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The genetic architecture of sexual dimorphism

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ACTA
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2015

ISSN 1651-6214
ISBN 978-91-554-9278-6
urn:nbn:se:uu:diva-258986

Dissertation presented at Uppsala University to be publicly examined in Lindahlsalen, Evolutionary Biology Centre, Norbyvägen 18, Uppsala, Friday, 18 September 2015 at 10:00 for the degree of Doctor of Philosophy. The examination will be conducted in English. Faculty examiner: Russell Bonduriansky (University of New South Wales).

Abstract

Griffin, R. 2015. The genetic architecture of sexual dimorphism. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1264. 56 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-554-9278-6.

Phenotypic differences between the sexes evolve largely because selection favours a different complement of traits in either sex. Theory suggests that, despite its frequency, sexual dimorphism should be generally constrained from evolving because the sexes share much of their genome. While selection can lead to adaptation in one sex, correlated responses to selection can be maladaptive in the other. In this thesis I use *Drosophila* to examine the extent to which the shared genome constrains the evolution of sexual dimorphism and whether the sex chromosomes might play a special role in resolving intralocus sexual conflict.

Gene expression data shows that intersexual genetic correlations are generally high, suggesting that genes often affect both sexes. The intersexual genetic correlation is negatively associated with sex-bias in expression in *D. melanogaster*, and the rate of change in sex-bias between *D. melanogaster* and six closely related species, showing that a sex-specific genetic architecture is a prerequisite for the evolution of sex difference. In further studies I find that genetic variance affecting lifespan is found in the male-limited Y chromosome within a population, which could offer a route to the evolution of further sexual dimorphism in lifespan, though the amount of variance was small suggesting adaptive potential from standing genetic variance is limited. Genetic variance on the X chromosome is also expected to be depleted once the sex chromosomes evolve, but here I find no evidence of depletion in either sex. Dosage compensation does not appear to double the male X-linked genetic variance, but this effect may be complex to detect. Finally, the X chromosome appears to be enriched for sex-specific genetic variance, and the consequences of this are explored using a variety of analytical methods to test biologically meaningful aspects of G-matrix structure.

In summary, this thesis suggests that the evolution of sexual dimorphism is generally constrained by the shared genome, but intralocus sexual conflict could be resolved by novel mutations on the Y chromosomes, and by standing sex-specific genetic variance on the X chromosome. It highlights a special role for the X chromosome in the evolution of sexual dimorphism.

Keywords: Drosophila melanogaster, evolution, intralocus sexual conflict, sex chromosomes, sexually antagonistic selection, sexual dimorphism

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ISSN 1651-6214
ISBN 978-91-554-9278-6
urn:nbn:se:uu:diva-258986 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-258986)

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I **Griffin, R. M.**, Dean, R., Grace, J. L., Rydén P., Friberg, U. (2013) The shared genome is a pervasive constraint on the evolution of sex-biased gene expression. *Molecular Biology and Evolution*, 30(9):2168–2176.

II **Griffin, R. M.**, Le Gall, D., Schielzeth, H., Friberg, U. Within-population Y-linked genetic variation for lifespan in *Drosophila melanogaster. In press, Journal of Evolutionary Biology*.

III **Griffin, R. M.**, Schielzeth, H., Friberg, U. Autosomal and X-linked additive genetic variation for lifespan and ageing: comparisons within and between the sexes in *Drosophila melanogaster*. *Submitted manuscript*.

IV **Griffin, R. M.**, Schielzeth, H., Friberg, U. Adaptation across the genome: multivariate decomposition indicates different roles for the X and autosomes. *Manuscript.*

Contents

Introduction

The evolution of sexual dimorphism

Phenotypic differences between the sexes are prevalent in nature, and while classic examples are often drawn from extremes such as the peacocks tail, sexual dimorphism is present to some extent throughout sexual species in physiological, morphological, behavioural, and life history traits. Because access to mates is often a limiting factor on male fitness, but not female fitness, selection within the two sexes often favours a different complement of traits. Sexual selection might favour males with extravagant traits, through male-male competition and female mate choice, while natural selection might select against such traits (Darwin 1871). For example, sexual selection caused by competition for access to females may favour male deer with larger antlers, but the effect of carrying larger antlers is selected against by natural selection in females (because of cost of production and maintenance, predation risk, effects on mobility). Sexually antagonistic selection, when the total effect of selection within each sex favours different trait values, is the major mechanism underlying the evolution of sexual dimorphism.

Phenotypic value is determined by a combination of genetic and environmental effects. Adaptive evolution occurs when selection at the phenotypic level increases the frequency of beneficial alleles, genetic variants that produce the favourable phenotype. The net effect of selection is the evolution of the male and female traits towards their respective phenotypic optimum, by spreading the alleles which best allow those phenotypes to be realised. However, much of the genome is common to both sexes, and the genes that affect the trait in one sex will often affect the trait in the other sex. As a result, when different phenotypes are favoured in the sexes, there is a potential for intralocus sexual conflict to occur. When this conflict does occur it results in an evolutionary tug-of-war with at least one of the sexes suffering reduced fitness. Research aimed at developing the understanding of how the evolution of sexual dimorphism is affected by a shared genome is an increasingly active area of evolutionary biology (for example: Lande 1980; Bonduriansky and Rowe 2005; Bonduriansky and Chenoweth 2009; Arnqvist and Tuda 2010; Innocenti and Morrow 2010; Lewis et al. 2011; Connallon and Clark 2014).

Variance in phenotype and its components are important concepts for the study of evolutionary biology. As selection affects the frequency of alleles in

a population it brings a change in the affected traits. The amount of genetic variance underlying a trait determines the rate at which that trait can respond to selection (Fisher 1930). Genetic variance occurs when there are different alleles responsible for the variance in phenotype. If there is genetic variance in a trait subject to selection, then selection should produce a change in the frequency of alleles from one generation to the next because those carrying beneficial alleles have higher reproductive success. The response to selection can be formalised using the multivariate breeders equation, referred to as the Lande equation (Lande 1979, 1980), where $\Delta\bar{z}$ is the response for a vector of traits (z) with length *m*, **G** is an $m \times m$ genetic variance-covariance matrix, and β is a vector of selection coefficients (partial regression coefficients of fitness on the traits included in the study) also of length *m*.

$$\Delta\bar{z} = \mathbf{G}\beta$$

Simply put, this equation shows the change that occurs in a trait is determined by both the selection imposed, and the genetic variance available. It is with the G-matrix that the effect of a shared genome can be properly understood. When genes affect more than one trait they act pleiotropically, and if we consider the male and female phenotype as separate traits, then genes which affect both are pleiotropic (Roff 1997). It is reasonable to expect that many of the genes affecting a trait present in one sex will frequently affect the analogous trait in the other sex. For example, it is likely that many of the genes determining wing length in female fruit flies are also affecting wing length in males. Pleiotropy, therefore, causes covariance between the sexes, and expanding the Lande equation in to male (M) and female (F) specific responses it is clear that selection in one sex can produce a correlated response in the other due to this covariance (Lande 1980; Agrawal and Stinchcombe 2009).

$$\Delta z_M = G_M\beta_M + G_{F,M}\beta_F$$
$$\Delta z_F = G_F\beta_F + G_{M,F}\beta_M$$

Furthermore, this illustrates why the evolution of sexual dimorphism from a shared genome is such a curious phenomenon. Given that sexual dimorphism predominantly evolves due to sexually antagonistic selection, the response to selection can be reduced, or even reversed, because of pleiotropy. The evolution of sexual dimorphism should require that selection can produce a response in one sex, without producing a response in the other, which requires a sex-specific genetic architecture.

Resolving intralocus sexual conflict

There are a number of routes through which a sex-specific genetic architecture can be attained. While much of the genome is shared, some components of the genome are sex-limited (Ellegren and Parsch 2007). Heteromorphic sex chromosomes, such as the X and Y chromosome, are found in numerous species (Bachtrog et al. 2014), and evolve following the cessation of recombination between them (Beukeboom and Perrin 2014). Y chromosomes are limited to males, being passed only from father to son. The Y could, therefore, offer a route to the evolution of sexual dimorphism through the genetic variance they harbour (Rice 1984). However, the unusual dynamics of the Y chromosome could place limits on adaptive potential.

Despite coming from a homologous ancestral pair of autosomes (Parisi et al. 2003; Beukeboom and Perrin 2014), the X and Y often show vastly different numbers of protein coding genes due to several population genetic processes, ultimately stemming from the cessation of recombination (Engelstädter 2008), that contributes to Y chromosome degeneration. Muller's ratchet, Hill-Robertson interference, background selection, selective sweeps, and genetic hitchhiking are all expected to make Y-linked genes poorly adapted, which eventually causes their shut-down and loss (Charlesworth and Charlesworth 2000, 2010; Beukeboom and Perrin 2014). The Y chromosome is not only expected to be depleted for genes, leaving limited opportunity for Y-linked genetic effects, but many of the same processes will reduce within-population variance in genes that remain. Genetic variance is a prerequisite of adaptation (Fisher 1930). Genetic variance within a population of Y chromosomes will also be reduced by genetic drift because the effective population size is smaller relative to the X and autosomes ($N_Y = \frac{1}{3}N_X = \frac{1}{4}N_A$), bottlenecks caused by large reproductive variance in males, and because hemizygosity exposes all mutations to selection (while autosomal alleles can be hidden from selection by recessivity).

Accordingly, it is a common observation that Y chromosomes feature both a small portion of the protein coding genes in a species and carry little genetic variation. In *Drosophila melanogaster*, for example, the X contains more than 150 times as many protein coding genes as the Y (Bachtrog 2013). Male *D. melanogaster* lacking a Y chromosome (XO) also remain viable and exhibit little phenotypic difference from XY males, though XO males are infertile (Bridges 1916). The *D. melanogaster* Y chromosome is also completely heterochromatic, which reduces transcription rates, and the few genes that do persist show low levels of nucleotide polymorphism (Larracuente and Clark 2013).

Although substantial Y-linked genetic variance within population has been shown to affect male fitness in fruit flies (Chippindale and Rice 2001), and some studies have shown that the Y chromosome also exerts a small effect on phenotypic variation within populations for some male-limited

traits (Carvalho et al. 1997; Montchamp-Moreau et al. 2001; Huttunen and Aspi 2003; Branco et al. 2013), it is only recently that the dogma of an insignificant role for adaptation through the Y chromosome has begun to be re-evaluated (Mank 2012). This paradigm shift was largely caused by a study of Y-linked variation among populations (Lemos et al. 2008). That study by Lemos and colleagues, along with several supporting follow-up studies, show that the Y can exert an influence on gene expression in potentially thousands of genes spread throughout the genome and, although many are testes-specific, many are expressed in both sexes (Lemos et al. 2008; Jiang et al. 2010; Sackton et al. 2011). This gives the Y a potential role in alleviating intralocus sexual conflict at loci throughout the genome where conflict is caused by sexually antagonistic selection over gene expression level, promoting the evolution of sex-biased genes (Ellegren and Parsch 2007). It has been suggested that the Y can affect gene expression by altering the heterochromatin landscape of the genome (Jiang et al. 2010; Lemos et al. 2010). Genes near heterochromatic regions in the rest of the genome can have gene expression reduced as a result of an "overflow" of heterochromatin effects. It is thought that the Y chromosome acts as a sink of heterochromatin in males, therefore genes near heterochromatin regions may have lower expression in females since the overflow effect does not occur in males.

While the Y chromosome is now known to generate variance for gene expression throughout the genome, when comparing Y chromosomes from multiple populations, and that small amounts of Y-linked genetic variance have been found within populations for male-limited traits, little is known about how much Y-linked genetic variance persists within populations for traits expressed in both sexes. Given the possible role of the Y chromosome in negating intralocus sexual conflict, it is more likely that it will exert influence on sexually dimorphic traits rather than sexually monomorphic traits. Considering Y-linked variance could stem largely from *trans*-acting regulatory effects, complex traits could also be more consistently affected (i.e. there should be less variance in the relative magnitude of Y-linked genetic variance among complex traits than simple traits) by having more potential regulatory targets. Furthermore, lifespan, which is frequently sexually dimorphic, and other related traits have known links to the heterochromatin landscape, through which the Y chromosome is known to exert at least some of its regulatory effects. Such traits would be prime candidates in which to assay within-population Y-linked genetic variance.

Despite having a potentially special role in resolving intralocus sexual conflict, by being sex-limited, the Y chromosome is expected to be generally inert, with limitations on its adaptive potential expected to be imposed by its inability to retain standing genetic variance. Sex chromosomes are also not ubiquitous to all dioecious species. It is likely, therefore, that much of the sexual dimorphism present in nature arises through other mechanisms.

Sex-biased gene expression can be regulated by the Y chromosome, but it is also possible for sex-biased gene expression to stem from the rest of the genome. Sex-specific modifiers can up- or down-regulate genes in one sex without producing a correlated response in the other sex, and those that increase fitness in either will spread through a population. Sex hormones in mammals, for example, bind to receptors allowing sex-specific gene expression to be regulated. Sex-biased genes are common, representing as much as 91.5% of the *D. melanogaster* transcriptome (Ellegren and Parsch 2007; Innocenti and Morrow 2010; but see Stewart et al. 2010) and are recorded in a broad range of other species (Ranz et al. 2003; Marinotti et al. 2006; Yang et al. 2006; Eads et al. 2007; Mank et al. 2008; Grath et al. 2009; Allen et al. 2013).

Intralocus sexual conflict does not just occur over the expression level of a locus. Two alleles might lead to different proteins being produced, with sexually antagonistic selection acting on these (Bonduriansky and Chenoweth 2009; Connallon and Clark 2011), and sex-biased gene expression will not completely resolve intralocus sexual conflict in such cases. Intralocus conflict over two alleles may be resolved by duplication and sex-limited expression of genes (Ellegren and Parsch 2007; Bonduriansky and Chenoweth 2009; Connallon and Clark 2011; Gallach and Betrán 2011b). Once duplicated, the derived and ancestral loci can be sequestered into sex-specific regulatory networks. It is likely, due to the complex machinery required to evolve such effects, that the evolution of sexual dimorphism occurs infrequently by this route (Stewart et al. 2010). It is also possible that pleiotropic effects of duplicated genes cause intralocus sexual conflict to remain unresolved (Hosken 2011, but see Gallach & Betrán 2011b for a response) because correlated responses to selection might still occur (Harano et al. 2010; Hosken 2011).

Alternative splicing of genes can produce different functional proteins (isoforms), by the retention of different exons in the mRNA, which could offer another potential source to resolving intralocus sexual conflict (Telonis-Scott et al. 2009). In *Drosophila* the sex determining cascade begins, when two X chromosomes are present, with the sex lethal gene (*Sxl*) causing female-specific splicing of the *tra* transcript, which in-turn causes female-specific splicing of the *doublesex* (*dsx*) and *fruitless* (*fru*) genes (Pomiankowski et al. 2004; Telonis-Scott et al. 2009). Eventually this cascade allows many genes to have sex-specific splicing or expression. In males, the *Sxl* transcript produces a non-functional protein, causing default-splicing of *tra*, *fru*, and *dsx* (Telonis-Scott et al. 2009). Evidence suggests that alternative isoforms show sex-bias in *D. melanogaster* (McIntyre et al. 2006; Telonis-Scott et al. 2009), while patterns are highly conserved across several *Drosophila* species (Telonis-Scott et al. 2009).

Finally, intralocus sexual conflict might be resolved by genomic imprinting. Females (males) that reproduce should generally possess genes which

will produce daughters (sons) which are fitter than the average female (male). Given sexually antagonistic mutations improve fitness in one sex and reduce fitness in the other, because of different phenotypic optima in the sexes, it makes sense that a parent will also produce opposite-sex offspring of lower than average fitness. Day and Bonduriansky (2004) suggest that this combination of conditions should favour the evolution of genomic imprinting as a mechanism to resolve intralocus sexual conflict. In this scenario selection would favour sex-specific patterns of genomic imprinting leading to daughters expressing alleles inherited from the mother, and sons expressing alleles inherited from the father. The only requirements would be sexually antagonistic selection and the capacity to perform genomic imprinting which leads to sex-specific expression of genes dependent upon the parent of origin. However, while genomic imprinting has been demonstrated in some mammals and plants, many other species have failed to show signs of imprinting when studied, including *Drosophila* (Coolon et al. 2012). This generates unfortunate challenges in studying this potential mechanism (Patten et al. 2014).

While intralocus sexual conflict is a single locus phenomenon, it is often measured and considered in complex quantitative traits, those determined by many loci. As such, sexual dimorphism at the phenotypic level may evolve by one or several of the mechanisms acting simultaneously at many of the underlying loci, making it complex to estimate how frequently, and to what extent, each mechanism resolves intralocus sexual conflict. Despite it being unclear what role each of the described mechanisms has to play in resolving intralocus conflict, it is clear that sex-specific genetic architecture can evolve, thus, it is possible to address the question of to what extent the shared genome constrains the evolution of sexual dimorphism.

Genetic variance across the genome

An increase in sex-specific genetic variance and a reduction of covariance between the sexes are required for sexual dimorphism to evolve. Intersexual genetic correlations (r_{MF}) capture the extent to which male and female forms of a trait are affected by the same genes. Correlations are determined by the additive genetic variance within the trait for each sex (V_{AM} and V_{AF}) and covariance among them (COV_{AMF}), and are predominantly created by pleiotropy among the male and female forms (Roff 1997).

$$r_{\mathrm{MF}} = \frac{COV_{\mathrm{AMF}}}{\sqrt{V_{\mathrm{AM}} V_{\mathrm{AF}}}}$$

The vast majority of traits are complex, or quantitative, meaning that they are determined by a large number of loci each with generally small effects on the phenotype, each with the potential for sex-limited effects. According-

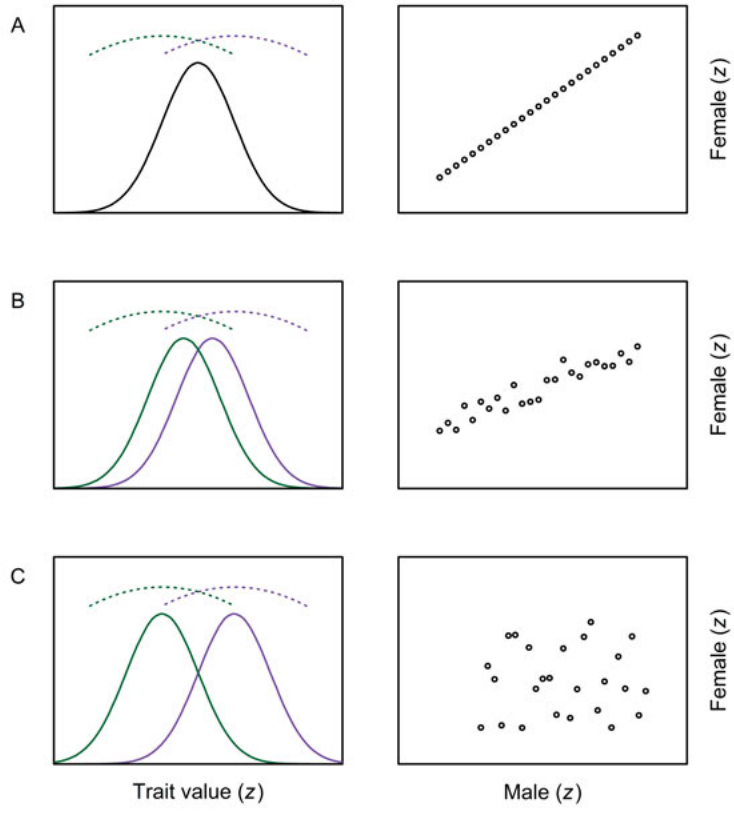

Figure 1. Intralocus sexual conflict and the genetic correlation. Lefthand figures: Sexually antagonistic sex-specific selection on males (green dashed lines) and females (purple dashed lines), and population frequencies of both sexes (black solid curve), males (green solid curve), and females (purple solid curve). Rigthhand figures: Scatterplots of male and female trait values illustrating intersexual genetic correlation, each point represent a genotypic value (e.g. male and female trait values for 25 genotypes). *Figures A, unresolved intralocus sexual conflict:* The distribution of male and female trait values are identical and genetic correlation is perfect because genetic effects are identical in either sex. *Figures B, partially resolved intralocus sexual conflict:* The two sexes show some sexual dimorphism but still do not reach sex-specific optima, and the intersexual genetic correlation is <1, some genetic effects are sex-specific. *Figures C, largely resolved intralocus sexual conflict:* The two sexes show significant sexual dimorphism, reaching sex-specific optima, and the intersexual genetic correlation is near 0, most or all genetic effects are sex-specific in their action. (Figure adapted from Bonduriansky and Chenoweth 2009).

ly the r_{MF} may be highly variable among traits. However, genetic correlations should, in general, be high because much of the genome is not sex-specific (Roff 1997; Lynch and Walsh 1998; Poissant et al. 2010; Stewart et al. 2010). For example, abdomen length in the male and female of a beetle

might be determined by the same 20 autosomal loci, each with the same expression in either sex, and one Y-linked locus, as a result the r_{MF} would be close to 1. Sexual dimorphism will typically be constrained from evolving by intralocus sexual conflict if genetic correlations are normally high. Traits under novel sexually antagonistic selection, when the r_{MF} is high, will not be able to evolve to sex-specific optima (Fig. 1A), resulting in one or both sexes having reduced fitness (Bonduriansky and Chenoweth 2009; Mank 2009). Over time, loci under sexually antagonistic selection should accumulate sex-specific modifiers, thus allowing the sexes to move towards sex-specific optima (Fig. 1B). As the genetic correlation is further eroded the sexes are better able to reach sex-specific optima (Fig. 1C). Increased levels of sex-specific genetic variance and reduced cross-sex covariance, resulting from evolution under sexually antagonistic selection, will lower the r_{MF} from previous levels. Once the sexes reach their optima it is, however, possible that the r_{MF} will increase as sex-specific genes fix in the population, decreasing the disparity between the variances and covariance.

Sexually antagonistic mutations, though a rare form of mutations (Morrow et al. 2008; Mallet et al. 2011; Mallet and Chippindale 2011), may persist more easily on the X chromosome than in the autosomes (Rice 1984). Under Rice's model, recessive male-beneficial female-deleterious mutations on the X chromosome will express their phenotype in males at the frequency of the allele, p, while they will express their phenotype in females at a frequency of p^2. Therefore, the beneficial effect in males does not have to outweigh the deleterious effect in females for the allele to persist. Furthermore, some sexually antagonistic mutations that are female-beneficial can also persist more easily in certain conditions. A dominant mutation X-linked mutation will almost always express its phenotypic effect more frequently in females than in males (a completely dominant allele gives the phenotype with the frequency $[2p]$-p^2 in females and p in males). Dominant sexually antagonistic mutations with female-beneficial effects can, therefore, persist even when the benefit to females does not outweigh the cost to males, and this disparity in fitness effects can be larger when the allele is rare. However, by allowing for sex-differences in dominance it is possible that the autosomes could, instead, be a relative hotspot for genetic variance (Fry 2010). There is some evidence that both the X (Rice 1992; Gibson et al. 2002; Pischedda and Chippindale 2006; Foerster et al. 2007) and the autosomes (Calsbeek and Sinervo 2004; Fedorka and Mousseau 2004; Delcourt et al. 2009) are hotspots for such variation. Strong evidence of the persistence of sexually antagonistic mutations on the X comes from transcriptome analysis which identified sexually antagonistic loci, and found an over-representation on the X chromosome (Innocenti and Morrow 2010).

Assuming the X chromosome is a hotspot for sexually antagonistic genetic variance, it is possible that the X chromosome will become a hotspot for sex-specific genetic variance as antagonistic loci attract sex-specific modifi-

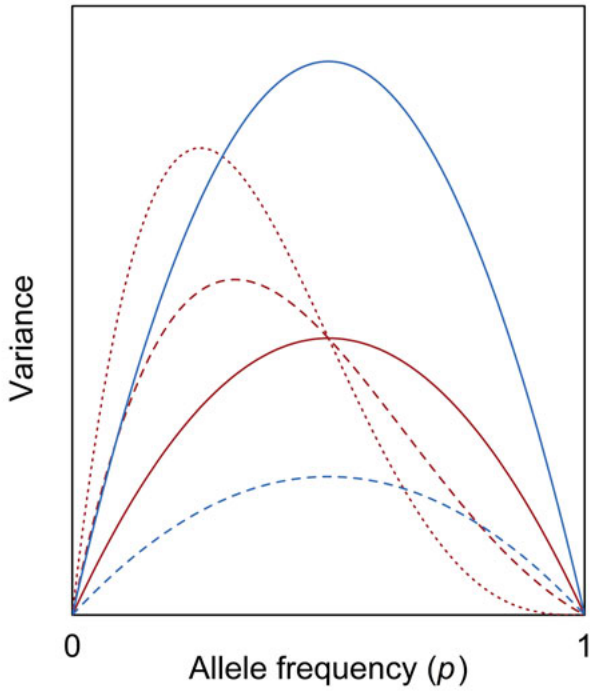

Figure 2. Additive genetic variance at X-linked loci. Dosage compensation and dominance complicate predictions about sex-specific additive genetic variance at the X chromosome. The effect of dosage compensation on male variance is illustrated by the blue lines, with (solid line) and without (dashed line) perfect dosage compensation. The effect of dominance (h), which only affects females in X-linked loci, is illustrated by the red lines with complete dominance (h = 1, dotted line), partial dominance (h = 0.75, dashed line), and an additive locus (h = 0.5, solid line).

ers (Rice 1984; Connallon and Clark 2010). If the mechanisms to resolve conflict more frequently evolve in close proximity to the locus, then the X should be enriched for sex-specific genetic effects. If the X chromosome does become enriched for sex-specific genetic variance it suggests that the X, in comparison with the autosomes, might play a special role in resolving intralocus sexual conflict.

However, genetic variance determines the rate at which adaptation can occur, and the cessation of recombination between the sex chromosomes does not only alter the population genetic settings of the Y chromosome. X chromosomes are hemizygous in males so the effective population size, assuming equal sex ratios, is three-quarters that of the autosomes ($N_X = ¾\ N_A$). Smaller populations are more prone to drift which leads to lower genetic variance. There are, however, a number of other factors which can complicate predictions about the variance of the X chromosome, but generally

these lead to predictions of further reductions in variance on the X relative to the autosomes (Ellegren 2009). Molecular diversity in *Drosophila* appears to agree with predictions where variance is lower on the X in synonymous sites of non-African populations (Hutter et al. 2007; Mackay et al. 2012), and all populations when only non-synonymous sites are considered (Langley et al. 2012; Campos et al. 2013). It remains unclear whether the reduced molecular diversity of the X chromosome translates in to reduced genetic variance and adaptive potential.

Furthermore, because of male hemizygosity of the X chromosome, it is common for dosage compensation mechanisms to evolve, which generally elevate male expression of the X chromosome. A consequence of this could be that X-linked genetic variance is elevated in males. At a polymorphic X-linked locus, a female can be homozygous or heterozygous, while a male only has one allele which becomes effectively homozygous (if male gene expression is doubled by dosage compensation), causing up to two times as much additive genetic variance within males (Fig. 2). While evidence for this phenomenon is mixed (Reinhold and Engqvist 2013; Wyman and Rowe 2014), there are reasons why the evidence might not be so clear cut. Firstly, if selection in males promotes elevated expression of X-linked loci then female expression may also increase as a correlated response leading instead to elevated female X-linked genetic variance (Mank et al. 2011; Wright and Mank 2012). Secondly, dosage compensation may not be perfect across all loci, thus male X-linked genetic variance might not be twice that of females (Allen et al. 2013). Thirdly, when intralocus sexual conflict is resolved through gene duplication, female-specific genetic variance may accumulate disproportionately on the X, and male-specific variance on the autosomes (Connallon and Clark 2011), causing a female bias in X-linked additive genetic variance. Finally, dominant X-linked alleles might elevate additive genetic variance in females, but not males, when $p < 0.5$ (Fig. 2).

Aims of this thesis

There are a number of reasons why both the amount and type of genetic (co)variance may differ among the X, Y, and autosomes. Because of the inherent importance of genetic variance for evolution by selection, the potential for adaptation to occur could vary across the genome, and interestingly some regions of the genome may better offer resolution to intralocus sexual conflict brought about by sexually antagonistic selection. The general aim of this thesis is to explore constraint on the evolution of sexual dimorphism, and to explore patterns in genetic variance and covariance which may affect the resolution of intralocus sexual conflict. Specifically, *Drosophila* are used throughout this thesis to:

1. Investigate the extent to which the evolution of sex-biased gene expression is constrained by the shared genome.

2. Estimate within-population standing genetic variation for lifespan, a complex sexually dimorphic trait, on the Y chromosome.

3. Study X-linkage of standing genetic variance, and covariance between the sexes, for lifespan and ageing.

4. Assess the relative adaptive potential of the X and autosomes under different forms of selection, particularly selection promoting the evolution of sexual dimorphism, in a multivariate framework.

Methods

Study species and populations

Throughout this thesis I principally use the fruit fly *Drosophila melanogaster*, a Dipteran insect long used as a model organism. In **paper I** data are also used from six other *Drosophila* species (*D. simulans*, *D. yakuba*, *D. ananassae*, *D. psuedoobscura*, *D. virilis*, and *D. mojavensis*).

The *D. melanogaster* gene expression data in **paper I** and lifespan data in **paper II** are both measured in lines from the Drosophila Genetic Reference Panel (DGRP) (Ayroles et al. 2009; Mackay et al. 2012). The DGRP lines are a series of highly inbred lines which were established by performing 20 generations of full-sib mating on isofemale lines collected from the wild in Raleigh, North Carolina (USA) (Ayroles et al. 2009; Mackay et al. 2012). Gene expression analysis was performed on 40 of these lines by Mackay et al. (2012) and data deposited online, and in **paper I**, this data is used to test for a correlation between sex-biased gene expression and the intersexual genetic correlation.

In **paper III** and **paper IV** data are collected from a laboratory adapted population called Dahomey, which was collected from what is now Benin, West Africa, over 40 years ago. This population has been kept as a large outbred population at constant conditions (12:12 light-dark cycle, 60% humidity, 25°C, and on a standard yeast-sugar diet) for that time, with overlapping generations. Flies used in the experiments in **papers II**, **III**, and **IV**, were performed in these same standard conditions unless otherwise stated.

The genome of *D. melanogaster* is comprised of the X and Y sex chromosomes, two major autosomes (AII and AIII) and the fourth "dot" autosomes (AIV) which contains <1% of the DNA, is largely heterochromatic, and highly degenerate (Bachtrog 2005, 2013). There are a number of features of *D. melanogaster* which also make them of particularly valuable use in this thesis. They exhibit sexual dimorphism in a plethora of traits, have XY sex chromosomes, and can be studied in large volumes with little logistical constraint. Having been used as a model organism for more than 100 years (e.g. Morgan 1910) and being one of the systems used at the forefront of developing tools for the genomics era, the genetics and genomics of this species are also among the most well understood. Furthermore, a key characteristic is the genome-wide absence of recombination in males, which, com-

bined with specialised "genetic tool" lines of flies, is utilised in this thesis to create chromosome substitution lines in **papers II**, **III**, and **IV**.

Gene expression data

Much of the DNA in the genome is shared in both sexes, but gene expression is the lowest level at which the sexes can begin to differentially use the genetic information in the genome, by sex-specific modification of gene expression levels. **Paper I** of this thesis uses multiple sets of gene expression data to test hypotheses regarding the intersexual genetic correlation and the evolution of sexual dimorphism. Estimates of the r_{MF} and sexual dimorphism are made using gene expression data from the DGRP lines downloaded from an online depository (Ayroles et al. 2009, EBI accession code: E-MEXP-1594). This data contains estimates of gene expression for >14000 transcripts in *D. melanogaster* for 40 DGRP lines, with two replicates pools of 25, 3–5 day old, flies per sex and line. Gene expression was measured using Affymetrix *Drosophila* 2.0 arrays (Ayroles et al. 2009), and normalised by the robust multi-array average method (RMA) (Irizarry et al. 2003).

Further data was collected from a second study where gene expression data was collected in parallel to a fitness assay, allowing the original authors to assign genes as having sexually antagonistic fitness effects (Innocenti and Morrow 2010). This data comes from another population of *D. melanogaster* (LHM), which is large, outbred, and laboratory adapted. Data was collected from the supplementary material provided with Innocenti and Morrow (2010), and used to assign fitness effect to genes (Categorical: Sexually antagonistic and not sexually antagonistic). A final set of data was used for gene expression data for sex bias in seven *Drosophila* species (*D. melanogaster*, *D. simulans*, *D. yakuba*, *D. ananassae*, *D. psuedoobscura*, *D. virilis*, and *D. mojavensis*) from the Gene Expression Omnibus (Zhang et al. 2007, GEO Accession code: GSE6640), which was also normalised by RMA. This data was used to give scores of sex-bias in gene expression for the six species related to *D. melanogaster*.

Y chromosome substitution lines

Genetic variance on the Y chromosome, using a sample of Y chromosomes from within a population, is estimated in **paper II**. To estimate this I use a chromosome substitution method which allows the focal Y chromosomes to be cloned into a large number of individuals, giving accurate estimates of the variance explained by the Y. Within each line of flies (Y-line), all individuals share the same Y chromosome, while the Y chromosome varies among lines. The genetic variance on the Y chromosome is then estimated by statistical decomposition of the variance components, and is equivalent to the line variance, because it is a hemizygous chromosome. To construct each line, I

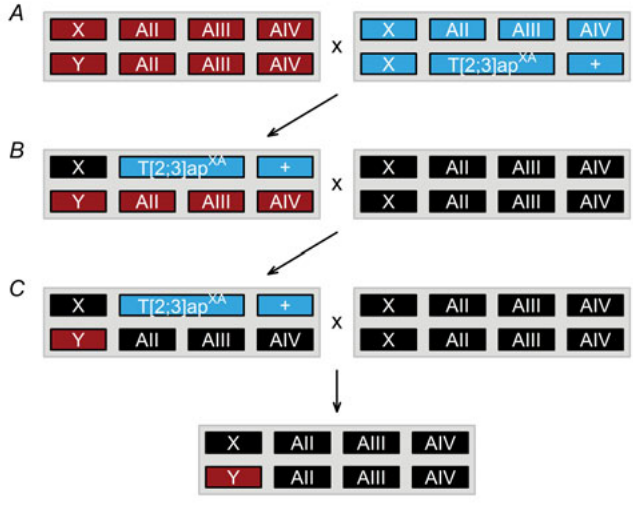

Figure 3. Crosses to produce Y-lines. Chromosomes indicated as X chromosome (X), Y chromosome (Y), autosomes 2-4 (AII, AIII, AIV), marked translocations of AII and AIII (T[2;3]ap^{XA}), and a Ci^{D} marked AIV (+). Red chromosomes indicate descent from source line (one of 33 DGRP lines), blue indicate those descended from the translocated female stock, and black chromosomes indicate those from the DGRP-486 line, which is identical in every Y-line. *A*) Males from each of 33 DGRP line crossed to virgin females carrying marked translocation to remove a haploid genome originating from the source line. *B*) Male offspring from *A* carrying marked translocations crossed to virgin DGRP-486 females to remove the autosomes from the source line. *C*) Male offspring from *B* carrying marked translocation crossed to virgin female DGRP-486 to produce focal males, with source-line Y chromosome in DGRP-486 background.

took males from 33 of the DGRP lines, and used back-crossing to clonally amplify the Y chromosome in to a single homozygous genetic background. Males from each line were crossed to females carrying a phenotypically marked translocation of the second and third autosome (T[2;3] ap^{XA}) and a phenotypically marked fourth chromosome (Ci^{D}) (Fig. 3A). The translocation forces autosomes AII and AIII to cosegregate (transmitted as a single unit from parent to offspring), allowing the homologous chromosome to be tracked by simply scoring phenotype.

For each proto-Y-line, male offspring from this first cross, displaying phenotypes indicating the presence of the marked chromosomes, were then mated to virgin females from a single randomly selected DGRP line (DRGP-486) (Fig. 3B). From this second cross I collected the male offspring which, again, displayed the phenotypic markers, indicating that the marked chromosomes and the Y had been inherited from the fathers and the homologous chromosomes from the DGRP-486 mothers. These males were mated to

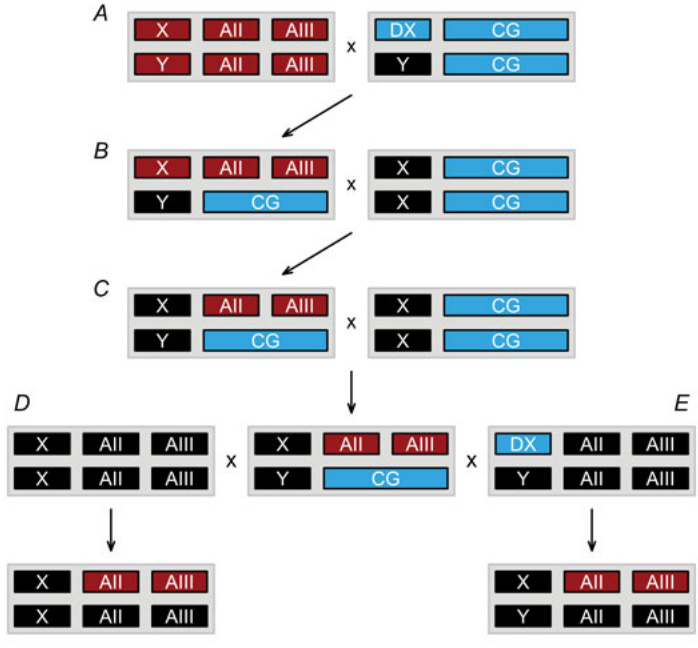

Figure 4. Crosses to produce A-lines. Chromosomes indicated as X chromosome (X), Y chromosome (Y), autosomes 2-3 (AII, AIII), joined-X chromosomes (DX), and translocated AII and AIII (CG). Autosome AIV not shown to ease visualisation. Red chromosomes indicate descent from the source male, blue chromosomes indicate descent from stock line (DXCG), black chromosomes represent random and variable Dahomey descended chromosomes, which vary among every fly. Dahomey chromosomes inserted in to DX and CG lines prior to these crosses by repeated largescale backcrossing to ensure variability. *A*) Dahomey male mated to virgin DXCG female to remove haploid genome. *B*) Male offspring from *A* heterozygous for the CG translocation crossed to virgin CG females to remove source X chromosome. *C*) Male offspring from *B* heterozygous for the CG translocation crossed to virgin CG females to *i*) maintain lines and *ii*) maximise X chromosome variance within lines. *D*) Male offspring from *C* heterozygous for the CG translocation crossed to virgin Dahomey females to replace the Y chromosome and translocation with variable Dahomey X and autosomes, to produce focal females. *E*) Male offspring from *C* heterozygous for the CG chromosomes crossed to virgin DX-D females to replace the Y chromosome and translocation with variable Dahomey Y and autosomes.

DGRP-486 virgins (Fig. 3C). This created the complete Y-line, with focal Y chromosomes all placed in to a single genetic background, and varying among lines at the Y chromosome only. Y-lines were maintained by crossing to DGRP-486 females, and large sample populations were easily produced by the same cross.

Autosome substitution lines

In **paper III** and **paper IV** the additive genetic variance was estimated for the major autosomes (AII and AIII). This was also done by substitution lines, similarly to the aforementioned Y-lines, to create lines (A-lines) of flies with clonally amplified sets of AII and AIII. Similarly to the Y-lines, the individuals within A-lines share an identical set of clonally amplified chromosomes (AII and AIII in this case) but, unlike the Y-lines, this is placed into an outbred random genetic background which varies within lines. This is an important feature of these lines. By measuring the phenotypic effect of a single haploid set of autosomes against a potentially infinite number of genetic backgrounds it is possible to get very precise estimates of breeding value of that set of autosomes, without dominance variation affecting the result, and only a minor level of potential interaction variance (Lehtovaara et al. 2013). Consequentially, the line variance estimated by statistical decomposition is caused by variance in the additive genetic effects of the autosomes.

These lines were initially formed by crossing Dahomey males to DXCG females (Fig. 4A). The DXCG (C[1]DX, *y*, *f*/Y; T[2;3] bw^d, *in*, p^p, *rdgC*, *ri*, *st*/T[2;3] bw^d, *in*, p^p, *rdgC*, *ri*, *st*) females carry phenotypically marked translocations of autosomes AII and AIII which, similarly to the translocations used to create the Y-lines, force the cosegregation of these autosomes, and in this case, allow the differential identification of offspring which are hetero- or homozygous for this translocation. The DXCG females also carry a joined pair of X chromosomes, which transmit to offspring as one unit, allowing females to also carry a Y chromosome; therefore male offspring of DXCG females inherit their Y chromosome from the mother, and their X chromosome from the father. From this cross I collected male offspring with the autosomal translocations and Y chromosome from the mother, and homologous chromosomes from the Dahomey father. These were mated to CG females (Fig. 4B), a stock of females homozygous for the same autosomal translocations in the DXCG stock, but carrying two wildtype Dahomey X chromosomes.

Multiple male offspring, heterozygous for the translocation (thus also carrying the focal autosomes), are collected and mated again to virgin CG females to maintain the A-lines in this state (Fig. 4C). Populations of focal females are produced by crossing males from each A-line, which are heterozygous for the translocation, to females from the Dahomey population (Fig. 4D). Populations of focal males are created by crossing to DX-D virgin females to replace the Y chromosome descended from the DXCG female in the first cross, and give genetic variance in the genetic background (Fig. 4E).

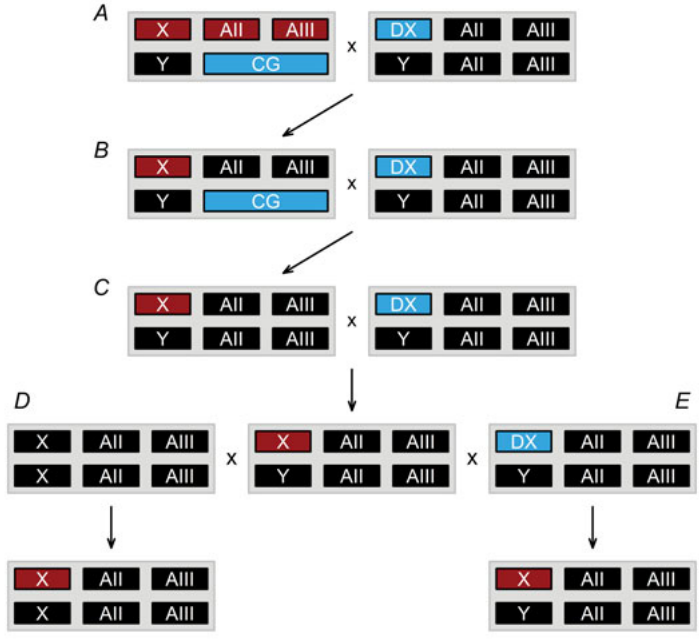

Figure 5. Crosses to produce X-lines. Chromosomes indicated as X chromosome (X), Y chromosome (Y), autosomes 2-3 (AII, AIII), joined-X chromosomes (DX), and translocated AII and AIII (CG). Autosome AIV not shown to ease visualisation. Red chromosomes indicate descent from the source male, blue chromosomes indicate descent from stock line (DXCG), black chromosomes represent random and variable Dahomey descended chromosomes, which vary among every fly. Dahomey chromosomes inserted in to DX lines prior to these crosses by repeated largescale backcrossing to ensure variability. *A*) Males formed in the same way as those in (Fig. 4A), heterozygous for the CG translocation, crossed to virgin DX-D females to remove source autosomes. *B*) Males from *A* heterozygous for the CG translocation crossed to virgin DX-D females to remove the CG translocation. *C*) Males carrying the focal X in Dahomey background crossed to DX-D virgin females to *i*) maintain lines and *ii*) maximise variance in the background. *D*) Males from *C* crossed to virgin Dahomey females to remove the Y chromosome, to produce focal females. *E*) Males from *C* crossed to virgin DX-D females to replace the Y chromosome, to produce focal males.

X chromosome substitution lines

The additive genetic variance of the X chromosome was also estimated in both **paper III** and **paper IV** of this thesis. To do this I constructed X chro-

mosome substitution lines (X-lines), and estimated variance explained by the X. Similarly to the A-lines, these place focal chromosomes in a variable genetic background. The variance explained by line in the statistical decomposition is equivalent to half the X-linked additive genetic variance in females, but is equivalent to the X-linked genetic variance in males due to male X chromosome hemizygosity.

To construct the X-lines I took 40 males from the Dahomey stock population and crossed them individually to virgin DXCG females, as was done in the first step of the cross to produce A-lines (see Fig. 4A). Male offspring from this cross, carrying one set of the translocation and a Y chromosome from the mother, with homologous chromosomes inherited from the Dahomey male, were collected and crossed to DX-D females (Fig. 5A). The DX-D female stock carries the joined pair of X chromosomes, variable Dahomey Y chromosomes, and variable Dahomey autosomes (C[1]DX, *y, f*/Y). Male offspring from this cross were sorted for those with a phenotype indicating that they were heterozygous for the translocation, showing that the donor-male origin autosomes had been removed, and were mated to further DX-D females (Fig. 5B). Male offspring from this cross were sorted for those with a phenotype indicating that the autosomes were all of Dahomey origin, which were then mated to further DX-D females (Fig. 5C). The males within each X-line at this point share the same X chromosome, and vary at the autosome and Y chromosome, while the entire genome is variable among X-lines. X-lines were maintained, and variance in the genetic background ensured, by repeating the final cross for several generations to large numbers of DX-D females. The phenotypic effect of the focal X chromosome was then estimated by amplifying the X chromosome in to large sampling populations of both males and females. Populations of focal females were produced by crossing to females from the Dahomey population (Fig. 5D), and populations of focal males were produced by repeating the cross to DX-D females (Fig. 5E).

Genetic variance

Phenotypes are, in general, determined by a combination of genetic and environmental effects. It is possible to decompose the variance in phenotype seen in a population (V_P) in to variance components explained by the genetic (V_G) and environmental effects (V_E). Genetic variance can be further decomposed in to additive genetic variance (V_A), dominance variance (V_D), and interaction variance (V_I).

$$V_G = V_A + V_D + V_I$$

In evolutionary biology, the additive genetic variance is of distinct importance. It is this component of the genetic variance that selection affects from generation to generation because this is the heritable effect of genes transmitted from parent to offspring under random mating (Conner and Hartl 2004). Additive genetic variance can be calculated using population genetic principles based on knowledge of allele frequency (p and q in a two allele locus) and the additive effect, a, which is, in essence, the average deviation of the homozygote from the heterozygote. If there are no dominance interactions at the locus, the additive genetic variance is maximised when $p = 0.5$, illustrating the relationship between the amount of heritable information and the allele frequency in the population. There is no heritable component when $p = 1$ or 0 (Fig. 2).

$$V_{\mathrm{A}} = 2pqa^2$$

While additive genetic variance has, for a long time, been estimated using parent-offspring regressions or breeding designs, such as full-sib and half-sib breeding designs, recent work has shown how the hemiclone technique can be used to give precise estimates of additive genetic variance (for example: Lehtovaara et al. 2013). The hemiclone technique uses the principles of chromosome substitution lines (Rice et al. 2005; Abbott and Morrow 2011), as described above, but applied to the whole genome (X, AII, and AIII combined). Chromosome substitution lines, like those used in this thesis, have some properties that make them particularly useful for quantitative genetic studies (Abbott and Morrow 2011). Lines can be maintained for extended periods so it is possible to conduct follow-up experiments on the same set of lines. Similarly, they can be used to perform experiments in many replicate blocks allowing more traits, environments, treatments, or lines to be assayed within each block than would normally be possible. Focal chromosomes can also be clonally amplified in to a theoretically infinite number of individuals, limited only by logistical constraints, which allows highly precise estimates of additive genetic variance.

The use of X-, Y-, and A-lines allows genetic variance to be decomposed and compared across these different components of the genome. This presents an opportunity to address questions regarding evolution, rates of adaptation, and the role of the sex chromosomes.

Exploring G-matrices

Chromosome substitution lines can also be used to estimate the covariance among-traits within-sexes, among-sexes within-traits, and among-traits between-sexes. As such, it is possible to construct genetic variance-covariance

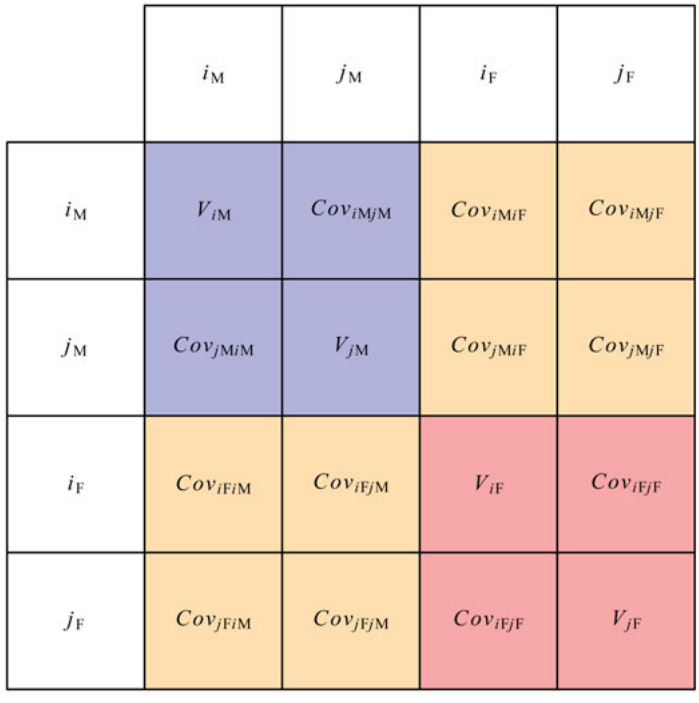

Figure 6. Structural composition of the G-matrix. The G-matrix, illustrated here with two traits (*i* and *j*) measured in both sexes (M and F), subdivided into the male-specific submatrix ($\mathbf{G}_M$, blue shaded cells), female-specific submatrix ($\mathbf{G}_F$, red shaded cells), and the cross-sex submatrices ($\mathbf{B}$ and $\mathbf{B}^T$, yellow shaded cells).

matrices (G-matrix) (Lande 1979, 1980) based on high precision estimates.

The G-matrix, herein referred to as **G**, is composed of four submatrices, the within sex genetic variance-covariance matrices ($\mathbf{G}_M$ and $\mathbf{G}_F$, for males and females respectively), and the cross-sex covariance matrices, **B** and **B**-transposed ($\mathbf{B}^T$) (Fig. 6). The diagonal elements of **G**, and thus the diagonal elements of $\mathbf{G}_M$ and $\mathbf{G}_F$, show the within-sex within-trait additive genetic variance, the off diagonal elements of $\mathbf{G}_M$ and $\mathbf{G}_F$ show the additive covariance among-traits within-sexes. The diagonal elements of **B** (and $\mathbf{B}^T$ herein collectively referred to as **B**) show the within-trait cross-sex additive covariance, while the off diagonals represent the among-trait cross-sex additive covariance.

G-matrices have a number of biologically important properties that can be described. Matrix shape is largely affected by the covariance structure, with covariance rotating the eigenvectors of the matrix, and uneven variance or asymmetrical (co)variance structure distorting the shape away from circular, affecting the eigenvalues. Large eigenvalues suggest that the variance is unevenly distributed and/or there is covariance among traits, thus responses

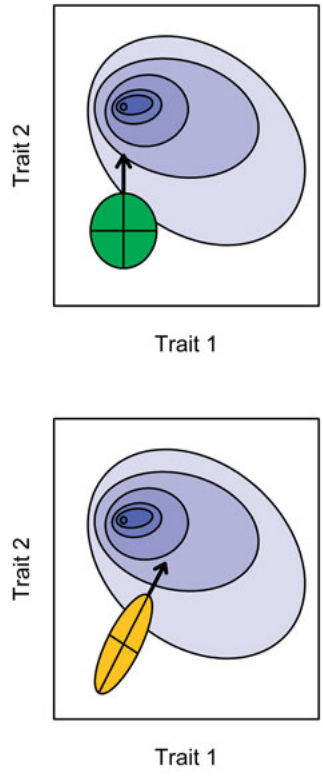

Figure 7. Illustrating the effect of ***G*** *on the response to selection with two traits.* Blue ellipses represent the fitness landscape going from low fitness (white) through to high fitness (dark blue). *Upper figure:* The G-matrix (green ellipse) has even genetic variation in all directions and no covariance among traits 1 and 2, allowing selection to cause a response towards the fittest point. *Lower figure:* The G-matrix (orange ellipse) contains covariance among traits 1 and 2 rotating the axes, and becomes more elliptical due to asymmetry in the covariance structure. The result is a deflected response to selection away from the fittest point of the landscape. Figure adapted from Steppan et al. (2002).

to selection will differ depending upon the direction of selection, with the best response seen in the direction of most variance (Fig. 7). Matrices that are very similar in shape will tend to respond to selection in similar directions, because genetic (co)variance is similarly distributed, and evolution will proceed along axes with more variation more easily. Matrices that differ in size will predominantly differ in the magnitude of the response to selection, because size is determined by the volume of genetic (co)variance, if one G-matrix contains little genetic variance the response to selection will be relatively small, and adaptation slowed.

In **paper IV** of this thesis I construct separate G-matrices for the X chromosome and autosomes to explore patterns in genetic variance and covari-

ance, and how this might affect adaptation, with particular focus on sexually antagonistic selection. To do this I employ a number of descriptors and comparison methods, briefly outlined here. No method yet describes all of the possible ways that two matrices can differ and application of multiple methods allows testing various interesting features of matrices (Roff et al. 2012).

A G-matrix can be decomposed to estimate the main axis of variation in multivariate space, g_{max}, which is the eigenvector along which the variance is most abundant (Schluter 1996; Wyman et al. 2013). Comparisons of the angle between g_{max} values of two matrices can illustrate how differently they align in multivariate space, thus how differently they are likely to affect the response to selection.

Mantel tests allow the shape of two matrices to be compared, with only a very minor effect of relative size of the matrices (Roff et al. 2012). Tests that give values of near one suggest that two matrices are very similar in shape, while scores near zero suggest very different shapes. Thus, high matrix correlations suggest the direction of the response to selection will be similar among the two, while selection will produce different directions of response in uncorrelated matrices.

Skewers analysis is a way to further explore the differences among G-matrices, with obvious intuitive links to questions frequently at the core of evolutionary biology. In principle, skewers analysis uses the Lande equation with the same vector(s) of selection applied to two matrices, and measures the mean angle of deflection away from the vector of selection. If a G-matrix contains genetic (co)variance that allows response in any direction the mean difference between the direction of selection and response should be low, while a highly structured G-matrix will frequently deflect the response away from the direction of selection, and the angle will be large.

Frequently, skewers are generated either by empirical estimation of the selection on the traits in the matrix (selection skewers) or by randomly assigning selection (random skewers) (Cheverud 1996; Roff et al. 2012). This latter method constructs many thousands of selection vectors (β in the Lande equation) generated by drawing from a uniform distribution. Each vector is applied to both matrices, and the average deflection can be compared, and it is also possible to compare the direction of deflection between to matrices, as the angle between response vectors. For both types of skewer analysis, large angles between the response vectors suggest the genetic (co)variance structure deflects the response in to very different direction, and evolution will proceed differently through the two. Here I also use modified versions of the random skewers method in two further semi-random methods.

Normal random skewers analysis draws the values from a uniform distribution between -1 and +1 (Cheverud 1996; Roff et al. 2012). In **paper IV**, two sets of semi-random skewers are used, one drawn such that selection is always concordant between the sexes, and one drawn such that selection is

always antagonistic between the sexes. These novel methods both test how matrices respond differently to two important and interesting forms of selection, given that responses to sexually antagonistic selection should be impeded by covariance in the B-submatrix, and improved under sexually concordant selection.

The effect of cross-sex covariances, the elements of the B-submatrix, on the response to selection can be explicitly tested by setting the B-submatrix to zero (Agrawal and Stinchcombe 2009). This method has been employed in a number of recent studies, to test whether cross-sex genetic covariances constrain evolution of sexual dimorphism in single G-matrices (Lewis et al. 2011; Gosden et al. 2012), and here we extend its use to compare the effect of removing cross-sex covariance in two matrices, comparing the change in the angle of deflection for either matrix. If, for a single matrix, the angle between the selection and the response reduces when **B** is set to zero then the cross-sex covariance constrains evolution. Comparing this change in angle between two matrices illustrates which is most affected by the cross-sex covariance, with the G-matrix experiencing the largest change being the one most affected by **B**.

Results

Paper I

This study used gene expression data from a number of sources to test the relationship between sex-biased gene expression, as a sexually dimorphic trait, and the intersexual genetic correlation. This indicates whether or not a disconnected genetic architecture between the sexes is required for sexual dimorphism to evolve, and how common such correlations are. Gene expression data is prone to noise therefore the genes for which a strong genetic effect (i.e. statistically significant genetic variance) for expression level were excluded from further analysis. The r_{MF} was found to be high when genes with non-significant genetic signal were removed (median $r_{MF} = 0.427$), and it is worth noting that this was even higher ($r_{MF} = 0.724$) under a very strict criterion of <20% of variance coming from residual variance (Fig. 8).

The genes identified by Innocenti and Morrow (2010) as being sexually antagonistic had a higher r_{MF} shown by a small but significant association between selection regime and r_{MF} (regression coefficient, $r = 0.096$) suggesting that a low intersexual genetic correlation reduces the effects of intralocus sexual conflict. Genes on the X chromosome had a lower intersexual genetic correlation, confirmed with a small but significant association between r_{MF} and chromosome type ($r = 0.020$).

Sex-bias was found to be negatively associated with the intersexual genetic correlation ($r = -0.125$) (Fig. 9). This result, combined with the finding that, both in this study, and in others (Bonduriansky and Rowe 2005; Poissant et al. 2010) the intersexual genetic correlation is generally high, suggests that the shared genome can frequently act as a constraint on the evolution of sexual dimorphism under novel sexually antagonistic selection. Finally the change in sex-bias between *D. melanogaster* and six other *Drosophila* species, which is the change in sexual dimorphism since divergence, was negatively associated with the intersexual genetic correlation. Significant negative regression coefficients were estimated between the extent of change in sex-bias and the r_{MF} for all six tests (correlation between *D. melanogaster* r_{MF} and change in sex-bias between *D. melanogaster* and other species; *D. simulans* = -0.074, *D. yakuba* = -0.192, *D. ananassae* = -0.066, *D. psuedoobscura* = -0.156, *D. virilis* = -0.128, *D. mojavensis* = -0.100). This result suggests that only genes with a low intersexual genetic correlation are able to become more or less sex-biased over longer evolutionary

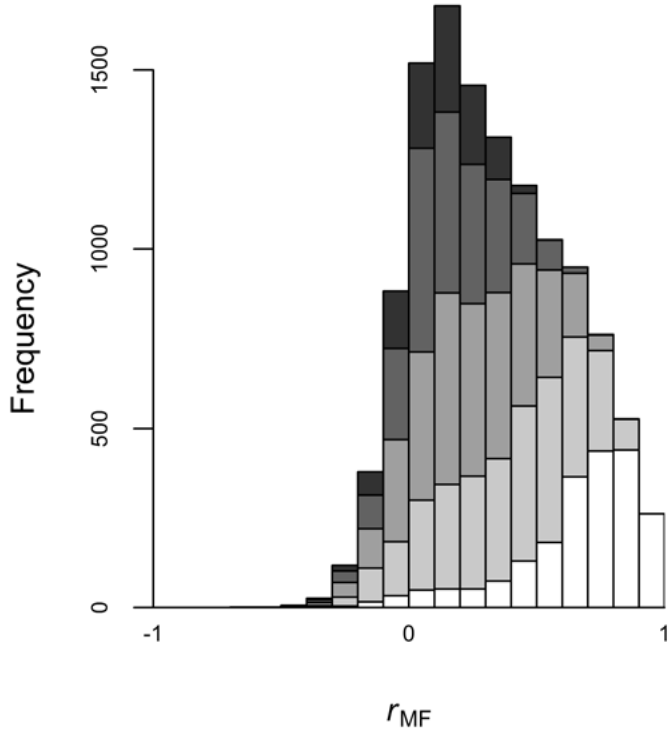

Figure 8. Distribution of r_{MF} estimates for gene expression. Bars from dark through light show all data and those with < 80%, < 60%, < 40%, and < 20% of total variance explained by the residual term as estimated in **paper I**.

time periods. However, this result is based on an assumption that the form of selection was not biased among the genes (i.e. existing sex-biased genes were not more prone to future sexually antagonistic selection), so should be interpreted cautiously.

Paper II

This study used the DGRP lines of *D. melanogaster* as sources of 33 Y chromosomes in Y-lines to test for standing Y-linked genetic variance for lifespan, a complex sexually dimorphic trait. This study tests for standing genetic variance within a population to provide an indication of the adaptive potential of the Y chromosome which could respond to male-specific selection, without causing correlated responses in females. A small but significant level of genetic variation was found on the Y chromosome (V_G = 0.65) (Fig. 10), and Y-linked variance explained 0.4% of the variance in lifespan. Lifespan was found to exhibit significant and large phenotypic variance (V_P = 153.97) relative to similar studies, while the coefficients of genetic and ph-

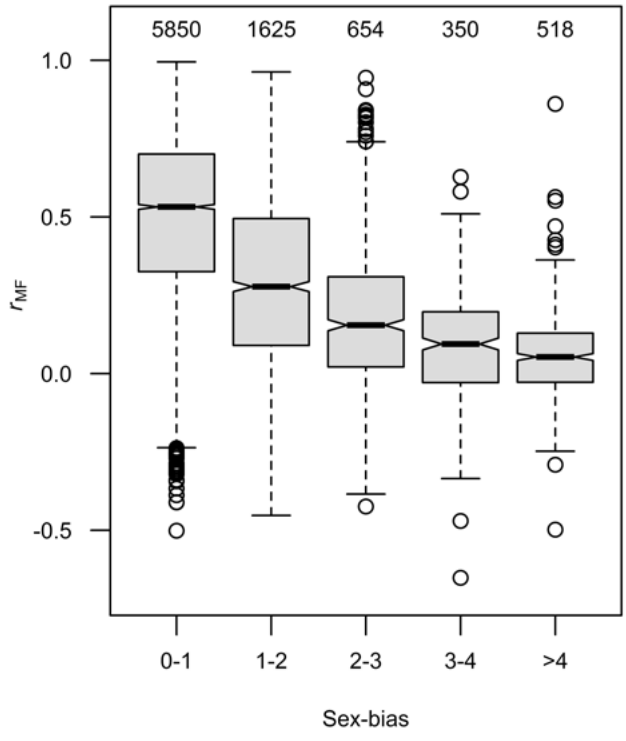

Figure 9. The relationship between sex-biased gene expression and the intersexual genetic correlation. Sex-bias in gene expression as absolute value of the fold difference between the sexes. Numbers above the boxes show the number of genes within each category, data used was that with significant genetic signal (n = 8,997).

enotypic variation were 0.012 and 0.190 respectively. This result shows that the Y chromosome does affect lifespan in *D. melanogaster* but the potential to evolve further sexual dimorphism from standing genetic variance is limited in this population.

Paper III

In this paper, X- and A-lines were used to estimate standing additive genetic variation, in each sex, for the X and autosomes separately in two complex sexually dimorphic traits; lifespan and ageing. The aim here was to explore *i)* differences in autosomal and X-linked genetic variance, *ii)* sex-bias in X-linked genetic variance, and *iii)* the genomic distribution of sex-specific genetic variance. This study uses the same base population and similar methods as a recent study which examined genetic variance in lifespan and ageing for hemiclones (Lehtovaara et al. 2013), estimating genome wide genetic variance.

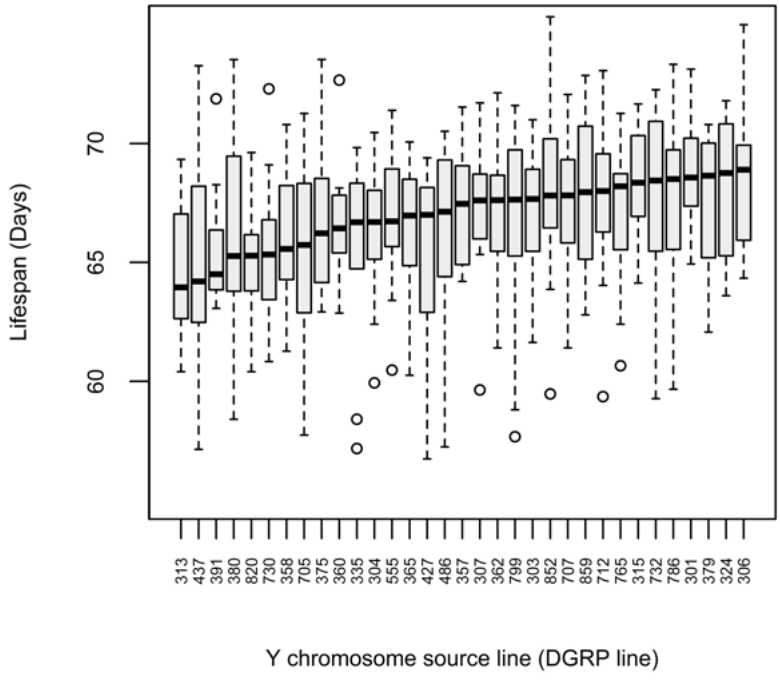

Figure 10. Lifespan of Y-lines. Boxplot of lifespan for 33 Y-lines derived from the DGRP population. Data plotted using vial means.

Population genetic theory predicts erosion of additive genetic variation on the X chromosome, relative to the autosomes. The X chromosome contains 15.6% of the protein coding genes and 18.8% of euchromatin in *D. melanogaster*, thus a depletion of additive genetic variation on the X would result in the X hosting less than these values. Interestingly, the analyses suggest that, for lifespan, the X is not depleted, and may well be enriched, for additive genetic variation (X-linkage of lifespan V_A in females = 21.0%, males = 21.6%). These results, however, do not rule out a slight depletion. Furthermore, the results suggest that additive genetic variance was also not depleted on the X chromosome for ageing, though the point estimates are quite different for the sexes, and again, neither preclude a depletion and credible intervals are broad (X-linkage of ageing V_A in females = 28.6%, males = 15.6%). Hemizygosity of the male X chromosome may complicate matters for these results, and conclusions may best be drawn from females.

Dosage compensation of the X chromosomes in males may act to increase standing genetic variance in males relative to females. The analyses show that males exhibit 1.32 times as much genetic variance in the X chromosome as females, but this was not significantly different from a 1:1 ratio. While this result does not suggest a sex-bias in X-linked genetic variance in either

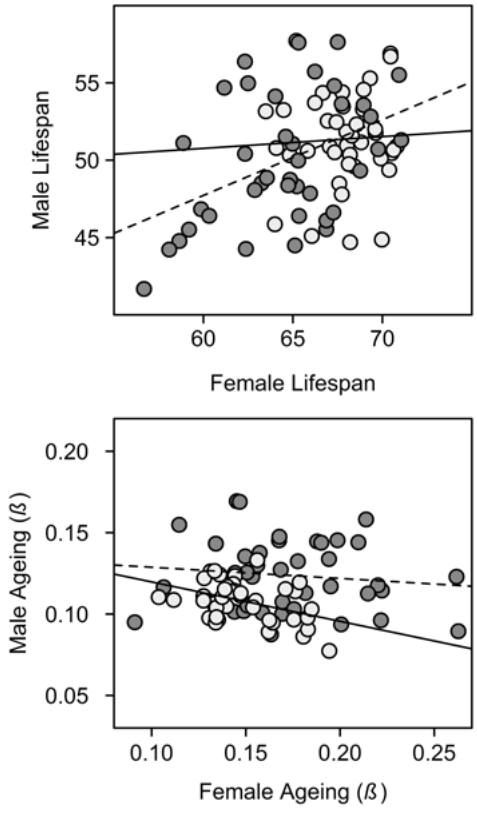

Figure 11. Intersexual genetic correlations for lifespan and ageing. Light grey points and solid lines represent the X chromosome, and dark grey points and dashed lines represent the autosomes. The plot is scaled such that the steepness of the regression slopes reflects the strength of the correlation.

direction, comparison of "raw" variance estimates is inappropriate because males and females show sexual dimorphism in lifespan. It is more correct to use the coefficient of additive genetic variation, which provides a scale free measure of the variances of each. The coefficient of variance was more suggestive of male-bias, and credible intervals only marginally overlap a ratio of no difference between the sexes (CV_{AM}/CV_{AF} = 1.49). A third measure, the ratio of X-linkage, also provides a scale free measure which also accounts for differences in the total amount of genome wide standing additive genetic variance. This was calculated as the ratio of X-linkage in males to the X-linkage in females, where X-linkage is the percentage of all genetic variance with sex which is X-linked. This ratio was not different from 1 for lifespan ($X\%_M/ X\%_F$ = 1.12), suggesting no sex-bias in X-linked variance.

Interestingly, in ageing the result appears to be reversed with both ratios of additive genetic variance (V_{AM}/V_{AF} = 0.20) and of the coefficient of addi-

tive genetic variance (CV_{AM}/CV_{AF} = 0.60) suggesting that there is a female-bias in additive genetic variance for ageing on the X chromosome. The ratio of X-linkage suggests, however, that there is no sex-bias ($X\%_M/ X\%_F$ = 0.64), though credible intervals are broad.

Finally this data was used to test if the X or autosomes are enriched for sex-specific genetic variance, by estimating intersexual genetic correlations for each component of the genome. The intersexual genetic correlation for lifespan was moderate in the autosomes ($r_{MF\text{-}A}$ = 0.50) and not different from zero in the X chromosome ($r_{MF\text{-}X}$ = 0.04) (Fig. 11). The genetic correlation in the X chromosome was not different from that of the autosomes ($r_{MF\text{-}A}$ - $r_{MF\text{-}X}$ = 0.46) (Fig. 11). It should be noted that the difference was nearly confirmed by this effectively two-tailed test, because only 61 of 2000 samples in the posterior distribution were less than zero (< 50 of 2000 would be required for difference to be confirmed), and intersexual genetic correlations are notoriously difficult to compare, requiring extremely large sample sizes (Lynch and Walsh 1998; Bonduriansky and Chenoweth 2009). For ageing the results were less compelling, with the intersexual genetic correlations in both components of the genome not being different from zero ($r_{MF\text{-}A}$ = -0.11; $r_{MF\text{-}X}$ = -0.31), and not different from one another ($r_{MF\text{-}A}$ - $r_{MF\text{-}X}$ = 0.20) (Fig. 11). The results obtained for ageing in this part of the analyses are perhaps unsurprising given the problems of sample size generated by ageing analyses, where ageing is a population-level trait (measured as ageing per vial of 50 flies, thus giving a sample size of 4 per sex and line), and the subsequent limitations on estimating genetic correlations (Lynch and Walsh 1998; Bonduriansky and Chenoweth 2009).

Paper IV

In this study G-matrices were constructed containing multiple traits measured in both sexes, for both the X-lines and A-lines, G-matrices are referred to as $\mathbf{G}_X$ and $\mathbf{G}_A$, respectively. These were 6×6 G-matrices (Table 1) composed of estimates for male and female additive genetic (co)variance in body size (BS), climbing ability or negative geotaxis (NG), and lifespan (LS). The data used to estimate lifespan is the data used in **paper III**, while a second assay was used to estimate (co)variance in climbing ability and body size, and both used the same 80 chromosome substitution lines. The main aim here was to test whether differences in the genetic variance-covariance structure of the X chromosome and autosomes bring about differences in response to selection, most importantly, in response to sexually antagonistic selection.

Significant additive genetic variance was found in all traits for both sexes, in both components of the genome. More additive genetic variance was found in the autosomes than the X chromosome for five of six sex-trait com-

Table 1. *G-matrices for A-lines and X-lines*. Within-trait genetic variances are the six diagonal elements of each matrix, intersexual genetic correlations are show in the lower triangle of each matrix (left and down of the diagonal) and covariances are shown in the upper triangles of each matrix (right and above the diagonal). Estimates with credible intervals not overlapping zero are marked with an asterisk.

A-lines		Female			Male		
		BS	**NG**	**LS**	**BS**	**NG**	**LS**
Female	**BS**	*0.59	0.01	-0.08	*0.29	-0.17	-0.01
	NG	0.02	*0.37	0.06	-0.02	*0.20	0.03
	LS	-0.15	0.15	*0.45	-0.11	0.11	*0.17
Male	**BS**	*0.51	-0.04	-0.21	*0.57	*-0.25	-0.02
	NG	-0.28	*0.42	0.21	*-0.44	*0.57	0.04
	LS	-0.03	0.06	*0.39	-0.04	0.07	*0.40

X-lines		Female			Male		
		BS	**NG**	**LS**	**BS**	**NG**	**LS**
Female	**BS**	*0.27	0.06	0.01	0.04	0.03	0.01
	NG	0.16	*0.47	-0.01	-0.01	*0.18	0.04
	LS	0.05	-0.05	*0.19	0.02	-0.02	0.00
Male	**BS**	0.21	-0.04	0.11	*0.17	0.00	0.02
	NG	0.12	*0.53	-0.09	-0.01	*0.24	0.04
	LS	0.05	0.16	0.02	0.14	0.12	*0.13

binations, while there was no difference in the amount of additive genetic variance on the X and autosomes for female climbing ability. The ratio of X-linkage for five of six traits was in the expected ranges that suggest no depletion or enrichment of genetic variance on the X chromosome, while there appeared to be significant X-linkage of additive genetic variance for female climbing ability. Sex-bias in genetic variance was found in climbing ability in both components of the genome, with female-bias in the X chromosome ($V_{A\text{-}FX}$ - $V_{A\text{-}MX}$ = 0.23), and male-bias in the autosomes of a similar magnitude, though this was not significant ($V_{A\text{-}FA}$ - $V_{A\text{-}MA}$ = -0.20). No other sex biases were found in the remaining four trait-chromosome type combinations.

Overall volume of the matrices (the sum of the diagonal elements of **G**) was significantly higher in the autosomes ($\mathbf{G}_X$ - $\mathbf{G}_A$ = -1.49), though the ratio ($\mathbf{G}_X$ / $\mathbf{G}_A$ = 0.51) of these did not support either depletion or enrichment of genetic variance on the X as a general pattern when allowing for relative gene or euchromatin content. There was a moderate matrix correlation betw-

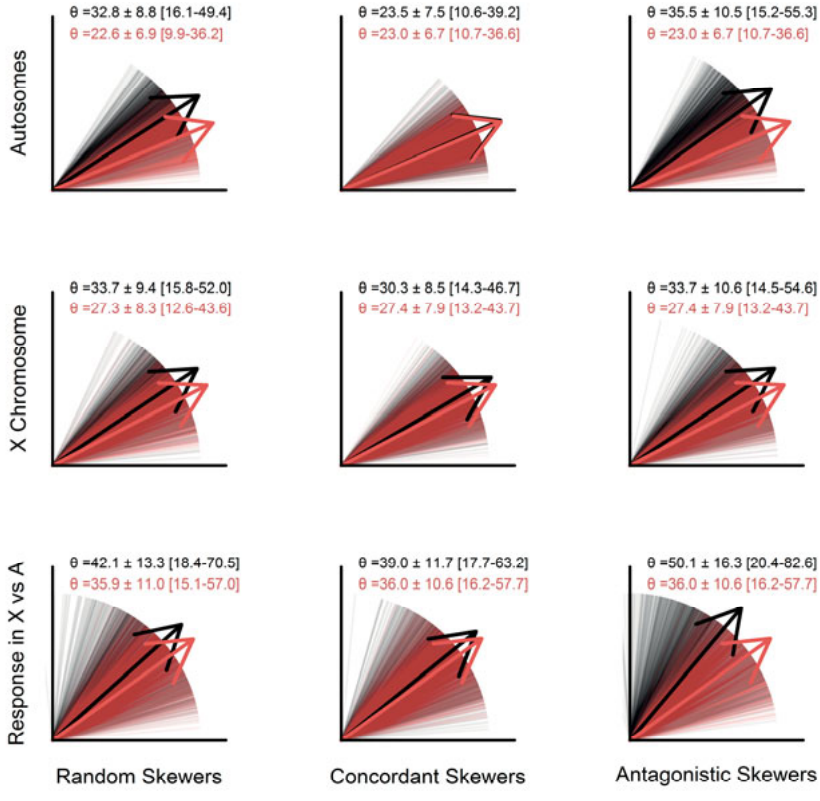

Figure 12. Random skewer analysis of deflection by different G-matrices. In the upper two rows of plots, each line shows the response to selection relative to the vector of selection (along the x-axis) for one random skewer while the large arrows show mean deflection angles (for A-lines in upper row and X-lines in middle row). Deflection by original matrices is shown in black, deflection by matrices with elements of **B** constrained to zero are shown in red. The lowest row of plot shows the difference in deflection between $\mathbf{G}_A$ and $\mathbf{G}_X$ matrices.

een $\mathbf{G}_X$ and $\mathbf{G}_A$ as measured by Mantel tests ($M_{GX\text{-}GA}$ = 0.54), therefore the shape occupied by these two matrices in multivariate spaces was not the same. This result is further supported by a large angle between $g_{max\text{-}X}$ and $g_{max\text{-}A}$ (69.9°).

Cross-sex covariance was found to be present in all three traits on the autosomes, while only climbing ability showed any significant cross-sex covariance in the X chromosomes. These four covariances all translate in to significant correlations between the sexes ($r_{MF\text{-}A}$ BS: 0.51; CL: 0.42; LS: 0.39; $r_{MF\text{-}X}$ NG: 0.53) suggesting that covariance between the sexes has the potential to constrain the evolution of sexual dimorphism through the autosomes in all three traits, and through the X in climbing ability. The effect of

the cross-sex covariance can be shown by setting the values of the B-submatrix to zero (Agrawal and Stinchcombe 2009; e.g. Lewis et al. 2011). Doing this increases the correlation between the matrices ($M_{GX0\text{-}GA0}$ = 0.70) suggesting that the cross-sex covariance is an important difference between the two components of the genome. Interestingly the angle between the dominant eigenvectors of $\mathbf{G}_X$ and $\mathbf{G}_A$ increase when **B** is set to zero (angle between $g_{max\text{-}X0}$ and $g_{max\text{-}A0}$ = 83.2°) but this result could be due to differences in the distribution of the (co)variances. Structure of **B** was different among the X and autosomes, with both the upper and lower segments indicating large angles between the dominant eigenvectors (angle between $g_{max\text{-}BUpX}$ and $g_{max\text{-}BUpA}$ = 63.0°; $g_{max\text{-}BLoX}$ and $g_{max\text{-}BLoA}$ = 62.4°). The structure of the upper and lower segments of **B** also differed from each other in both the X and autosomes. This was supported by Mantel tests in the autosomes ($M_{BUpA\text{-}BLoA}$ = 0.77), and significantly different alignments of the dominant eigenvector in both (angle between $g_{max\text{-}BUpX}$ and $g_{max\text{-}BLoX}$ = 32.1°; $g_{max\text{-}BUpA}$ and $g_{max\text{-}BLoA}$ = 36.2°).

Skewers analyses were used to characterise the effects of the overall differences between the two matrices (Fig. 12). The angle between selection and the response (Θ) is telling of how the (co)variance volume and structure affects evolution, with large angles showing that the G-matrix in question will generally offer poor responses to selection, while low angles suggest that adaptation is generally unconstrained by **G**. Using random skewers, which allows selection in any direction for each trait and sex, the mean angle between the selection and response was similar for both the X and autosomes (Θ_A = 32.8°, Θ_X = 33.7°) but the angle between the response vectors was large (angle between Θ_A and Θ_X = 42.1°) showing that the X and autosomes distort the response to selection in different directions. Constraining **B** to zero slightly reduces the angle between selection and response in both $\mathbf{G}_X$ and $\mathbf{G}_A$ suggesting that cross-sex covariances do constrain the response to selection in general. Given that this thesis revolves around the evolution of sexual dimorphism it is interesting to examine the effects of (co)variance on the response to specific forms of selection. With sexually concordant skewers analysis the response to selection was deflected further from the direction of selection more in the X than in the autosomes (Θ_A = 23.5°, Θ_X = 30.4°) and setting **B** to zero produced a slight improvement in the response. The mean angle between the responses of the X and autosomes under sexually concordant selection (39.1°) again indicates that differences in the (co)variance of the two constrain adaptation in different directions. Under sexually antagonistic selection, which should lead to the evolution of sexual dimorphism, the response to selection is, perhaps surprisingly, similarly deflected in both components of the genome (Θ_A = 35.5°, Θ_X = 33.8°). When **B** is constrained to zero the deflection away from the vector of selection is reduced in the autosomes and the X chromosome, suggesting that the surprisingly large angle of deflection with $\mathbf{G}_X$ is, at least in part, due to the co-

variance between the sexes. The largest difference between the X and autosomes direction of response was seen in the sexually antagonistic skewers analysis, supporting that these parts of the genome might play different roles in the evolution of sexual dimorphism.

Finally, in a third assay, I estimated sex-specific selection for all three traits. Using these values in the Lande equation for both components of the genomes shows that the autosomes respond better to selection than the X chromosome ($\Theta_A = 32.2°$, $\Theta_X = 40.1°$), while the angle between the response each component allows was large (47.5°). Constraining **B** to zero allowed improved response to selection for both components of the genome ($\Theta_A = 22.5°$, $\Theta_X = 35.4°$). Overall the results of **paper IV** suggest that the shared genome has the potential to constrain the evolution of sexual dimorphism, through both components of the genome but perhaps most severely in the autosomes, and this potential indeed appears to be affecting this population.

Discussion

The central theme of this thesis is the evolution of sex differences. I aim to test whether intersexual genetic correlations, which are caused by both sexes being affected by a largely shared genome, generally reduce the potential for adaptation under sexually antagonistic selection, and whether different parts of the genome have different roles to play in adaptive evolution. This research was conducted using a combination of transcriptomic methods, laboratory techniques, and quantitative genetic analytical approaches.

The work conducted in **paper I** provides an insight into patterns in the intersexual genetic correlation. Previous studies have indicated that the intersexual genetic correlation may be, in general, high or moderate, and rarely zero. Many studies measure just a handful of traits, a symptom of the large sampling effort required to obtain good quality estimates of the intersexual genetic correlation (Lynch and Walsh 1998; Bonduriansky and Chenoweth 2009). Meta-analysis also suggests that the intersexual genetic correlation is typically positive and high, and rarely zero (18 of 488 estimates were < 0 in Poissant et al. 2010).

By studying gene expression I was able to simultaneously generate estimates for the intersexual genetic correlation, from a single population measured in one environment, for thousands of traits. This is a robust way to gain insight into distributions of the r_{MF} but is not without its drawbacks. In the data used for estimating r_{MF}, gene expression was measured with low numbers of replicates, just two replicate pools (25 flies per pool) of each sex and line. Here, I removed genes for which significant genetic variance was not detected in an attempt to remove low quality estimates which would have an artificially low r_{MF}.

Genetic correlations between the sexes are expected to cause a constraint on the evolution of sexual dimorphism (Lande 1980). As such, a negative association is expected to occur between sexual dimorphism and the intersexual genetic correlation, with the most extreme sexual dimorphism only occurring in traits with a (near) zero r_{MF}. Negative correlations have been described between these two in a number of traits (Bonduriansky and Rowe 2005; Poissant et al. 2010) but evidence and support for the hypothesis is mixed and it is unclear how rapidly the r_{MF} can be eroded (Reeve and Fairbairn 2001). Given that the r_{MF} is found to be typically high in this study, and the constraint that this should theoretically bring, it seems that the evolution of sexual dimorphism should be constrained. In support of this I show a

negative association between the intersexual genetic correlation and sex-bias in gene expression. Furthermore, change in sex-bias between *D. melanogaster* and six other *Drosophila* species was also negatively associated with the r_{MF}, suggesting long-term constraint. Though this latter result appears to offer good support for a hypothesis of constraint, the selection that gene expression has been under is not known, and it could be that sex-biased genes have come under more frequent or powerful sexually antagonistic selection. The results of **paper I** support the hypothesis that the shared genome is a pervasive constraint on the evolution of sexual dimorphism.

Male-limited genetic variance contained within the Y chromosome has the potential to resolve intralocus sexual conflict that is generated by sexually antagonistic selection. Classically considered gene-poor and unimportant beyond male-limited traits, recent work has shown that the Y could regulate the expression of thousands of genes spread throughout the genome (Lemos et al. 2008; Jiang et al. 2010; Lemos et al. 2010). Furthermore, within-population genetic variance, which is widely expected to become depleted in the Y chromosome, has been shown to remain high on the Y chromosome for male fitness (Chippindale and Rice 2001). **Paper II** examines whether genetic variance for lifespan persists within a population. Lifespan was deliberately chosen as the focal trait for this study for three reasons. Y chromosome effects are expected to more frequently manifest in sexually dimorphic traits. Complex traits are more likely to be regulated by the Y chromosome by simple probability, due to their having more potential regulatory targets. Lifespan has known links to the heterochromatin landscape, and position effect variegation, related to heterochromatin on the Y, is a major mechanism through which the Y can exert regulatory effects.

Using chromosome substitution lines to place Y chromosomes sampled from a single population in a uniform genetic background I show that the Y chromosome contains a small amount of genetic variance within the population. Because this effect is tested within a single genetic background, it is unclear whether the genetic variance occurs through additive or epistatic effects, or a mixture of both. Although a significant signal is detected, it is a small amount of genetic variance. This highlights the need for large sampling effort and robust experimental design when looking for Y-linked genetic variance. Overall, because the genetic variance found explains just a fraction of the variance in lifespan, this result suggests that intralocus sexual conflict, induced by novel sexually antagonistic selection on lifespan, would probably only be partially resolved through standing Y linked genetic variance. However, because the Y chromosome does have an effect on lifespan, novel Y-linked mutations have the potential to also resolve conflict.

Genetic variance is not only expected to be affected in the Y chromosomes when sex chromosomes form. X chromosomes also change relative to the autosomes, with predictions generally suggesting reduced X-linked genetic variance. Molecular evidence supports this hypothesis, with relatively

lower molecular diversity in the X chromosome of *D. melanogaster*. It is, however, unclear how well molecular variance translates into variance at the phenotypic level (Dean and Mank 2014), and there are reasons to also expect a disconnect between molecular diversity and genetic variance at the X chromosome (Rice 1984; Fry 2010; Reinhold and Engqvist 2013). In **paper III** and **paper IV** chromosome substitution lines are used to investigate the genetic variance and covariance in the X and autosomes.

An immediate complication of testing for a depletion of X-linked genetic variance is that the X chromosome is a significantly smaller part of the genome. If the effect size of genes is equal for both X-linked and autosomal genes the expectation would be that the X chromosome host 15.6% of the standing genetic variance if there is no difference between the two components of the genome. In **paper III**, the estimates of genetic variance for lifespan and ageing indicate that the X chromosome is not depleted for standing additive genetic variance. While the credible intervals of all of these estimates could not preclude depletion, these intervals only marginally overlap 15.6% in both male and female X-linkage of lifespan variance. Additive genetic variance in ageing also appears to be enriched on the X chromosome for females, while in males it is neither enriched nor depleted. The ageing estimates though have larger credible intervals, and are more difficult to draw firm conclusions from. Larger credible intervals occur because ageing is measured on a population level basis, in this case, per vial of 50 flies, each line then has just 4 measures of ageing per sex, compared to 200 in the lifespan data.

Dosage compensation could explain why the X chromosome is not depleted for genetic variance. In *Drosophila* the X chromosome is almost perfectly upregulated in males relative to the expression of autosomal genes (Conrad and Akhtar 2012). Subsequently it is predicted that male X-linked genetic variance should be twice that of females (Fig. 2) (Reinhold and Engqvist 2013). In **paper III** it appears that the X chromosome is not enriched for additive genetic variance. However, lifespan is a sexually dimorphic trait which renders comparison of the additive genetic variance flawed. It is more correct to compare the sexes using the coefficient of variation which accounts for differences in the mean, giving a scale free comparison. By using this method there is still no significant sex-bias in the genetic variance, though it is suggestive of a male-bias. Nonetheless, the results also suggest that the X-linked genetic variance is not twice as high in males. It is possible that selection for dosage compensation in males, not only increases expression in males, but also in females by correlated responses. It is also possible that the X chromosome is enriched for female-specific genes, which would counteract the effect of dosage compensation on this ratio.

The X chromosome could also be enriched for genetic variance because sexually antagonistic mutations may persist more easily on the X (Rice 1984; but see Fry 2010). Loci under sexually antagonistic selection should

attract sex-specific modifiers which allow conflict to be resolved. The results in **paper III** and **paper IV** suggest that the X chromosome is enriched for sex-specific genetic variance. In **paper III** the results for the ageing analysis again show no difference between the X and autosomes, but this again suffers from low power caused by limited sampling (Lynch and Walsh 1998). The intersexual genetic correlation for lifespan was almost exactly zero in the X chromosomes, while it was substantial for the autosomes. This supports a previous study in this population which found an intersexual genetic correlation for lifespan which was slightly lower than that observed in the autosomes, when measured across the entire genome (Lehtovaara et al. 2013).

Covariance between the sexes should constrain the response to selection when selection is antagonistic. Given the difference between the X and autosomes in covariance structure (Table 1) these two components of the genome should produce different responses to selection. Indeed the structure of $\mathbf{G}_X$ and $\mathbf{G}_A$ was different in many ways which produced very different responses to selection in the skewers analyses. The constraint each G-matrix imposed also differed, with marginally less constraint in response to sexually antagonistic selection skewers in the X chromosome than in the autosomes. Furthermore, constraining **B** improved the response to selection in the autosomes more so than in the X chromosome when tested under sexually antagonistic selection, showing that the covariance between the sexes is a larger constraint to evolution through the autosomes than the X. It is noteworthy that the modified random skewers methods used here are a novel way to explore G-matrix structure in a biologically meaningful context. The results from the sexually antagonistic skewers analysis suggests that the X chromosome may play a special role in adaptive evolution when sexually antagonistic selection occurs. This contrasts with the results for the autosomes, and significant covariance between the sexes seen at the whole-genome level of a number of species (Barker et al. 2010; Lewis et al. 2011; Stearns et al. 2012; Ingleby et al. 2014).

Conclusion

This thesis set out to examine the extent of constraint on the evolution of sexual dimorphism in general, and then to explore the patterns of genetic (co)variance across the genome which might affect the role different components of the genome have to play in this constraint. Transcriptomic data was used to show that the genetic architecture underlying gene expression appears to be largely shared between the sexes, and that this constrains the sexes from exhibiting sex-bias. The Y chromosome is found to harbour a small amount of genetic variance affecting lifespan, suggesting that it has a limited potential to resolve intralocus sexual conflict from standing genetic variation, but the finding of any effect suggests that the Y can resolve intralocus sexual conflict in traits present in both sexes. The X chromosome is shown to host a surprisingly large amount of genetic variance in a number of traits, despite predictions based on the population genetic settings of the X. Dosage compensation does not appear to cause male-bias in X-linked genetic variance and a number of reasons are discussed. Finally the genetic variance-covariance structure of the X chromosome shows that the X has a potentially special role to play in the resolution of intralocus sexual conflict.

Svensk sammanfattning

Skillnader mellan könen är mycket vanligt förekommande i naturen. Det man kanske först kommer att tänka på är extrema exempel såsom påfågelhanens långa och vackra stjärt, storleksskillnaden mellan könen hos många däggdjur, eller horn och andra vapen som ibland förekommer hos hanar men inte honor. Skillnader förekommer dock med avseende på så gott som alla egenskaper och inkluderar morfologi, fysiologi, och beteende såväl som livshistoria. Det som ligger till grund för att könen skiljer sig åt, och då inte bara med avseende på reproduktionsorganen, är att hanar generellt producerar små könsceller (spermier) medan honor producerar stora (ägg). Av detta följer att hanar och honor ofta bäst bidrar med avkomma till nästa generation via olika strategier, vilket i sin tur selekterar för olika egenskaper hos könen. Hos vissa arter är det till exempel av stor vikt för en hane att ha egenskaper som gör det möjligt att försvara ett revir för att kunna attrahera honor, medan det hos andra kan vara en tjusig fjäderskrud eller en imponerande dans som honorna faller för. Många gånger fyller sådana egenskaper inte någon funktion hos honor, eller är rent av skadliga, då de har en negativ effekt på deras reproduktionsförmåga.

Medan det är relativt lätt att förstå att det ofta gynnar hanar och honor att ha olika egenskaper, är det desto svårare att förstå hur evolutionen skapar könsskillnader. Hanar och honor tillhör samma art och delar därför samma genom, vilket befinner sig 50 % av tiden i vardera kön. Om olika egenskaper gynnas beroende på vilket kön genomet befinner sig i skapas en konflikt mellan könen över genomets funktion. Eftersom könsskillnader är vanliga vet vi att genomet löser den konflikten på lång sikt, men till vilken grad begränsas evolution av könsskillnader av att hanar och honor delar samma genom, och har olika delar av genomet olika förutsättningar att skapa könsskillnader? Det är frågor som den här avhandlingen försöker besvara. För att undersöka detta har jag studerat graden av könsspecifik genetisk variation, en grundförutsättning för evolution av könsskillnader, i olika delar av genomet hos bananflugan *Drosophila melanogaster*. Jag har framför allt fokuserat på genetisk variation i genuttryck och livslängd.

Genomet är uppdelat på kromosomer som kan delas in i tre klasser *i)* autosomer, vilka det finns två kopior av i vardera kön och som nedärvs symmetriskt mellan könen, *ii)* X-kromosomen, vilken det finns två kopior av hos honor men endast en av hos hanar, och som därför nedärvs symmetriskt från honor men endast till döttrar från hanar, och slutligen *iii)* Y-

kromosomen, vilken endast finns i en kopia hos hanar, och som nedärvs strikt mellan far och son. Skillnader i antal kromosomer per kön, samt olika nedärvningsmönster, har lett till teorier om att olika delar av genomet skulle kunna ha olika förmåga att bidra till evolution av könsskillnader.

Enklast att skapa skillnader mellan könen torde Y-kromosomen ha, då den endast befinner sig i hanar och därför helt och fullt kan anpassas för att optimera egenskaper som premieras i det könet. Problemet med Y-kromosomen är att den hos bananflugan, och hos många andra arter, har förlorat det mesta av sitt funktionella DNA och endast innehåller ett fåtal gener. I en av mina studier undersöker jag om det finns genetisk variation på Y-kromosomen för livslängd inom en population. Studien visar att variation finns, men att den utgör en mycket liten del av den totala variationen. Trots det kan den fylla en viktig funktion, eftersom all variation är hanspecifik och därmed kan förändra hanar utan att ha någon effekt på honor.

I två andra studier utforskar jag skillnader mellan autosomerna och X-kromosomen. I den första av dessa tittade jag närmre på flera prediktioner från teoretiska modeller, varav en relaterar till evolution av könsskillnader. I och med att X-kromosomen bara uppträder med en kopia hos hanar antas den genetiska variationen på denna kromosom vara mindre än den på autosomerna. Anledningen är att mutationer måste uppträda i två kopior för att deras effekt ska slå igenom när de sitter på autosomerna, men alltid har en effekt om de befinner sig på en hanes enda X-kromosom. Detta gör att det naturliga urvalet mer effektivt kan sålla bort skadliga mutationer och selektera fram de som är fördelaktiga, vilket borde medföra en lägre grad av genetisk variation på X-kromosomen. Min studie visar dock att så inte verkar vara fallet, för vare sig livslängd eller åldrande, eftersom variationen på X-kromosomen för dessa egenskaper står i proportion till X-kromosomens storlek. En annan prediktion jag undersökte var om X-kromosomen uppvisar mer genetisk variation i hanar än hos honor. Då hanar endast har en X-kromosom uppregleras uttrycket av generna på den hos bananflugan, för att likställa uttrycket med de två X-kromosomer som finns hos honor. Om det finns två genetiska varianter, och honorna därför kan ha två av endera eller en av varje, kommer hanarna i praktiken alltid ha två av samma slag, då den enda de har uttrycks dubbelt. Hanar kan därför endast ha ytterligheterna i en fördelning där honorna också har ett mellansteg. Följden av detta är att hanar i de allra flesta fall borde uppvisa mer variation på X-kromosomen än vad honorna gör. Min studie visar dock förvånade att detta inte är fallet, vare sig för livslängd eller åldrande.

Den tredje prediktionen jag undersökte, och som kanske är nyckeln till de avvikande resultaten för de två andra delstudierna, är om X-kromosomen hyser relativt mer könsspecifik variation än autosomerna. Här finns det teori som stödjer detta, men också teori som predikterar det motsatta. Mina resultat pekar på relativt mer könsspecifik genetisk variation på X-kromosomen för livslängd. För åldrande föreligger dock ingen skillnad, då både X-

kromosomen och autosomerna endast verkar bestå av könsspecifik variation för denna egenskap. Detta resultat pekar på att evolution av könsskillnader lättare borde utvecklas på X-kromosomen än på autosomerna. Det faktum att all variation är könsspecifik på X-kromosomen är en möjlig förklaring till varför vi inte ser en reduktion av genetisk variation på X-kromosomen, och inte ser mer variation på X-kromosomen hos hanar jämfört med honor. Anledningen är att flera av de antaganden som prediktioner avseende den relativa storleken av genetisk variation på X-kromosomen vilar på förutsättningen att det är samma gener som påverkar variation i båda könen, vilket mina resultat motsäger.

I min andra studie om skillnader mellan X-kromosomen och autosomerna uppmätte jag genetisk variation samt hur det naturliga urvalet agerar på egenskaperna livslängd, kroppsstorlek samt flugornas respons att röra sig uppåt efter ett störningsmoment (negativ geotaxis). Här använde jag mer sofistikerade metoder för att se hur dessa egenskaper samvarierar inom och mellan könen, för att skatta till vilken grad X-kromosomen och autosomerna begränsar evolution av könsskillnader. Även denna studie pekar på att evolution av könsskillnader borde kunna ske lättare via X-kromosomen än via autosomerna.

I en fjärde studie fokuserade jag på genetisk variation i genuttryck av bananflugans alla gener. Tre generella slutsatser kan dras från den studien. För det första är det mesta av den genetiska variationen i genuttryck könsneutral, och det är endast något mer könsspecifik variation för uttryck av gener på X-kromosomen. För det andra så begränsar denna höga grad av könsneutral variation evolution av könsspecifikt genuttryck på både kort och lång sikt. Och för det tredje uppvisar gener vars uttryck i nuläget utsätts för urval i olika riktning i hanar och honor en högre grad av könsneutral variation, vilket är ett ytterligare bevis för att ett mellan könen delat genom begränsar evolution av könsskillnader.

Sammanfattningsvis visar denna avhandling att evolution av könsskillnader begränsas av att hanar och honor delar samma genom. Y-kromosomen är minst begränsad följd av X-kromosomen, och störst är begränsningarna på autosomerna. Väger man in den totala variationen, visar det sig dock att det är autosomerna som har störst kapacitet att bidra till evolution av könsskillnader, eftersom de utgör den större delen av genomet, vilket kompenserar för en högre grad av inbyggda begränsningar.

Acknowledgements

The work presented in this thesis represents more than four years of work, much of it done in the lab with the help of a number of people. I would like express my gratitude to those that helped me tackle some huge experiments; Emma Thorén, Markus Hiltunen, Fredrik Dölfors, and Therese Jonsson for their assistance on the G-matrix fitness assay, and Damien Le Gall and Aivars Cīrulis for their work on the Y chromosome project. Thanks to Jaime Grace and Rebecca Dean, also for help with the lab workload, as well as providing continued guidance about science, academia, and life in general. Without doubt, the substantial empirical elements of this thesis benefitted greatly from the assistance of Cindy Canton. Thank you for going way beyond what anyone could have asked you to do and making the lab a brighter place in the process, without you there the challenges these projects presented would have felt even more daunting!

Thanks to MD Sharma for getting me in to the fly lab when I was an undergraduate and showing me, by excellent example, that a PhD requires a huge effort. Thanks to those who have engaged with me in scientific discussion, particularly Göran Arnqvist *et al* for having me in your group meetings over the last year and a half, and the numerous officemates that I've had the pleasure of sharing an office with over the PhD, I apologise for the faint smell of fly food that followed me for much of it.

Some of the best experiences in my time as a PhD student came in Bielefeld. I want to thank everyone in the department there for great discussions and being so welcoming for my visits. Obviously thanks to Pablo, Bahar, Michael, Klaus, Matthias, and many others for numerous kicker games. In between games, I did get some work done, and I thank Holger Schielzeth for his part in that, and devoting so much time and effort to our projects.

I would like to thank the many friends I made out on the bike and those that joined me on rides over the last four years, especially Rado Kozma, Ludovic Dutoit, Johan Brändström - I had a lot of fun riding Vätternrundan (and training) with you this year! Thank you to Axel Danielsson and Andreas Elvin for our time in Ångkvarnsgatan and beyond, you guys have been so important to me and I appreciate the support you have given. And an extra thanks to Axel and his family who between them, over the last four years, have spent many hours standing on top of mountains patiently waiting for me to eventually ski to the top, and then spent many more hours waiting for me to ski back down! Also thank you to my housemates from university who

continue to be wonderful friends, I wish Jack Hollis, James Regan, Chris Perry, and Stuart Campbell all the best for the future. I also want to thank Eva Podgrajsek and Sebastian Bay for providing me with many fun times on Sturegatan. Thanks to the many friends within EBC, especially Blaize Denfeld and Monica Ricão Canelhas who started with me as PhD's back in 2011, you two are just brilliant and have made me laugh so many times. Thank you also to the mother's committee, Oddný Ósk Sverrisdóttir and Gunilla Kärf, who have cared for me like one of their own so many times, reminding me to eat and sleep, inviting me for Christmas when I was stuck here on projects, and taking every opportunity to embarrass me - you're just like my real mother, which is terrifying.

I want to thank Urban Friberg for his work as my supervisor, and Jon Ågren and Alexei Maklakov for co-supervision. Thank you for giving me the opportunity to come to Sweden, for the guidance you have given and for the work that you put in which made this thesis possible.

I would like to thank my family who have given me so much support and been so understanding for all the missed birthdays, holidays, and various other family moments. I promise I will start phoning more regularly now, and who knows, you might even get Christmas cards *before* Christmas day this year (but no promises). Thank you also to the Kauffeldt family for being so welcoming, I promise to get to work on my Swedish now. Finally I wish to thank Anna for all she has done to help get me to the end of this - thank you for all of the times you've managed to make me smile at the end of a difficult day and showing me the best bits of Sweden (but you can keep the Kalles kaviar), and for putting up with my bike being in the apartment.

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