GENETIC CONSIDERATIONS IN THE EVOLUTION OF SEXUAL DIMORPHISM

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Graduate Department of Ecology & Evolutionary Biology
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Abstract

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Sexual differences are dramatic and widespread across taxa. However, a common genome between males and females should hinder phenotypic divergence. In this thesis I have used experimental, genomic, and theoretical approaches to study processes that can facilitate and maintain differences between males and females.

I studied two mechanisms for the evolution of sexual dimorphism — condition-dependence and gene duplication. If sex-specific traits are costly, then individuals should only express such traits when they possess enough resources to do so. I experimentally manipulated adult condition and found that the sex-biased gene expression depends on condition. Second, duplication events can permit different gene copies to adopt sex-specific expression. I showed that half of all duplicate families have paralogs with different sex-biased expression patterns between members.

I investigated how current sexual dimorphism may support novel dimorphism. With regards gene duplication, I found that related duplicates did not always have different expression patterns. However, duplicating a pre-existing sex-biased gene effectively increases organismal sexual dimorphism overall. From a theoretical perspective, I investigated how sexually dimorphic recombination rates allow novel sexually antagonistic variation to invade. Male and female recombination rates separately affect invasion probabilities of new alleles.
Finally, I examined the assumption that a common genetic architecture impedes the evolution of sexual dimorphism. First, I conducted a literature review to test whether additive genetic variances in shared traits were different between the sexes. There were few significant statistical differences. However, extreme male-biased variances were more common than extreme female-biased variances. Sexual dimorphism is expected to evolve easily in such traits. Second, I compared these results to findings from the multivariate literature. In contrast to single trait studies, almost all multivariate studies of sexual dimorphism have found variance differences, both in magnitude and orientation, between males and females.

Overall, this thesis concludes that sexual dimorphism can evolve by processes that generate novel sexual dimorphism or that take advantage of pre-existing dimorphism. Furthermore, a common genome is not necessarily a strong barrier if genetic variances differ between the sexes. It will be an exciting challenge to understand how mutation and selection work together to allow organisms differ in their ability evolve sexual dimorphism.
Sancta Maria,
Sedes sapientiae,
ora pro nobis.
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Chapter 1

Introduction

The sexes differ most fundamentally with regard to reproduction. All phenotypic sexual dimorphism extends from and supports these reproductive differences. This dissertation examines several mechanisms that relate to the genetic underpinnings of sexual dimorphism in order that sex differences may originate and persist over time.

1.1 The implications of anisogamy

In *The Descent of Man and Selection in Relation to Sex*, Darwin (1871) begins his discussion of sexual selection by noting that males and females most obviously differ with regard to reproduction. By definition, females produce few large gametes and males produce many small gametes, a phenomenon referred to as anisogamy. Producing, harboring, and sometimes rearing, larger eggs represent a significant energetic cost to females. By contrast, making and dispersing numerous small sperm is relatively inexpensive for males. As a result, competition among the gametes of different males to fertilize female gametes is expected to be very strong, while competition among the gametes of different females to be fertilized is expected to be weaker.
Chapter 1. Introduction

This fundamental difference between the sexes in intensity of competition for mates will allow variance in male reproductive success to be greater than variance in female reproductive success. Bateman (1948) empirically demonstrated in *Drosophila melanogaster* that the majority of offspring were sired by a small number of males. As a result, 21% of males left behind no offspring at all; by contrast, only 4% of females left behind no offspring. He also showed that male reproductive success increases monotonically with each additional mate while female reproductive success does not. All of the sexual differences seen in morphology, behavior, physiology, development, and life history emerge from this basic expectation that male reproductive success is more variable than female reproductive success (Trivers 1972; Schärer et al. 2012). Indeed, phenotypic sexual dimorphism is likely both the resolution and affirmation of anisogamy.

Understanding that sexual differences ultimately emerge because of anisogamy, the next salient question becomes *how* they have evolved. Differences in gamete size and their consequences will inevitably invoke contrasting selection pressures in males versus females. This sex-specific selection will cause males and females to further diverge from one another. The fundamental issue is then how the sexes can diverge for a shared trait in light of the fact that they share many of the same genes for that same trait. Theory suggests that a shared genetic architecture will mean that the intersexual covariance is high. In effect, selection in one sex will evoke a correlated response in the opposite sex, preventing sex-specific adaptations from evolving and depressing population fitness (Lande 1980). This type of between-sex antagonism is referred to as intralocus sexual conflict; the term “intralocus” refers to the shared genes involved. It is difficult to imagine how sex-specific divergence might transpire in the shared traits. Yet, temporary resolutions to sexual conflict must be common, as sexual dimorphism is almost ubiquitous in taxa with separate male and female individuals or functions. In recent years, much theoretical and empirical progress has been made in understanding how to decrease the intersexual covariance to allow sexual dimorphism to evolve more quickly.
1.2 Mechanisms to evolve sexual dimorphism

Bonduriansky and Chenoweth (2009) summarized four genetic mechanisms that reduce intralocus sexual conflict and have empirical support: sex-linkage, sex-specific allelic effects, gene duplication, genomic imprinting. These four mechanisms may be further divided into two categories: those that rely either directly or indirectly upon sex-linkage and sex-determination.

Sex-linkage can foster sexually antagonistic variations through unequal chromosome number (Rice 1984). The sex with only one major X (or Z) sex chromosome (i.e., heterogametic sex) can express a rare recessive allele. However, the opposite sex with with two major sex chromosomes (i.e., homogametic sex) will likely be heterozygous for the rare recessive allele. This heterozygosity will shield the recessive allele from selection and allow it to spread — even if it benefits the heterogametic sex and harms the homogametic sex. Conversely, the homogametic sex can express rare dominant mutations that will spread even though their expression in heterogametic sex is always harmful. The sex chromosomes harbor a great many genes with sexually dimorphic expression in all of the model taxa so far examined (Gurbich and Bachtrog 2008). It may be that such excesses or deficits in the number of genes with male- and female- biased expression result from the dynamics of dominant and recessive alleles.

The sex chromosome can also indirectly foster sexual dimorphism by harboring factors that activate sex-specific genetic networks, triggering the expression of sexual dimorphism on autosomes. Sex-determination pathways can influence sex-specific patterns of alternative splicing (Lopez 1998; McIntyre et al. 2006) or make sex-specific modifications to cis- and/or trans- binding sites of autosomal genes (Williams and Carroll 2009) to manifest sex-specific allelic effects. Sex-determination pathways may also trigger sex-specific patterns of genomic imprinting. In general, genomic imprinting is the expression of only one of the two inherited alleles at a locus; this expression depends upon the allele’s parental origin (DeChiara et al. 1991). Although in classic genomic imprinting a particular locus
is always maternally or paternally imprinted, in sex-specific genomic imprinting, the imprinting status relies on the bearer’s sex (Hager et al. 2008; Gregg et al. 2010). Under the supposition that same-sex alleles are more advantageous on average, males are predicted to imprint, or suppress, maternally inherited alleles, and females are predicted to imprint paternally inherited alleles (Day and Bonduriansky 2004).

Gene duplication events may also provide the raw materials for sex-specific patterns of autosomal or sex-linked expression (Ellegren and Parsch 2007; Connallon and Clark 2011; Gallach and Betran 2011). Duplications produce extra gene copies whose functions are initially identical. As such, redundancy can release one of the copies from the original functional constraints to evolve new patterns of expression. If these new patterns of expression are acquired in a sex-specific manner, gene duplication can facilitate the evolution of sexual dimorphism. Again, the acquisition of sex-specific patterns of duplicate expression ultimately relies upon the upstream pathways that determine sexual fate, such as the presence of sex chromosomes.

In addition to these four genetically explicit mechanisms, condition-dependence may also be a fifth mechanism that can foster and maintain sexual dimorphism. Sex-specific adaptations can result in decreased fitness if they are expressed in the opposite sex. For instance, females should limit the expression of any costly display traits related to male mating success while males should limit the expression of any traits related to enhanced female fecundity. The same pleiotropic costs that favor sexually dimorphic trait expression can also favor condition-dependent expression in the benefitting sex (Rowe and Houle 1996; Bonduriansky and Rowe 2005; Bonduriansky 2007). In other words, the sex bearing the dimorphic adaptation should suppress trait expression when there are not enough resources to support full expression. So, males in higher condition should express expensive display traits while males in lower condition should not. Females in higher condition should express fecundity favoring traits while females in lower condition should not. The intersexual genetic correlation in the focal trait effectively decreases by
condition-dependent expression. As with the mechanisms of sex-specific allelic effects, duplication, and genomic imprinting, condition-dependence must ultimately rely upon sex-linkage or sex-determination.

All five mechanisms effectively decrease the intersexual covariance so that males and females no longer share perfectly overlapping genetic architectures. In effect, the genetic correlation between the sexes is less than one, so selection in one sex does not produce an exact corresponding response in the opposite sex. Once the intersexual genetic constraints are relaxed, the evolution of sexual dimorphism may proceed and sex-specific adaptations may permit increased population fitness (Lande 1980).

1.3 Justifications

Understanding how sexual dimorphism evolves over time is essential to understanding diversity in nature. The elaborate male structures and behaviors employed in courtship that he observed in the wild motivated Darwin (1871) to develop the theory of sexual selection, which was to contrast distinctly from his theory of natural selection (Darwin 1859). Natural selection quickly garnered strong support and formed the foundation of biology. And while sexual selection’s reception was initially tepid, it now constitutes one of the major branches of evolutionary biology. An analysis of selection in the wild goes as far as to conclude that selection for mates is stronger than selection for survival (Kingsolver et al. 2001; Hoekstra et al. 2001). And a recent primer on speciation also suggests that sexual selection, like natural selection, is a powerful engine of diversification (Coyne and Orr 2004). If access to mates is so fundamental to the origin, existence, and persistence of species, we would do well to try to understand how sexual differences might emerge.
1.4 Approaches

In order to understand how sexual dimorphism may evolve, I have adopted a variety of approaches to understand various genetic aspects of sexual dimorphism. I specifically studied condition-dependence and gene duplication as mechanisms that foster the evolution of sexual dimorphism. I also explored more broadly, how pre-existing sexual differences might support novel sexual dimorphism. In particular, I analyzed the role of sexually dimorphic recombination rates on the invasion of novel sexual dimorphism. Finally, I studied how often male and female genetic architectures actually differ and the implications of any such differences.

In Chapter 2, I addressed the hypothesis that an individual’s intrinsic quality, or condition, may affect sexually dimorphic gene expression. Much of the genome is expressed differently between the sexes and it was not clear at the time if these differences had functional implications. I experimentally manipulated the larval rearing environment to produce adults of high and low phenotypic condition in *Drosophila melanogaster*. I measured the effect of this manipulation and found that condition can be a significant source of variation in sex-biased gene expression. These results corroborate those found previously for traditional phenotypic sexually selected traits. Because of the breadth and unbiased selection of traits in this study (i.e., all genes), I was able to generalize the phenomenon of condition-dependence to the transcriptome.

In Chapter 3, I looked at how gene duplication might provide the raw material for sex-specific divergence in gene expression. A fundamental assumption of the evolution of sexual dimorphism is that a common genetic basis for a trait will impede selection favoring phenotypic divergence between the sexes. I addressed this hypothesis by comparing how related pairs of paralogs differ with respect to their sex-biased gene expression status. Because gene duplication provides extra gene copies, these copies may be requisitioned in a sex-specific manner, allowing the sex-specific genetic architectures to diverge. I found that about half of all paralog pairs have concordant expression patterns between mem-
bers; both members had male-biased only expression or female-biased only expression, or unbiased only expression. For the other half of paralog pairs, the members had discordant expression patterns. In particular, paralog pairs with one male-biased member and one unbiased member were common. These results suggest that gene duplication may be a way to help mitigate sexual conflict over expression and evolve sexual dimorphism.

Because the sexes might differ in functional aspects of genetics, in Chapter 4 I analyzed the implications of sexual dimorphism in recombination rates. Recombination rates can differ drastically between the sexes and the theoretical implications of these differences are poorly understood on autosomes. I found that the effect of sexually dimorphic recombination rates could extend beyond the simple sex-averaged rates. Furthermore, sexually dimorphic recombination rates can either impede or facilitate the invasion of novel sexually antagonistic variation. Thus I confirmed that the sexes can build upon pre-existing sexual dimorphism to facilitate the introduction of novel sexual dimorphism.

Finally, I took a quantitative genetics approach to the evolution of sexual dimorphism and explored the hypothesis that genetic architectures differ between males and females. Again, the theory underlying the evolution of sexual dimorphism suggests that a common genome will impede sexual divergence. In Chapter 5, I conducted an extensive meta-analysis to estimate sex-specific differences in additive genetic variance in single traits. I find surprisingly that few studies have analyzed and found statistically significant differences in male and female genetic variances. When combined with a high intersexual covariance, these data suggest that the potential to evolve sexual dimorphism in single traits is severely limited. Even so, the data show that biases in variance are prevalent – even after removing traits related to sexual reproduction. In particular, extreme variances tend to be male-biased rather than female-biased. This suggests that such traits may be under unrecognized male-specific selection or are correlated to traits under male-specific selection.

To extend the univariate results, in Chapter 6 I present the multivariate extension
of the view that male and female $G$ (variance-covariance) matrices differ and discuss its implications. The translation of sexual dimorphism in genetic variances into the multivariate formulation suggests that males and females can differ with regard to the absolute amount of available genetic variation, but also in the orientation of this genetic variation. Furthermore, the multivariate formulae suggest that high positive intersexual covariances can sometimes facilitate dimorphism, unlike the univariate formulae. I suggest that because the multivariate perspective is more general, it can confer more flexibility to evolve sexual dimorphism than a univariate perspective. Thus, even while the univariate results in Chapter 5 imply that sexual dimorphism is hard to evolve, a multivariate view suggests that this may not be the case. To understand why sexual dimorphism is so common, multivariate approaches may be necessary.
Chapter 2

Condition-Dependence of the Sexually Dimorphic Transcriptome in *Drosophila melanogaster*¹

2.1 Abstract

Sexually dimorphic traits are by definition exaggerated in one sex, which may arise from a history of sex-specific selection — in males, females, or both. If this exaggeration comes at a cost, exaggeration is expected to be greater in higher condition individuals (condition-dependent). Although studies using small numbers of morphological traits are generally supportive, this prediction has not been examined at a larger scale. We test this prediction across the transcriptome by determining the condition-dependence of sex-biased (dimorphic) gene expression. We find that high-condition populations are more sexually dimorphic in transcription than low-condition populations. High condition populations have more male-biased genes and more female-biased genes, and a greater degree of sexually dimorphic expression in these genes. Also, condition-dependence in

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male-biased genes was greater than in a set of unbiased genes. Interestingly, male-biased genes expressed in the testes were not more condition-dependent than those in the soma. By contrast, increased female-biased expression under high condition may have occurred because of the greater contribution of the ovary-specific transcripts to the entire mRNA pool. We did not find any genomic signatures distinguishing the condition-dependent sex-biased genes. The degree of condition-dependent sexual dimorphism \((CDSD)\) did not differ between the autosomes and the X-chromosome. There was only weak evidence that rates of evolution correlated with \(CDSD\). We suggest that the sensitivity of both female-biased genes and male-biased genes to condition may be akin to the overall heightened sensitivity to condition that life-history and sexually selected traits tend to exhibit. Our results demonstrate that through condition-dependence, early life experience has dramatic effects on sexual dimorphism in the adult transcriptome.

2.2 Introduction

Sexual dimorphism is ubiquitous in sexually reproducing organisms (Darwin 1871; Andersson 1994). Dimorphism in morphological and behavioral traits has long been apparent, yet only recently has the widespread extent of dimorphism in transcription been appreciated (Jin et al. 2001). In \(Drosophila melanogaster\), 15 to 70\% of known genes have sexually dimorphic expression (Jin et al. 2001; Parisi et al. 2003; Ranz et al. 2003; Gibson et al. 2004). Sexual dimorphism in gene expression also is taxonomically widespread, occurring in flies, worms, mammals, and birds (Jiang et al. 2001; Jin et al. 2001; Yang et al. 2006; Ellegren et al. 2007; Ellegren and Parsch 2007). At least for classic phenotypic traits, it is well-known that the extent of sexual dimorphism can vary dramatically within a single population (Darwin 1871; Andersson 1994), i.e., some males are phenotypically similar to females while other males are much different. Quantifying and explaining this variation in sexual dimorphism in the transcriptome (Meiklejohn et al. 2003; Baker et al.
2007), as well as in classic phenotypic traits (Fairbairn et al. 2007), remains a major challenge.

Sexual dimorphism evolves as a response to sex-specific selection—in males, females or both. Females are more fit if they limit the expression of any costly traits that primarily function in males to increase mating success (e.g., exaggerated display traits). Likewise, males are more fit if they limit the expression of any costly traits that function in females to enhance fecundity. Thus, much selection for dimorphism may occur because traits that benefit one sex carry pleiotropic costs that affect both sexes. The same pleiotropic costs that favor sexual dimorphism can also lead to condition-dependent expression of these traits (Rowe and Houle 1996; Bonduriansky and Rowe 2005; Bonduriansky 2007). Under a variety of assumptions, males in higher condition are expected to express these costly traits to a greater extent than males in lower condition. Although this process has been discussed most often in the context of exaggerated display traits, it applies to any traits with a history of sex-biased selection. Likewise, exaggeration of life history traits (e.g., female fecundity) may carry pleiotropic costs and may therefore evolve condition-dependent expression (Houle 1998). As a consequence of condition-dependence in sexually selected traits, and other dimorphic traits, the overall degree of sexual dimorphism is itself expected to be condition-dependent. Some studies of morphological characters have verified this prediction by finding higher levels of dimorphism when individuals are in higher condition (Bonduriansky and Rowe 2005; Bonduriansky 2007). This relationship between condition and the degree of dimorphism should hold broadly, applying to any costly trait that primarily benefits one sex.

Sex-biased gene expression represents a novel character set in which to test condition-dependent sexual dimorphism. Several lines of indirect evidence suggest that sex-specific selection was the driving force behind the evolution of sex-biased gene expression (Meiklejohn et al. 2003; Connallon and Knowles 2005; Reinius et al. 2008). Perhaps as a consequence of this sex-specific selection, sex-biased genes bear distinct evolutionary
signatures, which they share in common with other classic sexually dimorphic traits. In *Drosophila*, male-biased genes possess greater lineage-specific divergence than unbiased or female-biased genes with respect to coding-sequence (Zhang et al. 2004; Zhang and Parsch 2005; Pröschel et al. 2006; Haerty et al. 2007; but see Metta et al. 2006) and expression state (Meiklejohn et al. 2003; Ranz et al. 2003; Zhang et al. 2007). By contrast, female-biased genes demonstrate stronger conservation than unbiased or male-biased genes. These patterns mirror the phenotypic patterns showing that male traits often diversify while female traits appear more consistent across closely related taxa (Darwin 1871; Andersson 1994). If similar forms of sex-specific selection have shaped these parallels, then sex-biased gene expression may share in common other similarities with classic sexually dimorphic traits. In particular, sex-biased gene expression might respond to variation in condition.

We used microarrays to assess the condition-dependence of sexually dimorphic transcription. We reared larvae on diluted and concentrated sugar-yeast medium to produce “low” and “high” condition adult flies. We predicted that high condition individuals should be more sexually dimorphic in their expression patterns than low condition individuals. In particular, we expect male-biased gene expression, female-biased gene expression, and the total amount of sex-biased gene expression to be greater among high than low condition individuals. Only a handful of morphological studies have verified the condition-dependence of sexual dimorphism, and all of these studies have focused upon a small number of non-randomly selected traits (David et al. 2000; Cotton et al. 2004a,b; Bonduriansky and Rowe 2005; Bonduriansky et al. 2008; Boughman 2007; Punzalan et al. 2008). Testing condition-dependence in transcriptional sexual dimorphism across the entire genome reduces the potential for discovery bias while also extrapolating the prediction to the molecular level for the first time.
2.3 Methods

2.3.1 Microarrays

We used the two-channel Oligo 14kv1 microarrays printed by the Canadian Drosophila Microarray Center (CDMC) in Mississauga, Ontario. Arrays were synthesized with CMT-UltraGAPS slides using a SpotArray 72 microarrayer and the 65-69mer probes were based on release 4.1 of the *Drosophila melanogaster* genome from April 2005 (GEO accession # GPL3603). The array had 13,880 unique spots, representing *D. melanogaster* sequences (13,319 unique genes), blanks, buffer spots, and Arabidopsis controls; each spot was printed twice consecutively on the array. The CDMC handled all aspects of reverse-transcription, sample labeling, array hybridization, and slide scanning (see www.flyarrays.com for protocols). Our data are MIAME compliant and are available in the Gene Expression Omnibus repository.

2.3.2 Microarray experimental design

In the basic experimental block, condition was manipulated at two food levels (high or low) for both sexes (male or female). Each sex-by-diet combination was replicated twice within an experimental block for a total of eight biological samples. The within-block replication enabled each sex-by-diet combination to be labeled once with Alex647 and once with Alexa555 in a loop fashion, allowing us to account for dye-introduced variance. We used two genotypes; each genotype had three replicates of the basic experimental block. This resulted in 48 biological samples (2 sexes × 2 diets × 2 dyes × 2 genotypes × 3 experimental blocks) hybridized to 24 arrays.

2.3.3 Condition manipulation

In the high condition treatment, flies were reared on standard sugar-yeast medium. In the low condition treatment flies were reared on medium at 25% of the standard
sugar-yeast concentration. Size is a good proxy for condition in many insects including *D. melanogaster* because it correlates positively to the total energy reserves available at eclosion — corresponding to one aspect of “condition”. Our weight measurements confirmed that sex and larval diet treatments produced differences in adult size (Sex: $F_{1,116} = 491, P < 0.0001$; Diet: $F_{1,116} = 169, P < 0.0001$). Females were larger than males; high condition flies were larger than low condition flies. We observed sex × condition interactions on size ($F_{1,116} = 6.96, P = 0.009$). High condition females were 36% larger than low condition females; high condition males were 43% larger than low condition males. In other studies, we have found that reductions in larval nutrition have perceptible effects on adult sexual dimorphism, reducing fecundity in females and mating success in males (Sharp and Agrawal 2009).

2.3.4 Experimental treatments and rearing

Each genotype was the F1 hybrid offspring of two inbred lines founded from wild populations in North Carolina (courtesy of G. Gibson). The first genotype was the progeny of We61 × We29 (female × male); the second genotype was the progeny of We32 × We107. Each cross was performed only in one direction (no reciprocal crosses). We used these replicable genotypes for two reasons. First, the low larval diet treatment causes greater pre-eclosion mortality than the high larval diet treatment. Using individuals with the same genotype ensures that the survivors have the same genotype as the non-survivors. Thus, expression differences between the high and low condition individuals result directly from diet manipulation, rather than indirectly through differential selection. Second, inbred lines typically become homozygous for many loci but independent lines carry different alleles. Crossing two lines creates heterozygous individuals that are more representative of field-caught individuals (except for X-linked loci in males). Finally, we used two genotypes to expand the breadth of our results so that they are not confined to a single, perhaps unusual, genotype.
Virgin females from the inbred lines were mated to their respective males en masse in population cages containing grape-agar plates. Groups of 40 first instar larvae were picked from the grape-agar plates into 8-dram vials with 7.5ml of 25% or 100% medium and a small pellet of yeast. Virgin adult flies from high and low treatments were collected within 8h of eclosion and held in fresh vials with live yeast for 2 days. Adult flies from both larval diet treatments had access to food ad libitum. This ensured that any changes in adult gene expression could be attributed to larval, rather than adult diet. On the second day, 100 flies of the same sex and same diet treatment were placed in food bottles and allowed to mate with 100 control-mates from a separate outbred stock (Dahomey population collected from West Africa in 1970s) reared on 100% food. This “mating” bottle allowed the experimental flies to recognize and court members of the opposite sex – events that are integral to adult maturation and that significantly alter gene expression (Lawniczak and Begun 2004; McGraw et al. 2004; Mack et al. 2006; McGraw et al. 2008). We visually confirmed that flies from both diet treatments and of both sexes mated; we also confirmed that the newly mated females laid viable eggs. There were four bottle-level replicates of each sex-by-diet treatment. After 24 hours, the experimental flies were separated out and their mates were discarded. RNA was extracted from each group of ∼100 experimental flies using Trizol reagent (Invitrogen) according to the manufacturer directions.

2.3.5 Statistical analysis

To reduce noise, the intensity data were not background-corrected (Gibson and Wolfin-ger 2004). We performed a series of normalizations on the log base 2 intensity measurements to adjust for local, global, and array-specific effects (Quackenbush 2002) using the limma package (Smyth and Speed 2003) for R v. 2.7.1 (R Development Core Team 2008). We loess-normalized the intensities by print-tip-group (span = 0.4) and within arrays (span = 0.4) and across arrays with the quantile method. The normalized data
were analyzed with PROC MIXED in SAS v. 9 with gene-specific ANOVAs of the form:

\[ Y_{ijklmn} = \mu + \text{Array}(\text{Block}(\text{Genotype}))_{l(m(n))} + \text{Block}(\text{Genotype})_{m(n)} + \text{Genotype}_n + \text{Sex}_j + \text{Diet}_k + (\text{Sex} \times \text{Condition})_{jk} + \text{Dye}_i + \varepsilon_{ijklmn} \]  

(2.1)

where \( Y \) is the normalized expression for a gene labeled with dye \( i \) for sex \( j \) from condition \( k \) for array \( l \), nested in block \( m \), which is nested within genotype \( n \), with residual error \( \varepsilon \). Array, block, and genotype were random effects. Sex, diet, and dye were fixed effects.

This analysis allows us to assign an expression value to each gene for each treatment cell using the LSMEANS option in PROC MIXED (Gibson et al. 2004; Gibson and Wolfinger 2004; McGraw et al. 2008). The LSMEANS statement extracts the least-squares means, which we used to assess the significance of the sex and diet treatments by using the DIFFS option in PROC MIXED. A gene was considered sexually dimorphic in expression if the least-squares mean difference between males and females was statistically different from zero. This amounts to a t-test and is one method of expressing the extent of sexual dimorphism (Lovich and Gibbons 1992). To take into account multiple testing issues, we applied a false discovery rate (FDR) correction (Storey and Tibshirani 2003). We used a \( q \)-value cutoff of 0.01, which means that on average 1% of the genes reported as significant are truly null.

### 2.3.6 Technical considerations

Sexual dimorphism can occur in two ways. First, one sex may express a trait not found in the other sex. Second, each sex may express the same trait but in a different manner. Ideally, we would like to be able to distinguish between these two forms of dimorphism. However, this is difficult due to technical limitations of microarray data, where it is not possible to distinguish zero expression from very low-level expression. We considered genes showing a very large difference in expression between the sexes to
be likely to be sex-limited; specifically, we imposed a 10-fold cutoff to the difference of
the least-squares means between the sexes (Female − Male) to distinguish sex-biased
genes (i.e., expressed in both sexes) from sex-limited genes (i.e., expressed in one sex).
As sexual dimorphism is usually measured in shared traits with an index requiring male
and female measurements (Lovich and Gibbons 1992), we excluded sex-limited genes and
analyzed only sex-biased genes. This excluded 192 genes from our final analysis. While
using a high cut-off is a practical way to identify genes that are potentially sex-limited,
it is important to recognize that the 10-fold cutoff is an arbitrary distinction. This
arbitrariness is evident in the observation that even after applying the cutoff, three genes
which were classified as “sex-biased” under one condition were classified as “sex-limited”
in the other condition. Nonetheless, the use of such a cut-off at the very least provides
a rough distinction between sex-limited versus sex-biased genes. More importantly, our
main results remain unchanged whether we exclude genes identified as sex-limited from
our analyses, or analyze all genes.

2.3.7 Gonad-specific condition-dependence

The gonads harbor the majority of the sex-biased genes in the entire body (Parisi et al.
2003, 2004). It is therefore possible that any observed increase in sexual dimorphism un-
der high condition is entirely driven by changes in gonadal gene expression or in the
relative contribution of the gonad to the whole body transcript pool. To assess this pos-
sibility we compared the condition-dependence of genes expressed in the gonads to genes
expressed only outside of the gonads. We used previously published datasets (Parisi et al.
2003, 2004) to assign genes to one of four tissue types: ovaries, testes, female-soma minus
the ovaries (“female-soma”), and male-soma minus the testes (“male-soma”). We used
hybridizations that directly compared ovaries to gonadectomized females (GEO acces-
sions: GSM16554, GSM16555, GSM16542, and GSM16550), or testes to gonadectomized
males (GSM16569 and GSM16556).
A gene in the Parisi et al. (2004) data set was considered specific to the gonad or soma if the mean expression difference across arrays met a particular expression cutoff. We used four cutoffs, 2-fold, 4-fold, 8-fold, or 16-fold (Figs. 2.3, 2.5, 2.4 and 2.9). This enabled us to test how the results relied upon the cutoffs employed. We cross-referenced these tissue-assigned genes to the sexually dimorphic genes identified by our study. Based upon the expression values from our study, we then calculated the 95% confidence intervals for genes in each of the expression-by-tissue categories. After tissue-assignment and cross-referencing, some categories did not have any genes remaining; for instance, at the 16-fold cutoff there were no unbiased genes remaining in the testes. This effect occurs because we considered only genes whose expression was exclusive to each tissue type within a sex; there are still unbiased genes expressed in the testes but these are shared between the testes and the male-soma. To maximize the number of genes included in the analysis, we focused on the 2-fold cutoff, although the 3 more stringent cutoffs are presented in the supplementary material.

2.3.8 Characteristics of condition-dependent sex-biased genes

We looked at whether the extent of condition-dependent sexual dimorphism varied according to genomic location, rate of evolution, and functional categories for the genes identified as sex-biased under either of the two conditions. To test for differences in these features we constructed an index of condition-dependent sexual dimorphism ($CDSD = |Female_{High} - Male_{High}| - |Female_{Low} - Male_{Low}|$). For positive values, sexual dimorphism is greater under high condition; for negative values, sexual dimorphism is greater under low condition. For $CDSD = 0$, sexual dimorphism does not differ between the conditions. However, it is theoretically possible for a gene to reverse sex-biased expression (e.g., female-biased gene becomes male-biased), thereby making the interpretation of $CDSD$ problematic. For instance, equal but opposite reversals in sex-biased expression would also produce $CDSD = 0$. However, no genes statistically identified as
sex-biased showed such a reversal between conditions, precluding this issue. We chose CDSD to quantify the change in dimorphism over using the estimate of the interaction coefficient from the linear model. This is because the biological interpretation of the interaction coefficient depends on the values of the main effects. By contrast, CDSD has a simple sign with a simple interpretation that does not rely upon the main effects.

For genomic location, we calculated the mean CDSD according to chromosomal location and male- or female-biased expression. For rates of evolution, we regressed CDSD against ln($d_N/d_S$). Chromosomal locations and the pairwise $d_N/d_S$ values (for D. melanogaster – D. simulans) were obtained from the Sebida database (Gnad and Parsch 2006). For functional categories, we compared sex-biased genes in the top 25% of our index CDSD to sex-biased genes in the bottom 25% of CDSD for differences in the Gene Ontology (GO) Biological Processes category. GO Biological Process includes the most obvious terms relevant to sexual selection (e.g., mating and reproduction related functions). The two-tailed Fishers exact test provided through FatiGO, Babelomics 2008 (Al-Shahrour et al. 2006) analyzes over- or under-representation in functional terms between any two gene lists using $2 \times 2$ contingency tests (FDR-corrected).

2.4 Results

2.4.1 Changes in the number of sex-biased genes

We found strong condition effects on the total number of genes identified as sexually dimorphic in expression. Low condition flies had fewer genes that were sexually dimorphic than high condition flies when analyzing the difference of the least-squares means (Table 2.1). In high condition flies, 5763 genes show significant sex-biases ($Female_{High} - Male_{High}$): 2626 genes with male-biased expression and 3137 genes with female-biased expression. In low condition flies, only 5215 genes show significant sex-biases ($Female_{Low} - Male_{Low}$): 2357 genes with male-biased expression and 2858 genes
with female-biased expression. According to this metric, there was a \sim 10\% increase in the number of genes (n = 548) with sex-biased expression in high condition flies, and this difference was significant ($\chi^2_{df=1} = 46.84$, $P < 0.0001$). Although some genes that were identified as sex-biased in low condition flies lost their bias in high condition flies, more than twice as many genes acquired sex-biased expression under high condition than lost it ($\chi^2_{df=1} = 244.15$, $P < 0.0001$). Yet, diet manipulation did not affect the relative number of male- and female-biased genes in low versus high condition flies; the ratio of male-biased to female-biased genes did not depend upon treatment ($\chi^2_{df=1} = 0.1366$, $P = 0.71$).

The above analysis comparing the number of sexually dimorphic genes in high and low condition flies is sensitive to statistical power; a gene was only classified as sexually dimorphic if it had a significant q-value. However, if we had extremely large sample sizes, we would expect that almost all genes would be classified as sexually dimorphic in both conditions because very few genes might be expressed to exactly the same level by both sexes (e.g., increased sample sizes decrease the standard error about the estimate). Thus, we sought to confirm the pattern of more sexually dimorphic genes in high than low condition flies without using a statistical definition of sex-biased expression. Rather, we classified genes as sex-biased by setting a minimum threshold expression difference between the sexes. This minimum difference was gradually increased from zero (e.g., all genes were classified as sexually dimorphic) to 3.32 (e.g., only genes with at least a 10-fold difference between the sexes were classified as sexually dimorphic). We then asked whether high and low condition flies differed in the number of sexually dimorphic genes at a given threshold value (Fig. 2.1). Across this range, sex-biased gene number was greater in high than low condition flies (except at zero when the numbers are exactly equal).
Table 2.1: Number of sex-biased genes under low and high condition. Gene expression status could remain the same or change between the condition treatments. Values in parentheses indicate the percent of the total number of genes on the array. Sex-limited genes (as defined by our study) are not shown.

<table>
<thead>
<tr>
<th>Status under low condition</th>
<th>Status under high condition</th>
<th>Number of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unchanged</td>
<td>unbiased</td>
<td>7023 (52.7)</td>
</tr>
<tr>
<td></td>
<td>female-biased</td>
<td>2655 (20.4)</td>
</tr>
<tr>
<td></td>
<td>male-biased</td>
<td>2219 (17.6)</td>
</tr>
<tr>
<td>Lost sex-bias</td>
<td>female-biased</td>
<td>203 (1.5)</td>
</tr>
<tr>
<td></td>
<td>male-biased</td>
<td>138 (1)</td>
</tr>
<tr>
<td>Gained sex-bias</td>
<td>unbiased</td>
<td>482 (3.6)</td>
</tr>
<tr>
<td></td>
<td>female-biased</td>
<td>407 (3.1)</td>
</tr>
<tr>
<td></td>
<td>male-biased</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.1: Difference in the number of sexually dimorphic genes in high vs. low condition. We calculated the index of sexual dimorphism $D = |Female - Male|$ from the least-squares means for all genes on the microarray, under high and low condition separately. Within each treatment, we calculated $N[x]$ as the number of genes for which the level of dimorphism is greater than $x$ (i.e., $D > x$). This plot shows the difference between treatments in the number of genes meeting a specified threshold level of dimorphism (i.e., $N_{high}[x] - N_{low}[x]$). Across the range of threshold levels ($x$), the difference is positive. Thus, the number of sexually dimorphic genes is greater under high condition regardless of the threshold level used to classify a gene as dimorphic.
2.4.2 Changes in the extent of sex-biased gene expression

In addition to asking whether condition affects the number of dimorphic genes, we also asked whether condition affects the extent of dimorphism. Using only those genes classified by their q-value as dimorphic in at least one treatment, we performed a one-way ANOVA with the two-level factor condition as the independent variable and sexual dimorphism (|Female\text{High} - Male\text{High}| or |Female\text{Low} - Male\text{Low}|) as the dependent variable. These analyses included all genes identified as sex-biased in at least one treatment (high or low condition). The average extent of sex-biased expression was greater by \( \sim 10\% \) in high than low condition flies \((F_{1,12234} = 72.44, P < 0.0001)\). This increase occurred independently of the increase in sex-biased gene number; using only those genes that were sex-biased in both treatments we still find that the average extent of sex-bias is greater in high condition than low condition flies \((F_{1,9774} = 60.34, P < 0.0001)\).

While the absolute female-to-male difference in expression increased among the sex-biased genes, it was unclear if this occurred through expression changes in males, females, or both. We used a two-way ANOVA to quantify the concurrent effects of condition and sex on sex-biased expression. We performed this test separately for male- and female-biased genes. In the first analysis, we used genes identified as biased under either high or low condition. Within male-biased genes, there was a significant condition effect \((F_{1,11052} = 7.0435, P = 0.008)\) and a significant sex \(\times\) condition interaction \((F_{1,11052} = 5.3646, P = 0.021; \text{Fig. } 2.2A)\). Condition increased the expression of male-biased genes more in males than in females, accounting for the significant interaction term. For female-biased genes (\text{Fig. } 2.2C), there is no significant condition effect \((F_{1,13356} = 0.0624, \quad P = 0.8026)\). However, there was a significant sex \(\times\) condition interaction \((F_{1,13356} = 4.1467, \quad P = 0.0417)\); this interaction occurs because of the slight (but non-significant) up-regulation of female-biased gene expression in females and slight (but non-significant) down-regulation in males.
Confining our analysis only to those genes with sex bias under both conditions, we found similar results. There was a significant condition effect ($F_{1,8872} = 7.7983$, $P = 0.0052$) and sex × condition interaction ($F_{1,8872} = 4.2805$, $P = 0.0386$) for the male-biased genes (Fig. 2.2B); there was no condition effect ($F_{1,10616} = 0.0019$, $P = 0.9657$) or interaction ($F_{1,10616} = 3.3844$, $P = 0.0658$) for the female-biased genes (Fig. 2.2D).

## 2.4.3 Gonad-specific condition-dependence

At the 2-fold cutoff we find that male-biased genes expressed in the testes and the soma were more condition-dependent than unbiased genes (i.e., 95% confidence intervals do not overlap). Moreover, male-biased genes expressed in the male-soma responded similarly to diet as those in the testes; unbiased genes also responded similarly between the soma and testes (Fig. 2.3). These patterns persist at the 4-fold cutoff (Fig. 2.4). At the 8-fold cutoff the male-soma and the testes are still not distinct from each other for their average level of condition-dependence; however, the male-biased genes are no longer distinct from the unbiased genes for condition-dependence (although there is a trend). At 16-fold the male-soma and testes are again not different with regard condition-dependent male-biased gene expression (Fig. 2.4).

In contrast to male-biased genes, female-biased genes show some evidence that the average level of condition-dependence differs between the female-soma and the ovaries. At the 2-fold cutoff, female-biased genes in the ovaries appear to be more condition-dependent than female-biased genes in the female-soma, but this pattern breaks down at the 4-fold cutoff (Figs. 2.5, and 2.9). And in contrast to the male-biased genes, female-biased genes do not show greater condition-dependence than the unbiased genes on average for a given tissue, regardless of the cutoffs employed (Fig. 2.5).

In sum, these results suggest that the condition-dependent changes in expression for male biased genes are not entirely due to changes in gonad size. Male-biased genes show increased expression at high condition regardless of whether those genes are expressed...
Figure 2.2: Condition effects on sex-biased gene expression (log base 2). Sex-biased genes were pooled to assess condition and sex effects on expression. Genes were grouped according to male-biased (panels A and B) and female-biased (panels C and D) expression. Genes were also pooled according to whether they demonstrated sex-biased expression in at least one of the two condition treatments (A, C) or in both treatments (B, D). For both pools of male-biased genes (A, B) there were significant condition and sex × condition effects, resulting from an increase in expression of these genes at high condition in males that was greater in males than females. By contrast, there was no main effect of condition on the expression of female-biased genes in either pool of genes. When considering genes that were female-biased in at least one treatment, we observed a significant sex × condition interaction (C); this interaction occurred because of slight male down-regulation and slight female up-regulation of female-biased genes. When considering only those genes that were female-biased in both treatments, the interaction was no longer significant.
Figure 2.3: Extent of condition-dependence in the testes and male-soma. Condition-dependence (mean + 95% confidence intervals) is defined as $Male_{High} - Male_{Low}$. Values differ if 95% CI do not overlap among groups. A gene from the Parisi et al. (2004) data set was assigned to either the testes or the male-soma if it showed at least a 2-fold difference in expression between the two tissues (see text for details). Male-biased genes expressed in the testes and the male-soma share a similar degree of condition-dependence. This was true when the threshold specificity was increased (see Fig. 2.4).
Figure 2.4: Additional thresholds to define tissue specificity in male gene expression. We increased the threshold used to define a gene as being expressed in the male-soma versus the testes. Like the 2-fold cutoff (Fig. 2.3), at the 4-fold cutoff male-biased genes appear to be more condition-dependent on average than the unbiased genes (A). This pattern breaks down 8-fold (B) and 16-fold (C) cutoffs. Missing genes in the 16-fold cutoff (C) are due to considering only those genes that are specific to the testes or the male-soma. Finally, across all thresholds the testes and male-soma do not differ in their average level of condition-dependence.
Figure 2.5: Extent of condition-dependence in the ovaries and female-soma. Condition-dependence (mean + 95% confidence intervals) is defined as $Female_{High} - Female_{Low}$. Values differ if 95% CI do not overlap among groups. A gene from the Parisi et al. (2004) data set was assigned to either the ovaries or the female-soma if it showed at least a 2-fold difference in expression between the two tissues (see text for details). At the 2-fold cutoffs the ovaries and female-soma seem to differ slightly in their degree of condition-dependence. However, this relationship breaks down at higher thresholds (see Fig. 2.9). Female-biased genes do not seem more condition-dependent than the unbiased genes within a particular tissue type or within a given fold cutoff.
mostly in the testes or in the soma. This does not appear to be the case for female-biased genes. Female-biased genes in the ovaries appear to show increased expression under high condition but genes expressed mostly outside the ovaries do not. However, we have less power to make this comparison for female-biased genes than male-biased genes because there are fewer female-biased genes that meet our selection criteria.

2.4.4 Sensitivity to condition

The degree of sex-biased gene expression (for genes with sex-bias under either of the diet treatments) correlated with the degree of condition-dependence (i.e., $CD_{Female} = Female_{High} - Female_{Low}$ and $CD_{male} = Male_{High} - Male_{Low}$). However, the direction and strength of the correlation depended upon the sex in which male-biased (Fig. 2.6A,C) or female-biased expression (Fig. 2.6B,D) was measured.

The correlation was positive for male-biased genes expressed in males (Fig. 2.6A; $n = 2764; \text{slope} = 0.085, r^2 = 0.08, P < 0.0001$). The correlation was also positive for male-biased genes expressed in females (Fig. 2.6C; $n = 2764; \text{slope} = 0.044, r^2 = 0.03, P < 0.0001$). However, the slope and percent variance explained is greater in males than in females, showing that male-biased genes expressed in males responded more strongly to condition. Our non-parametric analyses (not shown) for these correlations were also significant. Condition-dependence of female-biased genes expressed in females was also an increasing function of sexual dimorphism (Fig. 2.6D; $n = 3340; \text{slope} = 0.054, r^2 = 0.02, P < 0.0001$). By contrast, female-biased genes expressed in males decreased with increasing sexual dimorphism (Fig. 2.6B; $n = 3340; \text{slope} = -0.0402, r^2 = 0.02, P < 0.0001$). Thus, while condition explains the same amount of variance in the extent of female-biased expression for both sexes, the sign of the correlation differs.

For each of the regressions listed above a component of the X variable is present in the Y variable (e.g., in Fig. 2.6A both X and Y variables contain the value $Male_{High}$ in order to calculate the difference in the least-squares means). It is possible that such reiterations
can result in spurious significant correlations (Jackson and Somers 1991). To test this possibility we performed a randomization test (for details see Supplementary Materials). We find that the observed slopes in Fig. 2.6 always fall well outside the distribution of the permuted slopes (Fig. 2.4.4). We conclude that these significant regressions (Fig. 2.6) are not spurious.

Overall, these results show that the sensitivity of sex-biased gene expression to condition depended upon the degree of sexual dimorphism as well as upon the sex in which they were expressed. Finally, male-biased genes were more sensitive to condition than female-biased genes regardless of the sex in which they were expressed.

### 2.4.5 Characteristics of condition-dependent sex-biased genes

The autosomes and X-chromosome differ slightly in their average extent of male-biased and female-biased gene expression (Parisi et al. 2003). We tested whether this difference in the extent of sex-bias was related to a difference in the degree of condition-dependent sexual dimorphism (\(CDSD\)). At a 2-fold cutoff to distinguish soma-specific versus gonad-specific genes, we did not find differences in \(CDSD\) between chromosome types (i.e., 95% confidence intervals did not overlap). This lack of a chromosome effect occurred whether or not we took into account tissue of expression (Fig. 2.8). Interestingly, female-biased genes expressed in the female-soma show a lower degree of condition-dependent sexual dimorphism than sex-biased genes in the other categories.

Condition-dependent sexual dimorphism may facilitate or hamper the rate of adaptive evolution. To test these alternatives, we regressed \(\ln(d_N/d_S)\) against \(CDSD\). We removed genes whose \(d_N/d_S > 2\) to correct for saturation effects. The correlation was negative in female-biased genes \((n = 2832; \text{slope} = -0.44; r^2 = 0.004, P = 0.0005)\) but positive in male-biased genes \((n = 2375; \text{slope} = 0.45; r^2 = 0.0049, P = 0.0004)\). We removed 293 female-biased genes and 140 male-biased genes with extremely small \(d_N/d_S\) values. However, the correlations were significant even when including these outliers. Non-parametric
Figure 2.6: Extent of sex-biased expression and condition-dependence within each sex. The extent of sex-biased expression was correlated to the extent of condition-dependence. The direction and strength of the correlation relied upon the sex in which it was measured. Male-biased genes expressed in males (A) and in females (C) had a strong positive correlation. Female-biased genes expressed in females had a positive correlation (D). However, female-biased gene expressed in males had a negative correlation (B).
Chapter 2. Condition-dependent sex-bias

Figure 2.7: Permutation analyses of regression slopes to test for bias. The X and Y variables in Fig. 2.6 share the same quantity (e.g., both variables contain Male$_{High}$). This commonality may cause an autocorrelation and spurious significance. To test for this bias, we performed a permutation analysis. We paired the least-squares mean for one sex for each gene with the least-squares mean for the opposite sex from a randomly chosen gene. We re-calculated the sexual dimorphism index (Female$-$Male) and paired the new index with the within-sex diet effect associated with the least-squares mean of one of the sexes (e.g., CD$_{Female} = $Female$_{High} - $Female$_{Low}$ or CD$_{male} = $Male$_{High} - $Male$_{Low}$). We then re-calculated the slope of the regression between the new sexual dimorphism index and the diet effect. Any correlations between these measures is due only to autocorrelation as the randomization procedure removes any true biological correlation. The randomization was done 1000 times to extract 1000 slopes and observe their distribution. In Fig. 2.6A, the female least-squares means were randomly paired with the male least-squares means; we re-calculated the sexual dimorphism index. Then we paired the new dimorphism calculation with the within-male diet effect associated with the male least-squares mean. We observe that the true slope from Fig. 2.6A falls well outside of the distribution of the permuted slopes. In other words, the autocorrelation between the X and Y variables is too small to account for the observed regression slope. The true slope falls well outside of the distribution of the permuted slopes for all four regressions present in Fig. 2.6. The significant regressions in Fig. 2.6 are real and not spurious.
rank tests also showed significant correlations for male- and female-biased genes. However, these correlations are weak and the percent variance in ln($d_N/d_S$) explained by the index of condition-dependence is small.

Because sex-biased genes varied in their degree of $CDS\text{D}$, we tested for differences in functional enrichment between sex-biased genes in the top versus bottom quartile of $CDS\text{D}$. We found GO Biological Process terms within the male-biased genes that were enriched in the bottom quartile of $CDS\text{D}$; no terms directly related to male reproduction were enriched in the top quartile of $CDS\text{D}$. In the female-biased genes, we found that genes in the top quartile of $CDS\text{D}$ were enriched for terms related to sexual reproduction and gametogenesis (Table 2.2).

2.5 Discussion

The degree of sex-biased expression can evolve within populations and species (Meiklejohn et al. 2003; Connallon and Knowles 2005; Baker et al. 2007; Zhang et al. 2007) and may correlate to the intensity of sex-specific selection (Reinius et al. 2008). Here we show that variation in condition can induce substantial variation in sexually dimorphic patterns of gene expression. Individuals reared under high condition had more male-biased gene expression, female-biased gene expression, and total sex-biased gene expression than individuals reared under low condition. These results corroborate the morphological data demonstrating that condition can modulate the degree of sexual dimorphism (Bonduriansky and Rowe 2005; Bonduriansky 2007).

2.5.1 Condition-dependent male-biased gene expression

Condition-dependent male-biased gene expression assumed two forms in this study. First, condition affected the total number of detectable male-biased genes. Second, high condition males increased expression of genes that were already male-biased under low
Table 2.2: Gene Ontology Biological Process. We compared sex-biased genes in the top 25% (T) of our index CDSD to sex-biased genes in the bottom 25% (B). This test looks for over- and under-representation in functional categories between gene lists. The sign indicates the list that is enriched for terms relative to the other list.

<table>
<thead>
<tr>
<th>Gene Ontology (GO) Biological Process</th>
<th>enriched gene list</th>
<th>adjusted P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female-biased genes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GO:0048869 Cellular developmental process</td>
<td>T &gt; B</td>
<td>0.0021</td>
</tr>
<tr>
<td>GO:0019953 Sexual reproduction</td>
<td>T &gt; B</td>
<td>0.0069</td>
</tr>
<tr>
<td>Level 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GO:0030154 Cell differentiation</td>
<td>T &gt; B</td>
<td>0.0019</td>
</tr>
<tr>
<td>GO:0007276 Gametogenesis</td>
<td>T &gt; B</td>
<td>0.0434</td>
</tr>
<tr>
<td>Level 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GO:0048468 Cell development</td>
<td>T &gt; B</td>
<td>0.0112</td>
</tr>
<tr>
<td>Level 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GO:0022607 Cellular component assembly</td>
<td>T &gt; B</td>
<td>0.0266</td>
</tr>
<tr>
<td><strong>Male-biased genes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GO:0050789 Regulation of biological process</td>
<td>T &lt; B</td>
<td>0.0158</td>
</tr>
<tr>
<td>GO:0048869 Cellular developmental process</td>
<td>T &lt; B</td>
<td>0.0469</td>
</tr>
<tr>
<td>Level 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GO:0009653 Anatomical structure morphogenesis</td>
<td>T &lt; B</td>
<td>0.0417</td>
</tr>
<tr>
<td>GO:0009790 Embryonic development</td>
<td>T &lt; B</td>
<td>0.0417</td>
</tr>
<tr>
<td>GO:0030154 Cell differentiation</td>
<td>T &lt; B</td>
<td>0.0417</td>
</tr>
<tr>
<td>GO:0046903 Secretion</td>
<td>T &lt; B</td>
<td>0.0417</td>
</tr>
</tbody>
</table>
Figure 2.8: Condition-dependence of sexual dimorphism (CDSD) on the X and autosomal chromosomes. CDSD is defined as $|Female_{\text{High}} - Male_{\text{High}}| - |Female_{\text{Low}} - Male_{\text{Low}}|$. Male- and female-biased genes are defined as those that were identified as biased in either of the condition treatments (see Table 2.1). Tissue location was based upon a 2-fold cutoff between the gonad and soma within each sex. Means are significantly different if 95% confidence intervals do not overlap. Among female-biased genes, there was no difference in CDSD among the chromosome types regardless of their expression in the female-soma or ovaries. There was no difference in CDSD among male-biased genes regardless of their chromosomal location or tissue as well ($F_{\text{soma}} = \text{female-soma}; M_{\text{soma}} = \text{male-soma}$).
condition (Figs. 2.2 and 2.3), resulting in a significant condition and sex × condition interaction. Even while there is no direct evidence that male-biased genes are generally under sexual selection, there is evidence that sex-specific selection can shape sex-biased gene expression (Zhang et al. 2004; Reinius et al. 2008) Male-biased genes bear the hallmarks of traditional sexually selected traits (Darwin 1871; Andersson 1994), evolving rapidly and showing strong divergence between closely related lineages (Meiklejohn et al. 2003; Ranz et al. 2003; Zhang et al. 2004, 2007). Insofar as their total number and extent of bias are condition-dependent, male-biased genes appear similar to other classic sexually selected traits in yet another respect.

As expected, male-biased genes were more condition-dependent than unbiased genes in the subset analyzed in the testes and male-soma (Fig. 2.3). Moreover, the correlation between degree of male-biased expression and degree of condition-dependence was significantly positive in males (Fig. 2.6A); genes with greater male-bias were more sensitive to condition. However, at present it is unclear if these patterns result because extremely male-biased genes impose greater costs (Rowe and Houle 1996), or for some unrelated reason. Curiously, these same male-biased genes expressed in females also showed a highly significant positive correlation between degree of male-biased expression and female condition-dependence (Fig. 2.6C), but the correlation was not as strong. Such a correlation may indicate unresolved intralocus conflict. Connallon and Knowles (2005) have shown that D. melanogaster male-biased genes are significantly overrepresented for genes that are upregulated in both males and females relative to unbiased genes. When male-biased genes evolve increased expression in males, increased expression also occurs in females. So condition-dependence may itself have a high intersexual correlation (Bonduiriansky and Rowe 2005), causing the response in females.

Our observation of greater expression of male-biased genes under high condition could simply reflect the relatively larger contribution of the testes to whole-body sex-biased gene expression under high condition. Overall, male-biased gene expression is known to
be greater in the testes than in the rest of the male-soma (Parisi et al. 2004). Moreover, testes size has a positive allometry with body size in *Drosophila* (Pitnick 1996; Bangham et al. 2002). However, we found that male-biased genes expressed mostly outside of the testes show the same elevated level of condition-dependence as genes expressed mostly in the testes (Fig. 2.3). While the testes may make a larger contribution to the total expression pool under high condition, this effect alone cannot explain the heightened expression of male-biased genes.

### 2.5.2 Condition-dependent female-biased gene expression

There was evidence that female-biased genes were condition-dependent. High condition females had more female-biased genes than low condition females. However, unlike for the male-biased genes, the extent of female-biased gene expression was only weakly affected by condition. For instance, while female-biased genes had a significant sex × condition interaction (Fig. 2.2C), there was no significant diet effect. High condition females do not significantly increase expression of female-biased genes; rather, slight female up-regulation and slight male down-regulation of female-biased genes accounts for the interaction. Furthermore, the interaction disappears when we consider only genes with female-biased expression under both condition treatments (Fig. 2.2D). This suggests that overall, female-biased gene expression in females shows greater resilience to variation in condition.

The positive correlation between the extent of female-bias and condition-dependence in females while significant (Fig. 2.6D), was weaker than the correlation between the extent of male-bias and condition-dependence in males (Fig. 2.6A). This positive correlation nonetheless suggests that genes with greater female-bias are more sensitive to condition. By contrast, the correlation for female-biased genes expressed in males was significantly negative (Fig. 2.6B). Relative to low condition males, high condition males appear to down-regulate the most strongly female-biased genes. High condition males appear to be
more masculine in male-biased gene number and expression while also being less feminine in female-biased gene expression, consistent with previous morphological work (Bonduriansky and Rowe 2005; Bonduriansky 2007). Finally, unlike for the male-biased genes, we were not able to rule out the effect of positive allometry on increases in female-biased gene expression. At the 2-fold cutoff, the female-biased genes in the ovaries appear to be more condition-dependent than the female-biased genes in the female-soma (Fig. 2.5). Unbiased genes in the ovaries are also more condition-dependent than unbiased genes in the female-soma; in fact, unbiased genes in the female-soma show decreased expression under high condition. Altogether, this suggests that the overall increase in female biased gene expression at high condition may be due to a larger contribution of the ovaries to the total expression pool. However, at the 4-fold cutoff, these patterns disappear and both female-biased and unbiased genes expressed in either the ovaries or female-soma are not condition-dependent (Fig. 2.9). Thus, the positive allometry interpretation for increased female-biased gene expression under high condition has some support but it remains inconclusive.

Female-biased genes may serve as the genomic analogues of life history traits. Phenotypic life history traits rely heavily upon condition and can experience strong directional selection without appearing to evolve, e.g., breeding date in birds (Price et al. 1988). In a corresponding manner, condition can affect the extent of female-biased gene expression in females (Fig. 2.5 and Fig. 2.6D) and exhibits signatures of evolutionary conservation compared to male-biased genes (Zhang et al. 2004). In support of this interpretation, we found that female-biased genes in the top quartile of $CDSD$ are relatively enriched for genes involved in reproduction and gametogenesis (Table 2.2).

2.5.3 Condition-dependent sexual dimorphism

Our results from the transcriptome demonstrate that sexual dimorphism itself is condition-dependent, corroborating earlier more limited studies of condition-dependence
Figure 2.9: Additional thresholds to define tissue specificity in female gene expression. We increased the threshold used to define a gene as being expressed in the female-soma versus the ovaries. Unlike in the 2-fold cutoff (Fig. 2.5), at the 4-fold cutoff genes expressed primarily in the ovaries are not more condition-dependent than genes expressed primarily in the female-soma (A). Also, across all cutoffs, we do not observe that female-biased genes are more condition-dependent than unbiased genes. Missing genes in the 8-fold (B) and the 16-fold (C) cutoffs are due to considering only those genes that are specific to the female-soma or the ovaries.
in phenotypic sexual dimorphism. In contrast to prior studies, which were confined to a few selected morphological phenotypes (David et al. 2000; Cotton et al. 2004a,b; Bonduriansky and Rowe 2005; Bonduriansky 2007; Boughman 2007; Punzalan et al. 2008), our study is both much greater in scope and less biased in its selection of traits. High condition increased the total number of sex-biased genes by several hundred genes. And while genes could lose sex-biased expression under high condition, overall, there were many more gains than losses of sex-biased expression. High condition also increased the overall expression difference between the sexes by ∼10%.

This work emphasizes that sex-biased gene expression is plastic. Perhaps the lack of selective constraint on sex-biased genes has facilitated both sex-biased gene expression and its sensitivity to condition. Yet, if condition-dependence were simply an outcome of the general flexibility in expression of sex-biased genes, the greater sexual dimorphism in high condition is not expected. Rather one might expect variance in condition to introduce random variance in sex-biased gene expression (e.g., as many genes being dimorphic under high condition as low condition). Furthermore, we found only very weak evidence that condition-dependent sex-biased genes are either free from selective constraint, or experience evolutionary constraint, as measured by $d_N/d_S$. Finally, condition-dependent sexual dimorphism ($CDSD$) did not demonstrate any unique genomic patterns across chromosomes (Fig. 2.8). There is no evidence that X-linked condition-dependent sexual dimorphism is stronger or weaker than autosomal condition-dependent sexual dimorphism. However, female-biased genes expressed in the female-soma have the lowest degree of $CDSD$ compared to the other gene categories. The reasons for this pattern are unclear.

There have been several previous attempts to connect changes in gene expression to diet manipulation in $D. melanogaster$ (Pletcher et al. 2002; Zinke et al. 2002; Carsten et al. 2005; Harbison et al. 2005; McGraw et al. 2007); two pertain to our study. Harbison et al. (2005) found that differences in current adult nutrition affected 12% of all sexually
Chapter 2. Condition-dependent sex-bias

Dimorphic genes – confirming that when organisms are tested at the same developmental stage as the diet treatment, gene expression changes are common (Endo et al. 2002; Pletcher et al. 2002; Zinke et al. 2002; Carsten et al. 2005). Our study differs from Harbison et al.s (2005) because we manipulated larval diet and measured changes in adult gene expression (keeping adults fed ad libitum). Our approach corresponds more closely to the life history definition of condition (Rowe and Houle 1996), and is not confounded by short-term responses to feeding. Like our study, McGraw et al.s (2007) reared larvae on different nutrient levels and tested the condition-dependence of adult gene expression. Their study focused on nine male-limited accessory gland genes (i.e., proteins present in seminal fluids), of which only one had detectable condition-dependent expression in adult males. By applying a cutoff to remove potentially sex-limited genes, our study shows that condition also more broadly affects the sex-biased genes.

2.5.4 Conclusions

It remains a challenge to decipher the relative importance of natural and sexual selection on sex-biased genes. There is much underlying variation that requires further study. Why are genes with greater sex-bias more condition-dependent? Theory predicts that the optimal level of condition-dependence will depend on the relationship between expression level and its costs and benefits (Rowe and Houle 1996; Houle 1998). Does the observed variation in condition-dependence reflect variation in these relationships or is it simply due to gene-specific constraints on sensitivity to condition? Addressing these questions will require a more detailed understanding of the function of these genes and the extent of selection and constraint on their expression.

2.6 Acknowledgments

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3.1 Abstract

Males and females share most of the same genes, so selection in one sex will typically produce a correlated response in the other sex. Yet the sexes have evolved to differ in a multitude of behavioral, morphological, and physiological traits. How did this sexual dimorphism evolve despite the presence of a common underlying genome? We investigated the potential role of gene duplication in the evolution of sexual dimorphism. Because duplication events provide extra genetic material, the sexes each might use this redundancy to facilitate sex-specific gene expression, permitting the evolution of dimorphism. We investigated this hypothesis at the genome-wide level in *Drosophila melanogaster*, using the presence of sex-biased expression as a proxy for the sex-specific specialization of gene function. We expected that if sexually antagonistic selection is a potent force acting upon individual genes, duplication will result in paralog families whose members differ.

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in sex-biased expression. Gene members of the same duplicate family can have different expression patterns in males versus females. In particular, duplicate pairs containing a male-biased gene occur more frequently than expected, in agreement with previous studies. Furthermore, when the singleton ortholog is unbiased, duplication appears to allow one of the paralog copies to acquire male-biased expression. Conversely, female-biased expression is not common among duplicates; fewer duplicate genes are expressed in the female-soma and ovaries than in the male-soma and testes. Expression divergence occurs more in older than in younger duplicates pairs, but expression divergence does not correlate with protein sequence divergence. Finally, genomic proximity may have an effect on whether or not paralogs differ in sex-biased expression. We conclude that the data are consistent with a role of gene duplication in fostering male-biased, but not female-biased, gene expression, thereby aiding the evolution of sexual dimorphism.

3.2 Introduction

The transcription of many genes differs between males and females, and this sexual dimorphism is widespread across the genomes of many taxa (Ellegren and Parsch 2007). However, the evolution of sexual dimorphism presents a practical difficulty: because males and females share most genes, selection in one sex can cause a correlated response in the opposite sex (Lande 1980). When phenotypic optima differ between the sexes, this correlated response can cause sexual antagonism over the evolutionary fate of those shared genes or traits, depressing overall fitness (Bonduriansky and Chenoweth 2009; van Doorn 2009).

Resolving this genomic conflict between the sexes can occur through mechanisms that rely directly or indirectly upon the major sex chromosomes. X- and Z-linkage can foster sexually antagonistic genetic variation (Gibson et al. 2002 for X-linked example) and help decrease the intersexual genetic correlation (Chenoweth et al. 2008 for X-linked
example). Unequal X or Z number and hemizygous expression in the heterogametic sex facilitate the spread of sexually dimorphic variation (Rice 1984; but see Fry 2010). More generally, the X and Z can possess sexually dimorphic genes in excess, with the relative enrichment of male- or female-biased genes depending upon the particular taxon (Gurbich and Bachtrog 2008). Even degenerate sex chromosomes like the Y possess genes for fertility, the essential basis of dimorphism (see reviews Carvalho 2002; Graves 2006).

In addition to directly coding for sexual dimorphism, all sex chromosomes can possess factors that influence sexual fate and activate sex-specific genetic networks, triggering the expression of autosome-based dimorphism. For example, sex-determination pathways can initiate sex-specific splicing that underlies dimorphic phenotypes (Lopez 1998; McIntyre et al. 2006). Alternatively, sex-specific modifications to cis- (or trans-) binding sites of autosomal genes can result in dimorphism (Williams and Carroll 2009). Genomic imprinting may foster dimorphism if allelic expression depends upon the offspring’s sex (Day and Bonduriansky 2004; Hager et al. 2008; Gregg et al. 2010). Finally, dimorphic trait expression can evolve if condition-dependent expression varies in a sex-specific manner due to the environment or genes (Bonduriansky and Rowe 2005; Bonduriansky 2007; Wyman et al. 2010). Breeding experiments, QTL studies, and gene expression data confirm that autosomes can indeed encode a great deal of dimorphism (Reinhold 1998; Parisi et al. 2003; Fitzpatrick 2004; Fry 2010). So while important, direct sex-linkage is not required for the evolution of dimorphism (Mank 2009a).

In addition to direct and indirect control by the sex chromosomes, the genome might rely upon gene duplication events that permit the partitioning of male and female expression patterns (Ellegren and Parsch 2007; Connallon and Clark 2011; Gallach and Betran 2011). Gene duplication provides a major source of evolutionary novelty (Ohno 1970), with the spontaneous rate of duplication being high enough to represent a powerful contribution to evolutionary change (Lynch and Conery 2000; Lynch et al. 2008; Watanabe et al. 2009; Lipinski et al. 2011). Duplications produce additional copies of genes whose
functions are expected to be initially identical. While one copy fulfills the ancestral workload, redundancy can release the other copy from its selective constraints. Mutation and selection can result in functional changes introducing a new function or specialization of old functions (Force et al. 1999); alternatively, pre-existing allelic variation can spread following duplication (Proulx and Phillips 2006). Thus, by providing extra genetic material, duplication might be the first step in the sex-specific specialization of genomes (Ellegren and Parsch 2007). Overall, we expect that if sexually antagonistic selection is a potent evolutionary force acting on individual genes, duplication will eventually produce gene family members that have discordant rather than concordant expression patterns.

In support of this idea, duplicates are common among sex-biased genes. Sex-biased gene expression itself suggests a past history of sex-specific or sexually antagonistic selection (Zhang et al. 2004; Connallon and Knowles 2005; Pröschel et al. 2006; Mank and Ellegren 2009; Innocenti and Morrow 2010; Wyman et al. 2010). When paralogs (i.e., within-species duplications) differ in expression, one or both of the sexes may have co-opted a gene copy to their own purposes in response to such selection. Alternatively, when paralogs have the same sex-biased expression type, selection may allow one copy to obtain even greater sex-bias than had previously been present in the original gene. In the fly and worm genomes, male-biased genes have more paralogs compared to unbiased genes (Cutter and Ward 2005; Gnad and Parsch 2006). In *Drosophila*, primary spermatocytes express basal transcription factors that are paralogs of conserved transcription factors that are expressed throughout the entire body (Li et al. 2009). In addition, male-biased functions seem common among duplicates acquired through reverse transcription (Bai et al. 2007, 2008), with sex-specific selection shaping the expression and location of retrotransposed genes (Betran et al. 2002; Meisel et al. 2009; Vibranovski et al. 2009; Zhang et al. 2010). Although poignant, these examples address neither the generality of this pattern nor the tendency of duplicates to adopt sex-specific patterns of expression. We do not know how often duplicate copies diverge in sex-biased expression patterns.
Finally, it is unclear what factors may prevent duplicates from developing sex-specific patterns of expression.

In this study we used microarray data to assess two aspects of sex-specific patterns of duplicate expression. First we tested the hypothesis that selection can co-opt duplicates in a sex-specific manner. We analyzed the number of paralog pairs with discordant versus concordant expression patterns. Discordant expression patterns would suggest a role for duplications in the evolution of sexual dimorphism. To corroborate the within-species patterns, we also compared the between-species expression patterns of the singleton ortholog in *D. ananassae* and the duplicates in *D. melanogaster*. We also looked at sexual dimorphism in the use of duplicates in the gonads versus the soma. Second, we tested for factors that might be associated with expression divergence. For example, since nucleotide changes can accompany changes in expression patterns (Wagner 2000; Castillo-Davis et al. 2004; Gu et al. 2004; Li et al. 2005) we analyzed whether duplicate genes with discordant expression states have elevated substitution rates relative to duplicate genes with concordant expression patterns. We also looked at genomic relocation to explore potential constraints on the evolution of dimorphism following duplication events. Co-regulation due to spatial clustering (Boutanaev et al. 2002; Cusack and Wolfe 2007; Mezey et al. 2008; Kaessmann et al. 2009; Gallach et al. 2010) may inhibit expression divergence between paralogs and thus prevent sex-specific specialization.

### 3.3 Materials and Methods

#### 3.3.1 Homology and sequence information

We used data from the recently sequenced 12 *Drosophila* genomes to identify genes that are paralogous in *Drosophila melanogaster* (Drosophila 12 Genomes Consortium 2007). We used only gene families containing exactly two members in *D. melanogaster* and excluded gene families containing more than two genes in our analyses. We used both
Chapter 3. Duplicates and sex-biased gene expression

*D. melanogaster* lineage-specific paralogs and *D. melanogaster* paralogs present in multiple *Drosophila* species. To find the lineage-specific duplicates we used phylogenies available from the Hahn lab website (http://sites.bio.indiana.edu/~hahnlab/Databases.html) and selected duplicates for which duplication appeared to have occurred after *D. melanogaster* diverged from the *D. sechellia* and *D. simulans* clade (Hahn et al. 2007). If the lineage-specific paralog families had more than 2 members, we included only the 2 duplicates at the tip of the gene tree. For all families, we removed genes that were potentially misidentified as paralogous; for example, a duplicate pair of genes could have different gene names but they might still share a common secondary Flybase ID. To prevent this ambiguity from biasing our results, such pairs were removed. If the gene had alternative splice forms, we used only the longest one.

For each duplicate pair we aligned the sequences against each other using T-Coffee (Notredame et al. 2000) and calculated the number of substitutions per silent (*S*) and replacement (*R*) site between the two sequences using PAML (Yang 1997). For each pair of duplicates we also calculated divergence in the region 1kb upstream of the start codon. To do this, we extracted the 5′ UTRs for each gene and then used the intergenic DNA to obtain additional upstream DNA. Using sequence information from FlyBase, the 5′ UTRs were designated as the region between the maximum location of the gene to the start codon for genes located on the positive strand (or from the minimum location to the start codon for genes on the negative strand). The 5′ UTR and intergenic DNA were masked using RepeatMasker (Smit et al. 2004) and the repeat and low complexity regions were removed. We aligned these upstream regions between the duplicate copies and calculated divergence using the Tajima-Nei correction method in the *distmat* function of the EMBOSS toolkit (Rice et al. 2000). To account for the neutral mutation rate, we divided the per-site divergence estimates by the value of *S* calculated from the corresponding coding region.
3.3.2 Sex-biased gene expression

Each pair of duplicate genes was put in one of 6 categories based on their joint expression pattern. Expression status could be the same between duplicate copies: both copies unbiased (UU), female-biased (FF), or male-biased (MM). Alternatively, expression could be dissimilar between copies: one copy unbiased and one female-biased (UF), one copy unbiased and one male-biased (UM), or finally, one copy male-biased and one female-biased (MF). In order to assign duplicate pairs into one of these groups, we used expression data from two studies (Ayroles et al. 2009; Wyman et al. 2010), which were chosen for their large sample sizes of male and female whole-body hybridizations. The detection of sex-bias depends on the statistical approach and power of a given experimental design, which may partly explain differences among studies in the number of sex-biased genes identified. Furthermore, because evolutionarily recent gene duplicates are expected to be more similar in expression, minor differences in expression are more likely to be detected when sample size is large. Next, we used the fact that these two studies did not always agree in their classifications of unbiased, male-biased, and female-biased expression for each gene as a way to understand expression variation. We compared how often expression patterns were similar between the Ayroles et al. (2009) and Wyman et al. (2010) datasets for duplicate versus singleton genes. If duplicates can be used for sex-specific expression and if duplicates in general show greater ability to vary in expression, these two studies should disagree more often in their categorizations of sex-bias in the duplicate genes than in the singletons. This variation is potentially interesting because selection can act upon it, leading to the evolution of new expression patterns between paralogs.

We recognize an important point regarding the limitations of microarray expression data for duplicates. If related duplicates have very similar sequences, the microarray probes may bind to the DNA from all recent duplicates, yielding an averaged expression value and inaccurate extent of sex-bias for that duplicate. The inability of microarrays
to distinguish minor sequence differences will underestimate the frequency with which duplication results in sex-specific expression. This makes our analyses conservative with respect to the role of duplications in the evolution of sexual dimorphism. However, we believe that cross-hybridization is a minor concern for our analysis: when using an array designed for one species with cDNA from another species, sequence divergence as low as \(~1\%\) will regularly show significant expression differences (Gilad et al. 2005). Expression divergence is only expected to increase with sequence divergence. Since the average sequence identity between paralogs (calculated as the percent of sites that were identical) in our study was \(~48\%\), there should be even greater binding specificity to the appropriate spot for related-paralogs within species than for orthologs from two different species. Finally, although categorizing duplicates on the basis of their sex-bias is a coarse measure of their functional divergence, this will again lead to an underestimate rather than overestimate of the amount of sex-specific specialization.

Finally, we looked more closely at the transitions in sex-biased expression between singletons from an outgroup species, *D. ananassae*, and paralogs from *D. melanogaster* to see whether between-species patterns corroborated the within-species patterns. We found duplicates specific to the melanogaster-subgroup (clade containing *D. yakuba*, *D. erecta*, *D. melanogaster*, *D. simulans*, and *D. sechellia*). We chose young gene families to limit the effect that factors like expression drift and turnover in the pattern of sex-specific selection might have on older paralogs. All expression data came from species-specific microarray data previously published for *D. melanogaster* and *D. ananassae* (Zhang et al. 2007); this analysis is independent of the analyses based upon the Ayroles et al. (2009) and Wyman et al. (2010) data.

### 3.3.3 Lack of differentiation between duplicates

Many duplicate pairs had concordant expression patterns, and hence no evidence for sex-specific expression divergence. We investigated genomic location of duplicates as a
potential reason for the lack of expression divergence. Tandem or segmental duplications are more likely to result in duplicates that are under the control of the same regulatory elements, constraining the evolution of new expression patterns. By contrast, if a duplicate copy moves from the original location to new location, expression may be free to diverge. To test for this possibility, we analyzed the number of chromosomal arm relocations in gene pairs with the same versus different expression patterns. The locations of all paralogs were obtained from FlyBase by parsing FASTA file headers.

3.4 Results

3.4.1 Sex-biased expression among singletons and duplicates

The Ayroles et al. (2009) dataset categorized a greater proportion of singletons as sex-biased (88%) compared to the Wyman et al. (2010) dataset (57%) (Table 3.1). Interestingly, the proportion of sex-biased genes was similar for the singletons and duplicates, within each dataset: sex-biased genes comprised 87% of the duplicate genes in the Ayroles et al. data and 57% of the duplicates in the Wyman et al. data (Table 3.1). The greater number of sex-biased genes in the Ayroles et al. data is due to the detection of more female-biased genes with relatively weak sex-bias (Table 3.1 and Figs. 3.1 and 3.2). This detection resulted in a greater number of female-biased than male-biased duplicates (Table 3.1) in the Ayroles et al. (2009) study.

While the proportion of sex-biased genes was similar between the duplicate and singleton pools, the relative proportion of male-biased and female-biased genes was not (Table 3.1). Both datasets had disproportionately more male-biased genes among the duplicates than among the singletons (Binomial tests: Ayroles et al.: $\chi^2_{df=1} = 61.41$, $P < 0.001$; Wyman et al.: $\chi^2_{df=1} = 74.35$, $P < 0.001$) and disproportionately fewer female-biased genes among the duplicates than among the singletons (Binomial tests: Ayroles et al. $\chi^2_{df=1} = 21.1$, $P < 0.001$; Wyman et al.: $\chi^2_{df=1} = 47.1$, $P < 0.001$). By contrast, the
relative proportion of unbiased genes did not differ significantly between the singletons and duplicates for either of the datasets.

### 3.4.2 Sex-specific expression divergence between duplicates

Among all of the paralog pair types (Table 3.2), we find that nearly half have discordant expression patterns: 44.3% in the Ayroles et al. (2009) and 49.8% in the Wyman et al. study. This suggests that there is potential for duplication to facilitate the evolution of dimorphism.

Because sex-biased gene expression can evolve rapidly within species (Meiklejohn et al. 2003; Ranz et al. 2003; Gibson et al. 2004; Baker et al. 2007), it is hard to discern an accurate null expectation for whether discordant pairs are overrepresented relative to concordant pairs. Following a duplication event, the daughter gene may have the same or different expression bias as the parent gene copy. In addition, one or both copies may undergo several subsequent transitions in sex-biased expression. It is currently unclear what these transition probabilities are and modeling such conditional probabilities is beyond the scope of this study. Rather, we sought to construct a relatively assumption-free null distribution by using a randomization approach similar to that used by Mikhaylova et al. (2008). We randomly constructed new gene pairs from the actual pool of duplicates and tabulated the resulting expression pair types (e.g., MM, UM, MF, etc.). We used the averaged proportions of the expression pair types from 10,000 randomizations as our null distribution. We find that FF, MM, and UU expression pairs are overrepresented in the Ayroles et al. (2009) dataset whereas only FF and MM pairs were overrepresented in the Wyman et al. (2010) dataset. Both datasets showed a deficit of UF and MF gene pairs (Table 3.2).
Figure 3.1: The rescaled extent of female-biased expression. We calculated the degree of female-bias for each female-biased gene, $F_{bias} = \text{Female} / \text{Male}$ using the normalized array expression values. To rescale each study we divided each $F_{bias}$ value by the mean $F_{bias}$ for each study respectively. This generated two distributions that could be compared, despite differences in normalization techniques. The Ayroles et al. (2009) study detected female-biased genes with relatively low female-biased expression compared to the Wyman et al. (2010) study both in terms of absolute and relative numbers (see percentage of genes in the first two bins).
Figure 3.2: Differences in the extent of sex-bias. We calculated an index of sexual dimorphism, $SDI = (\text{Female} - \text{Male}) / (\text{Female} + \text{Male})$, for all statistically quantified sex-biased genes in the Ayroles et al. (2009) and Wyman et al. (2010) studies. We used the absolute value of this index to compare the extent of sex-bias between the sexes. We standardized all SDI values by dividing by the mean SDI calculated across all sex-biased genes, within each study respectively. The distributions below demonstrate that the extent of sex-bias in the male-biased genes is greater than the extent of sex-bias in the female-biased genes. So while there are a greater number of female-biased genes across both studies, many of these genes have relatively weaker sex-biased expression. Conversely, while there are fewer male-biased genes, they have relatively greater sex-biased expression.
Table 3.1: Gene frequencies among singletons and duplicates. Both datasets show that the relative proportions of unbiased genes and sex-biased genes are the same among the singletons and among the duplicates. However, the relative proportions of the male-biased and female-biased genes are different. Both datasets show that duplicates have a higher proportion of male-biased genes and a lower proportion of female-biased genes compared to the singletons.

<table>
<thead>
<tr>
<th></th>
<th>Ayroles et al. 2009</th>
<th>Wyman et al. 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Singleton</td>
<td>Duplicate</td>
</tr>
<tr>
<td>Unbiased</td>
<td>814 (12%)</td>
<td>121 (13%)</td>
</tr>
<tr>
<td>Female-biased</td>
<td>4303 (66%)</td>
<td>459 (51%)</td>
</tr>
<tr>
<td>Male-biased</td>
<td>1456 (22%)</td>
<td>318 (35%)</td>
</tr>
</tbody>
</table>
Table 3.2: The observed and expected frequency of duplicate pair types. We randomized gene pairs to construct a null distribution to test against; see text for details. Percentages indicate the fractional abundance of that pair type within each dataset; parentheses are absolute numbers.

<table>
<thead>
<tr>
<th>Duplicate pair type</th>
<th>Ayroles et al. 2009</th>
<th>Wyman et al. 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Randomized expectation</td>
</tr>
<tr>
<td>Female-biased, Female-biased</td>
<td>34% (151)</td>
<td>26%</td>
</tr>
<tr>
<td>Male-biased, Male-biased</td>
<td>18% (83)</td>
<td>12.5%</td>
</tr>
<tr>
<td>Unbiased, Unbiased</td>
<td>4% (16)</td>
<td>2%</td>
</tr>
<tr>
<td>Female-biased, Unbiased</td>
<td>10% (47)</td>
<td>14%</td>
</tr>
<tr>
<td>Male-biased, Unbiased</td>
<td>9% (42)</td>
<td>9.5%</td>
</tr>
<tr>
<td>Female-biased, Male-biased</td>
<td>24% (110)</td>
<td>36%</td>
</tr>
</tbody>
</table>
3.4.3 Variation of sex-bias in singletons and duplicates

We observed that singleton genes have higher consistency in expression pattern between two different microarray datasets than duplicates genes do. The Ayroles et al. (2009) and Wyman et al. (2010) studies identified identical expression patterns for 69% of singletons (3628 out of 6119 genes), but the identical expression patterns for only 44% of genes that have paralogous copies (491 out of 884 genes). This is a significant difference (Binomial test: $\chi^2_{df=1} = 4.33$, $P = 0.038$) and suggests greater expression variability across studies for duplicates than singletons.

3.4.4 Duplicates in the melanogaster subgroup

We identified 15 duplicate families possessing exactly 2 paralogs that are specific to the melanogaster subgroup. We compared the statistically quantified expression status of the D. ananassae singleton orthologs to the expression status of the D. melanogaster paralogs (Table 3.4). We find that the between-species pattern supports the within-species pattern: unbiased- and male-biased singletons potentially give rise to male-biased genes in a disproportionate manner. By contrast, female-biased genes appear to be less common in the pool of singletons and paralogs, at least for this set of genes.

3.4.5 Lineage-specific duplicates

We compared the sex-bias of genes for recent D. melanogaster specific duplicates to older non-lineage specific duplicates. This can reveal whether more recent duplicate members share more similar expression patterns than older duplicates. We used the D. melanogaster lineage-specific duplicates found by another study (Hahn et al. 2007); if a duplicate family specific to D. melanogaster had multiple members, we used only the two most recent genes and assigned an expression status to the lineage-specific duplicates. Because of differences in platforms and genome annotation at the time of the
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studies, the Wyman et al. (2010) dataset had expression information available for only 23 lineage-specific duplicate pairs while the Ayroles et al. (2009) dataset had expression for 50 duplicate pairs (Table 3.3). Both datasets indicate that pairs derived from recent lineage-specific duplications more often have concordant rather than discordant patterns of expression. In the Ayroles et al. data, 86% of pairs were concordant among the lineage-specific duplicates, whereas 52% were concordant among the non-lineage-specific duplicates (Binomial test: \( \chi^2_{df=1} = 19.6, P < 0.0001 \)). In the Wyman et al. data, 61% of pairs were concordant among the lineage-specific duplicates; 46% were concordant among the non-lineage-specific duplicates, but this difference was not significant (Binomial test: \( \chi^2_{df=1} = 0.71, P < 0.39 \)). For \( S < 0.25 \), saturation effects are minimal and \( S \) is a reasonable proxy for the age of a duplication event (Lynch and Conery 2000, 2001). For this class of duplicates, we confirmed that the lineage-specific duplicates used in our study are younger (smaller \( S \) values) than the non-lineage specific duplications (\( F_{1,31} = 7.37, P < 0.011 \)).

3.4.6 Sequence divergence and expression

Because changes in gene expression might reflect changes in the underlying sequence, we tested the hypothesis that dissimilar expression patterns between duplicate genes are associated with higher substitution rates. The substitutions per replacement site \( (R) \), substitutions per synonymous site \( (S) \), and divergence \( (R/S) \) between the coding regions of the duplicate pairs were analyzed for the six categories. We used \( S \) as a proxy for age of the duplication event (Lynch and Conery 2000) (but we note that gene conversion may drive down \( S \), and hence the apparent age, in some gene pairs (Casola et al. 2010)). We noticed that the 516 duplicate pairs in this study fell into roughly three different age groups (Fig. 3.4.6). Sixty-six pairs were relatively recent duplications \( (S < 1) \); two hundred four pairs were old duplications \( (1 < S < 3) \). The remaining 246 pairs represented extremely ancient duplication events \( (S > 3) \). To avoid the problem
of saturation in the oldest duplicates, we assessed $R$ for pairs with $S_3$. We quantified variation in $R$ as a function of $S$ and duplicate pair type (e.g., MM, UM, MF, etc.) in a two-way ANOVA. $S$ correlated positively with $R$, as expected, for both datasets (Wyman et al.: $F_{1,191} = 70.72, P < 0.0001$; Ayroles et al.: $F_{1,191} = 70.69, P < 0.0001$). However, neither duplicate pair type nor its interaction with $S$ significantly explained variation in $R$ in either dataset.

We also investigated if some of the differentiation in expression bias between pairs could be attributable to cis-regulatory differences by looking at sequence divergence in the 1kb region upstream of the start codon for each duplicate gene. There was no difference in the divergence rates of the upstream region (corrected by the coding region $S$) among paralog pair expression types for either the Ayroles et al. (2009) or the Wyman et al. (2010) datasets (both $P > 0.10$). There was also no difference in the divergence rates between gene pairs with concordant versus discordant expression.

We calculated the degree of sex-biased expression ($SB = Male/(Female+Male)$) for all duplicate genes and looked at the relative contribution of $S$ and $SB$ of one duplicate on $SB$ of the related duplicate. Duplicate genes in a family were randomly assigned to be either the independent or dependent variable in a multiple regression analysis (results did not differ when designations were switched). Members of a paralog family correlate positively for $SB$ (Wyman et al.: $F_{1,229} = 84.26, P < 0.0001$; Ayroles et al.: $F_{1,205} = 70.26, P < 0.0001$). This is consistent with the overall observation that related duplicates can share categorical expression status (Table 3.2). There was a significant negative interaction between $S$ and $SB$ of the duplicate genes assigned as an independent variable (Wyman et al.: $F_{1,229} = 19.61, P < 0.0001$; Ayroles et al.: $F_{1,205} = 15.59, P = 0.0001$). As $S$ increases, the correlation of $SB$ between duplicates decreases. This is consistent with the observation that duplicate pairs with low $S$ are more likely to share the same expression status (Table 3.3).
Table 3.3: *D. melanogaster* duplications. We assigned an expression status to all of the *D. melanogaster* specific and non-lineage specific duplication events. The frequency of gene pairs with the same expression status is more common in the lineage-specific genes than in the set of duplicate genes that are not specific to *D. melanogaster*.

<table>
<thead>
<tr>
<th></th>
<th>Ayroles et al. 2009</th>
<th>Wyman et al. 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lineage-specific</td>
<td>Not lineage-specific</td>
</tr>
<tr>
<td>Female-biased, Female-biased</td>
<td>22 (44%)</td>
<td>129 (32%)</td>
</tr>
<tr>
<td>Male-biased, Male-biased</td>
<td>17 (34%)</td>
<td>66 (17%)</td>
</tr>
<tr>
<td>Unbiased, Unbiased</td>
<td>4 (8%)</td>
<td>12 (3%)</td>
</tr>
<tr>
<td>Female-biased, Unbiased</td>
<td>1 (2%)</td>
<td>46 (12%)</td>
</tr>
<tr>
<td>Male-biased, Unbiased</td>
<td>1 (2%)</td>
<td>41 (10%)</td>
</tr>
<tr>
<td>Female-biased, Male-biased</td>
<td>5 (10%)</td>
<td>105 (26%)</td>
</tr>
</tbody>
</table>
Table 3.4: Sex-bias in an outgroup species and in the melanogaster subgroup. Ten of the 15 gene families had unbiased expression in the *D. ananassae* singleton ortholog. Of these 10 orthologous groups, 5 families had male-biased expression in one of the *D. melanogaster* paralogs.

<table>
<thead>
<tr>
<th><em>D. ananassae</em> singleton ortholog</th>
<th><em>D. melanogaster</em> paralogs</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>unbiased</td>
<td>unbiased, male-biased</td>
<td>5</td>
</tr>
<tr>
<td>unbiased</td>
<td>unbiased, unbiased</td>
<td>5</td>
</tr>
<tr>
<td>male-biased</td>
<td>male-biased, male-biased</td>
<td>2</td>
</tr>
<tr>
<td>male-biased</td>
<td>unbiased, male-biased</td>
<td>1</td>
</tr>
<tr>
<td>male-biased</td>
<td>unbiased, unbiased</td>
<td>1</td>
</tr>
<tr>
<td>female-biased</td>
<td>unbiased, unbiased</td>
<td>1</td>
</tr>
</tbody>
</table>
3.4.7 Genomic location

We found evidence that genomic re-location affects the probability that gene members of a duplicate family retain the same expression pattern. In the Ayroles et al. (2009) dataset, members of a duplicate pair located on different chromosomal arms are more likely to have a different expression pattern than the same expression pattern (Binomial test: 76 gene pairs with different expression out of 122 pairs; \( P = 0.008 \)). In addition, members on the same chromosomal arm are more likely to have the same expression pattern than a different expression pattern (Binomial test: 204 gene pairs with same expression out of 327; \( P < 0.001 \)). These patterns were not statistically significant in the Wyman et al. (2010) dataset, but were nominally consistent with results from the Ayroles et al. (2009) dataset.

Oftentimes, autosomal paralogs show increased male-biased expression while the related X-linked paralogs show decreased male-biased expression (Betran et al. 2002; Wu and Xu 2003). It is possible that the effect of genomic location on expression divergence is driven by the presence of X-autosome duplicate pairs. To test this possibility we removed such gene pairs from the analysis. The Ayroles et al. (2009) dataset showed that related duplicates located on the same chromosomal arms were still more likely to have the same, rather than different expression pattern. (Binomial test: 167 gene pairs with same expression out of 275; \( P = 0.0004 \)). We also looked at the role of retrotransposition on the expression patterns; because regulatory regions are not usually copied during retrotransposition, daughter duplicates might be more likely to acquire new expression patterns. After we removed 29 gene families that had retroposed duplicates, genomic location had no effect on the probability of having the same expression pattern.

3.4.8 Tissue differences in duplicate use

We looked at sexual dimorphism in the use of duplicates in sex-specific tissues. We used expression comparisons of gonads and gonadectomized males and females (Parisi
Figure 3.3: Distribution of $S$ values calculated between paralogs. We used all of the duplicate families identified in D. melanogaster that had microarray expression information available. Only gene families with exactly two pairs of genes were used. The dashed line shows the mean $S$ across all 516 duplicate pairs in this study. The dotted line shows the mean $S$ among pairs with $S < 3$. 
et al. 2004). Genes were considered gonad- or “soma-” (whole body minus gonads) specific if there was at least a two-fold expression difference between the two tissues; two-fold cutoffs mimic patterns seen at higher threshold cutoffs (Wyman et al. 2010). Using this designation, we found that among all duplicates, 138 were male-soma-specific, 164 testis-specific genes, 79 female-soma-specific, and 10 ovary-specific genes; among these tissues, 8, 18, 3, and 1 duplicates respectively were retrogenes (according to those identified in Vibranovski et al. 2009a). Among the 1:1 singleton orthologs (shared among the 12 sequenced Drosophila species), we observe the same rank order number of genes as the duplicates. In D. melanogaster there are 631 male-soma-specific 1:1 orthologs, 760 testes-specific orthologs, 287 female-soma-specific orthologs, and 164 ovary-specific orthologs. The proportion of ovary-specific genes is smaller among the duplicates than among the 1:1 orthologs (Chi-square test: $\chi^2 = 24.28$, $P < 0.0001$). By contrast, the proportion of testes-specific genes is the same between the duplicates and orthologs (Chi-square test: $\chi^2 = 0.14$, $P = 0.91$). Finally, among the duplicate genes there were pairs with concurrent tissue-specificity: 28 male-soma-specific families, 35 testis-specific duplicate families, 15 female-soma-specific families, and 1 ovary-specific family.

### 3.5 Discussion

Gene duplication is a key step in the evolution of functional novelty. Here we have asked whether the sexes can use additional gene copies to facilitate the evolution of sexual dimorphism. This study represents a first general attempt at describing sex-specific expression by explicitly examining how paralogs are partitioned between the sexes across the genome. We find that some patterns of sex-biased expression of duplicate gene pairs in D. melanogaster are consistent with this process. Previous studies have shown that duplications are associated with the evolution of male-biased gene expression (Cutter and Ward 2005; Gnadt and Parsch 2006); we find additional support for this pattern through
the excess of MM and UM paralog pairs. By contrast, female-biased genes are not as common among duplicates, with MF and UF paralog pairs being underrepresented. Furthermore, we find that the patterns between-species support a pattern of mainly unbiased- or male-biased genes giving rise to more male-biased genes. Female-biased genes are nearly absent among the singleton orthologs looked at in this study (Table 3.4). We have corroborated prior reports that the testes appear to requisition duplicates readily (Parsch et al. 2005; Belote and Zhong 2009; Gallach et al. 2010). We have extended these previous studies by showing that the male body in general can co-opt duplicates; even the male-soma has more duplicate use compared to the female-soma or the ovaries. By looking at how related paralogs are partitioned between the sexes, we have confirmed that duplicates are more commonly used for male-biased expression and less commonly used for female-biased expression. These observations are consistent with the notion that gene duplication can provide the raw materials to relieve intralocus sexual conflict.

3.5.1 Sexual dimorphism in paralog expression

Although recent evidence suggests that not all sex-biased genes currently experience intralocus antagonism (Innocenti and Morrow 2010), it is hard to ascertain what proportion of sex-biased genes has experienced sexual conflict in the past. At least some of the antagonism could have been relieved by duplication events that permit sex-specific specialization. Our data show that \(~50\%\) of all duplicate pairs have discordant expression patterns (Table 3.2), which implies that intralocus sexual conflict could have had a large impact on duplicate expression divergence. We demonstrate that paralog co-regulation patterns can be looked at between the sexes, much like they have been analyzed for the testis vs. other tissues (Mikhaylova et al. 2008). We find that sex-specific duplicate use may have occurred through the increase in the number of male-biased genes, and a decrease in the number of female-biased genes. The pool of duplicates has proportionally
more male-biased genes and fewer female-biased genes compared to the pool of singletons (Table 3.1). We note that the Ayroles et al. (2009) dataset has a greater number of female-biased duplicates as compared with previous studies (Gnad and Parsch 2006). This is in part due to the detection of more genes with weak female-bias in the Ayroles et al. (2009) study compared to the Wyman et al. (2009) study (Figs. 3.1 and 3.2).

Overall, these results are consistent with previous studies that show male-biased and male-specific genes are associated with duplications (Cutter and Ward 2005; Gnad and Parsch 2006; Bai et al. 2007; Belote and Zhong 2009; Li et al. 2009). Interestingly, UF and MF pairs were underrepresented, suggesting that duplicate use for female functions is uncommon. Female-biased duplicates may be eliminated by selection more often (Zhang et al. 2007) or may have fewer opportunities to proliferate via mutation (Cardoso-Moreira and Long 2010). However, the presence of UM pairs may yet represent female specialization through the lack of differentiation. Many sexually dimorphic traits manifest their naturally selected (monomorphic) state in females while only males in high condition express the sexually selected (dimorphic) state (e.g., Rowe and Houle 1996; Bonduriansky and Rowe 2005; Wyman et al. 2010). Many studies have concluded that males and male-related functions appear to experience sex-specific selection more often and more strongly among individual genes (Meiklejohn et al. 2003; Hambuch and Parsch 2005; Pröschel et al. 2006; Ellegren and Parsch 2007; Zhang et al. 2007, 2010) and among phenotypic traits (Darwin 1871; Andersson 1994; Hoekstra et al. 2001; Kingsolver et al. 2001) than females and female-related functions. Sexual selection likely drives both patterns (Singh and Kulathinal 2005).

3.5.2 Duplicate expression in tissues

The degree of sex-biased expression correlates negatively with tissue breadth; sex-biased genes may have pleiotropic consequences limiting their widespread expression (Mank et al. 2008). It is therefore conceivable that duplication can mitigate sexual an-
tagonism by providing extra gene copies that can spatially or temporally specialize in male or female functions without disrupting existing expression networks (Force et al. 1999; Gu et al. 2004; Huminiecki and Wolfe 2004; Gallach and Betran 2011; but see also Hosken 2011). For example, in *D. melanogaster*, 12 out of 33 proteins that make up the proteasome (i.e., protein degrading machinery) expressed in male testes are paralogs of genes that have much broader expression (Belote and Zhong 2009). We confirm that more duplicate genes and gene families are requisitioned for specialization in the testes (Betran et al. 2002; Bai et al. 2008; Mikhaylova et al. 2008; Belote and Zhong 2009; Vibranovski et al. 2009b), a pattern also found in mammals (Kaessmann 2010). However, there are also more duplicates used in the male-soma compared to the female-soma and ovaries. If duplication can mitigate the constraints of pleiotropy, it is surprising that female body does not use duplicates to a greater extent. It may be that the stronger sexual selection on male function in *D. melanogaster* explains this pattern. These conclusions are tentative and require further functional characterization of duplicates (Gallach and Betran 2011), especially since we know that the female reproductive tract of other *Drosophila* species (Kelleher and Markow 2009; Kelleher and Pennington 2009) appear to requisition duplicates in the context of sexual antagonism.

### 3.5.3 Alternative explanations

Although we are interested in the role of duplications in intralocus sexual conflict, we acknowledge that sexual antagonism is only the most general explanation for expression divergence. Two particular examples of differential sex-specific selection include meiotic sex chromosome inactivation (MSCI) and dosage compensation. During male meiosis the X chromosome is transcriptionally silenced, discouraging the buildup of male-specific genes on the X chromosome (Wu and Xu 2003; Hense et al. 2007; Vibranovski et al. 2009a). As such, genes whose retrotransposed copies have left the X are common in flies (Betran et al. 2002; Meisel et al. 2009; Vibranovski et al. 2009b; Zhang et al. 2010).
and mammals (Emerson et al. 2004; Vinckenbosch et al. 2006). Some species equalize expression products in the heterogametic sex so that X:A = 1 despite having only one X chromosome. Such dosage compensation may deter the influx of newly duplicated genes onto the X if sexual antagonism over expression occurs on the X (Mank et al. 2011). Both MSCI and dosage compensation rely upon the particular selective forces acting upon the sex chromosomes. Yet sexually antagonistic selection over the expression of duplicates can occur whenever male and female optima differ.

Non-adaptive explanations that can account for expression divergence include differences in expression drift, mutation rates, and dosage sensitivities of paralogs. While we observed that older paralog pairs have more expression differences compared to younger lineage-specific pairs (Table 3.3), it is difficult to discern how much divergence may have occurred also by expression drift (Khaitovich et al. 2004). Similarly, the excess of male-biased paralogs may simply be a by product of the higher mutational rates that they experience (Cardoso-Moreira and Long 2010), suggesting that the excess of male-biased duplicates might not require invoking selection. Finally, while duplication may enable sex-biased genes to proliferate, the genome may better tolerate the acquisition of novel sex-bias in duplicates if sex-bias is non-essential (Mank and Ellegren 2009a). Such tolerance may be more common in duplicates, which like sex-biased genes show higher expression variance and flexibility (e.g., Gu et al. 2004; Huminiecki and Wolfe 2004). Thus, male-biased duplicate genes may have higher origination or fixation rates, or both (Gnad and Parsch 2006). Disentangling the relative contribution of adaptive and non-adaptive processes to the enrichment of male-biased genes and the time scales at which such processes operate (Long et al. 2003; Kaessmann et al. 2009; Kaessmann 2010) requires further work.
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3.5.4 Molecular evolution of paralog pairs

We hypothesized that gene expression differences between paralog members could be explained by underlying differences in sequence divergence. We found no compelling support for this effect. While several studies have found a correlation between expression divergence and sequence divergence between duplicates, the relationship is not a consistent one and requires additional study (Wagner 2000; Gu et al. 2002; Castillo-Davis et al. 2004; Li et al. 2005). However, we did observe that duplicate pairs found on different chromosomal arms are more likely to have distinct expression patterns. Conversely, duplicate pairs on the same arm tend to have equivalent patterns of sex-bias. These results are consistent with the spatial clustering of co-regulated genes and the greater expression divergence among re-located genes found in previous studies (Boutanaev et al. 2002; Miller et al. 2004; Bai et al. 2008; Mezey et al. 2008; Gallach et al. 2010). Yet, because our measure of functional divergence was so coarse (i.e., presence or absence of sex-bias), we still do not know how much divergence exists between genes with concordant expression patterns. Further characterization of individual duplicate pairs is required to assess functional divergence and sex-specific specialization.

3.6 Conclusions

We have compared patterns of duplicate use between males and females on a genome-wide scale. Male-biased gene expression is more common than female-biased gene expression among duplicates, opposite to the pattern observed in singletons. Dimorphism can increase through the acquisition or exaggeration of male-bias in paralogs compared to the singleton ortholog. Duplicates are used to a greater extent in the testes and male-soma compared to the ovaries and female-soma. Thus, duplication may primarily aid the evolution of male-biased expression rather than by allocating one gene copy for each sex. Duplication events have the potential to weaken the intersexual genetic correla-
tion, thereby aiding the evolution of dimorphism (Bonduriansky and Chenoweth 2009). However, it is still unclear whether duplication can completely resolve intralocus conflict (Hosken 2011). Furthermore, it is unknown how much sex-bias represents expression optimization. Understanding these processes and factors for duplicated genes may shed light on how dimorphism evolves in more complex polygenic phenotypes (Lande 1980).

3.7 Acknowledgements

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Chapter 4

The contribution of sex-specific recombination rates to the invasion of sexually antagonistic variation

4.1 Abstract

Because sex chromosomes are inherited in a sex-specific manner, the genetics underlying sexual dimorphism are easier to envision for sex-linkage than for autosomal linkage. However, much phenotypic sexual dimorphism resides on the autosomes. If this is the case, then it is possible that sexual dimorphism in genetic features found on autosomes can also foster phenotypic sexual dimorphism. In particular, recombination rate affects adaptation and can vary substantially between the sexes. It may be that sex-specific recombination rate differences can facilitate the introduction of autosomal alleles with different effects in males versus females. We find that sex-specific recombination rates

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1This chapter was the result of a collaboration with Mark Wyman. Minyoung J. Wyman conceived of the idea and wrote the chapter. Both authors wrote the mathematical model. Mark Wyman performed the perturbation analyses.
can affect the invasion outcome of sexually antagonistic alleles. In other words, the sex-averaged recombination rate is not necessarily sufficient to predict invasion. We confirm the general expectation that the average recombination rate needs to be lower in the sex benefitting from invasion and higher in the sex harmed by invasion. However, within the invasion space, male recombination rate can be greater than, equal to, or less than female recombination rate in order for male-benefit alleles at two loci to concurrently invade (or similarly for two female-benefit alleles). To complement our study of sex-specific recombination, we also looked at how sexual dimorphism in resident allele frequencies can impact the invasion of new sexually antagonistic alleles. We find that differences in allele frequencies between the sexes can affect the invasion outcome and that this effect can be at least as large as the effect of recombination. Our results suggest that reliably predicting invasion outcomes in actual populations will require understanding sex-specific differences in recombination and allele frequencies because they can be as important as differences in sex-specific selection.

4.2 Introduction

Males and females differ from each other in a panoply of behavioral, physiological, developmental, and morphological traits. However, because males and females share many of the same genes, selection in one sex may produce an unintended correlated genetic response in the opposite sex. Such a response can impede the fitness of the unselected sex, depressing overall population fitness (Lande 1980). Evolutionary biologists have sought genetic mechanisms that can explain the origins of sexual differences in spite of the shared underlying genetic structure.

Theoretical efforts have mainly focused upon genetic features that differ between males and females. Most obviously, the sexes have separate sex chromosomes. Rice (1984) pointed out that X-linked (or Z-linked) expression might facilitate the spread of
Chapter 4. Sexually dimorphic recombination

alleles that are sexually antagonistic alleles, i.e. beneficial to one sex but harmful to the other. Recessivity allows genes to be expressed in the heterogametic sex while often being hidden from selection in the homogametic sex. Dominance permits genes favorable to the homogametic sex to experience selection more often than the heterogametic sex (Rice 1984). Thus sex-linked expression can facilitate the spread of sexually antagonistic alleles despite costs to one sex. In a complementary model, Rice (1996) also proposed that the sex-determining locus linked to sexually antagonistic variation might gradually select for suppressed recombination along the Y chromosome, leading to the further accumulation of antagonistic alleles in a chain-reaction type scenario.

Despite the theoretical focus upon sex chromosomes, the data suggest that sexual dimorphism can also reside on autosomes (Mank 2009a; Fry 2010). For example, autosomal genes can be differentially expressed or spliced between the sexes. Autosomes may also harbor genes that have undergone gene duplication and subsequently diverged in sex-specific functions (Betran et al. 2004; Khil et al. 2005; Meisel et al. 2009; Connallon and Clark 2011; Wyman et al. 2012). Yet the underpinnings for an autosomal-based sexual dimorphism require further theoretical attention. Rice (1984) mentioned briefly that the invasion of a sexually antagonistic allele on an autosome only requires that the benefit to one sex exceed the cost to the opposite sex (Mandel 1971; Rice 1984). However, it is unclear how the invasion criteria might change for a slightly more realistic two-locus model, which can offer more biological insight. Previous studies have considered two-locus models of autosomal sexual dimorphism, but the two traits under consideration have mainly been sex-limited traits that may experience little or no direct sexual antagonism, e.g., female preference alleles and male trait alleles (e.g., Curtsinger and Heisler 1988; Gomulkiewicz and Hastings 1990; Heisler and Curtsinger 1990; Otto 1991; Albert and Otto 2005).

Connallon and Clark (2010) (hereafter, C&C 2010) have filled this theoretical void by explicitly examining a two-locus model with sexually antagonistic alleles. The
presence of two loci requires a recombination term that describes their association. As such, C&C (2010) were able to look specifically at the effect of sex-specific recombination rates on autosomal and sex-linked invasions. They found that recombination facilitates invasion of male- and female-benefit alleles on the X and Z, which recombine only in one sex, compared to autosomes, which recombine in both sexes. If only one sex recombines (i.e., achiasmy), invasion is easier on autosomes for alleles that benefit the non-recombining sex; by contrast, invasion will be easier on the sex chromosome for alleles that benefit the recombining sex. Yet as C&C (2010) were more interested in comparing invasion rates on the X versus autosomes, they did not explore the additional consequences of heterochiasmy itself (i.e., the phenomenon of sex-specific recombination). Thus, it is still unclear how sex-specific recombination rates affect the autosomes, where rates can vary more continuously in both sexes.

In this paper we explore the effects of male ($r_m$) and female ($r_f$) recombination rates on the probability of invasion of sexually antagonistic allele combinations. In general, recombination can hasten the rate of adaptation in finite populations by allowing favorable allele combinations to occur together more often. However, recombination can also hinder adaptation by breaking up the very same favorable combinations. Here we considered the operation of these two processes alongside each other in the two sexes. In other words, higher female recombination may break up, while lower male recombination may preserve, male-benefitting combinations. Do the individually considered sex-specific rates matter or does only their averaged effect matter for invasion? While this may seem a pedantic point, it is of practical importance since both the overall and local differences in recombination rate can be dramatic between the sexes (e.g., Mank 2009b; Lenormand and Dutheil 2005). Up to 75% of chiasmate species demonstrate >5% overall rate difference between the sexes (Burt et al. 1991; Lenormand 2003). Furthermore, recombination rates on a local scale can also vary substantially between the sexes (e.g., Hansson et al. 2005; Berset-Brändli et al. 2008; Kong et al. 2010). It is important to understand what,
if any, implications this sexual dimorphism in recombination might have on the evolution of phenotypic sexual dimorphism.

C&C (2010) and others (Strobeck 1975) suggest that the relative sex-specific rates can matter because their equations have different coefficients for $r_m$ and $r_f$. We perform a more systematic investigation of $r_m$ and $r_f$ in a model similar to that of C&C (2010). We find that both the sex-averaged recombination rate and their relative sex-specific rates contribute to the invasion of new alleles with sex-specific fitness effects. We also extend C&C’s (2010) model to allow for unequal allele frequencies in males and females; this further modifies the conditions for invasion, whether or not recombination is dimorphic. In sum, sexual dimorphism in the local recombination rates and in allele frequencies may help to explain the genome-wide variation in the enrichment of male- or female-biased genes observed on autosomes (Parisi et al. 2003; Harbison et al. 2005; Ram and Wolfner 2007). Because these processes are generic and do not require sex-linkage to foster sexual dimorphism, they merit further consideration in our understanding of how sexually antagonistic variation can appear and persist in populations.

4.3 Model

We constructed an autosomal two-locus diploid model with sex-specific fitnesses, similar to that of C&C (2010). Much of our notation is adopted from C&C (2010) to facilitate comparison. We label our alleles as $A_1$, $A_2$, $B_1$, and $B_2$. Whenever we consider a population with both alleles fixed, we take $A_1$ and $B_1$ to be the resident alleles ($p$ is the frequency of $A_2$ and $q$ is the frequency of $A_1$). There are 10 unique male and 10 unique female genotypes and associated fitnesses (see Table 4.1 for definitions); like C&C (2010), we do not include parent-of-origin effects. Recombination between the $A$ and $B$ locus occurs at a rate $r_m$ in males and $r_f$ in females. Discrete time recursion equations describing the frequency of the genotypes are identical to those in C&C (2010).
However, in our model we look more closely at recombination rates that are non-zero (i.e., no assumption of achiasmy) and different in each sex for the polymorphic equilibrium case (e.g., $A_1$ is not fixed upon the invasion of $B_2$). We first work with the general model and then plug in a specific fitness model (see Appendix A for the fitness model); however, any fitness model may be substituted. Finally, we also carefully studied the model’s behavior when allele frequencies are allowed to be different in the sexes.

4.4 Results and Discussion

4.4.1 Sex-averaged versus sex-specific recombination rates

We addressed the impact of sexual dimorphism in recombination rate, $r$, to the invasion of sexually antagonistic variation. At first glance, it may appear that only the sex-averaged recombination rate will matter for invasion (e.g., Hedrick 2007). After all, recombination shuffles loci in males and females, so that any particular allelic combination has approximately the same likelihood of appearing in both sons and daughters. These combinations experience sex-specific selection, but then get re-shuffled in males and females of the second generation. Since re-shuffling is inevitable, albeit at differing rates in each sex, the net effect over time may average out between the sexes.

However, the question of whether invasion can occur depends upon both short and long term effects. Because recombination occurs in an alternating sequence with sex-specific selection, the interplay of sexually antagonistic fitness and sex-specific recombination rates may lead to small but potentially consequential sex differences in allele frequencies and combinations. It may well be that such short term dynamics yield an outcome whose consequences differ from the long term expectation. In effect, the persistence of new sexually antagonistic alleles may depend more upon the short term dynamics.
Table 4.1: Shorthand for genotype fitnesses.

<table>
<thead>
<tr>
<th>Diploid genotype</th>
<th>Female fitness</th>
<th>Male fitness</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_1B_1 A_1B_1$</td>
<td>$f_{11}$</td>
<td>$m_{11}$</td>
</tr>
<tr>
<td>$A_1B_1 A_2B_1$</td>
<td>$f_{21}$</td>
<td>$m_{21}$</td>
</tr>
<tr>
<td>$A_1B_1 A_1B_2$</td>
<td>$f_{12}$</td>
<td>$m_{12}$</td>
</tr>
<tr>
<td>$A_1B_1 A_2B_2$</td>
<td>$f_{22C}$</td>
<td>$m_{22C}$</td>
</tr>
<tr>
<td>$A_1B_2 A_2B_1$</td>
<td>$f_{22R}$</td>
<td>$m_{22R}$</td>
</tr>
<tr>
<td>$A_2B_1 A_2B_1$</td>
<td>$f_{31}$</td>
<td>$m_{31}$</td>
</tr>
<tr>
<td>$A_1B_2 A_1B_2$</td>
<td>$f_{13}$</td>
<td>$m_{13}$</td>
</tr>
<tr>
<td>$A_2B_1 A_2B_2$</td>
<td>$f_{32}$</td>
<td>$m_{32}$</td>
</tr>
<tr>
<td>$A_2B_2 A_1B_2$</td>
<td>$f_{23}$</td>
<td>$m_{23}$</td>
</tr>
<tr>
<td>$A_2B_2 A_2B_2$</td>
<td>$f_{33}$</td>
<td>$m_{33}$</td>
</tr>
</tbody>
</table>
Chapter 4. Sexually dimorphic recombination

Is it possible that the relative differences in sex-specific recombination rates will have an invasion outcome that differs from the simple sex-averaged rate? In order to compare the long-term and short-term effects of sex-specific recombination, we conducted a stability analysis. The ability of a novel allele to invade a population is determined by a local stability analysis of the recursion equations that describe the population. We calculated the eigenvalues of the Jacobian matrix for the recursion equations of our discrete time model. Whenever the dominant eigenvalue describing an invasive allele is greater than one, the population is subject to invasion by that allele.

Here we treat the case of the A locus being polymorphic: we assume that $A_1$ ($q$) and $A_2$ ($p$) are both present in the population. The polymorphic case may be more interesting under the assumption that a novel mutation may appear on a background that is genetically variable throughout a population. However, the simpler fixed equilibrium eigenvalues may be recovered by setting $p = 0$. $B_1$ is the resident allele at the $B$ locus and $B_2$ is trying to invade. The dominant eigenvalue (here referred to as $\lambda_{CC}$) for the invasion of a novel $B_2$ allele when $p_m = p_f$ at the $A$ locus (see below for the case when $p_m \neq p_f$) is given by equation 6a in C&C (2010):

$$\lambda_{CC} = \frac{1}{2 w_f} (f_{32} p + f_{22} (1 - p)(1 - r_f)) + \frac{1}{2 w_m} (m_{32} p + m_{22} (1 - p)(1 - r_m)) \quad (4.1)$$

We can rewrite and interpret this eigenvalue. If we assume that all of the different genotypes (Table 4.1) have fitnesses that are close to 1, then we can define a new fitness variable as the deviation of the fitness from 1. For example, the $A_2 B_1 A_2 B_2$ ($f_{32}$) genotype can be re-expressed as:

$$f_{32} = 1 - u \delta f_{32}. \quad (4.2)$$

$\delta f_{32}$ is the small deviation of $f_{32}$ from 1, and $u$ is a counting parameter for doing a series expansion; so keeping track of the powers of $u$ will tell at a glance how large that term
is. For instance, terms with no $u (u^0)$ are larger than terms with $u^1$, and terms with $u^1$ are larger than terms with $u^2$, etc. We assume that recombination rates are small as well, and also multiply them by $u$. We may now rewrite equation 4.1 as a power series expansion in $u$:

$$
\lambda_{CC} \approx \frac{1}{2} \left( \frac{\bar{w}_f + \bar{w}_m}{\bar{w}_f \bar{w}_m} \right) + 
- \frac{u}{2 \bar{w}_f \bar{w}_m} \left( \bar{w}_f ((1 - p)\delta m_{22C} + p\delta m_{32}) + \bar{w}_m ((1 - p)\delta f_{22C} + p\delta f_{32}) \right) \quad (4.3)
+ (1 - p)(\bar{w}_m r_f + \bar{w}_f r_m) + \frac{u^2}{2 \bar{w}_f \bar{w}_m} (1 - p) (\delta f_{22C} \bar{w}_m r_f + \delta m_{22C} \bar{w}_f r_m) + \mathcal{O}(u^3).
$$

Despite its apparent complexity, this re-parameterization has a few nice features that help our understanding. The first line contains just the mean fitnesses and is approximately 1. The second line contains terms that are smaller by one power of the parameter $u$; the third line contains even smaller pieces (where $\mathcal{O}$ indicates subsequently higher orders of $u$). It is possible to make higher order corrections to this equation that improve the accuracy of the approximation. However, the second order term is sufficient to demonstrate the qualitative point that the relative differences in recombination rates between the sexes matter as well (the full expression is provided in online supplementary materials). In any case, we can make a further simplification by setting $\bar{w}_f = \bar{w}_m = 1$:

$$
\lambda_{CC} \approx 1 - \frac{u}{2} [(1 - p)\delta m_{22C} + p\delta m_{32} + (1 - p)\delta f_{22C} + p\delta f_{32} + (1 - p)(r_f + r_m)] 
+ \frac{u^2}{2} (1 - p) [\delta f_{22C} r_f + \delta m_{22C} r_m] + \mathcal{O}(u^3). \quad (4.4)
$$

We observe right away that the main recombination effect is indeed the sex-averaged recombination rate (the last term in the first line of equation 4.4), as is typically assumed (Hedrick 2007). However, whenever males and females differ in their fitness when carrying the combination $A_1 B_1 A_2 B_2$ – that is, whenever $\delta f_{22} \neq \delta m_{22}$ (as is expected when $B_2$ is sexually antagonistic) – the sex-averaged rate is adjusted by an additional
contribution, the one that appears on the second line. The recombination terms in the second line only affect the genotypes bearing $B_2$.

The contribution of the second line ($O(u^2)$) is usually smaller than the first line ($O(u^1)$). However, we can imagine a scenario when the various terms on the first line almost cancel out, allowing order 1 terms to be order 2 – that is, when

$$\frac{u}{2}[(1 - p)\delta m_{22} + p\delta m_{32} + (1 - p)\delta f_{22} + p\delta f_{32} + (1 - p)(r_f + r_m)] \approx u^2. \quad (4.5)$$

When is statement 4.5 possible? As a heuristic, one could imagine that $p = 0.25$ (as might be occur when $h = -1/2$, for the polymorphic equilibrium frequency $p = h/(2h - 1)$). Under this case, one can imagine that the $\delta m$ terms are all slightly negative (male-benefit) and the $\delta f$ terms are all slightly positive (female-detrimen), so that the magnitude of $\delta m_{22}$ is approximately equal to the magnitude of $\delta f_{22}$ and the magnitude of $\delta m_{32}$ is approximately equal to the magnitude of $\delta f_{32}$, but with the overall sum of the four terms having a slightly negative effect. In other words, the $A_2$ and $B_2$ alleles are only slightly male-benefit and slightly female-detrimen. Then the sum of the 5 terms within the square brackets of equation 4.5 may be very small, of $O(u^1)$, since $r_m$ and $r_f$ are of $O(u^1)$. As a result, this would make the size of the “first” order term the same or smaller magnitude as the second order term. In such a case, the second order term makes a substantial difference to the outcome of invasion and must be included to be consistent. This means that the sex-averaged rates and the relative rates contribute at least equally when considering alleles with minor sexually antagonistic effects. Such alleles may be important to study under the assumption that new mutations may have minor phenotypic effects in relation to the current phenotype, as we assume under a “Gaussian” model (Barton and Turelli 1987).

Thus, our conclusion is that a simple cost-benefit accounting is not quite enough to specify whether an allele invades in the two-locus autosomal case. Both the sex-specific recombination rates and the sex-specific fitnesses matter. The invasion outcome can
depend critically on the particular combination of parameter values assumed for fitness and recombination. We have presented the above example to demonstrate qualitatively how the terms in equation 4.5 may mathematically cancel out. Below we also present an example using a particular fitness model to highlight both the qualitative and quantitative implications of equation 4.5 to invasion.

4.4.2 Empirical implications of sex-specific recombination

The analysis above shows clearly that sex-averaged recombination rates do not alone determine invasion probabilities; the relative difference in recombination and selection between the sexes also matter. In order to show this analytic result graphically, we plug a fitness model (see Appendix A) into the equations and present their results. We determine whether $B_2$ can invade when present at very low frequencies, given that it interacts epistatically with the $A_2$ allele to increase only male fitness. Our fitness model has additive epistasis ($\epsilon$) and assumes linear dominance (parameters $h$ for $A$ locus, and $g$ for $B$ locus); however, any model of fitness can be easily substituted into the equations above. We study the scenario when locus $A$ is polymorphic and $B_1$ is fixed. In our model, positive $s$ and $t$ indicate a fitness detriment at the $A$ and $B$ loci, respectively; however, positive $\epsilon$ indicates a fitness benefit. For the $A$ locus we assumed equal dominance and selection coefficients between the sexes: $h_f = -0.1, h_m = -0.1, s_f = 0.1$, and $s_m = 0.1$. For the $B$ locus we also assumed equal dominance and selection coefficients $g_f = 0.5, g_m = 0.5, t_f = 0.05$, and $t_m = 0.05$. However, for epistasis we assumed, $\epsilon_m = 0.25$ and $\epsilon_f = 0$, so that having $A_2$ and $B_2$ together confers an overall benefit to males but not females. Thus, even though the $A_2$ and $B_2$ alleles themselves are not sexually antagonistic, their combination is. It is easy enough to make the alleles themselves antagonistic by setting selection to have an opposite signs between the sexes. However, doing so at the $A$ locus would violate the assumption of equal allele frequencies in males and females (see more below). Meanwhile, making the $B$ locus sexually antagonistic independently
of the \( A \) locus would reduce the dynamics to a one-locus model.

In Fig. 4.1a we notice a few obvious features of this model. The solid line represents the critical invasionary eigenvalue of one; invasion occurs to the left, and not to the right, of this line. First, we observe that the range of recombination rates required for the new \( B_2 \) allele to invade due to its epistatic interaction with \( A_2 \) differs between male and females. Males have a smaller range of recombination rates conducive to invasion than females. So, on average \( r_m < r_f \). C&C (2010) also show this result but present it in terms of the wait times for successful co-invasion of epistatically beneficial alleles; lower recombination rates have shorter wait times for invasion. In Fig. 4.1a, we see more directly the effect of the variation in sex-specific recombination rates. An intuitive explanation for this theoretical result rests in the fact that keeping \( A_2 \) and \( B_2 \) together in males requires suppressing recombination to some degree. By contrast, because \( B_2 \) does not benefit females with an \( A_2 \) background, a wider range of recombination values that can potentially break up the detrimental combinations actually benefits females. In support of this interpretation, when \( B_2 \) actually hurts females (\( t_m < 0 \) and \( t_f > 0 \)) in the case of sexual antagonism, this range of female recombination rates increases.

Secondly, we observe that within the overall invasion space (to the left of the solid line), \( r_m = r_f \), \( r_m > r_f \) and \( r_m < r_f \) all yield valid conditions for the successful invasion of \( B_2 \). This may explain why we might not expect to see a consistent fine-scale correlation between sex-specific recombination rates and the concentration of male- or female-benefit alleles – even though, the range of invasionary recombination rates differ between the sexes. In other words, while male-specific recombination rates should be lower on average in regions enriched with genes beneficial to males, in particular cases they may not be. Conversely, female recombination rates may not necessarily be lower in regions of female-benefit genes. Therefore, positive correlations between the concentration of sex-specific beneficial alleles and lower recombination rates may only be apparent in the most extreme circumstances: e.g., when male and female recombination
Figure 4.1: Sex-specific recombination rates. We assumed $h_f = -0.1$, $h_m = -0.1$, $s_f = 0.1$, and $s_m = 0.1$ at the $A$ locus and $g_f = 0.5$, $g_m = 0.5$, $t_f = 0.05$, and $t_m = 0.05$ at the $B$ locus. For epistasis we assumed, $\epsilon_m = 0.25$ and $\epsilon_f = 0$. See text for further explanation of parameters. 

a) The dashed line represents the sex-averaged recombination dependent term; it connects the same recombination value on the $r_f$ and $r_m$ axes. The solid line represents $\lambda_{CC}$ which provides the correction to the sex-averaged estimate. Because the respective recombination rates matter, the solid line does not connect the same values on both axes. Both lines represent the critical eigenvalue of one. To the right of the each line is when no invasion can occur ($\lambda_{CC} < 1$); to the left is when invasion can occur ($\lambda_{CC} > 1$). Interestingly, in order for recombination to facilitate the invasion of the second male-benefit, female-detriment allele, the range of male recombination rates has to be smaller than the range of female recombination rates. However, within the invasion space, $r_m = r_f$, $r_m > r_f$ and $r_m < r_f$ can all permit invasion. Also, note that lower male recombination values have a larger range of female recombination rates that permit the $B_2$ allele to invade (compare lengths of the double arrowheads), even though the overall range of female recombination rates have to be smaller than the range of male recombination rates (compare where the dotted lines touch the x- and y-axes).

b) In region II, the recombination rates ($r_m = 0.08$, $r_f = 0.012$) allow invasion of $B_2$ even though the sex-averaged rate would not predict invasion. In region I the recombination rates ($r_m = 0.01$, $r_f = 0.079$) do not allow invasion of $B_2$, even though the sex-averaged rate would predict invasion.
values are near the invasion border and when males do not recombine (i.e., along the x-axis of Fig. 4.1a). The lack of male recombination and the fact that the X-chromosome experiences selection more often in females than in males may explain empirically why the concentration of male-biased alleles is lower on the X chromosome of a male non-recombining species such as *Drosophila* – as has been shown theoretically by Connallon and Clark (2010). While it is still not clear whether any given male- or female-biased gene confers a male- or female-benefit, the current evidence suggests that in general they have fitness consequences (e.g., Innocenti and Morrow 2010; Wyman et al. 2010).

We note a third interesting conclusion from Fig. 4.1a. When recombination is too high in either sex, the new sexually antagonistic allele cannot invade (to the right of the solid line). The $\lambda_{CC}$ eigenvalue will always decrease as $r_m$ or $r_f$ increase. Nonetheless, within the invasion space, a lower male recombination rate, which is associated with a relatively higher female recombination rate, is actually more permissive to invasion than a higher male recombination, which is associated with a relatively lower female recombination rate (compare widths of the doubleheaded arrows in Fig. 4.1a). The data in *Drosophila* suggest that the male-limited genes like ACPs (accessory gland proteins) can have autosomal overrepresentation (Ram and Wolfner 2007). This empirical observation can seem confusing at first glance. Male-benefit, female-detriment genes can be enriched on autosomes because *Drosophila* males have no recombination, so that they are better able to keep male-benefit allelic combinations together. However, female recombination is simultaneously non-zero, which should break apart concentrations of male-benefit alleles. Which process dominates? The answer is not obvious without doing the actual calculation to see exactly how beneficial the allelic combination would have to be for males in order for invasion to occur (e.g., setting the eigenvalue ($\lambda_{CC}$) greater than one and plugging in values for recombination). Fig. 4.1a suggests that even though higher female recombination rates will break apart male-benefit allele combinations, a situation where more recombination occurs in females than in males is also more favorable for the
invasion of male-benefit, female-detriment alleles. So, whenever the net benefit to males is large enough, sex-limited alleles like ACPs with sexually antagonistic effects can invade even in the presence of female recombination.

A host of factors can affect variation in the rate of recombination among individuals – such as age, genetic background, and environmental stress (see Tedman-Aucoin and Agrawal (2011) for review). Intriguing sources of variation relevant to this study include re-mating rate (Priest et al. 2007) and male-genotype (Stevison 2012). Sexually antagonistic allele combinations may invade more easily in populations where males can induce lower average recombination rates in females, relative to the critical invasionary value. Conversely, sexually antagonistic allele combinations may invade less easily in populations where males cannot decrease female recombination rate or actually increase them.

The last feature of Fig. 4.1a to notice is that the correction (solid line) to the sex-averaged (dotted line) recombination rate makes the greatest impact near the border of invasion. Both lines indicate the slope of the critical eigenvalue; a value of one means invasion to the left of the lines and no invasion to the right of the lines. As we see, in region II, the sex-averaged rate will incorrectly predict the lack of invasion for this given set of parameters; conversely, in region I, the sex-averaged rate will incorrectly predict successful invasion. The numerical results support this mismatch between the two eigenvalues (Fig. 4.1b). Empirically this suggests that reliably predicting the persistence or disappearance of novel sexually antagonistic alleles on a polymorphic background will require that male and female recombination rates are well within or beyond this borderline.

4.4.3 Sexually dimorphic allele frequencies

In the previous section, we allowed for a polymorphic equilibrium at the A locus, but made the assumption that allele frequency in males and females were equivalent,
\( p_{\text{female}} = p_{\text{male}} \), in agreement with C&C (2010). While assuming equal allele frequencies in the sexes simplifies the math and is certainly a fair approximation, as a precise statement it may have limited biological relevance. After all, when an allele is sexually antagonistic, it is likely that allele frequencies will be slightly higher in the helped sex and slightly lower in the harmed sex, at least over the short term. To assess and estimate the importance of this mismatch between male and female allele frequencies, we returned to the recursion equations and found polymorphic equilibria for which \( p_{\text{male}} \neq p_{\text{female}} \).

We can write the equilibrium allele frequencies as:

\[
\begin{align*}
    x_1 &= 1 - p_f \\
    y_1 &= 1 - p_f - d_m \\
    x_2 &= p_f \\
    y_2 &= p_f + d_m
\end{align*}
\]  

(4.6)

where \( x_1 = 1 - p_f \) is the proportion of the ova carrying the \( A_1 \) allele, \( x_2 \) the proportion carrying \( A_2 \); \( y_1 \) is the proportion of sperm carrying \( A_1 \), \( y_2 \) carrying \( A_2 \). \( d_m \) is the difference in allele frequency between males and females, \( d_m = p_m - p_f \).

Plugging equations 4.6 into the recursion formulas and using the same expansion as above for fitnesses (i.e., \( f = 1 - u \delta f \)), and now expanding out the mean fitnesses \( \bar{w}_m \) and \( \bar{w}_f \), we found the following equilibrium solutions (keeping only terms of order \( u \), our expansion parameter):

\[
p_f = \frac{\delta f_{11} - \delta f_{21} + \delta m_{11} - \delta m_{21}}{\delta f_{31} - 2\delta f_{21} + \delta f_{11} + \delta m_{31} - 2\delta m_{21} + \delta m_{11}}
\]  

(4.7)

\[
d_m = 2u [\delta f_{31} - \delta f_{21} + \delta m_{31} - \delta m_{21}](\delta f_{21} - \delta f_{11} + \delta m_{21} - \delta m_{11}) \times \\
    \delta f_{11}(\delta m_{31} - \delta m_{21}) + \delta f_{31}(\delta m_{21} - \delta m_{11}) + \delta f_{21}(-\delta m_{31} + \delta m_{11})] \times \\
    [\delta f_{31} - 2\delta f_{21} + \delta f_{11} + \delta m_{31} - 2\delta m_{21} + \delta m_{11}]^{-3}
\]  

(4.8)
We notice that $p_f$ appears with no factors of $u$; this makes sense since allele frequencies can be large relative to recombination when $A$ is polymorphic. However, $d_m$ appears as a multiple of one $u$ (we now re-express the term $d_m$ as $d_m u$ to make obvious its size in our counting parameter $u$); we can see this because there are up to four factors of $\delta f$ or $\delta m$ in the numerator and three in the denominator for $d_m$. What this means is that the assumption $p_m = p_f$, in addition to its limited biological appeal, is not necessarily consistent – the difference between $p_m$ and $p_f$ has the same size effect on the eigenvalue as the sex-specific fitnesses do. Hence, when male and female fitnesses are dimorphic and reasonably strong, we should expect non-negligible sexual dimorphism in the allele frequencies.

We can use this result to calculate how the eigenvalue for invasion is modified from the C&C (2010) result when $d_m \neq 0$. We can write

$$\lambda = \lambda_{CC} + \Delta \lambda$$

(4.9)

where $\lambda_{CC}$ is the eigenvalue given in equation 4.1 and $\Delta \lambda$ is the modification to the C&C (2010) eigenvalue that comes from keeping $d_m$. $\Delta \lambda$ is found for by solving for the characteristic polynomial of the following (where $J$ is the Jacobian matrix and $I$ is the identity matrix):

$$\text{Det}(J - (\lambda_{CC} + \Delta \lambda)I).$$

(4.10)

Now, $\Delta \lambda$ is in general a very long expression. The most important new piece is the first order contribution to the eigenvalue from $d_m$. This is given by

$$\Delta \lambda = \frac{1}{2} d_m u + \mathcal{O}(u^2).$$

(4.11)

This shows us that invasion can be substantially impacted by including sexually dimor-
phic allele frequencies, as represented by $d_m$. Moving onto the next smaller set of terms, we find additional $O(u^2)$ contributions to the eigenvalue that affect $d_m$. However, since our conceptual focus is on how sexually dimorphic recombination rates modify the eigenvalue, we show only those few terms that include $r_m$ or $r_f$. We solve the characteristic polynomial order by order in $r_m$, $r_f$, and $d_m$, making the assumption that all three are of the same size to make the calculation tractable). We define fitness as before, $f = 1 - u \delta f$, to show:

$$\Delta \lambda \supset \frac{1}{4} d_m u^2 (-p_f r_f - 2 r_m + p_f r_m) + O(u^3) \quad (4.12)$$

Hence, we see that there is also an additional correction to the sex-averaged recombination rate on the eigenvalue whenever $d_m \neq 0$. In cases when the fitnesses that contribute to the $O(u^1)$ part of the eigenvalue nearly cancel out, we must reconsider $O(u^2)$ contributions to the eigenvalue. As a result, we must also keep these new parts of the eigenvalue that are proportional to $d_m$.

To interpret $d_m$, we can again plug in our fitness model (see Appendix A) into $p_f$ and $d_m$:

$$p_f = -\frac{h_f s_f + h_m s_m}{s_f - 2h_f s_f + s_m - 2h_m s_m} \quad (4.13)$$

$$d_m = -\frac{2(h_f - h_m)s_f s_m((h_f - 1)s_f + (h_m - 1)s_m)(h_f s_f + h_m s_m)}{(s_f - 2h_f s_f + s_m - 2h_m s_m)^3} \quad (4.14)$$

The equation for $d_m$ shows that when male and female dominance coefficients are equal, $d_m = 0$, so that male and female allele frequencies are identical. Fry (2010) demonstrates that non-equal dominance coefficients between the sexes can allow sexually antagonistic alleles to invade on the autosomes more easily than the sex chromosomes. Our results suggest that this may be because of the effect that sex-specific dominances have on
sex-specific allele frequencies. Sexual dimorphism in dominance can affect the invasion probabilities, in addition to the effect from sexual dimorphism in recombination rates.

In Fig. 4.2 we numerically confirm that $d_m$ has the potential to impact whether a sexually antagonistic allele $B_2$ can invade in the population at large. For the same averaged male and female allele frequencies at locus $A$, $B_2$ can eventually invade in one population (black points: $d_m = 0.005; h_m = -0.9, h_f = -0.1, s_m = 0.15, s_f = 0.05, g_m = 0.1, g_f = 0.2, t_m = 0.05, t_f = 0.2, \epsilon_m = 0.1, \epsilon_f = 0$) but not in the other population (grey points: $d_m = 0; h_m = h_f = -0.69, s_m = 0.1, s_f = 0.1, g_m = 0.1, g_f = 0.2, t_m = 0.05, t_f = 0.2, \epsilon_m = 0.1, \epsilon_f = 0$). This occurs despite the fact that both populations have the same recombination parameters ($r_m = r_f = 0.006$) and the same sex-averaged $p$. We note that in general, invasion depends on the size of $d_m$ (larger $d_m$ has a greater impact). Empirically this means that extremely dimorphic allele frequencies at one locus will facilitate the invasion of a novel sexually antagonistic allele at another locus.

4.5 Conclusions

Recombination is important for adaptation because it can shuffle loci and place favorable alleles together. However, recombination can also hinder adaptation by breaking up the very same favorable combinations. The model we have studied here suggests that sex-specific recombination rates affect the spread of sexually antagonistic alleles. In effect, the perquisites and drawbacks of recombination occur alongside each other, albeit in the different sexes. Furthermore, sex-specific recombination affects invasion in a manner distinct from simply the sex-averaged recombination rate. In general this occurs by allowing the helped sex to keep favorable sexually antagonistic combinations together by decreasing recombination relative to the invasion threshold, while allowing the harmed sex to break them apart by increasing recombination. However, our model highlights
Figure 4.2: Differences in allele frequencies of $A_1$ between the sexes. Invasion of the $B_2$ allele (males, upper black points; females, lower black points) can occur in a population which has sexually dimorphic frequencies of $A_1$, with $d_m = 0.005$ ($h_m = -0.9$, $h_f = -0.1$, $s_m = 0.15$, $s_f = 0.05$, $g_m = 0.1$, $g_f = 0.2$, $t_m = 0.05$, $t_f = 0.2$, $\epsilon_m = 0.1$, $\epsilon_f = 0$). However, invasion of $B_2$ does not occur (both sexes, grey points) when $A_1$ frequency is monomorphic, $d_m \approx 0$ ($d_m = 0$; $h_m = h_f = -0.09$, $s_m = 0.1$, $s_f = 0.1$, $g_m = 0.1$, $g_f = 0.2$, $t_m = 0.05$, $t_f = 0.2$, $\epsilon_m = 0.1$, $\epsilon_f = 0$). This difference in invasion outcome exists even though the sex-averaged allele frequencies were initially identical and even though recombination was the same in both populations ($r_m = r_f = 0.006$).
that successful invasion does not necessarily require that the benefitting sex have a lower recombination rate than the harmed sex. These results point to sexually dimorphic recombination as another potential mechanism that can foster sexual dimorphism on autosomes.

In the one locus case, the cost:benefit ratio can determine the outcome of invasion on an autosome when sex-specific dominances are equal (Rice 1984; Fry 2010). However, beyond one locus, the outcome is less obvious, relying upon the particular values for sex-specific recombination rates and fitnesses. We see this both in the nature of equations governing invasion (e.g., equation 4.5) and in the actual invasion space (Fig. 4.1a). While the sex-averaged recombination rate is the largest factor determining invasion, the relative rates have the potential to matter. In particular, the relative rates matter at the border of invasion; the sex-averaged eigenvalue and the corrected eigenvalue have different slopes (Fig. 4.1b) and different invasion outcomes. We also see the effect of the corrected eigenvalue in the overall range of male versus female recombination rates that permit invasion. The range is smaller in the benefitting sex, whereas the eigenvalue based upon the sex-averaged recombination rate would predict the same range. Such differences were not expected prior to this analysis (Hedrick 2007) and they suggest that the short term dynamics between selection and recombination can yield deviations from the long term expectation.

Secondly, within the invasion space, we find that any pair of male and female recombination rates can facilitate the spread of a sexually antagonistic allele. This suggests that we do not expect to see a consistent fine-scale correlation between recombination rates and the concentration of male-benefit or female-benefit genes on chromosomes. The correlations may only be obvious in the most extreme circumstances, e.g., sex chromosomes which recombine in only one sex and/or when one sex has no recombination at all. Furthermore, the association between sex-specific recombination rates and enrichment patterns may not be easily predicted unless there is also some knowledge about the
strength of sex-specific selection operating on the chromosomal region (Connallon and Clark 2010).

Finally, we find that sexual dimorphism in allele frequencies also affects the conditions for invasion. Because sexually antagonistic alleles benefit only one sex, allele frequencies may be higher in that sex. Interestingly, sex differences in allele frequencies at one locus impact invasion of novel alleles at a different locus (Fig. 4.2). We find that the magnitude of this effect can be as important as that of sex-specific recombination. These results suggest that in real populations, maintaining sexually dimorphic allele frequencies at one locus would be a way to initiate the spread of sexually antagonistic alleles at a second locus.

In sum, there seems to be a delicate interplay among sex-specific selection, sex-specific recombination rates, and sex-specific allele frequencies. Their cumulative effect could not be estimated without looking closely at the calculation. Because the data suggest that much phenotypic sexual dimorphism has at least some autosomal basis, we ought to also turn our focus to mechanisms that include factors besides sex-linkage. Investigating the genetic aspects of autosomes that are shared between the sexes will contribute to our understanding of how sexually dimorphic phenotypes arise in the face of a common genetic architecture.

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4.7 Appendix: Model of selection and dominance

The $m$ and $f$ prefix or subscript indicate male and female, respectively. The $h$ and $s$ terms describe dominance and selection at the $A$ locus. The $g$ and $t$ terms describe dominance and selection at the $B$ locus. Epistasis, $\epsilon$, is additive; having two copies of $B_2$ has twice the effect of having one copy.

\[
\begin{align*}
fA_1B_1A_1B_1 &= 1 \\
fA_2B_1A_1B_1 &= 1 - h_fs_f \\
fA_1B_2A_1B_1 &= 1 - g_ft_f \\
fA_2B_2A_1B_1 &= 1 - h_fs_f - g_ft_f + (1/2)\epsilon_f \\
fA_2B_1A_2B_1 &= 1 - s_f \\
fA_1B_2A_2B_1 &= 1 - h_fs_f - g_ft_f \\
fA_2B_2A_2B_1 &= 1 - s_f - g_ft_f + (1/2)\epsilon_f \\
fA_1B_2A_1B_2 &= 1 - t_f \\
fA_2B_2A_1B_2 &= 1 - h_f s_f - t_f + (1/2)\epsilon_f \\
fA_2B_2A_2B_2 &= 1 - s_f - t_f + \epsilon_f \\
mA_1B_1A_1B_1 &= 1 \\
mA_2B_1A_1B_1 &= 1 - h_m s_m \\
mA_1B_2A_1B_1 &= 1 - g_m t_m \\
mA_2B_2A_1B_1 &= 1 - h_m s_m - g_m t_m + (1/2)\epsilon_m \\
mA_2B_1A_2B_1 &= 1 - s_m \\
mA_1B_2A_2B_1 &= 1 - h_m s_m - g_m t_m \\
mA_2B_2A_2B_1 &= 1 - s_m - g_m t_m + (1/2)\epsilon_m \\
mA_1B_2A_1B_2 &= 1 - t_m \\
mA_2B_2A_1B_2 &= 1 - h_m s_m - t_m + (1/2)\epsilon_m \\
mA_2B_2A_2B_2 &= 1 - s_m - t_m + \epsilon_m
\end{align*}
\]
Chapter 5

Sex-specific differences in additive genetic variation

5.1 Abstract

Sexual differences are widespread among taxa and traits. However, the shared genetic architecture between males and females may prevent phenotypic divergence. Indeed, previous studies have shown that the extent of genetic correlation between the sexes is negatively associated with phenotypic sexual dimorphism. We analyzed this association more closely by asking whether genetic variances differ between males and females and whether this difference correlates to the extent of phenotypic sexual dimorphism. The extent of genetic variation in each of the sexes and their relative magnitudes can determine the extent of potential sexual dimorphism. We conducted a literature review to compile estimates of male and female genetic additive variances across 79 taxa including arthropods, vertebrates, and plants among morphological, behavioral, survival, developmental, and physiological traits. We targeted only those traits expressed in both sexes and excluded sex-limited traits. The mean difference in variation across all studies was

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1This chapter was written in collaboration with Locke Rowe.
zero as measured by coefficients of variation and heritabilities. However, the distribution of the sexual difference in additive variances had significant right skews, indicating that more extreme variances were male-biased. This pattern persists even after removing any traits related to reproduction. Many traits may be under undetected sexual selection or be correlated to traits under sexual selection. Furthermore, we find no instance of significant female-biased skews for the entire dataset or for the distributions analyzed separately by trait or organismal groups. Finally, we found a significant correlation between the extent of sexual dimorphism in additive genetic variances and the extent of phenotypic sexual dimorphism. This suggests that sexual dimorphism may have evolved because the genetic variation necessary for it also exists. Alternatively, dimorphism in genetic variation may exist because of strong sex-specific selection to produce phenotypic dimorphism - since strong selection can sometimes permit a trait to ally itself with the large genetic variation that is proper to condition. We conclude that sexual differences in additive genetic variance and selection, along with the intersexual genetic correlation, are all important factors to consider when describing the evolution of sexual dimorphism.

5.2 Introduction

In order for adaptive sexual dimorphism to evolve, three criteria must be met to varying degrees. First, selection should act differently in males than in females. Darwin (1871) largely attributed male-female differences to the action of sex-specific selection, particularly, sexual selection in the form of male-male competition or female-choice. However, even natural selection can shape sexual differences through sex-specific niche partitioning (e.g., Slatkin 1984; Shine 1989; Temeles 2000). Alternatively, natural selection may act upon aspects of fecundity or fertility, which are uniquely expressed in females (Darwin 1871; Fairbairn 1997).

Second, the intersexual genetic correlation \( r_{MF} \) should be lower than 1 (Lande
1980). If the sexes share a perfectly correlated underlying genetic structure, no amount of sex-specific selection can foster sexual dimorphism. Selection on one sex will inevitably evoke a correlated response in the opposite sex. This inextricable binding of evolutionary fates imposes a “gender load”, thus preventing either sex from achieving their respective phenotypic optima, and depressing population fitness (Lande 1980).

The intersexual genetic correlation is defined as,

$$r_{MF} = \frac{\text{Cov} (M, F)}{\sqrt{V_{A_m}V_{A_f}}}$$

(5.1)

where the covariance between males and females is divided by the square root of the product of the male-specific ($V_{A_m}$) and female-specific ($V_{A_f}$) variances. The covariance of a quantity with itself is simply the variance, allowing the correlation to equal 1. Empirically $r_{MF}$ has a negative correlation with phenotypic sexual dimorphism (e.g., Bonduriansky and Brassil 2005; Poissant et al. 2009). As expected, when the intersexual correlation is low or even negative, male-female differences can be extreme; when the correlation is close to 1, differences are muted or non-existent.

Third, the evolution of sexual dimorphism might also require the presence of additive genetic variation ($V_A$) for sexual dimorphism. This is an extension of the general principle that a response to selection requires additive genetic variation (Falconer and Mackay 1996). In other words, sex-specific selection is effective insofar as there is free genetic variation in one or both of the sexes to act upon. This point has received less emphasis compared to the intersexual genetic correlation, perhaps because $r_{MF}$ is already a measure of sex-specific additive genetic variances. That sex-specific variances can have an effect on phenotypic sexual dimorphism independently of $r_{MF}$ is made clear by examining the univariate equation governing the evolution of sexual dimorphism:

$$\Delta SD = \Delta \bar{Z}_m - \Delta \bar{Z}_f = \frac{1}{2} [h^2_m s_m - h^2_f s_f - r_{MF}h_m h_f (s_m - s_f)]$$

(5.2)
The change in sexual dimorphism ($\Delta SD$) is the difference between the change in the male trait ($\Delta \bar{Z}_m$) and the change in the female trait ($\Delta \bar{Z}_f$). The response to selection on males is determined by the male heritability ($h^2_m$) and the strength of male-specific selection ($s_m$). Heritability is the additive genetic variance ($V_A$) divided by the phenotypic variances ($V_P$). Likewise, the response to selection on females is determined by the female heritability ($h^2_f$) and the strength of female-specific selection ($s_f$). The difference between the first two terms in equation 6.3 is then modulated by the the third term, which describes how indirect correlated selection of males on females and of females on males (via $r_{MF}$), will alter $\Delta SD$.

To demonstrate heuristically the independent importance of the male- and female-specific additive genetic variances on the evolution of total sexual dimorphism, we can set $r_{MF}$ to zero by assuming that the intersexual covariance is zero. Then $\Delta SD$ depends on the magnitude of the male- and female-specific heritabilities as well as their relative difference (Cheverud et al. 1985).

It is also possible to see the contribution of sex-specific variances to the evolution of sexual dimorphism by making sexual dimorphism the trait of interest, measured as the male trait minus female trait ($Z_m - Z_f$), and by examining its additive variance:

$$V_A(Z_m - Z_f) = V_A(Z_m) + V_A(Z_f) - 2 \text{Cov}(Z_m, Z_f) \quad (5.3)$$

As long as twice the male-to-female covariance for trait $Z$ does not cancel out the sum of the sex-specific additive genetic variances (i.e., $r_{MF} = 1$), the size of the sex-specific variances matters. However, when the covariance is zero, only the magnitudes of the sex-specific variances matter.

Thus, even without knowing anything about the intersexual correlation (or intersexual covariance), it is possible to show that the extent of sexual dimorphism can rely strongly upon the difference in the male and female additive genetic variances. This
is because when additive variances are dimorphic, their raw difference describes the minimum amount of free variance that is available to sex-specific selection in a univariate context. If the intersexual covariance is negative, then the difference between $V_{Am}$ and $V_{Af}$ will actually be a conservative underestimate of the potential for dimorphism.

The current data suggest that sexually dimorphic variances exist. Several studies have shown that single traits can have heritabilities that differ between males and females (e.g., Jensen et al. 2003; Rolff et al. 2005; Holloway et al. 1993). Furthermore, other studies have demonstrated that the G matrix (e.g., variance-covariance matrix in a multivariate context) can have significantly different orientation and structure in males versus females (Holloway et al. 1993; Guntrip et al. 1997; Ashman 2003; Rolff et al. 2005; Steven et al. 2007; Campbell et al. 2011; Lewis et al. 2011; Gosden et al. 2012). While individual studies have looked at the size and orientation of genetic variation in males and females, we do not yet know how widespread this sexual dimorphism is.

In this paper we compiled estimates of sexual dimorphism in variances across a variety of taxa and traits by targeting traits expressed in both males and females. We find that additive genetic variances are on average monomorphic. However, extreme variances are present and tend to be male-biased. We also used our estimates of sex-specific variances to test the hypothesis that dimorphism in additive genetic variances can also predict the extent of phenotypic sexual dimorphism. We find that this correlation is positive, which complements the previous work showing a negative correlative between $r_{MF}$ and phenotypic sexual dimorphism. It is likely that differences in sex-specific selection acting upon sex-specific variances and the intersexual covariance have altogether contributed to the observable sexual dimorphism.
5.3 Methods

5.3.1 Literature search

We conducted a literature search to compile estimates of male and female additive genetic variances. Our search methods had three parts. First, we searched for “(male* OR female* OR dimorphi* OR monomorph*)” in ISI Web of Science. We targeted journals of interest to the evolutionary biologists: *Evolution, Journal of Evolutionary Biology, Proceedings of the Royal Society B - Biological Sciences, Heredity, Nature, Science, American Naturalist, Evolutionary Ecology, Evolutionary Ecology Research, Biology Letters, PLoS, and Current Biology*. Second, we conducted a search on the term “(heritabilit* and sex*)” to retrieve additional heritability estimates from a wider variety of journals. We explicitly ignored any etiological hits in the animal breeding or human medicine literature since disorders and diseases were not the focus of this review. Lastly, we searched the literature cited sections of review papers that have compiled additive variance estimates (Ashman 2003; Ashman and Majetic 2006; Poissant et al. 2009; Hansen et al. 2011).

We collected papers that published additive genetic variances (or sire-related variance), narrow-sense heritabilities ($h^2$) and/or coefficients of additive genetic variation ($CV_A$) for traits that are expressed in both males and females. Narrow-sense heritability was calculated as $V_A/V_P$ and $CV_A$ was calculated as $100\sqrt{V_A}/\bar{X}$, where $\bar{X}$ is the trait mean (Houle 1992). If a paper reported only the narrow-sense heritability and also provided the observed phenotypic variance (or phenotypic standard deviation, or standard error and sample size) on the raw data, we could calculate the additive genetic variance.

For animal models using mixed models, using the observed phenotypic variance may overestimate the actual amount of additive genetic variation if the mixed model included fixed effect; the measured phenotypic variance (i.e., square of the standard deviation) will be greater than the $V_P$ from the mixed model containing fixed effects.
(Wilson 2008). However, we do not expect that this overestimation should systematically affect one sex over the other sex. Even if males have greater total phenotypic variation and unexplained / residual variance (Pomiankowski and Moller 1995), it is not necessarily the case that their fixed effects explain less of the non-additive variance compared to females. There were 42 studies using the animal model approach, almost all of which had fixed effects. However, only 16 $CV_A$ pairs (5%) used in this study were calculated by multiplying the standard deviation squared to the heritability. Most $CV_A$s (98 pairs) from animal model studies were reported or could be calculated directly from reported $V_A$, $V_P$s and means. Considered altogether, the potential $V_A$ overestimation from using standard deviations to estimate $V_P$ will be of minor consequence in this study.

We did not calculate $CV_A$ from $h^2$ data based upon residuals and PC scores (because these data transformations have a mean zero). We did calculate $CV_A$ from heritabilities derived from z-transformed data as long as the phenotypic means and standard deviations were available. We did not use any estimates from studies that were ambiguous about the application of log or other transformations and scalings. Furthermore, since coefficients of variation are only meaningful for data on a ratio or log-interval scale, both of which are distinguished by having a true zero value (Zar 2010), we did not calculate or include $CV_A$ for any proportional or categorical data, even though it is possible to keep $h^2$ estimates for such data. We did not calculate or include $CV_A$ for any data with ambiguous units.

Because we were interested in sex-specific differences in variance, we did not include any sex-limited traits such as male-limited sexually selected traits. However, we did include a male sexually selected trait if a clear homologue existed in the female, e.g., male tail streamers and female tail feathers in barn swallows. We also did not include any fecundity-related traits, which can be measured in both sexes, but are actually a property of the female; so measures of reproductive success or fitness were not included. However, we did include traits such as copulation duration, which can be measured in
both sexes and represent a shared trait.

Our measures of $V_A$ came from parent-offspring regressions (i.e., mother-daughter, father-son, mother-offspring, and father-offspring), breeding experiments (e.g., half-sib designs and full-sib designs if dominance variance was justifiably low or non-existent), and animal-model studies based on pedigree information (i.e., sire associated variance). If a paper used more than one method to estimate additive genetic variance, we chose the one we deemed to be most appropriate. While heritabilities based upon mother-daughter (or mother-offspring) regression can be inflated due to maternal effects, we included such values to increase our sample size. In principle it is possible to estimate female heritability by the father-daughter regression, but in practice, these slopes were for the most part negative - which is not technically allowed for heritabilities (variances are always positive numbers). In any case, misidentified $V_A$ estimates that actually include some maternal variance can increase or decrease the potential $V_A$ difference between the sexes. For example, if the true male $V_A$ is greater than the true female $V_A$, positive maternal effects may decrease this difference. But if male $V_A$ is less than female $V_A$, positive maternal effects may increase this difference. When we look at the distribution of $h^2$ differences between the sexes (Fig. 5.4.1), we note that the mean difference in heritability ($Male\ h^2 - Female\ h^2$) is negative, suggesting maternal effects may be an issue. However, this mean is not significantly different from zero (see Results). Therefore, we felt justified in including heritabilities from single sex regressions. Maternal effects that decrease resemblance between mothers and daughters are also possible; however, since the overall mean in variance difference between the sexes was zero, we chose to ignore this effect for both negative and positive maternal effects.

In addition to additive variances, we compiled trait means measured separately in males and females in order to determine the degree of phenotypic sexual dimorphism. Some studies only provided a ratio of size as measured by the Lovich-Gibbons index (Lovich and Gibbons 1992). These values were entered to ensure that the male mea-
measurement was always in the numerator and the female measurement in the denominator. Several of our phenotypic dimorphism ratios also came from the supplementary file associated with Poissant et al. (2009).

Finally, in order to streamline the dataset, we culled any variance estimates for a trait that was measured by different publications (whether by the same or different set of authors). We decided to choose the paper which had the most complete information (e.g., reported sex-specific trait means, trait standard deviations, $V_A$, $V_P$, $h^2$, and $CV_A$), or the paper which had measured the greatest number of traits. When a study included a mean measure of additive variance across treatment groups, we used this value only. However, if a study measured additive genetic variance in more than one environment or population and did not provide overall means, we included all reported values. Although this is a type of pseudoreplication, we could not calculate $CV_A$s from averaged heritabilities because of differences in the phenotypic variance across treatments. In addition, it is possible to keep such pseudoreplicates under the possibility that genes can be expressed differently in different environments, strains, breeds, or populations, revealing different additive effects that effectively might be considered as different traits.

Because of the variety of traits and organisms, our estimates of additive variance are spread across a wide range of scales and dimensions. They formed four basic categories: survival & development, behavior, physiology and morphology. Although we could have had a life history category, because we had no fecundity or fertility data, we instead decided to group together traits related to maturity and the life cycle. Survival & development traits included longevity, growth rate, egg-adult maturation time, age of fastest growth, daily weight gain, and growth curve parameters. Behavioral traits included traits like dispersal distance, parental care, courtship behaviors, and locomotion. Physiological traits included fat stores, cuticular hydrocarbons, hormone levels, and immune or defensive response.

Within morphology, traits could be essentially linear or volumetric. Among the
linear traits we included count data, length and shape measurements, and area measurements. Although area is not linear, because there were only two of these traits, we included them with the linear traits. Among the volumetric traits, we included all mass measurements. Because coefficients of variation depend upon the variation along all three axes and correlation among axes, scaling is often recommended. For instance, several have suggested that the $CV$ of mass must be scaled downward by a factor of 3 to allow comparison with linear traits (Lande 1977; Houle 1992; Milner et al. 2000) as the added dimensions will inflate the variation. However, this downward scaling only makes sense if the correlation among the three dimensions scale perfectly (García-González et al. 2012). It may be that other factors make more sense. To eschew potentially erroneous scaling factors, we decided simply to divide morphological traits into linear and mass traits. Mass traits were analyzed separately under the general assumption that all mass measurements are comparable to each other but perhaps not to other trait types. Although not adjusting the mass $CV_A$s complicates comparisons among trait types, erroneous scaling may compound the issue.

5.3.2 Hypotheses and statistical analyses

Genetic variances can be measured by the dimensionless quantities $CV_A$ and $h^2$. Sexual dimorphism in genetic variances can be measured as the $Male - Female$ difference in the $CV_A$s and $h^2$s, which is dimensionless. If a study reported a negative $V_A$ or $h^2$, we set those values to zero, so as to be conservative with respect to the $Male - Female$ difference. If male variances are systematically higher, we predicted that there should be significant male-biased means and positive skews in the distribution of the $Male - Female CV_A$ and $h^2$ differences. By contrast, if female variances are systematically higher, female-biased means and negative skews should occur. We analyzed the distribution of $CV_A$ and $h^2$ differences between the sexes by studying means, skews, and kurtoses. We analyzed the distributions by looking at the combined data and by looking
at the data broken down by trait types. In particular, we compared the distributions for mass, morphological, behavioral, physiological, and survival & developmental traits. We also compared reproduction-related traits to all other traits. Tests for departures from normality in skew and kurtosis were conducted as recommended in Crawley (2007).

For differences among means we used one-way ANOVAs. However, the data were in general not normally distributed. As a result, we used only non-parametric tests or validated any parametric tests with non-parametric procedures when typical statistical assumptions were violated. We used the aovp() command in the R package lmPerm to conduct permutation tests to compare groups. To analyze means, we also randomized the original data to generate a distribution of new means. We noted where the observed mean fell in the randomized distribution and considered it to be significant if it exceeded 95% of the randomized means. However, we randomized the data to take into account that genetic variances are known to differ widely among groups (e.g., life history traits have higher levels of additive variance than morphological traits). So, when necessary, we randomized the allocation of “male” and “female” to each pair of variance values.

We also tested for difference in sexual dimorphism in genetic variance among organismal groups caused by sex-linkage. For a two-allele one-locus model, the heterogametic sex can express one of two extreme two states: recessive and dominant. By contrast, the homogametic sex can express one of three states: double homozygous or heterozygous. As a result, heterogametic sex will have higher additive variance than the homogametic sex (Lynch and Walsh 1998). We sought to test this prediction by comparing variance differences in XY versus ZW systems. XY systems should have greater male-specific additive variance whereas ZW systems should have greater female-specific additive variance.
5.3.3 Variance sexual dimorphism versus phenotypic sexual dimorphism

Sexual dimorphism can be measured as a ratio between the sexes so that the larger sex is expressed as a percentage of the smaller sex (Lovich and Gibbons 1992). Here we defined sexual dimorphism in means as the log (base 2) of the male : female ratio in phenotypic means. This is a dimensionless quantity because the units are eliminated by dividing the male units by the female units. We also compared sexual dimorphism in variances as the log (base 2) of the male : female ratio in additive variances, another dimensionless quantity. The extent of the sex-specific difference in additive variances can indicate the potential to evolve sexual dimorphism because the intersexual covariance can only be as large as either the male- or the female- specific additive variance. Thus, the greater the variance difference, the smaller the potential intersexual covariance, and the greater the potential for phenotypic sexual dimorphism. We predicted that the amount of variance dimorphism should correlate positively to the amount of phenotypic dimorphism in sex-specific trait means.

5.4 Results

We found 141 papers that provided male and female estimates of coefficients of variation and/or of heritabilities. After removing some studies and entries to avoid repetition, we had 291 $CV_A$ and 510 $h^2$ pairs from 121 studies representing 79 species. There were fewer $CV_A$ than $h^2$ pairs because many studies did not report enough information to calculate $CV_A$s in both males and females. In addition, there were many studies for which it is possible to calculate $h^2$ on residual, PC, percentage, proportional, or categorical data but for which calculating $CV_A$ would be invalid.

Most of the data came from insects and vertebrates. Of the 5 plant species used in this study, 2 species were dioecious, possessing separate male and female flowers.
However, 3 species were gynodioecious, possessing female and hermaphrodite flowers; we designated the hermaphrodite measurements as “male” (42 $CV_A$ pairs; 47 $h^2$ pairs). Table 5.7 in the Appendix details the references and the types of information that were available for each organism. The data used for all analyses in this study are available as Supplementary File S1.

5.4.1 **Overall variance dimorphism**

Surprisingly few studies have explicitly analyzed differences in genetic variances between the sexes. Of the 20 studies that have statistically tested for male and female differences in heritabilities, only 11 found significant differences in a subset of the traits examined (Mousseau and Roff 1989; Wilcockson et al. 1995; Mignon-Grasteau 1999; Ashman 1999, 2003; Jensen et al. 2003; Rolff et al. 2005; Fedorka et al. 2007; Zillikens et al. 2008; Gershman et al. 2010; Stillwell and Davidowitz 2010). Only 2 studies tested for and found sex-specific differences in the unstandardized additive variances (Zillikens et al. 2008; Rolff et al. 2005).

We therefore decided to use all of the heritabilities and variances reported in the 141 studies, whether they were reported as significantly different from zero or whether the sexes were statistically dimorphic. We analyzed variance differences between the sexes in a variety of ways. First, we analyzed the distribution of the $Male - Female$ differences in $CV_A$ and $h^2$ for the entire dataset (Fig. 5.4.1 and Table 5.1).

**Skew**

We removed two outliers with extremely female-biased $CV_A$s (e.g., difference less than -40). We felt justified in this removal because the outliers were more than 6 standard deviations away from the mean $CV_A$ difference for the unculled dataset (8 standard deviations for the culled dataset). Removing the two outliers also meant that all of the data occurred within equally sized ranges on the right and left sides of zero (e.g.,
Figure 5.1: Variance frequency distributions. A) Male CVA − Female CVA and B) Male $h^2$ − Female $h^2$. The black line represents the mean difference, which is not significantly different from zero for either distribution.
The resulting distribution of the $CV_A$ differences had significant right skew, indicating that more extreme $CV_A$s were more common in males than in females ($skew = 0.54, P = 0.0001, N = 289$). When the two female-biased outliers were included, there is a non-significant left skew ($skew = -1.54, P = 1, N = 291$), suggesting that these two points had a great deal of leverage and further warranting their removal. The distribution of the $h^2$ differences also had significant right skew, indicating that more extreme heritabilities are more common among males than among females ($skew = 0.57, P < 0.0001, N = 510$).

**Mean**

We analyzed whether the mean $CV_A$ and $h^2$ Male − Female differences (Fig. 1) were statistically different from zero across the entire data set with a randomization procedure. Both the $CV_A$ and $h^2$ datasets have a great deal of structure, meaning that each estimate is with respect to a specific trait measured in a specific organism. As such, we constructed a randomization test to preserve this structure. Instead of randomly pairing any two $CV_A$ (or $h^2$) values and calculating a randomized Male − Female difference, we took the original male and female data pair and randomly made their difference positive or negative. This procedure essentially re-allocates which value is “male” and which is “female”. We could then create a new data set over which to calculate a new mean of differences. We repeated this process 10,000 times to generate a distribution of all of the new means. The observed mean of the $CV_A$ differences ($\mu = 0.28$) had a probability of $P = 0.22$. The observed mean of the $h^2$ differences ($\mu = -0.01$) had a probability of $P = 0.869$. So, across the entire dataset the sexes do not differ in mean genetic variance as measured by either $CV_A$ or $h^2$. 


Table 5.1: Skew on distributions by groups. The skew was calculated on subsets of the \( Male \ CV_A - Female \ CV_A \) and \( Male \ h^2 - Female \ h^2 \) frequency distributions.

<table>
<thead>
<tr>
<th>Group</th>
<th>Male - Female ( CV_A )</th>
<th>p-value</th>
<th>Male - Female ( h^2 )</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trait Type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphology</td>
<td>0.972 (193)</td>
<td>&lt;0.0001*</td>
<td>0.334 (337)</td>
<td>0.006*</td>
</tr>
<tr>
<td>Mass</td>
<td>-1.769 (45)</td>
<td>1</td>
<td>-0.431 (45)</td>
<td>0.919</td>
</tr>
<tr>
<td>Behavior</td>
<td>-0.215 (11)</td>
<td>0.611</td>
<td>0.347 (32)</td>
<td>0.215</td>
</tr>
<tr>
<td>Survival &amp; Development</td>
<td>-0.435 (26)</td>
<td>0.813</td>
<td>1.651 (41)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Physiology</td>
<td>1.464 (12)</td>
<td>0.033*</td>
<td>-0.495 (31)</td>
<td>0.865</td>
</tr>
<tr>
<td><strong>Organism Type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant</td>
<td>0.157 (54)</td>
<td>0.320</td>
<td>0.696 (65)</td>
<td>0.0126*</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td>1.205 (9)</td>
<td>0.092</td>
<td>0.44 (22)</td>
<td>0.202</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>0.861 (36)</td>
<td>0.021</td>
<td>-0.826 (42)</td>
<td>0.983</td>
</tr>
<tr>
<td>Diptera</td>
<td>2.225 (31)</td>
<td>&lt;0.0001*</td>
<td>0.198 (110)</td>
<td>0.199</td>
</tr>
<tr>
<td>Bird</td>
<td>0.025 (65)</td>
<td>0.468</td>
<td>0.191 (98)</td>
<td>0.221</td>
</tr>
<tr>
<td>Orthopteran</td>
<td>0.618 (12)</td>
<td>0.201</td>
<td>0.621 (27)</td>
<td>0.100</td>
</tr>
<tr>
<td>Mammal</td>
<td>-0.489 (62)</td>
<td>0.939</td>
<td>-0.409 (92)</td>
<td>0.943</td>
</tr>
<tr>
<td>Fish</td>
<td>-0.874 (16)</td>
<td>0.912</td>
<td>1.615 (32)</td>
<td>0.0004*</td>
</tr>
<tr>
<td><strong>Combined Data</strong></td>
<td>0.536 (289)</td>
<td>0.0001*</td>
<td>0.571 (510)</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>
Kurtosis

We analyzed departures from normality by examining kurtosis. Under a normal distribution, kurtosis is expected to equal zero. We find that the distribution of the $h^2$ differences has a kurtosis of 2.71 ($P < 0.0001$) and that the distribution of the $CV_A$ differences has a kurtosis of 4.55 ($P < 0.0001$). In other words, both distributions are significantly leptokurtic, containing more values in the middle bins than would be expected under a normal distribution. Leptokurtosis was not due to setting negative variances to zero, as there were only a handful of such cases.

5.4.2 Variance dimorphism in reproduction-related traits

Several studies reported whether traits were under sexual selection or related to sexual reproduction. In animals, these were traits such as copulation duration, sexually selected trait size, age at first reproduction, mating frequency, and body size (when explicitly stated as under sexual selection). In plants, these were traits such as flower number, flower size, calyx size, and seed mass (in gynodioecious species). To see whether the significant right skews evident in Fig. 5.4.1 were attributable to the reproduction-related traits, we dropped all such trait pairs. We surprisingly find that skews are actually still significantly positive for both the $CV_A$ Male − Female difference (skew = 0.38, $P = 0.007$, $N = 247$) and the $h^2$ difference (skew= 0.24, $P = 0.02$, $N = 445$). The $CV_A$ mean difference became less male-biased ($\mu = 0.087$, $P = 0.38$) and the $h^2$ mean difference became more female-biased ($\mu = -0.02$, $P = 0.99$) in the filtered dataset compared to the whole dataset (Fig. 5.4.1) but neither mean difference was statistically different from zero by randomization tests. Furthermore, the mean $CV_A$ difference for the filtered dataset and the mean for the entire dataset were not significantly different from each other (parametric ANOVA: $F_{1,287} = 2.22$, $P = 0.14$; permutation test: $P = 0.22$, 365 iterations). However, the mean $h^2$ difference for the filtered dataset and the mean
for the entire dataset were significantly different from each other (parametric ANOVA: $F_{1,508} = 13.63, P = 0.0002$; permutation test: $P < 0.0001$, 5000 iterations). The permutation test was performed by using the lmPerm package in R to implement a one-way ANOVA using the command aovp(); the test works by reassigning all of the $CV_A$ (or $h^2$) difference values to each of the groups (e.g., reproduction-related and not reproduction-related) randomly and calculating the $F$ statistic for each iteration. The actual $F$ is then compared to the distribution of permuted $F$ statistics to see what percentage of permuted $F$-values exceed the observed $F$-value.

We also separately analyzed the distribution characteristics of the subset of reproduction-related traits. For the $CV_A$ Male − Female difference, the skew was not significant (skew = 0.25, $P = 0.26$, $N = 42$) and the mean was not significantly different from zero by the randomization test ($\mu = 1.4$, $P = 0.17$). However, for the $h^2$ Male − Female difference, the skew was significantly positive (skew = 0.61, $P = 0.02$, $N = 65$), and the mean was also significantly positive ($\mu = 0.075$, $P = 0.02$). So, heritabilities, but not coefficients of variation, suggest that male variances on average are greater than female variances among reproduction-related traits.

We also analyzed the reproduction-related traits by plants versus animals. We find that there is significant right skew in the $CV_A$ Male − Female difference among animal reproductive traits (skew = 1.29, $P = 0.027$, $N = 16$) but not among plant reproductive traits (skew = 0.017, $P = 0.49$, $N = 26$). However, mean of the $CV_A$ Male − Female difference among animals ($\mu = 1.36$) did not significantly differ from the mean of the Male − Female difference among plants ($\mu = 1.42$) (parametric ANOVA: $F_{1,63} = 0$, $P = 0.983$; permutation test: $P = 1$, 51 iterations). Finally, there were no significant right or left skews in the $h^2$ difference among either animal or plant reproductive traits; the mean of the Male − Female $h^2$ difference did not differ between animals and plants.
5.4.3 Variance dimorphism across trait types

Skew

Certain traits, such as those closely related to fitness, might be expected to have high amounts of additive variance in both sexes (Houle 1992; Rowe and Houle 1996). As a result, variance dimorphism may be smaller in life history related traits than in morphological traits. We therefore tested for differences in variance among trait groups. Table 5.1 shows that morphology and physiology have significant right skew when the variance difference between the sexes is measured by $CV_A$. However, only morphology has significant right skew when variance difference is measured by $h^2$. Some trait types show left skew (negative values in Table 5.1), but none of the left skews were significant. So, even on a trait by trait basis, the more extreme variance values are typically male rather than female.

Mean

When the entire dataset is broken down by trait type, we observed differences among groups for the degree of sexual dimorphism as measured by both $CV_A$ ($F_{4,282} = 3.842, P = 0.0047$) and $h^2$ differences ($F_{4,501} = 3.872, P = 0.0042$). The Tukey’s HSD test for post-hoc comparisons showed that mean $CV_A$ differences between behavioral and mass traits were significantly different (adjusted $P = 0.007$); mean differences between behavioral and survival & developmental traits were significantly different (adjusted $P = 0.038$). Post-hoc comparisons for $h^2$ differences showed that survival/developmental traits were significantly different from behavioral (adjusted $P = 0.034$), mass (adjusted $P = 0.01$), and physiological traits (adjusted $P = 0.02$).

However, while heritability differences were approximately normally distributed, the coefficients of variation were not. We therefore used the aovp() command to conduct a permutation test for both $CV_A$ and $h^2$ differences. $CV_A$ differences between the sexes
was significantly different among trait types ($P < 0.0001$, 5000 iterations). The $h^2$ differences between the sexes was significantly different among trait type ($P = 0.0084$, 5000 iterations).

We also used randomizations to analyze which traits had $CV_A$ and $h^2$ Male − Female differences significantly different from zero. Within a given trait type, we took each original male and female data pair and randomly made their difference positive or negative. We then created a new data set for which to calculate a mean difference. We repeated this process 999 times to generate a distribution of the new means that also included the original mean. For $CV_A$ differences, only morphology showed a mean difference between the sexes ($\mu = 0.698$) that was significantly different from zero ($P = 0.036$; Fig. 5.2). For $h^2$ differences, only survival & developmental traits showed a mean difference ($\mu = 0.079$) significantly different from zero ($P = 0.031$; Fig. 5.2).

### 5.4.4 Variance dimorphism among organismal groups

**Skew**

Because sex chromosome information was not available for all organisms, we proceeded under the justified assumption that certain groups of organisms are in general male heterogametic (e.g., mammals, flies) while others are female heterogametic (e.g., butterflies, moths, birds). Under these broad taxonomic classifications, we find little evidence consistent with the hypothesis that sex-linkage alone is enough to explain sex-specific variance differences (Table 5.1). For example, when measured by $CV_A$s, birds and Lepidopterans have non-significant right skew, whereas significant left skew would be predicted by female heterogamety. Furthermore, mammals show non-significant left skew, whereas significant right skew would be predicted. However, Dipterans show significant right skew in $CV_A$ differences (but not $h^2$ differences) as would be predicted by male heterogamety. For $h^2$ differences, right skews are significant in plants and fish; however,
Figure 5.2: Mean +/- 1 SE differences by trait types. A) \( Male \ CV_A - Female \ CV_A \) and B) \( Male \ h^2 - Female \ h^2 \).
as sex chromosomes are less studied and more labile in both these groups, their results are not appropriate for evaluating this particular hypothesis.

**Mean**

We tested for differences in mean sexual dimorphism in genetic variances among organismal groups (Fig. 5.3). We observed differences among broad taxonomic groups as measured by both $CV_A$ ($F_{7,275} = 2.744; P = 0.0091$) and $h^2$ ($F_{7,476} = 5.744; P < 0.0001$). The Tukey’s HSD post hoc comparisons showed marginally significant differences between plants and fish (adjusted $P = 0.08$) and between plants and mammals (adjusted $P = 0.069$) for $CV_A$ differences. For $h^2$ differences between the sexes, birds were significantly different from beetles (adjusted $P = 0.004$), fish (adjusted $P = 0.0006$), and plants (adjusted $P = 0.00002$); plants were also significantly different from mammals (adjusted $P = 0.02$).

Again, because the $CV_A$ were not normally distributed, we also used a `aovp()` command in the lmPerm package to redistribute the variance differences across the different organisms and compare the observed $F$ statistic to the distribution of permuted $F$ statistics. Both $CV_A$ differences ($P = 0.001$; 5000 iterations) and $h^2$ differences ($P < 0.0001$; 5000 iterations) were significantly different among groups.

We analyzed which organisms had $CV_A$ and $h^2$ Male $-$ Female differences that were significantly different from zero with a randomization test. Within a given organism, we took the original male and female data pair and randomly made their difference positive or negative for all data pairs. We repeated this procedure 1000 times to generate a distribution of the new means. The Male $-$ Female $CV_A$ difference was significantly different from zero for Dipterans ($\mu = 1.211; P = 0.028$) and plants ($\mu = 1.913; P = 0.056$). The Male $-$ Female $h^2$ difference was significantly different from zero for Coleopterans ($\mu = 0.052; P = 0.022$), plants ($\mu = 0.073; P = 0.005$), and fish ($\mu = 0.086; P = 0.05$). All significant differences in mean genetic variance between the sexes indicated by the
Figure 5.3: Mean +/- 1SE differences by organism types. A) Male CV\textsubscript{A} – Female CV\textsubscript{A} and B) Male $h^2$ – Female $h^2$. 
randomizations was always male-biased, whether measured by $CV_A$ or $h^2$.

The plants in this study were either dioecious or gynodioecious. Because of this distinction, we could compare whether having fully partitioned sexes could maintain more sexual dimorphism in genetic variances, i.e., potentially via balancing selection. We found no difference between the 2 dioecious and 3 gynodioecious species for differences in $CV_A$ (one-way ANOVA: $F_{1,52} = 1.585$, $P = 0.214$; permutation test: $P = 0.623$, 464 iterations) or differences in $h^2$ (one-way ANOVA: $F_{1,63} = 0.244$, $P = 0.623$; permutation test: $P = 0.486$, 107 iterations).

### 5.4.5 Variance sexual dimorphism versus phenotypic sexual dimorphism

We found a positive correlation between sexual dimorphism in variances and sexual dimorphism in means (Fig. 5.4). A non-parametric Spearman rank correlation test between the log, base 2 of the male : female variance ratios and mean ratios was significant ($N = 222; \rho = 0.23; P = 0.00014$) for the entire dataset. After removing four outliers with implausibly high sexual dimorphism in genetic variances or in means (over 16-fold difference), this correlation remained significant ($\rho = 0.21; P = 0.0013$). When the data are divided into traits with male-biased versus female-biased means, the correlation between variance dimorphism and mean dimorphism is only significant for the male-biased traits ($N = 120; \rho = 0.34; P = 0.00013$).

When the correlations are performed on a trait-by-trait basis, we find that only mass has a positive correlation between variance and mean dimorphism ($N = 43; \rho = 0.54; P = 0.0002$). Morphological ($N = 135; \rho = 0.14; P = 0.1$) and physiological ($N = 11; \rho = 0.51; P = 0.09$) traits had marginally nonsignificant correlations.

It is also possible to compare the relationship between variance dimorphism and phenotypic dimorphism by using the differences in the coefficients of variation and heritabilities. A Spearman rank correlation test between the $Male - Female CV_A$ and the
Figure 5.4: Phenotypic sexual dimorphism versus variance dimorphism. The amount of dimorphism in genetic variances is positively correlated with the extent of phenotypic sexual dimorphism (four outliers were removed; see text for details).
log base 2 of the male : female means was significantly negative \( (N = 232; \rho = -0.27; P < 0.0001) \). The correlation between the Male – Female \( h^2 \) and log ratio in means was also significantly negative \( (N = 251; \rho = -0.14; P = 0.03) \). When performing the same correlation but considering only the absolute value of the \( CV_A \) difference and the absolute value of the log ratios, the correlation is significantly positive \( (N = 232; \rho = 0.28; P < 0.0001) \). The correlation between the absolute value of the \( h^2 \) difference and the absolute value of the log ratios is also significant \( (N = 250; \rho = 0.15; P = 0.02) \).

### 5.4.6 Phenotypic and residual variances

There were 254 trait pairs with information about phenotypic variances in both sexes. Males and females were not significantly different for their coefficients of phenotypic variance \( (CV_P) \) considered across the entire dataset by a randomization test \( (P = 0.33) \). The mean Male – Female \( CV_P \) difference was 0.007. However, there is a significant right skew in the dataset, indicating that more extreme coefficients of phenotypic variance are male-biased \( (skew = 8.44, P < 0.0001) \). There was no difference among trait types in the Male – Female difference for coefficients of phenotypic variance \( (F_{3,207} = 0.55, P = 0.65) \).

We also broke up the entire dataset by reproduction and not reproduction-related traits. Neither group had a Male – Female \( CV_P \) that was significantly different from zero by randomization tests (reproduction-related: \( P = 0.426 \); not reproduction-related: \( P = 0.362 \)). However, both trait subsets had significant skew (reproduction-related: skew = 1.42, \( P = 0.003 \), \( N = 27 \); non-reproduction-related: skew = 9.04, \( P < 0.0001 \), \( N = 227 \)).

We analyzed coefficients of non-additive variance by subtracting \( V_A \) from \( V_P \) and calculating the coefficient of variation. There were no significant difference between the sexes for non-additive variance by a randomization test \( (P = 0.104) \). There were also no
significant skews in the Male – Female distribution for the coefficients of non-additive variance.

We re-analyzed the trait groups to include only the subset of data for which both heritabilities and coefficients of variation were available (Fig. 5.5). This made it possible to analyze why $h^2$ and $CV_A$ gave contrasting pictures about the overall sexual dimorphism in the levels of additive variance (i.e., positive versus negative sexual dimorphism). For instance, among survival & development traits the Male – Female $CV_A$ difference is negative but the $h^2$ difference is positive. This suggests that the additive variance in females may be higher than in males, but also that the phenotypic variance is higher in females. However, when we analyzed the mean male and female $V_P$, $V_A$, and means, we do not find such a simple pattern. For instance, among survival & development traits, the additive variance is higher in males than females (mean male $V_A = 175.3$; mean female $V_A = 114.9$). The phenotypic variance is also higher in males than females (mean male $V_P = 385.3$; mean female $V_P = 266.3$). The mean male trait is also smaller than the mean female trait (mean male 73.86; mean female 81.87). Thus the reversals in sign seen between $h^2$ and $CV_A$ are attributable to very subtle relationships between the numerators and denominators of $h^2$ and $CV_A$. These subtle relationships were also the cause of the dimorphism sign change for morphological traits.

5.5 Discussion

The study of variability between the sexes has long fascinated biologists. Darwin observed that much of this variation could be traced to differences in the intensity of sexual selection in males versus females. This insight has been affirmed by the observation that sexually selected traits often have higher amounts of additive genetic variation than non-sexually selected traits (Pomiankowski and Moller 1995; Rowe and Houle 1996). Here we present evidence which suggests that among shared traits the sexes do not differ in the
Figure 5.5: Mean +/- 1 SE differences by for traits with both $CV_A$ and $h^2$ estimates. A) Male $CV_A$ – Female $CV_A$ and B) Male $h^2$ – Female $h^2$. 
amount of additive genetic variation overall. However, when more extreme differences do occur, variances tend to be male-biased more often than female-biased. This pattern holds even after dropping all of the traits potentially under sexual selection. In addition we show that variance dimorphism is associated with phenotypic sexual dimorphism.

5.5.1 Variance dimorphism

In general, the distributions of the Male − Female coefficients of variation and heritabilities show significant right skew (Table 5.1). In other words, there are many more traits with extreme male-biased variances than female-biased variances. Furthermore, there were no cases when the difference distributions for either $CV_A$ or $h^2$ had significant left skews (female-biased variance dimorphism) - across the entire dataset or by traits or by organismal groups.

The mean Male − Female difference in the $CV_A$ and $h^2$ was not significant across the entire dataset (Fig. 5.4.1). However, this may be because both distributions were extremely leptokurtic, possessing many more differences close to zero, so that the overall mean difference is also zero. This clustering was perhaps inevitable because we targeted traits that were expressed in both sexes. It is perhaps not surprising for shared traits to have similar levels of additive genetic variation in both sexes. However, this analysis also revealed that even shared traits can have very high sexual dimorphism in genetic variances as significant skews were prevalent. Many of these traits may be under undetected sexual selection or be highly correlated to traits under sexual selection.

When the traits are divided by those related to reproduction and those that are not, the reproduction related traits have a higher male-biased mean than the rest of the traits. However, neither group had a Male − Female mean that was significantly different from zero. Furthermore, the reproduction related traits did not have a significant skew in the Male − Female $CV_A$ distribution. This contrasts with the Pomiankowski and Moller (1995) results which show greater mean $CV_A$s in male sexually selected traits than in
naturally selected traits. This discrepancy may be caused by differences in methodology. We specifically targeted only shared traits expressed in both sexes while Pomiankowski and Moller (1995) compared sexually selected and naturally selected traits that were not necessarily paired.

We observed some differences in the mean degree of variance dimorphism among traits. Randomization tests revealed that only morphological traits had a $CV_A$ mean difference that was significantly different from zero; likewise, only survival & developmental traits had a $h^2$ mean difference that was significantly different from zero. Both means were positive, suggesting greater levels of additive variance in males than females.

It is puzzling that the randomizations on the $CV_A$ differences did not have similar results as the randomization on the $h^2$ differences when analyzed on a trait-by-trait basis (Figs. 5.2, 5.5). This may be attributable to the fact that $CV_A$ and $h^2$ are not expected to be correlated to one another (Houle 1992; Hansen et al. 2011). Furthermore, as $h^2$ is expected to be a poor predictor of evolvability, it may be that only the significant difference seen among Male – Female $CV_A$ differences in morphology are meaningful. Finally, because we studied only traits expressed in both sexes, it is rather surprising that the mean difference in $CV_A$ for morphological traits was significantly different from zero. Shared traits might be expected to have similar levels of $V_A$ between the sexes.

This is the first systematic study of variances differences between the sexes to our knowledge. Interestingly, these results share interesting parallels with the genomic data from *Drosophila melanogaster*. Male-biased genes tend to have more variation in expression among genes than female-biased genes (Zhang et al. 2004). Most female-biased genes have less extreme sex-biased expression (Zhang et al. 2004; Wyman et al. 2012; Ayroles et al. 2009). Effectively, the phenotypic variance in expression is greater among male-biased genes than female-biased genes. Genomic data posses an unbiased selection of traits (i.e., genes) and represent an advantage over classic single trait phenotypic studies in this regard. In the same way, by being as broad as possible with regard trait
selection (e.g., expressed in both sexes, not just one), we have tried to be as unbiased as possible and show that more extreme male-biased variability may be common.

The greater general variability of males is sensible for two reasons. First, males have higher variance in reproductive success than females (Bateman 1948). It may be that this higher fitness variance is associated with higher variance in many other aspects of the male organism - e.g., resource acquisition, resource allocation, trait expression. Second, females are more often expected to experience stabilizing natural selection while males are more often expected to experience directional selection. The role of stabilizing selection is always to reduce the additive variance. By contrast, directional selection might decrease or increase the variance. Directional selection is expected to deplete variance by eroding genetic variation during the evolution of extreme phenotypes. However, directional selection can momentarily increase variation by increasing the frequency of rare alleles. In addition, directional selection can lead to the cooption of condition associated genetic variance (see more below).

Interestingly, recent studies suggest that the greater genetic variability of males is actually non-random. Even though males in Drosophila serrata are under directional selection to increase expression of sexually selected cuticular hydrocarbons, high fitness males have less additive genetic variance than low fitness males (McGuigan and Blows 2008; Sztepanacz and Rundle 2012). Overall, pleiotropy will generate apparent stabilizing selection and constrain the genetic variation of traits (McGuigan et al. 2011), so that dispersion about the optimum may indicate the strength of apparent stabilizing selection. In other words, the most successful males not only possess the most attractive traits but also have higher phenotypic integration. It is as yet unclear if such patterns occur in high versus low fitness females and whether the difference in their dispersion is as large.
5.5.2 Sex chromosome system and sexual differences in variance

We find very little support for the association between sex chromosome system and greater variance in the heterogametic sex. However, it may be that we did not have the ability or power to detect sex chromosome associated variances. It is likely that many, if not all, of the traits in this study likely have a complex polygenic basis. If so, the traits probably have both autosomal and sex-linked loci that govern expression. If there are many more autosomal than sex-linked loci (or traits) any signal of greater heterogamety-associated variance is expected to be weak. Thus, the data collected here may not have been sufficient to evaluate this particular hypothesis.

Furthermore, while many traits in our study were sexually dimorphic, suggesting potential sex-linkage, very few studies employed methods capable of detecting sex-linked additive variance. If a quantitative genetic design does not take into account heterogamety, the sire variance measured in females will be greater than the sire variance measured in males for XY species (Cowley and Atchley 1988; Cowley et al. 1986). However, despite this expected female-bias, XY-system Dipterans actually showed significant male-biased mean variances (by randomization tests) and significant male-biased skew in variances.

5.5.3 Multivariate caveats

We analyzed the $V_A$ differences between males and females in a univariate context because there are many more single trait studies than multivariate studies of sexual dimorphism. However, multivariate studies are becoming more common and may supply additional insights. While males and females may differ in magnitudes for $V_A$ in a univariate framework, under the multivariate formulation, the sexes may differ for both magnitude (i.e., eigenvalues) and orientation (i.e., eigenvectors). Both the scale and direction of the sex-specific $G$ matrix (variance-covariance matrix) will impact how the evolution of sexual dimorphism proceeds (e.g., Lewis et al. 2011; Gosden et al. 2012). By
and large, $G$ matrices appear to be conserved for their eigenvectors across populations, experimental treatments, species, and sexes (Arnold et al. 2008). However, distinct, unrelated eigenstructures are by no means uncommon, representing 22 - 26% of all studies. As more multivariate studies are conducted, we expect to understand how often the sexes differ with regard to direction, in addition to magnitude. For the time being, the insights derived from compilations of univariate estimates such as this one, hint at the potential for widespread sexual dimorphism in genetic architectures.

5.5.4 $V_A$ dimorphism versus phenotypic dimorphism

We found correlations between the extent of sexual dimorphism in additive variances and the extent of phenotypic sexual dimorphism. However, this correlation could be positive or negative, depending upon the measure of additive variance. The correlation between the $V_A$ ratio and the phenotypic mean ratio between the sexes is positive, suggesting that sexual dimorphism is biased toward the sex with the greater $V_A$ (Fig. 5.4.1). This accords with the prediction that male-specific selection produces male-biased dimorphism if there is male-biased variances (and vice versa for female-biased dimorphism).

Alternatively, it is also possible that strong sex-specific selection on phenotypic means has itself caused the increased sex-specific differences in $V_A$ via condition dependence. Life history and sexually selected traits are often expensive to produce. As a result, their expression is contingent upon the bearer’s intrinsic quality, or condition. Condition is a complex trait controlled by many different loci and therefore has a large amount of additive genetic variation. Sexually selected traits and life history traits effectively adopt this large pool of condition-specific additive variance, leading to their higher additive variance as well (Rowe and Houle 1996). In other words, it may be that sex-specific differences in selection on means has produced differences in sex-specific variance. Interestingly, this process would suggest that directional selection acting on just one sex would produce variance dimorphism. However, were selection to act on both sexes but
in different manners, i.e., via niche partitioning, variance dimorphism may not result.

However, the correlation between the $CV_A$ difference and the phenotypic dimorphism ratio is negative. The correlation between the $h^2$ difference and phenotypic dimorphism ratio is also negative. These results seem to suggest that male-biased dimorphism is associated with female-biased additive variances (and vice versa for female-biased dimorphism). Such a pattern is puzzling but may make sense under the condition that male-specific selection is stronger than female-specific selection among these traits.

Finally, we observed a significant positive correlations between the absolute value of the $CV_A$ Male – Female difference and the absolute phenotypic dimorphism. There was also a significant positive correlation between the absolute value of $h^2$ Male – Female and the absolute dimorphism. These patterns suggest that only overall variance dimorphism may matter to overall phenotypic dimorphism. Again, patterns of sex-specific selection may actually modulate how sex-specific additive variances are used.

## 5.5.5 Conclusions

The evolution of sexual dimorphism may require one or several of three conditions: sex-specific selection, low intersexual genetic covariances, and sex-specific additive variances. We have shown that variances can differ between males and females across traits and organisms and that such differences are also associated with the level of phenotypic sexual dimorphism. It remains to be seen how such differences in the magnitude of genetic variance between the sexes potentially interacts with differences in the orientation of the genetic variance. It will also be interesting to determine the cause of the greater sex-specific $V_A$ in traits not currently identified as experiencing sexual selection.
5.6 Acknowledgments

We are grateful to S. Qidwai for assistance with obtaining the papers. We thank J. R. Stinchcombe and M.C. Wyman for invaluable mathematical and conceptual discussions. We thank B. Gilbert, A.D. Cutter and A.F. Agrawal for additional comments and advice. This work was funded by NSERC grants to L. Rowe. M.J. Wyman was supported by University of Toronto Connaught Scholarship and Doctoral Completion Award.
## 5.7 Appendix table

Table 5.2: A list of the organisms, estimates, and references used in this study.

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<th>Organism</th>
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<th>number of $h^2$ pairs</th>
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Chapter 6

The differences between univariate and multivariate perspectives in the evolution of sexual dimorphism

6.1 Abstract

Sexual differences are often dramatic and widespread across taxa. Their extravagance and ubiquity can be puzzling since the common underlying genome between males and females is expected to impede rather than foster phenotypic divergence. Part of the discrepancy may be explained by the differences in emphases provided by the univariate and multivariate formulae governing the evolution of sexual dimorphism. In the univariate formulation, differences in genetic variances and a low intersexual genetic correlation ($r_{MF}$) can facilitate the evolution of sexual dimorphism. However, most studies that have analyzed sex-specific differences in heritabilities or genetic variances do not find significant differences. Furthermore, many of the reported estimates of $r_{MF}$ are very high and positive. Taken together these data suggest low potential to evolve sexual dimor-

\footnote{This chapter was written in collaboration with John R. Stinchcombe and Locke Rowe.}
phism on a trait-by-trait basis. By contrast, the multivariate formulation has greater
generality and more flexibility. First, sex-specific differences in the $G$ matrix not only
have a magnitude but also a direction. While the number of multivariate sexual dimor-
phism studies is low, almost all support sex-specific differences in $G$. Furthermore, the
sexes appear to vary with regard to the magnitude and/or direction of their $G$ matrices,
supporting greater sexual dimorphism overall. Second, whereas positive values of the
univariate quantity $r_{MF}$ only hinder sexual dimorphism, positive values in the $B$ (inter-
sexual covariance) matrix can either help or hinder sexual dimorphism. Furthermore,
of the handful of studies reporting $B$ matrices, all suggest that it is asymmetrical. Un-
like in the univariate breeder’s equation, the multivariate equations allow male-to-female
patterns of trait covariation to not equal female-to-male patterns, and allow positive in-
tersexual covariances to facilitate sexual dimorphism. Thus the $B$ matrix can affect one
sex more than the other even if selection is of the same strength and form. Even though
the multivariate view is often thought to constrain trait evolution more than a univariate
view due to extra covariances, in the evolution of sexual dimorphism, the multivariate
perspective may actually be more flexible after all.

6.2 Introduction

The evolution of sexual dimorphism has long fascinated evolutionary biologists.
Males and females obviously differ with regard to reproduction, but sometimes the sexes
can diverge so much in other aspects of the phenotype as to question whether the two
belong to the same species. Such differences spurred Darwin (1871) on to write an entire
volume on the evolution of sex-specific traits, focusing upon the role of sexual selection
in their evolution. Darwin considered two models for how sex-specific differences might
emerge and persist over time. In the first verbal model he conjectured that most new
sexual dimorphism must be sex-limited initially. In the second verbal model, he suggested
that sexual dimorphism could appear by a two-step process: novel traits might appear simultaneously in both sexes, and then natural selection would promote sex-limitation by suppressing expression in the non-benefitting sex (Darwin 1866). While *The Descent of Man* focused upon the importance of intra- and inter-sexual selection, these two verbal models also pointed to the roles of sex-specific genetic variation and the intersexual covariation in affecting sexual phenotypic divergence. Darwin himself suggested that the one-step process was more feasible since it relied upon already present differences in sex-specific inheritance to propagate sex-specific traits. In other words, the genetic basis or genetic variation would have to differ *ab initio* between males and females. By contrast, the two-step process would have to rely upon the eventual decrease over time in the male-female patterns of covariation. In these two processes, Darwin (1871) foresaw all of the parts necessary for the evolution of sexual dimorphism as we understand it today: differences in sex-specific selection, differences in sex-specific genetic variances, and a low intersexual genetic correlation ($r_{MF}$).

Although Darwin espoused the one-step model of sexual dimorphism, empirical support exists for both models. Sexual dimorphism appears to evolve by relying upon changes in the extent of intersexual covariation as well as upon standing sexual differences in genetic variation. Passerines such as finches and hummingbirds show evidence for initial male-limitation of plumage (Coyne et al. 2008). Yet, among tanagers, females experience secondary acquisition of ornamentation, which means that plumage was first male-limited, but then became shared (Burns 1998) – contrary to the hypothesis for female crypsis (Wallace 1889; Amundsen 2000). Indeed, larger surveys suggest great lability in the transitions between monochromatism and dichromatism among passerines indicating that secondarily acquired sex-limitation may not be as insurmountable a barrier as Darwin had envisioned (Price and Birch 1996). Among butterfly genera, both processes appear to operate as well. Transitions to monomorphism were more common than transitions to dimorphism, owing both to gains by the non-ornamented sex and
losses by the ornamented sex (Oliver and Monteiro 2011). However, within the genus *Papilio*, ornamentation manifests as female-limited Batesian mimicry. So, in another interesting turnabout, natural selection (not sexual selection) decorates, rather than divests, females in order to bestow the veneer of toxicity (Kunte 2008). Dragon lizards as well demonstrate that the sexes can don ornaments separately, or congruently, then followed by sex-specific suppression; surprisingly, ornament loss in males was more common in males than in females (Ord and Stuart-Fox 2006). Among horned beetles, females could gain horns concurrently with or independently of males (Emlen et al. 2005).

This bewildering lability is consistent with the diversity fueled by sexual selection. Strong sexual selection can drive the rapid evolution of seemingly arbitrary traits related to mate choice (Darwin 1871; Andersson 1994). The divergence among sexually selected traits in closely related species is almost guaranteed, even while other traits are relatively conserved. This pattern has long been recognized in morphological and behavioral traits, but has also recently been observed in genes as well. Male-biased and male-limited genes have high rates of evolution and demonstrate rapid turnover in function among related species and among populations compared to female-biased and unbiased genes (e.g., Ranz et al. 2003; Meiklejohn et al. 2003; Zhang et al. 2004; Zhang and Parsch 2005; Pröschel et al. 2006; Zhang et al. 2007).

Yet this consistently high diversity is at odds with the current theoretical emphases and empirical data on the evolution of sexual dimorphism. For instance, if the intersexual genetic correlation (i.e., measure of correspondence between male and female genetic variances) is high (Lande 1980), sex-specific selection will have limited efficacy — as Darwin (1871) had conjectured. Recent data confirm that the intersexual genetic correlation can be a powerful constraint on the evolution of dimorphism in single traits. Traits with low or negative intersexual correlations display more phenotypic sexual dimorphism than traits with a high correlation (Bonduriansky and Rowe 2004; Poissant et al. 2009). From the multivariate perspective, the patterns of intersexual covariation
can impose even stronger constraints (Lewis et al. 2011; Gosden et al. 2012). For instance, male antlers and female limbs can have a between-sex covariance, in addition to the covariance of male limbs to female limbs. All of these cross-sex, cross-trait covariances can severely restrict the independent evolution of any given male or female trait.

If it is the case that intersexual covariances have such a constraining role on the evolution of dimorphism, how can the sexes ever diverge to produce the overwhelming sexual differences seen in nature? Or how can the sexes achieve their respective optima? Rather than emphasizing intersexual covariances, it is possible to alter our focus in two ways. First, like Darwin (1871), we can recognize standing differences in sex-specific genetic variances, not just intersexual covariances. The sexes can express different sets of genes or express them in a sex-limited manner. Sex-linkage, sex-specific gene expression, gene duplication, genomic imprinting, and condition dependence may all allow the genetic architecture in the sexes to differ (Bonduriansky and Chenoweth 2009; van Doorn 2009). We observe empirically that male sexually selected traits often possess greater amounts of genetic variation compared to naturally selected traits (Pomiankowski and Moller 1995). If males primarily experience sexual selection and females primarily natural selection, then the sexes may differ in genetic variances. As a result, the sexes will respond differently to selection, even if it is of the same strength and form (Cheverud et al. 1985). It may well be that the differences in genetic variances seen between male sexually selected and female naturally selected traits is a more common phenomenon, possibly extending to many other monomorphic and dimorphic traits shared between the sexes. Although intersexual covariances and sex-specific genetic variances are ultimately related, as we describe below, the slight shift in focus from the former to the latter offers more mechanistic freedom to evolve sexual dimorphism.

Second, we ought to recognize some fundamental differences between the univariate and multivariate approaches to the evolution of sexual dimorphism. Under a univariate perspective, the absolute difference in size of the sex-specific genetic variances
matters. However, under a multivariate perspective, differences in both the size and orientation of the sex-specific variances matter. The additional dimension can mean that the sexes may diverge with respect to different sets of traits, resulting in greater overall sexual dimorphism. Furthermore, positive intersexual genetic covariances in a univariate framework only hinder the evolution of sexual dimorphism whereas positive intersexual covariances in a multivariate framework can hinder or facilitate sexual dimorphism. These differences suggest that overall, sexual dimorphism in multivariate space is mathematically easier than in a univariate model.

In this synthesis we describe the univariate and multivariate equations for the evolution of sexual dimorphism to show how potential constraints can operate mathematically. We also review the empirical data to gauge what support there is for the usual simplifications made to understand these equations. In particular, we review the current empirical state of knowledge on univariate and multivariate differences in genetic variances and covariances between the sexes. The mathematical differences between the univariate and multivariate formulations and the data itself suggest that evolving sexual dimorphism is not necessarily an arduous process. The facility provided by a multivariate perspective is also more consistent with the diversity in sexual traits observed in nature. These conclusions suggest that future work should focus upon understanding differences in sex-specific genetic variances, particularly in a multivariate framework.

6.3 Constraints in evolving sexual dimorphism

6.3.1 Univariate changes in sexual dimorphism

One sex can impede adaptation of the other sex. It is possible to describe this intersexual dependence mathematically by studying the breeder's equation, \( R = h^2 s \), which describes the phenotypic response to selection in a single trait. Heritability, \( h^2 \), is the additive genetic variance of the trait, \( \sigma_A^2 \), divided by the total phenotypic variance,
\( \sigma_p^2 \). The selection differential, \( s \), is defined as the difference in the phenotypes between the mean of the entire population and the mean of the reproducing population. Thus, the response to selection, \( R \), is the difference in the mean of the entire population before selection and the mean of the offspring of the selected parents (Falconer and Mackay 1996; Lynch and Walsh 1998).

The univariate breeder’s equation can be extended to predict the trait change in males (\( \Delta \bar{Z}_m \)) and females (\( \Delta \bar{Z}_f \)):

\[
\Delta \bar{Z}_m = \frac{1}{2}(h_m^2 \sigma_{P_m} i_m + h_m h_f r_{MF} \sigma_{P_m} i_f) \\
\Delta \bar{Z}_f = \frac{1}{2}(h_f^2 \sigma_{P_f} i_f + h_f h_m r_{MF} \sigma_{P_f} i_m) \tag{6.1}
\]

The coefficient \( 1/2 \) is introduced to take into account the autosomal contribution from each parent. \( h_m^2 \) and \( h_f^2 \) are the sex-specific heritabilities, which are the sex-specific genetic variances divided by the sex-specific phenotypic variances. \( i \) is the sex-specific selection intensity, which is the selection differential divided by the phenotypic standard deviation (\( s/\sigma_p \)).

The new quantity, \( r_{MF} \) is the intersexual genetic correlation and it describes the degree of correlation between the sexes. It is the covariance of the additive effects between males and females divided by the square root of the product of the standard deviations of the sex-specific additive variances:

\[
r_{MF} = \frac{Cov(M, F)}{\sqrt{\sigma_{AM}^2 \sigma_{AF}^2}} \tag{6.2}
\]

When \( r_{MF} \) is positive, selection in one sex will produce a similar response in the opposite sex, according to the strength of the correlation. When \( r_{MF} \) is zero or negative, selection on one sex will produce no response or a response in the opposite direction in the opposite sex. \( r_{MF} \) is simply a specific case of the formula for the correlation coefficient, \( \rho \), which
always ranges between +1 and -1, since the covariance is largest in magnitude when the variances are equal (i.e., the covariance of a quantity with itself is the variance).

The mean change in a male character ($\Delta \bar{Z}_m$) or female character ($\Delta \bar{Z}_f$) is governed by the sex-specific genetic variances and the degree of correlation between the sexes for the shared trait. The change in the sexual dimorphism ($\Delta SD$) may be described as the difference in the resulting male and female trait means:

$$\Delta SD = \Delta \bar{Z}_m - \Delta \bar{Z}_f = \frac{1}{2}[h^2_m \sigma_{P_m} i_m - h^2_f \sigma_{P_f} i_f + h_m h_f r_{MF} (\sigma_{P_m} i_f - \sigma_{P_f} i_m)]$$  (6.3)

As equation 6.3 is complicated, it is easier to see the effect of $r_{MF}$ by making a few simplifications. To illustrate, we follow Cheverud et al.’s (1985) decomposition of the breeder’s equation for sexual dimorphism, which assumed that heritabilities and phenotypic variances are monomorphic ($h^2_m = h^2_f = h^2$ and $\sigma_{P_m} = \sigma_{P_f} = \sigma_P$):

$$R_{SD} = \frac{1}{2} h^2 \sigma_P (i_m - i_f)(1 - r_{MF}).$$  (6.4)

Because of the negative sign in front of $r_{MF}$, large positive correlations always have a constraining effect on the evolution of sexual dimorphism, even if selection favors different trait sizes in the sexes (i.e., $i_m - i_f \neq 0$). The correlated response will keep dimorphism from responding to what it would have been otherwise. Several authors have noted that the absolute constraining effect of $r_{MF} = 1$ rests upon the assumption of identical genetic variances (Leutenegger and Cheverud 1982; Slatkin 1984; Cheverud et al. 1985; Leutenegger and Cheverud 1985; Reeve and Fairbairn 1996; Lynch and Walsh 1998; Reeve and Fairbairn 2001; Poissant et al. 2009; Bonduriansky and Chenoweth 2009). Yet monomorphic variances (i.e., $h^2_m = h^2_f$) do not necessarily say anything about the extent of covariation between males and females (definition of $\rho$; see also Fig. 1), which must be high to be a constraint.

The negative correlation between $r_{MF}$ and the extent of projected changes in
Figure 6.1: Male and female variances. Here are two imaginary datasets. In both datasets males and females have the same mean and variance ($\bar{x} = 0$ and $\sigma^2 = 1$). Even though $\sigma^2_m = \sigma^2_f$, monomorphic variances say nothing about the extent of covariation between the sexes, which can be zero (left) or complete (right). In a similar fashion, whether $G_m$ and $G_f$ are the same says little about the structure of $B$. Furthermore $G$ matrices have no influence on whether $B$ and $B^T$ are equivalent.
sexual dimorphism has received empirical support. A study of antler flies revealed an overall negative relationship between $r_{MF}$ and morphological sexual dimorphism (Bonduriansky and Rowe 2004). When the intersexual correlation was negative or low, the sexes were the most dimorphic. By contrast, when the intersexual correlation was positive and substantial, the sexes were the least dimorphic. A recent survey confirms that this negative relationship extends to a variety of taxa and trait types (Poissant et al. 2009). Interestingly, however, there is a great deal of scatter in this relationship, particularly at low sexual dimorphism values (e.g., Bonduriansky and Rowe 2004; Poissant et al. 2009). Low phenotypic dimorphism was associated with high or low $r_{MF}$. Such a pattern may occur because $r_{MF}$ may be small whenever the covariance is small, the sex-specific genetic variances are large, or both - suggesting why the correlation between $r_{MF}$ and phenotypic sexual dimorphism can sometimes be weak (Poissant et al. 2009). Simulations confirm that high positive $r_{MF}$ can be associated with both low and high sexual dimorphism (Reeve and Fairbairn 2001), owing to differences in the relative magnitudes of denominator and numerator of $r_{MF}$. Furthermore, although temporary decreases in $r_{MF}$ are necessary for the evolution of sexual dimorphism, the equilibrium magnitude of $r_{MF}$ ultimately depends upon differences in selection, mutation, and genetic architecture between the sexes (Bonduriansky and Chenoweth 2009). For instance, if sex-specific selection has successfully made certain loci sex-limited, those formerly sexually antagonistic genes no longer contribute to the intersexual covariance. Under this scenario only concordantly selected loci contribute to $r_{MF}$, raising its value. Such processes may also explain why $r_{MF}$ and sexual dimorphism have a low correlation.

The potentially confounding standardization introduced by $r_{MF}$ demonstrates that examining the unstandardized intersexual covariance also has utility. It is possible to recast the univariate equation 6.3 for sexual dimorphism by making the components
of $r_{MF}$ explicit:

$$\Delta SD = \Delta \bar{Z}_m - \Delta \bar{Z}_f = \frac{1}{2} \left[ \frac{\sigma^2_{Am}}{\sigma^2_{P_m}} s_m - \frac{\sigma^2_{Af}}{\sigma^2_{P_f}} s_f \right] - \frac{\text{Cov}(Z_m, Z_f)}{\sigma_{P_m} \sigma_{P_f}} (s_m - s_f) \right] \left( s_m - s_f \right) \right] \right] \right] \tag{6.5}$$

Because the sign before the covariance term is negative, positive values will inhibit sexual dimorphism while negative values will accentuate it. For the assumptions $\sigma^2_{Am} = \sigma^2_{Af} = \sigma^2_{A}$ and $\sigma^2_{P_m} = \sigma^2_{P_f} = \sigma^2_{P}$ (i.e., $h^2_m = h^2_f = h^2$), dimorphism depends upon $\sigma^2_{A} - \text{Cov}(Z_m, Z_f)$. Intuitively, if male and female additive variances are identical and of the same nature, the extent of intersexual covariation is also the additive variance.

As we will discuss below, $\sigma^2_{A} - \text{Cov}(Z_m, Z_f)$ is equivalent to Lande’s (1980) multivariate formalism that the difference between the matrix of genetic variances and the matrix of the intersexual covariances (i.e., $G - B$) dictates the degree of dimorphism.

Relatively few studies have explicitly examined sex-specific differences in additive genetic variances and have shown significant differences on a trait-by-trait basis. A recent meta-analysis (Wyman and Rowe in prep) showed that of the 20 studies that tested for differences in sex-specific heritabilities, only 11 found differences in a subset of all traits examined (Mousseau and Roff 1989; Wilcockson et al. 1995; Mignon-Grasteau 1999; Ashman 1999, 2003; Jensen et al. 2003; Rolff et al. 2005; Fedorka et al. 2007; Zillikens et al. 2008; Gershman et al. 2010; Stillwell and Davidowitz 2010). Furthermore, only 2 studies tested for and found sex-specific differences in the unstandardized additive variances (Zillikens et al. 2008; Rolff et al. 2005). These data suggest that assuming $h^2_m = h^2_f$ is reasonable. In addition, a recent meta-analysis has shown that about half of published $r_{MF}$ values are extremely close to 1 (Poissant et al. 2009). Taken together, these data suggest extreme genetic constraints are indeed prevalent under a univariate perspective. However, it is unclear how general this insight is since traits do not evolve in isolation, but often in concert with other traits (e.g., Walsh 2007).
6.3.2 Multivariate changes in sexual dimorphism

Here we move from a single trait view to the multivariate framework proposed by Lande and Arnold (Lande 1979; Lande and Arnold 1983). As the expression of a trait can be correlated to the expression of other traits within the same individual, selection on the focal trait can cause an indirect response to selection in other traits. The multivariable response to selection is modeled as:

\[
\Delta \bar{Z} = G\beta
\]  
(6.6)

where \( G \) is the additive genetic variance-covariance matrix for the traits under consideration while \( \beta \) is the vector of the selection gradients for each trait. The elements of \( \beta \) is the vector resulting multiplying the inverse of the phenotypic matrix, \( P^{-1} \) and the vector of selection differentials, \( s \).

Lande (1980) re-purposed this multivariate approach to consider the evolution of sexual dimorphism since trait expression can be correlated between the sexes. Between- and within-sex evolution of traits is modeled as:

\[
\begin{pmatrix}
\Delta \bar{Z}_m \\
\Delta \bar{Z}_f
\end{pmatrix} = \frac{1}{2} \begin{pmatrix}
G_m & B \\
B^T & G_f
\end{pmatrix} \begin{pmatrix}
\beta_m \\
\beta_f
\end{pmatrix}
\]  
(6.7)

\( \Delta \bar{Z}_m \) and \( \Delta \bar{Z}_f \) are vectors representing changes in trait means in males and females, respectively. \( G_m \) and \( G_f \) represent the sex-specific additive genetic variance-covariance matrices for autosomal traits. \( \beta_m \) and \( \beta_f \) are vectors of the sex-specific selection gradients. \( B \) is the matrix of the intersexual covariances; the \( ij \)th element of the \( B \) matrix is the additive genetic covariance for character \( i \) expressed in males and character \( j \) expressed in females. Unlike \( G \) or \( P \) matrices, \( B \) is not necessarily symmetrical: the off-diagonal elements are not equivalent on either side of the diagonal, e.g., \( \text{Cov(Trait } i^f, \text{ Trait } j^m) \neq \text{Cov(Trait } i^m, \text{ Trait } j^f) \), where the superscripts \( m \) and \( f \) indicate which sex expresses
traits $i$ and $j$. $B^T$ is the transpose matrix of the $B$.

The change in sexual dimorphism is defined as the difference in the changes of the male and female phenotypes $\Delta SD = \Delta \bar{Z}_m - \Delta \bar{Z}_f$. This equation unfurled results in:

$$\Delta SD = \frac{1}{2}[(G_m \beta_m + B \beta_f) - (G_f \beta_f + B^T \beta_m)]$$

(6.8)

Because of the complexity of this equation, Lande (1980) made a few simplifying assumptions to elucidate its meaning. First, he assumed monomorphic $G$ matrices. Second, he assumed that $B = B^T$, resulting in:

$$\Delta SD = \frac{1}{2}[(G - B)(\beta_m - \beta_f)]$$

(6.9)

In other words, the extent of change in sexual dimorphism is governed by the similarity of the $G$ and $B$ matrices, in addition to differences in selection. As in the univariate case, the degree of similarity between $G$ and $B$ will constrain the efficacy of selection differences between the sexes. Simply, if male and female $G$ matrices are the same and if the intersexual covariance is complete, the $B$ matrix is simply the $G$ matrix; under these circumstances, no divergence is possible. Thus, as in the univariate case, the extent of covariation between the sexes is the limiting factor in the evolution of sexual dimorphism. However, what, if any, kind of support is there for these two simplifying assumptions, and how do the implications of the multivariate case differ from the univariate case?

First, in the univariate formulation, a single variance value only has a magnitude whereas in the multivariate formulation, the $G$ matrix has both a magnitude (i.e., eigenvalues), and an orientation (i.e., eigenvectors). Thus, comparing $G$ matrices between the sexes is a little more involved than statistically comparing a pair of heritabilities or variance values. It is possible to do an element-by-element comparison of the heritabilities and genetic correlations in the male and female $G$ matrices (e.g., Steven et al. 2007; Leinonen et al. 2010). But better yet is to take advantage of the multivariate framework
and compare eigenvectors and eigenvalues between the sexes. Eigenvectors describe a direction in multi-trait space while eigenvalues describe the magnitude or speed along that direction. Eigenvectors reveal the direction where the genetic variance is oriented while eigenvalues reveal how fast the predicted response to selection along that direction will be (Walsh and Blows 2009).

A common approach for comparing eigenvectors and eigenvalues is the Flury hierarchy analysis which compares a set of eigenvectors and eigenvalues for their shapes and relative sizes (Phillips and Arnold 1999). This analysis proceeds by testing a nested set of hypotheses in an ascending manner, or by comparing the various hypotheses to the hypothesis of no relationship. \( G \) matrices may be identical, sharing the same eigenvalues and eigenvectors. Alternatively, they may be proportional, sharing the same eigenvectors but having eigenvalues that differ by a constant. Or \( G \) matrices may share the same eigenvectors but nonproportional eigenvalues. Finally, \( G \) matrices may not share any eigenvectors and be completely different.

Several individual studies have shown that sexual dimorphism is common in sex-specific \( G \) matrices (Holloway et al. 1993; Guntrip et al. 1997; Ashman 2003; Rolff et al. 2005; Steven et al. 2007; McGuigan and Blows 2007; Campbell et al. 2011; Lewis et al. 2011). When the Flury hierarchical testing procedure is applied, by and large, \( G \) matrices seem to be conserved for their eigenvectors across populations, experimental treatments, species, and sexes in 75-78% of all studies (Arnold et al. 2008). In other words, the sexes appear to share all or some principle components. The direction along which the response to selection may proceed seems to be conserved overall between males and females. However, the sex-specific \( G \) matrices are not necessarily equivalent nor do they necessarily share proportional eigenvalues. So even if selection is the same between the sexes, the magnitude of the responses will differ along the shared directions. Furthermore, distinct, unrelated eigenstructures are by no means uncommon, representing 22% of sex-specific \( G \) matrix pairs (Arnold et al. 2008). Sex-specific \( G \) matrices that do not share any
eigenvectors mean that the response to selection for sexual dimorphism can potentially proceed along completely different axes in each sex. Assuming that sex-specific selection is aligned with these sex-specific axes, changes in sexual dimorphism will occur but for different sets of traits for each sex or in different proportions.

Second, in the univariate formulation, a single intersexual covariance value has a magnitude, whereas the $B$ matrix will have both a magnitude and an orientation. The additional intersexual covariances in the $B$ matrix can effectively re-orient the response of the sex-specific $G$ matrices to selection (e.g., Lewis et al. 2011; Gosden et al. 2012). The analogous situation in the univariate equation is when a single intersexual covariance can modulate the response to selection acting upon just the sex-specific additive variance (equation 6.5). However, a key difference is that positive intersexual covariances may only diminish univariate dimorphism, whereas in the $B$ matrix, a positive intersexual covariance can either augment or diminish the final change in multivariate dimorphism. As such, some have recommended setting the $B$ matrix equal to zero to see whether the response to selection for sexual dimorphism increases or decreases to measure its overall effect (e.g., Lewis et al. 2011). So far, empirical inclusion of $B$ substantially decreases the predicted phenotypic divergence between males and females (Lewis et al. 2011; Gosden et al. 2012). Furthermore, this decrease is beyond that predicted by considering the univariate quantity $r_{MF}$ alone (Gosden et al. 2012).

Of the 14 studies reporting sex-specific $G$ matrices, only 5 also reported the complete $B$ intersexual covariance matrix (Meagher 1999; Steven et al. 2007; Campbell et al. 2011; Lewis et al. 2011; Gosden et al. 2012). Among those studies, there is little evidence for the second assumption, that $B = B^T$. For example, the values reported by Steven et al. (2007) and Campbell et al. (2011) show that $B$ can be far from symmetric: female-to-male covariance and the male-to-female covariance can differ by an order of magnitude or have completely different signs. In fact, this asymmetry is suspected to be the cause of the greater impact of $B$ on one sex compared to the other sex. Both Lewis
et al. (2011) and Gosden et al. (2012) show that including $B$ re-orient the response to selection away from the direction of selection (i.e., orientation of $\beta$) more for females than males because $B$ is more similar to $G_m$ than to $G_f$. Both studies also show that $B$ increases the magnitude of the response more in males than in females. It is not possible to observe this effect in the univariate perspective because the breeder’s equation only has the intersexual covariance specific to a single trait and has no capacity to measure changes in orientation.

6.4 Relaxation from constraint

Some of the original simplifications made to understand the equations governing the evolution of dimorphism have only partial empirical support. $G$ matrices can differ either with respect to eigenvalues and/or eigenvectors between the sexes. Furthermore, multivariate intersexual covariances are not necessarily symmetric (i.e., male-to-female covariation $\neq$ female-to-male covariation). Here we discuss some of the empirical and mathematical implications of deviating from the original simplifications.

6.4.1 Differences in genetic variances

How might sexual dimorphism in variances or $G$ arise in practice? As genetic variances can be imagined as the sum of locus-specific allelic variances, sexual dimorphism in allele frequencies, additive effects, and dominance effects can all contribute. Empirically, alleles can have different additive effects or different dominance coefficients in males versus females (Fry 2010). Furthermore, especially in the case of new mutations with sex-specific effects, allele frequencies can differ between the sexes (e.g., Rice 1984; Fry 2010).

It is also possible for the $G$ matrix to be dimorphic due to sex-linkage, even though both the breeder’s equation (eqn. 6.3) and the multivariate evolution equation
(eqn. 6.7) assume autosomal linkage of genes. Sex-linked genes alter genetic variances through differences in the number of sex chromosomes (X or Z) in each sex. As the heterogametic sex has only one allelic copy, it may only express the extreme phenotypes - e.g., dominant or recessive. By contrast, the homogametic sex can express all three states. As a result, the heterogametic sex has twice the additive genetic variance of the homogametic sex (Lynch and Walsh 1998). In XY and XO species, males should have higher genetic variance than females. Conversely, in ZW species, females should have higher variance than males. But whatever the influence of sex-linkage on the genetic variance, the effect would, of course, be modulated by the number of autosomal versus sex-linked loci affecting the trait. It is not yet clear whether the negative covariance engendered by sex-linkage (e.g., Chenoweth et al. 2008) will systematically decrease positive covariances brought on by autosomal linkage.

As described above, single trait variances and the multi-trait $G$ matrix differ because matrices can be redefined in terms of eigenvalues and eigenvectors. Understanding differences in the orientation of sex-specific $G$ matrices will become important as the number of such studies increase. Since $G$ matrices have as many eigenvalues as there are number of traits being measured, it is important to know which is the principle eigenvalue and how many of the other eigenvalues also describe a large (or statistically significant) proportion of the total variance. Recent reviews suggest that many $G$ matrices seem to be ill-conditioned such that most of the genetic variance is explained by the first 1 or 2 eigenvalues (Kirkpatrick 2009; Walsh and Blows 2009). Thus, while many traits may be measured, the response to selection may effectively only proceed along 1 or 2 dimensions. With respect to the evolution of sexual dimorphism, these effective dimensions can differ in the percent variance explained, in orientation, or in total number between males and females. If so, sexual dimorphism may evolve even without sex-specific selection differences - as long as the genetic variance in one sex is not totally misaligned with the selection vector. McGuigan and Blows (2007) compared male and female differences in
genetic dimensions and found that females possessed a greater number of effective dimensions than males in *Drosophila bunnanda*. In *Drosophila serrata*, multivariate selection affected the multivariate genetic variances in a sex-specific manner; different sets of axes were affected in males versus females with respect to population divergence (Chenoweth and Blows 2008).

That the number of traits examined and the number of effective dimensions differ is potentially worrisome since it suggests that selection may not proceed along all trait axes (Kirkpatrick 2009). But in terms of the evolution of sexual dimorphism, it may not be necessary for sex-specific differences to proceed along all potential directions. In fact, phylogenetic comparisons suggest that usually it is similar variations in the same trait that persist among related lineages - e.g., horns in cervids, horns in dung beetles, plumage in birds, color patterns in Lepidoptera - rather than variations among completely different traits among related lineages. Such patterns suggest that underlying differences in genetic architecture between the sexes is the only requirement for diversity, at least considered in the short-term timescales considered by the quantitative genetics equations. In other words, as long as those 1 or 2 dimensions differ in their lengths and/or orientations between the sexes, persistent sexual dimorphism may occur. The conservation of eigenvectors (Arnold et al. 2008) would suggest that sexual differences in eigenvalues may be more important to the diversity seen between homologous traits between males and females.

6.4.2 $B \neq B^T$

In the univariate breeder’s equation, positive intersexual covariances are always a constraint on evolving sexual dimorphism. By contrast, in the multivariate formulation, intersexual covariances can help or hinder changes in the extent of sexual dimorphism. It is possible to see this by applying equation 6.8 to a simple two trait example. Consider here the homologous characters $K$ and $L$, measured in males ($m$) and females ($f$). The
response to selection for sexual dimorphism in the shared homologous trait \( K \) evolves as:

\[
\Delta S D_K = \frac{1}{2} \{ \text{Var}(K^m) \beta^m_K - \text{Cov}(K^m, L^m) \beta^m_L \} - \text{Var}(K^f) \beta^f_K - \text{Cov}(K^f, L^f) \beta^f_L - \text{Cov}(K^m, K^f) \left[ \beta^m_K - \beta^f_K \right] - \text{Cov}(K^f, L^m) \beta^m_L + \text{Cov}(K^m, L^f) \beta^f_L \}
\]

The response in sexual dimorphism for trait \( K \) (\( \Delta S D_K \)) is very complicated even in the simple two trait example. For the moment setting aside the \( \beta \) terms, equation 6.10 can more simply be interpreted as terms that depend upon either genetic variances or genetic covariances. The equation consists of: the sex-specific genetic variances (\( \text{Var}(K^m) \), \( \text{Var}(K^f) \)); the cross-trait, within-sex genetic covariances (\( \text{Cov}(K^m, L^m) \), \( \text{Cov}(K^f, L^f) \)); the within-trait cross-sex genetic covariances (\( \text{Cov}(K^m, K^f) \), \( \text{Cov}(K^f, K^m) \)); and the cross-trait cross-sex genetic covariances (\( \text{Cov}(K^m, L^f) \), \( \text{Cov}(K^f, L^m) \)). The negative sign of term 6.10c means that when \( \text{Cov}(K^m, K^f) \) is strongly positive, it will decrease the total potential response to selection between the sexes (6.10a - 6.10b). When \( \text{Cov}(K^m, K^f) \) is strongly negative, it will increase the difference between the sexes; \( \text{Cov}(K^m, K^f) \) is also present in the breeder’s equation and is used to calculate \( r_{MF} \).

Because equation 6.10 describes two traits, it has additional intersexual covariance terms (6.10d), and their relative magnitudes will determine how the covariation between the sexes affects \( \Delta S D_K \). Interestingly, these terms can have a positive or negative sign, as a result, positive intersexual covariances can hinder or facilitate changes in sexual dimorphism. By contrast, in the breeder’s equation (6.3) positive covariances could only hinder. The difference between the last two terms (6.10d) is a measure of how asymmetrical the \( B \) matrix is. The larger this difference, the greater its impact on the changes in sexual dimorphism. Even though equation 6.7 is for traits with an
autosomal basis, the asymmetry between $\text{Cov}(K^f, L^m)$ and $\text{Cov}(K^m, L^f)$ may effectively be enhanced by factors ultimately emerging from the sex chromosomes (e.g., Williams and Carroll 2009). Phenomena such as sex-biased gene expression or sex differences in the number of expressed autosomal genes may drive this asymmetry.

At first it may seem mysterious that a positive intersexual covariation between two different traits, each of which is expressed in only one sex, can promote dimorphism. However, a familiar example is the positive intersexual covariation between a male indicator trait and female preference seen in Fisherian runaway selection (Fisher 1915, 1958; Lande 1981); an increase in female preference results in an increase in male trait size due to the between-sex genetic correlation. In the Fisherian runaway example, the covariance of a male trait and female preference is non-zero, but the covariance of a female trait and male preference is zero (or, does not exist). In other words, the difference between $B$ and $B^T$ is at its maximum. The inclusion of extremely sex-biased or sex-limited traits in general will enhance the asymmetry of $B$.

It may also be that the asymmetry observed so far actually points to the prior efficacy of sex-specific selection in producing sexual dimorphism. A recent analysis suggests that the correlational selection on two traits can produce genetic correlations of the same sign and direction as the selection (Roff and Fairbairn 2012). Effectively, correlational selection can cause the $G$ matrix to point in the same direction of the selection as it accumulates mutations oriented in this same direction. An analogous phenomenon may account for why $B$ matrices are asymmetrical, as the pattern of intersexual covariances has altered to facilitate sex-specific selection (Barker et al. 2010; Delph et al. 2011).

While the asymmetry of the $B$ matrix refers to the off-diagonal elements, it is also possible to analyze the covariation patterns of the on-diagonal elements. Mother-son and father-daughter patterns of intersexual covariations should not be different, leading to one value for the intersexual covariance of a single trait. However, there is some empirical evidence which suggests the opposite (Bonduriansky and Rowe 2004). Heritabilities
estimated through a single parent can sometimes yield different values for daughters versus sons. A sex-specific genomic imprinting process might cause altered patterns of resemblance through one set of male-female relatives than through the opposite set of female-male relatives (Day and Bonduriansky 2004); however, this phenomenon requires further study.

6.5 Discussion

Biologists have long wondered how sexual dimorphism might arise given that males and females share a common genetic architecture that ought to impede phenotypic divergence. A closer examination of the univariate and multivariate equations suggests that divergence may be less difficult than is currently believed. Dimorphism may evolve because genetic variances are sexually dimorphic. Dimorphism may also evolve when genetic variances are monomorphic, as long as the intersexual genetic covariance is small. A review of the data suggest that genetic variances can be dimorphic or monomorphic in single trait studies. However, multivariate studies indicate that $G$ matrices are mainly dimorphic and that $B$ is asymmetrical. Such differences in sex-specific genetic architectures can facilitate the evolution of sexual dimorphism if selection is aligned accordingly. We describe some implications of dimorphic genetic variances and asymmetrical covariance structures for the diversity of sexually dimorphic traits.

6.5.1 Sexual dimorphism in genetic variances

In a sense, Darwin’s one step model of sexual dimorphism was so simple that it did not ostensibly require theoretical attention. By contrast, the two-step model posed a conundrum. A novel trait appears in both sexes but benefits only one sex and harms the opposite sex. Such a process drags mean population fitness down: the positive intersexual covariance will permit expression in the non-benefitting sex which results
in selection against expression, preventing optimal trait evolution in the helped sex. This interference was elucidated mathematically by setting sex-specific genetic variances equal and focusing upon the intersexual covariance term (e.g., Lande 1980; Cheverud et al. 1985). Once males and females have the same genetic variances for a shared trait, the extent of phenotypic divergence will only be dictated by the intersexual covariance. However, if we allow dimorphism in genetic variances, the univariate and multivariate equations both show that sexual dimorphism also depends on how much genetic variance there is in each sex. In other words, sometimes there may be pre-existing genetic variance for sexual dimorphism, waiting to respond to sex-specific selection, much like Darwin’s de novo hypothesis for sexual dimorphism.

Multivariate differences in genetic variances between the sexes can be more stark than univariate differences. Single trait studies indicate that while heritabilities and variances can be sexually dimorphic, overall they are not (Wyman and Rowe in prep). By contrast, all studies that have measured sex-specific $G$ matrices, demonstrate that they are dimorphic (Holloway et al. 1993; Guntrip et al. 1997; Ashman 2003; Rolff et al. 2005; Steven et al. 2007; Campbell et al. 2011; Lewis et al. 2011; Gosden et al. 2012). Males and females generally seem to share the same eigenvectors, but their eigenvalues can differ. In addition, the sexes can differ in their respective effective number of dimensions. These biological differences between the univariate and multivariate perspectives is due to the fact that multivariate formulation can take into account how the genetic variance is oriented between the sexes. Previous authors have pointed out that an eigenvalue of the multivariate $G$ matrix will explain always more variance than any particular univariate genetic variance alone (except when all covariances are zero) (Mercer and Mercer 2000; Kruuk and Garant 2007; Chenoweth and Blows 2008). Thus, multivariate sexual dimorphism will possess more evolutionary potential than univariate sexual dimorphism by default. However, heeding the geometry of $G_m$ and $G_f$ as well as their effective number of dimensions will further accentuate the differences between males and females in their
Finally, while sex-specific genetic variances are important, they need not impose lasting constraints. A particular $V_A$ or $G$ matrix may evolve, thereby affecting its impact on the evolution of sexual dimorphism. Indeed, the factors supporting long term permanence of the $G$ matrix are not well understood (Arnold et al. 2008). While selection can change phenotypic means, selection also may alter genetic and phenotypic variances. For instance, male sexually selected traits oftentimes experience strong directional selection while homologous female traits are more likely experience stabilizing selection (Pomiankowski and Moller 1995; Rowe and Houle 1996). Strong directional selection can briefly increase the variance if the frequencies of rare alleles rise (Barton and Turelli 1987; Blows and Higie 2003). Yet, sustained selection (directional or stabilizing) is expected to deplete genetic variation. Each of these conditions alters the genetic variance in males. Conversely, in females, if stabilizing selection is weaker or stronger relative to males or relative to the past, by definition, variances differ (e.g., Rundle and Chenoweth 2011). Thus, even if initially the phenotypic and genetic variances are equivalent between males and females, following sex-specific selection, they may not be. As a result, the evolution of dimorphism may substantially differ before and after selection. Some theoretical work also suggests that while variance-covariance structures can determine the short-term responses to selection, ultimately long term responses rely only upon the type of operating selection (e.g., stabilizing versus directional) (Zeng 1998).

### 6.5.2 Intersexual covariances

Considering only the univariate quantity $r_{MF}$ can give a misleading picture of trait evolution between the sexes for two reasons. First, $r_{MF}$ is subject to issues of standardization. The correlation may be small when the intersexual covariance is small, or when the sex-specific genetic variances are large, or both — suggesting why $r_{MF}$ does not always have a negative correlation with sexual dimorphism (Reeve and Fairbairn...
Second, $r_{MF}$ misses all of the additional cross-trait, intersexual covariance terms. In the univariate formulation positive intersexual covariances may only decrease the response to selection for sexual dimorphism. By contrast, the multivariate formulation is more general and positive values may increase or decrease the response to selection for dimorphism.

The recent data suggests that $B$ has the potential to constrain sex-specific responses to selection. However, the impact of the constraint is stronger in one sex than the other owing to the asymmetrical nature of the $B$ matrix. Since complete monomorphism would suggest that male-to-female and female-to-male patterns of trait covariance are the same, the asymmetry of $B$ may indicate the role of prior or current sex-specific selection shaping the covariance structure. Recent experiments suggest that the intersexual covariances themselves can be subject to change due to selection (Delph et al. 2011). Furthermore, theory and data suggest that the $B$ matrix may be intrinsically more pliable than the $G$ matrix given that its values are not realized in any particular individual (Barker et al. 2010). $B$ matrices among populations are more variable for eigenstructure compared to $G$ matrices (Barker et al. 2010) indicating that they can change more easily. Finally, in principle $B$ should also be able to facilitate sex-specific adaptation even though the evidence is thus far lacking.

### 6.5.3 Diversity in sexual dimorphism

Allowing for dimorphism in genetic variances and lability in the intersexual covariance structure may account for widespread dimorphism among animal and plant taxa in a multivariate context. Dimorphic $G$ would ensure a dimorphic response to identical selection pressures in both sexes. Once a trait is nearly or completely sex-limited, the genetic variances are dimorphic. It is then entirely possible for sex-specific selection to repurpose the standing dimorphism in $G$ for additional alterations to the phenotype. So,
no matter how long it took to evolve dimorphic $G$ matrices, once in place, it may be easy to introduce further sexual dimorphism afterwards. In this case, both selection and $G$ facilitate phenotypic sexual dimorphism. Such a process may account for the how homologous ornaments across taxa can vary so widely, e.g., *Drosophila* genitalia, dung beetle horns, cervid horns, butterfly colors, bird plumage etc. Indeed, sexually selected traits often possess higher levels of additive genetic variance over ordinary metric traits (Pomiankowski and Moller 1995). The increased level of genetic variation may also explain why dimorphism exhibits so much phylogenetic lability (e.g., Price and Birch 1996; Burns 1998; Amundsen 2000; Coyne et al. 2008; Oliver and Monteiro 2011). Furthermore, it would be consistent with the fact that sexual traits can demonstrate astonishingly quick phenotypic changes over short phylogenetic time scales. Sexual selection may be constantly re-shaping characters that already differ between males and females in their underlying architecture because those are precisely the traits that represent the path of least genetic resistance. While it is possible for sexual conflict to occur over sex-limited traits (e.g., Harano et al. 2010), it is also possible that such traits can have within-sex covariance patterns in the $G$ matrix that can help mitigate this conflict.

Because of dimorphism in genetic variances, one could just as easily formulate hypotheses about their relationship with phenotypic sexual dimorphism: e.g., sexual dimorphism in genetic variances is expected to correlate positively with the degree of phenotypic sexual dimorphism. In other words, neither the theory nor the data yet suggest the sole importance of the intersexual covariance over genetic variances in determining the final extent of phenotypic sexual dimorphism. Intersexual covariances, sexual dimorphism in genetic variances, and selection gradients all matter together; their particular combinations in particular systems deserve further and more adequate consideration. It will be interesting to know how often sexual dimorphism exists simply because of sex-specific differences in genetic architecture, versus because sex-specific selection is acting upon already dimorphic genetic architectures.
The paradox of widespread sexual dimorphism in the face of a constraining genetic architecture is partly resolved by considering multivariate trait combinations. However, without some prior expectation, it will ultimately be difficult to assess how much sexual dimorphism failed to evolve within lineages. The quantitative genetics equations are best for short-term predictions as it is unclear how stable variances and covariances may be over longer time periods or how their relative importances shift. As such, even the apparently high levels of sexual dimorphism seen in nature may be far less than the amount expected under a scenario of widespread sexually antagonistic selection. Comparisons of realized and potential levels of sexual dimorphism will be necessary to assess whether or not sexual differences are indeed difficult to evolve.

6.5.4 Conclusions

Although it is reasonable to assume that sexual dimorphism in traits is hard to evolve, this is not an underlying feature of the equations describing their evolution. Multivariate sexual dimorphism is potentially easier to evolve than univariate sexual dimorphism, perhaps reconciling the fact that sexual differences are nearly universal despite the widespread prevalence of very high intersexual genetic correlations. Perhaps once early in the evolution of modern flora and fauna, the ur-male and ur-female literally only differed in reproduction so that it was easy to assume that intersexual covariances severely constrained their initial divergence in shared traits and that \( r_{MF} \) had near total explanatory power. Now that recent evidence weighs in against assuming homogeneity of variances and stability in the intersexual covariance structure, it is important to recognize that even while the sexes might temporarily impede the adaptation of the other, the declines in fitness need not be permanent. Additional work on the sex-specific selection, sex-specific \( G \) matrices and the \( B \) matrix will enable us to dissect the forces underlying the evolution of sexual dimorphism and arrive at a fuller understanding of how present differences have come to be.
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Chapter 7

Concluding remarks

“The cause of the greater general variability in the male sex, than in the female is unknown, except in so far as secondary sexual characters are extraordinarily variable and are usually confined to the males; and as we shall presently see, this fact is to a certain extent, intelligible.”

-Charles Darwin, p. 224, The Descent of Man, 2nd ed.

7.1 General summary

In this thesis I have examined several sources of variation governing the extent of sexual dimorphism among populations. I have asked a series of basic questions relating to the assumptions of a verbal model for the evolution of dimorphism. Because males and females are expected to share many genes, selection in one sex will cause a correlated response in the opposite sex. As a result, phenotypic sexual divergence may be hindered. I have analyzed this verbal model by 1) exploring two mechanisms to weaken the inter-sexual covariance, 2) studying how novel sexual dimorphism might build upon current
sexual dimorphism, and 3) asking whether genetic variances do actually differ between the sexes.

### 7.2 Summary of major findings

In Chapter 2 I found that condition can be a significant source of variation in the extent of sexual dimorphism in gene expression. Gene expression, like many other morphological and behavioral traits, is sexually dimorphic: male-biased genes are up-regulated in males and female-biased genes are up-regulated in females. I show for the first time that the extent of this up-regulation is contingent upon an individual’s quality.

Because Chapter 2 showed that sex-biased gene expression may be costly, relying upon condition, it is important for males and females to express all genes in a manner adaptive for each sex separately. In Chapter 3 I investigated how gene duplication might be used as a way to mitigate conflicts over sex-biased gene expression. I show that about half of all paralog pairs have expression patterns that differ between members. This means that selection might requisition redundancy to facilitate sex-specific divergence in expression.

In Chapter 3 I also found that many paralog pairs had concordant expression patterns. In particular, there was an excess of gene duplicates with two male-biased genes, suggesting that duplication events can heighten pre-existing phenotypic sexual dimorphism. In Chapter 4 I also studied this principle of existing sexual dimorphism forming the basis for additional sexual dimorphism by analyzing the effects of sexually dimorphic recombination. Because recombination is important to the adaptation, I asked whether sexually dimorphic recombination could facilitate the invasion of novel variation with sexually dimorphic fitness effects. I find that this is possible and that dimorphism in recombination is potentially important to the introduction of new sexually antagonistic alleles.
Finally, in Chapters 5 and 6 I studied whether genetic variances differed in males and females and the implications of such differences. By conducting a meta-analysis I was able to determine that male and female genetic variances are, by and large, monomorphic for shared traits. Combined with a high intersexual genetic correlation, this suggests that common genetic architectures will be a strong impediment to phenotypic divergence between the sexes in single traits. However, the distribution of $Male - Female$ variances had significant right skews, indicating that there was an excess of traits with male-biased variances. So, even while on average males and females did not have significantly different additive variances, in certain instances they could. Such traits could be under strong male-biased selection, or be related to such traits, or be unconstrained because they are neutral with respect to fitness.

When studied from a multivariate perspective, it is possible that there is more freedom to evolve sexual dimorphism. The multivariate formulae are more general and allow us to see that positive intersexual covariances can both facilitate and hinder sexual dimorphism. Furthermore, while genetic variances have a magnitude, in the multivariate formulation they also have an orientation. Differences in magnitude, orientation, or both can facilitate or hinder the evolution of male-female differences.

### 7.3 Future avenues of study

I have examined a number of different mechanisms for the evolution of sexual dimorphism, each of which suggests that generating male-female differences may not be so difficult after all. However, I did not explore many other aspects.

Some specific follow-up questions to this dissertation remain. In the realm of condition-dependence, it is not clear whether those sex-biased genes that responded the most strongly to condition are indeed the most closely allied to sex-specific fitness compared to the most weakly responding genes. In addition, it is known that gene duplication
can permit tissue specialization in extra copies. In particular, in *Drosophila* old copies are often widely expressed while new copies are expressed in testes (e.g., Belote and Zhong 2009). Can this type of specialization occur in a sex-specific manner for non-sexual organs and tissues?

Some more general questions also require attention. There are relatively few theoretical studies of how the genetic covariance between the sexes weakens over time to evolve sexual dimorphism. We only understand that it must weaken by genetic mechanisms such as gene duplication, genomic imprinting, sex-specific allelic expression, and condition-dependence. However, we do not know which mechanisms are the most common and how quickly each mechanism can act. Presumably, alleles that decrease the intersexual covariance are selected over the course of the evolution of sexual dimorphism (Delph et al. 2011). The process of selection can sometimes allow the persistence of new mutations which permit the genetic variance to be oriented in the direction of selection. However, we do not know how high the mutation rates that alter intersexual covariances must be in order to facilitate any of the mechanisms to evolve dimorphism. Variation in such mutation rates may provide the ultimate reason for why certain species are more sexually dimorphic overall compared to other species.

### 7.4 Final remarks

The bewildering array of sex-specific adaptations present in nature was the starting point for Darwin. Their remarkable and consistently high diversity had inspired the theory of sexual selection and continues to inspire evolutionary biologists today. However today, the availability of high-throughput technological methods have made it possible to catalog and study sexual dimorphism in traits formerly invisible to the naked eye. Thus, recent data have revealed that traits like nucleotide sequence and gene expression can differ starkly between male- and female-specific genes, surprising many at the incredible
breadth and scope of sex-specific selection. All this is “intelligible”, as Darwin (1871) remarks, under the premise that male reproductive success is more variable than female reproductive success as in Bateman’s (1948) principle.

Because the existence of separate sexes (or of separate male and female functions) is so widespread, this dissertation is broadly relevant to a variety of taxa. The differences due to anisogamy are basic ones. As long as gamete sizes are uneven and as long as one sex has more to invest than the opposite sex, the intensity and direction of selection will differ between males and females. It remains an exciting challenge to understand how and why organisms differ in their extent of sexual dimorphism from gametes to gene expression, to higher level phenotypes such as morphology and behavior.
Bibliography


