THE GENOMIC CONSEQUENCES OF SEXUAL SELECTION

by

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Abstract

Sex-differences in phenotype, development, and life-history can alter the strength of selection experienced by males and females. In particular, theoretical models have demonstrated that differences in the strength of selection between the sexes can influence the deleterious mutation load of populations. Stronger selection acting on males, via the action of sexual selection, could lower the mutation load for females if deleterious mutations tend to be harmful for both sexes. The necessary data to evaluate these models have been lacking, however, as most empirical studies of mutations have overlooked the fundamental differences between the sexes. Using the IV laboratory-adapted population of *Drosophila melanogaster*, I have measured the sex-specific impact of mutations in both sexes. This was done both for mutations naturally segregating in IV and for new mutations occurring on the X-chromosome. For both classes of mutations, males suffered a greater selective cost than females, and the mutations responsible were deleterious in both sexes. Further characterization of the male fitness phenotype revealed widespread decline in sexually selected characters combined with an increase in the correlation between sexually selected traits and viability, indicative of pleiotropy between new mutations. My work establishes the necessary conditions for sexual selection to reduce the mutation load of females, and helps fill a crucial gap in our understanding of the consequences of deleterious mutations.
Co-Authorship

Chapters 2, 3, and 4 have previously been published along with co-authors, as follows:


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Chapter 1

The genomic consequences of sexual selection

1.1 Overview
From the earliest formulations of selection theory, sexual selection, arising from the struggle for reproductive success, was thought to be separate from natural selection, arising from the struggle for survival (Darwin, 1859). The ornaments and armaments of males, which are frequently the more elaborate of the two sexes and seem to have no obvious survival value, were hypothesized to function solely to attract females or to aid in male-male agonistic interactions. In turn, females would ‘gain’ nothing from mating with ornamented males save more attractive or successful sons (e.g. Fisher, 1930, Huxley, 1938, although the phrase “sexy sons” was not coined until much later in Weatherhead & Robertson, 1979). Females preferring successful males would transmit these preferences along with the alleles responsible for male ornamentation, driving trait exaggeration until counterbalanced by the obvious survival disadvantage conferred by ostentatious displays (Fisher, 1930).

Zahavi (1975) is credited with championing the idea that turned this narrative on its head, suggesting that females should prefer the most ornamented males precisely because they were able to overcome the survival handicap conferred by their adornments. In other words, male sexually selected characters served as indicators of overall genetic quality.
(the underlying assumption being that the majority of the genome is geared towards optimizing performance under natural selection), and display intensity should be positively associated with net fitness, not just reproductive fitness. This line of reasoning was at first strongly criticized because Fisher’s fundamental theorem states that fitness should have no additive genetic variation, and thus no heritability, at equilibrium (Andersson, 1994a). Potential resolutions to this problem were proposed, for example fitness variance could be continuously generated by co-evolution with parasites (Hamilton & Zuk, 1982) or by immigration between locally adapted populations (Endler, 1983), but these were not universally accepted. Thus, it was long argued that male ornamentation could not signal overall quality (reviewed in Andersson, 1994a). Over the past two decades this view has been gradually overturned, however, aided by a series of theoretical insights and empirical results which unified problems found in the study of both natural and sexual selection: the riddle of the maintenance and elaboration of traits under continued strong selection.

The proposed solution posits that the pool of loci available in the response to sexual or natural selection is much larger than previously thought and that sexually and naturally selected traits share a common genetic architecture. This concept was recently found to have bearing on another longstanding problem, that of the maintenance of sexual reproduction itself, because populations experiencing sexual selection may purge deleterious mutations more effectively (Agrawal, 2001, Siller, 2001). On the other hand,
a counterpoint to the view of sexual selection as advantageous to populations has emerged in the study of sexual conflict (Arnqvist & Rowe, 2005), which focuses on the harmful effects of sex. With the recognition of potentially far-reaching effects on sexual selection on the genome, considerable effort is currently being expended on elucidating the consequences of sexual selection for processes normally associated with natural selection (e.g. Radwan et al., 2004, Rundle et al., 2006, Hollis et al., 2009, MacLellan et al., 2009). Here I give a brief review of the seminal works in this area of research, leading up to the subject of this dissertation.

1.1.1 Genetic variation in life-history traits
What maintains genetic variation for fitness? This is one of the oldest and most fundamental questions in population genetics. While selectively neutral traits may accumulate variation unencumbered by selection, we expect much less variation in those traits closely associated with fitness. Early studies seemed to verify this prediction, and found that, indeed, fitness-related traits appeared to support less variation. For example, one early study on the collared flycatchers of Gotland found a negative correlation between the heritability of several characters and their estimated contribution to fitness (Gustafsson, 1986). Certain morphological traits like body size showed the most heritability while lifetime breeding success, which should be nearly equivalent to organismal fitness, registered heritabilities not significantly different from zero.
Fisher’s theorem, however, applies to additive genetic variation only and not to heritability *per se* (Price & Schluter, 1991). Although related to additive genetic variation, heritability also includes the contribution of environmental variation. A difference in heritability between two traits is therefore only diagnostic of a difference in additive genetic variation if the environmental contribution is the same for both traits. This will not always be the case: in particular, traits closely associated with fitness are often life-history traits (i.e. lifetime fecundity, juvenile survival etc.), which are of a very different nature than metric traits. Price and Schulter (1991) argued that life-history traits should actually be expected to show lower heritability than metric traits even at equilibrium, because they are ‘further up the causal chain’ and thus are subject to more environmental variance. In other words, variation in life-history traits is caused by variation in metric traits with the addition of environmental variance proper to them, so their heritability should always be equal to or lower than the underlying traits. However, they also stated that no clear theoretical prediction could be made as to the expected levels of additive genetic variation in life-history traits at equilibrium, because they are influenced by multiple metric traits under varying intensities of selection whose phenotypic and selective effects may combine in complex ways.

Houle (1992) attempted to ascertain levels of genetic variation in life-history traits empirically by compiling data from over 200 quantitative-genetics studies of animals to amass 400 independent estimates of trait means, variances and heritabilities. He argued
that comparing genetic variation between traits is best done using the standardized coefficient of additive genetic variation (CV\textsubscript{A}), the standard deviation of additive genetic variation in a trait divided by its mean, which he expressed as a percentage. By comparing heritabilities of life-history traits with their underlying components (\textit{i.e.} additive genetic variance and environmental variance), the low heritabilities of the former were best explained by high contributions from environmental variance, rather than a dearth of genetic variation. Using this metric the opposite conclusion about variability in life-history traits was reached: they in fact tend to have \textit{more} standardized genetic variation than metric traits.

This observation required explanation, conflicting as it did with prevailing expectations. In a later paper, Houle (1998) identified four potential explanations for differences in the genetic variability of life history and metric traits. These were 1) differences in the prevalence of balancing selection, which tends to maintain variation 2) differences in the dynamics of selective sweeps or 3) differences in the effectiveness of selection in purging deleterious mutations, where more effective selection is liable to reduce standing variation, and 4) differences in mutational target size, which is to say the number of loci which can affect a trait. Although the data necessary to discriminate between these four possibilities was (and still is) limited, the amount of variation contributed by mutation was found to be highly correlated with the standing variation of traits as estimated by CV\textsubscript{A}, and was highest for life-history traits. Similarly the effect of mutational target size,
which is related to the number of loci in which mutations can cause a change in the trait of interest, was investigated. Mutational target size was estimated by assigning a value on a scale of 1-5 based on a combination of previously published experimental data and qualitative considerations. For example, one study suggested that approximately 5000 genes are capable of causing homozygous lethality in *Drosophila melanogaster* (Judd et al., 1972), a trait with high mutational variability and standing variation. Mutational target size was thus found to be both positively correlated with standing variation and highest for life history traits. Regardless of the precise balance of the potential variance-generating mechanisms, it seemed apparent from Houle’s analysis that mutation plays a large part in determining levels of genetic variation segregating in populations, including the main components of fitness.

As Price and Schluter (1991) noted, this could be because life-history traits (*e.g.* fecundity) are affected by a larger number of genes than morphological traits, being typically caused by many of them. For example, a trait such as female fecundity might depend not only on body size, but also be affected by additional factors under genetic control such as immunocompetence, various resource allocation trade-offs (*e.g.* size vs. number of eggs), and even other life-history traits such as longevity. Because independent mutations in any one of these constituent traits could influence life-history traits, their ‘effective’ mutation rate is expected to be much higher. Although the
contribution of each locus would be small, they would be numerous enough to enable high levels of standing variation at mutation-selection balance in life-history traits.

1.1.2 Genetic variation in sexually-selected traits
An analogous problem to the maintenance of genetic variance for fitness is the so-called “lek paradox” of sexual selection. The lek paradox arose from the prediction that sexual ornaments, metric traits that are attractive to the opposite sex, should show very little genetic variation at equilibrium because they are under persistent strong directional selection (Borgia, 1979). Female preferences have in many systems been shown to be costly either in terms of missed opportunities for survival-improving endeavours (Byers et al., 2005) or through harming effects from copulation (Borgia, 1981). A combination of these costs and diminishing genetic benefits should therefore lead to the erosion of the strength of female preferences, along with the winnowing of variation in male sexually-selected traits. Despite this, female preferences have been demonstrated in a variety of study systems and are often strong (Downhower & Brown, 1980, Noonan, 1983, Kodric-Brown, 1985). However, sexually selected traits apparently possess ample genetic variation, even more than non-sexually selected metric traits (Pomiankowski & Moller, 1995) which presumably experience weaker selection. A general mechanism by which this might occur was elaborated by Rowe and Houle (1996) as the theory of genic capture through condition-dependence. Like life-history traits, sexually selected traits may present a larger mutational target than other metric traits.
The genic capture model has two simple requirements. First, the expression of sexually selected traits should be costly, and therefore dependent on male condition. Condition, while not precisely defined by Rowe and Houle, can be thought of as the pool of resources available for allocation to fitness-related traits (Rowe & Houle, 1996). This requirement has ample empirical support. Conspicuous ornaments have been shown in many systems to impart a cost to males, usually in terms of survival, the tail of the barn swallow being a prominent example. When the tail is experimentally lengthened, mortality is higher, with further exaggeration entailing greater cost. When it is shortened, mortality is reduced (reviewed in (Møller et al., 1998). In other systems, sexual ornamentation has been found to impose a cost to males in terms of parasite resistance (Moore & Wilson, 2002) and as a physiological stress (Andersson, 1994b).

The second requirement of the genic capture model is that condition itself should exhibit high levels of genetic variation. While condition is likely to have a large environmental component, there must surely also be substantial genetic variation in resource storage/acquisition as it will be affected by life-history, morphological, and physiological traits. Variation in condition can be further exacerbated by competition between individuals, and in this way genetic variation for competitive ability in general can also be captured in the genetic architecture of sexually selected traits (Wolf et al., 2008). As traits become further exaggerated, they should become more costly and thus more
effectively capture variation in condition, especially if the marginal cost of further trait exaggeration is higher for low-condition males (Wolf et al., 2008).

The genic capture hypothesis provides a potential explanation for the existence of substantial genetic variation in sexually selected traits as well as a mechanism by which sexually selected traits serve as indicators of overall genetic quality. While the genic capture narrative is primarily oriented towards explaining how genetic variation in condition causes variation in sexually selected traits, it uses similar reasoning to the ideas underlying the maintenance of variation in life-history traits. Genic capture thus forms a two-way bridge, as it opens the potential for sexual selection to act not only on the few loci directly responsible for the development of sexual ornaments, but also on the many loci that contribute to overall genetic quality.

1.1.3 The cost of sexual reproduction
The preceding two problems (the maintenance and origin of variation in life-history and sexually selected traits), have recently been connected to another longstanding conundrum in evolutionary biology: the maintenance of sexual reproduction in the face of the putative advantages to asexual reproduction. The ‘two-fold’ cost of sex, also known as the cost of producing males (Maynard Smith, 1971), arises because an obligate sexual female generally produces equal proportions of males and females. This effectively halves her reproductive output relative to a putative asexual competitor who bears only
reproductive young and would therefore rapidly outcompete the sexual in the absence of other advantages to sexual reproduction.

Genetically, the most obvious difference between sexual and asexual lineages is the ability for sexual lineages to form new recombinants. Early on, it was recognized that recombination affects the way sexual and asexual lineages deal with new mutations and this has been invoked to help explain the persistence of sexual reproduction. For example, recombination might allow beneficial mutations occurring in different individuals to be rapidly brought together in the same genome (Muller, 1932), while asexual lineages may fall victim to the gradual accumulation of deleterious mutations, a process known as Muller’s ratchet (Muller, 1964). The way in which different loci interact also affects the consequences of recombination. Most notably, if the effects of multiple deleterious mutations are worse than their independent effects (synergistic epistasis), groups of deleterious mutations will be more efficiently purged and recombination will be advantageous (Kondrashov, 1988).

Although the role of sexual reproduction in reducing the deleterious mutation load was already well recognized, the contribution that sexual selection might play had largely been ignored. This changed with the works of Agrawal (2001) and Siller (2001), which were independently published in the same issue of Nature. Both of these papers proposed models which quantified the benefits that sexual selection might have for the entire
genome, an idea which had been made more plausible by the genic capture hypothesis. Briefly, sexual selection often appears to create stronger net selection on males relative to females: if true, females choosing the best males will also be choosing the males with the lowest deleterious mutation load. Their offspring will benefit from lower mutation load, and so long as males remain in sufficient supply to ensure the insemination of all the females, this selection will have no direct cost to female fitness. If the mutation rate is sufficiently high (on the order of approximately 1 deleterious mutation per diploid genome per generation) and the average strength of selection is approximately double on males than on females, then sufficient mutations can be purged to allow equilibrium mean fitness in females to be more than double that of an otherwise similar asexual lineage. Thus, sexual selection could help repay the cost of sex. At the very least, sexual lineages with strong sexual selection on males are expected to show higher mean fitness than sexual lineages experiencing little or no sexual selection.

### 1.1.4 Sexual conflict
Simultaneously, another theme had arisen in sexual selection research, that of sexual conflict. This field experienced major growth from the early 1990’s onward, and aimed to provide a novel perspective on the evolutionary consequences of intersexual interactions. This theory comes from the simple but profound observation that males and females often have divergent fitness-maximizing strategies. The result can be sexual conflict, on multiple fronts. That males suffer harm from intrasexual competition has long been known, and work over the past decade highlights the costs to females as well. For
example, direct harm to females can occur as a result of the costs of mating and the cost of sexual interactions. In many systems males physically harm females, for example male bean weevil genitalia have spines that damage females (Crudgington & Siva-Jothy, 2000). Other forms of harm can be manifest through the chemical compounds exchanged during copulation (Chapman et al., 2000) or simply through the harassment typical of many courtship rituals across a variety of taxa (Ojanguren & Magurran, 2007), for example. This type of conflict, with males harming females to increase their fitness relative to other males and females evolving to resist male harm, can cause an intragenomic arms race between the loci responsible for mate harm and resistance to harm, analogous to the well-known predator-prey cycles of adaptation and co-adaptation, or parasite-host cycles of co-evolution (Rice & Holland, 1997).

Intersexual conflict can also be detected within a single locus, when an allele has opposite fitness effects on the sexes (Rice, 1992). Although theorized much earlier (Lande, 1980), the presence of so-called sexually antagonistic fitness variation was perhaps most clearly demonstrated by experiments in Drosophila showing a negative intersexual genetic correlation for adult fitness (Chippindale et al., 2001). This is quite a remarkable observation, as it implies that the majority of fitness variation within each sex is harmful to the other, such that the fittest males frequently produce the least fit daughters, and vice-versa. Measuring intersexual genetic correlations has thus become a useful tool to assess the overall prevalence of sexually antagonistic fitness variation in a
population. This approach has revealed intralocus sexual conflict in mammalian and avian taxa (Brommer et al., 2007, Foerster et al., 2007), and its presence is clearly a problem for good-genes models of sexual selection. Clearly, females will gain little for their daughters from mating with high-fitness males if the variation affecting male quality is primarily sexually antagonistic. Evidence of sexual conflict has led some to question whether sexual selection produces any net benefits to females at all (Tregenza et al., 2006).

1.1.5 Goals
The revival of good-genes models of sexual selection with the genic capture hypothesis, along with the explosion of sexual conflict research, has resulted in considerable uncertainty about the impact of sexual selection on fitness. Much of this uncertainty stems from the difficulty of studying net fitness, which requires considerable cost and effort in most study populations, and in finding populations that meet the theoretical assumptions of genetic models. For example, the relatively simple prediction that there should be little genetic variance for net fitness at equilibrium requires both the measurement of net lifetime fitness and a population at selective equilibrium, which will be difficult to find in nature.

Not only must the standing genetic variation of a population be studied, but so too must new mutational variation. The genetic variation present in a population at any given time is not necessarily a true reflection of the input of new mutations. In particular, new
sexually antagonistic fitness variation and sexually concordant variation may have very different fates and/or allele dynamics. Sexually antagonistic variation is expected to persist for longer than its fitness consequences for each sex would predict, and can even be maintained as a stable polymorphism (Connallon et al., 2010). By comparison, sexually concordant variation will tend to go to fixation or elimination relatively rapidly, especially if males experience strong selection. Thus, even a negative intersexual fitness correlation in the standing genetic variation of a population could belie extensive concordant fitness effects at loci across the genome.

To ascertain the overall impact of sexual selection on mean population fitness, we must disentangle the contributions of sexually antagonistic alleles and sexually concordant variation. First, we must examine both the standing genetic variation of a population and new variation due to mutation. For both types of variation, the intersexual correlation should be measured. This involves separately measuring the fitness effects of same genotypes in both males and females. Also of importance is the distinction between sex chromosomes and autosomes, because they too may be enriched for different types of variation. In particular, sex chromosomes are expected to be enriched in sexually antagonistic variation due to the differing amounts of time spent in each sex, and because of differences in expression caused by heterogamety. The differential penetrance of sex-specific mutations due to the hemizygosity of the sex chromosome in the heterogametic
sex will mean that a recessive SA allele which benefits the hemizygous sex can reach a higher equilibrium frequency (Rice, 1984).

1.2 The IV population
Lab-adapted populations of *Drosophila melanogaster* form a powerful model system to examine these questions. Long-term lab-adapted populations make it possible to have a well-defined environment in which to define fitness in a biologically sensible way (*i.e.* as the study organism experiences selection). Short generation time also helps, for two reasons. First, selective equilibrium is more rapidly reached: populations having been founded several decades ago have already experienced hundreds of generations of laboratory adaptation. Second, multi-generational procedures such as mutation-accumulation are (barely) feasible within the span of a graduate degree. Drosophila also has a small number of chromosomes (four), of which the X chromosome is particularly large (~20% of the genomes). This makes addressing the question of the extent of sexual antagonism on sex chromosomes much more easily testable, as they may contribute a large proportion of genetic variance for fitness. Third, recently developed genetic tools allow for the manipulation of nearly the entire genome, allowing us to test these theories at the appropriate genetic scale. Specifically, I adapted the clone-generator (CG) system developed by Rice and Chippindale, which allows us to extract, propagate and manipulate haploid genomes from a study population for use with the IV population. This approach is increasingly being used as a quantitative-genetic tool in *Drosophila* to address a variety of questions (Abbott & Morrow, 2011).
The IV population is a long-term lab-adapted population that was first brought into the lab in 1973 in South Amherst, Massachusetts (Rose & Charlesworth, 1981). They are representative of a wild population that was the object of study from the early 1930’s by Philip T. Ives, who thought them to be continuous and overwintering in the area. Several studies show evidence that the IV population is under continuous selection and that it has reached a selective equilibrium, as far as life-history evolution goes. This stability is not due to a loss of standing genetic variation, as the IV population has been used for numerous experimental evolution studies in which selectable variation for many aspects of its biology was demonstrated, including reproductive lifespan, development time, and starvation resistance (Teotónio & Rose, 2000). Populations derived from IV having undergone experimental evolution also tend to revert back to the IV phenotype once returned to the ancestral conditions (Teotónio & Rose, 2000). Thus, any reasonable measure of fitness using the IV population should attempt to replicate its culture conditions as closely as possible, as the genetic architecture and variance present in the population is only interpretable in the selective context that shaped its evolution.

Fortunately their culture regime (Figure 1.1) made it possible for me to design large-scale measurements of fitness. Although several hypotheses related to the advantages of sexual selection are not easily tested in the IV system (for example, the role of sexual selection in disease resistance, or as a response to environmental variation), it is an excellent candidate for distinguishing between the classical ‘good genes’ models and the role of sexual antagonism.
Figure 1.1: *IV* population culture protocol. The *IV* population is cultured on a 14-day cycle, at 50% relative humidity on a 12-hour light-dark photoperiod. On Day 0, adults are removed from the culture vials and mixed together under CO$_2$ anaesthesia. The adults are transferred to fresh vials containing banana-corn syrup medium to oviposit until a target density of 80-120 eggs is reached (approximately 30 minutes). At this point the adults are removed from the vials and the eggs are left to develop. After six days, larvae begin to crawl out of the spent media and pupate on the sides of the vials. Peak eclosion occurs on the morning of Day 9, when approximately 80% of the pupae eclose. For the remaining 5 days the cultures are left undisturbed and adult competition occurs. On Day 14/Day 0, the cycle is repeated.
I used the CG protocol to generate reference panels of genomic haplotypes. These panels represent ‘snapshots’ of the genetic variation segregating in the IV population, and served as the raw genetic material for my experiments. Two major lines of experimentation were carried out, to study both the standing variation and new mutational variance. First, a haplotype library was used to create homozygous lines, which could be subsequently either outcrossed or inbred. This reveals the nature of the intersexual genetic correlation in the standing variation for the IV population, and allows for the disentangling of the effects of dominance from selection. Second, a mutation-accumulation (MA) experiment was carried out, using a genetically variable population of haplotypes, to determine the consequences of several generations of relaxed selection for both sexes. Comparing the intersexual correlation of pre- and post-MA haplotypes allows us to infer the overall character (sexually concordant or sexually antagonistic) of new mutations, as well as the relative strength of selection on males and females.

1.3 References


Chapter 2

Inbreeding reveals stronger net selection on Drosophila melanogaster males: implications for mutation load and the fitness of sexual females

2.1 Abstract

Stronger selection on males has the potential to lower the deleterious mutation load of females, reducing the cost of sex. However, few studies have directly quantified the strength of selection for both sexes. Because the magnitude of inbreeding depression is related to the strength of selection, we measured the cost of inbreeding for both males and females in a laboratory population of *Drosophila melanogaster*. Using a novel technique for inbreeding, we found significant inbreeding depression for both juvenile viability and adult fitness in both sexes. The genetic variation responsible for this depression in fitness appeared to be recessive for adult fitness (h=0.11) and partially additive for juvenile viability (h=0.29). Inbreeding depression was identical across the sexes in terms of juvenile viability but was significantly more deleterious for males than females as adults, even though female X-chromosome homogamety should predispose them to a higher inbreeding load. We estimated the strength of selection on adult males to be 1.24 times greater than on adult females, and this appears to be a consequence of selection arising from competition for mates. Combined with the generally positive intersexual genetic correlation for inbred lines, our results suggest that the mutation load of sexual females could be meaningfully reduced by stronger selection acting on males.
2.2 Introduction

The power of sexual selection to shape traits directly related to reproduction is well established, but the consequences of sexual selection for the rest of the genome have only recently begun to emerge. The genic capture hypothesis suggests that mutations throughout the genome could be affected by sexual selection, as the overall health and vigor of the organism may alter the expression of traits preferred by females (Rowe & Houle, 1996, Tomkins et al., 2004). In addition, other sexually selected traits such as mate-finding, coercion, and endurance rivalry (Andersson & Iwasa, 1996) are likely candidates for condition-dependence. At the same time, theory predicts that stronger sexual selection on males can lower the number of deleterious mutations affecting females at shared sexually-selected loci (Agrawal, 2001, Siller, 2001). Taken together, these ideas imply that male-biased selection could be a force that improves mean female fitness on a genome-wide scale (Kodric-Brown & Brown, 1987, Whitlock & Agrawal, 2009). Under some conditions (e.g. a diploid genomic mutation rate of 1 and selection on males being approximately twice as strong as selection on females), the mean fitness of females can be double that of females in asexual populations (Agrawal, 2001, Siller, 2001), potentially accounting for the cost of sexual reproduction. Although populations enjoying increased female fitness through this mechanism may not be resistant to invasion by new asexual mutants, who in the short term benefit from both past bouts of selection and freedom from the cost of producing males (Salathé, 2006), reductions in mutation load will nevertheless have important consequences for populations. For example, reducing the number of segregating mutations will reduce inbreeding depression for female fitness: a substantial contributor to extinction risk (Frankham,
Whitlock and Agrawal (2009) have recently pointed out that measuring the total strength of selection on both sexes can be used to predict the effect of stronger selection on males for female mutation load, but they also highlight a lack of relevant empirical data. One approach to quantifying differences in net selection between the sexes is to independently quantify their response to inbreeding. Greater inbreeding depression for one sex may be a fundamental feature of most sexual populations for at least three reasons. First, the heterogamety of sex chromosomes predisposes each sex towards different costs of inbreeding. In XY sex-determination systems, one expects greater inbreeding depression for females than for males simply because the X chromosome is always effectively homozygous for males. In addition, mutations on the X with female-limited effects are not exposed to selection when expressed in males (Demuth & Wade, 2007); the reverse would be true in ZW systems. Second, the presence of widespread sexually antagonistic variation will result in sex-specific inbreeding depression (ID), as inbreeding would fix alleles having opposite fitness effects in each sex. Finally, if one sex experiences stronger net selection, then the cost of inbreeding will be greater for that sex for a given mutation. By comparing the cost of inbreeding for the same genotype expressed in both males and females, we will gain insight into the intensity of selection upon each sex at the whole-genome scale.

Meaningful comparisons of ID between the sexes based on current data is difficult, however, in part because few researchers have explicitly set out to compare inbreeding
load for males and females, and ID is environment-sensitive. The latter may make it
dubious to compare inbreeding load from different studies, even within the same species.
Where compared, however, the majority of results point to males as the more fragile sex.
For example, in wild populations a higher sensitivity to inbreeding for males has been
suggested for red deer (Slate et al., 2000), although in an isolated population of song
sparrows inbreeding depression for lifetime reproductive success was greater for females
(Keller, 1998). In the laboratory, male flour beetles experienced stronger ID than females
did (Pray & Goodnight, 1995), and male virility in Drosophila, an important component
of male fitness, appears to be more strongly affected by inbreeding than female fertility
(Brittnacher, 1981). Similarly, Miller et al. (1993) made the second chromosome
completely isogenic in Drosophila melanogaster and found that inbreeding had a stronger
impact on male virility than on female fecundity. Meagher et al. (2000) showed that
housing mice under competitive, semi-natural conditions increased the average
magnitude of inbreeding depression by 4.5 times compared to standard laboratory
conditions. Most of this change came from a dramatic reduction in male reproductive
success under semi-natural conditions, accounting for virtually all of the increase in
average inbreeding depression.

Although suggestive, these data are not sufficient to evaluate the hypothesis that females
can benefit from stronger selection on males: it is also necessary to demonstrate that the
alleles responsible for the inbreeding response of males have the same directional effect
on females. Measurements of the strength of selection acting on each sex must therefore
be supplemented by an estimate of the intersexual genetic correlation, because sex-
limitation and sexually antagonistic alleles can cause net selection to be stronger for males with no benefit to females.

In order to obtain a meaningful estimate of selection on both sexes, as well as the intersexual genetic correlation for fitness, we must express the same genotypes in each sex and measure their fitness in a common environment. Establishing a controlled environment that is defined and reproducible, however, often comes at the cost of relevance to the natural environment to which the study organism is actually adapted, which in most cases is highly complex and variable. The use of laboratory-adapted populations that have been maintained under relatively constant conditions for hundreds of generations alleviates some of these concerns, because estimates of evolutionary parameters can be performed in the relevant selective environment.

We therefore created a set of inbred lines using a novel extension of the *Drosophila melanogaster* clone-generator system developed by Rice and colleagues (Chippindale et al., 2001). The approach, which we dubbed ‘directed inbreeding’, allowed us to make a set of genomic haplotypes extracted from a large laboratory-adapted population homozygous, and then express them in both sexes, in both the inbred and outbred state. We then characterized performance throughout the fly’s life cycle, measuring both juvenile viability and adult reproductive success, under conditions to which the population had adapted for approximately 800 generations. This approach has some of the same limitations of traditional studies with balancer chromosomes, notably an averaging effect over many loci and resulting inability to resolve the effects of individual
loci, but several advantages as well. Specifically, it allowed us to shed all artificial
genetic aberrations and markers in the experimental generation, to recreate the ancestral
competitive environment in almost every detail, to measure the fitness of each sex
independently in the same experiment, as well as to estimate the intersexual genetic
correlation for the genomes studied.

We show that the effects of inbreeding vary strongly by sex and life-history stage, further
highlighting the importance of accounting for sex differences in studies of fitness and
mutation load. Specifically, we confirm results from previous studies suggesting that
there is a substantially higher cost of inbreeding for *Drosophila* males, we show that this
additional cost is strongest in the adult stage, and demonstrate that male and female
inbred fitness is largely positively correlated. Taken together, these results satisfy the
theoretical requirements for male-biased selection to cause a reduction in female
mutation load.

2.3 Methods

2.3.1 Stocks and culture conditions
The focal population was the Ives (IV) population of *Drosophila melanogaster*. *IV* was
isolated from a wild-caught sample of 200 males and 200 females in Amherst,
Massachusetts (Rose & Charlesworth, 1981). At the time, the south Amherst population
was thought to be continuous and overwintering, and had been monitored since at least
1931 (Ives, 1970). From 1981 onwards, the *IV* laboratory population has been maintained
as a large outbred stock at a minimum population size of 1000 individuals at 25°C, 50%
relative humidity, on a 14 day, discrete generation cycle with moderate densities of 60-120 individuals per 10mL of banana/agar/killed-yeast medium (Rose, 1984). On Day 14/Day0, the population is placed under CO$_2$ anaesthesia, mixed, and transferred to new vials to oviposit until ~100 eggs are laid in each vial. This usually takes approximately 30 minutes, and represents the only opportunity for offspring production.

In 2004, a replica of the $IV$ population was created by backcrossing the $IV$ population to a population bearing the recessive $bw^l$ (brown eyes) allele, and denoted $IV_{bw}$. The $IV_{bw}$ population serves as an outbred, genetically similar population for use as competitors against $IV$-derived individuals in measurements of fitness. This marker has few deleterious side effects and the $IV_{bw}$ stock is vigorous. Periodically, $IV$s is introgressed into $IV_{bw}$ to prevent drift between the focal ($IV$) and competitor ($IV_{bw}$) populations.

### 2.3.2 Inbred lines

The 18 inbred lines used for this study were generated using a novel application of the clone-generator system of markers and chromosome rearrangements (Chippindale et al., 2001). A selection of haploid genomes derived from the $IV$ population and known to possess significant genetic variation for fitness in each sex provided the raw material for the creation of the inbred lines. These genomic haplotypes were made homozygous by mating males bearing the haplotype of interest in the heterozygous state along with a marked translocation ($T(2:3)rdge st in ri p^p bw$) to wild-type females, discarding the marked translocated autosomes in the female progeny. These females, having received a
genomic haplotype from their father, were collected as virgins and again crossed to males with the haplotype of interest, along with the marked translocation, for 10 generations (Figure 2.1). With each successive cross, the proportion of genes identical to the founding line’s haplotype increases by 50%. Subsequently, these lines were maintained at population sizes of ten females and six males to minimize genetic variation. A reduced sex ratio was employed to reduce mate-harm to females, minimizing the effective population size while maintaining productivity. Periodically, they were backcrossed again to their founding lines. Because the final result of these crosses is the homozygous version of a specific genomic haplotype, we named this method directed inbreeding (DI).

The directed inbreeding method has several advantages over traditional inbreeding methods, such as brother-sister mating. First, DI is less susceptible to the stochastic loss of mutations resulting from the inbreeding process. Brother-sister mating exposes mutations to both drift and selection during the inbreeding process, which could result in genomes purged of a fraction of their deleterious mutation load. DI reintroduces all of the mutations present in the founding genome in each generation of inbreeding, preventing the loss of deleterious mutations. The exception, common to all inbreeding approaches, is that one cannot fix sterile or lethal mutations within a line; these are thought to contribute to approximately half of the total mutation load in *D. melanogaster* (Lynch & Walsh, 1997).
Figure 2.1: Breeding schemes used in this experiment. (a) Generation of hemiclone lines. A single male from the IV population possessing an unknown genotype (black) was crossed to virgin clone-generator females possessing a compound X (C(1)DX, y, f, depicted by DX symbol), a Y chromosome, and a marked translocation of chromosomes II and III (T(2 : 3)rdgc st in ri p° bw, depicted by solid bar spanning the two major autosomes because these chromosomes cosegregate in surviving offspring). The resulting male offspring possess one of four possible genotypes, consisting of a set of X plus wild-type autosomes inherited paternally, with a Y and marked translocation inherited maternally. (b) A single male is then randomly selected to again cross to clone-generator females, thus fixing a genomic paternal haplotype within a line. The resulting hemiclone line is propagated by crossing males heterozygous for the marked translocation with clone-generator females. (c) Directed Inbreeding. Males from a given hemiclone line are first crossed to virgin wild-type IV females with an unknown genotype (black). Virgin females are collected from this cross, which are now heterozygous for the founding hemiclonal haplotype. These females are again crossed to hemiclone males, and the process is repeated ten times to yield lines inbred for the founding haplotype. (d) Generation of experimental flies. Inbred females from a given line are crossed to either inbred males (blue) or outbred males (black) to yield outbred and inbred flies of both sexes.
Second, the directed inbreeding approach has the advantage of capturing all three of *D. melanogaster*’s major chromosomes in the same experiment. With this species, recombination-suppressing balancer chromosomes are restricted to capturing and manipulating at most two of the major chromosomes simultaneously. Manipulating the whole genome should give a more accurate picture of the population consequences of genome-wide processes, while leaving open the potential for later deconstruction and analysis at the chromosomal level using balancer techniques.

Each inbred line was used to experimentally generate both inbred and outbred flies for use in measurements of fitness (Figure 2.1). Outbred individuals were created by crossing females from the line of interest with randomly selected wild-type males from the IV population. Inbred individuals were created by crossing these females with males from the inbred line. In this manner, both outbred and inbred individuals have inbred mothers with the same genotype, eliminating differences between outbred and inbred experimental flies due to maternal effects. While the potential for inbreeding depression exists along the entire genome for females, it was restricted to the autosomes for males, as both outbred and inbred males of a given line express the same X chromosome hemizygotically.

**2.3.3 Viability Assay**

Juvenile viability was assessed by placing 50 eggs from a given genotype in a vial along with 50 eggs from a standard competitor (*IVbw*), mimicking standard culture densities.
After twelve days, sufficient for virtually all adults to emerge (confirmed by visual inspection), egg-to-adult viability was assessed by counting and scoring progeny for both sex and eye color. Each genotype/treatment combination was replicated five times.

2.3.4 Adult Fitness Assay

Inbred or outbred individuals of the sex/genotype of interest were collected during peak emergence (Day 9 from date of oviposition) and singly transferred to a vial containing an age-synchronized culture of $IV_{bw}$ that was reared at standard culture densities (approximately 100 eggs/vial). Five days later (Day 14), experimental vials were anaesthetized with CO$_2$ for 2.5 min and transferred to fresh vials to allow for oviposition (30 min), mimicking standard culture conditions. The adults were then removed from the vials and the sex/number of progeny from the target individuals (distinguishable by their red eyes) was scored twelve to fourteen days later. Each genotype/treatment/sex combination was replicated thirty times.

2.4 Results

2.4.1 Sex-specific inbreeding depression in the $IV$ population

For both juvenile and adult life-history stages, inbreeding caused a measurable depression in fitness. Based on analysis of line means, mean viability in the outbred treatment was 61.2% for males (95% C.I. = [55.1%, 67.3%]) and 61.7% for females (95% C.I. = [57.0%, 66.3%]), while it was 78.9% for the $IV_{bw}$ competitors (male 95% C.I. = [78.0%, 82.4%], female 95% C.I. = [74.9%, 79.5%]). The lower viability of outbred $IV$ individuals, relative to the $IV_{bw}$ competitors, is likely the result of maternal effects from
their inbred mothers. Inbreeding depression for juvenile viability was 35% for both sexes, with inbred male viability at 40.3% (95% C.I. = [32.8%, 47.9%]) and female viability at 40.0% (95% C.I. = [31.3%, 48.7%]). The total number of IVbw competitors in the vials containing inbred flies was unchanged (oneway ANOVA, p-value = 0.25), suggesting that the reduction in viability in the inbred lines was not due to competitive exclusion by IVbw individuals. Indeed, no correlation was found between the number of red-eyed progeny and the number of brown-eyed progeny within a vial (p-value = 0.55, r² = 0.002, type I slope = 0.124). A three-factor ANOVA was performed on the viability data (using line as a random effect and sex, and degree of inbreeding as fixed effects), which shows significant genetic variation for viability and a significant effect of inbreeding in the IV population but no sex x inbreeding interaction, despite the potential for females to express ID on the X chromosome (Table 2.1).

For adult fitness, males and females differed in both their outbred and inbred mean fitness. Based on analysis of line means, males had higher outbred fitness (male mean = 2.69, 95% C.I. = [2.09, 3.28], female mean = 1.99, 95% C.I. = [2.25, 1.72]) but lower inbred fitness (male mean = 0.267, 95% C.I. = [0.152, 0.382], female mean = 0.719, 95% C.I. = [0.546, 0.892]). The higher mean for outbred males could be due to an advantage of red eyes in mate competition against bw-bearing
Table 2.1: Mixed-effects ANOVA on juvenile viability

<table>
<thead>
<tr>
<th>Source</th>
<th>Mean Square</th>
<th>Numerator Degrees of Freedom</th>
<th>F-ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex</td>
<td>0.0261</td>
<td>1</td>
<td>0.0016</td>
<td>0.97</td>
</tr>
<tr>
<td>line</td>
<td>141.02</td>
<td>16</td>
<td>2.48</td>
<td>0.043</td>
</tr>
<tr>
<td>treatment</td>
<td>2363.5</td>
<td>1</td>
<td>43.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>sex x line</td>
<td>15.851</td>
<td>16</td>
<td>1.14</td>
<td>0.40</td>
</tr>
<tr>
<td>sex x treatment</td>
<td>0.652</td>
<td>1</td>
<td>0.047</td>
<td>0.83</td>
</tr>
<tr>
<td>line x treatment</td>
<td>54.96</td>
<td>16</td>
<td>3.94</td>
<td>0.0046</td>
</tr>
<tr>
<td>sex x line x treatment</td>
<td>13.94</td>
<td>16</td>
<td>1.17</td>
<td>0.29</td>
</tr>
</tbody>
</table>

All sources of variance that included line were treated as random-effects terms.
males. Inbreeding depression for adult fitness was therefore 90.1% for males and 63.9% for females, respectively. Because male and female outbred means were different and inbreeding depression is a relative measure, male and female adult fitness was divided by their outbred group means before testing for sex-specific declines in fitness due to inbreeding by a three-way ANOVA, which showed a significant sex x inbreeding interaction (Table 2.2). The three-way interaction between clone, sex, and degree of inbreeding was also significant, and this is due to a significant clone x inbreeding interaction for males, but not for females. We also directly tested for sex-specific ID in adult fitness by creating 5,000 bootstrap replicates of the ratio of inbred to outbred fitness for each sex, using line means. This gave us an estimate of 90.1% (95% CI = [0.865, 0.933]) for male ID and 64.0% (95% CI = [0.586, 0.689]) for female ID. Males thus experienced 1.41 times more ID than females (95%CI = [1.30, 1.56]). The higher cost of inbreeding to adult male fitness was observed despite males having a smaller genomic target for inbreeding depression, and despite the expectation that inbred experimental females would likely suffer an additional reduction in fitness due to reduced viability in their progeny compared to the offspring of outbred experimental females.
### Table 2.2: Mixed-effects ANOVA on adult fitness

<table>
<thead>
<tr>
<th>Source</th>
<th>Mean Square</th>
<th>Numerator Degrees of Freedom</th>
<th>F-ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex</td>
<td>6.51</td>
<td>1</td>
<td>0.51</td>
<td>0.48</td>
</tr>
<tr>
<td>line</td>
<td>17.82</td>
<td>17</td>
<td>1.42</td>
<td>0.32</td>
</tr>
<tr>
<td>treatment</td>
<td>1479.9</td>
<td>1</td>
<td>190.51</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>sex x line</td>
<td>14.01</td>
<td>17</td>
<td>1.41</td>
<td>0.24</td>
</tr>
<tr>
<td>sex x treatment</td>
<td>143.1</td>
<td>1</td>
<td>15.73</td>
<td>0.0009</td>
</tr>
<tr>
<td>line x treatment</td>
<td>8.41</td>
<td>17</td>
<td>0.85</td>
<td>0.63</td>
</tr>
<tr>
<td>sex x line x treatment</td>
<td>9.92</td>
<td>17</td>
<td>3.26</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

All sources of variance that included line were treated as random-effects terms.
2.4.2 Inbreeding load and the dominance coefficient

The inbreeding load and average degree of dominance for mutations segregating in our experiment were estimated separately for each life-history stage and sex, using line means (Table 2.3). Many experimental designs involving inbred lines create outbred individuals by crossing two inbred lines, and can estimate the average dominance coefficient by regressing outbred fitness on the sum of the parental inbred fitness values (e.g. Willis, 1999). Because we created outbred flies by expressing each focal genome against a collection of random wild-type backgrounds we instead used the estimator \( A/(2(A+B)) \) (where \( A = -\ln(W_{OUT}) \), \( B = -\ln(W_{IN}/W_{OUT}) \), and the reported values are based on the average across lines). This provides a reasonable estimate of the arithmetic mean dominance for segregating alleles (Lynch & Walsh, 1997 but see Fernández et al., 2004). Estimates for dominance were very similar between the sexes, with genetic variation affecting adult fitness being more recessive than variation with deleterious effects for juvenile viability. This results are similar to those of (Mackay, 1985), who found evidence for additivity for viability but an overall dominance coefficient for net fitness of 0.13 in a different population of *Drosophila*. 
Table 2.3: Dominance and load estimates for both sexes and developmental stages

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>A+B</th>
<th>h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male viability</td>
<td>0.42</td>
<td>0.91</td>
<td>0.27</td>
</tr>
<tr>
<td>Female viability</td>
<td>0.43</td>
<td>0.91</td>
<td>0.26</td>
</tr>
<tr>
<td>Male adult fitness</td>
<td>2.31</td>
<td>2.88</td>
<td>0.10</td>
</tr>
<tr>
<td>Female adult fitness</td>
<td>1.02</td>
<td>1.37</td>
<td>0.13</td>
</tr>
</tbody>
</table>

B is $-\ln(w_I/w_O)$ and A is $-\ln(w_O)$. B is a measure of inbreeding load and the number of lethal equivalents in a genome is bounded by B and A+B. To calculate A for adult fitness, $w_O$ was calculated relative to the highest outbred line mean, which underestimates h if higher fitness genotypes exist.
2.4.3 Variance for fitness across sexes and life-stages

We tested for significant additive genetic variance among genomes in the IV population by fitting a random-effects ANOVA for each sex/developmental stage/inbreeding level combination, using line as the only factor. A significant effect of line was found for every treatment combination, except for female outbred viability (Table 2.4). For viability the coefficient of phenotypic variation ($CV_P$) was corrected for the number of flies placed in each vial, and the coefficient of environmental variation ($CV_E$) was estimated from the residual variance ($V_P - V_A$). In all cases, the coefficient of additive genetic variation ($CV_A$) increased with inbreeding, consistent with the exposure of recessive variation by inbreeding. Male $CV_A$ was generally higher than female $CV_A$, except for inbred juvenile viability. Residual variation ($CV_E$) also increased with inbreeding, suggesting a higher susceptibility of inbred flies to environmental effects.

2.4.4 Intrasexual correlations for juvenile viability and adult fitness

Inbred line means tended to be correlated with outbred line means. We found a significantly positive correlation for outbred viability regressed on inbred viability for female line means ($p = 0.016, r^2 = 0.33$, slope = 0.31), but no significant relationship between mean outbred and inbred male line means ($p = 0.23, r^2 = 0.095$, slope = 0.25). For adult fitness, mean inbred fitness was significantly correlated with outbred fitness for both sexes (males: $p = 0.0495, r^2 = 0.22$, slope = 2.43, females: $p < 0.0001$, $r^2 = 0.62$, slope = 1.21).
Table 2.4: Random-effects ANOVA for fitness components

<table>
<thead>
<tr>
<th></th>
<th>$CV_A$</th>
<th>$CV_P$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viability</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outbred Males</td>
<td>0.15</td>
<td>0.30</td>
<td>0.0020</td>
</tr>
<tr>
<td>Inbred Males</td>
<td>0.34</td>
<td>0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Outbred Females</td>
<td>0.10</td>
<td>0.26</td>
<td>0.0557</td>
</tr>
<tr>
<td>Inbred Females</td>
<td>0.41</td>
<td>0.49</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Adult Fitness</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outbred Males</td>
<td>0.38</td>
<td>1.09</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Inbred Males</td>
<td>0.65</td>
<td>2.92</td>
<td>0.0015</td>
</tr>
<tr>
<td>Outbred Females</td>
<td>0.20</td>
<td>0.80</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Inbred Females</td>
<td>0.39</td>
<td>1.40</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
2.4.5 Intersexual correlations for juvenile viability and adult fitness

For both juvenile viability and adult fitness, inbreeding was associated with an increase in the intersexual correlation (Figure 2.2). For both developmental stages, there was no significant correlation between outbred male and female fitness values (viability: \( p = 0.14, r^2 = 0.14, \) slope = 0.29, adult fitness: \( p = 0.54, r^2 = 0.024, \) slope = 0.12). For the inbred lines, there was a highly significant positive intersexual relationship for juvenile viability (\( p < 0.0001, r^2 = 0.82, \) slope = 1.04), but not for adult fitness (\( p = 0.39, r^2 = 0.045, \) slope = 0.54). The lack of significant intersexual correlation for inbred adults was due to a single inbred line, which had both the highest female fitness and one of the lowest male fitness estimates (\( W_{IN}/W_{OUT} = 0.01 \) for males, \( W_{IN}/W_{OUT} = 0.58 \) for females). Its status as a statistical outlier was confirmed using the Mahalanobis Distance (3.08), Jacknife Distance (4.96) and the \( T^2 \) (9.51) criteria, and removing this line from the analysis results in a significantly positive and nearly isometric intersexual relationship for inbred adult fitness (\( p = 0.032, r^2 = 0.27, \) slope = 1.03).

2.4.1 Life-history correlations

Within each sex, we found no relationship between line means for viability and adult fitness for either outbred (males: \( p = 0.21, r^2 = 0.10, \) slope = 0.67; females: \( p = 0.49, r^2 = 0.032, \) slope = 0.34) or inbred (males: \( p = 0.89, r^2 = 0.0014, \) slope = 0.012; females: \( p = 0.482, r^2 = 0.034, \) slope = 0.13) flies, indicating no detectable tradeoffs or pleiotropy between juvenile and adult performance in terms of whole-genome effects.
Figure 2.2: Intersexual correlations for juvenile viability and adult fitness under inbred and outbred experimental treatments. Outbred line means (solid circles) are connected to their corresponding fitness value as inbred lines (white triangles) by arrows. Solid lines represent the slope of the intersexual correlation for outbred lines, and dashed lines show the slope of the intersexual correlation for inbred lines. The slope shown for inbred adult fitness was determined after removal of an outlier (see text).
2.5 Discussion

We present the first measurements of the cost of inbreeding to both sexes across the entire genome and life-history for a population of *Drosophila melanogaster*, extending the findings of previous studies having only examined fitness proxies, a fraction of the genome, a limited portion of the life-cycle, or a single sex. While we found that both sexes suffered equal reductions in juvenile viability, males experienced much stronger declines in adult fitness, resulting in a substantially greater overall cost of inbreeding for males than for females. Because the coefficient of dominance was the same between the sexes, we attribute greater inbreeding depression in males to stronger selection. Importantly, we recorded positive intersexual correlations for inbred fitness, indicating that selection against segregating mutations generally operates in the same direction for both sexes. Our data thus provide the first direct empirical support for the hypothesis that stronger selection on males has the potential to benefit females by reducing the number of deleterious mutations segregating in sexual populations.

2.5.1 Comparison to previous studies

The magnitude of inbreeding depression in populations is relevant to many areas of evolutionary biology, and has been the subject of considerable study. Although remarkably few studies have explicitly set out to examine the sex-specificity of ID, there are many individual estimates of ID in *Drosophila* for the fitness components reported here. (Lynch & Walsh, 1997) summarized the results for several studies measuring ID in *Drosophila melanogaster*. Although the estimates were variable, mean ID for egg-to-
adult viability was, with complete inbreeding, 0.36 (n=3) for the studies having employed *Drosophila melanogaster*; almost identical to the ID of 0.35 reported here. Estimates of adult fitness were also quite variable, and no study simultaneously measured both male and female major fitness components. Mean ID for female fertility (n=3) was 0.45 (0.64 in this study), and mean ID for male mating ability (n=3) was 0.73 (0.90 in this study), which is in good qualitative agreement with our results. These similarities are especially striking in light of the differences in environments and populations between all studies.

### 2.5.2 Estimating the strength of selection on the sexes for individual loci

Previous studies have shown that sexual selection can augment purifying selection for single mutations, but have generally been unable to directly compare the total strength of selection acting on males and females (reviewed by Whitlock & Agrawal, 2009)). Genome-level measures of sex-specific selection help to fill this critical gap, but it is nonetheless desirable to relate genome-wide measurements to the processes operating at individual loci. Most models attempt to capture the effects of sexual selection on the fitness consequences of segregating mutations (expressed as mutation load) by considering the relative strength of selection on males versus females (α) at individual loci, given by

\[
\alpha = \frac{s_m}{s_f}
\]
When an individual is homozygous for a single mutation, the fitness of its genotype in males and females can be expressed as

\[ w_f = 1 - s_f \]
\[ w_m = 1 - \alpha \cdot s_f \]

And we can directly calculate

\[ \alpha = \frac{1 - w_m}{1 - w_f} \]

For a given inbred line, which might contain many mutations, and for which we have fitness data for both sexes, we can calculate in a similar fashion:

\[ A = \frac{1 - W_m}{1 - W_f} \]

If the fitness effects of mutations are multiplicative (i.e., no epistasis) we will have:

\[ W_f = (w_f)^n \]
\[ W_m = (w_m)^n \]
Where \( n \) is the number of mutations. If \( s_f \) and \( \alpha \) are variable across loci, we can replace \( w_m \) and \( w_f \) by their geometric means (\( \bar{w}_m \) and \( \bar{w}_f \)), and define:

\[
\hat{s}_f = 1 - \bar{w}_f \\
\hat{s}_m = 1 - \bar{w}_m \\
\hat{\alpha} = \frac{\hat{s}_m}{\hat{s}_f} 
\]

Substituting these values yields:

\[
A = \frac{1 - (1 - \hat{\alpha} \cdot \hat{s}_f)^n}{1 - (1 - \hat{s}_f)^n} 
\]

It is apparent that \( A = \hat{\alpha} \) only when \( n = 1 \), and \( A \) tends towards 1 as the number of mutations in a given genome increases. Thus, \( A \) underestimates \( \hat{\alpha} \) when \( \hat{\alpha} > 1 \) (this study), and would overestimate it if \( \hat{\alpha} \) were smaller than one. For this reason, measurements of sex-specific selection at the genome level are likely to underestimate the strength of selection at individual loci, and \( A \) can be considered equivalent to \( \hat{\alpha}_{\text{min}} \).

2.5.3 Stage- and sex-specificity of inbreeding depression

We found that females did not suffer a greater cost of inbreeding at the juvenile stage \( A = 1.02 \), as measured by egg-to-adult viability. This result is perhaps surprising, because of the expected additional cost of inbreeding to females resulting from the X
chromosome (approximately 20% of the total gene content). Combined with the strongly positive intersexual correlation for juvenile viability, this result suggests that the X chromosome harbors few deleterious mutations affecting viability in the IV population. This finding is consistent with an earlier study of a different, wild population of D. melanogaster, which found low X-linked viability load along with a positive intersexual correlation for the variation in viability on the X (Eanes et al., 1985). We speculate that viability selection on males, where the X is expressed hemizygotously, has reduced the inbreeding load for females, thus suggesting a specific instance in which natural selection on males has resulted in fewer mutations segregating in the population, with viability benefits for females.

For adult fitness, the magnitude of inbreeding depression was much higher for males than for females. A single line was exceptional in that the cost of inbreeding was extremely high for males (A for this line was 2.34, 3.5 standard deviations away from the group mean), and was identified as a bivariate outlier in the inbred group (see Results) due to its combination of unusually high inbred female fitness and low inbred male fitness. For any given individual genome, a stronger decline in relative fitness in males could be due to the presence of sexually antagonistic alleles, the presence of alleles with sex-limited effects on fitness, or to a higher coefficient of selection on shared alleles. The relative importance of these three modes of allelic effect across the genome will determine the general magnitude and sign of the intersexual genetic correlation for fitness. Without
detailed genetic and phenotypic characterization it is unfortunately impossible to speculate on the reason for a specific line’s departure from the group response, but for the remaining 17 lines, the intersexual genetic correlation ($r_{MF}$) in inbred flies was estimated to be 0.52, with a slope of nearly one and net selection on males being greater than on females ($A = 1.24$). Because we observed very little adult mortality, the observation of stronger net selection acting upon male fitness was most likely the result of reduced mating or postcopulatory fertilization success. We therefore attribute greater inbreeding depression for males to stronger selection on the characters underlying success in sexual selection.

Our estimate of $A$ applies to autosomal mutations affecting adult fitness, because the X chromosome does not contribute to inbreeding depression in males. X-linked mutations affecting adult female fitness will cause our measure of $A$ for autosomal loci to be conservative, as they will increase the effect of inbreeding to females beyond the cost of autosomal loci, minimizing the difference between the sexes. Substantial X-linked inbreeding depression for adult female fitness has previously been reported in another population of *D. melanogaster* (Wilton & Sved, 1979). Alpha may also be underestimated if the offspring of inbred experimental females suffer reduced viability relative to the offspring of outbred experimental females as a result of maternal effects.

The generally positive genetic correlation we observed would seem to preclude the presence of large amounts of segregating sexually antagonistic variation in the $IV$
population, or a dominant role for alleles with sex-limited effects. These results differ notably from studies by Chippindale et al. (2001) and from Innocenti & Morrow (2010), using the LH\textsubscript{M} population of \textit{D. melanogaster}, who found a negative intersexual correlation for fitness (\textit{e.g.}, \( r_{MF} = -0.52 \), from Innocenti & Morrow, 2010). The extent of intralocus sexual conflict may therefore vary considerably between populations, and the very high levels of sexually antagonistic variation segregating in the \( LH_M \) population may not be a general feature of \textit{D. melanogaster} populations. Nevertheless, the intersexual genetic correlation did appear weaker for adult fitness than for juvenile survival, which may indicate that sex-limited or sexually antagonistic alleles segregate in the \textit{IV} population.

### 2.5.4 Inbreeding load across life-history

We did not detect any correlation between juvenile and adult fitness, implying that benefits accrued by females from selection in adult males would not manifest themselves as viability gains in the offspring. Thus, the strength of selection on adult relative to juvenile fitness will determine the potential for stronger selection on adult males to affect the net fitness of females. In our sample of genomic haplotypes, inbreeding load was much higher for adult fitness than for juvenile fitness for both sexes. For females, adult load represented 67% of the total inbreeding load, whereas for males it was 84%, implying stronger net selection overall for adults, and particularly for males. This stronger net selection may reflect a direct increase in the strength of selection active on adult reproductive success relative to juvenile viability (adult mortality is very low in the
IV population), or may be caused by a larger number of adult-specific segregating mutations. Very few transcripts in *D. melanogaster* appear to be adult specific (Arbeitman et al., 2002), and most genes in this organism appear to be developmentally regulated (Stolc et al., 2004), which suggests that for the majority of genes the opportunity for selection exists across the entire life-history. Because the total inbreeding load appears to be dominated by the reproductive success of adults, we expect that a reduction in the numbers of mutations affecting adult female fitness as a result of sexual selection would also result in a meaningful increase in their mean fitness.

The potential for sexual selection to reduce total genetic load is likely to be taxon-specific, as it depends on the relative strength of selection on adult versus juvenile fitness components as well as on the correlation between the two. There has been considerable interest in measuring the relative strength of selection across life-history stages: for a review see (Hoekstra et al., 2001), who suggests that for many populations the strength of sexual selection, broadly defined, is generally greater than that of natural selection.

**2.5.5 Predicted benefits of sexual selection to adult female fitness**

The results in (Agrawal, 2001) assume that mutations act additively within a locus and that the sexual populations are completely outbred, but most mutations are recessive and moderate levels of inbreeding are the norm for sexual populations. Because both recessivity and population structure can separately yield benefits to sexual reproduction, the nature of which depends on the mutation rate (Agrawal & Chasnov, 2001), estimates
of all these parameters are necessary to make quantitative predictions of benefits to females arising from sexual selection. To our knowledge, however, there have been no attempts to incorporate sexual selection, population structure, the dominance of mutations, and mutation rate in one analysis. We provide a simple program to carry out and plot numerical simulations while varying all these parameters using R, a free statistical package (Appendix A).

Using a recent estimate of the diploid genomic deleterious mutations rate in *Drosophila* (U = 1.2, Haag-Liautard *et al.*, 2007) as a proxy for the mutation rate in the IV population, the average dominance coefficient for segregating mutations affecting adult fitness obtained in this study (h=0.11), and our estimate of $\hat{\alpha}_{\min}$ (1.24), we speculate on the potential gain in adult female mean fitness for differing levels of population structure relative to a sexual population experiencing no sexual selection (Appendix A). With no population structure ($f$=0) equilibrium mean fitness is predicted to be 29% higher than the equivalent sexual population experiencing no additional selection on males and is 14% higher with complete inbreeding ($f$=1). We also provide a contour plot of mean female fitness for a variety of combinations of mutation rate and $\hat{\alpha}$ at a moderate level of inbreeding ($f$=0.2), which shows the potential for substantial gains in fitness from sexual selection, especially at high mutation rates (Appendix A). Lower mutation loads in sexual populations could contribute to a lower extinction risk (Frankham, 2005). Stronger selection on males could be a reason for male-biased dispersal (Pusey, 1987), and may
also have implications for breeding schemes aimed at improving fitness in captive populations (Wedekind, 2002).

2.5.6 Future Directions

In addition to estimates of net selection on males and females for standing genetic variation (this study), estimates of the relative strength of selection for \textit{de novo} mutations would be desirable. Segregating genetic variation may not reflect the underlying distribution of mutations and is expected to be enriched in certain classes of variation, including sexually antagonistic variation (Rice, 1984) and various forms of balancing selection (Peng et al., 1991). Other classes of mutation will be underrepresented and can only be studied with the input of new genetic variation. For example, we detected no inbreeding depression for X-linked mutations affecting juvenile viability, presumably due to a lack of segregating variation. Although we inferred that strong viability selection on hemizygous X chromosomes was the probable cause, a direct measure would be preferable. Deleterious mutations experiencing strong sexual selection in males may be rapidly purged, and estimates of the relative strength of selection in males and females might therefore be higher for new mutations than for segregating variation. We view estimates of sex-specific selection on segregating and \textit{de novo} mutations as complementary, and are currently engaged in a mutation-accumulation experiment to address some of these outstanding questions.
Theoretical models for the benefit of stronger selection on males to sexual population have been presented (Agrawal, 2001, Siller, 2001, Whitlock & Agrawal, 2009), and our work suggests further avenues for both empirical and theoretical work. Stronger selection on males will further amplify the benefits of mild inbreeding in purging deleterious mutations from sexual populations; we provide numerical estimates here but analytic predictions await a theoretical treatment. The potential for selection on males to reduce the deleterious mutation load affecting females is likely be even greater than current models predict.

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### 2.7 References


Chapter 3

Experimental mutation-accumulation on the X chromosome of *Drosophila melanogaster* reveals stronger selection on males than females.

3.1 Abstract

Sex differences in the magnitude or direction of mutational effect may be important to a variety of population processes, shaping the mutation load and affecting the cost of sex itself. These differences are expected to be greatest after sexual maturity. Mutation-accumulation (MA) experiments provide the most direct way to examine the consequences of new mutations, but most studies have focused on juvenile viability without regard to sex, and on autosomes rather than sex chromosomes; both adult fitness and X-linkage have been little studied. We therefore investigated the effects of 50 generations of X-chromosome mutation accumulation on the fitness of males and females derived from an outbred population of *Drosophila melanogaster*. Fitness declined rapidly in both sexes as a result of MA, but adult males showed markedly greater fitness loss relative to their controls compared to females expressing identical genotypes, even when females were made homozygous for the X. We estimate that these mutations are partially additive ($h \sim 0.3$) in females. In addition, the majority of new mutations appear to harm both males and females. Our data helps fill a gap in our understanding of the consequences of sexual selection for genetic load, and suggests that stronger selection on males may indeed purge deleterious mutations affecting female fitness.
3.2 Introduction

Understanding the properties of new mutations is critical to a broad range of evolutionary theory, including models relating to the maintenance of genetic variation in the face of selection (Lande, 1975, Barton, 1986, Keightley & Halligan, 2008), the persistence of small populations (Lande, 1995), and the advantages of sexual reproduction (Agrawal & Chasnov, 2001, Salathé et al., 2006, Haag & Roze, 2007). Accordingly, spontaneous mutation has been the focus of numerous experimental studies (reviewed in Simmons & Crow, 1977, Keightley, 1996, Drake et al., 1998, Keightley & Halligan, 2008, Halligan & Keightley, 2009) particularly with the fruit fly, Drosophila melanogaster. Mutation-accumulation (MA) experiments, in which new mutations are allowed to fix by removing selection, have typically measured changes in juvenile viability (egg-to-adult survival) as an indicator of total fitness. Adult survival and reproductive success (adult fitness) will often be important contributors to total fitness, yet have been much less studied in MA experiments.

Adult fitness is important to our understanding of mutations for several reasons. For many populations, it is thought that sexual selection is a stronger force than viability selection (Hoekstra et al., 2001). Moreover, variation in juvenile growth, rather than survival, can have carryover effects to adult size, condition and the realization of adult fitness. This would imply that mutation pressure on total fitness could be much greater than studies examining juvenile fitness alone would imply. Because reproductively mature individuals typically express the most pronounced sex-differences in phenotype,
implying divergence in selection pressures, the consequences of new mutations could be sex-specific. On the other hand, the expression of sexually selected traits may still share a common genetic basis between the sexes, as their expression is thought to depend on the overall health and vigor of the individual (the genic capture hypothesis) (Rowe & Houle, 1996, Tomkins et al., 2004). Thus, sexual selection on males could yield a correlated response in female fitness.

Indeed, sex differences in the impact of mutation have been shown to potentially shape the deleterious mutation load, with important consequences. For example, stronger selection on males is expected to improve the mean fitness of females for a given mutation rate, provided mutations have the same directional effect on fitness in each sex (Whitlock & Agrawal, 2009). Thus, a reduction in mutation load due to sexual selection may reduce the cost of sexual reproduction and the severity of inbreeding depression. However, the degree to which new mutations have concordant effects is unknown, and several recent studies have demonstrated the existence of alleles with opposite effects on adult fitness in each sex. These sexually antagonistic genes may actually create a cost of sexual selection for females (Chippindale et al., 2001, Brommer et al., 2007, Foerster et al., 2007). Whether sexual selection improves or degrades the mean fitness of females depends on the properties of new mutations as well as on the concordance of mutational effects between the sexes.
A few studies have attempted to measure the effects of MA on components of adult fitness in *Drosophila melanogaster*, with conflicting results. One study (Fernández and López-Fanjul, 1996), using MA lines derived from repeated brother-sister mating, examined female fecundity under non-competitive conditions and found, surprisingly, that it increased on average when compared to a large random-mating isogenic stock. Others (Houle et al., 1994), found that female fecundity declined with MA but found no significant effect on male mating ability, probably due to a small sample size; this study also suffered from a lack of concurrently measured controls. Two other studies either found very small negative effects on female fecundity after 30 generations of MA (Shabalina et al., 1997), or deleterious effects on both male mating ability and female fecundity after 30 generations of MA with stronger effects on male mating ability than female fecundity (Mack et al., 2000). Given the lack of consensus from these studies, further experimentation is clearly warranted. In addition to estimating the magnitude of selection on males and females, estimating the extent to which mutations have similar directional effects on each sex is also of interest.

MA experiments in *Drosophila* have typically been performed on the autosomes, and most frequently on the second chromosome (Halligan & Keightley, 2009). Very few studies have explicitly examined the X chromosome (Gong et al., 2005, Gong et al., 2006), despite it accounting for around 20% of the total gene content, and none of these studies measured adult fitness. The X chromosome has a number of distinctive features
particularly relevant in the context of the study of adult fitness. First, males are functionally homozygous for the X chromosome. We therefore expect selection to act more efficiently when mutations are expressed in males, the result of which could be reduced genetic load on the X. Second, the X chromosome appears to be dimorphic in terms of expression pattern, containing a relative paucity of genes with male-biased expression and an excess of genes with female-biased expression (Ranz et al., 2003). This might lead to the expectation that the fitness consequences of MA on the X chromosome are greater for females. Third, the X chromosome is predicted to be the genomic location most likely to harbour sexually antagonistic alleles. This is due to its expression pattern, with recessive male-benefit alleles being sheltered from selection in females and partially dominant female-benefit mutations enjoying the advantage of being expressed in females two-thirds of the time (Rice, 1984) but see (Fry, 2010). This latter prediction was tested in one population of *Drosophila* by measuring the intersexual correlation for adult fitness across a sample of X chromosomes. A significant negative correlation indicated that X-chromosomes favored in females were disfavored in males, and vice-versa, and that X is a major contributor to the negative intersexual correlation for adult fitness reported in a genome-wide assay (Gibson et al., 2002).

The way in which mutation and selection interact to shape the genetic load of populations for the X chromosome is unclear. On one hand, the greater effectiveness of selection on males due to the hemizygous expression of the X, which may be further reinforced by
sexual selection, is expected to lower the mutation load for females at shared loci. On the other hand, the presence of widespread intralocus sexual conflict would impose a net cost to females. The overall tendency for new mutations to cause sexually concordant effects, sex-independent effects, or sexually antagonistic effects will therefore determine whether the X chromosome is a liability or an asset to female fitness. Allowing new mutations to accumulate, and determining their average effect in each sex, is the best way to ascertain the overall mutational character of the X chromosome.

We therefore sought to quantify the effects of MA on the adult fitness of males and females in a laboratory-adapted population of *Drosophila melanogaster*. The Ives (IV) population has been maintained as a large population on a fixed culture protocol for several decades and is therefore likely to be at mutation-selection balance. This population’s stable environment also defines the relevant selective environment in which to measure fitness for both sexes. These features, combined with the inherent advantages of *Drosophila* as a model system, make the IV population an attractive study system to study the mutational process, as many of the simplifying assumptions used in models of mutation likely hold.

We carried out a MA experiment on a genetically variable sample of X chromosomes from the IV population. After 50 generations of MA we expressed these chromosomes, along with their controls, in males and females. For females we expressed the MA
chromosomes in both the heterozygous state to mimic the normal condition of expression for new mutations in an outbreeding population, and in the homozygous state to directly compare the strength of selection to hemizygous males. We found that the magnitude of mutational effects was higher in males than in females. In addition, the intersexual correlation for fitness in the MA lines was positive, suggesting that females may indeed benefit from stronger selection in males.

3.3 Methods

3.3.1 Stocks and culture conditions
All flies sampled for this experiment were derived from the same lab population – the Ives (IV) population. This lab-based population was established from a wild-caught sample of 200 females and 200 males in Amherst, Massachusetts in 1975 (Rose & Charlesworth, 1981). From 1981 onwards, the IV laboratory population has been maintained as a large outbred stock at a minimum population size of 1000 individuals at 25°C, 50% relative humidity, on a 14 day, discrete generation cycle with moderate densities of 60-120 individuals per vial with 10mL of banana/agar/killed-yeast medium (Rose, 1984). On Day 14, the population is placed under CO₂ anaesthesia, mixed and redistributed into new vials to oviposit until ~100 eggs are laid in each vial. This usually takes approximately 30 minutes, and represents the only opportunity for offspring production.

The IVbw population, which was created by backcrossing a recessive brown-eye colour marker (bw¹) into the IV population, served as an outbred, genetically similar population
for use as competitors against IV flies for measurements of fitness. This population is maintained under a culture protocol identical to the IV population, and is periodically backcrossed to the IV population to prevent drift between the focal and competitor populations.

Two additional stocks were created in order to express X chromosomes of interest in males and females. The DX-IV population is a copy of the IV population into which a compound X-chromosome (C(1)DX y f) has been introgressed. This compound-X (DX) chromosome forces the normal pattern of sex-chromosome inheritance to be reversed: males crossed with DX-bearing females pass on their X chromosome to their sons, and receive a Y chromosome from their mother. The FM-IV population is a copy of the IV population into which an X-balancer chromosome was introgressed (FM7a).

3.3.2 Mutation-accumulation protocol
A sample of 19 genetically variable X chromosomes from the IV population was obtained by singly crossing males from the IV population to virgin females bearing DX chromosomes. Males from these crosses were fixed for the X chromosomes of their fathers and used to simultaneously found two initially identical groups.

For the mutation-accumulation (MA) population, each of the 19 lines was taken through a single-X bottleneck every generation (Figure 3.1). To accomplish this, three males descended from the same father were separately mated to groups of five virgin DX-
bearing females to prevent line loss. These groups were housed in ‘conditioning vials’ with supplemental live yeast for two days, after which they were transferred to fresh vials and allowed to oviposit overnight. The conditioning vials were kept and used as a backup in case of failure in the oviposition vials. The oviposition vials were reared under reduced density (approximately 40 individuals) to minimize competition. After twelve days, three males were again chosen from a single vial to start the next cycle, so that for each generation of MA all of the males selected descended from the same father. By creating a single X-chromosome bottleneck each generation, selection on new germ-line mutations was minimized, except against those mutations causing death or sterility in males.

The control (C) lines were maintained in the same fashion as the MA lines except that each X-chromosome line was maintained by crossing 8-12 males and 16-20 females in two vials (16-24 males per population), keeping rearing densities at approximately 100 individuals and mixing between vials each generation (Figure 3.1). By maintaining the C lines in relatively small populations without recombination we hoped to prevent the possibility of adaptation in the control lines, a problem that has plagued the interpretation previous MA studies (Keightley, 1996, Keightley et al., 1998), while allowing for sufficient selection to prevent significant depression in fitness due to MA. This method of
Figure 3.1: Generation of mutation-accumulation lines and experimental flies. (A) Mutation-accumulation protocol. A single male bearing an IV-derived X chromosome was mated to multiple DX (C(1)DX y f)-bearing females. A single son, bearing new mutations (white stars), was randomly selected to found the next MA generation. Each generation, triplicate crosses were performed to guard against line lost. (B) Maintenance of control lines. Control lines were initially founded from the same X chromosomes used to create the MA lines. Each generation, males from two vials were mixed together and then split into two vials, each containing 8-10 males and 16-20 DX bearing females. (C) Generation of experimental MA flies. The autosomes from the C and MA males were substituted with a set of marked translocated autosomes ((T(2 : 3)rdgc st in ri p^p bw) (grey bars) and crossed to DX-IV females. The resulting males were subsequently crossed to both DX-IV females and FM (FM7a)-IV females to yield males fixed for the MA-X chromosome and females with a balanced MA-X chromosome. Females carrying a balanced C or MA X chromosome in the IV autosomal background were crossed to either random IV males to generate heterozygous females or were crossed to males bearing MA X-chromosomes to generate homozygous females. Males bearing MA chromosomes were collected from both crosses. The sequence of crosses was identical to generate control experimental flies.
maintaining control lines is likely to be ineffective in preventing mutations of very small effect to fix, however, and will make our estimates of the total effect of MA conservative.

3.3.3 Creation of experimental lines

For fitness assays, X chromosomes from the C and MA lines were placed in a random outbred IV autosomal background (Figure 3.1). The autosomes from the C and MA males were first substituted with a set of marked translocated autosomes ((T(2 : 3)rde st in ri p^p bw). Eight to ten C or MA males from each line were then crossed to virgin DX-IV females. Males from this cross carried X-chromosomes from their parent lines, an IV Y-chromosome from the DX-IV females, a set of translocated autosomes, a set of random IV-derived autosomes, and were subsequently crossed to both DX-IV females and FM-IV females. Males from the DX-IV cross were fixed for the X chromosome of interest and possessed a wild-type set of autosomes, while females from the FM-IV cross carried balanced X chromosomes along with a set of random IV autosomes.

Virgin females carrying a balanced C or MA X chromosome in the IV autosomal background were crossed to either random IV males to generate heterozygous-X females or were crossed to males bearing C and MA X-chromosomes in an IV autosomal background to generate homozygous-X females. Males bearing the C and MA chromosomes in an IV autosomal background were collected from both crosses. Both heterozygous and homozygous females were therefore produced from the same maternal
genotype, to remove the possibility of confounding maternal effects. The normal pattern of sex-chromosome inheritance was also preserved in the production of experimental flies.

3.3.4 Fitness Assay

The effects of mutation are known to change as a result of both the physical environment and the genetic environment (Houle et al., 1994, Shabalina et al., 1997). The IV population-genetic structure has been shaped by virtually unchanging selection pressure for over 700 generations: the effects of mutations in this genetic background are therefore best interpreted in the environment to which the population has adapted. Our measure of adult fitness was designed to capture the outcome of adult competition under IV culture conditions, while making the results of such competition tractable.

We transplanted experimental flies from the C or MA lines using light CO₂ anesthesia during the period of peak adult eclosion (Day 9 post-oviposition) in same-sex groups of 5 to an age-synchronized culture of IVbw reared under standard conditions. For five days, the experimental flies were allowed to acquire resources and mates in the competition vials. On Day 14 each vial was individually subjected to 2.5 minutes of CO₂, to simulate the amount of gas normally received when IV vials are mixed, and placed into vials containing fresh medium for oviposition to standard culture densities (25-30 minutes). The adults were then removed from the vials and the sex/number of progeny from the target individuals (distinguishable by their red eyes) was scored twelve to fourteen days
later, sufficient time for all of the adults to emerge. The number of progeny present in the vials measures the success of their parents in the previous generation. There will also be an influence of juvenile viability, but this will make our results conservative with respect to \( \hat{\alpha} \) (see Discussion). Each treatment/line/sex combination was replicated 20 times for a total of 2,280 vials.

**3.3.5 Statistical Analysis**

Parameter estimates were derived using the normalized likelihood method (Shcherbinin, 1987), using the R statistical package (R Development Core Team, 2010). Normalized likelihoods satisfy frequentist principles of inference but are also equivalent to Bayesian analyses using flat priors (Shcherbinin, 1987, Walley, 2002). The normalized likelihood distribution of a parameter \( \theta \) given data \( Y \) is equal to:

\[
\frac{L(\theta | Y)}{\int L(\theta | Y) d\theta}
\]

Where the denominator is simply a normalizing constant such that the likelihood distribution has unit area (or sum, in the discrete case). The subsequent posterior distribution (or likelihood density) can be used for point and interval estimation of \( \theta \), and numerical methods readily yield estimates for various functions of \( \theta \). Where the likelihood function also depends on other parameters (for example, the likelihood for the mean in a normal distribution also depends on the standard deviation), we take the marginal likelihood taken over all values of the second parameter.
We calculated the posteriors for the rate of red-eyed offspring production (number of red-eyed offspring produced in the oviposition period) for each line/sex/treatment combination using the Negative-Binomial likelihood function.

\[
L(\lambda | Y) = \prod_{i=1}^{n} \frac{\Gamma(y_i + \phi)}{\Gamma(\phi) + y_i!} \cdot p^\phi (1 - p)^{y_i}
\]

Where the \(y_i\) are the numbers of red-eyed offspring in each vial of a particular line/sex/treatment combination, \(\phi\) is a dispersion parameter and \(p\) is the probability of success, such that \(p = \frac{\phi}{\phi + \lambda}\) and \(\lambda\) is the mean offspring production for those flies. We took the exponent of the log-likelihood function to simplify calculation. At high values of \(\phi\), the negative-binomial distribution approaches a poisson distribution with mean \(\lambda\).

We estimated \(\phi\) separately for males and females, because exploratory analysis suggested that the male data was more dispersed than the female data.

We then evaluated the likelihood function at 5 000 x 5 000 grid spanning a large interval of \(\lambda(10^{-10} \leq \lambda \leq 30)\) and \(\phi(10^{-10} \leq \phi \leq 100)\) prior to normalization and marginalization in order to obtain accurate posteriors. Because the dispersion parameter is strictly positive and the resulting distribution asymptotically approaches a poisson distribution at high values, we will tend to over-estimate overdispersion by cutting off the likelihood surface at 100 (this was done for computational reasons). If the true distribution is poisson-distributed, our confidence intervals will be somewhat wider, and our p-values will be
We estimated parameters depending on multiple line means (for example, the group MA male mean) by numerical methods. For each line we first sampled 10 000 means according to their posterior probabilities and then combined them according to the desired function of the $\lambda$. For example, the point estimate and 95% confidence interval for the group MA male mean was calculated by taking 10 000 averages of the 19 MA male line means, where each MA line mean is a randomly sampled value from the posterior distribution for that line. The mean and 95% confidence interval of the resulting distribution corresponds to the point estimate and 95% confidence interval for the group MA male mean. P-values were estimated in a similar fashion, by calculating the area of the empirical distribution corresponding to the desired test. The main advantage of this method is the relative ease with which point and interval estimates for parameter that are complicated functions of the data (for example, $\hat{\alpha}$ and $\hat{h}$) can be obtained, without having to first derive the appropriate sampling distribution.

3.4 Results

3.4.1 Declines in fitness due to mutation-accumulation

Nineteen X-chromosome MA lines, along with a set of Control lines, were expressed in both sexes and assayed for fitness. X-chromosomes subjected to mutation-accumulation were less fit than their controls when expressed in both sexes. Based on analysis of line means, vials containing females expressing homozygous MA-X chromosomes had 4.10
red-eyed offspring, on average (95% CI = (3.89, 4.32)), whereas vials from C lines contained an average of 5.34 red-eyed offspring (95% CI = (5.09, 5.60)). Vials with males with the same MA-X chromosomes contained 8.94 red-eyed offspring on average (95% CI = (8.41, 9.51)): vials with males from the C lines contained 13.35 red-eyed offspring (95% CI = (12.66, 14.11).

In terms of relative fitness, MA females had 23.2% fewer offspring (95% CI = (17.4, 28.5%), p < 0.0001), and males from the MA population produced 33.1% fewer offspring than their controls (95% CI = (27.4%, 38.2%), p < 0.0001) (Figure 3.2). The effects of mutation-accumulation were much less pronounced for females expressing MA-X chromosomes heterozygously. Vials with females expressing heterozygous MA-X chromosomes contained an average of 8.98 offspring (95% CI = (8.57, 9.41)), and females with heterozygous C-X chromosomes produced an average of 9.62 offspring (95% CI = (8.48, 10.10)). Translated to relative fitness heterozygous MA females declined by 6.8% (95% CI = (1.6%, 11.6%), p = 0.01). The relative fitness of males bearing MA-X chromosomes ($W_m$) was significantly lower than the relative fitness of homozygous females with the same pool of mutations ($W_f$) (mean $W_m/W_f = 0.87$, 95% CI = (0.78, 0.97), p = 0.013), and homozygous MA females had significantly lower relative fitness than their heterozygous counterparts (mean =0.82, 95% CI = (0.75, 0.90), p = 0.0003).
Figure 3.2: Decline in relative fitness with MA. Heterozygous MA females experienced the smallest decline in fitness relative to their controls, followed by homozygous females and hemizygous males. Boxes span the interquartile range, and the whiskers extend 1.5 times this distance from the box.
3.4.2 Inbreeding depression for female fitness
Making the X chromosome homozygous had detrimental effects on fitness for females expressing X chromosomes from the both the C and MA populations. For the C-X chromosomes, inbreeding was associated with a 44.1% decline in fitness (95% CI = (40.7%, 47.4%), p < 0.0001). The effect was larger for MA-X chromosomes, where inbreeding was associated with a 54.0% decline in fitness (95% CI = (50.8%, 56.9%), p < 0.0001). For the C lines, there was no correlation between heterozygous and homozygous female fitness (p = 0.46, r² = 0.16, slope = 0.06). For the MA lines, however, we observed a significant correlation between inbred and outbred line means (p = 0.0048, r² = 0.38, slope = 0.20) (Figure 3.3). We tested for a difference in the slope and correlation between the MA and C lines by performing 5,000 bootstrap replicates, which did not reject the null hypothesis for either the slope (p = 0.30) or the correlation (p = 0.21).

3.4.3 Genetic variation for fitness and heritability
We estimated genetic variance for fitness by fitting a random-effects ANOVA (using line as the only factor) to each treatment/sex combination (Table 3.1). Significant genetic variance for fitness was found at all levels, and the MA lines had both greater levels of genetic variation and higher heritability than the C lines. CV_E and CV_P were corrected for the number of flies in each vial and these corrected estimates were used to infer heritability at the individual-level. This was done by multiplying residual variance
Figure 3.3: Correlation between inbred and outbred female fitness. Outbred and inbred relative fitness were not correlated in the C lines (black circles, solid regression line) and were positively correlated in the MA lines (white circles, dashed regression line), indicating increased dominance of new mutations. Fitness values were calculated relative to the heterozygous control population mean.
**Table 3.1:** Heritability, and coefficients of additive (CV\(_A\)) environmental (CV\(_E\)) and phenotypic (CV\(_P\)) variation for each sex-treatment combination. P-values are for the random-effects ANOVA used to estimate variance components.

<table>
<thead>
<tr>
<th></th>
<th>(h^2)</th>
<th>CV(_A)</th>
<th>CV(_E)</th>
<th>CV(_P)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C Females (heterozygous)</td>
<td>0.010</td>
<td>0.099</td>
<td>1.00</td>
<td>1.00</td>
<td>0.011</td>
</tr>
<tr>
<td>MA Females (heterozygous)</td>
<td>0.023</td>
<td>0.15</td>
<td>0.98</td>
<td>0.99</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C Females (homozygous)</td>
<td>0.089</td>
<td>0.39</td>
<td>1.26</td>
<td>1.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MA Females (homozygous)</td>
<td>0.16</td>
<td>0.65</td>
<td>1.49</td>
<td>1.63</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C Males (hemizygous)</td>
<td>0.007</td>
<td>0.12</td>
<td>1.51</td>
<td>1.51</td>
<td>0.041</td>
</tr>
<tr>
<td>MA Males (hemizygous)</td>
<td>0.042</td>
<td>0.37</td>
<td>1.76</td>
<td>1.80</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
estimates by the number of flies in each vial and then subtracting variance due to genotype.

### 3.4.4 Intersexual correlations
We estimated the intersexual genetic correlation for adult fitness in the IV population, for the C- and MA-X chromosomes. For the C lines, we recorded no significant genetic correlation between the line means of females expressing X chromosomes homozygously and the line means of males (p = 0.10, $r^2 = 0.15$, slope = 0.67). In the MA lines, fitness was positively correlated between homozygous MA females and MA males (p = 0.015, $r^2 = 0.30$, slope = 0.99) (Figure 3.4). We tested for a difference in the slope and correlation between the MA and C lines by performing 5,000 bootstrap replicates, which did not reject the null hypothesis for either the slope (p = 0.48) or the correlation (p = 0.54).

### 3.4.1 Estimating mutational effects on fitness
If we assume new mutations interact multiplicatively (i.e., no epistasis), fitness will decline by a fixed percentage each generation, corresponding to a factor $1 - \left( \frac{W_m}{U} \right)^U$ for males and $1 - \left( \frac{W_f}{U} \right)^U$ for females. Based on our observed values of $W_m$ and $W_f$ we estimate this per-generation rate of decline to be 0.53% for females (95% CI = (0.38%, 0.67%)) and 0.80% in males (95% CI = (0.63%, 0.96%)) for the X chromosome. Male fitness thus declined at a rate that was 1.52 times faster than females (95%CI = (1.09, 2.19), p = 0.013). The mean rate of decline for heterozygous female fitness was much lower, at 0.14% (95% CI = (0.03%, 0.25%)).
Figure 3.4: Correlation between male and homozygous female fitness. Male and female relative fitness were not correlated in the C lines (black circles, solid regression line) and were significant correlated in the MA lines (white circles, dashed regression line), indicating that new mutations were concordant in the direction of selection. Fitness values were calculated relative to mean control fitness.
3.4.2 Estimating the relative strength of selection on males vs. females

Assuming multiplicative effects on fitness and a constant coefficient of selection, the fitness of a genome homozygous for $n$ mutations will be:

$$W = w^n$$

where $w = (1 - s)$.

If selection varies across loci, we will have instead, on average:

$$\bar{W} = \bar{w}^n$$
$$\bar{w} = (1 - \hat{s})$$

where $\bar{W}$ is the geometric mean fitness at individual loci, and $\hat{s}$ serves as an estimator for the genome-wide selection coefficient ($\hat{s}$ is not the geometric mean of $s$). The per-generation haploid genomic mutation rate is

$$U = \frac{n}{t}$$

The fitness of a genome homozygous for new mutations after $t$ generations will then be, on average:

$$\bar{W} = (1 - \hat{s})^{\frac{n}{t}}$$
If coefficients of selection vary among loci for males and females, at the $i^{th}$ locus we will have:

$$w_m^i = 1 - s_m^i$$
$$w_f^i = 1 - s_f^i$$

and we can define:

$$\alpha^i = \frac{s_m^i}{s_f^i}$$

The fitness of populations of males and females expressing the same pool of mutations after $t$ generations can then be expressed as:

$$\overline{W}_f = (1 - \hat{s}_f)^{U^t}$$
$$\overline{W}_m = (1 - \hat{s}_m)^{U^t} = (1 - \hat{\alpha} \cdot \hat{s}_f)^{U^t}$$

Which we can rewrite as

$$\overline{W}_f^{\frac{1}{U^t}} = 1 - \hat{s}_f$$
$$\overline{W}_m^{\frac{1}{U^t}} = 1 - \hat{\alpha} \cdot \hat{s}_f$$

$$\hat{\alpha} = \frac{1 - \overline{W}_m^{\frac{1}{U^t}}}{1 - \overline{W}_f^{\frac{1}{U^t}}} = \frac{1 - \overline{W}_m^{\frac{1}{20U}}}{1 - \overline{W}_f^{\frac{1}{20U}}}$$
Our estimator for the overall relative strength of selection on males and females ($\hat{\alpha}$) thus also depends on the haploid genomic mutation rate ($U$), which is not known with much precision for any population. For *Drosophila*, experiments typically place the diploid genomic mutations rate ($2U$) in the range of 0.1-1.5 (Haag-Liautard et al., 2007, Halligan & Keightley, 2009), which would correspond to a mutation rate on the X chromosome of between 0.01 and 0.15 assuming that mutation rate is uniform across the genome. Using these two extreme values we obtain estimates of $\hat{\alpha} = 1.34$ (95% CI = (1.06, 1.77)) and $\hat{\alpha} = 1.50$ (95% CI = (1.09, 2.16)), respectively. Thus, variation of over an order of magnitude in $U$ has comparatively little effect on our estimate of $\hat{\alpha}$ given the duration of the MA experiment and the observed values of $W_m$ and $W_f$ for this experiment.

### 3.4.3 Dominance of new mutations

The dominance of mutations will influence the extent to which population size and structure modulate the consequences of MA. For females, the fitness of heterozygous and homozygous MA populations relative to their controls can be written as

\[
\overline{W}_f^{\text{Het}} = (1 - \hat{h}\hat{s}_f)^U t
\]

\[
\overline{W}_f^{\text{Hom}} = (1 - \hat{h}\hat{s}_f U t
\]

Where $\overline{W}_f^{\text{Het}}$ and $\overline{W}_f^{\text{Hom}}$ represent mean fitness for females after $t$ generations of MA relative to their controls in the heterozygous and homozygous states, respectively, and $\hat{h}$ serves as an estimator for genome-wide dominance coefficient. These equations can be rearranged to give:
As for \( \hat{\alpha} \), \( \hat{h} \) is not very sensitive to changes in \( U \) in the range of 0.01-0.15 per X chromosome per generation, given the values of \( W_m \) and \( W_f \) obtained in this experiment (\( U_x = 0.01: \hat{h} = 0.32, 95\% CI = (0.08, 0.56), U_x = 0.15: \hat{h} = 0.27, 95\% CI = (0.06, 0.51) \).

3.5 Discussion

Despite decades of research on the properties of new mutations, we know relatively little about their effect on adult fitness. By quantifying the consequences of new X-chromosome mutations for the reproductive success of males and females, we have begun to address this gap. Our results indicate that selection against new mutations differs in magnitude between the sexes, though the direction of change appears broadly concordant. This may be a fundamental feature of many populations due to the ubiquity of sexual selection.

3.5.1 Decline in adult fitness with MA

We estimated that the rate of decline in adult fitness due to MA was 0.53\% per X chromosome per generation in homozygous females, 0.80\% per X chromosome per generation in males, and 0.14\% per X chromosome per generation in heterozygous females. Scaled up to the entire haploid genome, this would imply a 2.6\% decline in fitness per generation in homozygous females, and a 3.9\% decline per generation in homozygous males. The heterozygous female decline is predicted to be much lower, at
0.7% per haploid genome per generation. Thus, the MA treatment was associated with a rapid decline in fitness. Our estimates for the rates of mutational decline in adult fitness are greater than estimates from both classical (Mukai et al., 1972) and recent MA studies using viability (Shabalina et al., 1997, Caballero & Keightley, 1998, Avila & García-Dorado, 2002). This is consistent with previous work performed with the IV population (Mallet & Chippindale, 2011), which found that inbreeding depression for total fitness was mainly due to depression in adult fitness. If the IV population is at mutation-selection balance, it is possible that stronger inbreeding depression for adult fitness is reflective of increased mutational pressure. In any case, our results demonstrate that the total mutational load of populations could be much greater than measurements of viability alone would imply.

### 3.5.2 Potential sources of error

Potential sources of error in estimating the rate of mutational decline in fitness for a population include confounding factors that bias the rate of mutation-accumulation during the MA protocol and factors that bias the measured impact of mutation during fitness assays. We accumulated mutations on hemizygous X chromosomes in *Drosophila* males. In *Drosophila*, the rate of sequence change at neutral sites suggests that the mutation rate is not distinguishable from parity between the sexes (Vicoso & Charlesworth, 2006), so we expect that the baseline rate of sequence change in our study was a fair representation of the normal mutation rate. Because we eliminated sexual selection by passing each MA line through single-X bottlenecks, the opportunity for
selection within the MA lines was limited to differences in viability between siblings resulting from a single generation of MA. Under the low competition conditions employed, we expect little viability selection, and even less impact on the rate of mutation-accumulation for genes affecting adult fitness in the MA population.

The control lines were kept as small, effectively asexual populations (i.e. no recombination between X chromosomes within a C-line) to minimize the possibility of adaptation. Adaptation in the control population would artificially inflate the measured decline in fitness due to MA and has been cited as a potentially major source of bias in estimating mutational parameters in other studies (Keightley, 1996, Keightley et al., 1998). Control-X chromosomes were expressed hemizygotously and the opportunity for sexual selection existed in these populations. While this, along with a larger population size, will slow down the rate of MA, we cannot eliminate the possibility that some deleterious mutations have fixed in these lines. The presence of mutation-accumulation in the control lines will cause us to underestimate the rate of erosion in fitness due to MA. MA in the control population could also affect our estimates for the relative strength of selection in males and females, if these mutations have sex-specific effects on mean fitness. In particular, mutations in genes with female-limited expression could accumulate freely under our experimental design because C-line chromosomes are only exposed to selection in males. If mutations with larger effects on females had accumulated in the C-lines, this would diminish differences between control and MA females, making the male
differential appear larger and inflating $\hat{\alpha}$. However, there is very little evidence for widespread female-limitation of gene-expression in the *D. melanogaster* genome (Connallon & Clark, 2011), and the results presented here indicate that most mutations are selected against in both sexes. In addition, direct observations from experiments on the maintenance of control lines over several dozen generations show no evidence of female-specific mutational decline (Appendix B).

Another important consideration stems from the observation that the environmental conditions under which fitness assays are performed can profoundly affect the perceived decline in fitness due to MA. As an example, it is well known that highly competitive conditions exaggerate differences between the control and MA populations: the decline in viability with MA can be nearly 10-fold greater under harsh competitive conditions (Shabalina et al., 1997), and diluting the food has also been found to affect the relative performance of MA flies (Houle et al., 1994). Many MA studies have used wild-caught or recently domesticated populations; the fitness assays used are unlikely to encapsulate the relevant selective environment. The use of populations in novel environments may also increase the probability of adaptation in the control lines. When measuring the selective effects of mutations in a particular population, it therefore seems sensible to restrict our measures to the conditions that shaped its population-genetic structure. The *IV* population has been maintained under consistent culture conditions for over 700 generations, and we emulated the culture protocol in almost every detail for our fitness
assays. We therefore do not anticipate substantial bias in our estimates of fitness decline resulting from the environmental conditions used.

Because we measured the performance of experimental flies as adults by counting their progeny, viability effects may have influenced our measurement of MA for adult fitness. In particular, the offspring of adults from the MA treatment may have suffered in terms of reduced viability, which would inflate our estimate of the effects of MA on adult fitness. This effect would be most pronounced for homozygous MA females, who pass on a full copy of their MA-X chromosomes to their sons, and who may also contribute adverse maternal effects to their offspring. A significant viability effect on the offspring of MA females would make our estimates of $\hat{\alpha}$ conservative. Strong differences in viability due to MA-X chromosomes should manifest themselves in terms of skewed sex ratios, but we found no differential effect of MA on offspring sex ratio.

3.5.3 Estimating the strength of selection on males vs. females
We estimated that the magnitude of mutational effects, X-chromosome wide, was approximately 1.4 times stronger in males than in females (1.06-2.14, a range that includes both experimental error and uncertainty around U). In addition, the intersexual genetic correlation for the MA lines was significant and positive. This suggests that, for the majority of new mutations on the X chromosome, selection operates in the same direction for both sexes. This is interesting because the X chromosome is the genomic location most likely to show mutations with sexually antagonistic or sex-independent
effects (Rice, 1984) but see (Fry, 2010). While we do not dispute this, our results
nevertheless seem to indicate that most of the mutations we assayed had sexually
concordant effects on adult fitness. However, even if all mutations had concordant effects
on fitness, the range in \( \hat{\alpha} \) presented here (1.06-2.14) could be consistent with anything
from modest benefits of sexual selection to females to a greater than two-fold fitness
advantage compared to a hypothetical asexual competitor, depending on the mutation
rate, so refining estimates for \( \hat{\alpha} \) through further study will be critical.

We found that the effects of new mutations were positively correlated between males and
females when females expressed MA-X chromosomes homozygously, but new mutations
will most often be expressed heterozygously in females. In the MA lines, we found a
positive association between homozygous and heterozygous female fitness values
suggesting that new mutations are partially additive. We estimate that the population-
wide dominance coefficient for new mutations is about 0.3. As new mutations will be
expressed hemizygously in males and heterozygously in females, the effectiveness of
selection will be much greater for males. Based on the rate of heterozygous female
decline in fitness, we estimate the ‘effective’ \( \hat{\alpha} \) to be greater than 5 for the X
chromosome.

The hemizygosity of males should result in more efficient selection on recessive alleles
on the X chromosome. When these alleles have concordant directional effects across the
sexes, this will result in reduced genetic load, an expectation corroborated by the absence of detectable inbreeding depression for juvenile viability in several populations of *Drosophila melanogaster* (Eanes et al., 1985, Mallet & Chippindale, 2011). For adult fitness, however, our results indicate that there remains substantial standing deleterious genetic variation on the X chromosome, as evidenced by the presence of substantial inbreeding depression for female fitness in the control group.

### 3.5.4 Genetic load on the X chromosome

There are several possible explanations for the high genetic load for adult fitness found on the X chromosomes of the *IV* population. First, adult fitness might represent a larger mutational target than juvenile viability. We believe this is likely because adult fitness will be influenced both by juvenile traits not captured by viability (for example larval condition upon pupation) and by mate competition. Second, sexually antagonistic alleles, though in the minority, may nonetheless exert considerable effects on net fitness because the genetic load associated with them tends to be greater than for concordantly selected alleles (Connallon et al., 2010). In a separate population of *Drosophila* (*LH*), the amount of sexually antagonistic variation on the X chromosome was sufficient to cause a negative intersexual genetic correlation for adult fitness (Gibson et al., 2002). In that study, the X chromosome was estimated to account for about 45% of the total genetic variation in fitness, and nearly all of the sexually antagonistic variation. Even so, most new mutations in the *LH* population are predicted to be under concordant selection
(Morrow et al., 2008), although the intersexual genetic correlation for fitness was not measured.

Similarly, the X chromosome appears to harbour a disproportionate amount of fitness variation in the IV population. Previous work with the IV population suggested that completely inbred IV-derived females were 36% as fit as their outbred counterparts (Mallet & Chippindale, 2011). In this study, females inbred for the X chromosome were 57% as fit as outbred females. Assuming multiplicative fitness effects, we infer that females completely inbred for the autosomes would be 63% as fit as outbred females. The X chromosome therefore seems to contribute more than half of the total inbreeding depression for adult female fitness, despite accounting for only a fifth of the gene content. The presence of segregating sexually antagonistic alleles on the X chromosomes in the IV population would be consistent with the disproportionate amount of genetic load on this chromosome, and is supported by a lack of positive intersexual correlation in the control lines.

Moderate amounts of sexually antagonistic variation have the potential to reduce the benefits of sexual selection, but the extent to which it does so depends on the relative amount and intensity of sexually concordant and sex-specific selection on the genomic scale (Connallon et al., 2010). Our results suggest that, at least for the X chromosome, the majority of mutations have sexually concordant effects, as our estimate of $\hat{\alpha}$ is a global
estimate. As long as the fraction of sexually antagonistic alleles generated by mutation is small, our results suggest that sexual selection could still yield net benefits to females, though estimating the precise fraction of mutations that are concordantly selected vs. sexually antagonistic should be a priority.

3.5.5 Conclusions
No study yet designed has been able to estimate all of the relevant properties of new mutations. Molecular methods are increasingly being used (Haag-Liautard et al., 2007), but can only directly measure the total rate of sequence change; without contemporary fitness data these methods provide only indirect estimates of the deleterious mutation rate. Conversely, fitness estimates on their own, while providing important insight into the consequences and character of new mutations, do not produce reliable estimates of the mutation rate. Mutation-accumulation in well-defined and replicable experimental populations such as the IV population, combined with advances in sequencing, could provide a much clearer picture of the fate of new mutations in populations than either technique in isolation. For example, one approach could involve sequencing MA lines to obtain the actual changes having occurred during MA, and then allowing replicated populations to purge these mutations in the standard laboratory environment. The rate at which mutations are eliminated would permit estimation of the strength of selection against them.
Our data highlight the importance of quantifying adult fitness and incorporating the distinctive features of X-linkage to understanding the consequences of mutation. Erosion of adult fitness due to MA on the X-chromosome was high, and the finding of sex-specificity in the strength of selection against deleterious mutations adds a new dimension to the problem of the maintenance of sexual reproduction. Direct estimates of the deleterious mutation rate, the range of variation in alpha between mutations, and the fraction of sexually antagonistic mutations will be important in quantifying the net cost/benefit of sexual reproduction for populations. Our data represent a critical first step in this direction: they suggest that most mutations are concordantly selected in the two sexes, and so the potential exists for female fitness to improve as the result of selection on males.

3.6 Acknowledgements
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3.7 References


Chapter 4

Susceptibility of the male fitness phenotype to spontaneous mutation

4.1 Abstract
Adult reproductive success can account for a large fraction of male fitness, however we know relatively little about the susceptibility of reproductive traits to mutation-accumulation (MA). Estimates of the mutational rate of decline for adult fitness and its components are controversial in Drosophila melanogaster, and post-copulatory performance has not been examined. We therefore separately measured the consequences of MA for total male reproductive success and its major pre-copulatory and post-copulatory components: mating success and sperm competitive success. We also measured juvenile viability, an important fitness component that has been well studied in MA experiments. MA had strongly deleterious effects on both male viability and adult fitness, but the latter declined at a much greater rate. Mutational pressure on total fitness is thus much greater than would be predicted by viability alone. We also noted a significant and positive correlation between all adult traits and viability in the MA lines, suggesting pleiotropy of mutational effect as required by ‘good genes’ models of sexual selection.

4.2 Introduction
The effects of individual mutations may be too small to detect individually, however their impact on total fitness is a fundamental quantity in populations genetics. Mutation-
accumulation (MA) experiments, where selection is relaxed to allow new mutations to fix, can reveal their cumulative effects (Halligan & Keightley, 2009). In *Drosophila melanogaster*, the most commonly used organism in MA studies, experiments have typically examined a single fitness trait: juvenile survival. In comparison, a handful of studies have measured adult fitness, with conflicting results (Houle et al., 1994, Fernández & López-Fanjul, 1996, Fry et al., 1998, Mack et al., 2000, Mallet et al., 2011). In the IV population of *D. melanogaster*, adult fitness is disproportionately important for males, accounting for 84% of inbreeding depression for net fitness on the autosomes (Mallet & Chippindale, 2011). In the same population, new X-linked mutations are deleterious for both sexes but have a stronger impact on males than females (Mallet et al., 2011). Unfortunately, little is known about the susceptibility of specific reproductive traits like sperm competitive success and mating success to MA in any population, despite their importance for male fitness (Harshman & Clark, 1998, Rybak et al., 2002).

The magnitude and pattern of mutational change in these traits informs us about their relative importance to fitness and their genetic architecture. For example, a decrease in multiple traits accompanied by stronger genetic correlation between traits indicates pleiotropy. The correlation between male reproductive traits and juvenile viability has been of particular interest in sexual selection research: a positive correlation is the most common test of additive benefits of sexual selection to offspring (Kokko et al., 2006). We therefore performed 50 generations of MA on *D. melanogaster* haploid genomes from an
outbred laboratory-adapted population and measured its impact on juvenile viability, lifetime male reproductive success and mating success as well as providing the first estimates of mutational effects for post-copulatory traits. The effects of MA were assessed genome-wide, in the normal condition of expression for new mutations in males (hemizygous on the X, heterozygous on the autosomes).

4.3 Methods
Haploid genomes originated from *Ives* (*IV*), a long-term laboratory-adapted population, and were isolated with the *Drosophila* hemiclone system (reviewed in (Abbott & Morrow, 2011)) (Figure 4.1A). The same set of 21 hemiclone lines was used to found both control (C) and mutation-accumulation (MA) groups. For the MA lines we reduced the effective population size to a single haploid genome per generation, propagated without recombination (Figure 4.1). The same crosses with larger population sizes were used to maintain the C lines. We kept the controls as moderate-sized populations without recombination to limit adaptation, a persistent concern in MA experiments (Keightley, 1996, Keightley & Halligan, 2008). This may allow some MA in the controls, and will make our estimates of mutational impact conservative.
Figure 4.1: Crosses employed. A) Random IV hemiclones were isolated by crossing wild-type males individually to groups of clone-generator (CG) females bearing attached-X chromosomes (DX) and homozygous for a marked autosomal translocation ((T(2 : 3) rdgc st in ri pp bw, grey bars). A single son from these crosses (white genotype) was then selected to fix a different haploid genome within each hemiclonal line. The MA and C populations were founded from the same initial group of hemiclones. B) Mutation-accumulation (3 generations shown). A single male from each line was mated to a group of CG females, creating a single-genome bottleneck and fixing the mutations present in the parent (black asterisks). Three sons from this cross were each mated to CG females in separate vials, one of which was randomly chosen to found the next generation (the other vials are kept as back-ups). Controls were maintained using identical crosses, but at larger population sizes (16-25 males) to allow selection. C) Generation of experimental flies. MA or C hemiclones were crossed to DX females with wildtype autosomes. Hemiclonal males with MA or C hemiclones heterozygous for a set of random IV genotypes (1.) were used to assay performance. Females without any C or MA chromosomes were used to standardize viability (2.)
For viability assays we generated C/MA males by crossing males from 19 lines with $DX$-$IV$ females, allowing them to lay approximately 100 viable eggs. The expected yield is 25% hemiclonal males, and 25% brown-eyed females that do not carry C/MA-derived chromosomes (Figure 4.1). The females were thus used to standardize viability. We measured 10 vials per line/treatment combination, 380 in total.

To measure adult fitness we transferred single hemiclonal C/MA males from 19 lines to age-synchronized vials of a competitor ($IV_{bw}$) reared under standard conditions (~100 individuals/vial, 25°C, 50% relative humidity), during peak eclosion (Day 9). The vials were left undisturbed for five days. On Day 14 the entire population was placed under 2.5 min CO$_2$ to simulate normal culture, then transferred to oviposition vials until approximately 100 eggs were laid (25-30 minutes). Red-eyed progeny emerging from these vials represent the lifetime reproductive success of the hemiclonal males under normal $IV$ culture conditions. We measured 20 males for each line/treatment combination, 760 in total.

Mating success was measured by competing hemiclonal C/MA males from 20 lines with $IV$ males for virgin $IV$ females. We collected virgin hemiclonal males, as well as virgin $IV$ females and competitor $IV$ males on Day 9 post-oviposition in same-sex groups. On Day 11, the males were transferred to medium containing red or blue food-dyed yeast paste, which colours their underbelly. We then transferred pairs of opposite-coloured
competitors (C/MA males with IV males) to female vials without anaesthesia, observing until mating took place. We performed 10 trials for each line/treatment/colour combination, 800 in total, including reciprocal dye treatments of all male genotypes.

For post-copulatory success, we collected virgin IV bw females, males, and C/MA males from 20 lines on Day 9. On Day 12, groups of 18 males (P1) were combined with 12 virgin females and allowed to interact for 1.5 hours: nearly all females mate once under these conditions. The first mates were removed using light CO₂ and 12 males (P2) were added after a 30-minute female recovery period. The flies interacted overnight (18 hours), and we then placed females in individual 13x100mm test tubes containing fresh media to oviposit for 20 hours. Progeny were scored for paternity 11-14 days later. Male performance was divided into two components: P1 and P2, depending on whether the focal males (C/MA) or the competitor (IV bw) males mated first. We assessed paternity in 50 females for each line/treatment/order combination, 3,800 in total.

Statistical inferences were performed using normalized likelihoods (Shcherbinin, 1987) using R 2.12.0 (R Development Core Team, 2010). Normalized likelihoods are equivalent to Bayesian analyses using flat priors, and can also be used to generate standard p-values and confidence intervals (Walley, 2002) (Appendix C). All statistics were based on line means.
4.4 Results

Results for all performance measures are summarized in Table 4.1. Line means and between-line variance estimates along with their confidence intervals are presented in Appendix C. For mating success, we verified that the ratio of red to blue-dyed success was not significantly different from unity for the MA and C males before combining them (MA red/blue = 1.10 (0.88-1.39), C red/blue = 0.85 (0.71-1.02)). For P2, where we did not observe matings over the entire 18-hour interaction window, we excluded females having produced no offspring from the P2 male before calculating line means to ensure that sperm competition occurred. Females mated to MA males had slightly but significantly larger broods. This was true whether the MA males were the P1 (MA/C = 1.05, 95% CI = (1.02, 1.08), p < 0.0001) or P2 (MA/C = 1.04, 95% CI = (1.01, 1.07), p = 0.01) male. Correlations between adult traits and viability are shown in Table 4.2.
Table 4.1: Fitness declines associated with MA, based on group means (95% confidence-intervals in brackets). Per-generation rates of declines were calculated assuming multiplicative fitness effects between mutations.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean</th>
<th>% decline</th>
<th>per-generation decline (%)</th>
<th>p-value (two-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viability</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>85.1%</td>
<td>30.8</td>
<td>0.73</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>(80.5-89.9)</td>
<td>(24.9-36.1)</td>
<td>(0.57-0.89)</td>
<td></td>
</tr>
<tr>
<td><strong>Adult Fitness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3.06</td>
<td>53.6</td>
<td>1.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>(2.89-3.24)</td>
<td>(48.5-58.2)</td>
<td>(1.32-1.73)</td>
<td></td>
</tr>
<tr>
<td><strong>Mating Success</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>45.6%</td>
<td>20.8</td>
<td>0.46</td>
<td>0.0032</td>
</tr>
<tr>
<td></td>
<td>(41.0-50.2)</td>
<td>(7.2-32.5)</td>
<td>(0.15-0.79)</td>
<td></td>
</tr>
<tr>
<td><strong>P1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>10.1%</td>
<td>42.1</td>
<td>1.09</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>(9.5-10.7)</td>
<td>(36.5-47.3)</td>
<td>(0.90-1.27)</td>
<td></td>
</tr>
<tr>
<td><strong>P2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>85.9%</td>
<td>21.6</td>
<td>0.48</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>(85.2-86.5)</td>
<td>(22.9-20.2)</td>
<td>(0.45-0.52)</td>
<td></td>
</tr>
<tr>
<td><strong>Total fitness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.60</td>
<td>67.8</td>
<td>2.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>(2.40-2.82)</td>
<td>(63.3-71.8)</td>
<td>(1.98-2.50)</td>
<td></td>
</tr>
<tr>
<td>MA</td>
<td>0.84</td>
<td>(0.75-0.93)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2: Correlations between male fitness traits and viability, based on line means (95% confidence-intervals in brackets).

<table>
<thead>
<tr>
<th>Traits</th>
<th>C</th>
<th>MA</th>
<th>P-value for difference between C and MA (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>0.14</td>
<td>0.43</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>(-0.13, 0.42)</td>
<td>(0.23, 0.61)</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>0.21</td>
<td>0.57</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>(-0.07, 0.47)</td>
<td>(0.45, 0.67)</td>
<td></td>
</tr>
<tr>
<td>Mating success</td>
<td>0.13</td>
<td>0.42</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>(-0.22, 0.47)</td>
<td>(0.17, 0.64)</td>
<td></td>
</tr>
<tr>
<td>Adult fitness</td>
<td>0.098</td>
<td>0.49</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>(-0.16, 0.38)</td>
<td>(0.31, 0.65)</td>
<td></td>
</tr>
</tbody>
</table>
4.5 Discussion

Adult male fitness declined significantly with MA. We previously estimated that the X chromosome depressed adult fitness by 0.8 % per generation (Mallet et al., 2011), assuming multiplicative effects of mutations on fitness, so the heterozygous autosomes contributed roughly 0.7% per generation. To our knowledge this is the first reported estimate for the effects of MA on heterozygous male reproductive success. Adult male fitness declined at more than double the rate of viability, accounting for roughly two-thirds of the rate of decline in total fitness. The cost of mutation for $IV$ males is thus much greater than viability alone would predict, as others have hypothesized (Shabalina et al., 1997, Fry et al., 1998).

We separately measured components of male reproductive success, and found that all of them declined with MA. MA males were on average 20% worse than C males at obtaining matings, indicating deleterious mutational effects on attractiveness and/or male-male competition. We are aware of only one other estimate of pre-copulatory mating success: (Houle et al., 1994), using a closely related $IV$ population, did not find a significant reduction in male mating ability on homozygous second chromosomes after 44 generations of MA but this was attributed to a lack of experimental power.

We show for the first time that MA is associated with a decline in post-copulatory success, for both P1 and P2. Our P2 measure excludes males that failed to produce any offspring: while ensuring that sperm competition did occur, this tends to underestimate
the decline due to MA. We attribute the decline in post-copulatory success to competitive exclusion rather than reduced survival of MA male offspring, because females mated to MA males did not produce smaller broods. In fact, females mated to MA males tended to have slightly more progeny, whether the males were in P1 or P2. Given that this increase was similar regardless of male position, and that the exposure time to P1 and P2 males was very different, we suggest that this result is unlikely to be caused by a reduction in male harassment/vigour with MA. Instead, the ejaculate of MA males might be less harmful. One possible mechanism is that MA males produce fewer harmful accessory peptides, indicating a trade-off between post-copulatory success and mate-harm.

Viability has been well characterized in MA studies using *D. melanogaster* (Halligan & Keightley, 2009). Most studies have measured homozygous effects on a single autosome and extrapolated to haploid genomes: these estimates are usually 0.3-1% per generation. Considering that new mutations were heterozygous for 80% of the genome in our experiment, our estimate of 0.73% seems somewhat high. Using a different experimental design, (Shabalina et al., 1997) noted a 1% per generation decline on larval survival in outbred populations. Their result is comparable to ours although their experimental conditions were much harsher, with mean larval survivals of only ~10%. Overall, our result supports high mutation pressure on viability.
For all traits in the control lines, there was no significant association between male performance and viability. We (Mallet & Chippindale, 2011), and others (e.g. Promislow et al., 1998, Janhunen et al., 2011), have interpreted this as suggesting a lack of viability benefits to offspring resulting from sexual selection. In the MA lines, however, we noted a significantly positive relationship between each of the male performance traits and viability, resulting in a significant difference in the correlations between C and MA for adult fitness and P2 success. New mutations thus appear to have pleiotropic effects on viability and male reproductive performance, representing one avenue for offspring to realize additive genetic benefits from sexual selection.

We thank members of the Chippindale Lab for help with data collection. Funding was provided by NSERC. We also thank the editor and anonymous reviewers for their helpful comments.

4.6 References


Chapter 5

General Discussion

5.1 Overview

The current surge of interest in elucidating the consequences of sexual selection for the net fitness of populations has brought together previously disparate traditions of theoretical and empirical work. By re-expressing ‘good genes’ models of sexual selection in the context of condition dependence, (Rowe & Houle, 1996) simultaneously provided an explanation for the high levels of standing genetic variation in sexually selected traits and provided an avenue for sexual selection to affect large swaths of the genome, perhaps even the majority. While the genic capture model is intuitively very appealing, the recently established existence of significant sexually antagonistic variation in populations challenges the view that sexual selection is unconditionally beneficial in terms of mean fitness.

Establishing whether or not sexual selection improves mean fitness depends crucially on the properties of mutations. The results presented in the previous chapters using the IV population of Drosophila melanogaster provide some of the strongest evidence to date that the overall strength of selection on deleterious mutations can be greater for males than females, on the scale of the whole genome. When adult and juvenile fitness were compared for selection on standing genetic variation (Chapter 2), males were found to experience stronger selection in terms of adult fitness only. Males also experience
stronger selection on adult fitness than females for new mutations on the X chromosome (Error! Reference source not found.), and new mutations generally affect adult male fitness more than they do male juvenile viability (Chapter 4). While male reproductive traits were strongly affected by mutation-accumulation (MA), mortality differences are minimal over the timescale of IV culture (C. Kimber, personal communication), allowing us to implicate sexual selection as the driver of stronger net selection on males.

In addition to my own work, several recent papers have been recently published using different model organisms or alternative empirical approaches than presented here, highlighting the timeliness of this research. In the following section, I briefly review some of these studies and comment on their contribution to our knowledge of the consequences of sex for mean fitness. Finally, I conclude with a discussion of the gaps remaining to be filled with the IV experimental system I’ve developed, as well as areas where I think new ground can be broken.

5.2 Recent empirical work
The following four studies represent very recent work addressing the same questions that I have attempted to answer here. These papers were not cited in the previous data chapters because of they were published simultaneously to my work. Because of their relevance to my own results, I briefly discuss them here.
5.2.1 Sexual selection reduces extinction risk in bulb mites

If populations experiencing sexual selection have lower mutation loads, it stands to reason that their risk of extinction should be lower relative to an equivalent population without sexual selection. Lower mutation loads should also result in a reduced cost of inbreeding, to the extent that inbreeding depression is caused by deleterious recessive variation. Jarzebowska & Radwan (2010) set out to test these predictions by maintaining small populations of 5 breeding pairs of the bulb mite, *Rhizoglyphus robini*, with and without the opportunity for sexual selection by experimentally enforcing monogamy. Starting out with 100 lines (50 with sexual selection and 50 without), nearly 50% of the lines were lost after 6 generations in the treatment where sexual selection was removed. In the lines experiencing sexual selection, only 27% of the lines were lost. In addition, populations experiencing sexual selection had lower inbreeding depression than populations in the monogamy treatment, indicating that their mutation load was lower. Although they were not able to specifically link the effect of sexual selection to males and the possibility remained that higher inbreeding due to smaller population sizes could have contributed to the purging of mutations in the sexual selection treatment (Agrawal & Chasnov, 2001, Jarzebowska & Radwan, 2010), the result from this study are consistent with the predictions of the male-selective-sieve hypothesis.

5.2.2 Inbreeding depression in wild-caught *Drosophila melanogaster*

As I argued in Chapter 2, the severity of inbreeding depression (ID) across the sexes can be used to infer the overall strength of selection acting within each one, to the extent that
ID is caused by deleterious mutations. Enders and Nunney investigated the magnitude and sex-specificity of ID in a population of recently wild-caught *Drosophila* in different environmental treatments (Enders & Nunney, 2010). Environmental stress has often been assumed to increase the strength of selection against deleterious variation (but see Agrawal & Whitlock, 2010), and sex differences in the strength of selection between the sexes could be exacerbated in stressful environments. Enders and Nunney manipulated diet quality by diluting the food medium by two-thirds. This treatment was equated with higher larval competition, although no evidence was provided that the increased mortality was dependent on the presence of competitors. The magnitude of ID for larval survival was greater in stressful conditions, approximately doubling (depending on the measure of competitive success used) in the low-nutrient vials. Adults emerging from these low-nutrient vials had lower fecundity as females and lower mating success as males. Importantly, in both treatments male mating ability showed the highest ID and the difference in ID between the sexes was the same in both environments. This result is broadly concordant with the results presented in Chapter 2, but Enders and Nunney did not find a significant correlation between inbreeding depression for male fitness and any female trait.

This latter observation is seemingly at odds with the significant correlation I observed between adult fitness in males and females in my experiment. I believe that limitations of the experimental design may have influenced the accuracy of their estimate of the
intersexual genetic correlation for fitness. First, the use of wild-caught *Drosophila*
unavoidably led to a novel and to some extent arbitrary environment for the
measurements of fitness. At least one study has reported that intersexual correlation for
fitness can be affected by changes in diet, becoming more negative in a novel food
environment in a population of *D. serrata* (Delcourt et al., 2009). Second, they did not
estimate the intersexual correlation between inbred lines as they did for outbred families.
Rather, they calculated the correlation between male and female inbreeding depression
(*i.e.* whether the relative decline in outbred/inbred lines means is correlated across sexes).
This will not only be affected by error in estimating both the inbred and outbred line
means, making the test less powerful, but will also depend on whether there is variance in
the coefficients of dominance both between mutations and between the sexes. While I
estimated that the average coefficient of dominance was similar for males and females,
the amount of variation in dominance across the sexes for individual mutations is
unknown. Third, the fitness measures used may not have been comprehensive enough.
For example, male mating success was measured over a two-hour time-window as a
scramble competition for virgin females. Female fecundity was measured for females
held in isolation for up to 16 days after a 48-hour exposure to males. Important aspects of
fitness that may be more likely to be shared between the sexes in the adult phase could
have been missed in these assays. These might include traits such as longevity, resource
competition, and sperm competitive ability (which may be more condition-dependent
than mating ability males owing to the considerable expense of ejaculate in *Drosophila*, see Chapter 4).

**5.2.3 Mutation-accumulation with and without sexual selection in *Drosophila serrata***

McGuigan et al. (2011) performed a mutation-accumulation study to test the effectiveness of sexual selection in removing mutations using *Drosophila serrata*. Rather than performing a classical MA experiment and measuring sex-specific fitness, the approach we employed in *Error! Reference source not found.*, they performed MA on 100 replicate lines with and 100 lines without the opportunity for sexual selection. Relatively few genetic markers are available with this species, so MA was carried out by repeated brother-sister mating. In the standard MA treatment, a single female was randomly paired with one of her brothers each generation to propagate the lines (replicate crosses were kept as backups). In the sexual-selection MA treatment females were instead exposed to up to five different brothers (3-4, on average) until a mating occurred, after which the unmated males were removed. Thus, sexual selection in these lines was limited to pre-copulatory selection only. At various time points throughout MA (up to generation 26) male mating success was measured in several ways, the number of surviving lines was recorded as a proxy for viability, and a line productivity assay was performed at the end of the experiment.
While only marginally significant (0.06 < p < 0.07), there were more lines from the sexual selection MA treatment that survived after 26 generations of MA, consistent with the results of (Jarzebowska & Radwan, 2010). Also nearly significant was a higher proportion of vials with nonzero productivity in the sexual selection lines. Both male and female adult traits, such as fecundity in females and ejaculate quality in males contributed to these measures of ‘non-sexual’ fitness, and the results provide weak evidence in support of a role for sexual selection in reducing mutation load. The among-line variance was lower in the sexual selection MA treatment, however, suggesting fewer mutations had accumulated. Male mating success showed no consistent pattern in the evolution of the mean between the two MA treatments, however the mutational variance increased using the standard MA treatment, whereas it showed no consistent evolution over time in the sexual selection treatment. This is also consistent with fewer mutations accumulating in the selection MA lines. In addition, the correlation between productivity and male mating success was positive in the standard MA treatment, but significantly lower in the sexual selection MA lines. This suggests that mutations with pleiotropic effects were removed in the lines having experienced sexual selection. Although I find the lack of evolution in mean performance concerning, the results do appear consistent with a role for mate choice in reducing mutation load.

5.2.4 The purging of deleterious mutations with and without sexual selection in *Drosophila melanogaster*
Rather than allowing new mutations to accumulate in the presence or absence of sexual selection, Hollis & Houle (2011) exposed *D. melanogaster* to ethyl methanesulfonate (EMS), a well-known mutagen, and allowed the population to purge these mutations with and without sexual selection. The initial EMS treatment was associated with a weak decline in fitness (*i.e.* a 15% decline in viability, no decline in fecundity, and a non-significant decline in male mating success). Nevertheless, the populations were then propagated for 60 generations with (S+) and without (S-) sexual selection to purge these induced mutations. In the S- populations, virgin females were randomly paired with males, and spent 2 days in monogamy before being transferred into oviposition bottles for 3 days without males. In the S+ treatment, groups of 5 females spent 2 days with groups of 5 males before the females were transferred to the oviposition bottles.

After 60 generations of experimental evolution, lines were tested for fecundity, viability, and ‘net productivity’. No overall difference was found for fecundity or egg-to-adult viability between the S+ and S- flies, which is unsurprising given the modest declines present in these traits as the result of mutagenesis at the beginning of the experiments. There was a slight but significant difference in net fecundity (~7%) between the two lines, however, with more offspring emerging from S- vials after 3 days of egg-laying. It is unclear whether or not difference in initial egg densities may have contributed to this difference. S+ flies lay about 10% more eggs than S- flies when their parents are monogamous, which was the case for the net fecundity assay. Given that the densities in
the assays were high enough that egg number was the most significant predictor of egg-
to-adult viability in their analyses, it is possible that overcrowding in the S+ vials due to
higher fecundity may have contributed to their lower net productivity. In addition, the
gen. 60 experiments were performed in the absence of competition between treatments
(either direct competition between S+ and S-, or comparisons across a standard
competitor), further complicating interpretation of the results.

Trans-generational effects of parental mating environment on fecundity were noted, with
S+ flies producing more offspring if their parents were monogamous. Given that the S+
and S- populations were genetically variable to begin with (and were thus likely variable
in sexually antagonistic alleles), the authors attribute these effects to a reduction in male
harm in the S- treatments, an effect previously noted in experimental evolution treatments
involving enforced monogamy (Holland & Rice, 1999). This hypothesis is difficult to
evaluate without data on male mating success, which should have evolved if an evolved
reduction in male harm in the S- lines accounts for the apparently higher cost to
polygamy in the S+ lines.

5.2.5 Summary
The studies presented here represent the most recent attempts at measuring the
effectiveness of sexual selection in reducing the mutation load of populations, and
demonstrate the widespread interest in this area of research. These studies collectively support the hypothesis that sexual selection on males improves net fitness by purging deleterious mutations, although no single study demonstrated all of the necessary conditions for this to occur. Jarzebowska & Radwan (2010) found that sexual selection was associated with a lower extinction risk and lower inbreeding depression, but could not attribute this improvement to males. Enders & Nunney (2010) found that sexual selection was stronger on males, but did not find significant correlations between male performance and other traits. On the other hand, McGuigan et al. (2011) did find evidence for pleiotropy between sexual and ‘non-sexual’ fitness but found no improvement in mean performance with sexual selection. The results of Hollis & Houle (2011) were largely inconclusive, and I suspect this is because the initial mutagenesis treatment did not depress fitness sufficiently to allow for a meaningful test of the effectiveness of sexual selection. The differences in experimental results could represent real differences between populations or species of experimental organism, either in terms of the levels of sexual antagonism or the effectiveness of sexual selection, but important experimental limitations in each of these studies place limitations on the strength of their inferences. My experiments using the clone-generator system in the IV population of *Drosophila melanogaster* have been unique in providing almost unequivocal support for the idea that mutations are more harmful to males and that selection is stronger on males should benefit females. Clearly, however, careful experimental work in other study systems remains to be done to test the generality of the results presented here.
5.3 The population-genetic consequences of stronger selection on males.

The work presented in the previous chapters conclusively demonstrates that spontaneous mutations are predominantly deleterious for both sexes in the IV population, but that selection against deleterious mutations is stronger on males, on the balance. These are critical preconditions for sexual selection to purge mutations and reduce the deleterious mutation load of females, however further experimentation to determine the magnitude of these benefits to requires further analysis.

The magnitude of the benefits of sex to mean fitness in a population will depend on a large number of parameters, for example population structure (i.e. level of inbreeding, and variation in $f$), mutation rate, the dominance characters of new mutations, and the magnitude of differences in selection between males and females at individual loci. Determining any one of these parameters is a considerable empirical challenge, even in relatively tractable laboratory populations such as IV, which explains why so little data is currently available to explicitly test theoretical models. In Chapter 2, I provided some R scripts (Appendix A) that aim to help explore this parameter space, albeit with some simplifying assumptions. This exercise supported a significant advantage for sexual populations experiencing sexual selection versus sexual populations experiencing no sexual selection. For example, with a total diploid mutation rate of 0.5 (half that of recent molecular estimates) and a modest 15% increase in the strength of selection on males (less than the estimates in my data chapters), populations experiencing sexual selection still benefit from a 5% increase in mean fitness at equilibrium.
Ultimately, however, assessing the benefit of sexual selection for any particular population is a challenge best addressed empirically. A multi-generation fitness recovery experiment, where populations are allowed to purge either experimentally induced or naturally accumulated deleterious mutations, is perhaps the most intuitively appealing and direct way to quantify the benefits of sexual selection. Great pains in the experimental design must taken to ensure that the populations are comparable in all respects other than the strength of sexual selection, including population size, juvenile rearing conditions, and in environmental conditions. This is because experimental evolution protocols are notorious for producing unexpected results, often due to the population adapting to minute differences in the selection regimes (Chippindale et al., 2003). The possibility that the evolved populations of Hollis and Houle may have been adapted to aspects of the selection treatment itself (e.g. reduced mate harm in the monogamy treatment) illustrates this point. Performing what is essentially an MA experiment in reverse would be an ideal complement to the single-generation assays I’ve performed to date.

5.4 What causes stronger selection in males?

Phenotypic characterization of MA males indicates that the consequences of new mutations are widespread, affecting all the traits measured (Chapter 4). In addition, each of the measured reproductive traits (success in P1, P2, mating success and total reproductive success) became significantly correlated with viability in the MA lines.
while they were uncorrelated in the C lines, suggesting that new mutations have pleiotropic effects on viability. A general decline in performance combined with higher genetic correlations is consistent with the predictions of the genic capture hypothesis (Chapter 1). By what pathways do new mutations cause such widespread declines in performance? Further phenotypic and genetic characterization of the MA lines in both sexes may help to answer these questions, and perhaps better define condition itself.

Males may decline more in fitness than females given the same mutations because they are physiologically more susceptible to genetic injury. This hypothesis requires that the same mutations alter male traits to a greater extent than comparable female traits. Why might the male phenotype be more sensitive? Males presumably make greater investments in sexually selected traits than females. Mutations that cause a reduction in condition may have a greater phenotypic impact on males because they are already living life ‘on the edge’. A second possibility is that sexual selection on males (imposed by female choice or male-male competition) causes steeper selection gradients on male traits, such that the same change in trait value has a greater impact on males. Of course this explanation is not exclusive from the idea that males are more phenotypically sensitive to mutations. Indeed, stronger selection may drive allocation of male traits towards sexually selected characters and drive phenotypic sensitivity.
These ideas can be explored with the MA and C lines. Comparing the declines in traits with MA between males and females will reveal whether or not males are phenotypically more sensitive to mutation. This comparison should be made between the sexes for shared traits, but also within each sex between sexual and non-sexual traits. This has been done in other experimental systems by manipulating physical condition through reductions in diet quality (e.g. Bonduriansky & Rowe, 2005), however this is an indirect test as there is very little empirical evidence to show that the phenotypic effects of physiological manipulations are equivalent to declines in condition due to lower genetic quality. The clone generator system also readily allows the estimation of selection gradients: multiple individuals carrying the same haploid genome can be phenotypically characterized and then they (or their hemiclone siblings) can be placed in competition to determine the correlation between these traits and either fitness or some other performance measure.

I am currently engaged in a collaboration with Howard Rundle (University of Ottawa) and Russell Bonduriansky (University of New South Wales), to address some of these questions. We characterized both C and MA flies in males and females in terms of their morphology and cuticular hydrocarbons (CHCs), and measured the strength of pre-copulatory sexual selection acting on both suites of traits. We repeated these experiments on flies raised in diluted food medium, to test whether or not this environmental manipulation has similar phenotypic effects to MA and are still collecting data.
5.5 The properties of spontaneous mutations

Many of the theoretical models used to predict the benefits of sexual selection to both sexes depend on more detailed information about the properties of mutation than the estimates I provided. At minimum, estimates of the mutation rate and average effect of mutation are important, and ideally we would have detailed information about the distribution of mutational effects in each sex in the common currency of fitness. This information is notoriously difficult to obtain, and the estimates that have been published have generally been controversial (Halligan & Keightley, 2009). I have refrained until this point on speculating on these parameters, despite the availability of statistical methods designed to estimate mutational properties.

The ‘classic’ estimator for the rate and effect of mutations was obtained by (Bateman, 1959) and used by (Mukai, 1964) in his landmark mutation-accumulation study. Briefly, if we assume mutations act additively, each generation we will have a decline in fitness corresponding to UE(a), where U is the mutation rate and E(a) is the average mutational effect, and the dispersal of these mutations according to a poisson distribution will cause the variance to increase by a factor UE(a²) per generation. Assuming that mutations have a constant effect (i.e. no variation in a), we can obtain upper and lower bounds for E(a) and U. There are several problems with this approach. First, there is likely to be much variation in the strength of selection against new mutations and in mutation rate between
populations. Comparisons between estimates of $U$ or $E(a)$ between studies, traits, or even sexes therefore do not inform us about differences in the actual rate of mutation or average effect without prior information about which of these two parameters is causing the differences, which is clearly begging the question. For different traits we can expect $U$ to differ, as the ‘effective’ mutation rate is dependent on the number of loci affecting that trait. The average effect of mutations and the distribution of mutational effect is also expected to vary for different traits. While we could speculate on the patterns obtained with the classical Bateman-Mukai patterns, little direct evidence about mutation rates or their effect is likely to be gleaned with this approach. While alternative statistical methods, allowing for the variation in mutational effects, have been developed they generally have little power to distinguish few mutations of large effect from many mutations of small effect (Halligan & Keightley, 2009).

These statistical methods are rapidly being supplanted by molecular methods which can directly observe new mutations (Haag-Liautard et al., 2007, Keightley et al., 2009). Molecular methods have a major disadvantage, however, which is that they only provide estimates for the rate of sequence change, not the effects of mutations on fitness. Only experimental data can provide this information. No study yet has attempted to employ a combination of molecular and experimental methods to identify and infer the strength of selection acting on individual mutations in a multicellular organism, although I believe this is the ‘holy grail’ of MA research. In the meantime, clever experimental design may
increase the inferential power of statistical techniques in the absence of molecular data. Employing QTL mapping techniques to MA lines is potentially a very powerful approach that has been successfully employed in a virus (Burch et al., 2007), although in multicellular organisms obtaining the necessary sample sizes to detect mutations of small effect will prove difficult without large throughput assays of fitness.

5.6 Conclusions
The work presented in this thesis highlights the importance of sex-specific processes in our understanding of the evolution of sexual populations. Quantities of fundamental interest, such as the consequences of mutation for fitness, take very different values depending on whether mutations are expressed in males or females. My work establishes the conditions for sexual selection to improve female fitness, which could lead to lower extinction risk and higher competitive success in populations experiencing sexual selection. The experimental system I’ve developed can be used to address important gaps in our knowledge, such as understanding the effects of reducing genetic quality on the phenotypic and genetic architecture of both sexes, and can be adapted to complement modern analytic genomics methods.

5.7 References


Appendix A

Supplementary Materials for Chapter 2

**Figure A1:** (Left panel) Relative fitness of sexual females against a diploid asexual competitor in a population experiencing sexual selection (alpha=1.24, red line) and no sexual selection (alpha = 1, blue line), for various levels of population structure. (Right Panel) Fitness of sexual females in a population experiencing sexual selection (alpha=1.24) relative to sexual females in a population experiencing no sexual selection (alpha = 1), for various levels of population structure. To generate these values, the dominance of mutations was taken to be 0.11, and the genomic mutation rate was 1.2.
**Figure A2:** Relative fitness of sexual females in a population experiencing sexual selection relative to sexual females in a population experiencing no sexual selection (alpha = 1), for a range of genomic mutation rates and intensities of sexual selection. To generate these values, the dominance of mutations was taken to be 0.11 and the level of inbreeding was f=0.2.
R scripts
# this function calculates fitness relative to an asexual competitor: to obtain relative fitness between sexual competitors at different levels of sexual selection take ratio of two simulations with differing levels of alpha, but holding all other parameters constant.

R_compute = function(h=0.13,f=0,alpha=1.4,u=.00001,nloci=100000)

# values in the function definition corresponds to the default parameter values
# total genomic mutation rate is u*noci, results are essentially the same whether u or nloci are altered to yield a given total mutation rate, provided the per locus mutation rate is less than 10^-3. Results in figure s1 were obtained by keeping nloci at 100000 and altering u.

{
  sf=.05  # results do not depend on the exact value of sf, only on relative strength of selection in males and females
  sm=alpha*sf
  sa = sf
  time = 10000

  # allele freq after selection and mutation, Pm = freq in males, Pf= freq in females, Pa = freq in asexuales
  Pm = rep(NA,time)
  Qm = rep(NA,time)
  Pf = rep(NA,time)
  Qf = rep(NA,time)
  Pa = rep(NA,time)
  Qa = rep(NA,time)

  # allele freq after selection and mutation,after mating
  P = rep(NA,time)
  Q = rep(NA,time)

  Pm[1] = 1
  Qm[1] = 1-Pm[1]
  Pf[1] = 1
  Qf[1] = 1-Pf[1]
  P[1] = (Pm[1]+Pf[1])/2
  Q[1] = 1-P[1]

  # genotype freqs, after mating
  AAm = rep(NA,time)
  Aam = rep(NA,time)
  aam = rep(NA,time)

  AAf = rep(NA,time)
  Aaf = rep(NA,time)
  aaf = rep(NA,time)

  AAA = rep(NA,time)
  Aaa = rep(NA,time)
aaa = rep(NA, time)
fAAm = rep(NA, time)
fAam = rep(NA, time)
faam = rep(NA, time)
fAAf = rep(NA, time)
fAaf = rep(NA, time)
faaf = rep(NA, time)
fAAa = rep(NA, time)
fAaa = rep(NA, time)
faaa = rep(NA, time)

# genotype fitnesses
WAAm = l
WAam = l-sm*h
Waam = l-sm

WAAf = l
WAaf = l-sf*h
Waaf = l-sf

WAAa = l
WAaa = l-sa*h
Waaa = l-sa

# "number" after selection
AAm[1] = ((P[1]^2+P[1]*Q[1]*f)*WAAm)
Aam[1] = ((2*P[1]*Q[1]*(1-f))*WAam)

AAf[1] = (P[1]^2+P[1]*Q[1]*f)*WAAf
Aaf[1] = (2*P[1]*Q[1]*(1-f))*WAaf
aaf[1] = (Q[1]^2+P[1]*Q[1]*f)*Waaf

AAa[1] = l*WAAa
Aaa[1] = 0*WAaa
aaa[1] = 0*Waaa

# frequency after selection


Pa[1] = fAAa[1]+0.5*FAaa[1]
Qa[1] = 1-Pa[1]

143
for (i in 2:time)
{
    fAAa[i] = fAAa[i-1]*(1-u)^2
    fAaa[i] = fAaa[i-1]*(1-u)+fAAa[i-1]*(2*(1-u)*u)
    faa[i] = faa[i-1]+fAaa[i-1]*(u+fAAa[i-1]^(u^2))

    Pa[i] = (fAAa[i]+0.5*fAaa[i])
    Qa[i] = 1-Pa[i]

    # frequency after selection and mutation
    Pm[i] = (fAAa[i-1]+0.5*fAaa[i-1])*(1-u)
    Pf[i] = (fAAa[i-1]+0.5*fAaa[i-1])*(1-u)
    P[i] = (Pm[i]+Pf[i])/2

    Qm[i] = 1-Pm[i]
    Qf[i] = 1-Pf[i]
    Q[i] = 1-P[i]

    AAm[i] = (P[i]^2+P[i]*Q[i]*f)*WAAm
    Aam[i] = (2*P[i]*Q[i]*(1-f))*WAAm
    aam[i] = (Q[i]^2+P[i]*Q[i]*f)*Waam

    AAf[i] = (P[i]^2+P[i]*Q[i]*f)*WAAf
    Aaf[i] = (2*P[i]*Q[i]*(1-f))*WAaf
    aaf[i] = (Q[i]^2+P[i]*Q[i]*f)*Waaf

    Aaa[i] = fAaa[i]*WAAa
    Aaa[i] = fAaa[i]*WAaa
    aaa[i] = faaa[i]*Waaa

    # frequency after selection
    fAAm[i] = AAm[i]/(AAm[i]+Aam[i]+aam[i])
    fAam[i] = Aam[i]/(AAm[i]+Aam[i]+aam[i])
    faam[i] = aam[i]/(AAm[i]+Aam[i]+aam[i])

    fAAf[i] = AAf[i]/(AAf[i]+Aaf[i]+aaf[i])
    fAaf[i] = Aaf[i]/(AAf[i]+Aaf[i]+aaf[i])
    faaf[i] = aaf[i]/(AAf[i]+Aaf[i]+aaf[i])

    fAaa[i] = Aaa[i]/(Aaa[i]+Aa[i]+aaa[i])
    fAaa[i] = Aaa[i]/(Aaa[i]+Aa[i]+aaa[i])
    faa[i] = aaa[i]/(Aaa[i]+Aa[i]+aaa[i])
}

}

R_compute(h=.5,f=0,alpha=1,u=1.2e-05,nloci=1e+05)

#Example varying only 1 parameter (population structure)
F = seq(0,1,by=.1)
y1 = rep(NA, length=length(F))

for (i in 1:length(F))
  {y1[i] = R_compute(f=F[i], h=.11, alpha=1, u=1.2e-05, nloci=1e+05)}

y2 = rep(NA, length=length(F))

for (i in 1:length(F))
  {y2[i] = R_compute(f=F[i], h=.11, alpha=1.24, u=1.2e-05, nloci=1e+05)}

#Figure S1
par(mfrow=c(1,2))
plot(F,y2,type="o",col="red",xlab="Frequency of inbreeding",ylab = "Mean fitness relative to asexual competitor" ,ylim=c(1,4),pch=19)
points(F,y1,type="o",col="blue",pch=19)
abline(h=2) #level at which sexual populations completely compensate for the cost of sex

plot(F,y2/y1,type="o",pch=19,xlab = "Frequency of inbreeding",ylab = "Mean fitness relative to sexual population with no sexual selection",ylim=c(1,1.3))
abline(h=1)

#calculating the z matrices for contour plots can take a very long time (hours).

wrapper = function(x, y, ...)
  {sapply(seq(along = x), FUN = function(i) R_compute(u=x[i], alpha=y[i], ...))
}

u = seq(0.01e-05,1.5e-05,length=10)

alpha = seq(1,4,along.with=u)
z1 = outer(u,alpha,wrapper,h=.11,f=.2)

alpha1 = rep(1,length(u))
z2 = outer(u,alpha1,wrapper,h=.11,f=.2)

quartz()
par(mai=c(1,1,.75,.75))

contour(u*(1e05),alpha,z1/z2,xlab="Mutation Rate",ylab="Alpha",levels=seq(1.25,by=.05)) #figure S2
Appendix B

Supplementary Materials for Chapter 3

**Figure S1:** Relative fitness of whole-genome control lines, expressed as both females (red) and males (blue) over several generations of maintenance according to the protocol described in the Methods. Mean fitness of each point represents fitness of control populations, relative to the most fit control line within each assay. The estimates for g5 and g32 come from a separate set of lines than those used for the estimates at g12, g18, g25, g35, and g50. The slope of the regression was not significant when the control genomes were expressed either as females (slope = -.003, R2 = 0.27, p = 0.225) or as males (slope = -.004, R2 = 0.12, p = 0.23).
### Appendix C

#### Supplementary Materials for Chapter 4

**Table C1:** Line Means and 95% confidence intervals for all traits measured.

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<th>Line</th>
<th>Relative Viability (%)</th>
<th>Reproductive Success (%)</th>
<th>Mate Success (%)</th>
<th>P1 (%)</th>
<th>P2 (%)</th>
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148
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<th>Trait</th>
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<th>Var[ln(performance)]</th>
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<td>(0.82-0.93)</td>
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<td>(0.19, 0.29)</td>
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<td>0.61</td>
<td>0.45</td>
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<td></td>
<td>(0.57, 0.65)</td>
<td>(0.33, 0.61)</td>
<td>(0.44, 0.58)</td>
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<td>2.35</td>
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<td>(1.77, 3.09)</td>
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<td>(0.44, 0.57)</td>
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<td>0.84</td>
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<td>(0.77, 1.41)</td>
<td>(0.56, 1.33)</td>
<td>(0.63, 0.82)</td>
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<td>(0.086, 0.31)</td>
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<td>(0.37, 2.53)</td>
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<td>0.20</td>
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<td>(0.15, 0.27)</td>
<td>(0.39, 0.51)</td>
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<td>0.80</td>
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<td>(0.0011, 0.0019)</td>
<td>(0.41, 2.48)</td>
<td>(0.57, 0.73)</td>
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<td><strong>P2</strong></td>
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<td>0.0036</td>
<td>0.0051</td>
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<td>(0.0029, 0.0045)</td>
<td>(0.0049, 0.0065)</td>
<td>(0.062, 0.078)</td>
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<td>MA</td>
<td>0.040</td>
<td>0.13</td>
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<td></td>
<td>(0.035, 0.46)</td>
<td>(0.11, 0.18)</td>
<td>(0.28, 0.32)</td>
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<tr>
<td><strong>Total fitness</strong></td>
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<td>C</td>
<td>2.69</td>
<td>0.42</td>
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<td></td>
<td>(2.47, 2.94)</td>
<td>(0.31, 0.56)</td>
<td>(0.46, 0.65)</td>
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<td>MA</td>
<td>1.01</td>
<td>1.75</td>
<td>1.01</td>
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<td>(0.90, 1.14)</td>
<td>(1.32, 2.35)</td>
<td>(0.89, 1.16)</td>
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Estimation using normalized likelihoods.

The normalized distribution of a parameter $\theta$ is equal to:

$$\frac{L(\theta | Y)}{\int L(\theta | Y) d\theta}$$

Where $\frac{L(\theta | Y)}{\int L(\theta | Y) d\theta}$ is simply a normalizing constant such that the normalized likelihood has unit area (or sum, in the discrete case). In Bayesian terms, this is equivalent to estimations where the prior does not contribute to the posterior distribution. The subsequent posterior distribution (or likelihood density) can be used for point and interval estimation of $\theta$, and numerical methods readily yield estimates for various functions of $\theta$.

For example, we calculated parameter estimates for the mean rate of red-eyed offspring production (number of red-eyed offspring produced during oviposition) for the focal males and competitor males from each experimental line and treatment in the assays where we performed progeny counts (i.e. the P1, P2, reproductive fitness and viability assays) using the Poisson likelihood function.

$$e^{n\lambda + (\sum_{i=1}^{n} x_i) \ln(\lambda) - \sum_{i=1}^{n} \ln(x_i)}$$

Where the $x_i$ are the numbers of red-eyed offspring in each vial of a particular line/ treatment combination and $\lambda$ is the mean offspring production for those flies. Given the data, we then calculate the likelihoods for various $\lambda$ over a sufficiently large interval so
as not to preclude any high-likelihood values. We took the exponent of the log-likelihood function to simplify calculation, then evaluated the function at 5 000 points over a large interval of $\hat{\lambda}(10^{-10} \leq \hat{\lambda} \leq 30)$ prior to normalization. We also visually inspected each posterior, to confirm that the range of $\hat{\lambda}$ chosen included all values supported by the data. We performed a similar procedure for calculating the number of brown-eyed offspring per line.

To calculate the likelihoods for binomial traits like the proportion of successes in mating trials, we used the binomial likelihood function:

$$e^{\ln\binom{n}{k} + k \ln(p) + (n-k)\ln(1-p)}$$

Again, using the exponent of the log-likelihood to simplify calculations. We estimated parameters depending on multiple line means (for example, the group MA male mean for P1) by numerical methods. For each line we first sampled 20 000 values of $\hat{\lambda}$ according to their posterior probabilities and then combined them according to the desired function of the $\hat{\lambda}$. For example, the point estimate and 95% confidence interval for the group MA male mean for P1 was calculated by taking 20 000 averages of the 20 MA male line means, where each MA line mean is a randomly sampled value from the posterior distribution for that line. The median and 95% confidence interval of the resulting distribution corresponds to the point estimate and 95% confidence interval for the group MA male mean (or credibility intervals, in Bayesian terms). P-values were estimated in a
similar fashion, by calculating the area of the empirical distribution corresponding to the
desired test. Sample R scripts used to carry out these procedures, along with a brief
example, are included in this appendix.
R scripts demonstrating statistical methods.

# poisson loglikelihood
poisloglik = function(data, lambda) {
    sum(log(dpois(data, lambda)))
}
# sums up the individual loglikelihoods given lambda, using the built-in R function dpois to give total log likelihood for dataset given lambda.

binomloglik = function(data, prob) # data is a vector of 0s and 1s {
    sum(log(dbinom(sum(data), length(data), prob)))
}
# sums up the individual loglikelihoods given prob, using the built-in R function dbinom to give total log likelihood for dataset given prob.

# Example

# range of means to consider
meanrange = seq(0.0000000001, 30, length = 5000)
probrange = seq(0.0000000001, .9999999999, length = 5000)

data1 = rpois(100, lambda = 3) # sample data
data2 = rpois(100, lambda = 3.75)

data1loglik = sapply(meanrange, poisloglik, data = data1) # finds the loglikelihood associated with each point in meanrange
data2loglik = sapply(meanrange, poisloglik, data = data2)

plot(meanrange, exp(data1loglik), type = "l")
points(meanrange, exp(data2loglik), type = "l", col = "red")

data1lik = exp(data1loglik)/sum(exp(data1loglik)) # normalize the likelihood distribution
data2lik = exp(data2loglik)/sum(exp(data2loglik))

sum(data1lik)
sum(data2lik) # both sum to 1

plot(meanrange, data1lik, type = "l")
points(meanrange, data2lik, type = "l", col = "red")

mean(data1)
mean(data2)

abline(v = mean(data1), col = "black")
abline(v = mean(data2), col = "red") # maximum likelihood is at the mean, as expected.

# to construct significance tests by numerical approximation we sample from the probability distributions for each sample
\begin{verbatim}
data1mean = sample(meanrange,20000,replace=T,prob=data1lik)
data2mean = sample(meanrange,20000,replace=T,prob=data2lik)

hist(data1mean)

hist(data2mean)

quantile(data1mean,probs=c(0.025,.5,0.975))
t.test(data1)

quantile(data2mean,probs=c(0.025,.5,0.975))
t.test(data2)

quantile(data1mean-data2mean,probs=c(0.025,.5,0.975))
t.test(data1,data2) #t.test shown for comparison

#To find the p-value we find the quantile for which the pdf overlaps the null hypothesis of interest

#Arbitrary functions of the data can be estimated, and the p-values are coherent (i.e. probability that the difference in means is 0 is the same as the probability that the ratio of the means is 1)
quantile(data1mean/data2mean,probs=c(0.025,.5,0.975))

#The same procedure is used for the binomloglik function, except using probrange as the sample space instead of meanrange.
\end{verbatim}