The genetic architecture of sexual dimorphism

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Abstract

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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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 peacock’s 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; Arnvist 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 $\beta$ is a vector of selection coefficients (partial regression coefficients of fitness on the traits included in the study) also of length $m$.

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

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 \( \left( N_Y = \frac{1}{3}N_X = \frac{1}{4}N_A \right) \), 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).

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

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). Right hand 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 $2p^2$ 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; Piscchedda 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 ($N_X = \frac{3}{4} N_A$). 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]apX), and a CiD 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]apX) and a phenotypically marked fourth chromosome (CiD) (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 into 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^v, rdgC, ri, st/T[2;3] bw^d, in, p^v, 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, fY). 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 ($V_P$) into variance components explained by the genetic ($V_G$) and environmental effects ($V_E$). Genetic variance can be further decomposed into 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).

\[
V_A = 2pq\alpha^2
\]

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 (\( G_M \), blue shaded cells), female-specific submatrix (\( G_F \), red shaded cells), and the cross-sex submatrices (\( B \) and \( B^T \), yellow shaded cells).

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, $g_{\text{max}}$, which is the eigenvector along which the variance is most abundant (Schluter 1996; Wyman et al. 2013). Comparisons of the angle between $g_{\text{max}}$ 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 ($\beta$ 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
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 \textit{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 \textit{i}) differences in autosomal and X-linked genetic variance, \textit{ii}) sex-bias in X-linked genetic variance, and \textit{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 ($r_{MF-A} = 0.50$) and not different from zero in the X chromosome ($r_{MF-X} = 0.04$) (Fig. 11). The genetic correlation in the X chromosome was not different from that of the autosomes ($r_{MF-A} - r_{MF-X} = 0.46$) (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 ($r_{MF-A} = -0.11$; $r_{MF-X} = -0.31$), and not different from one another ($r_{MF-A} - r_{MF-X} = 0.20$) (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 $G_X$ and $G_A$, 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 shown 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.

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Combinations, 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-FX} - V_{A-MX} = 0.23*), and male-bias in the autosomes of a similar magnitude, though this was not significant (*V_{A-FA} - V_{A-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 (*G_X - G_A = -1.49*), though the ratio (*G_X / 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 GA and GX matrices.

Seen GX and GA as measured by Mantel tests (MGX-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 gmax-X and gmax-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 (rMF-A BS: 0.51; CL: 0.42; LS: 0.39; rMF-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 \( M_{G_{X0}-G_{A0}} = 0.70 \) 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 \( G_X \) and \( G_A \) increase when \( B \) is set to zero (angle between \( g_{\text{max-}X0} \) and \( g_{\text{max-}A0} = 83.2^\circ \)) 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 \( g_{\text{max-}B_{\text{Up}X}} \) and \( g_{\text{max-}B_{\text{Up}A}} = 63.0^\circ; \) \( g_{\text{max-}B_{\text{Lo}X}} \) and \( g_{\text{max-}B_{\text{Lo}A}} = 62.4^\circ \)). 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 \( M_{B_{\text{Up}A}-B_{\text{Lo}A}} = 0.77 \), and significantly different alignments of the dominant eigenvector in both (angle between \( g_{\text{max-}B_{\text{Up}X}} \) and \( g_{\text{max-}B_{\text{Lo}X}} = 32.1^\circ; \) \( g_{\text{max-}B_{\text{Up}A}} \) and \( g_{\text{max-}B_{\text{Lo}A}} = 36.2^\circ \)).

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 (\( \Theta \)) 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 \( (\Theta_A = 32.8^\circ, \Theta_X = 33.7^\circ) \) but the angle between the response vectors was large \( (\Theta_A = 32.1^\circ, \Theta_X = 42.1^\circ) \) 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 \( G_X \) and \( G_A \) 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 \( (\Theta_A = 23.5^\circ, \Theta_X = 30.4^\circ) \) 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 \( (39.1^\circ) \) 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 \( (\Theta_A = 35.5^\circ, \Theta_X = 33.8^\circ) \). 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 \( G_X \) is, at least in part, due to the co-
variance between the sexes. The largest difference between the X and autoso-
mes 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 (Θ_A = 32.2°, Θ_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 (Θ_A =
22.5°, Θ_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 $G_X$ and $G_A$ 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.

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 tjuasig fjäderskrud eller en imponerande dans som honorna faller för. Många gånger fyller sådana egenskaper inte någon funktion hos honor, eller är rent av skadlig a, 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.


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 mest 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 upptäcker 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.
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The Shared Genome Is a Pervasive Constraint on the Evolution of Sex-Biased Gene Expression

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Abstract

Males and females share most of their genomes, and differences between the sexes can therefore not evolve through sequence divergence in protein coding genes. Sexual dimorphism is instead restricted to occur through sex-specific expression and splicing of gene products. Evolution of sexual dimorphism through these mechanisms should, however, also be constrained when the sexes share the genetic architecture for regulation of gene expression. Despite these obstacles, sexual dimorphism is prevalent in the animal kingdom and commonly evolves rapidly. Here, we ask whether the genetic architecture of gene expression is plastic and easily molded by sex-specific selection, or if sexual dimorphism evolves rapidly despite pervasive genetic constraint. To address this question, we explore the relationship between the intersexual genetic correlation for gene expression ($r_{MF}$), which captures how independently genes are regulated in the sexes, and the evolution of sex-biased gene expression. Using transcriptome data from Drosophila melanogaster, we find that most genes have a high $r_{MF}$ and that genes currently exposed to sexually antagonistic selection have a higher average $r_{MF}$ than other genes. We further show that genes with a high $r_{MF}$ have less pronounced sex-biased gene expression than genes with a low $r_{MF}$ within D. melanogaster and that the strength of the $r_{MF}$ in D. melanogaster predicts the degree to which the sex bias of a gene’s expression has changed between D. melanogaster and six other species in the Drosophila genus. In sum, our results show that a shared genome constrains both short- and long-term evolution of sexual dimorphism.

Key words: sexual dimorphism, genetic constraint, sex-biased gene expression.

Introduction

In most species, male and female fitness is optimized through different strategies, which selects for phenotypic differences between the sexes (Arnqvist and Rowe 2005; Bonduriansky and Chenoweth 2009; Van Doorn 2009). Traits that show such sexual dimorphism are common in nature (Fairbairn et al. 2007), and they typically evolve rapidly (Darwin 1871; Meyer 1997; Arnvist 1998; Civetta and Singh 1998; Omland and Lanyon 2000; Emlen et al. 2007). On the one hand, this is expected, because sexual characters are often exposed to strong sex-specific selection (Badyaev and Martin 2000; Hoekstra et al. 2001; Kingsolver et al. 2001). On the other hand, it is a paradox, because males and females share the same genome, apart from a few genes found on the Y and W chromosomes. With most genes shared between the sexes, the evolution of sexual dimorphism should be constrained, because selection on one sex should result in a correlated response in the other. Theory confirms this verbal argument and shows that evolution of sexual dimorphism proceeds exceedingly slowly when the genetic architecture is very similar in the sexes (Lande 1980, 1987; Reeve and Fairbairn 2001).

As males and females have largely the same genes, sexual dimorphism often cannot evolve through sequence differences between the sexes. Instead, the evolution of sexual dimorphism is restricted to sex-specific expression (Rinn and Snyder 2005; Conallon and Knowles 2005; Ellegren and Parsch 2007) and splicing of genes (McIntyre et al. 2006; Telonis-Scott et al. 2009). Genomic studies of the transcriptome have revealed that a large fraction of genes in model organisms have evolved sex-biased expression (Jin et al. 2001; Rinn and Snyder 2005; Yang et al. 2006; Ellegren and Parsch 2007; Mank, Hultin-Rosenberg, Webster, et al. 2008; Reinius et al. 2008; Jiang and Machado 2009) and that sex-biased genes, particularly those with male-biased expression, undergo rapid evolution (Ranz et al. 2003; Meiklejohn et al. 2003; Khatovich et al. 2005; Voolstra et al. 2007; Zhang et al. 2007; Grath et al. 2009; Jiang and Machado 2009, Parsch and Ellegren 2013). Given the rapid evolution of sexual dimorphism on all levels of phenotypic organization, does this take place despite strong constraints, or is the genetic architecture in males and females free to evolve independently?

The intersexual genetic correlation ($r_{MF}$) is a scaled measure of the extent to which genetic variation covaries between the sexes and ranges from $-1$ to $1$. An $r_{MF}$ of one means that the genetic variation for a trait has exactly the same genetic basis in males and females, whereas an $r_{MF}$ of zero indicates that it has a sexually independent genetic foundation. If the evolution of sexual dimorphism is constrained by a shared genetic architecture, a negative association between the
degree of dimorphism and the strength of the \( r_{MF} \) is predicted (Lande 1980; Bonduriansky and Rowe 2005; Fairbairn and Roff 2006). Such a relationship can arise in two different ways, either because traits that initially have a low \( r_{MF} \) respond faster to novel sex-specific selection or because sex-specific selection causes mutations with sex-specific effects to accumulate over time, reducing the \( r_{MF} \) (Fairbairn et al. 2007). Several mechanisms have been proposed, which should allow for evolution of sex-specific genetic variance. These include gene duplication, where each sex sequesters one of the paralogous genes (Rice and Chippindale 2001; Stewart et al. 2010; Connallon and Clark 2011; Gallach and Betran 2011a, 2011b; Hosken 2011; Wyman et al. 2012), recruitment of sex-specific transcription factor binding sites (reviewed in Williams and Carroll 2009), sex linkage (Rice 1984), and genomic imprinting (Day and Bonduriansky 2004). However, it is noteworthy that rapid fixation of alleles with sex-specific effects could mitigate the build-up of a negative association between the \( r_{MF} \) and the degree of sexual dimorphism (Meagher 1992; Reeve and Fairbairn 1996). In this scenario, sexual dimorphism evolves but leaves no lasting signature on the \( r_{MF} \).

Empirical studies testing for an association between sexual dimorphism and the \( r_{MF} \) using traits at a high level of phenotypic organization (i.e., morphological, behavioral, and physiological) have given mixed results within the population level. A negative correlation has been documented in waltzing flies (Bonduriansky and Rowe 2005), water striders (Preziosi and Roff 1998; Fairbairn et al. 2007), a moss (McDaniel 2005), and a dioecious plant (Delph et al. 2004, 2010), whereas no such associations have been documented in fruit flies (Cowley et al. 1986; Cowley and Atchley 1988; association reported in Fairbairn and Roff 2006) and sticklebacks (Leinonen et al. 2011). A meta-analysis of plant species also failed to find a negative association (Ashman and Majetic 2006). However, a more extensive meta-analysis, compiling data from both animals and plants, did find a marginally significant negative correlation (Poissant et al. 2010).

Little is known about the extent to which the genetic architecture at the lowest level of phenotypic organization, gene expression, constrains the evolution of sexual dimorphism. To address this question, we used gene expression data from Drosophila melanogaster and contrasted it to gene expression in D. simulans, D. yakuba, D. ananassae, D. pseudoobscura, D. virilis, and D. mojavensis. We show that the \( r_{MF} \) for gene expression in general is high and that genes currently expressed to divergent selection on gene expression in the sexes have a higher \( r_{MF} \) than other genes. We further show a negative association between the \( r_{MF} \) and the degree of sex-biased gene expression within D. melanogaster and that the \( r_{MF} \) of a gene in D. melanogaster predicts the extent to which sex bias has evolved within D. melanogaster and other Drosophila species. In sum, our results provide several lines of independent evidence that the shared genome represents a pervasive constraint on the evolution of sex-biased gene expression.

### Results

**Estimates of the Intersexual Genetic Correlation**

Across all genes in the D. melanogaster genome, the median \( r_{MF} \) was only 0.295 (95% confidence interval [CI] = 0.287–0.302, fig. 1, black bars). However, genetic correlations are determined by how tightly the genetic variances of two traits are associated (in this case male and female gene expression). When genotypic values are estimated with poor precision, this will, on average, reduce the association between traits and bias the estimate of the genetic correlations toward zero. We used the data from the Drosophila Genetic Reference Panel (DGRP) study of Ayroles et al. (2009) to calculate the \( r_{MF} \) across the genome of D. melanogaster. This data set is unique with respect to its extensive sampling of genome-wide gene expression across 40 genotypes from one population but limited in that it consists of “only” two samples per sex and genotype. Low sampling combined with potentially high levels of noise, typically associated with gene expression estimated through microarrays, thus suggest that estimates of the \( r_{MF} \) from this data set, on average, will be biased downward (given that the \( r_{MF} \) of most genes is positive).

In an attempt to reduce this problem, we applied two statistical approaches to filter out genes with high levels of sampling variation and genes without a genetic component associated with the variation (the \( r_{MF} \) is not defined for genes that lack genetic expression variation). After normalizing expression variation for each gene in each sex (\( \hat{X} = 0, \sigma = 1 \)), we fitted a linear mixed effects model to each gene with the fixed factor Sex and the factors Genotype and Sex × Genotype defined as random effects. In our first approach, we classified genes according to the percentage (≥20%, ≥40%, ≥60%, and ≥80%) of the sum of random effects variation and residual variation (“total”) that had a genetic component (captured by the random effects). Our second approach used the same model as defined earlier and employed log-likelihood ratio testing to generate \( P \) value estimates for both random effects. Genes were retained if either or both of the random effects were significant where \( P < 0.01 \). These genes are herein referred to as having significant genetic variation (\( n = 8,997 \)). The unfiltered set consisted of 12,572 genes.

Gradually removing genes, from those for which the genetic variance was a small component of the total variance, resulted in a steady increase in the \( r_{MF} \) (fig. 1, shaded bars). When we retained only the genes for which the genetic variance explained 80% or more of the total variance, the median \( r_{MF} \) was 0.274 (95% CI = 0.192–0.374, fig. 1, white bars), including only genes with significant genetic variation resulted in a median \( r_{MF} \) of 0.427 (95% CI = 0.419–0.435).

**\( r_{MF} \) and Sexually Antagonistic Selection**

Sexual antagonism is resolved through the evolution of sexual dimorphism. Genes whose expression levels are currently under sexually antagonistic selection should be moving toward higher sex bias. If the \( r_{MF} \) is high, then the evolution
of sex bias will proceed more slowly. Accordingly, we expect that sexual antagonism will persist for longer and genes presently experiencing sexually antagonistic selection should have a higher $r_{MF}$ than other genes. To test this, we first gathered information on a gene’s selective regime from the study of Innocenti and Morrow (2010) and $r_{MF}$ values based on calculations from the data of Ayroles et al. (2009). Genes currently exposed to sexually antagonistic selection (SA) genes and genes under no or another form of selection (Other). Only the genes with significant genetic variation are included. Notches on the boxes represent approximate 95% CIs. Numbers above the boxes show how many genes each box represents.

$r_{MF}$ and Sex Linkage

Theory predicts that sexual dimorphism should evolve more easily through genes located on the X-chromosome (Rice 1984; but see Connallon and Clark 2010). Following from this theory, it has been suggested that the X-chromosome should host more sex-specific genetic variation than the autosomes (Fairbairn and Roff 2006; Husby et al. 2013). We therefore tested whether X-linked genes have a lower $r_{MF}$ compared with autosomal genes. X-linked genes had a small and marginally significant reduction in the $r_{MF}$ compared with autosomal genes when only genes having significant genetic variation were included (estimated coefficient for chromosome type $cct = 0.020$, $P = 0.025$; fig. 3). Similar results were found when all genes were included ($cct = 0.032$, $P < 0.0001$; fig. 3).

$r_{MF}$ and Evolution of Sex-Biased Gene Expression

The presence of genetic constraint for evolution of sex-biased gene expression should result in a negative association between the $r_{MF}$ and the degree of sex bias. Sex-biased gene expression was indeed negatively associated to the $r_{MF}$ for genes in D. melanogaster when only significant genes were included (estimated coefficient for sex-biased expression $c_{sb} = -0.125$, $P < 0.0001$; fig. 4), as well as when all genes were included ($c_{sb} = -0.100$, $P < 0.0001$).

We also tested for an association between the $r_{MF}$ in D. melanogaster and the degree to which genes have changed their sex-biased expression between D. melanogaster and six other Drosophila species, to test whether the genetic architecture in D. melanogaster is informative of the extent to which genes can change in their sex bias. In all cases, we found a negative association between the $r_{MF}$ and the degree of change in sex-biased expression, both when only significant genes were included (estimated coefficient for $\Delta_D.D. melanogaster - D. simulans$ $c_{\Delta D\ D. melanogaster - D. simulans} = -0.074$, $P = 0.015$; $c_{\Delta D\ D. yakuba} = -0.192$, $P < 0.0001$; $c_{\Delta D\ D. ananassae} = -0.066$, $P < 0.0001$).

![Fig. 1. Distributions of the $r_{MF}$ value estimates.](image1)

![Fig. 2. $r_{MF}$ for genes with expression under sexually antagonistic selection (SA) and genes under no or another form of selection (Other). Only the genes with significant genetic variation are included. Notches on the boxes represent approximate 95% CIs. Numbers above the boxes show how many genes each box represents.](image2)

![Fig. 3. $r_{MF}$ for X-linked and autosomal genes.](image3)
to be the case as the estimated median $r_{MF}$ for gene expression level across the *D. melanogaster* genome is only approximately 0.3. However, when we gradually filtered out genes for which the $r_{MF}$ was estimated with poor precision, a different pattern emerged, which suggests that the true $r_{MF}$ for gene expression probably approaches the high value found for typical phenotypic traits.

If a shared genetic architecture poses a constraint for sex-specific evolution, intralocus sexual conflict over expression level caused by sexually antagonistic selection, should remain unresolved longer for genes with a high $r_{MF}$. From this, it follows that genes with expression levels currently exposed to sexually antagonistic selection should have a higher $r_{MF}$ than other genes. Our analyses give strong support for this prediction, despite that the $r_{MF}$ estimates of each gene came from one population (Raleigh, North America) and assignment of selective regimes for the same genes came from another population (Modesto, North America). The fact that our predicted relationship holds between these two populations, that have been separated by more than 500 generations (Rice et al. 2005), indicates that the shared genetic architecture continues to constrain evolution for at least hundreds of generations, and that it is not rapidly broken down by sexually antagonistic selection (but see Delph et al. [2011] for an example where artificial selection for a reduced $r_{MF}$ was successful over just a few generations).

A further prediction, with respect to the genetic architecture and its role in constraining the evolution of sexual dimorphism, is that there should be a negative association between the degree of sexual dimorphism and the strength of the $r_{MF}$ (Lande 1980; Bonduriansky and Rowe 2005; Fairbairn and Roff 2006). This is expected to result if only traits with an initially low $r_{MF}$ can respond to sex-specific selection or if genes with long-term exposure to sexually antagonistic selection evolve a reduced $r_{MF}$. This prediction has received mixed support in previous analyses on traits at a higher phenotypic level, that have been separated by more than 500 generations (Rice et al. 2005; McDaniel 2005; Ashman and Majetic 2006; Fairbairn and Roff 2006; Fairbairn et al. 2007; Poissant et al. 2010; Leinonen et al. 2011). A possible explanation for this discrepancy is that previous studies have suffered from low power as they have either dealt with a limited number of traits or compiled data from many different studies and taxa. Here, we take advantage of the fact that gene expression can be viewed as a phenotypic trait and that thousands of phenotypes can be studied simultaneously through whole genome transcriptome analysis. By analyzing variation in gene expression in a population of *D. melanogaster*, and comparing it with several species in the *Drosophila* genus, we provide strong and manifold evidence that a shared genetic architecture causes a severe constraint on the evolution of sexual dimorphism.

A high $r_{MF}$ should constrain the evolution of sexual dimorphism, and the fact that traits at a high phenotypic organizational level (morphological, physiological, behavioral, and life-history traits) have a median $r_{MF}$ of approximately 0.8 (reviewed in Poissant et al. 2010) indicates that most traits should be constrained. As traits at a high level of phenotypic organization are composed of phenotypes at a lower level, logic suggests that the $r_{MF}$ should be of similar magnitude also for these traits. In our study, this, at first glance, does not seem to be the case as the estimated median $r_{MF}$ for gene expression level across the *D. melanogaster* genome is only approximately 0.3. However, when we gradually filtered out genes for which the $r_{MF}$ was estimated with poor precision, a different pattern emerged, which suggests that the true $r_{MF}$ for gene expression probably approaches the high value found for typical phenotypic traits.

### Discussion

Although theory predicts that a shared genome should pose a severe constraint on the evolution of sexual dimorphism (Lande 1980, 1987), empirical studies have given mixed support for this prediction (Delph et al. 2004, 2010; Bonduriansky and Rowe 2005; McDaniel 2005; Ashman and Majetic 2006; Fairbairn and Roff 2006; Fairbairn et al. 2007; Poissant et al. 2010; Leinonen et al. 2011). A possible explanation for this discrepancy is that previous studies have suffered from low power as they have either dealt with a limited number of traits or compiled data from many different studies and taxa. Here, we take advantage of the fact that gene expression can be viewed as a phenotypic trait and that thousands of phenotypes can be studied simultaneously through whole genome transcriptome analysis. By analyzing variation in gene expression in a population of *D. melanogaster*, and comparing it with several species in the *Drosophila* genus, we provide strong and manifold evidence that a shared genetic architecture causes a severe constraint on the evolution of sexual dimorphism.

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If a shared genetic architecture poses a constraint for sex-specific evolution, intralocus sexual conflict over expression level caused by sexually antagonistic selection, should remain unresolved longer for genes with a high $r_{MF}$. From this, it follows that genes with expression levels currently exposed to sexually antagonistic selection should have a higher $r_{MF}$ than other genes. Our analyses give strong support for this prediction, despite that the $r_{MF}$ estimates of each gene came from one population (Raleigh, North America) and assignment of selective regimes for the same genes came from another population (Modesto, North America). The fact that our predicted relationship holds between these two populations, that have been separated by more than 500 generations (Rice et al. 2005), indicates that the shared genetic architecture continues to constrain evolution for at least hundreds of generations, and that it is not rapidly broken down by sexually antagonistic selection (but see Delph et al. [2011] for an example where artificial selection for a reduced $r_{MF}$ was successful over just a few generations).

A further prediction, with respect to the genetic architecture and its role in constraining the evolution of sexual dimorphism, is that there should be a negative association between the degree of sexual dimorphism and the strength of the $r_{MF}$ (Lande 1980; Bonduriansky and Rowe 2005; Fairbairn and Roff 2006). This is expected to result if only traits with an initially low $r_{MF}$ can respond to sex-specific selection or if genes with long-term exposure to sexually antagonistic selection evolve a reduced $r_{MF}$. This prediction has received mixed support in previous analyses on traits at a higher phenotypic level, that have been separated by more than 500 generations (Rice et al. 2005; McDaniel 2005; Ashman and Majetic 2006; Fairbairn and Roff 2006; Fairbairn et al. 2007; Poissant et al. 2010; Leinonen et al. 2011). In this analysis, at the level of gene expression variation, we find substantial support for this prediction, as genes with a high degree of sex-biased expression, in general, show a substantially lower $r_{MF}$ than genes with a more similar expression in the sexes.

A negative association between the $r_{MF}$ and the degree of sex-biased expression can possibly also arise through genomic imprinting. Males and females that have successfully reached the mating stage will probably have a phenotype that suits their sex better than the average male and female phenotype in the population. Sons would therefore benefit from expressing their father’s phenotype, and daughters from their mother’s, rather than the average phenotype of their parents. Day and Bonduriansky (2004) have suggested that this problem can be solved through genomic imprinting, where sons primarily express the haploid genome they inherit from their father and daughters the haploid genome they inherit from their mother. If this were the case, imprinted genes would display a higher level of sexual dimorphism and a reduced
realized $r_{MF}$, compared with nonimprinted genes. This process could thus give rise to a negative association between the $r_{MF}$ and the degree of sex-biased gene expression. However, although this is a plausible scenario, we do not think it applies to the negative association we document here for two reasons. First, there is very little evidence for genomic imprinting in *Drosophila* (Coolon et al. 2012). Second, in this study, we use gene expression data from inbred individuals. Males and females from the same inbred line thus had a mother and a father of the exact same genotype. It is therefore not possible for sons to express different alleles than daughters, even if sons would only express genes inherited from their father and daughters only from their mother.

The evidence we present for how a shared genetic architecture constrains the evolution of sexual dimorphism is based on both within- and between-population comparisons. However, if a shared genetic architecture is a true obstacle for the evolution of sex-specific phenotypes, constraints should remain over long periods of time. We find support for this hypothesis as $r_{MF}$ values in *D. melanogaster* predict the extent to which evolutionary change in sex bias has occurred between *D. melanogaster* and its closest relative, *D. simulans*, as well as the more distantly related species in the *Drosophila* genus we tested here. There is no obvious trend in terms of how the strength of the negative association between the $r_{MF}$ and the degree of change in sex-biased expression change with phylogenetic distance. We nevertheless suggest that a plausible scenario is that the change in sex bias between closely related populations is often very small, since drift and novel selection has not had the time to move traits far from their values at time of divergence. The negative association between the $r_{MF}$ and change in degree of sex bias would then probably increase with time and reach a minimum at some point, after which it should revert back toward zero as the predictive value of the genetic architecture of a distantly related relative becomes less informative. These data presented here do not corroborate such a U-shaped relationship. The lack of support for this hypothesis may be because none of the species we studied have had enough time to completely dissociate their genetic architecture from *D. melanogaster*, although *D. melanogaster* and *D. virilis/ D. mojavensis* are estimated to have separated approximately 60 Ma (Tamura et al. 2004). Alternatively, sex bias evolving by drift with constant mutation rates and stabilizing selection would cause the relationship between the change in sex bias and $r_{MF}$ to remain more stable over large phylogenetic

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**Fig. 5.** $r_{MF}$ and the degree of change in gene expression sex bias between *Drosophila melanogaster* and six other species in the *Drosophila* genus, for genes with significant genetic variation that were present in all six species. Notches on the boxes represent approximate 95% CIs. Numbers above the boxes show how many genes each box represents.
differences (Bedford and Hartl 2009). Our results nevertheless provide strong evidence that a shared genetic architecture can constitute a long-term constraint on the evolution of sex-biased expression.

Theory predicts that sexual dimorphism should more easily evolve on the X-chromosome (Rice 1984). However, empirical studies that have tested this hypothesis for traits at a higher organizational level have been inconclusive (Reinhold 1998; Fitzpatrick 2004; Chenoweth et al. 2008; Manik 2009; Husby et al. 2013). In the case of the genomic distribution of sex-biased genes, the X-chromosome plays a special role, but usually it is only overrepresented with genes biased in either the female or the male direction and not in both (reviewed in Ellegren and Parsch 2007). A corollary to the above prediction is that the sex chromosomes should host more sex-specific genetic variation than the autosomes (Chenoweth et al. 2008; Fairbairn and Roff 2006) and thus that X-linked genes should have a reduced $\rho_{MF}$ compared with autosomal genes. We find some support for this prediction, but the effect is rather small. These results hence appear not to offer support of a strong role for the X-chromosome with respect to sex-specific genetic variation. A potential caveat with this conclusion is that our $\rho_{MF}$ values are estimated from variation among inbred lines. When the $\rho_{MF}$ for X-linked genes is estimated from inbred genotypes in D. melanogaster, males and females essentially have the same genotype, because, dosage compensation makes males produce as much gene product as females from their single X. When genetic correlations are estimated from outbred genotypes this may not be the case, as females are heterozygous, whereas males are effectively homozygous for X-linked loci. This contrasts to the autosomes where both sexes will have the same levels of heterozygosity and, as such there is more potential for X-linked than autosomal sex-specific variation. Similarly though, despite substantial inbreeding in the DGRP lines, residual heterozygosity could also contribute to our observation of a slightly lower $\rho_{MF}$ on the X-chromosome.

Collectively, our results provide strong evidence that the shared genome is a pervasive constraint on the evolution of sexual dimorphism. Previous attempts to show this have given equivocal results, which is surprising given that intralocus sexual conflict seems ubiquitous in both laboratory and wild populations (Chippindale et al. 2001; Rand et al. 2001; Fedorka and Mousseau 2004; Pischedda and Chippindale 2006; Foerster et al. 2007; Brommer et al. 2007; Cox and Calusbeek 2009; Mainguy et al. 2009). The pressing question then becomes; how are generally strong genetic constraints compatible with rapid evolution of sexual dimorphism, on both the trait (Darwin 1871; Meyer 1997; Arnqvist 1998; Civetta and Singh 1998; Omland and Lanyon 2000; Emlen et al. 2007) and gene expression level (Coulthart and Singh 1988; Civetta and Singh 1995; Meiklejohn et al. 2003; Zhang et al. 2004; Zhang and Parsch 2005)? Although strong sex-specific selection acting on genes with a moderate to high $\rho_{MF}$ probably contributes to resolving this paradox to a small extent, the main explanation is probably different. Sex-specific selection primarily targets specific sets of genes and it is plausible that the $\rho_{MF}$ values for a subset of these have evolved over time to become relatively low. These genes would then have the capacity to rapidly respond to shifts in sex-specific selection and could hence contribute largely to the rapid diversification of sexual traits between species. One such example could be genes affecting cuticular hydrocarbon (CHC) profiles. In Drosophila, the CHC profiles is sexually dimorphic (Fervuer and Cobb 2010) with low $\rho_{MF}$ (Sharma, Mitchell, et al. 2012) and respond rapidly to selection (Sharma, Hunt, et al. 2012). The subsets of genes regularly exposed to novel sex-specific selection do probably still frequently contribute to intralocus sexual conflict, at least transiently, because the $\rho_{MF}$ of most of these genes is slightly positive and sex-specific optima probably change rapidly. Genes that primarily contribute to intralocus sexual conflict are, however, more likely to be found among pleiotropic genes (Manik, Hultin-Rosenberg, Zwahlen et al. 2008), and genes that, for other architectural reasons, are constrained from evolving a reduced $\rho_{MF}$.

**Materials and Methods**

**Gene Expression Data**

We used published data from three different sources in this study. To estimate the $\rho_{MF}$ and the degree of sex-biased expression for each gene in D. melanogaster, we used data from the study by Ayroles et al. (2009). These data consist of whole body microarrays from 40 inbred genotypes, all derived from a single population. The raw data were downloaded from http://www.ebi.ac.uk/arrayexpress/experiments/E-MEXP-1594 (last accessed July 23, 2013) and normalized using RNA (Irizarry et al. 2003). To test whether genes currently exposed to sexually antagonistic selection have a higher $\rho_{MF}$ than other genes, we gathered information on the selection regime that gene expression is under from a study of a different population of D. melanogaster (Innocenti and Morrow 2010). In this study, the authors measured fitness and genome-wide gene expression in males and females for a set of genotypes derived from one outbred population and used regression analysis to establish which genes were exposed to sexually antagonistic selection for gene expression. Data were collected from an online depository (http://www.plosbiology.org/article/info%3Adoi%2F10.1371%2Fjournal.pbio.1000335#s4, last accessed July 23, 2013). To calculate the extent to which genes have changed with respect to their degree of sex bias between D. melanogaster and other Drosophila species, we used whole body microarray data from the study of Zhang et al. (2007). Data were downloaded from the Gene Expression Omnibus, GEO accession: GSE6640 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6640, last accessed July 23, 2013) and normalized using RNA (Irizarry et al. 2003).

**Estimation of the Intersexual Genetic Correlation**

The intersexual genetic correlation ($\rho_{MF}$) (Lynch and Walsh 1997) was estimated for each gene using the mean value of the two microarray samples for each sex (Ayroles et al. 2009).
The 95% CIs around the median of each described category were estimated by bootstrapping the data 10,000 times.

\[ r_{\text{MF}} \] and Evolution of Sex-Biased Gene Expression

All analyses were conducted on two data sets: one including all genes \((n = 12,572)\) and one including only those genes for which there was significant genetic variation \((n = 8,997)\) (discussed earlier). In all analyses, we used linear regression to test for associations between the \( r_{\text{MF}} \) and the various variables we were interested in (gene selective regime, chromosome linkage, degree of sex-biased expression within \( D. \) melanogaster and degree of change in sex-bias expression between \( D. \) melanogaster and other \( Drosophila \) species). In all these analyses, we included expression level \((\mu)\) and tissues specificity \((\tau)\) as covariates, because these two variables have been shown to influence various aspects of sequence and expression evolution (Nuzhdin et al. 2004; Larracuente et al. 2008). For example, the relationship between \( r_{\text{MF}} \) and sex bias \((s_b)\) was modeled as

\[
E[r_{\text{MF}}] = \alpha + \beta_1 s_b + \beta_2 \mu + \beta_3 \tau.
\]

We defined \( \mu \) as mean expression level across the sexes in the \( D. \) melanogaster data from the study by Ayroles et al. (2009), and \( \tau \) was estimated as

\[
\tau = \frac{\sum_i \left[ 1 - \left( \log_i(t_i) / \log_i(t_{\text{mean}}) \right) \right]}{(n - 1)},
\]

where \( i \) is expression in tissue \( i \) and \( t_{\text{mean}} \) is the expression in the tissue with the highest gene expression. Values of expression level in each tissue were taken from the FlyAtlas database (Chintapalli et al. 2007). Expression level and tissue specificity were both positively and significantly related to the \( r_{\text{MF}} \) in all analyses. Removing these covariates from the analyses did, however, have only a very small effect on the association between \( r_{\text{MF}} \) and any of the focal variables. We report only on the coefficient of interest and the corresponding \( P \) value.

Sex-biased gene expression was estimated as

\[
| \log_2(M/F) |,
\]

and the degree to which genes have changed with respect to sex-biased expression between \( D. \) melanogaster and \( D. \) simulans, \( D. \) yakuba, \( D. \) ananassae, \( D. \) pseudoobscura, \( D. \) virilis, or \( D. \) mojavensis was estimated by

\[
| \log_2(M/F) |_{D. \text{ melanogaster} - D. \text{ simulans}}.
\]

In these analyses, only genes that were present in all species \((\text{all genes } n = 5,857, \text{significant genes } n = 4,550)\) were used in the pair wise comparisons. We took this approach to not change the power with which we tested for an association between the \( r_{\text{MF}} \) and the change in sex bias with phylogenetic distance. All figures were produced in the R software environment (R Development Core Team 2011) and all statistical analyses were conducted in S-plus.

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References


Within-population Y-linked genetic variation for lifespan in *Drosophila melanogaster*

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*Keywords*: intralocus sexual conflict, longevity, sex chromosomes, sexual dimorphism, Y chromosome
Abstract

The view that the Y chromosome is of little importance for phenotypic evolution stems from early studies of *Drosophila melanogaster*. This species’ Y chromosome contains only 13 protein coding genes, is almost entirely heterochromatic, and is not necessary for male viability. Population genetic theory further suggests that non-neutral variation can only be maintained at the Y chromosome under special circumstances. Yet, recent studies suggest that the *D. melanogaster* Y chromosome *trans*-regulates hundreds to thousands of X and autosomal genes. This finding suggests that the Y chromosome may play a far more active role in adaptive evolution than has previously been assumed. To evaluate the potential for the Y chromosome to contribute to phenotypic evolution from standing genetic variation, we test for Y-linked variation in lifespan within a population of *D. melanogaster*. Assessing variation for lifespan provides a powerful test because lifespan *i)* shows sexual dimorphism, which the Y is primarily predicted to contribute to, *ii)* is influenced by many genes, which provides the Y with many potential regulatory targets, and *iii)* is sensitive to heterochromatin remodelling, a mechanism through which the Y chromosome is believed to regulate gene expression. Our results show a small but significant effect of the Y chromosome, and thus suggest that the Y chromosome has the potential to respond to selection from standing genetic variation. Despite its small effect size, Y-linked variation may still be important, in particular when evolution of sexual dimorphism is genetically constrained elsewhere in the genome.
Introduction

The potential for adaptive evolution of phenotypic traits through the Y chromosome is currently being re-evaluated (Mank 2012). Once a pair of neo Y and X chromosomes stops recombining, the Y chromosome becomes exposed to a range of degenerative processes (Charlesworth and Charlesworth 2000; Bachtrog 2013). These include Müller’s ratchet, Hill-Robertson interference, background selection, and genetic hitchhiking (Charlesworth and Charlesworth 2000; Kaiser and Charlesworth 2010). In concert with the small effective population size of the Y, these processes act to decrease the efficacy of selection, which eventually should result in a gradual shut down, and later loss, of genes on the Y chromosome (Rice 1996; Bachtrog 2005; Zhou and Bachtrog 2012). Population genetic models also predict that the Y chromosome can only maintain non-neutral genetic variation under very special circumstances (Clark 1987, 1990). According to theory, a mature Y chromosome should hence have a very limited capacity to maintain standing genetic variation for phenotypic traits.

In accordance with above scenario, the Y chromosome of *Drosophila melanogaster* features just 13 protein coding genes (Carvalho et al. 2001; Carvalho and Clark 2005; Koerich et al. 2008; Vibranovski et al. 2008; Krsticevic et al. 2010), which all exhibit very low levels of nucleotide polymorphism within populations (Zurovcova and Eanes 1999; Larracuente and Clark 2013). The Y chromosome is, furthermore, completely heterochromatic (densely packed DNA which typically suppresses expression) (Hoskins et al. 2002), and while males which lack a Y chromosome (XO) are infertile, they are viable and only have minor changes to their phenotype (Bridges 1916). For these reasons the *D. melanogaster* Y chromosome was long considered a genetic desert, with the exception of its importance for fertility (Francisco and Lemos 2014).

Despite both theory and the above empirical observations suggesting that the *D. melanogaster* Y chromosome should have a very limited potential to contribute to adaptive evolution, there is evidence that suggests the opposite. The chromosome has remained large and constitutes as much as 13% of the male genome (Hoskins et al. 2002), and while the vast majority of the chromosome is made up of seemingly non-functional repetitive DNA and transposable elements (Hoskins et al. 2002) this class of DNA actually displays substantial molecular variation (Lyckegaard and Clark 1989; Clark 1990). Over the last decades a few studies of *D. melanogaster*, and its close relatives, have also suggested that the Y chromosome harbours genetic variation for phenotypic traits including geotaxis (Stoltenberg and Hirsch 1997), suppression of X-linked gametic drive (Carvalho et al. 1997; Montchamp-Moreau et al. 2001; Branco et al. 2013), courtship song (Huttunen and Aspi 2003), thermal sensitivity (Rohmer et al. 2004) and fitness (Chippindale and Rice 2001). There are also a few findings in other taxa which point to an
effect of the Y or W chromosome (the equivalent of the Y in ZW sex determination systems) on colour traits (e.g. Lindholm et al. 2004; Postma et al. 2011; Evans et al. 2014). None of these findings were, however, able to fully challenge the perception that the Y is a largely inert chromosome.

Recent findings have, however, strongly called into question the long held view of the Y as a passive chromosome. In a study of Y chromosomes collected from multiple globally dispersed populations of D. melanogaster, Lemos et al. (2008) showed that the Y chromosome affects the expression of hundreds, potentially thousands, of genes spread throughout genome. This finding has now been thoroughly replicated by a number of studies (Paredes and Maggert 2009; Jiang et al. 2010; Lemos et al. 2010; Paredes et al. 2011; Sackton et al. 2011). Because the Y chromosome is only inherited from father to son, it is predicted to primarily affect genes and traits which are sex limited or show sexual dimorphism. The fact that the set of genes which the Y chromosome regulates is enriched for testis-specific genes supports the hypothesis that the Y chromosome’s gene regulatory effect is adaptive (Lemos et al. 2008; Jiang et al. 2010; Sackton et al. 2011).

The finding that the Y chromosome has a substantial capacity to regulate gene expression warrants further investigations into its effect on phenotypic traits. Of particular interest are those which show sexual dimorphism, as the Y chromosome masculinizes the transcriptome (Lemos et al 2008). Lifespan shows sexual dimorphism in many species (Maklakov and Lummaa 2013), including D. melanogaster (e.g. Lehtovaara et al. 2013). In addition to being sexually dimorphic there are also other aspects of lifespan which suggest it should be a good candidate trait to assess for Y-linked genetic effects. First, lifespan is a life history trait, and therefore is presumably affected by a large number of genes. This should provide the Y chromosome with ample targets for gene regulation, despite likely having a limited set of mechanisms through which it can regulate expression (Sackton and Hartl 2013; Francisco and Lemos 2014). The Y chromosome is furthermore seemingly enriched for variation affecting metabolism and mitochondrial function (Lemos et al. 2008; Lemos et al. 2010; Paredes et al. 2011; Sackton et al. 2011), which should have links to lifespan (Balaban et al. 2005). In addition it has been shown that lifespan is sensitive to modulations of the heterochromatin landscape (Larson et al. 2012), which is the main mechanism through which the Y chromosome is believed to exert its gene regulatory effect (Sackton and Hartl 2013; Francisco and Lemos 2014).

Here we assess the influence of the Y chromosome on within-population genetic variation for lifespan in D. melanogaster. To accomplish this we cloned and amplified a set of Y chromosomes, which we expressed in a common genetic background. This allowed us to measure the effect of the Y chromosome independent from all other genomic components. We detect a small, yet statistically significant, effect of the Y chromosome. Our study thus shows that the Y chromosome does contribute to phenotypic variation,
and that it has the potential to influence the evolution of sexual dimorphism from standing genetic variation, but only to a limited extent since the estimated variance is small.

Materials and Methods

Y chromosome substitution lines
We studied within-population genetic variation in lifespan among a set of 33 Y chromosomes, all derived from the Drosophila Genetic Reference Panel (DGRP). The DGRP lines were created through 20 generations of sister-brother mating from a set of flies collected in 2003 from Raleigh, North Carolina (Mackay et al. 2012). The flies were kept under standard conditions throughout the experiment (12:12 light-dark cycle, 60% humidity, 25°C and on a standard yeast-sugar diet). By a series of backcrosses (Fig. 1), each of the focal Y chromosomes were placed in a common homozygous genetic background from the same population (DGRP-486, Bloomington Stock Number 25195). In this way studied lines only differed genetically with respect to their Y chromosome, and any variation among lines thus has to be linked to this chromosome.

Lifespan assay
Focal males were produced by pairing 20 males from each Y-line with 40 virgin DGRP-486 females, in multiple vials over three consecutive blocks. Vials were trimmed to contain approximately 150 eggs, in order to standardise larval competition. Ten days after oviposition we collected multiple vials of 30 males per line, under a light CO₂ anaesthesia (<4 minutes of exposure). Males were housed without females, since we have shown in a previous experiment that housing males with other males or females only have a limited effect on average lifespan (~10%) and has no detectable effect on the magnitude of genetic variation (Lehtovaara et al 2013). Experimental males were transferred without anaesthesia to fresh food on day 1, 2 and 5, and every 2 days thereafter, until all flies had died. At each transfer we scored deaths and discarded dead flies. On average we assayed the lifespan of 411 (SD = 81) focal males per line, and 29.7 (SD = 1.5) flies per vial.

Statistical analysis
Variation in lifespan was analysed using mixed-effects models fitted by Markov chain Monte Carlo (MCMC) sampling, using the MCMCglmm package (Hadfield 2010) in R version 3.1.2 (R Core Team 2014). A random
Figure 1. Crosses to produce Y chromosome insertion 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]apXΔ), and a CiD 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.

effects model assuming Gaussian error distributions was used with lifespan as the response variable, block as a fixed effect and vial and line (DGRP line of origin) as random effects. Parameter expanded priors, suited for estimation of variances which are expected to be small, were used to estimate variances for the random effects, with the prior defined as prior variance (V) of 1, a belief parameter (nu) of 1, prior mean (alpha.mu) of 0, and prior covariance (alpha.V) of 1000 (Hadfield 2010, personal communication J. Hadfield). A weak prior was used for the residual variance where V = 1 and nu = 0.002 (Hadfield 2010). Results were robust to alternative values of V and nu. Two independent MCMC chains were run for 500 000 iterations, with a burn-in of 100 000 iterations, and a thinning interval of 100 iterations. Further, to ensure that the line variance estimate represents a true signal, rather than an artefact introduced by the sampling algorithm when estimating variances near zero, we randomised each vial’s assignment with respect to Y-
line, and generated 20 additional chains, one for each of 20 independent randomisations. The posterior distributions of line variance were then compared to the original distributions. This processing confirmed that we had detected a true signal of the Y chromosome (see Results), because the observed data does not stack values at zero, while the randomised does (Fig. 2). Furthermore, results were confirmed using restricted maximum likelihood (REML) in the R package lme4 (Bates et al. 2015), and are reported with standard deviations and p-values. Convergence was checked visually for each parameter and replicate MCMC chain. From the MCMC chains we extracted mean lifespan and estimates for each variance component, as well as standard errors and 95% credible intervals for each estimate.

Results

Mean male lifespan across all 33 Y lines was estimated to 66.85 days (±0.31 SE, 95% CI [66.23-67.50]). The variance explained by Y-line (variance among Y chromosomes) was 0.65 (± 0.37 SE, 95% CI [0.09-1.52], Fig. 2) and the total phenotypic variance was estimated to 153.97 (± 1.93 SE, 95% CI [150.21 - 157.73]). The vial variance was 4.42 (±0.66 SE, 95% CI [3.21-5.81]), and block variance was 0.89 (±0.27 SE, 95% CI [0.42-1.47]). Variance among Y chromosomes therefore explained 0.4% (± 0.2% SE, 95% CI [0.2% - 1.0%]) of the total phenotypic variance in male lifespan. The genetic and phenotypic coefficients of variation were 0.012 (± 0.004 SE, 95% CI [0.005 - 0.019]) and 0.190 (± 0.001 SE, 95% CI [0.187 - 0.193]), respectively. Using REML we show similar levels of genetic (0.58 ± 0.76, \( p = 0.009 \)), vial (4.35 ± 2.09, \( p < 0.001 \)), and phenotypic variance.

Two earlier assays of the DGRP lines have measured the total genetic variance for lifespan across the whole genome. They estimate genetic variance to be 93.75 (Ivanov et al. 2015) and 104.34 (Ayroles et al. 2009). Dividing our estimate of the Y-linked genetic variance through these estimates suggests that the Y chromosome explains approximately 0.65% (0.69%, 0.62%) of the total genetic variation, though experimental conditions were not identical.

Discussion

Motivated by the newly discovered large gene regulatory capacity of the Y chromosome (Lemos et al. 2008; Paredes and Maggert 2009; Jiang et al. 2010; Lemos et al. 2010; Paredes et al. 2011; Sackton et al. 2011), and the possibility that the Y chromosome might play a larger role in phenotypic evolution than previously appreciated, we here assessed the Y chromoso-
Figure 2. Plots of the posterior distributions for estimates of line variance. Distributions obtained from the analyses with the observed data (red) and the randomised vial data (black/grey), see methods for details. Randomisation of vial label causes lower, and often zero, estimates of variance, while the observed data produce variance estimates which are higher and do not stack against estimates of zero variance.

me’s impact on within-population genetic variation for lifespan. In support of the emerging view we find that the Y chromosome harbours genetic variation for this trait. The effect is small, but suggests that the Y has the potential to contribute to phenotypic evolution from standing genetic variation.

The evolution of sexual dimorphism is constrained by males and females sharing the same genome (Lande 1980; Bonduriansky and Rowe 2005; Bonduriansky and Chenoweth 2009; Poissant et al. 2010; Lewis et al. 2011; Gosden et al. 2012; Griffin et al. 2013; Pennell and Morrow 2013; Ingleby et al. 2014). This constraint does not, however, concern the Y chromosome, which is free to accumulate male specific adaptations independently of their effect in females, due to its strict inheritance from father to son. Proof of this principle recently gained support from a study of the W chromosome in chickens, where the expression level of W-linked genes rapidly responded to
female limited selection (Moghadam et al. 2012). From the perspective that evolution of sexual dimorphism in general is constrained, the Y-linked genetic variation found here may thus be important in facilitating evolution of sex differences, despite being small in its effect size. It is also possible that the effect of the Y chromosome detected here have larger pleotropic effects on other key traits in males.

The small Y-linked effect we report here is not in conflict with the relatively larger effects on gene expression and fertility observed at the between-population/species level (Lemos et al. 2008; Sackton et al. 2011). Population genetic models suggest selected variation should only rarely be maintained at the Y chromosomes (Clark 1987, 1990), while differences at the between-population level can rapidly accumulate through fixation of slightly deleterious mutations, because the Y chromosome does not recombine and have a relatively small effective population size. What probably helps maintain a small amount of variation is that the Y presumably has a larger mutational input than previously thought, where the whole chromosome acts as a single locus determining the amount of heterochromatin at other chromosomes, which should shift the equilibrium frequency towards more variation at mutation-selection-drift balance.
Among the genes that the Y chromosome regulates, those interacting with mitochondrial genes or associated with metabolism are over-represented (Lemos et al. 2008; Lemos et al. 2010; Paredes et al. 2011; Sackton et al. 2011). The idea that there is an association between metabolism and lifespan, mediated through the ‘rate of living hypothesis’, has been around for a long time, but the empirical evidence for this connection is weak at best (Speakman 2005). More direct evidence has been established for a link between mitochondrial function and lifespan (e.g. James and Ballard 2003; Trifunovic et al. 2004; Maklakov et al. 2006). This link appears especially strong in males (Camus et al. 2012), presumably because mutations with adverse effects on males, and neutral effects on females, are free to accumulate in mitochondria (Frank and Hurst 1996; Friberg and Dowling 2008; Innocenti et al. 2011). To reduce the effect of such male detrimental mutations males appear to evolve counter adaptations (Yee et al. 2013). It is thus not improbable that the Y chromosome plays a role in this context (Rogell et al. 2014), and that this is part of how the Y mediates the variation in male lifespan detected here.

We are only aware of one other study testing for an effect of the Y chromosome on lifespan in *Drosophila*. In this study males having either a *D. sechellia* or *D. simulans* Y chromosome, placed in a *D. simulans* genetic background, were compared (Johnson et al. 1993). The estimated difference was sizable (14%) but marginally non-significant, potentially due to a relatively small sample size. For guppies, a within-population effect has been reported (Brooks 2000), and in a study between two populations of seed beetle no effect was detected (Fox et al. 2004).

Our approach of placing Y chromosomes in a standardised genetic background provides a powerful test for Y-linked within-population genetic variation. The drawback with this method is that we are unable to discern whether the variation is additive, or largely locked into epistatic interactions with the rest of the genome. Previous studies of the *D. melanogaster* Y chromosome have emphasised the prevalence of Y by genetic background interactions, for both gene expression (Jiang et al. 2010) and fitness (Chippindale and Rice 2001), although theory suggests such should rarely maintain variation (Clark 1987, 1990). However, the mitochondria, which shares many of its characteristics with the Y chromosome (haploid genome selected exclusively in one sex with small effective population size), only displays interactions with the genetic background for females fitness within a population of *D. melanogaster* (Dowling et al. 2007), while the same set of mt-types displayed additive genetic variation for female lifespan (Maklakov et al. 2006).

In conclusion our study provides support for Y-linked standing genetic variation in lifespan, but the effect is small and required high sample size to detect. Given the facts which are lined up in favour of finding a Y-linked effect on lifespan (see Introduction), it is plausible that the effect on other
sexually dimorphic traits is frequently even smaller, but the reverse may apply to male limited traits on which the Y chromosome may have a larger gene regulatory influence. This may explain why a Y-linked effect on within-population genetic variation only rarely has been reported. Our data nonetheless supports that the Y chromosome could have a small but distinct capacity to contribute to phenotypic evolution from standing genetic variation, especially for traits where sex-specific evolution is constrained elsewhere in the genome.

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References


Autosomal and X-linked additive genetic variation for lifespan and ageing: comparisons within and between the sexes in *Drosophila melanogaster*

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Abstract

Theory makes several predictions about differences in genetic variation between the X chromosome and the autosomes, due to male X hemizygosity. The X chromosome should typically i) show relatively less standing genetic variation than the autosomes, ii) exhibit more X linked variation in males compared to females, and iii) be prone to the accumulation of sex-specific genetic variation. Here we address each of these predictions, by disentangling X linked and autosomal additive genetic variation for lifespan and ageing in *Drosophila melanogaster*. We do not find evidence for either a depletion of X linked genetic variation (as measured through females), or for male-bias in X linkage of genetic variation, while our results do suggest an enrichment of sex-specific genetic variation for lifespan. Our results thus partly differ both from what is commonly expected, and also from observations made at the molecular level. We discuss several possibilities which could have caused the unexpected patterns, including incomplete dosage compensation and X linked enrichment of sex-specific genetic variation. Collectively our results suggest the X chromosome has reduced capacity to respond to sexually concordant selection, while the ability to respond to sex-specific selection should be enhanced.
Introduction

The X chromosome is present only as a single copy in males. Together with an unusual inheritance pattern, this presumably exposes the X chromosome to population genetic parameter settings which, in many aspects, differ from those of the autosomes (Vicoso & Charlesworth, 2006; Ellegren, 2009). As a result, both the amount and the type of molecular variation are expected to change on the X relative to the autosomes, once a pair of X and Y sex chromosomes stops to recombine. The direction of this change does, however, depend on a range of factors (Ellegren, 2009), and furthermore, it is not obvious how well changes observed at the molecular level translate to the phenotypic trait level (Dean & Mank, 2014).

Theory typically predicts that, relative to the autosomes, the X chromosome should become depleted for genetic variation. This prediction simply follows from hemizygosity of the X chromosome in males, which reduces the effective population size of the X to $\frac{3}{4}$ that of the autosomes, and results in more efficient selection on X linked mutations (Avery, 1984; Charlesworth et al., 1987). However, because the X chromosome spends two-thirds of its time in females, there are many other factors with the potential to either increase, or further decrease, the relative genetic variation at the X chromosome (reviewed in Ellegren, 2009). These include sex differences in mutation rate (Li et al., 2002; Ellegren & Parsch, 2007; Xu et al., 2012), recombination rate (Charlesworth et al., 1993; Slatkin, 1995; Orr & Betancourt, 2001; Cutter & Payseur, 2013), variance in reproductive success (Caballero, 1995), and philopatry (Laporte & Charlesworth, 2002). Population bottlenecks and expansions can also influence the relative X to autosomal genetic variation (Pool & Nielsen, 2007; 2008).

Empirical investigations into the relative molecular variation at the X chromosome and the autosomes of Drosophila melanogaster have shown mixed results. In general, the X chromosome hosts less molecular variation in non-African populations (Hutter et al., 2007; Mackay et al., 2012), while this effect is less pronounced, and commonly reversed, in ancestral African populations (Hutter et al., 2007). With respect to non-synonymous sites, which are presumably subject to selection, lower molecular variation on the X chromosome is, however, consistently reported in all populations studied so far (Langley et al., 2012; Campos et al., 2013).

It seems reasonable to expect that reduced X linked variation at functional molecular sites should also reduce X linked phenotypic variation. In a comparison between the X chromosome and the autosomes there are, however, reasons to believe that the amount of molecular genetic variation does not perfectly reflect the amount of genetic variation seen at the phenotypic level. One type of mutations which could potentially increase X linked standing genetic variation in phenotypic traits are those with sexually antagonistic
effects. Population genetic models show that the parameter space for a balanced polymorphism for these mutations is much wider on the X chromosome than on the autosomes (Rice, 1984; Gibson et al., 2002; but see Fry, 2010), and the *D. melanogaster* X chromosome accordingly appears enriched for such mutations (Gibson et al., 2002). While sexually antagonistic mutations may be relatively uncommon (Morrow et al., 2008; Mallet et al., 2011; Mallet & Chippindale, 2011), they could still have large phenotypic effects (Chippindale et al., 2001; Gibson et al., 2002). They could thus shift the sources of genetic variation in phenotypic traits towards the X chromosome, with only little effect on the molecular variation. It has further been suggested that the presence of sexually antagonistic mutations should be followed by modifiers, which suppress the deleterious effect of these mutations in the suffering sex (Rice, 1984; Connallon & Clark, 2010). Mutations in such modifiers can also disproportionally contribute to X linked phenotypic variation, provided that the X chromosome is enriched for sexually antagonistic mutations and that the modifiers evolve in close proximity (*cis*) to these. The reason is that modifier are selected primarily in one sex, and that mutations in these may therefore drift to relatively high frequencies, and/or have larger effect size than other mutations (Morrow & Connallon, 2013). A prediction which follows from this latter scenario is that the X chromosome should exhibit a lower intersexual genetic correlation than the autosomes.

A second factor which complicates the link between genetic variation at the molecular and the phenotypic trait level, specific to differences between the autosomes and the X chromosome, is dosage compensation. When complete, dosage compensation should normally result in an elevated contribution of the X chromosome to standing genetic variation in males compared to females, because the male population effectively only consists of homozygous individuals with respect to the X chromosome (Reinhold & Engqvist, 2013). Dosage compensation may, however, also increase X linked genetic variation in females, if selection for higher gene expression in males increases expression in females to some extent as a correlated response (Xiong et al., 2010; Mank et al., 2011; Wright & Mank, 2012). If there is no dosage compensation, males should typically exhibit lower X linked variation than females, and with partial dosage compensation any male to female variation ratio is possible (Fig. S1).

In this study we separately estimate X linked and autosomal additive genetic (co)variance within and between the sexes, for lifespan and ageing in *D. melanogaster*. By focusing on standing genetic variation for two key life-history traits we shift the focus from the molecular to the phenotypic level. This allows us to test the extent to which patterns of molecular variation at the X chromosome connect to additive genetic variation at the phenotypic level, and to compare the evolutionary potential of the X and the autosomes from standing genetic variation. In particular we address the following three
questions: i) does the X chromosome show reduced levels of additive genetic variation, ii) does the X chromosome maintain more additive genetic variation in males compared to females, and iii) does the X chromosome harbor relatively more sex-specific additive genetic variation than the autosomes? This study expands on a previous study of the same population, which reported substantial sex-specific genetic variation for both lifespan and ageing, when genetic variation across the whole genome was studied (Lehtovaara et al., 2013).

Materials and Methods

Experimental population

In our experiment we used a laboratory adapted population of *D. melanogaster* (Dahomey), originating from a sample of wild caught flies collected in Benin (Africa) over 40 years ago. Dahomey has since been kept as a large outbred population, with overlapping generations and in constant conditions (12:12 light-dark cycle, 60% humidity, 25°C and on a standard yeast-sugar diet). All flies in this experiment were kept under these standard conditions throughout.

Construction of X and autosome substitution lines

The genome of *D. melanogaster* is composed of the sex chromosomes (X and Y), two major autosomes (AII and AIII), and the small 4th dot chromosome (AIV, < 1% of the genome). To study the autosomal contribution to additive genetic variance for lifespan and ageing we randomly sampled 40 copies of chromosomes AII and AIII, and clonally amplified them as haploid pairs into random genetic backgrounds. Within each autosome substitution line (A-line), all individuals share an identical copy of AII and AIII, while all other chromosome copies vary randomly between individuals (Fig. 1). To study the contribution of the X chromosome to additive genetic variance for lifespan and ageing we randomly sampled 40 copies of the X chromosome, and clonally amplified them into random genetic backgrounds. Within these X chromosome substitution lines (X-lines) all individuals share an identical X chromosome and vary randomly with respect to all other chromosomes (Fig. 1). Because the genotypic value for each randomly sampled and cloned chromosome copy (or cloned set of chromosomes) was tested in a large number of random genetic backgrounds in each sex, variation among lines can be used to calculate the additive genetic variance separately for each sex and chromosome type. These estimates are devoid of dominance variation, but could include a minor component of variation caused by epistatic inter-
actions, within and between cloned chromosome copies (Rice et al., 2005; see Lehtovaara et al., 2013 for discussion).

A-lines and X-lines were constructed by first taking 80 randomly selected Dahomey males and crossing them individually to virgin DXCG females (C[1]DX, y, f/Y; T[2;3] bw\(^d\), in, p\(^r\), rdgC, ri, st/T[2;3] bw\(^d\), in, p\(^r\), rdgC, ri, st) (see Fig S2 and S3 throughout). Sons from this cross inherited one wildtype copy of the X and each of the autosomes from their father. From their mother they inherited a Y chromosome and a phenotypically marked translocation between the major autosomes, which forces the homologous AII and AIII chromosomes to cosegregate. To construct the A-lines we took one male offspring from 40 of the above crosses, and mated them individually to virgin CG females from a population homozygous for the aforementioned autosomal translocation, but with genetically variable wildtype Dahomey X chromosomes. This procedure replaced the one X chromosome associated with the founder male with randomly sampled Dahomey X chromosomes, and cloned the focal sets of AII and AIII chromosomes. Each A-line was maintained at a size of 40 males, mated to 80 CG females for three generations prior to producing focal flies.

To construct the X-lines we took multiple sons from each of the remaining 40 initial crosses (sons from one cross carry the same X chromosome copy), and mated them to virgin DX-D females, a population where females carry the aforementioned compound X chromosome (C[1]DX,y,f)/Y) placed with genetically variable wildtype Dahomey autosomes, to remove the autosomes associated with the founder male. Sons from each of these crosses

\[ \text{X}_1 \quad \text{X}_2 \quad \text{X}_3 \quad \ldots \quad \text{X}_{40} \]

\[ \text{Males} \]

\[ \begin{array}{cccc}
\text{X} & \text{Y} & \text{X} & \text{Y} \\
\text{AII} & \text{AIII} & \text{AII} & \text{AIII} \\
\text{AII} & \text{AIII} & \text{AII} & \text{AIII} \\
\text{AII} & \text{AIII} & \text{AII} & \text{AIII} \\
\end{array} \]

\[ \begin{array}{cccc}
\text{Females} \\
\text{X} & \text{X} & \text{X} & \text{X} \\
\text{AII} & \text{AIII} & \text{AII} & \text{AIII} \\
\text{AII} & \text{AIII} & \text{AII} & \text{AIII} \\
\text{AII} & \text{AIII} & \text{AII} & \text{AIII} \\
\end{array} \]
were mated to virgin DX-D females to remove the autosomal translocation. This procedure replaced autosomes associated with the founder male with randomly sampled Dahomey autosomes. Each X-line was maintained at a size of 40 males mated to 80 DX-D females for three generations before focal flies were produced. The crossing scheme to produce and maintain the X- and A-lines are described in detail in the supplementary material (Fig. S2, and Fig. S3).

Lifespan and ageing assay

Lifespan and ageing were both estimated from 200 focal flies of each sex and line, split equally among 4 replicates, totalling ~32000 focal flies. For each replicate, focal flies were produced by either crossing 45 males from each X- and A-line to i) 90 virgin Dahomey females across 3 vials to produce focal females, or ii) to 90 virgin DX-D females across 3 vials to produce focal males. Once the parental flies were transferred from the oviposition vials, the number of eggs was manipulated to standardize number of viable larvae to 150 per vial.

Ten days after egg laying, virgin focal flies were collected under light CO$_2$ anaesthesia (<4 minutes of exposure) into vials of 56 individuals per sex and line. These were paired with 56 opposite sexed flies homozygous for a recessive dark body pigment mutation (ebony - earlier introgressed into the Dahomey background), and allowed to interact and mate for 72 hours. Ebony flies were subsequently removed and discarded, and 50 of the focal flies were randomly selected (after removing dead flies) and transferred to a fresh vial under light CO$_2$ anaesthesia. After 24 hours the flies were transferred to fresh food without anaesthesia. Every 48 hours, from this point onwards, we transferred the focal flies to fresh vials without anaesthesia, scored mortality, and discarded dead flies.

Statistical analysis

Lifespan data was analysed separately for the two line types (X- and A-lines), using mixed-effects models fitted by Markov chain Monte Carlo (MCMC) sampling as implemented in the MCMCglmm package (Hadhfield, 2010) in R 3.1.2 (R Core Team, 2014). Lifespan data were modelled assuming Gaussian error distributions with lifespan in each sex treated as separate response variables. This multi-response model approach allowed us to efficiently estimate intersexual genetic correlations. Line and replicate (vial identity) were fitted as random effects and sex-specific fixed effects were fitted to account for the four batches of replicates. Fixed effect dummy variables were centered, such that the intercept estimates the global mean (Schielzeth, 2010). Unstructured variance-covariance matrices were formed, each containing variance-covariance estimates for both sexes, with one 2x2
matrix for the A-lines and one 2x2 matrix for the X-lines. Vial and residual variance-covariance matrices had off-diagonal elements constrained to zero, because each vial and fly can only represent one sex and line-type, and therefore has the covariance structure undefined. The final model in R code was MCMCglmm(cbind(LSf,LSm) ~ trait – 1 + trait:batch2 + trait:batch3 + trait:batch4, random = ~ us(trait):Line + idh(trait):Vial, rcov = ~ idh(trait):units, family = rep("gaussian", 2)), where LSf and LSm are individual lifespans of females and males, respectively, and batch2, batch3 and batch4 are the dummy-coded and centred identifiers for replicate batches 2, 3, and 4 respectively.

We used Inverse-Wishart priors for the variance-covariance matrices of the random effects and residual variances, where diagonal elements equal the specific phenotypic variances for each sex divided by the number of variance components (line, vial and residual) and a degree of the belief parameter $\nu = 1$ (i.e. equal to the dimensions of the variance-covariance matrix minus 1). We checked the robustness with respect to alternative choice of $\nu$, including $\nu = 0.002$ and $\nu = 2$, which resulted in slightly lower estimates of all variance estimates with lower $\nu$, but this had only very minor effects on ratios and differences. Four independent MCMC chains, two for each line type, were run for 110,000 iterations, with a burn-in of 10,000 iterations and a thinning interval of 100 iterations. Convergence was checked visually and using the Gelman-Rubin criterion, applied to two independent chains for each line-type (all upper CI of potential scale inflation factors $\leq 1.05$).

Gompertz mortality functions of the form $\mu(t) = \alpha e^{\beta t}$ (where $\mu(t)$ is the rate of mortality at age $t$) allow decomposing lifespan in a component $\alpha$, the initial mortality, and $\beta$, the rate of ageing. We estimate these two parameters at the level of the vial, using the WinModest program (Pletcher, 1999). Four estimates of each parameter, one per replicate block, were made for each of the 160 combinations of line and sex. The two parameters $\alpha$ and $\beta$ were strongly negatively correlated ($r = -0.94$, 95% CI -0.93--0.95, $P < 10^{-15}$ with $\alpha$ log transformed to account for the highly skewed distribution) and we decided to model only the ageing parameter $\beta$ in multi-response models similar to the lifespan models described above, but without random effect of vial as there was only one estimate of $\beta$ per vial. We also implemented a bivariate, non-linear mixed model in OpenBUGS 3.2.3 (Lunn et al., 2009) with parameter $\beta$ allowed to vary and covary between lines and sexes, but the model did not converge for the critical parameter of the genetic correlations. Hence, we present the results of the two-step analysis here (using WinModest estimates of $\beta$ as data in the MCMCglmm model as described above).

We estimated the line variance ($V_L$), the vial variance ($V_V$) and the residual variance ($V_R$), separately for the two sexes, and the line covariance among sexes ($\text{Cov}_{\text{MF}}$). The total phenotypic variance ($V_P$) was reconstructed as the sum $V_P = V_L + V_V + V_R$, again separately for the two sexes. Since lines were
cloned for haploid chromosomes, additive genetic variance was calculated by multiplying the line variances by 2, with the exception of the male X-lines (because the X is hemizygous in males). The line covariance was converted to an intersexual additive genetic correlation by $r_{MF} = \frac{Cov_{MF}}{\sqrt{V_{LF}} \sqrt{V_{LM}}}$. One of the key advantages of the MCMC sampling approach is that we can form sums, ratios and differences of (co)variances for the entire chain and thus get samples from the posterior distribution of these quantities. We summarize posterior distributions by their means, standard error (SE) and 95% credible intervals (95% CI).

Visual examination of the mean female lifespan per vial revealed a bimodal distribution, with a small group of vials hosting unusually short-lived females, suggesting that a strong extrinsic factor (e.g. disease) affected survival in these vials. Due to the nature of the distribution, vials presumably affected were easily separated out, having an average lifespan of less than 51 days (Fig. S4). Since we were interested in genetic variation between lines we tested if there was a genetic component to the low scoring vials. To do this we first removed the low scoring vials and then tested whether female lifespan of lines not having a low scoring vial was larger than lines which had a low scoring vial. No difference between these groups of lines were detected (mean difference [lines w/o low scoring vial – lines w low scoring vial] (days): X-lines = 0.14, $t_{38} = 0.22$, $P = 0.41$; A-lines = 0.36, $t_{38} = 0.25$, $P = 0.40$; all lines = -0.19, $t_{78} = -0.23$, $P = 0.59$, all P-values one-tailed). Hence there was no indication that lines with outlier vials were more short-lived than other lines due to their genotype. Visual inspection of the distribution of 400 female and 400 male vials from a previous study (Lehtovaara et al., 2013), where the same population was studied under similar experimental conditions, showed no excess of low scoring vials. Taken together this suggests that the small group of low scoring vials represent true outliers. Therefore, we present results from analyses excluding these vial. Results including all vials are reported in Table S1.

Results

The estimates of line variance, and hence also the estimates of additive genetic variance (see Methods), were all significantly different from zero, for all four combinations of sex and chromosome type for both lifespan and ageing (Table 1). Line averages were approximately normally distributed for both lifespan and ageing within each combination of chromosome substitution type and sex.
Comparing X to autosomal additive genetic variance

To evaluate if the contribution of the X chromosome to additive genetic variance is different than what is expected from its size, we focus on females, since the relative contribution of the X is complicated by dosage compensation in males. Since the constitution and gene content potentially varies between chromosomes, it is not obvious what constitutes the best unit for calculating the proportion of the active genome which is X linked, but two metrics that should provide good approximations are the proportion of euchromatin and the proportion of genes on X chromosome. In *D. melanogaster*, the X chromosome hosts 18.8% of the euchromatin and 15.6% of the genes (*D. melanogaster* genome Release 5.30). The observed levels of X linkage of the additive variance for lifespan and ageing were not different from expectations based on the size of the X chromosome, but both did, if anything, suggest enrichment rather than depletion of X linked additive genetic variance (female X linkage of lifespan $V_A = 21.0\% \pm 6.5\%, 95\% \text{ CI} = 10.3\% - 35.2\%$; female X linkage of ageing $V_A = 28.6\% \pm 11.5\%, 95\% \text{ CI} = 11.3\% - 56.4\%$) (Fig. 2).
Figure 3. Male to female ratios for various metrics of genetic variation. The dashed horizontal line indicates equal genetic variation in males and females. Values above (below) this line are male (female)-biased. Vertical lines around each estimate are standard errors. $V_A =$ additive genetic variance, $CV_A = \text{coefficient of additive genetic variance}$, $\%X = \text{percentage of total sex-specific additive genetic variance on the X.}$

Comparing X linked additive genetic variance in males and females

To test if X chromosome hemizygosity and associated dosage compensation causes males to have more X linked $V_A$ than females, we first compared X linked $V_A$ in males ($V_{AXM}$) and females ($V_{AFX}$) (where subscript F and M denote female or male respectively, and subscript X denotes X chromosome). The ratio of male to female X linked $V_A$ was not different from 1 for lifespan ($V_{AXM} / V_{AFX} = 1.32 \pm 0.55$, 95% CI = 0.56 - 2.68), and was significantly lower than 1 for ageing ($V_{AXM} / V_{AFX} = 0.20 \pm 0.11$, 95% CI = 0.06 - 0.48) (Fig. 3). However, these comparisons do not take into account that males and females display sexual dimorphism for lifespan and ageing (Table 1), and, since the variance is expected to scale with the mean, this has to be taken into account. The coefficient of additive variation ($CV_A$) provides a mean-standardized, scale free, measure of variation and therefore provides more suitable estimates for comparison. The ratio of the male to female $CV_A$ for the X chromosome is not significantly larger than 1 for lifespan ($CV_{AXM} / CV_{AFX} = 1.49 \pm 0.30$, 95% CI = 0.98 - 2.15), though the broad credibility interval only marginally overlap 1, and again is significantly lower than 1 for ageing ($CV_{AXM} / CV_{AFX} = 0.60 \pm 0.15$, 95% CI = 0.35 - 0.95) (Fig. 3). Any indication of more X linked variance in males compared to females for lifespan is, however, not restricted to the X chromosome, as similar ratios for $V_A$ and $CV_A$ also are observed for the autosomes ($V_{AMA} / V_{AFA} = 1.22 \pm 0.39$, 95% CI = 0.61 - 2.12; $CV_{AXM} / CV_{AFX} = 1.41 \pm 0.22$, 95% CI = 1.02 - 1.90)
Figure 4. Scatterplot of male and female X- and A-line means 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.

(Fig. 3). To take the autosomes also into account when evaluating if males have comparatively more X linked $V_A$ than females we calculated the ratio of sex-specific X linkage ($V_{AMX}/(V_{AMA} + V_{AMX})$) to $V_{AFX}/(V_{AFA} + V_{AFX})$, which does not have a scaling problem. This ratio is not different from 1 for lifespan ($1.12 \pm 0.46$, 95% CI = 0.47 - 2.24) (Fig. 3), and thus does not suggest that males have relatively more X linked $V_A$ for lifespan. A similar comparison for ageing also suggests that males do not have proportionally less X linked $V_A$ than females, as this ratio is also not different from 1 ($0.64 \pm 0.46$, 95% CI = 0.17 - 1.76) (Fig. 3).
Table 1. Mean and variance data for lifespan and ageing. Estimates for which the credibility intervals do not overlap zero are marked in bold. Values provided are the estimates from the model, followed by the standard error, and 95% credibility intervals below. Mean lifespan is given in days, and mean ageing given for values of $\beta$, with estimates of line ($V_L$), additive genetic ($V_A$), Coefficient of additive genetic ($CV_A$), phenotypic ($V_P$), and vial ($V_V$) variance. All values for ageing have been multiplied by 100, excluding $CV_A$, to ease visualisation.

<table>
<thead>
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<th>X chromosomes</th>
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Comparing the $r_{MF}$ between the X and the autosomes

To test if the X chromosome is enriched for sex-specific additive genetic variance we calculated, and compared, the intersexual additive genetic correlation ($r_{MF}$) for the X chromosome and the autosomes. For lifespan the $r_{MF}$ of the autosomes was moderate and significantly greater than zero ($r_{MF-A} = 0.50 ± 0.13, 95% CI = 0.21 - 0.73$), while it was low and not significantly different from zero for the X chromosome ($r_{MF-X} = 0.04 ± 0.20, 95% CI = -0.35 - 0.45$). The correlation for the autosomes was not different from the X chromosomes, though the broad credibility intervals only marginally overlap zero (lifespan $r_{MF-A} - r_{MF-X} = 0.46 ± 0.24, 95% CI = -0.02 - 0.90, 61 of 2000 posterior samples were ≤ 0, Fig. 4). The intersexual genetic correlation for ageing was not significantly different from zero both for the autosomes ($r_{MF-A} = -0.11 ± 0.27, 95% CI = -0.62 - 0.43$) and the X chromosome ($r_{MF-X} = -0.31 ± 0.24, 95% CI = -0.72 - 0.20$), and these were not different from one another ($r_{MF-A} - r_{MF-X} = 0.20 ± 0.36, 95% CI = -0.53 - 0.92, Fig. 4).
Discussion

Hemizygosity of the X chromosome in males has been suggested to influence a range of properties of the X chromosome relative to the autosomes, such as the amount and type of standing genetic variation, and the evolutionary potential (Haldane, 1937; Avery, 1984; Rice, 1984; Charlesworth et al., 1987; Orr & Betancourt, 2001; Vicoso & Charlesworth, 2009; Mank et al., 2010; Orr, 2010; Connallon et al., 2012). The question of how standing genetic variation differs between the X chromosome and the autosomes has recently been addressed empirically, by studies of genetic variation at the DNA sequence level (Hutter et al., 2007; Ellegren, 2009; Langley et al., 2012; Campos et al., 2013). Here we take this question to the level of the phenotype, by separately studying the relative contributions of the X and the autosomes to additive genetic variation in lifespan and ageing of *D. melanogaster*. In particular we address *i*) if additive genetic variation is depleted on the X chromosome, *ii*) if males show relatively more additive X linked variation than females, and *iii*) if the X hosts relatively more sex-specific genetic variation. Our results point to no depletion of X linked variation and no excess of X-linked variation in males for both lifespan and ageing. With respect to sex-specific genetic variation our results suggest enrichment on the X chromosome for lifespan, but not for ageing.

Comparing X to autosomal additive genetic variance

Lifespan and ageing are life history traits and should hence be under directional selection, although this might be complicated by trade-offs with other life-history components (Price & Schluter, 1991). Thus, most mutations which affect lifespan and ageing are likely to be deleterious to fitness, and the expected amount of genetic variation should be approximately proportional to their frequency at mutation-selection balance. Assuming that the dominance and selection coefficients (*h* and *s*), and the mutation rate (*μ*), are all the same for the X and the autosomes, and that dosage compensation is complete, the equilibrium frequency at the X (*3μ/[s+2hs]*) is always lower than on the autosomes (*μ/sh*), when *0 ≤ h ≤ 1* (Charlesworth & Charlesworth, 2010). This should result in lower genetic variation on the X chromosome compared to the autosomes, as is observed with respect to polymorphisms in non-synonymous sites in *D. melanogaster* (Langley et al., 2012; Campos et al., 2013).

Two previous studies have indirectly tested for reduced X linkage of variation at the level of the phenotype in *D. melanogaster* (Cowley et al., 1986; Cowley & Atchley, 1988). In these studies the X linkage of additive genetic variation was, on average, 19.6% across 28 morphological traits, estimated in females for which dosage compensation should not directly influence variation. This is not less than what would be predicted from the relative size
of the X chromosome (15.6 and 18.8% based on the proportion of genes or euchromatin, respectively [D. melanogaster genome release 5.30]). Our data therefore corroborate a lack of depletion of X linked additive genetic variation, with estimated values of approximately 21% and 29% for female lifespan and ageing respectively, although the credibility intervals of these estimates do not preclude a slight to moderate depletion of X linked variance.

Several factors could potentially explain why a reduction in genetic variation observed at the molecular level does not necessarily translate into a reduction at the phenotypic level. One possibility is that the underlying genes of the traits we study have a genomic distribution which is shifted towards the X chromosome. However, given the wide array of morphological traits surveyed in the studies by Cowley and co-authors (Cowley et al., 1986; Cowley & Atchley, 1988), and the fact that lifespan and ageing are traits which should be affected by a large number of genes, it seems unlikely that these traits would all have an over-representation of coding factors on the X chromosome. On the other hand it has been suggested that traits which show sexual dimorphism, such as the ones studied here and most of the traits studied by Cowley and co-authors, should primarily be linked to the X chromosome (Rice, 1984; Fairbairn & Roff, 2006; Mank, 2009). Another possibility is that idiosyncrasies of the X allow this chromosome to accumulate more trans-regulatory functionality than the autosomes, and that this increases its impact on genetic variation (Wayne et al., 2007; Stocks et al., 2015). A further possibility is that balancing selection generated by sexual conflict maintains allelic variants with large phenotypic effects on the X chromosome (Rice, 1984), or that the relative number of sexually antagonistic mutations with large phenotypic effects and long transition time are more common on the X than the autosomes (Connallon & Clark, 2012). A final possibility is that the X chromosome is enriched with genetic variants with sex-specific effects, which appears to be the case for lifespan in the population we study. Such genetic variants are exposed to less efficient selection, being selected in only one sex, and can therefore maintain more phenotypic variation (Day & Bonduriansky, 2004; Bonduriansky & Chenoweth, 2009; Hosken, 2011; Morrow & Connallon, 2013). Simply limiting selection to one sex converts the equilibrium frequency of a deleterious mutation at mutation-selection balance at the X to $3\mu/(2hs)$ in females, which is always larger than the corresponding value for the autosomes ($\mu/sh$), assuming $\mu,s$ and $h$ are the same on the X chromosome and the autosomes.
Comparing X linked additive genetic variance in males and females

When the X chromosome is fully dosage compensated, X linked genes are effectively homozygous in males. From this it follows that X linked variance should typically be higher in males than females (Fig. S1) (Reinhold & Engqvist, 2013). This hypothesis has received mixed support from empirical examination by studies comparing total male and female genetic variation across a broad range of species (Reinhold & Engqvist, 2013; Wyman & Rowe, 2014; Nakagawa et al., 2015). With respect to *D. melanogaster*, Cowley and co-authors did, however, find more X linked additive genetic variance in males for 20 out of 22 morphological characters (Cowley et al., 1986; Cowley & Atchley, 1988).

Taken together, our results suggest there is no male excess of X linked additive genetic variation for either lifespan or ageing. There are many potential factors which could have caused these patterns, but the most parsimonious explanation appears to be lack of complete dosage compensation of involved X linked genetic factors. We come to this conclusion because ratios of male to female X linked variation are largely matched by the ratios of male to female autosomal variation, especially for lifespan, and to a certain degree for ageing. Incomplete dosage compensation reduces male X linked variation (from its full potential), but leaves the variation untouched in females (Fig. S1), which is largely what we observe here. The possibility that dosage compensation is incomplete for the traits examined here provides an alternative explanation to why we observe no depletion of X linked variation in females (see above). If incomplete dosage compensation causes deleterious mutations to have smaller phenotypic effects in males, and this causes them to have smaller selection coefficients in this sex, the total strength of selection against such mutations will be reduced and result in elevated X linked genetic variation under mutation-selection balance in females (Stocks et al., 2015). An alternative explanation to the lack of excess of male X linked variation is X linked enrichment of dominant mutations at low frequency. Such mutations have the effect that they cause female X linked variation to exceed male variation (Fig. S1), and they could be more common on the X because theory predicts enrichment of dominant female-beneficial-male-detrimental mutations on the X chromosome (Rice, 1984). Finally, the low \( r_{MF} \) for lifespan and ageing itself raises the possibility that the number and effect sizes of loci contributing to these traits differ between the sexes, which can counteract the effect of dosage compensation and create any possible pattern of sex-bias in additive genetic variation. An indication of slightly higher (lower) amounts of variation in male lifespan (ageing), taking the whole genome into account and adjusting for sex differences, has previously been observed for the population studied here (Lehtovaara et al., 2013).
Comparing the $r_{MF}$ between the X and the autosomes

Lifespan shows sexual dimorphism in most populations of *D. melanogaster*, including the one studied here (Lehtovaara et al., 2013; Zajitschek et al., 2013). For sexual dimorphism to evolve, a genetic architecture which is, at least partially, decoupled between the sexes is required (Lande, 1980; Bonduriansky & Rowe, 2005; Bonduriansky & Chenoweth, 2009; Poissant et al., 2010; Lewis et al., 2011; Gosden et al., 2012; Griffin et al., 2013; Ingleby et al., 2014). This has been observed for lifespan in our population (Lehtovaara et al., 2013) and other populations of *D. melanogaster* (Nuzhdin et al., 1997; Curtsinger et al., 1998; Vieira et al., 2000; Leips & Mackay, 2002; Forbes et al., 2004; Wilson et al., 2006), as well as in other species (Fox et al., 2004; Mühlhäuser & Blanckenhorn, 2004; Zajitschek et al., 2007; Berg & Maklakov, 2012). Theory predicts that sexual dimorphism should primarily develop on the X chromosome (Rice, 1984; Fairbairn & Roff, 2006; but see Fry, 2010; Connallon & Clark, 2012). For lifespan our results supports this prediction, since the estimated $r_{MF}$ for the X chromosome is close to zero (0.04), while moderate (0.50) for the autosomes. The difference between these estimates was marginally non-significant, but is nonetheless supported by the intermediate $r_{MF}$ values found for the whole genome, in our previous study of this population ($r_{MF} = 0.29$ and $r_{MF} = 0.43$ in two different social environments, Lehtovaara et al., 2013). It is noteworthy that testing for differences between genetic correlations requires exceptionally high sample sizes (Lynch & Walsh, 1998; Bonduriansky & Chenoweth, 2009).

In an earlier study of this population the $r_{MF}$ for ageing was estimated across the whole genome to be close to zero (-0.11 and 0.10 in two different social environments, Lehtovaara et al. 2013). Provided this low value was correct, there would not be much opportunity for the X chromosomes and autosomes to show values departing far from zero. In line with the previous results we also estimate the $r_{MF}$ for the X chromosome (-0.31) and the autosomes (-0.11) to not differ from zero. The $r_{MF}$ values for ageing are, however, estimated with less power than those for lifespan, since lifespan can be measured for individuals, while each single estimate of the rate of ageing requires a cohort of individual flies.

A reduced $r_{MF}$ in the X chromosome in *D. melanogaster* has previously been deduced from studies of morphological traits (Cowley et al., 1986; Cowley & Atchley, 1988), expression of cuticular hydrocarbons (Chenoweth et al., 2008) and, to a small degree, for gene expression (Griffin et al., 2013). Reduction of the $r_{MF}$ on the X chromosome relative to the autosomes, as seen for lifespan here, suggests enrichment of genetic factors with sex-specific effects. However, reduction of the $r_{MF}$ of the X chromosome is, to a moderate degree, inevitable even in the absence of allelic variants with sex-specific effects. This results from the special inheritance patterns of the X chromo-
some. While an autosomal mutation with a sexually concordant effect always affects the mean value of sons and daughters to the same extent, independent of parent of origin, the same mutation placed on the X chromosome will change the phenotype exclusively in daughters when paternally transmitted, and sons more extensively than daughters when maternally transmitted. An autosomal sexually concordant mutation can hence never reduce the $r_{MF}$, while the same mutation placed at the X chromosome will reduce the $r_{MF}$ to $\sqrt{2} \approx 0.71$, independent of dominance and allele frequency (James, 1973). This fact could explain some of the difference in the $r_{MF}$ between the X and the autosomes, observed for lifespan here and other traits in other studies, but is unlikely to explain the whole difference. The low $r_{MF}$ of the X chromosome could, in theory, also result from over- or under-dominant mutations with sexually concordant effects, since such mutations will cause a negative $r_{MF}$ on the X chromosome at some frequencies. However, genes with over- or under dominant alleles are probably rare (but see Chase et al., 2005 for a possible example).

On the relative potential for the X and the autosomes to contribute to adaptive evolutionary change

Theory predicts that X hemizygosity should result in faster adaptive change from novel beneficial mutations at the X chromosome than the autosomes, whenever mutations are at least partly recessive (Charlesworth et al., 1987; Orr & Betancourt, 2001; Connallon et al., 2012; Meisel & Connallon, 2013). With respect to adaptive evolution from standing genetic variation the evolutionary rate is predicted to follow the opposite pattern, because more efficient selection due to male hemizygosity prevents genetic variation from building up on the X chromosome to the same extent as on the autosomes (Orr & Betancourt, 2001). Current evidence favors more rapid evolutionary change on the X chromosome (Meisel & Connallon, 2013), but is unable to discern if this results from novel mutations or standing genetic variation. Our results, for lifespan and ageing, suggest additive genetic variation is not depleted on the X chromosome (as measured through females). Taken alone this suggests a larger scope for evolution through standing genetic variation on the X, compared to what is normally implied. The fact X linked variation is not elevated in males, in both lifespan and ageing, as expected under complete dosage compensation, does however speak against an enhanced possibility for faster X through standing genetic variation. If a lack of excess male X linked variation is caused by incomplete dosage compensation, associated with mutations of smaller phenotypic effects and selection coefficients in males compared to homozygous females, this does, however, speak against rapid adaptive evolutionary change also through novel recessive beneficial mutations, as these experience less of a selective advantage when X linked.
A factor which influences the rate of adaptation is genetic correlations (Lande, 1980; Agrawal & Stinchcombe, 2009). Positive genetic correlations between the sexes can enhance the response to selection when the sexes are selected concordantly, but can also have the opposite effect when selection is sexually antagonistic. Similarly a low genetic correlation impedes the rate of adaptation of traits selected concordantly in the sexes, while it allows for more rapid evolution of traits subject to sexually antagonistic selection. In this respect our observation of a supposedly lower $r_{MF}$ for the X chromosome than the autosomes for lifespan (and a range of other traits found in earlier studies, see Cowley et al., 1986; Cowley & Atchley, 1988; Chenoweth et al., 2008), suggests that adaptive evolution from standing genetic variation would proceed relatively faster on the X chromosome when driven by sexually antagonistic selection. This conclusion fits the observation that the strongest evidence for a faster X effect has been observed for genetic factors with sex-biased expression (reviewed in Meisel & Connallon, 2013). In summary our results thus suggests that the X chromosome may provide a relatively poor environment for rapid evolution of traits exposed to sexually concordant selection, but a favourable environment for traits selected for sex-specific adaptations, which constitute a significant part of phenotypic diversity.

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References


Figure S1. Total and additive X linked genetic variance in males and females. 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). The figure is adapted from Reinhold and Engqvist (2013) and the additive genetic variation is calculated after James (1973). In the figure genotypic values were set to $A_2A_2 = 0$, $A_2A_1 = 2\alpha h$, $A_1A_1 = 2\alpha$ in females and $A_2 = 0$, $A_1 = (DC+1) \times \alpha$ in males, where $\alpha$ was set to 2 and $DC$ (dosage compensation) was set to either 0 (absent) or 1 (complete).
Figure S2. 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.
Figure S3. 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. S3), 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.
Figure S4. Distribution of mean lifespan per vial for X- and A-lines in males and females. The figures show that a small fraction of vials, within the female X- and A-lines, have a substantially shorter average lifespan than the rest of the population of vials. Vials to the left of the vertical line were excluded from the final analyses (see methods).
Table S1. Mean and variance data for lifespan and ageing with all vials included in analysis. Estimates for which the credibility intervals do not overlap zero are marked in bold. Values provided are the estimates from the model, followed by the standard error, and 95% credibility intervals below. Mean lifespan is given in days, and mean ageing given for values of $\beta$, with estimates of line ($V_L$), additive genetic ($V_A$), Coefficient of additive genetic ($CV_A$), phenotypic ($V_P$), and vial ($V_V$) variance. All values for ageing have been multiplied by 100, excluding $CV_A$, to ease visualisation.

<table>
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<tr>
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<th>Autosomes</th>
<th>X chromosomes</th>
<th>Autosomes</th>
<th>X chromosomes</th>
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<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td>Difference (F - M)</td>
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<tr>
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<td>50.0 ± 0.7</td>
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<td>48.7 - 51.4</td>
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<td>$V_L$</td>
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<td>17.8 ± 4.8</td>
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<td></td>
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<td>10.7 - 29.5</td>
<td>-12.9 - 15.7</td>
<td>4.5 - 31.2</td>
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<td>$V_A$</td>
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<td>35.6 ± 9.5</td>
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<td></td>
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<td>21.4 - 59.0</td>
<td>-25.8 - 31.4</td>
<td>9.0 - 62.5</td>
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<tr>
<td>$CV_A$</td>
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<td>0.06 - 0.12</td>
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<td>$V_P$</td>
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<td>$V_V$</td>
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Paper IV
Adaptation across the genome: multivariate decomposition indicates different roles for the X and autosomes

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Abstract

Understanding the evolution of complex phenotypes is a major goal in evolutionary biology. This requires information on selection and additive genetic variation. However, since traits rarely are selected in isolation, evolutionary change also depends both on the selection upon, and covariation with, other traits. Frequently the sexes do not optimise fitness in the same way, causing sexually antagonistic selection, while genetic correlations between the sexes are often high. A predictive model of phenotypic evolution thus has to include the selection on traits and (co)variation of traits both within and between the sexes. Further understanding of how the genetic architecture governs evolutionary change can be gained by studying different components of the genome. In this respect the X chromosome is of special interest, since theory suggests that it may host variation which differs in both amount and type from the autosomes, and because it has been proposed to play a special role with respect to the evolution of sexual dimorphism. To examine differences in adaptive potential across the genome we use G-matrices which we generate separately for the X and the autosomes in *Drosophila melanogaster*. Using a variety of descriptive measures, and both empirically estimated and simulated regimes of selection, we show that differences in the structure of $G$ across the genome can affect evolution in specific ways. Our results suggest that the X chromosome has a genetic architecture which is more favourable than the autosomes for the evolution of sexual dimorphism.
Introduction

Two fundamental goals of quantitative genetics are to predict and understand evolution of complex phenotypes through selection and genetic variation. The genetic variance in a trait determines the rate at which adaptation evolution can occur (Fisher 1930) and the breeder’s equation \( R = h^2 S \) captures this relationship, predicting the short-term response to selection \( R \) from the strength of selection \( S \) while accounting for the heritability of a trait \( h^2 \).

However, traits are rarely genetically independent of other traits, and evolution within a trait is therefore also affected by selection on connected traits. Lande and Arnold (Lande 1979, 1980; Lande and Arnold 1983) extended the breeder’s equation into a framework allowing the response to selection at the level of a multivariate phenotype to be given by

\[
\Delta Z = G \beta \quad \text{(Eq. 1.1)}
\]

where \( \Delta Z \) is a \( m \)-dimensional column vector of the response to selection (where \( m \) is the number of traits under consideration within the multivariate phenotype), \( G \) is an \( m \times m \) genetic variance-covariance matrix, and \( \beta \) is a column vector of length \( m \) containing the partial directional selection gradients. The Lande equation (Eq. 1.1) thus incorporates selection on correlated traits with respect to the response in a focal trait, and makes it possible to assess the effect of correlations on the rate of adaptation in the multivariate phenotype. It is clear, as demonstrated by a simple two-trait scenario (traits denoted by \( i \) and \( j \)) that when the sign of the selection coefficients on traits \( i \) and \( j \) are equal and there is positive covariance (\( G_{ij} \) in the following example), genetic covariance can promote more rapid adaptation in a trait (Eq. 1.2) (Agrawal and Stinchcombe 2009):

\[
\Delta Z = G \beta + G_{ij} \beta_j \quad \text{(Eq. 1.2)}
\]

Under certain scenarios, however, selection favouring traits to evolve in different directions in multivariate space is expected to be more common (i.e. when the sign of the selection coefficients does not match), leading to constraint on adaptation when a shared genetic architecture is present. Male and female forms of homologous traits are likely to have a highly connected genetic architecture but could come under frequent antagonistic selection because of the different routes by which the sexes optimise fitness. Sex differences evolve once the genetic correlation among the sexes reduces, indicating some sex-specific genetic architecture (Lande 1980; Bonduriansky and Chenoweth 2009). Despite the frequency with which sexual dimorphism, the result of sexually antagonistic selection, is observed, several recent studies have highlighted how a shared genome can impede the evolution
of sexual dimorphism (Bonduriansky and Rowe 2005; Poissant et al. 2010; Lewis et al. 2011; Gosden et al. 2012; Stearns et al. 2012; Griffin et al. 2013), and others have stressed the importance of incorporating selection on both sexes and genetic covariance for proper assessment of phenotypic change (Lewis et al. 2011; Gosden et al. 2012; Wyman et al. 2013; Ingleby et al. 2014). Less emphasis has, however, been placed on examining how genetic correlations affect the response to selection in general, and how these may be beneficial under different modes of selection (Bonduriansky and Chenoweth 2009; Hosken 2011).

It is possible that differences in both the amount and type of genetic variance can occur among different components of the genome. The sex chromosomes have unusual population dynamics in comparison to the autosomes which could erode the genetic variation found in this part of the genome (reviewed in Ellegren 2009), reducing the adaptive potential of the X chromosome. While many studies report depleted levels of molecular variation at the X chromosome (Hutter et al. 2007; Langley et al. 2012; Mackay et al. 2012; Campos et al. 2013), the relative contribution to phenotypic variation made by the X is not necessarily reduced (Cowley et al. 1986; Cowley and Atchley 1988, Griffin et al unpublished data). Genetic variance may, despite the predictions made by population genetic theory, persist more easily on the X chromosome, because of a more permissive parameter space for the persistence of sexually antagonistic mutations (Rice 1984; Fry 2010). Some evidence suggests that the X chromosome is enriched for sexually antagonistic mutations in Drosophila (Gibson et al. 2002; Innocenti and Morrow 2010) despite the expected rarity of such mutation events (Morrow et al. 2008; Mallet et al. 2011; Mallet and Chippindale 2011). Sexually antagonistic mutations should attract sex-specific modifiers to resolve intralocus sexual conflict, which will reduce the intersexual genetic correlation in this part of the genome, for which there is some evidence (Griffin et al. 2013, Griffin et al unpublished data)(Griffin et al. 2013, Griffin et al unpublished data). Overall there is a prediction that the X is enriched for sex-specific genetic variance, but suffers a possible depletion of genetic variance overall, and it is unclear how this might affect adaptation.

Here we use chromosome substitution lines in Drosophila melanogaster to generate G-matrices for the X chromosome and autosomes separately, for one behavioural, one morphological, and one life history trait measured in both sexes. We then examine the potential for adaptation of the multivariate phenotype in each genome component using both simulated selection regimes and empirical fitness data. The use of chromosome substitution lines allows us to generate high precision estimates of the individual contributions to genetic variance and covariance from a large sample of the X and autosome, and from an outbred genetic background. While chromosome substitution lines are not the only way in which X and autosome specific contributions to genetic variance can be estimated (e.g. double first-cousin, Fairbairn
and Roff 2006), these lines are a simple method to sample a large number of genotypes and do so with the power required for such quantitative genetic studies. Our results show that there is generally a lower covariance between the sexes on the X chromosome, and are supportive of different adaptive potential across the genome. The differences we find suggest a possible special role for the X chromosome in adaptive evolution in conditions favourable for sexual dimorphism.

Methods

Experimental population
The flies used in this experiment were taken from a long term laboratory adapted population (Dahomey) of *D. melanogaster*. This population originates from flies caught in Africa over 40 years ago, and has since been maintained in a large outbred population of overlapping generations in constant conditions (12:12 light-dark cycle, 60% humidity, 25°C and on a standard yeast-sugar diet).

Construction of X and autosome substitution lines and estimation of additive genetic variance
The *D. melanogaster* genome consists of the X and Y sex chromosomes, two major autosomes (AII and AIII) and the 4th dot chromosome (< 1% of the genome). The construction of the 40 X chromosome substitution lines (X-lines) and 40 autosome substitution lines (A-lines) is described in detail in the supplementary material of paper III (Griffin et al unpublished). Briefly, each line was constructed using stocks of *D. melanogaster* with specific properties and phenotypic markers which make it possible to track and clonally amplify focal chromosomes into a large number of random genetic backgrounds. All flies within each of the 40 X-lines, regardless of sex, share one identical copy of an X chromosome, while varying at all other chromosomes. All flies within each of the 40 A-lines, regardless of sex, share one identical copy of AII and one identical copy AIII, while varying at all other chromosomes.

Since each cloned X chromosome copy, and each cloned pair of autosome copies, is placed in an individual with a random genetic background, also sourced from the population we study, the average effect of each clone, taken over many individuals, corresponds to the chromosomal clone’s breeding value. Because we clone haploid copies of parts of the genome, the variance among lines equals one half of the additive genetic variance, for the focal component of the genome, for all lines except male X-lines. Hemizygosity of
the X chromosome in males mean the line variance itself is equal to the additive genetic variance. These estimates of line variance include no dominance variation, but a small component of epistatic variation could be included due to interactions between genetic elements within cloned chromosomal parts (Rice et al. 2005; see Lehtovaara et al. 2013 for discussion).

Genetic variance-covariance assay: lifespan

Lifespan estimates were made from 200 flies per sex and line, split among four replicate blocks, totalling 32000 focal flies. Focal X-line females were produced by taking 45 sires carrying the focal X chromosome in a Dahomey genetic background and mating them to 90 virgin Dahomey dams spread equally across three vials. Focal X-line males were produced by taking a further 45 sires, also carrying the focal X chromosome in a Dahomey genetic background, and crossing them to 90 virgin DX-D dams, a stock where dams carry a double-X chromosome (C[1]DX, y, f/Y;) in a Dahomey genetic background causing sons to inherit a Y chromosome from their mother and the focal X chromosome from their father, spread equally across three vials. Focal A-line females were produced by taking 45 sires from the A-lines, which carry a haploid set of the focal autosomes and a set of translocated and phenotypically marked autosomes (T[2;3] bw<sup>d</sup>, in, p<sup>a</sup>, rdgC, ri, st/T[2;3] bw<sup>d</sup>, in, p<sup>a</sup>, rdgC, ri, st) which co-segregate as a single unit, and crossing them to 90 virgin Dahomey dams, spread equally across three vials. Focal A-line males were produced by taking 45 sires also carrying a haploid copy of the focal autosomes, and the translocated autosomes, and crossing them to 90 DX-D dams, spread across three vials. Parental flies were allowed to oviposit in vials for 24 hours, after which we manually adjusted the number of eggs in the vial to equalise larval density at approximately 150 larvae per vial, accounting for inviability induced by the genetic tools. We repeated this procedure four times, to produce four replicate blocks.

Ten days after oviposition the vials were cleared of emerged flies and we collected vials of 56 freshly eclosed virgin focal flies under a light CO<sub>2</sub> anaesthesia (<4 minutes of exposure). These flies were then paired with 56 opposite sex flies carrying a recessive dark body pigment mutation (ebony - earlier introgressed in to the Dahomey background), and allowed to mate for 72 hours. The ebony flies were removed and we collected 50 randomly selected focal flies from each vial, under a light CO<sub>2</sub> anaesthesia, and transferred them to a fresh vial. These flies were transferred to fresh vials, while scoring deaths and removing dead flies, every 48 hours from this point onwards until all flies were dead. A small number of vials (38 of 640, 5.9%) showed unusually short average lifespan in females (< 51 days) and we suspect that they have been affected by some strong environmental effect (e.g. a disease). These vials were removed after careful consideration (see details in Griffin et al unpublished, paper III).
Genetic variance-covariance assay: negative geotaxis and body size

To produce focal flies, for assays of negative geotaxis and body size for the 40 A- and X-lines, we employed a protocol similar to the one described above for the lifespan assay. In this assay we produced four further blocks of focal flies for measurement, and production protocols were identical. We collected vials of 25 focal flies for each sex and line in each block and allowed them to mature for 2 days at standard conditions. Flies were then transferred to vials containing no food, where we tapped them firmly on the desk three times and allowed three seconds for the flies to climb the sides of the vials to assay negative geotaxis (see RING protocol in Nichols et al. 2012), and imaged them using a Sony α200 DSLR camera. Each vial was assayed 12 times, with six repeats two hours and six repeats five hours after the start of the light cycle, leading to 1200 measures of negative geotaxis, from 100 flies per line and sex. Images were uploaded to ImageJ v1.48 (Schneider et al. 2012) and measurements of climbing distance were taken for each fly that was clearly visible. We analysed average climbing distance per images as a trait, because it was impossible to follow individual flies across images. However, this means that within-image phenotypic variation was not modelled, but the relevant numbers are shown in Table 1.

Once the negative geotaxis experiment had been performed we stored the focal flies at -20°C. The thorax of 5 flies per line, sex, and replicate block were later dissected. These were imaged from the dorsal plane using a Leica MZ8 stereomicroscope mounted with a Lumenera Infinity 2-2C digital CCD camera and Infinity Analyze v6.1.0 (Lumenera Corporation) image capturing software. Mesothorax length, herein referred to as body size, and here defined as the anterior tip of the mesothorax to the posterior tip of the scutellum (Bergland et al. 2008), was measured using ImageJ v1.48 (Schneider et al. 2012).

Selection assay

To measure selection acting on lifespan, negative geotaxis and body size, in males and females separately, we scored an index of fitness for 200 Dahomey flies of each sex. Focal flies were generated by placing 80 oviposition vials in to Dahomey population cages and collecting 150 eggs (manually adjusted) per vial. Ten days after the first eggs were collected, the oviposition vials were cleared of emerged flies one hour before the beginning of the light cycle and, over the next six hours, we collected 200 virgin flies of each sex into individual vials. After being allowed to mature for 48 hours we performed a negative geotaxis assay as described above, collecting 12 images per focal fly (sets of six measures, each two and five hours after the begin-
ning of the light cycle). Images and measurements were taken as described above.

After completion of the negative geotaxis assay we transferred focal individuals into fresh vials with food containing 9 same sex and 10 opposite sex competitor flies, all homozygous for the recessive body colour mutation ebony giving these flies a dark body colour. Flies were then transferred to fresh vials three times a week until the focal fly died, and remained in vials for 3, 1 and 3 days. The vial which housed flies for 24 hours (fitness vial) was kept for 12 days, after which the offspring that emerged from the fitness vial was counted and scored for body colour to assess reproductive success of the focal flies. This gave a measure of reproductive success of the focal flies for one day per week throughout their life. Every 14 days the set of competitor flies were replaced under a light CO₂ anaesthesia (<30 seconds), to provide a constant competitor environment throughout the life of the focal flies. When a focal fly died it was stored at -20°C and later measured for its mesothorax length.

Estimation of (co)variances

The data were analysed using mixed-effects models fitted by Markov chain Monte Carlo (MCMC) sampling as implemented in the MCMCglmm package (Hadfield 2010) in R 3.1.2 (R Core Team 2014). Each trait in each sex was standardized to zero mean and unit variance while pooling data from A- and X-lines. Standardization was done to facilitate comparisons across the three traits that were measured in very different units and simultaneously accounted for differences in variances that arise from scaling with the mean in sexually dimorphic traits. However, we used the pooled data across A and X, such that differences among (co)variances in A to X comparisons cannot arise from differences in the standardization.

All six trait-by-sex combinations were treated as separate response variables and were modelled assuming multivariate normal error distributions. Such multi-response models allowed us to efficiently estimate genetic variances and covariances and thus to assemble complex G-matrices. Line and replicate (vial identity) were fitted as random effects, and trait and sex-specific fixed effects were fitted to account for the four batches of replicates. We fitted separate models for A-lines and X-lines, because these data were unconnected by our sampling design and there is hence no benefit in fitting a combined model.

For the line random effect and the residual variation, we formed 6×6 variance-covariance matrices, but since measurements for the three traits arose from separate pools of individuals, off-diagonal elements (i.e., the covariances) for the residuals were not identifiable in our dataset such that these elements were constrained to zero. For the line variance-covariance matrices, however, the data contained sufficient information for estimating all ele-
ments. Negative geotaxis and body size for males and females was measured from the same vials, while lifespan was quantified for flies from different vials. Hence, we fitted separate 2×2 matrices for the vial random effect that allowed us to estimate 6 vial variances (3 traits × 2 sexes) and two vial covariances (between negative geotaxis and body size in males and females, respectively).

We used the default normal priors with large variance for the fixed effects and Inverse-Wishart priors for the variance-covariance matrices of the random effects and residual variances. The Inverse-Wishart distribution is a multivariate generalization of gamma distributions and a conjugate prior for positive-definite variance-covariance matrices. The distribution is determined by two parameters, the scale matrix $\Psi$ and the degrees-of-freedom $\nu$. We set the diagonal elements of $\Psi$ equal to 1/3 (unit variance after standardization divided by the number of variance components line, vial and residual) and the degrees-of-freedom to $\nu = 5$ (equal to the dimensions of the variance-covariance matrix minus 1). Four independent MCMC chains, two for each line type, were run for 220,000 iterations, with a burn-in of 20,000 iterations and a thinning interval of 100 iterations. Convergence was checked visually and using the Gelman-Rubin criterion, applied to each of the independent chains generated.

We estimated the line variance ($V_L$), the vial variance ($V_V$) and the residual variance ($V_R$), each separated for the two sexes and traits, and the line covariance among sexes and traits. Since lines were cloned for haploid chromosomes, additive genetic variance was calculated by multiplying the line variances by 2, with the exception of the male X-lines (because the X is hemizygous in males). Line covariances were converted to additive-genetic correlations by $r_{xy} = \sigma_{xy}^2 / \sqrt{\sigma_x^2 \cdot \sigma_y^2}$. One of the key advantages of the MCMC sampling approach is that we can form sums, ratios and differences of (co)variances for the entire chain and thus get samples from the posterior distribution of these quantities. When estimating the posterior distribution of differences among A and X, we linked the two independent chains after randomizing one against the other, such that any remaining autocorrelation was erased. We summarise posterior distributions by their means, standard error (SE) and 95% inter-quantile range (95% CI), and extract P values that represent the fraction of the posterior samples beyond a specified threshold (two-tailed if required).

**G matrix assembly and notation**

The 6x6 G matrix can be decomposed into four 3×3 submatrices such that $G_F$ captures the female-specific variances and covariances among the three traits, $G_M$ captures the males-specific variances and covariances and $B$ contains the covariances across sexes both within traits (diagonal elements of $B$) and among traits (off-diagonal elements of $B$):
\[
G = \begin{bmatrix}
G_F & B^T \\
B & G_M
\end{bmatrix}
\]

The \(G_F\) and \(G_M\) are symmetrical, while \(B\) can be asymmetrical if cross-trait covariances differ by which sex expresses which trait. We follow (Gosden and Chenoweth 2014) in analysing the upper and lower triangles of \(B\) separately by constructing symmetrical matrices \(B_{up}\) and \(B_{lo}\) with diagonal elements equal to the diagonal elements of \(B\) and off-diagonal elements mirrored from the upper or lower triangle, respectively.

We index matrices, variances and the summary statistics described below by trait name (BS = body size, NG = negative geotaxis, LS = lifespan), sex (F = females, M = males), and chromosome type (A = autosomal lines, X = X-chromosomal lines) as required. Within submatrices, we order the three traits in the sequence (top left to bottom right): body size, negative geotaxis, lifespan. Since we were particularly interested in assessing the influence of the cross-sex covariances (i.e. the \(B\) submatrices) on the structure of \(G\) and hence on the potential for influencing evolutionary change, we employed a range of matrix comparisons with the estimated \(B\) (sub)matrices and with \(B\) matrices that had all their elements constrained to zero. We refer to those constraint matrices as \(G_0\).

Statistical comparisons of \(G\) matrices

Due to their inherent multivariate nature, \(G\)-matrices differ in multiple aspects and a large number of indices and matrix comparison methods have been used to inspect particular properties of \(G\)-matrices (reviewed in Roff et al. 2012). We here employ a series of different \(G\)-matrix analyses that shed light on multiple important properties. We include some meaningful element-wise comparisons that allow us to evaluate the trait-wise differences in sex-chromosome linkage and trait-specific sex-differences in genetic variance. Similarly, the comparison of cross-sex genetic correlations on a trait-by-trait basis allows us to evaluate trait differences and facilitates comparisons with univariate studies.

Specifically, we use the following summary statistics for (sub)matrices:

1) Eigenvolume: We summarize the total genetic variances across all traits as the trace of a matrix, i.e., the sum of the variances in the diagonal equal to the sum of the eigenvalues. We denote the volume of a matrix \(\sum \lambda\).
2) Main axis of variation: The first eigenvector after spectral decomposition describes the main axis of multivariate variation with a matrix
and hence the main axis of genetic variation. We refer to this vector as \( g_{\text{max}} \).

3) Dominance of first eigenvector: The ratio of the first eigenvalue \( \lambda_1 \), i.e., the amount of variation explained by the first eigenvector, to the total genetic variance within a matrix (\( \sum \lambda \)) as a measure of concentration of multivariate variation in a single dimension. We denote the dominance \( D_1 \).

4) Angles among vectors: One way to summarize the similarity of two vectors in multivariate space is the angle between them. Vectors that we compare are the main axis of variation between two matrices or a vector of predicted response to selection to the vector of selection as a measure of deflection by the genetic variance-covariance matrix. Vectors of main axis of genetic variation are unsigned, such that the range of angles is 0 (complete alignment) to 90° (completely orthogonal). Vectors of selection and response to selection, however, are signed and the range of angles is thus 0 (complete alignment) to 180° (completely opposing). We denote the angles between two vectors as \( \theta \).

5) Matrix correlations: We use Mantel tests (Goodnight and Schwartz 1997) to compare the structure (sub)matrices. The test statistic range -1 to 1, where 1 indicates perfect positive correlation and 0 indicates a complete absence of a correlation. The test is mostly sensitive to the shape of the matrices, in particular the off-diagonal elements, but also to the relative size of the variances in the diagonal. We denote Matrix correlations by Mantel tests as \( M \).

Random skewer analysis

The effect of a genetic variance-covariance matrix on the response to selection can be quantified by the deflection angle (possible range 0-180°) between a vector of directional selection gradients \( \mathbf{\beta} \) and the response vector \( \Delta \mathbf{z} \) as predicted by the Lande equation (eq 1.1). We use random skewers to study the adaptive potential and the constraint imposed by genetic covariance (Cheverud 1996). Random skewers are randomly generated vectors of selection gradients that simulated unpredictably arbitrary selection in all possible directions. Random skewers are generated by randomly generating elements of \( \mathbf{\beta} \) (drawn from a uniform distribution \( U(-1,1) \)) followed by standardization of \( \mathbf{\beta} \) to unit length such that the strength of selection is constant, but the direction varies in multivariate trait space (Roff et al. 2012). However, selection is unlikely to be entirely arbitrary with respect to its direction in multivariate trait space. Two particularly important special cases are sexually concordant and sexually antagonistic selection. We hence modified the random skewers approach to accommodate these two special cases (a) by selecting three selection gradients for the three traits that are identical
in magnitude and direction for males and females to simulate sexually concordant selection and (b) by selecting three selection gradients for the three traits that are identical in magnitude, but opposite in direction for males and females to simulate sexually antagonistic selection.

Multivariate estimation of selection
We quantified directional, non-linear, and correlational selection based on our fitness assays following the multiple regression approach (Lande and Arnold 1983). Following standardization of fitness to relative fitness within sexes, we used relative fitness as a response and body size, negative geotaxis and lifespan (all standardized to mean of zero and unit variance), their squared terms and their two-way interactions as predictors. The regression coefficients for the three main effects are the standardized selection gradients $\beta$, the coefficients for the squared terms give (after doubling; Stinchcombe et al. 2008) the standardized non-linear selection gradients and the coefficients for the cross-trait interaction terms give the correlational selection gradients. The latter two types can be assembled in the non-linear selection matrix $\gamma$, a symmetrical matrix with non-linear selection in the diagonal and correlational selection in the off-diagonal. We calculated standard errors of selection gradients based on non-parametric bootstrapping and calculated the predicted response to directional selection by applying the Lande equation with the empirical estimates of $\beta$.

Posterior distributions of derived statistics
All derived statistics could be conveniently calculated on each multivariate sample from the posterior distribution in the MCMC chain such that we get the full posterior distribution for all derived statistics based on the 2 x 2000 MCMC samples. These distributes were summarised as described above. When forming comparing estimates among statistics for the autosomal and X chromosomal lines (for which we fitted separate MCMC models), we randomized the samples from one chain against the other as described above.

Results
All traits were sexually dimorphic and phenotypically variable (Table 1). We found substantial additive genetic variance within-trait for all three traits, in both sexes, in both the X chromosome and autosomes (Table 1). Furthermore, we found significant vial variance within traits for all three traits in both sexes, indicating shared environmental influences on flies within vials (Table 1).
Table 1. Variance components. Sources of variances for three traits measured separately in the two sexes in D. melanogaster. BS = body size, NG = negative geotaxis, LS = lifespan. For negative, SD = sexual dimorphism. Phenotypic mean and variance are calculated from the raw data, while variance components are estimated after variance-standardization (using the following devisors, for Females: BS 0.0253, NG 4.85, LS 9.17, and for males: BS 0.0215, NG 5.85, LS 10.52). The original-scale variances can be reconstructed by multiplication with the square of the devisor. For geotaxis phenotypes we report the raw phenotypic mean and variance as for the other traits, but the variance components and the heritability were estimated based on mean values of approx. 25 flies per image (between-image variance of 11.04 in female A lines, 11.51 in female X lines, 9.80 in male A lines and 9.54 in male X lines were thus excluded from the analysis).

\[ V_P = \text{phenotypic variance}, \ SD = \text{sexual dimorphism}, \ V_L = \text{line variance}, \ V_V = \text{vial variance}, \ V_R = \text{residual variance}, \ h^2 = \text{heritability}. \]

<table>
<thead>
<tr>
<th>Autosome</th>
<th>Mean</th>
<th>( V_p )</th>
<th>SD</th>
<th>( V_L )</th>
<th>( V_V )</th>
<th>( V_R )</th>
<th>( h^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>BS</td>
<td>1.05 ± 0.00</td>
<td>7.29·10^-4</td>
<td>0.13</td>
<td>0.29 ± 0.09</td>
<td>0.36 ± 0.06</td>
<td>0.59 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>NG</td>
<td>26.29 ± 1.29</td>
<td>282.3</td>
<td>0.13</td>
<td>0.19 ± 0.05</td>
<td>0.12 ± 0.02</td>
<td>0.74 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>64.76 ± 1.09</td>
<td>92.9</td>
<td>0.22</td>
<td>0.22 ± 0.06</td>
<td>0.07 ± 0.01</td>
<td>0.88 ± 0.01</td>
</tr>
<tr>
<td>Male</td>
<td>BS</td>
<td>0.91 ± 0.00</td>
<td>4.84·10^-4</td>
<td>0.28 ± 0.08</td>
<td>0.10 ± 0.03</td>
<td>0.76 ± 0.04</td>
<td>0.49 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>NG</td>
<td>30.21 ± 1.70</td>
<td>371.0</td>
<td>0.29 ± 0.08</td>
<td>0.16 ± 0.03</td>
<td>0.66 ± 0.02</td>
<td>0.29 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>50.02 ± 1.25</td>
<td>110.4</td>
<td>0.20 ± 0.05</td>
<td>0.05 ± 0.01</td>
<td>0.81 ± 0.01</td>
<td>0.38 ± 0.08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>X chromosome</th>
<th>Mean</th>
<th>( V_p )</th>
<th>SD</th>
<th>( V_L )</th>
<th>( V_V )</th>
<th>( V_R )</th>
<th>( h^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>BS</td>
<td>1.05 ± 0.00</td>
<td>5.51·10^-4</td>
<td>0.12</td>
<td>0.13 ± 0.04</td>
<td>0.18 ± 0.04</td>
<td>0.63 ± 0.04</td>
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<tr>
<td></td>
<td>NG</td>
<td>27.00 ± 1.35</td>
<td>294.3</td>
<td>0.06</td>
<td>0.24 ± 0.06</td>
<td>0.08 ± 0.02</td>
<td>0.76 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>67.85 ± 0.85</td>
<td>70.1</td>
<td>0.24</td>
<td>0.10 ± 0.03</td>
<td>0.07 ± 0.01</td>
<td>0.72 ± 0.01</td>
</tr>
<tr>
<td>Male</td>
<td>BS</td>
<td>0.92 ± 0.00</td>
<td>4.31·10^-4</td>
<td>0.17 ± 0.05</td>
<td>0.09 ± 0.03</td>
<td>0.77 ± 0.04</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>NG</td>
<td>28.73 ± 1.64</td>
<td>358.1</td>
<td>0.24 ± 0.07</td>
<td>0.13 ± 0.02</td>
<td>0.62 ± 0.02</td>
<td>0.13 ± 0.03</td>
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<tr>
<td></td>
<td>LS</td>
<td>51.36 ± 1.24</td>
<td>110.3</td>
<td>0.13 ± 0.03</td>
<td>0.04 ± 0.01</td>
<td>0.89 ± 0.01</td>
<td>0.12 ± 0.03</td>
</tr>
</tbody>
</table>

Comparison of genetic variance between A- and X-lines

Pairwise comparisons showed that the additive genetic variance is higher in the autosomes than the X chromosome for all three traits, in both sexes, except for female negative geotaxis where variation was equally affected by the X chromosome and autosomes (Fig. 1). Given that the X chromosome contains approximately 15-19% of the genetic material in D. melanogaster, a reasonable null expectation is that the X chromosome will explain this much variation in traits. We find that the trait-specific X-linked genetic variance explains percentages above this range for all traits (Table S1). Summing the total genetic variances across traits separately in the \( G_F \) and \( G_M \) submatrices, the X chromosome hosts 34% and 18% of the total genetic variance in females and males, respectively (Table 2). Consequently, summing across traits and sexes (i.e. the trace of the entire G-matrices), the X chromosome contained approximately 25% of the additive genetic variance (Table 2) suggesting that the X chromosome does carry a disproportionately large density.
of the additive genetic variance, albeit credible intervals are large and include the range of 15-19% predicted by DNA content.

Comparison of genetic variance between the sexes

Sex-biases in genetic variance can affect the evolution of sexual dimorphism (Bonduriansky and Chenoweth 2009) and we therefore compare the genetic variance within line types between the sexes. We find no sex-bias in the genetic variance for both body size and longevity, both in the X chromosome and autosome (Fig. 1). However, we find a suggestive amount of male-bias in the genetic variance for negative geotaxis in the autosomes, and a significant and similar magnitude of female-bias in genetic variance for negative geotaxis in the X chromosome (Fig. 1). Overall, X chromosome additive genetic variance showed a bias toward female-specific additive genetic variance ($G_{XF}$ vs $G_{XM}$, Table 2), while the autosomes showed no significant bias in the distribution of additive genetic variance towards either sex ($G_{AF}$ vs $G_{AM}$, Table 2). This finding is opposite to what would be expected based on complete dosage compensation in males, because dosage compensated males are effectively homozygous and should hence show more X chromosomal genetic variation.

Structure in $G_F$ and $G_M$ caused by trait-covariances

We next inspect the within-sex among-trait covariances, i.e., the off-diagonal elements of $G_F$ and $G_M$. The $G_{AM}$ submatrix shows significant covariance between body size and negative geotaxis within males, but other cross-trait genetic covariances (including all those in $G_{AF}$, $G_{XF}$ and $G_{XM}$) were low and non-significant (Table 3). Comparisons between sex-specific submatrices within line types by Mantel tests show high correlations between the male and female submatrices in A-lines ($G_{AF}$ vs $G_{AM}$, $M = 0.87$) and a subtly lower estimate with larger uncertainty for X-lines ($G_{XF}$ vs $G_{XM}$, $M = 0.83$,

<table>
<thead>
<tr>
<th>Volume</th>
<th>Shape</th>
<th>Proportion</th>
<th>Ratio</th>
<th>Mantel</th>
<th>Angle</th>
<th>Dominance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G_A &amp; G_X$</td>
<td>1.49 ± 0.47</td>
<td>0.25 ± 0.05</td>
<td>0.51 ± 0.10</td>
<td>0.54 ± 0.14</td>
<td>69.9 ± 13.7</td>
<td>0.02 ± 0.09</td>
</tr>
<tr>
<td>$G_{OA} &amp; G_{OX}$</td>
<td>-0.48 ± 0.30</td>
<td>0.34 ± 0.08</td>
<td>0.68 ± 0.17</td>
<td>0.66 ± 0.23</td>
<td>5.9 ± 17.8</td>
<td>0.05 ± 0.09</td>
</tr>
<tr>
<td>$G_{AF} &amp; G_{XF}$</td>
<td>-1.01 ± 0.29</td>
<td>0.18 ± 0.04</td>
<td>0.36 ± 0.09</td>
<td>0.98 ± 0.59</td>
<td>51.6 ± 20.3</td>
<td>-0.01 ± 0.11</td>
</tr>
<tr>
<td>$B_{Ap} &amp; B_{Xp}$</td>
<td>-0.23 ± 0.46</td>
<td>63.0 ± 17.5</td>
<td>0.26 ± 2.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$G_{AM} &amp; G_{XM}$</td>
<td>0.14 ± 0.31</td>
<td>0.56 ± 0.12</td>
<td>1.12 ± 0.24</td>
<td>0.86 ± 0.21</td>
<td>51.5 ± 19.5</td>
<td>0.05 ± 0.09</td>
</tr>
<tr>
<td>$G_{AF} &amp; G_{AM}$</td>
<td>0.14 ± 0.31</td>
<td>0.56 ± 0.12</td>
<td>1.12 ± 0.24</td>
<td>0.86 ± 0.21</td>
<td>51.5 ± 19.5</td>
<td>0.05 ± 0.09</td>
</tr>
<tr>
<td>$B_{Ap} &amp; B_{Xp}$</td>
<td>-0.23 ± 0.46</td>
<td>63.0 ± 17.5</td>
<td>0.26 ± 2.06</td>
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</tr>
<tr>
<td>$B_{Xp} &amp; B_{Xlo}$</td>
<td>-0.77 ± 0.46</td>
<td>36.2 ± 20.2</td>
<td>0.03 ± 0.20</td>
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</table>
Figure 1. Relevant element-by-element comparison of G-matrices. Estimated differences and their SE are shown the direction of the error shows the direction of the comparison.

Table 2). Nevertheless, the angle of the dominant eigenvectors was large in particular in A-Lines ($G_{AF}$ vs $G_{AM}$, $\theta = 51^\circ$) and, to a lesser degree, in X-lines ($G_{XF}$ vs $G_{XM}$, $\theta = 40^\circ$, Table 2). These results illustrate little cross-trait genetic correlation with only moderate differences in overall structure among within-sex genetic covariances both between the sexes and between the two components of the genome.

Covariance between the sexes

We next inspect the cross-sex genetic correlations within and among traits. The diagonal elements of the $B$-submatrix show significant cross-sex covariance and intersexual genetic correlation ($r_{MF}$) between the sexes for all three traits in the diagonal of $B_A$ (Table 3). In contrast, only one of the diagonal elements of $B_X$, that of negative geotaxis, shows significant cross-sex covariance and correlations, while no other significant covariances were estimated in the X-lines (Table 3).

Mantel tests show only moderate correlations among $G_X$ and $G_A$ matrices ($M = 0.54$, Table 2). When constraining the cross-sex genetic covariances in $B_X$ and $B_A$ to zero, the correlation among $G_{0A}$ and $G_{0X}$ is increased ($M = 0.70$, Table 2), indicating that an important difference among autosomal and X chromosomal genetic variance lies in the cross-sex genetic covariances. Somewhat contradictory, the angle between $g_{\text{max},A}$ and $g_{\text{max},X}$ increased from $70^\circ$ to $83^\circ$ when constraining $B$. 


Table 3. *G*-matrices for *A*-lines and *X*-lines. Within-trait genetic variances are the six diagonal elements of each matrix, intersexual genetic correlations are shown 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 bold text.

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th></th>
<th>Male</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td></td>
<td>BS</td>
<td>NG</td>
<td>LS</td>
<td>BS</td>
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<tr>
<td><strong>G</strong></td>
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<tr>
<td><strong>A</strong></td>
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</tr>
<tr>
<td>Female</td>
<td>0.59 ± 0.18</td>
<td>0.01 ± 0.10</td>
<td>-0.08 ± 0.10</td>
<td>0.29 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>0.02 ± 0.19</td>
<td>0.37 ± 0.10</td>
<td>0.06 ± 0.08</td>
<td>-0.02 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>-0.15 ± 0.18</td>
<td>0.15 ± 0.18</td>
<td><strong>0.45 ± 0.11</strong></td>
<td>-0.11 ± 0.10</td>
</tr>
<tr>
<td><strong>X</strong></td>
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</tr>
<tr>
<td>Female</td>
<td>0.51 ± 0.15</td>
<td>-0.04 ± 0.18</td>
<td>-0.21 ± 0.17</td>
<td><strong>0.57 ± 0.16</strong></td>
</tr>
<tr>
<td></td>
<td>-0.28 ± 0.18</td>
<td><strong>0.42 ± 0.15</strong></td>
<td>0.21 ± 0.17</td>
<td>-0.44 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>-0.03 ± 0.18</td>
<td>0.06 ± 0.18</td>
<td><strong>0.39 ± 0.15</strong></td>
<td>-0.04 ± 0.17</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.27 ± 0.08</td>
<td>0.06 ± 0.07</td>
<td>0.01 ± 0.04</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>0.16 ± 0.19</td>
<td><strong>0.47 ± 0.13</strong></td>
<td>-0.01 ± 0.06</td>
<td>-0.01 ± 0.05</td>
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<tr>
<td></td>
<td>0.05 ± 0.18</td>
<td>-0.05 ± 0.18</td>
<td><strong>0.19 ± 0.05</strong></td>
<td>0.02 ± 0.03</td>
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<tr>
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<td>LS</td>
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<tr>
<td><strong>X</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.21 ± 0.18</td>
<td>-0.04 ± 0.19</td>
<td>0.11 ± 0.18</td>
<td><strong>0.17 ± 0.05</strong></td>
</tr>
<tr>
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<td>0.13 ± 0.19</td>
<td><strong>0.53 ± 0.13</strong></td>
<td>-0.09 ± 0.18</td>
<td>-0.01 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>0.05 ± 0.18</td>
<td>0.16 ± 0.17</td>
<td>0.02 ± 0.17</td>
<td>0.14 ± 0.17</td>
</tr>
</tbody>
</table>

Both, upper and lower segments of the **B**-submatrices compared between line types show significant differences between the A-lines and X-lines (angles of 63° in both cases, Table 2), further illustrating the difference between the chromosome types in **B**. Mantel correlations of B-submatrices among line type as well as between upper and lower sections of **B** within line types showed immense sample variation (Table 2). The angle between the dominant axis of variation when comparing upper and lower sections of **B** within line types showed lower angles (**B** _Up_ vs. **B** _Lo_ θ_A = 36° and θ_X = 31°; Table 2), indicating only moderate asymmetry in **B**.

**Adaptive potential**

Based on 4000 random skewers, we find that the angle between random skewers and predicted response was similar in the A-lines (**G_A**, θ = 32.8°) and in the X chromosomes (**G_X**, θ = 33.7°) and these are aligned differently (**G_A** vs. **G_X**, θ = 42.1°, Fig. 2). When setting the elements of the **B**-submatrix to zero we find that the mean angle between selection and response reduces slightly in the autosomes (**G_0A**, θ = 22.6°) and in the X chromosomes (**G_0X**, θ = 27.3°), and these two matrices remain differently aligned (**G_0A** vs **G_0X**, θ = 35.9°, Fig. 2). Sampling variation, however, precluded individual differences from being statistically significant.

When focusing on sexually concordant selection we find that all angles are slightly reduced relative to random skewers, indicating more efficient
Table 4. Standardized directional and correlational selection coefficients. For directional selection gradients, Model1 refers to estimates from a model with only linear terms, while model2 refers to estimates of directional selection gradients from a model that includes all linear and non-linear terms. Estimates that are statistically different from zero are shown in bold.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Directional selection gradients $\beta$</th>
<th>Non-linear and correlational selection gradients $\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model1</td>
<td>Model2</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS</td>
<td>-0.02 ± 0.05</td>
<td>-0.02 ± 0.05</td>
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<tr>
<td>NG</td>
<td>0.03 ± 0.05</td>
<td>0.03 ± 0.05</td>
</tr>
<tr>
<td>LS</td>
<td>0.18 ± 0.04</td>
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</tr>
<tr>
<td>Males</td>
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<td></td>
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<tr>
<td>BS</td>
<td>-0.04 ± 0.04</td>
<td>-0.04 ± 0.04</td>
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<tr>
<td>NG</td>
<td>0.15 ± 0.04</td>
<td>0.13 ± 0.05</td>
</tr>
<tr>
<td>LS</td>
<td>0.10 ± 0.04</td>
<td>0.03 ± 0.05</td>
</tr>
</tbody>
</table>

and more aligned response to selection (Fig. 2). Furthermore, in all three cases, the angles of full G-matrices and G-matrices with B elements constrained to zero were more similar to each than under random skewers analysis (Fig. 2). Sexually antagonistic selection produced the opposite pattern with larger angles and greater differences between G-matrices with B constrained and unconstrained in the autosomes, while this result is similar to the random skewers analysis in the X chromosome (Fig. 2). Antagonistic skewers produce the largest difference in direction of response between the X and autosomes (Fig. 2).

Empirical quantification of selection

We find no significant selection on body size, neither in males nor in females. Selection gradients were estimated positive for negative geotaxis in both sexes, but significantly so only in males, while lifespan is under significant positive selection in both females and males (Table 4). Non-linear and correlational selection was generally weak and non-significant, with the exception of a component of selection for reduced variance in males (Table 4).

We also use the selection coefficients observed in our fitness assay, applied in the multivariate breeders equation, to test the evolutionary potential of the X chromosomes and autosomes under the selection experienced within this population. The mean angle between the direction of selection and the response was subtly lower in the autosomes ($G_A, \theta = 29.6^\circ \pm 7.5^\circ \pm 44.4^\circ$) than in the X chromosomes ($G_X, \theta = 36.3^\circ \pm 7.5^\circ \pm 50.5^\circ$) suggesting that the autosomes are better able to respond to the selection we measured in this population. These responses are also differently aligned ($G_A$ vs $G_X, \theta = 42.9^\circ \pm 10.0^\circ \pm 62.8^\circ$), suggesting that they will respond to selection differently. Forcing the B-submatrix to zero reduces the angle between the response and selection in both the autosomes ($G_{0A}, \theta = 20.8^\circ \pm 6.4^\circ \pm 33.6^\circ$) and the X chromosome ($G_{0X}, \theta = 31.6^\circ \pm 7.0^\circ \pm 45.5^\circ$).
and they remain similarly (un)aligned relative to each other ($G_A - G_X = 38.4° \pm 9.9° [18.9° - 57.8°]$). A larger shift in the autosomes suggests that the B-submatrix distorts the response to evolution more so there than it does in the X chromosomes, and therefore, that evolution through the X chromosome is less severely constrained by the covariance between the sexes.

**Discussion**

The X chromosome differs from the autosomes because it only occurs with one copy in males. This simple fact has motivated several theoretical investigations, which have concluded that the X chromosome’s special selective environment may alter both the amount and the type of mutations that accumulate relative to the autosomes (e.g. Haldane 1937; Avery 1984; Rice 1984; Charlesworth et al. 1987; Connallon and Clark 2010; Fry 2010). Empirical investigations into gene content, gene expression and molecular variation have confirmed that differences occur (Vicoso and Charlesworth 2006; Ellegren 2009), but our knowledge of what effect these differences have on phenotypic variation has been understudied (but see Cowley et al. 1986; Cowley and Atchley 1988; Gibson et al. 2002; Chenoweth et al. 2008). Here we report on a study comparing G-matrices estimated separately for the X chromosome and the autosomes in *D. melanogaster*, spanning a morphological, a behavioural, and a life history trait, paying special attention to the evolution of sexual dimorphism, which should be constrained by the shared genome.

One reason we chose to study body size, negative geotaxis and lifespan, is because all these traits can be assumed to be highly polygenic. This includes also the behavioural trait negative geotaxis (here defined as the speed by which a genotype orients and climbs upward after exposure to a stressor), because the speed part of the trait can be considered a performance trait (Gargano et al. 2005) which should be affected by many genes. The distribution of genes affecting these traits over the X and the autosomes is thus expected to closely match their ‘equilibrium’ distribution, as the chance for substantial stochastic chromosomal over- or under-representation should be limited. Genetic variation and covariation, between traits and the sexes, should hence be representative for the X chromosome and the autosomes. A second reason for choosing these traits is that they all display sexual dimorphism, which theory suggests could show a bias with respect to its X chromosome versus autosome location (Rice 1984; Fairbairn and Roff 2006; Connallon and Clark 2010; Fry 2010).
Figure 2. 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 \( G_A \) and \( G_X \) matrices.

General structure of the G-matrix at the X chromosome and the autosomes

As expected we found additive genetic variation for all three traits in both sexes. Covariation between traits within each sex was low, with the exception of body size and negative geotaxis which co-varied negatively in males. Given that lifespan is a life history trait, and thus should be the product of the phenotype of many underlying traits (Price and Schluter 1991; Merila and Sheldon 1999), we find it surprising that neither body size nor negative geotaxis co-vary with lifespan. In particular since the phenotype of these traits probably relate to general genetic condition. The negative autosomal
genetic association between body size and negative geotaxis in males could simply result from that body size interferes with a fly’s capacity to move upwards. However, since no such negative association was present for the X chromosome in males, and for none of the chromosome types in females, a genetic association appears to be the most likely explanation. All three traits showed positive covariation between the sexes for the autosomes, while only negative geotaxis co-varied between the sexes at the X chromosome. A result which indicates a genetic architecture which is less shared between the sexes at the X chromosome, and thus supports a larger potential for the X chromosome to mediate intralocus sexual conflict.

The X chromosome is a smaller part of the genome than the autosomes. Accordingly, the volume of $G_X$ was substantially smaller than $G_A$, accounting for 25% of the total additive genetic variation. This value is, however, somewhat larger than expected based on the size of the X chromosome, which is 15.6 or 18.8% of the genome (Genome Release 5.30) when estimated through the number of genes or the amount of euchromatic DNA, respectively. Theory suggests that selected variation should be reduced at the X chromosome, because of more efficient removal of deleterious mutations at this chromosome at mutation selection balance (Charlesworth and Charlesworth 2010). This argument applies to molecular variation in both sexes, and genetic variation for phenotypic traits in females, but the situation is different for genetic variation in males. In *D. melanogaster*, male X-linked genes are expressed to the double amount compared to females (Conrad and Akhtar 2012), to equalise the expression ratio to the autosomes between the sexes. This doubling of gene expression effectively causes all males to be homozygous for X-linked genes, which in most cases should result in a doubling of the additive genetic variation in males compared to females (Reinhold and Engqvist 2013). Surprisingly we observe the opposite to the above predictions. When we estimate the proportion of the volume explained by the X for each sex separately ($G_{XM}/[G_{XM}+G_{AM}]$ and $G_{XF}/[G_{XF}+G_{AF}]$), we find that the X in females explains about twice (34%), or more, compared to what is expected, and that the X explains less (18%) than expected in males. These results thus indicate that genetic variation is not depleted on the X chromosome for the traits we study here, as measured through females where dosage compensation does not influence the comparison to the autosomes. These results further indicate that dosage compensation may not be complete for genetic variants which cause variation in these traits. A lack of dosage compensation will, under simplified assumptions, halve the additive genetic variation in males compared to females (Reinhold and Engqvist 2013). This latter result contradicts the findings by Cowley and co-authors (Cowley et al. 1986; Cowley and Atchley 1988), which showed more male X linked variation in 20 out of 22 morphological (head, wing and thorax) characters.
Adaptive potential through the X and autosome under different selective regimes

To investigate if the structural differences observed between $G_X$ and $G_A$ have an influence on the adaptive potential of the two chromosome types we modelled the phenotypic response to selection. Under the current selection regime, as estimated through a fitness assay, the autosomal and X chromosome responses were rather similarly aligned relative to the selection vector, if anything indicating that $G_A$ is somewhat better oriented to accommodate phenotypic change in the selected direction. However, comparing the X and autosomal response directions to each other reveals that they differ significantly, indicating that the two chromosome types under the current settings have distinct roles in how they respond to selection, by promoting adaptation in different directions. Differences between $G_X$ and $G_A$ are also found using a number of descriptive tests, such as Mantel tests, further suggesting that the two components of the genome will affect adaptation differently. Forcing the B-submatrices to zero, to test the degree to which a shared genetic architecture influences the response to selection, reduces the point estimate of the angle between selection and response directions for both the X chromosome and the autosomes. This difference in response was, if anything larger, for the autosomes, suggesting that phenotypic change is more constrained due to due to higher cross-sex genetic covariance in the autosomes.

While predicted adaptation in response to selection measured in the base Dahomey population is interesting, it is of limited use for describing overall differences between $G_A$ and $G_X$. Accordingly we utilised the random skewers method (Cheverud 1996), and made further novel expansion of this method to model the response to selection in two biologically meaningful contexts. The random skewers analyses show that the autosomes and X chromosome generally deflect the response to selection to a similar degree, but in different directions, and the autosomes are more greatly affected by covariance in $B$. Under sexually concordant selection the autosomes respond better to selection, producing a response more aligned with the direction of selection, although this response is surprisingly not improved by covariance in $B$. The X chromosomes produced a response to sexually concordant selection which was more deflected away from selection, and although this response was marginally improved by constraining $B$ to zero, doing so still left the X chromosomes less able to respond. This result suggests that structure outside of $B$, variance-covariance in the $G_F$ and $G_M$ submatrices, also constrains the response to sexually concordant selection. Finally we exposed the G-matrices to sexually antagonistic skewers. Response to selection was marginally better in the X chromosome, and was greatly improved by removing cross-sex covariance in the autosomes, and to a lesser extent, in the X. Under sexually antagonistic selection the two components of the genome allowed
adaptation in very different directions, and the covariance between the sexes was largely responsible for this. Overall, these results suggest that the autosomes may have a genetic architecture which constrains evolution of sexual dimorphism more than the X chromosome.

Conclusions

Collectively our results show structural differences between the genetic architecture of the X chromosome and the autosomes. Differences include shape and orientation of the G-matrices, and how the two chromosomes mediate phenotypic change through the current selection regime. Perhaps surprisingly, there is more genetic variation on the X chromosome in females than in males, while the presence of dosage compensation should result in the opposite pattern. The X chromosome also seems to have more genetic variation than expected given its relative size, a finding which counteracts current theoretical predictions, and molecular evidence. It also appears as if the genetic architecture is more shared between the sexes at the autosomes, and that selection towards different optima in males and females may be more constrained on the autosomes as a consequence.

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References

allometric relationship of ovariole number and thorax length in *Drosophila melanogaster*. Genetics 180:567-582.


Table S1. Trait-specific X-A ratios and proportions of volumes.

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<th>Volume</th>
<th>Proportion</th>
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<td>BS</td>
<td>F</td>
<td>0.32 ± 0.09</td>
<td>0.50 ± 0.22</td>
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<td>NG</td>
<td>0.56 ± 0.09</td>
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Table S2. Length and dominance of first eigenvector.

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