments, that are not detectable in the experiment.

Most theories on the maintenance of sex are based on the ability of recombination to increase variance allowing populations to adapt to new conditions much more quickly and efficiently than clonal lines (table 1.2)(Bell 1982, Koella 1993, Fisher 1930, Muller 1932, Otto and Lenormand 2002). If the results of an experiment show that the recombinant lineage has an increased variance over the non-recombinant lineage, the effects of recombination are grounded in increasing the variance of a population. An increase in variance is caused by breaking down negative linkage disequilibrium, $LD<0$. 
Increased variance for a recombinant line over a non-recombinant line allows for the assessment of many of the current theories. For example, in the Fisher Muller Hypothesis, recombination persists because it increases variance and spreads beneficial mutations (Fisher 1930, Muller 1932). Alternately, if the results of the experiment are that the recombinant line has less variance than the non-recombinant line, it is deducible that linkage disequilibrium is positive, $LD > 0$, because a decrease in variance is the result of when extreme types are converted to intermediate types.

Recombination may affect the mean as well as the variance. It is possible that recombination may cause a decrease in mean fitness in the recombinant lineage relative to the non-recombinant line. While some theories do not make predictions about the short-term consequences of recombination, those that do, and have a decrease in mean fitness as part of their hypothesis, are supported by this outcome (table 1.2). Conversely, if the results of an experiment demonstrate that the recombinant lineage has a higher mean fitness over the non-recombinant lineage this is informative because recombination provides a short-term fitness advantage to a population. The only way for there to exist a short-term advantage is when linkage disequilibrium and epistasis are of opposite sign (table 1.1). Observing an increase in the mean fitness of a recombinant line is supportive of theoretical models such as the Red Queen hypothesis that is predicated on epistasis and linkage disequilibrium being of opposite sign (Peters and Lively 1999). Determining why a population has linkage disequilibrium and epistasis of opposite sign, however, is beyond the scope of this type of experiment.

Finally, along the same line, it is having the two results of mean fitness and variance together that is most informative about the effects of recombination. If there is
an increase in the mean fitness as well as a change in variance, then the sign of both epistasis and linkage disequilibrium can be deduced (see table 1.1). As I have previously discussed, if there is an increase in variance this is the result of recombination acting on negative linkage disequilibrium, \( LD < 0 \). In addition to the increase in variance, if there is an increase in mean fitness, which requires that epistasis and linkage disequilibria are of opposite sign, there is positive epistasis. The same argument can be made for a decrease in variance which equates to positive linkage disequilibrium and, coupled with an increase in mean fitness, there is negative epistasis. These results make possible an evaluation of current theoretical hypotheses by comparing their predictions, based on the current classification, with the actual outcome (table 1.2).

**Experimental evidence**

Current research on the effects of recombination on the mean and variance in a population is scarce. Four experimental studies have been published on the effects of recombination, however the studies have limitations. Charlesworth and Charlesworth (1975) developed three lines of recombinants, non-recombinants and highly recombinants from *Drosophila melanogaster* taken from the wild. The recombinant and highly recombinant lines are problematic since the parental females were captured in the wild, already inseminated, and reared their offspring in a laboratory setting. Thus, the associations that were built up in the wild were broken down in the lab under, what may be, completely different selection pressures. The results of this study include that, while the lineage with increased recombination has a higher variance than the recombinant line, the non-recombinant line has a higher variance than both. In addition, they find decreasing mean fitness with increasing recombination. These results are difficult to
interpret given the conditions under which linkage disequilibrium was generated and broken down.

Another set of studies exists in which the changes in variance are also difficult to interpret since the species was inseminated in the wild and reared in laboratory conditions. *Daphnia pulex* research undertaken by Deng and Lynch (1996) finds an increase in variance using several life history traits when evaluating a sexual lineage in comparison to an asexual lineage. Further complicating matters is that the variance results of this study are contrary to an earlier work on *Daphnia pulex* by Lynch and Deng (1994) in which they find variance decreases in the sexual lineage in comparison to the asexual lineage. The means of the life history traits in both studies decline in the sexual lineage in comparison to the asexual lineage (Deng and Lynch 1996, Lynch and Deng 1994). Given that both experiments involved insemination in the wild and rearing in a laboratory, inferences about the genetic associations are undeterminable.

An increase in variance and a decrease in mean fitness are in another article contrasting sexual versus asexual lines of *Chlamydomonas reinhardtii* (Kaltz and Bell 2002). In this experiment, the genetic associations that were broken down in the laboratory setting originated from a variety of geographic locations. As I asserted earlier, migration and selection can affect the linkage disequilibrium of a population. Consequently, mixing individuals exposed to different selection pressures makes it extremely difficult to make conclusions on exactly what happened to the associations in the recombinant and non-recombinant lines.

In stark contrast to the aforementioned studies, Kelley, Antonovics and Schmitt (1988) used *Anthoxanthum odoratum* to determine that a sexual lineage has a mean
fitness 1.43 times higher than an asexual counterpart. This result is in opposition to the previously noted works of the same nature. Unfortunately, the authors do not report the variance. Moreover, the effects of segregation and recombination cannot be disentangled from such a study, thus complicating conclusions.

As a result of the limitations of the aforesaid studies, an empirical undertaking testing the effects of recombination on mean and variance is not only warranted in evolutionary biology but essential to start unraveling the mystery for the persistence of sex.

I designed an experiment to test the effects of recombination in a lab-adapted population via a recombinant and non-recombinant line of *Drosophila melanogaster* (figure 1.3). I sampled a lab-adapted population for the recombinant and non-recombinant lines. I suspended recombination by the use of a balancer chromosome for the third chromosome. I performed a series of crosses to yield offspring from the same father in both the recombinant and non-recombinant lines and thus minimize any genetic noise. In similar earlier works, assaying the effects of recombination is confounded by the effects of segregation (Kelley et al. 1988). This is controlled for in the current experiment because I used phenotypically detectable chromosomes such that the wild type offspring in the third cross have one possible combination of chromosomes that parallel its counterpart in the alternate lineage. I used these offspring to obtain fitness measures by either mating them to for fecundity counts or weighing them, as fitness is often strongly correlated to body size.
Materials and Methods

The population

Using a controlled homozygous second chromosome obtained from a lab adapted stock population and a third chromosome that originated from 150 different lab adapted lineages, I created a density controlled cage population. I set up a new cage every two weeks and this initial set up day is considered day one of a new generation. I placed 30 vials of 100% yeast food with a sprinkle of yeast pellets in the cage for a 24 hour period; this was collection a. After the 24 hour period, I placed 15 vials, of 100% food with a few fresh yeast pellets on the surface, in the cage for 2.5 hours after which time I changed them for another 15 vials until 60 vials had been rotated into the cage; this was collection b. These time limitations assured that the vials were not overcrowded or suffer from density problems. I then maintained the cage on three to five bottles until I set up the next generation. On day 14, I set up a new cage, the next generation, using collection b that I carefully sorted by sex and counted. I controlled this cage to house no less than 1500 to no more than 2500 flies at one time. I repeated the process with 30 vials for 24 hours, and 60 vials in alternating shifts of 15 vials for 2.5 hours to start the proceeding generation.

The balancers

I collected males from lab-adapted stocks with phenotypically detectable markers of TM3Ser/+ and TM3SerSb/+ . From the population collection a, I collected virgin females (+/+) on day 8 through 11 of emergence and I collected TM3Ser/+ and TM3SerSb/+ males on day 11. I mated these males to the virgin females on day 14 to assure that the content on the chromosomes, other than the balancer chromosome, was from the current cage population. I repeated the process every generation so that the cage
generation was continually re-established and the males with the balancers were continually crossed with current cage population. I maintained vials for each line of balancers at five vials per generation and, after the tenth generation, I expanded these collections to 25-50 vials per generation. *Drosophila melanogaster* males are naturally non-recombinant and through the use of this feature and the balancers, I suppressed recombination in the non-recombinant lineage for one generation in both the female and male. I also used balancers to suppress recombination in females to isolate the recombinant and non-recombinant chromosomes in the second cross (figure 1.3). Using a balancer at this stage meant that the father’s exact chromosome to the offspring was phenotypically detectable which allowed me to select female offspring with only wild type chromosomes (+/+) in the third stage of the experiment. By selecting females with only wild type (+/+) I ensured that both sets of offspring from both treatments inherited the same chromosome from the father. As a result, any differences in the lineages are solely attributable to the mother.

The experiment

I collected Tm3Ser/+ virgin females and males and generation 15 (+/+) virgin females and males between day 8 and 11. On day 12, I set up 150 vials of 100% yeast food for the recombinant line containing a single virgin Tm3Ser/+ male and a single virgin Generation 15 (+/+) female. This cross yielded a recombinant female offspring for cross 2 (figure 1.3). For the non-recombinant line, I crossed a single Tm3Ser/+ female with a single Generation 15 (+/+) male per vial for 150 vials of 100% yeast food. This cross yielded non-recombinant offspring since recombination was suspended in the female by the use of the balancer (figure 1.3). I cleared both sets of crosses on the
seventh day of mating. To minimize environmental effects, I randomized the vials daily.

On day 10 through 13 from the initial day of mating, I collected virgin Tm3Ser/+ females from the recombinant and non-recombinant lines and I kept them in individual vials clearly demarcated with the emergence date. On average, 50% of the vials produced offspring. For Tm3Ser/+ females from non-recombinant and recombinant lineages, I randomly assigned a partner from the opposing lineage that had emerged a day later; half of the non-recombinant females were assigned a recombinant female from the following day’s emergence and the other half of recombinant females were assigned a non-recombinant female from the next day emergence (for example, if a female emerged on day 10 her partner was from day 11). I collected Tm3SerSb/+ males from the balancer lines and I mated a single male to the earliest emerging partner for 24 hours and then I paired him with the second partner in a new vial for 48 hours after which time I discarded him. The longer lay period with the second female avoided any effects of sperm depletion. I cleared the females from the vials on the seventh day after introduction. I randomized the vials daily to minimize environmental effects. Offspring began to emerge on the eighth day and I collected six wild type (+/+) females from day 8 through 12. For the purpose of measuring body weight, I froze three of these females immediately and the other three I kept for mating. On day 15, I mated the reserved females for one week with Generation 17 (+/+ ) males that I had collected on day 11 of a cage setup. I took fecundity counts on day 11 and day 15 from the initial day of mating in the vials. For the weighing, I baked the females at 65-72 degrees for a 24-hour period at which point I transferred them to an incubator maintained at 24 degree and 70% humidity for 24 hours before
weighing. I repeated the experiment for five blocks, for the fourth block I set up 250 vials in stage one for each line instead of 150.

Results

I conducted five blocks of the experiment. The first block of the experiment I dropped from analysis and deemed it a preliminary trial due to adjustments made to the protocol of the experiment after this block. I used Blocks 2-5 for analysis. Blocks 2, 3, 5 contained 150 females for each treatment at the first cross and block 4 contained 250 females from each treatment at cross 1 (figure 1.3). In total, I set up 700 females for the F1 crosses to make the recombinant lineages (+/+ female by Tm3Ser/+ male) and non-recombinant lineages (Tm3Ser/+ female by +/- male). Since these crosses contained a single female to a single male, many of the crosses had no offspring either due to male sterility or female infertility. Also, when few eggs were present in the vial, I have some trepidation that the yeast pellet overgrew the eggs and larvae. Approximately 50% of these vials contained no offspring, thus, there was 375 usable vials from each treatment for the second stage of experiment, cross 2 (figure 1.3). I have presented the results and statistical analyses in tables 1.3-1.11 and figures 1.4-1.8. I performed statistical analysis using JMP software.

Matched pairs

Of the 375 offspring from cross 1, there is data for 78 pairs for offspring weights from both mothers, while there is data on 142 pairs for fecundity. If in cross 3 the mother from cross 2 did not have enough female offspring for both tests, I assigned the female offspring to the fecundity portion of the experiment, hence the higher number of pairings.
This was done because weight is used as a proxy for fitness while fecundity is a direct measure of fitness.

The mean fitness for the recombinant line is 0.3565 for offspring weights and 73.24 for fecundity (table 1.3). For the non-recombinant line, the mean fitness is 0.3647 for offspring weight and 76.17 for fecundity (table 1.3). For offspring weights, the mean difference between the lineages is 0.00814, which is statistically insignificant ($t=1.23$, $p=0.2209$; table 1.4a, figure 1.4). For fecundity, the mean difference in offspring counts is 2.92 offspring between the lineages and this is also statistically insignificant ($t=0.8687$, $p=0.3865$; table 1.4b, figure 1.5). For neither offspring weights nor fecundity are the difference in means statistically different from zero, thus, these measures of fitness for matched pairs have no differences between the lineages.

The variance for the non recombinant lineage is 0.001585 for offspring weights and 1145 for fecundity (table 1.3). For the recombinant lineage, the variance is 0.002212 for offspring weights and 1004 for fecundity (table 1.3). As determined by four tests of unequal variance, there is no difference in the variance between the recombinant and non-recombinant lines (minimum $p=0.29$, for offspring weights d. f. num: 1, d.f. den: 154; for fecundity d.f. num: 1, d.f. den. 282).

To better understand the factors influencing the means of the lineages and to test the difference in means, I performed an ANOVA using block and treatment as fixed factors for offspring weight. For fecundity I performed an ANOVA using block and treatment as fixed factors in addition, whether the individual female was the first to be mated to the male at cross 2 was included as a factor. The results demonstrate that the effect of block is statistically significant (for offspring weights $p<0.0001$, d.f. num: 3, d.f.
den: 153; for fecundity p=0.065, d.f. num: 3, d.f. den: 281). Thus, each block of the experiment has significantly different results for weights and fecundity than other blocks. This is an experimental limitation. Since each block is statistically different from each other, I performed a Z-test in which each block was tested individually for their significance and assigned an appropriate weight. A Z-test is also called for because one of the blocks, block 4, had a much larger sample size in the fecundity pairs (four times that of block 2 and double that of blocks 3 and 5) and thus must be weighted appropriately in its contribution to the p-value. In addition, a weighted Z-test is more sensitive to small p-values being obscured by hefty p-values and is deemed more appropriate for this form of analysis (Whitlock 2005). In this method, I calculated a one-tailed p-value for each block and then weighted it by the reciprocal of its squared standard error of the effect size for the block (Whitlock 2005). From the Z-test, offspring weights are not statistically significantly different between the lineages (p=0.12; table 1.5a). In addition, the results of the Z-test indicate that the recombinant and non-recombinant lineages do not have differences in fecundity that are statistically significant (p=0.385; table 1.5b). Therefore, the mean fitness for the recombinant and non-recombinant lineages in this experiment are similar.

Unmatched individuals

Recall that I developed the experiment using single females for the first two stages and, as I have discussed, approximately 50% of the females had no offspring for cross 2. It is easy to envisage that, after cross 2 the number of complete data set pairs, in which both females bore offspring for cross 3, is a fraction of the original values. Because of this problem, I analyzed the data independently for differences in means and
variance between the lineages. Thus, for the recombinant line the data for offspring weights is from 180 females while the non-recombinant lineage has data for 149; this is more than double the number of data points available from the matched pairs comparison. For fecundity, the non recombinant lineage has data from 184 females while the recombinant lineage data is from 223 individuals. While I acknowledge that the data is not independent, I analyzed the data as independent values to investigate if there is a statistically significant signature in the treatments.

For the recombinant lineage the mean fitness is 0.3635 for offspring weights and 73.59 for fecundity (table 1.6). The non recombinant lineage has a mean fitness of 0.3664 for offspring weights and 74.97 for fecundity (table 1.6). I performed an ANOVA to test whether the means of the lineage were statistically different from each other and for fixed factors I used block, treatment and, for fecundity, I also included whether the female was first mated to the male in cross 2. For offspring weights the mean fitness of the recombinant and non recombinant lineage are not statistically different from each other (F: 0.3145, p=0.7304, d.f. num: 1, d.f. den: 327; table 1.7a, figure 1.6). This lack of differences between the lineages is present when I tested the means based on fecundity (F:0.9476, p=0.3885, d.f. num: 1, d.f. den: 405; table 1.7b, figure 1.7). Therefore the lineages are not different from each other based on mean fitness. Because the females experiences must be identical, I tested the effect of the partner assignment 2. Statistically, whether the female was mated first to the Tm3SerSb/+ male at cross 2, over the opposing partner, has no effect on fecundity counts (F: 0.5588, p=0.4552, d.f num: 1, d.f. den: 405; table 1.7b). There is an effect of block as seen in the matched pairs analysis (for offspring
weights F: 20.38, p>0.0001, d.f. num: 3, d.f. den 326; for fecundity F: 5.86, p=0.0006, d.f. num: 3, d.f. den: 404; table 1.7).

The variance values for the recombinant lineage are 0.0023 for offspring weights and 989.3 for fecundity (table 1.6). The variance for the non recombinant lineage is 1.0019 for offspring weights and 1330.7 for fecundity (table 1.6). Four tests for unequal variance on the offspring weights analysis revealed no significant differences between the lineages for unmatched individuals (minimum p-value=0.2, d.f. num:1, d.f. den: 327). There is, however, a statistically significant difference in the variance of fecundity counts as the non-recombinant lineage has a larger variance than the recombinant lineage (maximum p=0.0369, d.f. num: 1, d.f. den: 405; table 1.9a). In order to ascertain whether this difference in variance is due to environmental noise or an actual genetic contribution, I evaluated the data for the quantity of offspring contributing to each data point. The experimental design I set up is for two measures using six offspring, three offspring for weight and three for fecundity. Using three offspring aids in converging on the mean from each mother from cross 2. If the non-recombinant lineage, with the higher variance, has less offspring per female per test than the recombinant lineage, the difference in variance may be due to increasing sampling error with smaller samples. Indeed, in the non-recombinant lineage I scored significantly fewer number of offspring for fecundity (p=0.0448; table 1.8). As a result, I attempt to control for the difference in variance.

For each female from cross 2, I collected six female offspring where possible. Females were assigned fecundity crosses and assigned a letter a, b or c. Where only one female offspring was available, I assigned her letter a. For an initial attempt to control for differences in variance, I dropped the data for offspring b and c and only offspring a was
used to calculate variance, mean fitness and tested for unequal variance. If offspring $a$
failed to yield fecundity count, I used the values from either offspring $b$ or $c$, depending
on which was available. For only one offspring, four tests of unequal variance reveal that
the recombinant and non recombinant lineages are not statistically different from each
other (minimum $p=0.2294$, d.f. num: 1, d.f. den: 405; table 1.9b) I also performed this
test using a requirement of two offspring. In this case as well, differences between
variance of treatments are statistically insignificant (minimum $p=0.1999$, d.f. num:1, d.f.
den: 363; table 1.9c). The only case of significance is when all offspring are considered
(maximum $p=0.0369$, d.f. num: 1, d.f. den: 405; table 1.9a).

I conducted power analyses on both matched and unmatched data. Given the
current values for means for either measure of fitness, I queried how many flies are
necessary for these values to be significantly different from each other at an alpha level
of 0.05. The analysis revealed that, for matched pairs data, the experiment has to be at
least six times as large to yield significant results in offspring weights while one order of
magnitude higher in sample size is required for statistically significant results in
fecundity counts (table 1.10a, 1.10b). For unmatched data, the experiment must be an
order of magnitude larger for statistically significant results in offspring weights and at
least three times the size for statistically significant results in offspring counts (table
1.10c, 1.10d).

**Discussion**

There are no statistically significant differences in variance and mean fitness
between the recombinant and non recombinant lineages. One possible reason for this is
that the advantages of recombination are not evident in a population over one generation.
As I have discussed in the introduction, if no genetic associations exist in a population recombination has nothing to act on (figure 1.2c). Lack of genetic associations can be the result of two things: a lack of genetic variation such that each genotype is essentially the same or, a lack of associations between alleles such that no genetic combinations are disproportionately common or uncommon given the allele frequencies in the population. In light of the results, I will consider both possibilities.

A lack of genetic variance in this experiment is not plausible as I conducted a test for genetic variance using quantitative genetics to detect additive genetic variance using a sib-ship model. Genetic variance was significantly detected in body weights among male and female offspring (p<0.0001; table 1.11).

In the second possibility, there may be no genetic associations in the population, $LD=0$. Many population geneticists assert that infinitely large random mating populations with no selection should have no linkage disequilibrium because all combinations of alleles are present (Otto and Barton 2001). While this is conceivable, linkage disequilibrium is observed in similar lab maintained populations of *Drosophila melanogaster* with significantly more linkage disequilibria than natural populations on the third chromosome (Smit-McBride et al. 1988). A lack of genetic associations then, while possible, might not be the cause of having no differences in the recombinant and non-recombinant lineages. However, a lack of associations is not the only explanation that is plausible.

Power analyses demonstrate that the sample size of the experiment must be extensively larger, on the order of one to six magnitudes, before the differences that were detected between the lineages might be statistically significant. Alternatively, it is
possible that there are much larger differences between the treatments but that the sample size was so small that these differences were lost by chance. I sampled a large (1500<x>2500), density controlled population for each block of tests and the blocks were relatively small, ranging from 150 to 250 individuals per lineage. In addition, about 50% of all matings were lost at cross 2 of the experiment where recombinant and non-recombinant females were paired with the same male (figure 1.3). One female laying per vial, losing 50% of the matings and small sample size, increase the risk of losing detectable differences between the treatments by random chance. As a result, having no differences between the lineages may be due to experimental limitations.

Thus far, loss of information due to random chance, a lack of statistical power due to small sample size and an absence of genetic associations are reasonable explanations for a lack of significant results. However, if recombination is maintained because of its effects on the linkage disequilibrium of mutations, this may not have been detectable in this experiment because recombination was suppressed for only one generation. If it were possible to suspend recombination in Drosophila melanogaster for multiple generations without introducing outside chromosomes, significant differences in mean fitness and variance in fitness between a non-recombinant and a recombinant treatment may possibly be larger and easier to detect.

Detecting the effects of recombination in a constant environment is important because this is when sex should give way to parthenogenesis (Bell 1982). Theoretical stances on the persistence of sex, such as lottery models and the Hitchiker model, rely on the contributions of mutation which requires a changing environment in either time or space (table 1. 2) (Bell 1982). These theories have some support from studies on
heterogeneous environments (Lenormand and Otto 2000, Gordo and Campos 2008). The recombinant and non-recombinant lines having similar means and variance in fitness may be indicative that recombination is advantageous in situations where selection fluctuates. If such is the case, testing for the effects of breaking down linkage disequilibrium in fluctuating environments must be made in a heterogeneous environment after testing these same effects in a constant environment.

**Conclusion**

Empirical evidence is necessary to shed light on the theoretical stances for the evolution of sex. Something so simple as detecting whether the advantages of recombination are short-term or long-term has been illusory for too long in evolutionary biology. In this experiment, I investigated whether recombination brought about a change in variance and a change in mean fitness in a recombinant versus non-recombinant lineage of *Drosophila melanogaster*. The experiment resolved into undetectable differences between the recombinant and non-recombinant lines; this does not imply that the experiment is uninformative. It must be acknowledged that such a situation may be the result of experimental limitations such as small sample size in which the effects were undetectably lost by chance. Alternately, suppressing recombination for one generation may simply not be long enough to render differences in the lineages. Power analyses revealed a need for a much larger sample size. It is also possible that the population lacked genetic associations, however, this is, theoretically, most unlikely. Conversely, it is possible that the absence of significant differences may be representative of some theoretical premises and needs to be furthered by testing for the effects of breaking down linkage disequilibrium in fluctuating environments after testing for the same effects in a
constant environment. More empirical evidence is required in order to make inferences about the effects of recombination on mean fitness and variance in fitness. Such findings will help to shed the veil on the theories for the evolution of sex.
References:


*referred to in table 1.2.
Figure 1.1. Fitness and epistatic interactions

<table>
<thead>
<tr>
<th>Fitness: $W_{bf} = 1$, $W_{bf/Bf} = 1 - s_b - s_f$</th>
<th>$W_{bf} = (1 - s_b)(1 - s_f) + \epsilon$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Negative Epistasis:</td>
<td>If $\epsilon &lt; 0$ then the $bf$ fly is even less fit than expected. In this case, where body size is correlated to fitness, the smallest fly is smaller than expected. $W_{bf} &lt; (1 - s_b)(1 - s_f)$</td>
</tr>
<tr>
<td>![Negative Epistasis Image]</td>
<td>![Image showing flies with different body sizes]</td>
</tr>
<tr>
<td>b) Positive Epistasis:</td>
<td>If $\epsilon &gt; 0$ then $bf$ fly is more fit than expected. In this case where body size is correlated to fitness, the smallest fly is not as small as expected. $W_{bf} &gt; (1 - s_b)(1 - s_f)$</td>
</tr>
<tr>
<td>![Positive Epistasis Image]</td>
<td>![Image showing flies with different body sizes]</td>
</tr>
<tr>
<td>c) No Epistasis:</td>
<td>If $\epsilon = 0$ $W_{bf} = (1 - s_b)(1 - s_f)$ and $bf$ is as fit as expected.</td>
</tr>
<tr>
<td>![No Epistasis Image]</td>
<td>![Image showing flies with different body sizes]</td>
</tr>
</tbody>
</table>

Two alleles controlling for body size: $B$ and $F$. $BF$ are large flies, $Bf$ and $bF$ are intermediate, $bf$ are small flies.
Figure 1.2. The effects of recombination on associations

Two alleles controlling for body size: \( B \) and \( F \). \( BF \) are large flies, \( Bf \) and \( bF \) are intermediate, \( bf \) are small flies. Different situations create different measures of fitness and variance.

<table>
<thead>
<tr>
<th>Fitness: ( W_{BF} = 1, W_{bF/Bf} = 1 - s_b, 1 - s_f, W_{bf} = (1 - s_b)(1 - s_f) + \epsilon )</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) If we only have big flies and little flies then we have positive genetic associations. Recombination will break down these associations and cause intermediate flies in the next generation, thus reducing variance.</td>
</tr>
<tr>
<td>b) If we have only intermediate flies then we have negative genetic associations, as ( B ) is never found with ( F ) and ( b ) is never found with ( f ). Recombination will break these associations and form the extreme body sizes in the next generation, thus increasing genetic variance.</td>
</tr>
<tr>
<td>c) If all combinations of fly body sizes are found equally in a population then there exists no genetic associations and recombination will have no effect on the population.</td>
</tr>
</tbody>
</table>
Figure 1.3. Experimental design

<table>
<thead>
<tr>
<th>Recombinant:</th>
<th>Non Recombinant:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cross 1</strong></td>
<td></td>
</tr>
<tr>
<td>♀ wt/ +/+</td>
<td>♀ wt/ +/+</td>
</tr>
<tr>
<td>♂ Tm3Ser/+</td>
<td>♂ Tm3Ser/+</td>
</tr>
<tr>
<td><strong>Cross 2</strong></td>
<td></td>
</tr>
<tr>
<td>♀ +/Tm3Ser</td>
<td>♂ Tm3SerSb/+</td>
</tr>
<tr>
<td>♂ +/+</td>
<td>♀ Tm3Ser/+</td>
</tr>
<tr>
<td><strong>Cross 3</strong></td>
<td></td>
</tr>
<tr>
<td>♀ +/+</td>
<td>♀ +/+</td>
</tr>
</tbody>
</table>

3 +/+ female offspring weighed, 3 +/+ female offspring mated for fecundity counts

Crosses represent the different steps of the experiment. Chromosomal combinations are indicated in colored lines next to the fly, these are for the third chromosome only. Arrows indicate cross.
Figure 1.4. Matched pairs: offspring weights histogram of differences

Differences based of matched pairs of 78 individual from recombinant and non-recombinant lineages.
Mean 0.00814, standard deviation 0.0582.
Test statistic value 1.23, p =0.2209.
Figure 1.5. Matched pairs: fecundity histogram of differences

Differences based on matched pairs of 142 individuals from recombinant and non-recombinant lineages. Mean 2.92, standard deviation 40.1. Test statistic 0.8687, p=0.3865.
Figure 1.6. Unmatched individuals: ANOVA test of offspring weights by treatment

Non recombinant (N), recombinant (R).
Graph based on 148 individuals for the non-recombinant lineage and 180 individuals for the recombinant lineage.
F: 0.3145, p=0.7304 d.f. num:1, d.f den: 326.
Figure 1.7. Unmatched individuals: ANOVA test fecundity by treatment

Graph based on 184 individuals for the non-recombinant lineage and 223 individuals for the recombinant lineage.
F: 0.9476, p = 0.3885 d.f. numerator: 1, denominator: 405.

Figure 1.8. Unmatched individuals: fecundity: test for unequal variance by treatment

Results based on 184 individuals for the non-recombinant lineage and 223 individuals for the recombinant lineage.
Test results in table 1.19. maximum p = 0.0.369 for F: 4.35, d.f. num: 1, d.f. den: 405.
Table 1.1. Summary of the effects of recombination

<table>
<thead>
<tr>
<th>Associations and interactions</th>
<th>Genotypes before→after recombination</th>
<th>Effects of Recombination</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε&gt;0 LD&lt;0</td>
<td>$B_f+b_F→B_F+b_f$</td>
<td>Increases mean fitness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increases variance</td>
</tr>
<tr>
<td>ε&lt;0 LD&gt;0</td>
<td>$B_F+b_f→B_f+b_F$</td>
<td>Increases mean fitness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreases variance</td>
</tr>
<tr>
<td>ε&gt;0 LD&gt;0</td>
<td>$B_F+b_f→B_f+b_F$</td>
<td>Decreases mean fitness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreases variance</td>
</tr>
<tr>
<td>ε&lt;0 LD&lt;0</td>
<td>$B_f+b_F→B_F+b_f$</td>
<td>Decreases mean fitness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increases variance</td>
</tr>
<tr>
<td>ε =0 LD&gt;0</td>
<td>$B_F+b_f→B_f+b_F$</td>
<td>Decreases variance</td>
</tr>
<tr>
<td>ε =0 LD&lt;0</td>
<td>$B_f+b_F→B_F+b_f$</td>
<td>Increases variance</td>
</tr>
<tr>
<td>ε &lt;&lt;0 W_{BF}&gt;(W_{B_f}+W_{b_F})/2 LD&lt;0</td>
<td>$B_f+b_F→B_F+b_f$</td>
<td>Decrease mean fitness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increase variance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Long term advantage from producing the most fit can outweigh immediate detriment of producing the least fit</td>
</tr>
<tr>
<td>LD=0</td>
<td>$B_f+b_F+B_F+b_f→B_f+b_F+B_F+b_f$</td>
<td>No effect</td>
</tr>
<tr>
<td>Hypothesis</td>
<td>Situation</td>
<td>Mean Fitness after 1 round of Recombination</td>
</tr>
<tr>
<td>------------</td>
<td>-----------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Fisher-Müller Hypothesis (Fisher 1930, Muller 1932, Bell 1982)</td>
<td>Deleterious mutations accumulate in population such that (through drift/mutation) the most fit genotype/load is lost and the population is unable to restore that lost genotype.</td>
<td>Decreases because of the creation of many misfits but chance creation of extremely fit</td>
</tr>
<tr>
<td>Ratchet (Muller 1964, Bell 1982, Koella 1993)</td>
<td>Chance creation of genotypes with extremely high fitness which will be present disproportionately in the next generation.</td>
<td>Possibility of decreased, on average, as recombination increases genetic load</td>
</tr>
<tr>
<td>Best Man Hypothesis (Williams 1966, Bell 1982)</td>
<td>The more combinations you have, the better the chance of having one that’s extraordinarily fit in each environment (at some point, in all of the possible combinations one is achieved that is extraordinarily fit). Parents must contribute &gt;1 offspring, and at least one to each environment and only 1 offspring of all will survive.</td>
<td>Under strict conditions (greater than 5 environments and parents contributing to environments other than the one in which they live) can provide short term advantage thus increase in mean</td>
</tr>
<tr>
<td>Sib-Competition Model (Maynard Smith 1976)</td>
<td>Opposite of lottery, only one genotype has zero fitness.</td>
<td>Decreased as least fit genotype is recreated</td>
</tr>
<tr>
<td>Sib-Competition Lottery Model (Bell 1982)</td>
<td>Opposite of lottery, only one genotype has zero fitness.</td>
<td>Opposite of lottery, only one genotype has zero fitness.</td>
</tr>
<tr>
<td>Runt Model (Bell 1982)</td>
<td>The rate at which an allele will propagate depends on the fitness of the chromosome/individual it arises in. Modifier of recombination will link to high fitness chromosome. An allele causing an increase in recombination is advantageous because it allows flexibility in responding to selection.</td>
<td>Decreased (sex retards the process of obtaining the most fit genotype)</td>
</tr>
<tr>
<td>Hitchhiker (Bell 1982)</td>
<td>A clone will proliferate and be at maximal fitness in an environment it is adapted to, but if the environment is heterogeneous in space then it will be maladapted to some regions and its sexual rival can spread and force the clone to extinction.</td>
<td>Possibly decreased</td>
</tr>
<tr>
<td>Red Queen (Peters and Lively 1999)</td>
<td>Based on negative frequency dependent selection. Host-parasite coevolution: selection for parasite genotype that can infect the most common host genotypes, thus rare host genotypes become more fit and increase in frequency, after which parasites that are adjusted to the newly emerged common host will be selected for.</td>
<td>Decreased when parasite is adapted to common genotype, increased when parasite is adapted to new common genotype. Under the right fluctuating conditions this can always be beneficial in the short term</td>
</tr>
<tr>
<td>Mutation Deterministic Hypothesis (Kouyos et al. 2007)</td>
<td>The deleterious mutations are purged from populations by breaking down the linkage disequilibrium built by epistasis.</td>
<td>Decreased (while certain conditions can increase LD and LD&gt;0)</td>
</tr>
</tbody>
</table>
Table 1.3. Matched pairs basics: fitness measures

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Offspring weights</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non Recombinant</td>
<td>0.3647</td>
<td>0.001585</td>
</tr>
<tr>
<td>Recombinant</td>
<td>0.3565</td>
<td>0.002212</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecundity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non Recombinant</td>
<td>76.17</td>
<td>1145</td>
</tr>
<tr>
<td>Recombinant</td>
<td>73.24</td>
<td>1004</td>
</tr>
</tbody>
</table>

Table 1.4. Matched pairs differences: fitness measures

|                  | Number | Mean   | St Dev | Test Stat | Prob>|t| |
|------------------|--------|--------|--------|-----------|------|
| Offspring weights | 78     | 0.00814| 0.0582 | 1.23      | 0.2209 |
| Fecundity        | 142    | 2.92   | 40.1   | 0.8687    | 0.3865 |

Table 1.5. Matched pairs differences: weighted Z-test

<table>
<thead>
<tr>
<th></th>
<th>block</th>
<th>ttest</th>
<th>Z value</th>
<th>st error</th>
<th>weight (1/(stdE)^2)</th>
<th>weighted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Offspring weights</td>
<td>2</td>
<td>0.3215</td>
<td>0.4635</td>
<td>0.01</td>
<td>9944.39</td>
<td>4609.23</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.3726</td>
<td>0.325</td>
<td>0.0118</td>
<td>7241.609</td>
<td>2353.52</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.0405</td>
<td>1.745</td>
<td>0.0167</td>
<td>3580.913</td>
<td>6248.69</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.1138</td>
<td>1.207</td>
<td>0.0096</td>
<td>10826.28</td>
<td>13067.32</td>
</tr>
<tr>
<td></td>
<td>√(wi)^2</td>
<td>16773.89</td>
<td>1.567</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>combined p-value (two tailed):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>block</th>
<th>ttest</th>
<th>Z value</th>
<th>st error</th>
<th>weight (1/(StdE)^2)</th>
<th>weighted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecundity</td>
<td>2</td>
<td>0.3272</td>
<td>0.448</td>
<td>8.3117</td>
<td>0.01448</td>
<td>0.006487</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.4259</td>
<td>0.187</td>
<td>7.6122</td>
<td>0.01726</td>
<td>0.003228</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.3042</td>
<td>0.512</td>
<td>5.2526</td>
<td>0.03624</td>
<td>0.018555</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.2677</td>
<td>0.62</td>
<td>6.8912</td>
<td>0.02106</td>
<td>0.013057</td>
</tr>
<tr>
<td></td>
<td>√(wi)^2</td>
<td>0.047586</td>
<td>0.86846</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>combined p-value (two tailed):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.385</td>
</tr>
</tbody>
</table>
Table 1.6. Unmatched individuals basics: fitness measures

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Means</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non Recombinant</td>
<td>148</td>
<td>0.3664</td>
<td>0.0019</td>
</tr>
<tr>
<td>Recombinant</td>
<td>180</td>
<td>0.3635</td>
<td>0.0023</td>
</tr>
</tbody>
</table>

Table 1.7. Unmatched individuals: ANOVA

<table>
<thead>
<tr>
<th></th>
<th>d.f. num</th>
<th>d.f. den</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Prob&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>3</td>
<td>326</td>
<td>0.1089</td>
<td>0.0363</td>
<td>20.38</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>treatment</td>
<td>1</td>
<td>327</td>
<td>0.0013</td>
<td>0.00066</td>
<td>0.3145</td>
<td>0.7304</td>
</tr>
</tbody>
</table>

* mated first was not applicable for weights

Table 1.8. Unmatched individuals: number of offspring scored

<table>
<thead>
<tr>
<th></th>
<th># of offspring scored</th>
<th>t-test on # of scored</th>
<th># of offspring scored</th>
<th>t-test on # of scored</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non Recombinant</td>
<td>2.41</td>
<td>p=0.6879</td>
<td>2.35</td>
<td>p=0.0448</td>
</tr>
<tr>
<td>Recombinant</td>
<td>2.43</td>
<td></td>
<td>2.52</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.9. Unmatched individuals: test for unequal variance in fecundity

<table>
<thead>
<tr>
<th>Test</th>
<th>F</th>
<th>Prb&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) For all offspring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O'Brien</td>
<td>6.57</td>
<td>0.0108</td>
</tr>
<tr>
<td>Brown-Forsythe</td>
<td>4.68</td>
<td>0.0312</td>
</tr>
<tr>
<td>Levene</td>
<td>6.34</td>
<td>0.0122</td>
</tr>
<tr>
<td>Bartlett</td>
<td>4.35</td>
<td>0.0369</td>
</tr>
<tr>
<td>d.f.</td>
<td>Numerator:1, denominator 405</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F</th>
<th>Prb&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>0.0588</td>
</tr>
<tr>
<td>O'Brien</td>
<td>1.44</td>
</tr>
<tr>
<td>Brown-Forsythe</td>
<td>1.16</td>
</tr>
<tr>
<td>Levene</td>
<td>1.35</td>
</tr>
<tr>
<td>Bartlett</td>
<td>0.756</td>
</tr>
<tr>
<td>d.f.</td>
<td>Numerator:1, denominator 405</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F</th>
<th>Prb&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>0.3651</td>
</tr>
<tr>
<td>O'Brien</td>
<td>1.6495</td>
</tr>
<tr>
<td>Brown-Forsythe</td>
<td>0.3799</td>
</tr>
<tr>
<td>Levene</td>
<td>0.5860</td>
</tr>
<tr>
<td>Bartlett</td>
<td>1.0807</td>
</tr>
<tr>
<td>d.f.</td>
<td>Numerator: 1, denominator: 363</td>
</tr>
</tbody>
</table>
Table 1.10. Unmatched individuals: power analysis

<table>
<thead>
<tr>
<th></th>
<th>α</th>
<th>σ</th>
<th>δ</th>
<th>Least Significant Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Offspring weights (matched pairs)</td>
<td>0.05</td>
<td>0.0436</td>
<td>0.0041</td>
<td>442.13</td>
</tr>
<tr>
<td>b) Fecundity (matched pairs)</td>
<td>0.05</td>
<td>32.78</td>
<td>1.46</td>
<td>1935.6</td>
</tr>
<tr>
<td>c) Offspring weights (unmatched)</td>
<td>0.05</td>
<td>0.04585</td>
<td>0.00201</td>
<td>3136.52</td>
</tr>
<tr>
<td>c) Fecundity (unmatched)</td>
<td>0.05</td>
<td>33.8</td>
<td>2.3</td>
<td>1292.9</td>
</tr>
</tbody>
</table>

Table 1.11. Test for variance: Offspring weights from 3 crosses per Sire

<table>
<thead>
<tr>
<th></th>
<th>Offspring Weighed</th>
<th>Mean</th>
<th>St Dev</th>
<th>F</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>42 sires</td>
<td>212</td>
<td>0.217</td>
<td>0.024</td>
<td>3.295</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>d.f. Numerator: 41 Denominator: 171</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 sires</td>
<td>321</td>
<td>0.301</td>
<td>0.03</td>
<td>3.141</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>d.f. Numerator: 39 Denominator: 281</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 2

Recombination rate and variable genotypes
Introduction

Recombination has an important role in evolutionary biology because it alters the relationship between genes in a population. Namely, changes in recombination affect the linkage disequilibrium of a population and alter how the genes are packaged. If selection is working to create favorable genotypes why break them up? There are many theories that discuss the benefits of recombination; however, few situations exist in which recombination is advantageous. To shed light on why recombination persists, the basic biology of recombination must be understood. One particular aspect of the biology of recombination is plasticity, or the capacity to modify recombination rate. Reasons for the persistence of this flexibility may help explain the advantages of recombination.

Theoretically, plasticity in recombination rates is selectively neutral in diploid populations (Agrawal et al. 2005). Experimental work, which has been ongoing for nearly a century, demonstrates that recombination is not simply a fixed value, but that it might be flexible and influenced by multiple phenomena, such as an increase in recombination rate due to stress (Plough 1917, Lawrence 1958). This leads to the question of whether changes in recombination rate reflect a general response to poor condition. Currently, little empirical evidence is available to substantiate whether internal influences on condition can impact recombination rates; specifically, the effect of variable genotypes (Zhuchenko et al. 1986, Tucic et al. 1981, Lawrence 1963). In this chapter I will investigate whether recombination can be influenced by variable condition caused by a variety of dominant mutations with known fitness effects.

Of the phenomena studied for its effects on recombination, stress predominates. An increase in recombination due to stress is posited as a means of adaptation (Parsons
1987). The basis of this reasoning is that high fitness genotypes suppress recombination and pass on their fitness effects to their offspring, whereas a low fitness genotype increases recombination to make many genetically variable offspring to potentially create one or some that are more fit. Several forms of stress are addressed in the literature, but few incorporate variable genotypes (see table 2.1) (Tucic et al. 1981, Lawrence 1963, Zhuchenko et al. 1986, Marinkovic et al. 1980.)

**Environmental stress: external stressors**

Environmental stress is examined in a variety of ways to determine its effects on the rate of recombination. Multiple studies find that environmental stress impacts the frequency of recombination (Parsons 1988). The effect of temperature on recombination rate is the prime environmental stress examined in the literature. Significant effects of temperature on recombination are found across several species including tomato plants, *Drosophila* and *Caenorhabditis elegans* (Zhuchenko et al. 1986, Hayman and Parsons 1960, Graubard 1932, Plough 1917, Plough 1921, Lawrence 1958, Rose and Baillie 1979, Kovalchuk et al. 2003, Lebel et al. 1993, Tracey and Dempsey 1981). The majority of these studies find that increasing environmental stress causes an increase in the rate of recombination.

In a vast array of taxa, other environmental stressors significantly influence recombination (see table 2.1). Stress in the form of crowding caused an increase in crossing over in male mice (Belyaev and Borodin 1982). Larval starvation in *Drosophila melanogaster* caused an increase in recombination (Neel 1941). Nutritional stress was also found to activate increased recombination in yeast (Abdullah and Borts 2001). Pathogen stress was demonstrated to increase recombination in *Arabidopsis* (Kovalchuk
Temporary increases in recombination were found with additional mating bouts in *Drosophila melanogaster*, analogous to environment-induced recombination (Priest et al. 2007). Ionizing radiation, the addition of motomycin C and heat shock increased the frequency of recombination in tobacco plants (Lebel et al. 1993). Not all experiments, however, provide significant results. Indeed, Plough (1917) detected effects of neither moisture content nor quantity of food fermentation on recombination rates in *Drosophila*. Despite these studies, the flexibility of recombination in response to environmental stress is well established in evolutionary biology.

**Genotype: internal stressors**

The studies on environmental stress are informative and supportive of the idea of plastic recombination; however, they lead to a deep-seated question: Is increased recombination a general response to poor condition? If so, the results should be reproducible by making individuals in poor form genetically. Herein the term genomic stress is used to identify poor condition caused by a genotype. Because it is necessary to uncover what is happening at the very basic biological level, examining the genotype and its impact on recombination is essential. The relation of fitness to changes in recombination makes the biology of recombination accessible because fitness is a measure of the extent of stress. Recombination acts on linkage disequilibrium in a population; increasing or decreasing recombination will alter how the genotype itself is distributed in the following generation. Accordingly, if recombination rate changes in response to condition created by variable genotype, this may help to explain the advantages of recombination.
For the effects of genotype on recombination, experimental evidence is scant. Studies demonstrate that changes in genomic structure cause an increase in recombination rates; these studies include the use of inversions and ploidy levels (Schultz and Redfield 1951, Francis et al. 2007, Rendel 1957, Grell 1978). The effects on recombination rates of structural changes have also been studied in combination with genotypic elements. Hayman and Parsons (1960) attempted to test the effect of age, the presence of an inversion and a change in temperature on recombination rates in *Drosophila melanogaster*; but the data was too heterogeneous to make inferences about the interaction of the three. Graubard (1932) combined an inversion with temperature changes and found no effect of temperature with the presence of an inversion. However, unlike the environmental stressors that I have previously discussed, inversions have a direct effect on recombination rate. Given that inversions suppress recombination in a region of the genome, it is compensated for in another region of the genome. Thus, structural elements cause an increase in recombination by directly altering recombination rate. In the current study, I attempt to uncover the indirect effects on recombination by changing the condition of the organism. Similarly, some studies use different conditions to evaluate the effects of variable genotypes on recombination rate.

In tomato plants, a change in environment affected recombination rate depending on the climate to which the plant was adapted; cold-resistant forms in cold climates had no change in recombination rate but increased temperature led to a 12% increase in recombination and vice versa for non-cold resistant plants with an 8% increase in recombination at cold temperature (Zhuchenko et al. 1986). This work suffers two problems: First, the authors perform their analysis on several hundred cells per plant, but
the unit of replication is an individual plant. Second, the data cannot be readily
generalized because only a single lineage was used in each treatment (Zhuchenko et al.
1986). The experiment is, nonetheless, informative because neither genotype nor
environment was sufficient to modify recombination rate; instead, it had to be a specific,
maladaptive, combination of the two. Indeed, Parsons (1987) asserts, the most effective
way to maximize the manifestation of genetic variability is by stimulation from extreme
environmental stress. But, as evidenced by this study, the environmental stress must be
genotype specific.

In another series of studies, the influence of genetic content on recombination
rates was investigated (Lawrence 1958, 1963). Five different inbred lines were used to
test the effect of both crosses between the lines and the lines themselves on the frequency
of recombination at two different temperatures (Lawrence 1963). At low temperatures,
the different lineages varied significantly in recombination rates, increasing as much as
25% in some regions (table 2.1). Specifically, the differences in recombination rate
between the lines are attributable to additive genetic variance, or the additive effect of
substituting one allele for another (Lawrence 1963). Thus, the genotype itself was
responsible for the differences in the recombination rates. This conclusion is applicable
only at stressful temperatures for the experimental lineages as there are no significant
differences between the lineages at normal temperature (Lawrence 1963). Fitness is not
reported for either experiment. Therefore, even though the differing genotypes exhibited
variable recombination rates in response to stress, the extent of this relationship cannot be
evaluated.
What if an experiment solely tested the effects of genomic stress on recombination? Tucic et al. (1981) tested the influence of a variety of wild-type chromosomes of *Drosophila melanogaster* on rates of recombination. They found a negative correlation between fitness and recombination; females with the highest fitness had the lowest recombination rates and vice versa (Tucic et al. 1981). However, they used natural variation without knowing the source of that variation. Within the different chromosomes there can be genes that: directly control recombination, influence DNA repair ability or interact with the markers used to measure recombination. Nonetheless, these possibilities should obscure the data rather than provide a false positive result. For the purposes of the current experiment, I will assume that no confounding factors are present in their analysis. Accordingly, the results should be reproducible by applying genes with known fitness. What happens to recombination if the only thing that is variable is the genotype?

I designed a *Drosophila melanogaster* experiment to address how a known mutational complement alters recombination rates (figure 2.1). I designed the experiment to address several other related features including: the relationship between the fitness effects of the mutational complement and the rate of recombination and the influence of the mutational complement on a known recombination to age relationship. I tested the effects of dominant markers on one chromosome with respect to recombination rate on a different chromosome. I backcrossed all of the markers with a lab adapted stock population. As a result, aside from the mutations themselves, there is a similar genetic background in the remainder of the chromosomes between the genotypes. I constructed control lines, with no mutations, to have the same genetic background as the dominant
markers (table 2.2). Based on the results of the aforementioned studies, I queried whether bearing the dominant marker, or having a bad genotype, causes an increase in recombination relative to not bearing that marker.

**Materials and Methods**

**Overview**

I conducted the experiment on two different sets of genotypes. For the first set, herein referred to as \( C2 \), I used eight different dominant markers on the third chromosome to influence recombination between three recessive markers on the second chromosome. In the second set of the experiment, herein referred to as \( C3 \), I used six dominant markers on the second chromosome to manipulate recombination between three recessive markers on the third chromosome. For the experiment, I crossed females heterozygous for the triple recessive mutations and the dominant marker, or the control, to two homozygous triple recessive mutant males. I transferred the females with their males into new vials ever two to three days. In all, I bred 100 females from each genotype and each wild type. For the dominantly marked genotypes, I divided the offspring into dominantly marked and unmarked piles. I counted the dominantly marked offspring as a whole but I did not score them for recombination because of the possibility of fitness interactions between the dominant marker itself and those markers used to measure recombination. For the unmarked offspring pile, herein referred to as the scorable offspring, I divided the individuals into phenotypically distinct groups and recorded the number of offspring for each group. For the control lines, I divided the offspring by phenotype and I counted each phenotypically distinct group. I calculated a recombination score based on the distribution of offspring.
Recessive mutations: triple mutants

I created two homozygous recessive triple mutant genotypes through a series of crosses (table 2.3). For the first set of recessive markers, C2, I constructed a genetic combination of markers on the second chromosome composed of the recessive mutations black (b), cinnabar (cn) and vestigial wing (vg). For the second set of recessive markers, C3, I constructed a combination of recessive markers on the third chromosome containing the markers sepia (se), blistery (by), and spineless (ss). The map location of markers on the second chromosome is: b at 48.5, cn at 57.5 and vg at 67.0. The map location for the markers on the third chromosome include: se at 26.0, by at 48.7, and ss at 58.5. Because recombination about the centromere is exacerbated by stress, I selected the markers to span the centromere (Lawrence 1963, Suzuki and Parry 1964). In addition, I selected small recombination regions in order to reduce undetectable double crossovers.

Treatment: dominant mutations

C2

For C2, I used eight dominant mutations on the third chromosome to generate poor condition in an attempt to alter recombination between the recessive markers of the second chromosome. The eight markers on the third chromosome were Prickly (Pr), Glued (Gl), Rough (R), Stubble (Sb), Drop (Dr), Kinked (Ki), Antennapedia (Antp) and Kidney (Kd). I repeatedly backcrossed all of the dominant marker genotypes to a lab adapted stock population for a minimum of ten generations. The genotypes, thus, are homogeneous for their genetic background on the second chromosome (+/+). M will forthwith be used to denote any and all dominant markers.
To avoid including any dominantly marked offspring in the scorable offspring, I determined whether a recessive marker could mask the appearance of the dominant marker. I tested the mutations in a cross by first crossing males bearing the dominant marker (+/+ ; +/M) to females homozygous for the triple recessive markers, (b cn vg/b cn vg ; +/+). I collected the offspring from these crosses bearing the dominant marker on the third chromosome (b cn vg/+ ; +/M) and I crossed them to each other to yield a variety of offspring. The recessive markers did not mask the dominant mutations to which they were crossed.

C3

In C3, I created poor condition using six dominant mutations on the second chromosome in an attempt to change recombination between recessive markers of the third chromosome. These six markers include: Additional Vein (Adv), Glazed (Gla), Blackoid (Bkd), Narrow Blade (nwB), Upturned (U) and Freckled (Frd). For all of these markers, I repeatedly backcrossed them to a lab adapted stock population, for a minimum of ten generations. The markers, then, are on similar genetic background on the third chromosome (+/+). As I have previously discussed, I ran a test cross to ensure that the recessive markers did not mask the dominant markers.

Controls

I extensively crossed the dominant markers and recessive markers with a lab adapted stock population to homogenize the backgrounds of the genomes. Thus, between any two dominant markers the only difference between the genomes is the marker. Since the goal of the experiment is to test the effect of the dominant mutation on recombination rate, it is important to ensure that the only thing that could alter recombination is that
dominant marker. I built wild type controls to obtain individuals homogeneous for the genetic background of the dominant markers. Essentially, since nothing considerably detrimental is in their genome that is not present in the remaining dominant markers, these are high condition lines. I built wild type controls on the pertinent chromosome by crossing and reciprocally crossing two dominant mutations not being used in the experiment (table 2.2). I collected virgin females and males, bearing the two dominant mutations, eleven days after the initial set up from both crosses (table 2.2). I combined the offspring from the cross and reciprocal cross and I crossed them in sets of six females to four males (table 2.2, see F1). For the third cross, I mated virgin individuals with opposing mutations, that I collected from the offspring of the F1 double mutant cross (table 2.2, see F2). From these crosses, I collected wild type males and females from which I maintained control lines (+/+ ; +/+).

Creating the combinations

I mated two to three males, from each dominant mutation, to two to five triple recessive homozygous females, per vial in sets of 16 to 25 vials on 100% cornmeal food. For C2, I crossed males (+/+ ; +/M) to females (b cn vg/b cn vg ; +/+). For C3, I crossed males (+/M ; +/+ to females (+/+ ; se by ss/se by ss). I conducted the same cross for the control lines consisting of crossing males (+/+ ; +/+ to triple recessive mutant females (b cn vg/b cn vg ; +/- for C2, or +/- ; se by ss/se by ss for C3). I checked the vials daily. When a male or female died, I replaced them immediately. I transferred the matings into new vials after three days. I watered all of the vials on the third day and again on the sixth day, where necessary. I collected virgin females bearing a dominant mutation on day ten or day eleven, depending on which day yielded the most females. For C2, I collected
females with the heterozygous dominantly marked genotype (*b cn vg/+ ; +/M*). For C3, I collected females with the heterozygous dominantly marked genotype (*M/+ ; +/se by ss*).

**The experiment**

For each block, I used females that emerged on the same day. I used no more than three females from any one source vial. For each of the dominant mutations and the control lines, I setup 15-35 females per block, depending on the number of females collected from the aforementioned cross. I setup each vial three days after I collected the females. In each vial I placed one female in addition to two homozygous recessive triple mutant males (see figure 2.1 for experimental design). Accordingly for C2, I crossed a marked female (*b cn vg/+ ; +/M*) to two homozygous males (*b cn vg/b cn vg ; +/+*).

Whereas for C3, I crossed a female (*M/+ ; +/se by ss*) to two males (*+/+ ; se by ss/se by ss*). For the first two weeks of matings, I transferred the three individuals of a vial to a new vial every second day. After which time I transferred the three individuals into new vials every third day of mating. I replaced males by aspiration if they were between seven and nine days of age or sooner if they died. Females were mated until they died. In order to keep egg production and nutrient availability ongoing while avoiding excessive yeast growth, I applied a yeast dilution of 2 g of yeast pellets to 5 ml of water on the 100% cornmeal food using an ultra thin paintbrush prior to transferring the trios.

I repeated the blocks until 100 females from each dominant marker or control genotype was completed. For C2, I performed five overlapping blocks starting in December of 2007 (last block started in February of 2008). For C3, I conducted five overlapping blocks starting in March of 2008 (last block started in June of 2008). As *Frd* has low offspring counts, I mated 130 females of this dominant mutation to ensure the
availability of data for this genotype. I watered vials on the fifth day after the female’s introduction into the vial.

**Scoring**

12 to 15 days after a female occupied a vial, I performed an initial scoring of the vial, time permitting. Since density decreased after the eighteenth day of mating, I did not conduct an initial score after the ninth vial. For scoring, I divided the offspring into two piles: a dominantly marked pile and a pile lacking a dominant marker. For the marked pile, I counted the offspring as a whole and discarded them (see figure 2.1). I did not use these flies to measure recombination in case of the occurrence of interactions between the dominant markers and the recessive markers. For the pile lacking dominant markers, or the scorable offspring, I divided the offspring into eight different phenotypic combinations of recessive markers and I recorded the number of offspring in each of these piles. For control lines, I omitted the first step and I divided the offspring among eight possible combinations of recessive markers. On the seventeenth day from a female’s introduction into a vial, I scored the vials in the same manner as the initial score for a second and final time. For vials that I had not scored on day 12-15, I scored them for their first and only time on day 17. I kept all vials in trays in the same environmental chamber and I randomized them within their respective trays every transfer that they were directly involved in and on day five when I watered them. I randomized the trays every day or every second day in the environmental chamber.

**Results**

I performed statistical analysis using JMP software.
Fitness and variation

For the dominantly marked females, I measured fitness using fecundity and viability. For the lines with dominant markers, I doubled the number of scorable offspring and I used this value for fecundity because this is the number of offspring that are produced if there is no selection penalty incurred by the marker. For wild type markers, I measured fecundity as the total number of offspring produced by a female in her lifetime. I performed an ANOVA to determine that the fecundity means of the genotypes are variable (for $C_2$, $F:37.26$, $p<0.0001$, d.f. num: 13, d.f. den: 940; for $C_3$, $F:20.66$, $p<0.0001$, d.f. num: 11, d.f. den: 714; figure 2.2 and figure 2.3). I conducted a Tukey-HSD test to evaluate which of the genotypes are significantly different from each other; in $C_2$ there are six statistically significant groupings and in $C_3$ there are four significant groupings (figure 2.2 and figure 2.3). I measured viability using the frequency of the dominantly marked offspring to calculate selection for each genotype and then I subtracted this selection cost from one. I assigned a viability value of one to wild type controls. I conducted an ANOVA which revealed that the viability means of the genotypes are significantly variable (for $C_2$, $F: 173.16$ $p<0.0001$ d.f. num 13, d.f. den: 940; for $C_3$, $F: 121.51$ $p<0.0001$, d.f. num 11, d.f. den: 714). These analyses demonstrate that the lines with dominant markers and the control lines are significantly variable with respect to fitness.

Recombination and variation: rates, locations, and recessives

I calculated recombination rates in two manners. From the different genotypes, I quantified recombination based on the average number of recombination events required to produce a given phenotype among the scorable offspring. I assigned a zero for each
offspring produced with no recombination, a one for each offspring produced with one
recombination event and a two for each offspring produced with two recombination
events (see figure 2.1). For C2, with recessive markers on the second chromosome, I
assigned a female a zero for every non-recombinant offspring (+/b cn vg or b cn vg/b cn
vg), a one for an offspring with a single recombination event (b++/b cn vg, b cn +/b cn vg,
+ cn vg/b cn vg, or + + vg/b cn vg), and a two for a double recombinant offspring
(+cn+/b cn vg or b + vg/b cn vg). For C3, with recessive markers on the third
chromosome, I assigned a female a zero for every non-recombinant offspring (+/se by ss
or se by ss/se by ss), a one for a single recombinant offspring (se ++/se by ss, se by +/se
by ss, + by ss/se by ss, + + ss/se by ss), and a two for a double recombinant offspring (se
+ ss/se by ss or + by +/se by ss). To obtain a recombination rate, I summed these
assignments over a vial and divided by the total number of scorable offspring. Since
males have no recombination events, the phenotypically detectable genotypes are
reflections of recombination events in females only.

I calculated average lifetime recombination rate for each genotype by summing
the scores of offspring produced by a female across all vials that she occupied. I tabulated
a score in the same manner as previously described where each offspring genotype was
weighted by the number of recombination events necessary to create the phenotypic
combination. I performed an ANOVA and determined the genotypes are significantly
variable with respect to recombination rates (for C2 F: 7.86, p<0.0001, d.f. num: 14, d.f,
den: 870; for C3, F: 12.56, p<0.0001, d.f. num 11, d.f. den: 678; figure 2.4 and figure 2.5).
I examined the differences between the genotypes using a Tukey-HSD test; for C2, there
are 3 statistically significant groupings and for C3, there are six statistically significant
groupings (figure 2.4 and 2.5). Because of these analyses, there is significant variation between the different genotypes with respect to recombination rates.

In both sets of the experiment, I investigated two regions of recombination. For $C_2$, these regions consisted of region one, between the recessive markers $b$ and $cn$, and region two between recessive markers $cn$ and $vg$. For $C_3$, region one spanned recessive markers $se$ to $by$ and region two spanned recessive markers $by$ to $ss$. I subtracted the number of recombination events in region two from the number of recombination events in regions one in order to compare the distribution of recombination events for each genotype (figure 2.6 and figure 2.7). Across the majority of genotypes, more recombination events occur in region one than the second region and I used an ANOVA to determine that there is significant variation between the genotypes (for $C_2$, F: 4.33, p<0.0001, d.f. num: 13, d.f. den: 904; for $C_3$, F: 8.19, p>0.0001, d.f. num: 11, d.f. den: 705).

I investigated coincidence and interference using the two regions of recombination in each of the experimental sets. Coincidence is a measure of recombination occurring in two regions concurrently. Interference is the ability of one region to suppress recombination in another therefore, increased coincidence is negative interference and decreased coincidence is positive interference. I tabulated coincidence by taking the number of observed occurrences of double recombinants and subtracting the number of expected based on single recombination events. Coincidence values for each genotype are significantly variable as I determined from conducting an ANOVA (for $C_2$, F: 5.96, p<0.0001, d.f. num: 13, d.f. den: 865; for $C_3$, F: 4.43, p<0.0001, d.f. num: 11, d.f. den: 690; figure 2.8 and figure 2.9). I used a Tukey-HSD test to reveal the
significance of the differences among genotypes; for C2, there are 5 significant groupings, for C3, there are 3 significant groupings (figure 2.8 and figure 2.9).

In relation to fitness: recombination rates, location and recessives

To analyze the relationship between recombination rate and fitness, I examined the means of average recombination rate for each genotype as a function of the three different measures of fitness including fecundity, viability and the product of viability and fecundity (figure 2.10, 2.11 and 2.12 for C2, and figure 2.13, 2.14 and 2.15 for C3). For C2, there is a slightly positive correlation in all graphs, however it is not statistically significant (for recombination to fecundity, correlation=0.42, p=0.227, d.f. 8; for recombination to viability, correlation=0.20, p=0.58, d.f. 8; for recombination to viability by fecundity, correlation=0.39, p=0.26, d.f. 8). The correlation is dominated by one genotype, Gl, between two of the relationships, recombination and fecundity as well as recombination and the product of viability and fecundity (figure 2.10 and 2.12). I calculated the correlation without the values for this genotype and this causes a reduction in the already weak correlations (for recombination to fecundity, correlation=-0.054, p=0.89, d.f. 7; for recombination to viability by fecundity, correlation=0.14, p=0.72, d.f. 7). For C3, the correlation of recombination rate to fitness is negative, although not significantly so for any of the graphs (for recombination to fecundity, correlation=-0.42, p=0.3, d.f. 6; for recombination to viability, correlation=-0.484, p=0.22, d.f. 6; for recombination to viability by fecundity, correlation=-0.51, p=0.20, d.f. 6). For all of the relationships in C3, the correlations are dominated by one genotype, Frd, and so I tabulated the correlations without this genotype (for recombination to fecundity,
correlation=-0.1, p=0.8, d.f. 5; for recombination to viability, correlation=-0.28, p=0.5, d.f. 5; for recombination to viability by fecundity, correlation=-0.31, p=0.54, d.f. 5).

For the difference between the recombination events in the first and second region as a function of fitness there was a slightly negative correlation for both sets of data, however it was not statistically significant (C2 correlation=–0.27, p=0.45, d.f. 8, C3 correlation=–0.26, p=0.53, d.f. 6).

I examined coincidence as a function of the three fitness measures of each genotype: fecundity, viability and the product of fecundity and viability (figure 2.16, 2.17 and 2.18 for C2; and figure 2.19, 2.20 and 2.21 for C3). A significant positive correlation is present in each of the coincidence to fecundity graphs (for C2, correlation=0.83, p=0.003, d.f. 8; for C3, correlation=0.72, p=0.044, d.f. 6). Coincidence as a function of viability as well as coincidence as a function of the product of viability and fecundity are positively correlated in both data sets, however, only significantly so for C3 (for recombination to viability, correlation=0.75, p=0.03, d.f. 6; for recombination to viability by fecundity, correlation=0.77, p=0.02, d.f. 6).

**The genotype, coincidence, recombination and age**

As coincidence decreases with age in some studies, I investigated the coincidence to age relationship using repeated measures ANOVA based on the first 17 days of a female’s lifespan (Bridges and Morgan 1919, Plough 1921). That the genotypes were variable with respect to coincidence is evident because the effect of marker is statistically significant (for C2, F: 2.47, p=0.009, d.f. num: 9, d.f. den: 400; for C3 F: 2.0 p=0.05; d.f. num: 7, d.f. den: 366; table 2.6). The coincidence values of the genotypes, however, did not vary in a predictable manner with time as the effect of time by marker is insignificant.
(for $C_2$, $F: 1.24$, $p=0.1$, d.f. num: 63, d.f. den: 2225; for $C_3$, $F: 1.105$, $p=0.288$, d.f. num: 49, d.f. den: 1832). To additionally examine this relationship, I used a linear least squares regression model where coincidence values are weighted by the reciprocal of the variance in coincidence. The effect of age is slightly positive for $C_2$ and slightly negative for $C_3$ however, neither value is significant (for $C_2$, $F: 0.39$, $p=0.53$, d.f. num: 1, d.f. den: 5379; for $C_3$, $F: 0.86$, $p=0.35$, d.f. num: 1, d.f. den: 4910). Therefore, changes in coincidence are not age dependent.

Historically, a well-known relationship in *Drosophila melanogaster* is that recombination decreases with age (Bridges 1915). To examine whether genotype can impact this recombination to age relationship, I evaluated the longevity profile for each genotype. For this relationship to hold, genotypes with a shorter lifespan must exhibit higher recombination rates overall because they only survive in the area of the graph where recombination rates peak. For $C_2$, $Gl$ is deviant from other genotypes with a shorter lifespan (see figure 2.22). Alternately, $Frd$ is deviant in $C_3$ with a shorter lifespan than other genotypes (see figure 2.23). These two genotypes must exhibit higher recombination to age profiles if all *Drosophila melanogaster* have the same recombination to age relationship.

I assessed whether genotype could impact the recombination to age relationship. To calculate the recombination to age relationship for each genotype, I used a linear least squares regression analysis based on the recombination rate, weighted by the reciprocal of the variance in recombination, per vial per female. I employed this analysis twice to the females of each genotype: for the first 14 days of life, and for the entire lifespan. The slopes of the lines for each genotype are not significantly variable within each data set.
therefore recombination changed with age similarly for the genotypes of a given set (for 
\textit{C2} for all: F: 1.11, p=0.35, d.f. num: 9, d.f. den: 4767; for \textit{C3} for all: F: 0.74, p=0.63, d.f. 
um: 7, d.f. den: 6724). For both data sets, the intercepts of lines are significant meaning 
that the genotypes are variable in recombination rates at the outset (for \textit{C2}, F: 2.94, 
p=0.0035, d.f. num: 9, d.f. den: 122; for \textit{C3}, F: 29.05, p<0.0001, d.f. num: 7; d.f. den: 112).

Because the females died at different rates, I examined the senescence data to 
account for some of the variability in the recombination to age profiles. For senescence to 
contribute to any observed relationship of recombination to age there must be a 
correlation between the slope or intercept of the mortality data to the recombination data. 
I used Gompertz hazard modeling to evaluate these relationships. There is no correlation 
between the intercept of the recombination to age linear estimates and the slope and 
intercept of the mortality curves (for \textit{C2}, minimum p=0.23 for correlation=-0.42, d.f. 8, 
for Gompertz slope to least square intercept; for \textit{C3} minimum p=0.28 for correlation - 
0.43, d.f. 6, for Gompertz slope to average recombination rate).

From the data I assessed whether the tenet that recombination decreases with age 
was reproducible. For \textit{C2}, recombination decreases with age (figure 2.24 and figure 2.25). 
The tenet that recombination decreases with age is not reproduced in the results of \textit{C3} 
(figure 2.26 and 2.27). Indeed, the age parameter is positive for this data set (t=1.80, 
p=0.39, d.f. num: 1, d.f. den: 6726).

Because fitting the data on the recombination to age profile is not necessarily best 
fit by a straight line, I tested the factors affecting the variability of recombination to age 
relationship using repeated measures ANOVA based on the first 20 days of life. For
repeated measures ANOVA, the program assumes each female as a single sampling unit and each measure not independent as she is measured under different conditions; in this case the condition is age. The effect of marker is significantly different between the genotypes for both C2 and C3 therefore the genotypes varied in recombination rates (for C2, F: 2.06, p=0.03, d.f. num: 9, d.f. den: 303; for C3, F: 12.54, p<0.0001, d.f. num: 7, d.f. den: 333; table 2.4). The effect of time was significant in C3 and close to significant in C2 therefore recombination changed over time (for C2, F: 1.83, p=0.07, d.f. num: 8, d.f. den: 296; for C3, F: 9.57, p<0.0001, d.f. num: 8, d.f. den: 326).

**Discussion**

**Variation**

The goal of the experiment is to test whether genotypes with varying fitness can impact recombination rates. In order to evaluate these effects, there must be variation between the genotypes with respect to fitness and recombination. For both measures of fitness, fecundity and viability, statistically significant variation exists amongst the genotypes (figure 2.2 and 2.3). For average lifetime recombination rates, genotypes are significantly variable (figure 2.4 and 2.5).

I determined that the genotypes are variable in a number of other ways. The physical location of recombination events varies significantly with genotype (figure 2.6 and 2.7). Accordingly, the physical distance of recombination events changes across genotypes. Thus, even if genotypes have similar recombination rates, the locations of recombination events are different. Finally, coincidence is significantly different between the genotypes as well (figure 2.8 and 2.9); therefore, genotypes vary in the extent to which a recombination event in one region interferes with a recombination event in
another region. In summary, there is significant variation among the genotypes with respect to recombination-related traits.

**Fitness correlations: recombination rates**

As I have discussed, the goal of the experiment is to analyze the impact of genotypes with differing fitness on recombination rate. The fitness measures in this experiment are: fecundity, viability and the product of viability and fecundity. Correlations between recombination rates and each of the three measures of fitness are slightly positive for C2, and slightly negative in the results of C3; none of the correlations are significant (figures 2.10 to 2.15). For C2, the genotype Gl dominates the recombination correlations to fecundity and viability by fecundity; Frd in C3, is dominant in the correlation of all fitness measures to recombination. I calculated correlations without these driving genotypes and the correlations decreased greatly. Therefore, for both sets of data, there is no discernible relationship between recombination and fitness. How does this relate to existing studies?

Parsons (1987) posited, theoretically, that flexibility in the response of recombination is advantageous because it enables low fitness genotypes to increase recombination in order to create variable offspring in the hopes that one or more is fit. In light of the current results, existing experimental evidence for such a theory has two problems: either the extent of the stress can not be evaluated because fitness is not reported in the study (table 2.1), or the experimental results are inconsistent with the results of the current experiment.

That there is no correlation of fitness and recombination in the current experiment is in stark contrast to other experiments of this nature (Tucic et al. 1981,
Cvetkovic and Tucic 1986, Marinkovic et al. 1980). There are multiple reasons for these differences. Tucic et al. (1981) used 70 wild type second chromosomes to impact recombination between recessive alleles on the second chromosome. Thus, the genotypes impacting recombination were heterozygous for the chromosome in question. Flies homozygous for the wild type chromosomes were used to measure fecundity (Tucic et al. 1981, Cvetkovic and Tucic 1986). Any recessive genes, therefore, contributing to fitness in homozygous flies, did not equivalently contribute to the recombination rates in heterozygous flies. Clearly, there are recessives genes as 15 chromosomes were excluded in fitness estimates due to the presence of recessive lethals (Tucic et al. 1981). In the current study, fitness and recombination are measured on the same individual and precautions have been taken to avoid incorporating interactions between the dominant markers and the recessive markers used the measure recombination. The results of the current study, then, do not suffer the same limitations as earlier works.

For the aforesaid studies, the chromosomes were extracted from a natural population, thus, the source of variation between the genotypes was unknown (Tucic et al. 1981, Cvetkovic and Tucic 1986). Without knowing the source of variation, genes or structural elements with direct effects on recombination can not be discounted. Undeniably, structural rearrangements directly impacting recombination rates are well known (Graubard 1932, Hayman and Parsons 1960, Rendel 1957). Effects other than condition, consequently, may confound the correlation of fitness to recombination rate. In the current study, the dominant mutations have no known direct effects on recombination (www.flybase.org). Any differences in recombination between the genotypes are solely
attributable to condition therefore the results of the current study are not confounded by uncontrolled sources of variation.

In addition to the experimental limitations of the Tucic et al. (1981) article, sample sizes between their study and the current one, are substantially different. For each second chromosome extracted from the natural population, Tucic et al. (1981) measured the fitness of ten females. Recombination rates were estimated from 16547 offspring (Tucic et al. 1981). In the current study, I estimated fitness using 100 females from each genotype and it was the offspring of the same 100 females that I used to estimate recombination rate. For C2, I scored upwards of 237 000 offspring; for C3, I scored upwards of 270 000 offspring. Therefore, the results reported in this paper are more robust. As a result of the robust and concise nature of the current experiment, there is no relationship between recombination and fitness.

In addition to experimental works investigating the relationship between recombination and fitness, theoretical modeling is used to investigate the advantages of recombination. A common theoretical model is fitness-associated recombination that is predicated on a negative correlation of recombination to fitness (Hadany and Beker 2003). However, finding no correlation between fitness and recombination in the current experiment negates this hypothesis. Indeed, detectable poor condition created by the genotypes that vary in fitness is not sufficient to cause an increase in recombination.

**Fitness correlations: coincidence**

Recombination in one region often interferes with a recombination event in the second region and vice versa. In some species recombination events are independent of events in other regions; however, coincidence is historically well observed in *Drosophila*
Previous studies on recombination demonstrate positive interference and accordingly, occurrences of coincidence should be less than expected based on the frequency of single recombination events (Francis et al. 2007, Grell 1978, Plough 1917, Graubard 1932, Hayman and Parsons 1960, Rendel 1957). Conversely, this prediction is not supported by the results of the current experiment. This most striking result is the significant positive correlation of coincidence and fitness (figures 2.16 to 2.21); all genotypes, with the exception of Frd, exhibit negative interference. The current results are from high fitness genotypes creating double recombinants at the expense of single recombinants.

Mathematically, if the frequency of recombination is 35% in one region and the frequency of recombination in a second region is 30%, then the expected frequency of double recombinants is the product of the frequencies of single recombination events, 0.35*0.3=0.105, or 10.5%. Therefore, double recombinants are the rarest genotype and increasing the frequency of double recombinants increases the variety of offspring that an individual produces. Possible explanations for these results include: the distance between the markers, the location of the markers, a stress induced reaction, a manifestation of an artifact of variance in recombination rate and a response age. I will consider each source.

Graubard (1932) observed that chromosomal distance affects the frequency of double recombination events. Indeed, other studies find interference increasingly more common with decreasing chromosomal lengths (Rendel 1957, Bridges 1915, Esch and Weber 2002, Auger and Sheridan 2001, Salamini and Lorenzoni 1970, Mather 1938). The distances between the markers, however, in the current study are not unlike the distances in the previous studies reporting positive interference (Francis et al. 2007, Grell...
1978, Plough 1917, Graubard 1932, Hayman and Parsons 1960, Rendel 1957). Because studies reporting positive interference use the same span of markers as the current experiment, the presence of negative interference can not be attributed to distance.

Some studies find negative interference in short centromere regions about the third chromosome (Morgan et al. 1925). Plough (1921) observed that certain regions of the chromosome have increased coincidence; the markers of the current study are in the proposed regions of higher coincidence. Graubard (1932) noted that regions closer to the centromere demonstrate increased incidence of double recombinants but did not provide an explanation to the cause. As I have previously discussed, the regions of the current study span the centromere. Green (1975) and Sinclair (1975) denote negative interference about the centromere because of gene conversion rather than double crossovers. Dennell and Keppy (1979), however, provide evidence that the detected negative interference is due to double crossovers and eliminate the possibility of gene conversion. Instead, they suggest that negative interference is characteristic of the region (Dennell and Keppy 1979). If negative interference was solely the result of the characteristics of the region, then all genotypes sharing the same set of recessive markers, or all of the genotypes in each set of this experiment, must have near same values of coincidence. But the results of the current experiment demonstrate that coincidence increases with fitness, thus, these results can not be explained by location of the recessive markers in the chromosome.

Some existing studies find increasing temperature with increased coincidence (Hayman and Parsons 1960, Graubard 1932, Plough 1917). As previously stated, Parsons (1987) noted that the least fit genotype will produce the most variable offspring in hopes that one will be more fit. If poor condition causes an increase in the quantity of double
recombinants, increased coincidence values will result from decreased fitness. This runs counter to the observations in the current study and it is not evident why a high fitness genotype produces the most variable offspring and vice versa for the least fit genotype.

Negative interference values may occur as an artifact of a large variance in the recombination rate of a female (Sall and Bengtsson 1989). Females who exhibit both extremes in recombination rates, both high and suppressed recombination rates, will mathematically create negative interference as an artifact of this variance. This is possible in *Drosophila* by producing offspring at an early age and at a late age. The known recombination to age relationship for *Drosophila melanogaster* is that recombination rate decreases with age. Thus, offspring born early to a female will be highly recombinant and those born towards the end of a female’s life will have minimal recombination. In accordance, genotypes exhibiting negative interference, must have a wide spread of when the majority of their offspring emerged. I investigated this phenomenon in the data from *C2* and *C3* by examining the distribution of offspring from each genotype as a function of the age of the female (figure 2.28 for *C2*, and 2.29 for *C3*). All genotypes except *Gl* have a similar pattern of offspring distribution for *C2*; for *C3*, *Bkd* and *Frd* have notable variation in the distribution of offspring. *Frd* and *Gl* only produce offspring in the early part of their lifetime and this is not conducive to the phenomenon. In addition, these genotypes have lower coincidence values than other genotypes. *Bkd*, another genotype with a deviant distribution of offspring, has a lull in offspring production around day 23; *Bkd*, additionally, has increased coincidence values (figure 2.23). For the high coincidence values of *Bkd* to be artifact of the variance in recombination rate, the genotype must have a sustained lull in offspring production and, following this lull,
produce a large amount of its total offspring late in life. This is not apparent in the offspring distribution pattern of \textit{Bkd}. The distribution of offspring that is necessary to produce increased coincidence as an artifact of variance is not present in the current data. Therefore, the increased coincidence values for both sets of the current experiment are not the result of an artifact of variance in recombination rates.

Some studies posit that coincidence increases with age (Plough 1921, Bridges and Morgan 1919). Genotypes with longer lifespan’s must exhibit increased coincidence values for this tenet to explain the variance in coincidence values between the genotypes. The current study, however, does not substantiate this relationship based on repeated measures ANOVA and a linear least square regression model (table 2.6). A predictable pattern between coincidence and age is not evident in the current data. Therefore, from the data in both studies, variability in coincidence is not age dependent.

It is not evident what causes an increase in coincidence with increasing fitness. Green (1975) observed that genotype has no impact on the amount of coincidence. But this is incompatible with the results of the current study. Not only is there negative interference, in addition, the quantity of negative interference is correlated to fitness. I combined the results of the two data sets using an unweighted \(Z\) test and the positive correlations between coincidence and fitness are significant (for recombination and fecundity \(p=0.0032\), d.f. 14; for recombination and viability \(p=0.028\), d.f. 14; for recombination and viability by fecundity \(p=0.026\), d.f. 14). Complex relationships between organisms and coefficients of coincidence have been previously observed (Copenhaver 2005). For the current results, however, there is a clear pattern. I have exhausted existing explanations for increased values of coincidence including: the
distance between the markers, the location of the recessives markers in the chromosome, the impact of stress, an artifact of variance in recombination rate and the relationship with age. In the current study, coincidence is positively correlated to fitness and the explanation, for why coincidence increases with fitness, remains elusive.

The genotype and recombination to age

For *Drosophila melanogaster*, the known recombination to age relationship is that recombination rate decreases with age (Neel 1941, Schultz and Redfield 1951, Bridges 1927). In the current experiment, I tested the data to query: whether all *Drosophila melanogaster* have the same recombination to age relationship, whether the genotypes could impact this known relationship, and whether the tenet that recombination decreases with age is upheld by the current data.

If the recombination to age relationship is the same for all *Drosophila melanogaster*, genotypes with a shorter lifespan must have the highest recombination rate over their lifespan because they only produce offspring in the initial part of the recombination to age relationship. To estimate this relationship for genotypes of the current experiment, I investigated the longevity profiles of the genotypes. Many of the genotypes are similar in the distribution of surviving females (figure 2.22 and 2.23). In C2, however, less than 60% of the offspring for Gl survive to day 11 whereas, for most other genotypes, 90% of the females are still alive by this point. In comparison to other genotypes, Gl has a decreased recombination rate even though it has a shorter lifespan (figure 2.24). A short lifespan with a reduced recombination rate is not possible if all *Drosophila melanogaster* have the same recombination to age relationship. In C3, Frd females have a markedly shorter lifespan than the other genotypes. In comparison to
other genotypes, Frd has a high recombination rate (figure 2.26). Gla, however, has a higher recombination rate than Frd, yet Gla has a longevity profile similar to genotypes with low recombination. A longer lifespan with increased recombination rate violates the predictions of a common recombination to age relationship (figure 2.23 and 2.26). Therefore, the current genotypes do not conform to predictions of the theory that all Drosophila melanogaster have the same recombination to age relationship. Indeed, genotypes of both data sets are significantly variable in their intercepts of the recombination to age graphs. Therefore, from the outset, genotypes vary in their levels of recombination over lifespan. These differences may be caused by the impact of the genotype on the recombination to age relationship, but other sources of variation must be eliminated.

Genotypes senesce at different rates, consequently, the differences between the genotypes in the recombination to age relationship may be a result of this. To investigate this phenomenon, I used Gompertz hazard models to examine the relationship between the senescence data and the significant results of the recombination to age profiles for each genotype (table 2.5). There are no correlations between recombination rate to age profile in relation to mortality and lifespan data. Thus, the impact of the genotype on the recombination to age relationship is independent of senescence.

The genotypes differ in their recombination rate to age profiles. In reference to the known recombination to age relationship for Drosophila melanogaster, only the data from C2 reproduced the known relationship that recombination decreases with age for the linear regression analysis of the recombination to age relationships (figure 2.24 and 2.25). For C3, age effect is positive (figure 2.26 and figure 2.27).
Consequently, for \( C3 \), recombination rate does not decrease, as flies get older. Therefore, the tenet that recombination decreases over time is not upheld by one of the data sets of this experiment. Studies reporting the relationship that recombination decreases over time focus on the initial eleven days of life because recombination is most susceptible to stress in this time period (Neel 1941, Bridges 1927, Priest et al. 2007, Parsons 1988). Lifetime recombination studies, extending beyond eleven days, demonstrate two peaks in recombination rates over the lifetime; one in the first days of life and a second after day fifteen (Rendel 1958, Parsons 1987, Neel 1941, Redfield 1966). As a result, I performed repeated measures ANOVA for the first 14 days of life to analyze how the recombination rate fluctuates and whether the differences in the lineages were significant if they were not fit by a straight line. Recombination rates change significantly over time and genotypes fluctuate in recombination rates over time and this was significantly genotype specific (table 2.4). The effect of age remains positive for \( C3 \). Therefore, the tenet, that recombination decreases with age, is inconsistent with the results of this experiment.

**Limitations**

Because selection against recessive markers can bias recombination estimates, I investigated the frequency of the homozygous recessive markers (Clark 1981). I tabulated the frequency of homozygous recessive markers as the quantity of the recessive markers divided by the number of scorable offspring. Homozygous recessive individuals are less common than heterozygotes; thus, there is some form of selection against the recessive markers (figure 2.30 for \( C2 \), and figure 2.31 for \( C3 \)). If the recessive markers suffer different viability effects, this can alter the tabulated recombination rates. I investigated the viability of each marker using the log-linear model as outlined by Clark (1981). The
markers in each data set have differing viabilities (for C2, b: 0.956, cn: 0.901, vg: 0.990; for C3, se: 1.031, by: 0.741, ss: 0.870). These are well within the acceptable ranges for minimal viability effects on recombination rate as proposed by Clark (1981). Because 18 genotypes are involved in the current study, the concern over viabilities is whether the selection costs are equivalent in the different genotypes.

Given that the goal of the experiment is to compare the recombination rates of the genotypes, the recombination rates must be affected in the same manner by biases. If recombination, or recombination related traits, is affected differently by biases, then any differences between genotypes may be an artifact of that bias. I analyzed the relationship of the biases of the recessive markers in relation to the genotypes to account for such discrepancies. As a function of fecundity, the homozygous recessive markers are frequently with genotypes with lower fecundity (for C2, minimum correlation: -0.8, p=0.0065, d.f. 8; for C3, minimum correlation: -0.12, p=0.79, d.f. 6; figure 2.32 and figure 2.33). The largest statistically significant difference between genotypes is Gl, the least fit genotype, and RLy+ type, the fittest genotype in C2. Selection against recessive markers can bias recombination rate estimates in different directions, but requires epistasis. If the sign of epistasis is associated with density, such that low density vials experience negative epistasis and high density vials have positive epistasis, this can complicate correlations between measures of fitness and measures of recombination. The concern in the present study is the relationship, or positive correlation, of coincidence to fitness; therefore, I used regression modeling to test the effect of fitness and selection on the least square means of coincidence for each genotype. I tabulated selection using the average of the frequency of the homozygous recessive markers. For C2, the effect of
fitness was significant and remained significant when selection was added as a model effect (F: 5.59, p<0.0001, d.f. 9; F: 7.85 p=0.026, d.f. 9, respectively). For C3, the effect of fitness was significant and was close to significant when selection was added as a model effect (F: 2.29 p=0.013, d.f. 7; F: 6.07 p=0.057, d.f. 7). Therefore the relationship of coincidence and fitness for each genotype is not driven by the selection against the recessive markers.

Recombination and recombination related traits must be carefully interpreted given variable selection against the recessive markers used to measure recombination. Nonetheless, the viability effects of the recessive markers are minimal in the current study because: the ranges of the viability effects of each recessive marker are within the acceptable ranges proposed by Clark (1981), and the significant correlation of coincidence to fitness is still significant when the genotype specific selection costs of the recessive markers are incorporated as model effects.

**Conclusion**

The advantages of recombination must be determined in order to help resolve the paradox of sex. The advantages of recombination are elusive especially in regards to the plasticity of recombination rates; that recombination rate can change in response to condition is not selectively advantageous in diploid populations. In this chapter, I investigated whether organisms respond to poor condition by altering recombination. Existing literature demonstrates that stressors such as temperature, parasites, and other external factors that make an individual in poor condition, cause an increase in recombination rate. This, theoretically, occurs because an unfit genotype produces the most variable genotypes in hopes that one is more fit. Along the same lines, fitness-
associated recombination is a means by which disadvantageous combinations are broken up while advantageous ones are maintained. However, much of the existing literature does not report the fitness of studied genotypes and thus the extent of the stress cannot be evaluated. Research studies demonstrating the relationship of changes in fitness to changes in recombination rate are problematic. The current study set out to test this hypothesis using variable genetic mutations, with no direct recombination effects, to manipulate condition genetically. The results of this study are inconsistent with aforementioned studies. Genotypes are variable with respect to fitness; however, the recombination rates of the genotypes are not correlated with fitness. Thus, the precept that recombination rate and fitness are correlated is disproved. In reference to fitness then, recombination is not a response to poor condition. Coincidence, alternately, is significantly positively correlated with fitness; however, the reasons for this are elusive. In previous works of this nature positive interference has predominated. The varying genotypes impact the recombination to age relationship and, thus, the tenet that recombination decreases with age is not upheld.
References


Figure 2.1. Experimental design

Crossing design for C2. Three chromosomes are represented. Recessive markers are on the second chromosome and dominant markers are on the third chromosome. M represents any dominant marker used in the cross. For wild type genotypes the non-scorable offspring did not exist and all offspring were scored in the manner indicated here (note that offspring could have been male or female, both were scored). 3 possible kinds of recombination events are phenotypically detectable among the offspring. For C3 the crossing design is the same except that the recessive markers are located on the third chromosome and the dominant markers are on the second chromosome.
Fecundity based on the total number of offspring produced over a female’s lifetime and pooled over genotype. For dominant markers, fecundity is based on twice the amount of scorable offspring produced. For wild type markers, fecundity is the number of offspring produced. Least squares regression model: ANOVA $F: 37.26$ and $p < 0.0001$, d.f. num: 13, d.f. den: 940; effect of marker $F: 42.67$, $p < 0.0001$, d.f. num: 9, d.f. den: 944. Effect test Tukey-HSD test reported as letter assignment above the column, columns not sharing the same letter(s) are significantly different from each other.
Figure 2.3. C3 Fecundity

Fecundity based on the total number of offspring produced over a female’s lifetime. For dominant markers, fecundity is based on twice the amount of scorable offspring produced. For wild type markers, fecundity is the number of offspring produced. Least squares regression model: ANOVA F: 20.66 and p <0.0001, d.f. num: 11, d.f. den: 714; effect of marker F: 31.24, p <0.0001, d.f. num: 7, d.f. den: 718. Tukey-HSD test reported as letter assignment above the column, columns not sharing the same letter(s) are significantly different from each other.
Average recombination rate based on the total number of offspring produced over a female’s lifetime. Females that produced less than 40 offspring are omitted. Recombination rate is pooled over all females of a genotype. For dominant markers, recombination rate is based on the amount of scorable offspring produced, or offspring not carrying the dominant marker. For wild type markers, recombination rate is based on all of the offspring produced. Least squares regression model weighted by the inverse of the variance in recombination rate. ANOVA F: 7.86 and p <0.0001, d.f. num: 14, d.f. den: 870; effect of marker F: 5.67, p <0.0001, d.f num: 9, d.f. den: 875. Tukey-HSD test reported as letter assignment above the column, columns not sharing the same letter(s) are significantly different from each other.
Figure 2.5. C3 Average lifetime recombination

Average recombination rate based on the total number of offspring produced over a female’s lifetime. Females that produced less than 40 offspring are omitted. Recombination rate pooled over all females of a genotype. For dominant markers, recombination rate is based on the amount of scorable offspring produced, or offspring not carrying the dominant marker. For wild type markers, recombination rate is based on all of the offspring produced. Least squares regression model weighted by the inverse of the variance in recombination rate. ANOVA F: 12.56 and p < 0.0001, d.f. num: 11, d.f. den: 678; effect of marker F: 19, p < 0.0001, d.f. num: 7, d.f. den: 682. Tukey-HSD test reported as letter assignment above the column, columns not sharing the same letter(s) are significantly different from each other.
Figure 2.6. C2 Region 1 versus region 2 per genotype

Recombination rate per region is based on the total number of offspring produced over a female’s lifetime. For dominant markers, recombination rate per regions is based on the amount of scorable offspring produced, or offspring not carrying the dominant marker. For wild type markers, recombination rate is based on all of the offspring produced. Here recombination events in region one (R1) are subtracted from recombination events in region two (R2), therefore the values plotted is the difference in recombination between the two regions. Least squares regression model: ANOVA F: 4.33 and p < 0.0001, d.f. num: 13, d.f. den: 904; effect of marker F: 3.95, p < 0.0001, d.f. num: 9, d.f. den: 908. Tukey-HSD test reported as letter assignment above the column, columns not sharing the same letter(s) are significantly different from each other.
Recombination rate per region is based on the total number of offspring produced over a female’s lifetime. For dominant markers, recombination rate per regions is based on the amount of scorable offspring produced, or offspring not carrying the dominant marker. For wild type markers, recombination rate is based on all of the offspring produced. Here recombination events in region one (R1) are subtracted from recombination events in region two (R2), therefore the values plotted is the difference in recombination between the two regions. Least squares regression model: ANOVA F: 8.19 and p <0.0001, d.f. num: 11, d.f. den: 705; effect of marker F: 12.16, p <0.0001, d.f. num: 7, d.f. den: 709. Tukey-HSD test reported as letter assignment above the column, columns not sharing the same letter(s) are significantly different from each other.
Coincidence values based on the average of the cumulative totals per female per genotype. Coincidence calculated as the number of observed double recombinants less the number of expected double recombinants based on the number of single recombination events. Linear least squares regression model weighted by the inverse of the variance in coincidence. ANOVA: F: 5.96, p < 0.0001, d.f. num: 13, d.f. den: 865; effect of marker F: 6.35, p < 0.0001, d.f. num: 9, d.f. den: 869; effect of block F: 4.59, p = 0.0011, d.f. num: 4, d.f. den: 874. Results of Tukey-HSD test is reported above each column, columns not sharing the same letter assignment(s) are significantly different from each other.
Coincidence values based on the average of the cumulative totals per female per genotype. Coincidence has been calculated as the number of observed double recombinants less the number of expected double recombinants based on the number of single recombination events. Linear least squares regression model weighted by the inverse of the variance in coincidence. ANOVA: F: 4.43, p <0.0001, d.f. num: 11, d.f. den: 690; effect of marker F: 6.35, p <0.0001, d.f. num: 7, d.f. den: 694. Results of Tukey-HSD test is reported above each column, columns not sharing the same letter assignment(s) are significantly different from each other.
Figure 2.10. C2 Recombination and fecundity

Results based on the total number of offspring produced in a female’s lifetime. Correlation: 0.42, p= 0.227, d.f. 8; correlation without Gl: -0.054, p=0.89 d.f. 7.
Figure 2.11. C2 Recombination and viability

Results based on Viability(V)_m=1-S_m where Frequency of the dominantly marked offspring (F_m)= Number Dominantly Marked/all offspring produced, Selection (S_m)=(1-2F_m)/(1-F_m). Correlation=0.20 p=0.58, d.f. 8.
Figure 2.12. *C2* Recombination and viability by fecundity

Fecundity based on the total number of offspring produced in a female’s lifetime. Viability based on $(V)_m=1-S_m$ where frequency of the dominantly marked offspring $(F_m)=\frac{\text{number dominantly marked}}{\text{all offspring produced}}$, Selection $(S_m)=(1-2F_m)/(1-F_m)$.

Correlation=0.395, $p=0.26$, d.f. 8; correlation without $Gl$=0.14, $p=0.72$, d.f. 7.
Figure 2.13. C3 Recombination and fecundity

Results based on the total number of offspring produced in a female’s lifetime. Correlation= -0.42, p=0.3, d.f. 6; correlation without Freckled= -0.1, p=0.8, d.f. 5.
Figure 2.14. C3 Recombination and viability

Results based on Viability(V)_m=1-S_m where Frequency of the dominantly marked offspring (F_m), number dominantly marked/all offspring produced, Selection (S_m)=(1-2F_m)/(1-F_m). Correlation=-0.484 p=0.22, d.f. 6; correlation without Frd=-0.282 p=0.5, d.f. 5.
Figure 2.15. C3 Recombination and viability by fecundity

Fecundity based on the total number of offspring produced in a female’s lifetime. Viability based on $(V)_m=1-S_m$ where frequency of the dominantly marked offspring $(F_m)$=number dominantly marked/all offspring produced. Selection $(S_m)=(1-2F_m)/(1-F_m)$.

Correlation=$-0.51$ p=$0.20$, d.f. 6; correlation without $Frd$=$-0.31$ p=$0.54$, d.f. 5.
Figure 2.16. C2 Coincidence and fecundity

Coincidence as a function of fecundity. Correlation: 0.83, p=0.003, d.f. 8; correlation without Gl 0.72, p=0.019, d.f. 7.
Figure 2.17. C2 Coincidence and viability

Viability based on \((V)_m = 1 - S_m\) where frequency of the dominantly marked offspring \((F_m)\), number dominantly marked/all offspring produced, Selection \((S_m) = (1 - 2F_m)/(1 - F_m)\). Correlation = 0.54 \(p = 0.11\), d.f. 8.
Figure 2.18. C2 Coincidence and viability by fecundity

Fecundity based on the total number of offspring produced in a female’s lifetime. Viability based on $V_m = 1 - S_m$ where frequency of the dominantly marked offspring ($F_m$) is number dominantly marked/all offspring produced, Selection ($S_m$) = $(1-2F_m)/(1-F_m)$. Correlation = 0.53, $p=0.11$, d.f. 8.
Figure 2.19. C3 Coincidence and fecundity

Coincidence as a function of fecundity. Correlation: 0.72 $p = 0.044$, d.f. 6; correlation without $Frd = 0.031 p = 0.94$, d.f. 5.
Figure 2.20. C3 Coincidence and viability

Viability based on \((V)_m = 1 - S_m\) where frequency of the dominantly marked offspring \((F_m)\), number dominantly marked/all offspring produced, dSelection \((S_m) = (1 - 2F_m) / (1 - F_m)\). Correlation = 0.75, \(p = 0.03\), d.f. 6; correlation without Frd = 0.40, \(p = 0.37\), d.f. 5.
Figure 2.21. *C3* Coincidence and viability by fecundity

Fecundity based on the total number of offspring produced in a female’s lifetime. Viability based on \((V)_m = 1 - S_m\), where frequency of the dominantly marked offspring \((F_m)\) = number dominantly marked/all offspring produced, Selection \((S_m) = (1 - 2F_m)/(1 - F_m)\). Correlation = 0.77, \(p = 0.02\), d.f. 6; correlation without Frd = 0.34, \(p = 0.46\), d.f. 5.
Figure 2.22. C2 Longevity profile

Longevity profile for each of the genotypes. Longevity was calculated as the percentage of surviving females for a given day.
Figure 2.23. C3 Longevity profile

Longevity profile for each of the genotypes. Longevity was calculated as the percentage of surviving females for a given day.
Recombination to age relationship based on a fit least squares regression model for the lifespan of the females. Recombination rates are weighted by the reciprocal of the variance in recombination rate. Model effects include marker, block, female with marker and block nested and random effects, age and a marker by age interaction. Significant effect of block, $F: 7.96$, $p=0.001$, d.f. num: 4, d.f. den: 51; and effect of age: $F:12.74$, $p =0.0004$, d.f. num: 1, d.f. den: 4766; and effect of marker: $F 2.94$, $p=0.035$, d.f. num: 9, d.f. den: 122. Marks are to demarcate genotypes, not actual data points.
Recombination to age relationship based on a fit least squares regression model for the first 14 days of the females. Recombination rates are weighted by the reciprocal of the variance in recombination rate. Model effects include marker, block, female with marker and block nested and random effects, age and a marker by age interaction. Significant effect of block, F:8.36, p <0.0001, d.f. num: 4, d.f. den: 53; and effect of age: F:13.67, p =0.0002, d.f. num: 1, d.f. den: 4715; and effect of marker: F 3.19 p=0.0017, d.f. num: 9, d.f. den: 124. Marks are to demarcate genotypes, not actual data points.
Figure 2.26. *C3* Recombination to age relationship (all vial transfers)

Recombination to age relationship based on a fit least squares regression model for the lifespan of the females. Recombination rates are weighted by the reciprocal of the variance in recombination rate. Model effects include marker, block, female with marker and block nested and random effects, age and a marker by age interaction. Significant effect of marker: $F_{29.05} < 0.0001$, d.f. num: 7, d.f. den: 112. Marks are to demarcate genotypes, not actual data points.
Figure 2.27. C3 Recombination to age relationship (first 14 days of vial transfers)

Recombination to age relationship based on a fit least squares regression model for the first 14 days of the females. Recombination rates are weighted by the reciprocal of the variance in recombination rate. Model effects include marker, block, female with marker and block nested and random effects, age and a marker by age interaction. Significant results: effect of age $F:35.35, \ p<0.0001$, d.f. num: 1, d.f. den: 4707; effect of marker $F:22.4 \ p<0.0001$, d.f. num: 7, d.f. den: 92; effect of age by marker $F:3.35 \ p=0.0014$, d.f. num: 7, d.f. den: 4706. Marks are to demarcate genotypes, not actual data points.
Figure 2.28. C2 Offspring distribution

Distribution of offspring born to a female based on all offspring produced by a female in her lifetime.
Figure 2.29. C3 Offspring distribution

Distribution of offspring born to a female based on all offspring produced by a female in her lifetime.
Figure 2.30. C2 Homozygous recessive markers

Frequency of homozygous recessive markers per genotype. White columns represent $b/b$, grey $cn/cn$, and black $vg/vg$. Expected frequency for markers is 0.5. The largest difference between genotypes as reported by a Tukey-HSD test is between $RLy^+$ and $Gl$, as indicated above the columns. Linear least squares regression results: $b/b$ ANOVA: $F: 5.22$ p$<0.0001$, d.f. num: 13, d.f. den: 871; effect of marker: $F: 2.5$ p$=0.008$, d.f. 9; $cn/cn$ ANOVA: $F: 27.7$ p$<0.0001$, d.f. num: 13, d.f. den: 871; effect of marker: $F: 3.43$ p$=0.0004$, d.f. 9; $vg/vg$ ANOVA: $F: 29.99$ p$<0.0001$, d.f. num: 13, d.f. den: 871; effect of marker: $F: 4.52$ p$<0.0001$, d.f. 9.
Figure 2.31. C3 Homozygous recessive markers

Frequency of homozygous recessive markers per genotype. White columns represent se/se, grey by/by, and black ss/ss. Expected frequency for markers is 0.5. The largest difference between genotypes as reported by a Tukey-HSD test is between RLy+ and Gl, as indicated above the columns. Linear least squares regression results: se/se ANOVA: F: 6.69 p<0.0001, d.f. num: 11, d.f. den: 707; effect of marker: F:9.3 p=0.008, d.f. 7; by/by ANOVA: F: 3.47 p<0.0001, d.f. num: 11, d.f. den: 707; effect of marker: F:3.08 p=0.0033, d.f. 7, ss/ss ANOVA: F: 5.06 p<0.0001, d.f. num: 11, d.f. den: 707; effect of marker: F:3.66 p=0.0007, d.f. 7.
Figure 2.32. C2 Homozygous recessives and fecundity

Frequency of homozygous recessives as a function of fecundity. Correlation $b/b=-0.83$ p=0.003; d.f. 8; $cn/cn=-0.88$ p=0.0007, d.f. 8; $vg/vg=-0.8$ p=0.0065, d.f. 8.
Figure 2.33. *C3* Homozygous recessives and fecundity

Frequency of homozygous recessives as a function of fecundity. Correlation $se/se=-0.43$, $p=0.29$, d.f. 6; $by/by=-0.27$, $p=0.5$, d.f. 6; $ss/ss=-0.12$, $p=0.79$, d.f. 6.
<table>
<thead>
<tr>
<th>Article</th>
<th>organism</th>
<th>region</th>
<th>recombinant recombination effects</th>
<th>observation number or number of offspring scored</th>
<th>recombination rate (or proxy)</th>
<th>additional change in recomb rate</th>
<th>stress</th>
<th>variance</th>
<th>variation between lines (pentaploids)</th>
<th>note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavell et al. 2007</td>
<td><em>A. thaliana</em></td>
<td>R1:16.4 map units, R2:23.1 map units</td>
<td>R1: 91981, R2 8919, (40799 tetrads scored)</td>
<td>R1: 1.65 at 19, R2: 22.7 at 19</td>
<td>0.3 \pm 2.25, 0.4%</td>
<td>0.2 at 25.5, 11%</td>
<td>0.3 at 28, 20%</td>
<td>0.3 \pm 22.5, 2.4%</td>
<td>0.25 at 26.5, 3.5%</td>
<td>0.24 at 28, 8.8%</td>
</tr>
<tr>
<td>Faci et al. 1981</td>
<td><em>Drosophila melanogaster</em></td>
<td>Second chromosome R1:12.6, R2:35.5, R3:6</td>
<td>four females for each genotype (16471 offspring scored)</td>
<td>R1: 6.4 map units, R2: 23.1 map units</td>
<td>0.4 (ranges from 59% to 44%)</td>
<td>0.3 (ranges from 38% to 47%)</td>
<td>0.4</td>
<td>observed much less doubles than expected 2:19, 51:304, 60:190 based on single recombination events not around centromere</td>
<td>not reported</td>
<td></td>
</tr>
<tr>
<td>Struhl 1978</td>
<td><em>Drosophila melanogaster</em></td>
<td>23 regions over chromosome regions on the X, 2L, 2R, 3R</td>
<td>7481 offspring for X, 9046 offspring for 2L, 7933 offspring for 2R, 7396 offspring for 3R</td>
<td>X: 4.5 \pm 2.0%</td>
<td>2L: 3.4 \pm 6.7%</td>
<td>2R: 5.7 \pm 7.1%</td>
<td>3R: 5.1 \pm 6.8%</td>
<td>3R: 6.1 \pm 11.4%</td>
<td>3R: 5.8 \pm 10.8%</td>
<td>not reported</td>
</tr>
<tr>
<td>Rose and Baillie 1979</td>
<td><em>Carassius auratus</em></td>
<td>2 regions between genes on chromosome 1, 1 region between genes on chromosome 4</td>
<td>60159 offspring scored</td>
<td>recombination rate increased 300% with increasing temperature (max increase 1.98 to 2.84)</td>
<td>lowest temperature did not cause a change in recombination rate</td>
<td>70 second chromosomes from a natural population</td>
<td>not reported</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ballyew and Bonnich 1982</td>
<td><em>Mus musculus</em></td>
<td>1 telomer region in chromosome 2</td>
<td>35 male mice</td>
<td>20.05 \pm 7.01, 7.0% increase ( p&lt;0.01 )</td>
<td>recombination decreases with parental age</td>
<td>not discussed</td>
<td>not reported</td>
<td>crosses between four highly inbred strains</td>
<td>stress of crowding (30 males)</td>
<td></td>
</tr>
<tr>
<td>Plough 1977</td>
<td><em>Drosophila melanogaster</em></td>
<td>chromosome 2 R1: 5.5 m.u., R2: 19.5 m.u.</td>
<td>5 fasts, (10181 offspring scored)</td>
<td>R1: 22.7 and R2: 22.2</td>
<td>0.13 \pm 14.9, 80%</td>
<td>0.31 \pm 31.5, 73%</td>
<td>0.31 \pm 31.7, 36%</td>
<td>0.31 \pm 28.7, 10%</td>
<td>not discussed</td>
<td>not reported</td>
</tr>
<tr>
<td>Lucht et al. 2002</td>
<td><em>A. thaliana</em></td>
<td>used recombinant reporter transgenes</td>
<td>258 infected, 232 control</td>
<td>0.23 \pm 41, 80%</td>
<td>not discussed</td>
<td>not reported</td>
<td>no variation</td>
<td>temperature at 13 and 31</td>
<td>using Plough's raw data to calculate expected coincidence can be assessed: at 22: expected 57 observed 51 at 13: expected 105 observed 52 at 15: expected 180 observed 164</td>
<td></td>
</tr>
<tr>
<td>Kosak et al. 2003</td>
<td><em>Nicotiana tabacum</em></td>
<td>used recombinant reporter transgenes</td>
<td>83 ( \times ) 4 spots per plant</td>
<td>16.3 ( \pm ) 7.2; 239%, signal only, 137%</td>
<td>not discussed</td>
<td>not reported</td>
<td>no variation 34b stock</td>
<td>infection and signal of infection</td>
<td>tobacco mosaic virus or tobacco mosaic virus, temperature on response (not significant)</td>
<td></td>
</tr>
<tr>
<td>Neel 1941</td>
<td><em>Drosophila melanogaster</em></td>
<td>4 regions on chromosome 2</td>
<td>58 control of which 6 were used, 171 survivor of which 17 were used, (4088 scored offspring)</td>
<td>range from 0.49 to 0.54</td>
<td>range from 0.47 to 0.28, except at beginning started always higher than control from 0.15%</td>
<td>over time periods, (3) experimental is higher than control (5) 0.01% to 0.00%</td>
<td>not discussed</td>
<td>no variation 34b stock</td>
<td>nutritional stress (starvation)</td>
<td>not one of the ratios of recombination differ from the rest significantly though recombination was distributed along the entire chromosome. Suggestion of decreased interference but claims it is not possible over the centromere (Neel 1941)</td>
</tr>
<tr>
<td>Abdullah and Barta 2001</td>
<td><em>Saccharomycyes cerevisiae</em></td>
<td>7 different loci containing detectable genes</td>
<td>40 strains</td>
<td>presence of nutrients can alter crossovers and gene conversion (max increase 15% decrease 15%)</td>
<td>Transcripton activation results in an increase of gene conversion &amp; crossovers (max 53% decreases)</td>
<td>not discussed</td>
<td>not reported</td>
<td>20 different strains</td>
<td>metabolic stress (presence/absence of a transcription activator)</td>
<td>not discussed</td>
</tr>
<tr>
<td>Article</td>
<td>Organism</td>
<td>Region</td>
<td>Recombination effects (chromosomes or regions)</td>
<td>Observation number or number of offspring scored</td>
<td>Change in Rec rate (max) Increase in Recombination</td>
<td>Additional change in Rec rate</td>
<td>Fitness</td>
<td>Variance</td>
<td>Variation between lines (genotypes)</td>
<td>Stress</td>
</tr>
<tr>
<td>---------</td>
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<td>-----------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>------------------------------------------------</td>
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<td>-------</td>
</tr>
<tr>
<td>Graulard 1952</td>
<td>Drosophila</td>
<td>X regions on chromosome 2</td>
<td>6096 offspring scored, normal amount</td>
<td>Presence of inversion suppressed recombination (less than 1% of its original value)</td>
<td>Temperature effects in the presence of an inversion failed to induce recombination</td>
<td>Not discussed</td>
<td>Not reported</td>
<td>Different inversions (on different arms of chromosome 2)</td>
<td>Inversions and temperatures</td>
<td>The number of double crossovers is less than expected, average number of double crossovers (frequency) range from 0.13 to 0.49 when it should be close to 1. Correlation values are higher at more stressful temperatures.</td>
</tr>
<tr>
<td>Hayman and Porosur 1960</td>
<td>Drosophila melanogaster</td>
<td>X chromosome region 1: 17.5 m.u., region 2: 11.5 m.u.</td>
<td>38,738 offspring scored across 8 treatment combinations</td>
<td>At 20: 29.74 Cy: 32.45 X-ray: 32.25</td>
<td>At 30: 36.62%, initial Cy: 27.88%, wild: 28.11%, -14.7%</td>
<td>Combinations of the inversions gave undeterminable results</td>
<td>Not discussed</td>
<td>Not reported</td>
<td>One genotype</td>
<td>Temperature, age, and an inversion (Cy) 8 combinations</td>
</tr>
<tr>
<td>Randel 1957</td>
<td>Drosophila melanogaster</td>
<td>Regions on the X chromosome ranging from 16.5 to 58.5 m.u.</td>
<td>23,331 offspring scored</td>
<td>Without the inversion: 0.314, with the inversion: 0.433, 37% increase</td>
<td>Not discussed</td>
<td>Not reported</td>
<td>One genotype</td>
<td>Presence of an inversion (Cy)</td>
<td>With the inversion, levels of coincidence (while still &lt;&lt;1) were higher than without the inversion (although less than expected given the increased map distance brought by the inversion thus not determined on length alone).</td>
<td></td>
</tr>
<tr>
<td>Plough 1921</td>
<td>Drosophila melanogaster</td>
<td>X chromosome regions 3a and X regions</td>
<td>X chromosome: 970 offspring scored, X chromosome: range from 0.1 to 16.1</td>
<td>Coincidence spikes at age 23 to negative interference (C: 1.38) and drops off with age</td>
<td>No significant changes in any region with age or heat (range 6.2 to 8.3).</td>
<td>Not discussed</td>
<td>Not reported</td>
<td>X chromosome one genotype</td>
<td>Temperature and age</td>
<td>Coincidence spikes at age 23 to negative interference (C: 1.38) and drops off with age.</td>
</tr>
<tr>
<td>Cvetkovic and Tosic 1966</td>
<td>Drosophila melanogaster</td>
<td>Second chromosome, R1: 12.9 R2: 38, R3: 46 R4: 47</td>
<td>3-10 females from 67 chromosomes</td>
<td>Low fecundity (5.92), longevity (62.85) and viability (0.22) had high recombination (&gt;72.76%).</td>
<td>High fecundity (11.78), longevity (57.68) and viability (0.85) had low recombination (&lt;63.03%).</td>
<td>Not discussed</td>
<td>Not reported</td>
<td>X chromosome one genotype</td>
<td>Temperature and age</td>
<td>In chromosome 3, the third region is noted that there is no significant effect of Cy or age.</td>
</tr>
<tr>
<td>Labeil et al. 1993</td>
<td>Nicotiana tabacum</td>
<td>Genomic cell copies of recombination substrates</td>
<td>18 X 19% protoplasts for X-ray and mitomycin C, 12 X 19% protoplasts for temperature</td>
<td>Embryony: 819%, 90 temperature: 559%, 85%</td>
<td>Any decrease viability to 53%, and heat shock reduced viability to 85%</td>
<td>Not discussed</td>
<td>Not reported</td>
<td>One genotype</td>
<td>Coating radiation, mitomycin C, heat shock, X-ray</td>
<td>Correlation reported but not apparent when 36 degrees of freedom came from when only 3 of chromosomes had data.</td>
</tr>
<tr>
<td>Lawrence 1958</td>
<td>Drosophila melanogaster</td>
<td>2 regions on the X chromosome, R1: 11.9, R2: 12.7</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Significantly different between the two lines at all temperatures</td>
<td>Significant differences between the reciprocal crosses in recombination rate in region one</td>
<td>Not discussed</td>
<td>Not reported</td>
<td>Two inbred lines and reciprocal crosses</td>
<td>4 temperatures, double crossovers not reported.</td>
</tr>
<tr>
<td>Lawrence 1963</td>
<td>Drosophila melanogaster</td>
<td>8 markers on the X chromosome</td>
<td>All young and each one crossed to the others (25 total), 20 cultures per line</td>
<td>280 offspring scored per line</td>
<td>At 25 degrees no differences between the lines. Frequency: 2/0–9, 9/0–14, 14/0–25</td>
<td>Centromeric region were the most sensitive to temperature change, 46-18 degrees; 2/0–10, 20%, 9/0–16, 16%, 14/0–15, 25%</td>
<td>Not discussed</td>
<td>Not reported</td>
<td>One inbred line</td>
<td>2 temperatures</td>
</tr>
<tr>
<td>Zhuchenko et al. 1986</td>
<td>Tobacco plants</td>
<td>The average frequency of chiasmata per cell</td>
<td>4-7 plants, 86,478 cells per plant examined</td>
<td>Cold resistant: 25-16.44, 16.44, 14.24, 14.24, 16.74, 16.74, 15.07, 15.07</td>
<td>Cold resistant: 25-16.44, 16.44, 14.24, 14.24, 16.74, 16.74, 15.07, 15.07</td>
<td>Interfacial dispersion also changed with changing temperature significantly</td>
<td>Not discussed</td>
<td>Not reported</td>
<td>Different cold resistance</td>
<td>Temperature</td>
</tr>
</tbody>
</table>
Table 2.2. Crossing design for wild type constructs

<table>
<thead>
<tr>
<th></th>
<th>Chromosome 3 (to use with Chromosome 2 recessives, lineage a)</th>
<th>Chromosome 2 (to use with Chromosome 3 recessives, lineage z )</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Control</td>
<td>(RLy+)</td>
<td>First Control</td>
</tr>
<tr>
<td>Second Control</td>
<td>(WBsb+)</td>
<td>(GlaPin+)</td>
</tr>
<tr>
<td></td>
<td>(bwDXSco+)</td>
<td></td>
</tr>
<tr>
<td>R++</td>
<td>++Ly</td>
<td>Gla++</td>
</tr>
<tr>
<td>++</td>
<td>+++</td>
<td>++Pin</td>
</tr>
<tr>
<td>F1</td>
<td>R++</td>
<td>bwD++</td>
</tr>
<tr>
<td>xx</td>
<td>X</td>
<td>x</td>
</tr>
<tr>
<td>++Ly</td>
<td>++Ly</td>
<td>++Sco</td>
</tr>
<tr>
<td>F2</td>
<td>R++</td>
<td>bwD++</td>
</tr>
<tr>
<td>xx</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>+++</td>
<td>+++</td>
<td>++Sco</td>
</tr>
<tr>
<td>F3</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Plus symbols indicate wild type chromosome from series of backcrosses with lab adapted stock population. Diagram represents a one way cross whereas reciprocal crosses were also performed and mixed with the offspring at F2 thus creating multiple possibilities for the outcome in F3.
Table 2.3. Construction of recessives

<table>
<thead>
<tr>
<th>Generation</th>
<th>Chromosome 2</th>
<th>CH2</th>
<th>Chromosome 3</th>
<th>CH3</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>b vg</td>
<td>X</td>
<td>se ss</td>
<td>by</td>
</tr>
<tr>
<td></td>
<td>b vg</td>
<td></td>
<td>by</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cn</td>
<td></td>
<td>se ss</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b vg</td>
<td></td>
<td>by</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cn</td>
<td></td>
<td>se ss</td>
<td></td>
</tr>
<tr>
<td></td>
<td>by</td>
<td></td>
<td>cn</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>b cn vg</td>
<td>X</td>
<td>se by ss</td>
<td>se ss or by</td>
</tr>
<tr>
<td></td>
<td>b vg or cn</td>
<td></td>
<td>by</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b cn vg</td>
<td></td>
<td>se ss or by</td>
<td></td>
</tr>
<tr>
<td></td>
<td>se ss or by</td>
<td></td>
<td>by</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b cn vg</td>
<td></td>
<td>by</td>
<td></td>
</tr>
<tr>
<td>F3/F4</td>
<td>b cn vg</td>
<td></td>
<td>se by ss</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b cn vg</td>
<td></td>
<td>se by ss</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.4. Repeated measures ANOVA, recombination to age

<table>
<thead>
<tr>
<th>Set</th>
<th>Effect of Block</th>
<th>Effect of Time</th>
<th>Effect of Marker</th>
<th>Effect of Time by Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C2 over 20 days</strong></td>
<td>F: 2.48, p=0.044</td>
<td>F: 1.83, p=0.07</td>
<td>F: 2.06, p=0.03</td>
<td>Wilks’ Lambda:</td>
</tr>
<tr>
<td></td>
<td>d.f. num: 4, d.f. den: 303</td>
<td>d.f. num: 8, d.f. den: 296</td>
<td>d.f. num: 9, d.f. den: 303</td>
<td>F: 1.18, p=0.146</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>d.f. num: 72, d.f. den: 1808</td>
</tr>
<tr>
<td><strong>C3 over 20 days</strong></td>
<td>F: 0.94, p=0.44</td>
<td>F: 9.57, p &lt;0.0001</td>
<td>F: 12.54, p &lt;0.0001</td>
<td>Wilks’ Lambda:</td>
</tr>
<tr>
<td></td>
<td>d.f. num: 4, d.f. den: 333</td>
<td>d.f. num: 8, d.f. den: 326</td>
<td>d.f. num: 7, d.f. den: 333</td>
<td>F: 1.27, p=0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>d.f. num: 56, d.f. den: 1761</td>
</tr>
</tbody>
</table>

Calculations based on the number of offspring produced in the first 29 days of female’s lifespan. Effect of time indicates that recombination rate within each marker changes overtime. Effect of marker indicates that the change in recombination rates between the genotypes is significant. Effect of time by marker indicates that the change at each time point in recombination rate is genotype dependent.
Table 2.5. Hazard model for mortality

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Gompertz Hazard Model</th>
<th>Linear Least Squares Regression</th>
<th>Correlations:</th>
<th>Correlation: P-value:</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antp</td>
<td>0.20262</td>
<td>0.00065</td>
<td>20.91</td>
<td>0.194</td>
</tr>
<tr>
<td>Dr</td>
<td>0.21705</td>
<td>0.00012</td>
<td>26.43</td>
<td>0.192</td>
</tr>
<tr>
<td>Gl</td>
<td>0.28934</td>
<td>0.00102</td>
<td>14.97</td>
<td>0.164</td>
</tr>
<tr>
<td>Kd</td>
<td>0.19165</td>
<td>0.00037</td>
<td>24.42</td>
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<td>26.02</td>
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<tr>
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</tr>
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<tr>
<td>Adv</td>
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<td>0.00044</td>
<td>38.78</td>
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<tr>
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<td>0.00018</td>
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<tr>
<td>GP+</td>
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<td>0.00012</td>
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Table 2.6. Repeated measures ANOVA, coincidence to age

<table>
<thead>
<tr>
<th>Set</th>
<th>Effect of Block</th>
<th>Effect of Time</th>
<th>Effect of Marker</th>
<th>Effect of Time by Marker</th>
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<tbody>
<tr>
<td>C2 over 17 days</td>
<td>F: 0.649, p=0.63, d.f. num: 4, d.f. den: 400</td>
<td>F: 1.38, p=0.22, d.f. num: 7, d.f. den: 394</td>
<td>F: 1.05, p=0.403, d.f. num: 9, d.f. den: 400</td>
<td>Wilks’ Lambda:</td>
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<td>F: 1.73, p=0.0006, d.f. num: 63, d.f. den: 2225</td>
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<tr>
<td>C3 over 17 days</td>
<td>F: 1.87, p=0.115, d.f. num: 4, d.f. den: 366</td>
<td>F: 1.66, p=0.116, d.f. num: 7, d.f. den: 360</td>
<td>F: 2.00, p=0.05, d.f. num: 7, d.f. den: 366</td>
<td>Wilks’ Lambda:</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>F: 1.105, p=0.288, d.f. num: 49, d.f. den: 1832</td>
</tr>
</tbody>
</table>

Calculations based on the number of offspring produced in the first 17 days of female’s lifespan. Effect of time indicates that coincidence within each marker changes overtime. Effect of marker indicates that the change in coincidence between the genotypes is significant. Effect of time by marker indicates that the change at each time point in coincidence is genotype dependent.
Thesis Conclusion

The persistence of sex is a pervasive mystery in evolutionary biology. Some of this mystery may be uncovered by investigating the advantages for one of the mechanisms of sex, specifically recombination. To further the understandings of recombination in evolutionary biology, I investigate recombination in this thesis.

In the first chapter, I explore the effects of recombination on the mean fitness and variance in fitness in a population. I distinguish the theories for the evolution of sex based on their predictions about changes in mean fitness and variance in fitness due to recombination. Such a classification is previously lacking in the literature, but necessary because these are quantifiable fundamentals upon which recombination acts. I survey the possible outcomes and the implications thereof for experiments that measure the effects of recombination on fitness and variance. Although the results for the experiment of chapter one were statistically insignificant, the chapter itself provides a foundation on which other research can build.

In the second chapter, I explore the effect of differing condition on recombination; in particular, deleterious mutations with known fitness effects are used to test whether recombination rate responds to the poor condition caused by these mutations. Existing literature reports changes in recombination in response to stressful conditions; however, few studies focus specifically on genotype and even fewer report fitness, which facilitates the evaluation of the extent of the stress. In the few existing studies, recombination is negatively correlated to fitness. In chapter two, recombination is not correlated to fitness. As a result, the poor condition created by the markers does not invoke the response of recombination previously demonstrated in the literature. In
addition, coincidence, or the ability of recombination events to interfere with each other, is positively correlated with fitness, although the explanations for this are elusive.

Both chapters are instrumental to furthering the understanding of recombination in evolutionary biology. Chapter one provides a foundation on which the theories for the evolution of sex can be tested and evaluated. Using this framework, further research can help solidify the advantages of recombination. Chapter two demonstrates previously unattained results that counter some the existing hypotheses for the persistence of recombination. Further exploration of these may reveal advantages of recombination.