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UNIVERSITY OF CALIFORNIA
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The Quantitative Genetics of Sexual Differences: New Methodologies and an Empirical Investigation of Sex-Linked, Sex-Specific, Non-Additive, and Epigenetic Effects

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Matthew Ernest Wolak

December 2013

Dissertation Committee:
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For my parents

ABSTRACT OF THE DISSERTATION

The Quantitative Genetics of Sexual Differences:
New Methodologies and an Empirical Investigation of Sex-Linked, Sex-Specific, Non-Additive, and Epigenetic Effects

by

Matthew Ernest Wolak

Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, December 2013
Dr. Daphne Fairbairn, Chairperson

Phenotypic differences between females and males are widely observed in organisms with separate sexes. How these sexual dimorphisms evolve despite the sexes sharing a majority of their genome remains an unresolved issue in evolutionary biology as theoretical models often disagree over the genetic mechanisms that are predicted to facilitate the evolution of sexual dimorphism. In this dissertation, I develop quantitative genetic methods for estimating sex-specific non-additive genetic and sex-linked additive genetic (co)variances. I then empirically quantify the genetic effects underlying population differences in sexually dimorphic traits using the water strider *Aquarius remigis*.

I show that differences between the sexes in genetic architectures bias estimates of additive genetic variance if these differences are improperly incorporated into quantitative genetic analyses. I then develop the **nadiv** software package for the R statistical program to facilitate estimation of non-additive genetic (co)variances using the "animal model". Next, I use simulations to demonstrate that estimates of sex-specific additive genetic variances and between-sex additive genetic correlations are biased when sex-linked additive genetic variance is ignored.

I create a genetic model of a sex-linked locus to derive general expressions for the covariance between relatives due to sex-linked genes applicable under any form of global sex

chromosome dosage compensation. These expressions lead to the development of formulae and algorithms (incorporated into **nadiv**) to create sex-linked relatedness matrices for use in animal model analyses. I further show that the way by which sex-linked relatedness matrices under the various forms of dosage compensation differ from one another implies that unbiased estimates of sex-linked additive genetic variance can still be obtained even when the particular form of dosage compensation is unknown.

Using population crosses of water striders, I show the net genetic effects contributing to sexually dimorphism differ between female and male water striders. I demonstrate that the magnitude of this difference in epistatic and dominance maternal genetic effects positively covaries with the magnitude of sexual dimorphism across a range of morphological traits. This is the first study to show that sex-specific non-additive genetic effects correlate with the degree of sexual dimorphism across traits.

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INTRODUCTION TO THE DISSERTATION

Within a species, female and male organisms often differ from one another in morphology, physiology, behavior, and/or life history (Darwin 1874; Fairbairn 2013). Explaining how these differences, called sexual dimorphisms, can arise and vary among populations presents a conundrum to evolutionary biologists. How do populations evolve differences between the sexes when the sexes share the same genes? The answer to this question not only contributes to the advancement of evolutionary theory, but also potentially affects the way plant and animal breeders manage agricultural populations and how we approach the study of sex-specific genetic disorders in humans.

The key assumption is that a genetic conflict between the sexes occurs when the phenotype that maximizes fitness for one sex differs from that which maximizes fitness for the other sex (Roff 1997). Darwin (1874) was the first to explain how this could come about when he argued that natural and sexual selection often act differently in the two sexes. However, sex-specific responses to these selection pressures are constrained by the genes shared between the two sexes. For example, females that have traits that are well adapted to maximize female fitness will pass on genes for these traits to the detriment of their sons and similarly for fathers passing on genes to their daughters.

Differences in the location and interaction of genes can arise to mitigate genetic sexual conflicts. Because the chromosomes involved in sex determination differ between females and males (XX/XY or ZZ/ZW), evolutionary theory has long predicted that genes responsible for sexually dimorphic traits will be located on the sex chromosome that both sexes share (sex-linkage; Fisher 1931). However, it is also widely observed that sexual dimorphism is present and often quite extreme in many species without chromosomal sex determination (e.g., some fish and reptile species do not have sex chromosomes at all). Therefore, identifying the genetic

mechanisms that facilitate the evolution of sexual dimorphism necessitates the investigation of other components of the genetic architecture besides sex-linkage.

Genetic conflict between the sexes can also be resolved if genes located on the autosomes (non-sex chromosomes) interact. A non-additive genetic interaction causes the phenotypic expression of an allele to be contingent upon the genetic background of that allele. If instead an allele is sensitive to the sex in which it is expressed (one can think of sex as a genetic background), this will lead to differential expression in one sex versus the other (i.e., sex-specific non-additive genetic effects). Testing the predictions of sex-linkage or sex-specific non-additive effects requires disentangling genetic effects located on sex chromosomes from those on the autosomes as well as the ability to discern additive effects of alleles from sex-specific interactive effects.

The field of quantitative genetics provides a useful framework for identifying the genetic effects contributing to phenotypic evolution while, at the same time, maintaining relevance to the fundamental unit of selection - the individual. Quantitative genetics uses statistical inferences from the similarity of phenotypes between relatives to elucidate the underlying genetic basis of phenotypic variation at the population level. It assumes that quantitative traits are polygenic (influenced by many genes of relatively small effect) and that the population phenotypic variance in a trait can be explained by different fractional contributions from components of the genetic architecture and the environment (Roff 1997).

The field of quantitative genetics has been established for over a century and remains a modern, expanding field (e.g., Yule 1906; Fisher 1918; Lande 1979, 1980; Steppan et al. 2002; Jones et al. 2003; Kruuk 2004; Kruuk et al. 2008). However, despite the widespread use in many fields, including evolutionary biology, there exist very few empirical studies seeking to elucidate the quantitative genetic bases of sexually dimorphic traits. This is partly due to a few

methodological and theoretical hurdles that must be overcome before we can test the most recent theory regarding the genetic architecture of sexually dimorphic traits. In my dissertation, I seek to develop new quantitative genetic methodologies and expand upon existing ones to enable empirical evaluation of theory predicting either sex-linkage of additive genetic effects or sex-specific non-additive genetic effects facilitating the evolution of sexual dimorphisms. Further, I empirically evaluate the theoretical predictions for the type of interaction (i.e., additive vs. non-additive) and location (i.e., autosomal vs. sex chromosomal) of genetic effects that underlie sexually dimorphic traits using the water strider *Aquarius remigis*.

In chapter one, I demonstrate that even simple quantitative genetic analyses to estimate additive genetic variation must consider the differences between the sexes in genetic architecture. I address a common misunderstanding in quantitative genetics regarding the biological assumptions underlying the use of "fixed effects" in analytical models that generally account for differences in phenotypes between two classes in a population. The ideas presented in this chapter apply generally to any situation where there are two discrete classes present in the population and are not just restricted to differences between the two sexes. I demonstrate the conditions under which treatment of differences between two classes as a fixed effect does not sufficiently account for differences among classes at the genetic level and leads to biased estimates of additive genetic variance. Despite the widespread occurrence of this practice, the potential for bias is not commonly known nor has it been formally demonstrated. The work goes beyond a simple technical comment by deriving equations to predict the bias in estimates of additive genetic variance when practitioners mistakenly implement statistical assumptions which are incongruent with the biology of their study organisms. This simple derivation of predictive equations is strengthened by its universal applicability to various methods used to estimate additive genetic variance.

In the second chapter, I develop methodological tools to be combined with animal model software programs to estimate non-additive genetic variances in populations for which a complete family history (pedigree) has been recorded. Many of the quantitative genetic software programs commonly used by ecologists and evolutionary biologists lack the option to construct the matrices required to estimate non-additive genetic variances. However, the software programs are still able to make these estimates if the proper matrices are supplied. The R statistical software package I develop in this chapter, **nadiv**, efficiently creates the necessary relatedness matrices to be used in an animal model. The work presented in this paper details a significant contribution to the toolkit used by evolutionary quantitative geneticists as well as providing an overview of the important aspects to the types of analyses now available because of this package.

The third chapter highlights the importance of explicitly accounting for sex-linked additive genetic variances and covariances in quantitative genetic analyses. To date very few studies have estimated or even considered genetic variation located on the sex chromosomes. In this chapter, I consider the common assumption that additive genetic variance on the shared sex chromosome (i.e., X or Z) is either assumed to be negligible and therefore ignored or that it is sufficiently modeled by autosomal covariances between relatives. Although this assumption has been made either overtly or implicitly for almost all estimates of additive genetic variance to date, this is not widely appreciated nor has its potential for introducing bias been measured. I show how sex-linked additive genetic (co)variation differs from its autosomal counterpart and demonstrate the bias in estimates of female and male additive genetic variances and between-sex correlations that arise from assuming an autosomal only model. As sexual conflict is predicted to facilitate the evolution of genes on the sex chromosomes, I emphasize how these results might affect interpretations of empirical estimates of sexually antagonistic (co)variances.

After the demonstration in chapter three of differences between autosomes and sex chromosomes in the expected covariance between relatives, I make a much more detailed investigation into the matrix of expected sex-linked covariances among relatives (sex-linked relatedness matrix) in my fourth chapter. Specifically, many taxa are thought to exhibit some mechanism of global sex chromosome dosage compensation as a means to equalize the phenotypic effect of alleles expressed in the homogametic sex (two copies of the shared sex chromosome) with that of the heterogametic sex (one copy of the shared sex chromosome). These forms of dosage compensation alter the sex-linked relatedness matrix and have yet to be incorporated into genetic models used to construct such matrices. I develop a single locus genetic model that is flexible enough to model the expected genetic covariance between relatives due to sex-linked genes under any form of dosage compensation. Additionally, I derive the formulae and algorithms necessary to construct sex-linked relatedness matrices for use in quantitative genetic analyses using animal models. These algorithms are available in the **nadiv** software package introduced in my second chapter.

In my final chapter, I present results from an empirical study that quantifies the genetic effects underlying a range of morphological traits in the water strider *Aquarius remigis* that vary in magnitude of sexual dimorphism. I quantify the net genetic effects underlying phenotypic differences among populations which enables me to draw inference on the relative importance of different types of genetic effects and where they are located in the genome (autosomes versus sex chromosomes). In this chapter, I find that female and male water striders differ in the magnitude of genetic effects contributing to population differences in sexually dimorphic traits. Further, the magnitudes of between-sex differences in non-additive genetic effects (i.e., epistatic and dominance maternal genetic effects) increase across traits in relation to the degree of the sexual dimorphism. Together, the chapters in this dissertation advance the field of evolutionary genetics

by providing new methodologies, theoretical advancements, and empirical evaluations of predictions from evolutionary theory.

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CHAPTER 1

Biased accounting for within-population differences in quantitative genetic models

Abstract

Populations often contain discrete classes or morphs (e.g., sexual dimorphisms, wing dimorphisms, trophic dimorphisms), which can be considered as different environments within which traits are expressed. Theory predicts the evolution of genotype-by-environment interactions as a consequence of selection favoring different trait combinations in each environment. When analyses consider the quantitative genetic architecture of a trait as being perfectly correlated between the two environments or morphs within a population, estimates of additive genetic variance can be biased. We illustrate the effects of modeling the distribution of breeding values between two morphs within a population as a fixed difference. We demonstrate that unless the between-morph additive genetic correlation is one, solely accounting for fixed differences between the two morphs results in an underestimation of the additive genetic variance. This result is derived for three widely used quantitative genetic variance partitioning methods. We highlight the specific case where the two morphs are the two sexes and discuss the potential biases present in estimates of additive genetic variance when between-sex correlations are less than one.

Introduction

Partitioning phenotypic variation into contributions from additive genetic as well as non-additive genetic and environmental variances is the central paradigm in evolutionary quantitative genetics (Roff 2006). Although recent work has highlighted other contributions to phenotypic variance, such as common environment, maternal genetic, and spatial autocorrelation among relatives (Kruuk et al. 2001; MacColl and Hatchwell 2003; Charmantier et al. 2004; Wilson et al. 2005; Kruuk and Hadfield 2007; Stopher et al. 2012), estimates of additive genetic variance are of paramount importance for predicting population responses to artificial selection (reviewed in Hill and Caballero 1992) or natural selection (Kruuk et al. 2008) using the breeder's equation (Falconer 1989; Lynch and Walsh 1998) or the Secondary Theorem of Natural Selection (Robertson 1966; Price 1970).

An individual's average genetic effect for a polygenic trait in a population is known as its breeding value. Because breeding values are expressed as an individual's average deviation from the population mean, the mean breeding value equals zero and the variance in breeding values is the additive genetic variance (Falconer 1989). Both breeding values and additive variances are defined within the context of a specific population and environment. However, traits are often expressed in two different environments or phenotypic classes within a single population. This is true of dimorphic traits such as disease incidence, wing dimorphisms, protective dimorphisms, trophic dimorphisms, mating dimorphisms, and life cycle dimorphisms (reviewed in Roff 1996), and also of traits differing between the sexes, for example sexual dimorphisms in behavior, morphology, physiology, and life history (reviewed in Fairbairn et al. 2007; Fairbairn 2013). Morph can be considered as an environment which interacts with genes to alter the average genetic effects. Polygenic traits occurring in both phenotypic classes (morphs) are often exposed to different selective environments within each morph, leading to selection for different average

allelic effects in each morph and ultimately to the evolution of morph-specific genetic effects that can be modeled as genotype-by-environment interactions (Roff 1997). If there are only two morphs (environments), the genotype-by-morph interaction can be expressed as a genetic correlation between morphs (Falconer 1952). This genetic correlation summarizes the relationship between the ranks of breeding values expressed in one morph relative to the rank in the other.

Investigators making statistical inferences on breeding values commonly treat dimorphic variation by including the morph as a fixed effect in statistical models to remove the average difference between the morphs (e.g., Wilson et al. 2010; see also WAMWiki at http://www.wildanimalmodels.org/tiki-index.php). Although this is necessary to control for fixed differences in phenotypic means between the morphs, it does not affect the correlation between morphs in breeding values (i.e., between-morph additive genetic correlation). By itself, using a fixed effect of morph invokes the biological assumption of a perfect additive genetic correlation between the two morphs. In this paper, we use breeding values to describe bias in the estimates of additive genetic variance that arises in analyses that consider the additive genetic effects of a trait as being perfectly correlated between two morphs in a population. We find that whenever the between-morph additive genetic correlation is less than one, the additive genetic variance for the morphs combined will be underestimated when only a fixed effect of morph is specified. Further, we consider how to estimate the magnitude of this bias for a variety of quantitative genetic variance partitioning methods (e.g., offspring-parent regression, half-sib ANOVA, and mixed effect models of pedigreed populations). To illustrate our point, we narrow our focus to one widely encountered dimorphism, sexual dimorphism.

Morph-specific quantitative genetic parameters

Breeding values

In practice, breeding values can be estimated using a combination of phenotypic information and the relatedness among individuals within a population. Although an individual can never be simultaneously measured for both phenotypes in a dimorphism, each individual carries genes that will contribute to both phenotypes. Therefore, breeding values for phenotypes that are never expressed can still be measured. A common example of this is milk production in dairy cattle, where bulls cannot be measured for milk yield (e.g., Mrode 2005). However, bull breeding values for milk yield can be estimated for the purposes of determining which bulls will produce daughters with the highest milk yield. Information for the bull's breeding value is gathered from female relatives that share some proportion of genes that the bull carries for the milk yield trait.

In a hypothetical population, if every individual mates with every other individual and offspring are produced from each mating, then breeding values can be estimated as two times the deviation of an individual's average offspring phenotype from the population mean phenotype (p. 73, Lynch and Walsh 1998). This concept of breeding values is useful for examining the effect of genotype-by-morph interactions on the distribution of breeding values for each morph within a population. If the average genetic effect of an allele differs between morphs, the breeding values of the two morphs will also differ. For example, consider height in an imaginary population of dimorphic organisms. A genotype's breeding value for height in morph M1 is defined as the average genetic effect of its genes on height when expressed in morph M1. The breeding value for the same genotype in morph M2 is defined as the average genetic effect of its genes on height when expressed in morph M2. For this one genotype, breeding values are estimated as two times the phenotypic deviation between the average phenotype of morph M1 offspring from the morph M1 population mean, and similarly for morph M2.

The distribution of breeding values ($\mathbf{a_1}$, $\mathbf{a_2}$) for the trait in the two morphs of a population can be described by a bivariate normal distribution, where each has a mean of zero, a variance according to the morph-specific variance in trait breeding values [$Var(\mathbf{a_1})$ and $Var(\mathbf{a_2})$], and some correlation between breeding values in the two morphs (i.e., $r_{\mathbf{a}\text{-}1,2}$; Figure 1.1). However, when the effect of morph on average genetic effects is ignored, the breeding values for a trait are defined as the average genetic effects when a genotype is expressed in both morph M1 and morph M2 (i.e., the average of morph M1 and M2 breeding values). The distribution of these average breeding values ($\mathbf{a_u}$) can be described by a univariate normal distribution with a mean of zero and variance equal to the variance in average breeding values, $Var(\mathbf{a_u})$ (Figure 1.1). The variance in $\mathbf{a_u}$ can be predicted from the general formula for the variance of two random variables averaged together:

$$Var(\mathbf{a_u}) = Var(\tfrac{1}{2}\mathbf{a_1} + \tfrac{1}{2}\mathbf{a_2})$$
$$= (\tfrac{1}{2})^2 Var(\mathbf{a_1}) + (\tfrac{1}{2})^2 Var(\mathbf{a_2}) + 2(\tfrac{1}{2})(\tfrac{1}{2}) Cov(\mathbf{a_1},\mathbf{a_2}) \quad (1)$$

Illustrations of the morph M1, morph M2, and average breeding value distributions, using random draws from each respective distribution, are shown in figure 1.1. When the morphs have the same additive genetic variance [$Var(\mathbf{a_1})=Var(\mathbf{a_2})$], an algebraic rearrangement of equation 1 shows that the variance in average breeding values, $Var(\mathbf{a_u})$, will be less than both $Var(\mathbf{a_1})$ and $Var(\mathbf{a_2})$ whenever the between-morph additive genetic correlation is less than unity. This is seen in figure 1.1, where the spread of points is greater for the breeding values of morphs M1 and M2 than it is for the spread in average breeding values (Figure 1.1C) and the probability distributions for breeding values of morphs M1 and M2 are wider than the probability distribution of the average breeding values (Figure 1.1B).

Assuming that $Var(\mathbf{a_1}) \geq Var(\mathbf{a_2})$, a rearrangement of the right hand side of equation 1 shows that the variance in average breeding values, $Var(\mathbf{a_u})$, will be less than the either of the two additive variances whenever:

$$r_{\mathbf{a}-1,2} < \frac{3Var(\mathbf{a}_2) - Var(\mathbf{a}_1)}{2\sqrt{Var(\mathbf{a}_1)Var(\mathbf{a}_2)}} \quad (2)$$

In figure 1.2, we have evaluated equation 2 over the range of possible between-morph genetic correlations when $Var(\mathbf{a_2})$ is 10% less than $Var(\mathbf{a_1})$.

The heritability, or ratio of the additive genetic variance to the phenotypic variance, is central to predicting the amount by which the average phenotype in a population will change from one generation to the next using the breeder's equation (Falconer 1989). Assuming equal residual variances for both morphs, if the heritability in morph M1 equals the heritability in morph M2, equation 1 can be extended to show that the heritability of the morphs combined will underestimate both morph M1 and M2 heritabilities whenever the between-morph additive genetic correlation is less than one. If the heritabilities in morphs M1 and M2 are not equal, then by a similar rearrangement to the one which produces equation 2, an equation can be derived to predict the between-morph genetic correlation at which the heritability of the morphs combined will be less than both of the heritabilities in the two morphs.

In practice, true breeding values are unknown and thus the additive genetic variance must be estimated using known contributions of additive variance to the phenotypic resemblance between relatives. For many breeding designs, the additive genetic variance is estimated as a fraction of the covariance between offspring and parent phenotypes or as a fraction of the sire or dam variance components (Falconer 1989). Alternatively, additive genetic variance estimates can

be obtained from mixed effect statistical models which simultaneously consider all pair-wise relationships. This latter approach enables estimation of additive genetic variance in non-standard breeding designs and wild populations for which a population pedigree is available. Below, we consider each approach separately.

Offspring-parent and half-sib models

Methods of estimating the additive genetic variance (i.e., the variance in breeding values) from the covariance between offspring and parents, or the variance among half-sib families also depend on there being no genotype-by-morph interaction for breeding values expressed in two different morphs (in addition to other assumptions regarding random mating, non-additive genetic effects, and inbreeding; Falconer 1989; Lynch and Walsh 1998). If these assumptions hold, the additive genetic variance equals two times the covariance between offspring and parent phenotypes, and four times the variance among sire- or dam-family (nested within sire) phenotypes in a nested half-sib breeding design (Falconer 1989; Lynch and Walsh 1998). When equation 1 is multiplied by ½ or ¼, it also predicts the offspring-parent covariance or the sire/dam variance, respectively. Figure 1.2 can be interpreted as depicting the line that predicts either the joint offspring-parent covariance or joint sire/dam variances over a range of between-morph additive genetic correlations when the (co)variance in one morph is 10% less than the other. For example when the two morphs are two sexes, equation 2 predicts that the mid-offspring on mid-parent covariance will be less than the sire on male offspring covariance of 90 when the female offspring on dam covariance is 100 and the between-sex additive genetic correlation is approximately 0.89 (Figure 1.2, grey vertical line).

Morph-specific offspring-parent regressions or nested linear models are therefore necessary when the between-morph additive genetic correlation is less than one or the additive

genetic variances differ between the morphs. Bivariate statistical models, where the phenotypes in the two morphs are treated as separate traits, can also be utilized to obtain morph-specific observed (co)variance components. The additive genetic (co)variances can then be estimated from sire, dam, and within-family (co)variances (e.g., Cowley et al. 1986).

Animal models

The range of organisms and populations for which researchers can obtain predictions of breeding values and make inferences about the additive genetic variance in populations has broadened since the adoption of the mixed effects linear model commonly known as the 'animal model' (Henderson 1973; Lynch and Walsh 1998; Kruuk 2004). Animal models have become popular tools in evolutionary ecology because of their potential to disentangle confounding sources of similarity between relatives, simultaneously consider relationships beyond offspring-parent or half- and full-siblings in the estimation of variance components, and obtain unbiased estimates of model parameters when selection has occurred during a given study (Lynch & Walsh 1998; Kruuk 2004).

Here, we consider the effect of the between-morph additive genetic correlation on joint estimates of variance components in animal models. Estimating one additive variance for both morphs in an animal model assumes no genotype-by-morph interactions and, therefore, a between-morph additive genetic correlation of one. A univariate analytical model incorporating these assumptions can be specified as:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z}_a\mathbf{a} + \mathbf{e} \qquad (3)$$

(Lynch and Walsh 1998; Mrode 2005). When every individual has only one measurement, **y** is a *n*x1 vector of phenotypes in a population of *n* individuals and **X** is a *n*x2 design matrix (i.e., contains 0s and 1s) with 1s in the first column at rows occupied by morph M1 (corresponding to rows in **y**) and 1s in the second column at rows occupied by morph M2. The matrix **X** relates the observation in **y** to the appropriate fixed effect (mean) of morph in **β**. The matrix $\mathbf{Z_a}$ is an *n*x*n* design matrix which associates the phenotypic observation in **y** to the breeding value in **a**. The variables **a** and **e** are the *n*x1 vectors of additive genetic effects and environmental effects, respectively. The random variables **a** and **e** are assumed normally distributed with means of zero and variances of $Var(\mathbf{a})=\mathbf{G_a}\otimes\mathbf{A}$, where **A** is the additive genetic relationship matrix ($\otimes$ symbolizes the direct product between two matrices), and $Var(\mathbf{e})=\mathbf{R}\otimes\mathbf{I}$, where **I** is an identity matrix (*n*x*n*, with 1s along the diagonal). In this model, $\mathbf{G_a}=\sigma^2_a$ where σ^2_a is the additive genetic variance in the base population and $\mathbf{R}=\sigma^2_e$, the environmental variance. Thus, the assumption regarding the relationship between morph M1 and M2 breeding values for the univariate model in equation 3 is that all breeding values are modeled from a univariate distribution of random effects.

Alternatively, the phenotype of interest can be modeled as a different trait for each morph (e.g., Mrode 2005, p.106) by estimating morph-specific variances. This approach is analogous to estimating additive genetic variance in two environments (Roff 1997; Roff and Fairbairn 2011). In practice, this is carried out by specifying a bivariate model where the two traits modeled represent the phenotype as expressed in morph M1 and morph M2. In such a model, only morph M1 traits are expressed in morph M1 and only morph M2 traits are expressed in morph M2. Accordingly, all morph M1 individuals will have missing phenotypes for the morph M2 trait and vice versa for morph M2 individuals. Trait is included as a fixed effect (analogous to morph in a univariate model of both morphs) to account for a difference in the means of un-standardized

phenotypes for morphs M1 and M2. This approach makes a residual covariance between the two morphs impossible to define since no individual can express the trait in both morphs (i.e., morph M1 phenotypes cannot be expressed in morph M2; e.g., Mrode 2005). The bivariate description is preferred over separate univariate models for each morph, because it allows for estimation of the between-morph genetic correlation and increases the precision with which BLUPs for the breeding values are obtained. The latter point arises from the additional information used to determine the breeding values for one morph derived from the expression of the phenotype in opposite morph relatives (analogous to the above example where one can estimate a bull's milk yield breeding value; Mrode 2005). This model can be written in matrix notation as:

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix}=\begin{bmatrix}\mathbf{X}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\beta_1\\ \beta_2\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_{a1} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_{a2}\end{bmatrix}\begin{bmatrix}\mathbf{a}_1\\ \mathbf{a}_2\end{bmatrix}+\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix} \quad (4)$$

In equation 4, $\mathbf{a}$ (the bivariate distribution of $\mathbf{a}_1$ and $\mathbf{a}_2$) and $\mathbf{e}$ (the bivariate distribution of $\mathbf{e}_1$ and $\mathbf{e}_2$) are assumed to represent random effects described by multivariate normal distributions. Consequently, $Var(\mathbf{a})=\mathbf{G}_a\otimes\mathbf{A}$, but here $\mathbf{G}_a$ is the 2x2 matrix:

$$\mathbf{G}_a=\begin{bmatrix}\sigma^2_{a-1} & \sigma_{a-1,2}\\ \sigma_{a-2,1} & \sigma^2_{a-2}\end{bmatrix} \quad (5)$$

When an animal model only includes morph as a fixed effect, the separate distributions of breeding values for the two morphs are assumed perfectly correlated (i.e., $r_{a\text{-}1,2}=1$). Thus, $\mathbf{G}_a$ in equation 5 is forced to satisfy $\sigma^2_{a\text{-}1}=\sigma^2_{a\text{-}2}=\sigma_{a\text{-}1,2}$ ($r_{a\text{-}1,2}=1$ when this occurs) and the model in

equation 4 is equivalent to the univariate model in equation 3. When these assumptions are valid (i.e., $\sigma^2_{a\text{-}1}=\sigma^2_{a\text{-}2}=\sigma_{a\text{-}1,2}$), mixed effect models treating any differences between the morphs as a fixed difference (i.e., morph as a fixed effect and jointly modeling the morphs) will produce unbiased estimates of the additive genetic variance in the population. However, if the between-morph additive genetic correlation is less than unity ($r_{a\text{-}1,2}\neq 1$) as illustrated in figure 1.1, the univariate model in equation 3 will produce a biased estimate of additive genetic variance as predicted by equation 1 (Figure 1.2).

Sexual dimorphism as an example

Increasingly, researchers have proposed that it is necessary to consider the quantitative genetic architecture of a trait separately for each sex, based on the argument that sex-specific differences exist (e.g., Fedorka et al. 2007). In agricultural breeding, the approach has often been to compare differences in parameter estimates from models that do or do not consider the sexes separately (e.g., Garrick et al. 1989; Rodríguez-Almeida et al. 1995; Lee and Pollak 1997; Van Vleck and Cundiff 1998; Näsholm 2004). These studies report differences in estimates only when the between-sex genetic correlations are significantly less than unity. Consequently, recommendations as to the separate or combined consideration of the sexes are proffered on a study by study basis.

To date, Roff and Fairbairn (2011) provide the best empirical investigation of the dynamics described by equations 1 and 2. In a study of the genetic basis of life-history trade-offs, Roff and Fairbairn (2011) analyzed five traits in two wing morphs of the cricket, *Gryllus firmus*. They initially estimated heritability when the sexes or wing morphs were combined as well as for each sex and wing morph separately. For a number of traits, the combined estimates of heritability were lower than the sex-specific or wing-morph specific estimates. They postulated

that these traits had between-sex or between-morph genetic correlations less than one. In agreement with their predictions, and our predictive equations above, Roff and Fairbairn (2011) confirmed the presence of genetic correlations between-sexes or wing-morphs significantly less than one in the same traits where they found the combined heritability estimate was lower than the sex-specific or wing-morph specific estimates. Roff and Fairbairn's results highlight that morphs within a population, particularly the two sexes, often have different distributions of breeding values that reflect the different selective pressures experienced by the morphs.

As a consequence of divergent reproductive roles between the sexes, female and male organisms have different optimal phenotypes which we recognize as dimorphisms in primary and secondary sexual traits (Darwin 1874; Fairbairn et al. 2007; Fairbairn 2013). The differences between sexes are presumed to reflect sex-specific evolutionary responses toward different fitness optima (Hedrick and Temeles 1989; Fairbairn et al. 2007; Fairbairn 2013).

Correlations between the sexes in the expression of shared alleles constrain the evolution of sexual dimorphism (Lande 1980; Reeve and Fairbairn 2001), creating sexual antagonism between the sexes (Parker 1979; Arnqvist and Rowe 2005). Because males and females share their autosomal genomes, between-sex genetic correlations for homologous traits are generally predicted to be high and close to unity (Roff 1997, p.247 table 7.4; Poissant et al. 2010). However, alleles with opposing effects in females and males evolve in response to sexually antagonistic selection and contribute disproportionately to genetic variance in fitness (Charlesworth and Hughes 1999; Connallon and Clark 2012), a pattern often seen in many empirical studies of plants and animals (e.g., Chippindale et al. 2001; Fedorka and Mousseau 2004; Brommer et al. 2007; Foerster et al. 2007; Cox and Calsbeek 2010; Delph et al. 2011). Consequently, the magnitude of sexual dimorphism is predicted to negatively covary with the between-sex genetic correlation (Fisher 1958; Lande 1980; Rice 1984; Fairbairn and Roff 2006;

Poissant et al. 2010; but see Meagher 1992). In addition to changes in the between-sex correlation, sex-specific gene expression will lead to differences between the sexes in their relative amounts of additive genetic variance (e.g., Preziosi and Roff 1998; Jensen et al. 2003; Coltman et al. 2005; Brommer et al. 2007; Walling et al. 2008).

Taken together, empirical support for separate estimates of sex-specific additive genetic variances (e.g., Roff and Fairbairn 2011) and evolutionary theory both promote adoption of a null model that considers the quantitative genetics of the sexes as different. Only by first rejecting this model should the sexes be combined in further analyses.

Discussion

Estimates of additive genetic variance are used by plant and animal breeders as well as evolutionary biologists to answer general questions regarding (*i*) the evolutionary forces that shape additive variance and (*ii*) population responses to selection. Discrete morphs or phenotypic classes occur in some species by virtue of different patterns of gene expression. There is no *a priori* reason to assume that the patterns of variances within and covariances among traits should be the same for traits expressed in these two genetic environments. Therefore, initial estimates of variances and covariances should consider the separate morphs or classes as distinct with the potential for genotype-by-morph interactions between them. As demonstrated above, when such genotype-by-morph interactions are not explicitly considered the resulting variance in the joint distribution of breeding values will be less than the variance in breeding values for either class. This downward bias can lead to erroneous inferences regarding the magnitude of evolutionary forces and to underestimation of expected evolutionary responses in mean phenotype.

Downward biases in estimates of additive genetic variance further exacerbate problems with quantitative genetic inference in limited datasets (e.g., some wild populations). Such

datasets often do not have the sample size or informative relationships necessary to disentangle additive variance from other sources of phenotypic resemblance among relatives. These issues are compounded when the statistical model attributes less of the phenotypic variance to additive genetic effects then it should because of the biases discussed above. The extent of this problem will, in part, be dictated by the additive genetic correlation between the trait values in the morphs.

Although the arguments made above have been framed within a single trait context for simplicity and ease of interpretation, the results extend to multivariate trait relationships where the pattern of covariances among traits will often differ between classes as well. This point is particularly salient for predicting evolutionary change using the statistical relationship between breeding values of a trait and of relative fitness (Secondary Theorem of Natural Selection; Robertson 1966; Price 1970). For example, studies of sexually dimorphic traits often find differences between the sexes in among-trait covariance matrices (e.g., Preziosi and Roff 1998; Jensen et al. 2003; Fedorka et al. 2007; Steven et al. 2007; Walling et al. 2008; Roff and Fairbairn 2011) which have been shown to impact predicted responses to selection (Fedorka et al. 2007).

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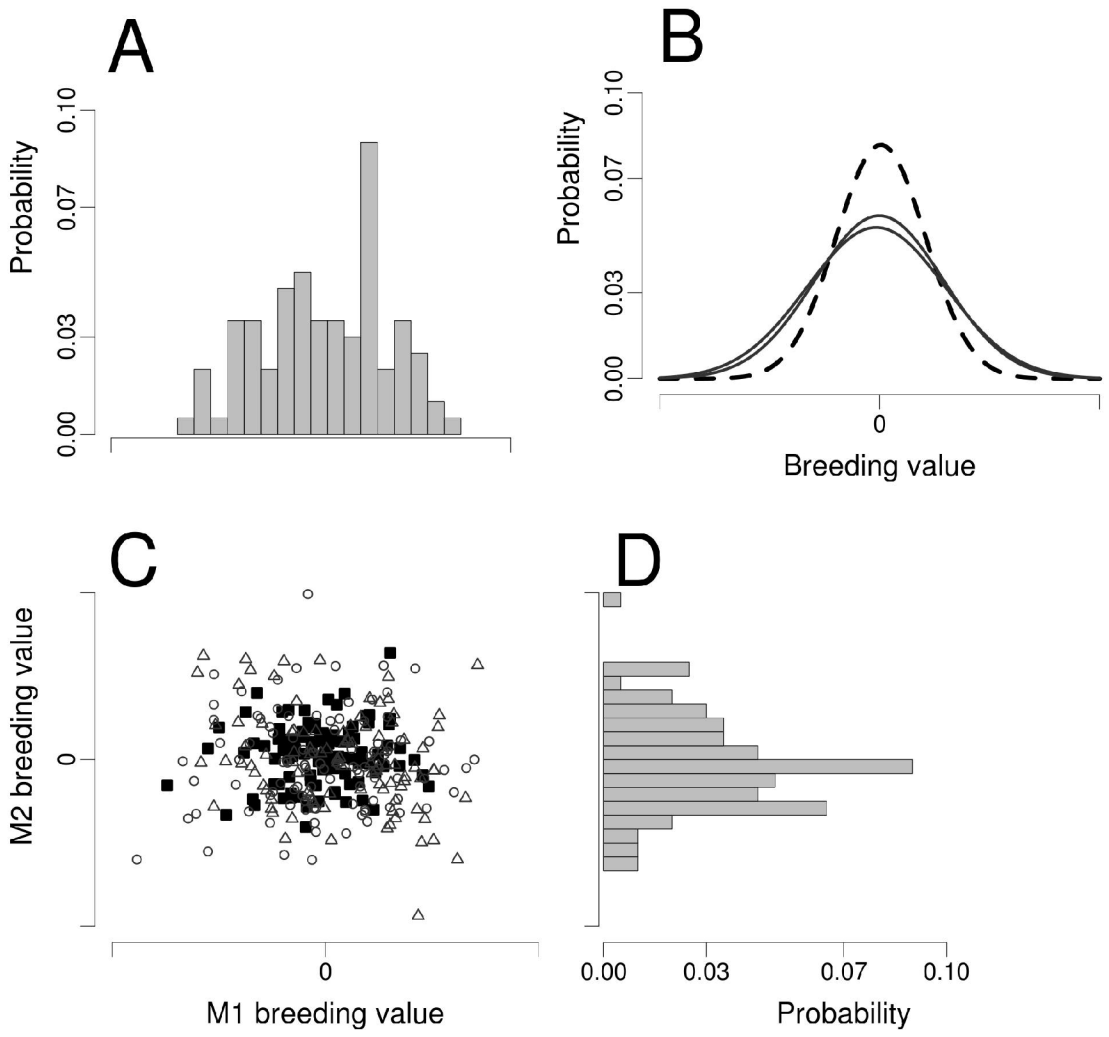

Figure 1.1. The distribution of breeding values when the variances for morphs M1 and M2 equal 50 and the between-morph correlation equals zero. A) the probability distribution of breeding values for morph M1, B) the probability distributions of the average breeding values (black dashed line) and those for of morph M1 and M2 (black solid lines) C) a scatter plot of the average breeding values (black filled squares) and the breeding values for morphs M1 (open triangles) and M2 (open circles), and D) the probability distribution of breeding values for morph M2.

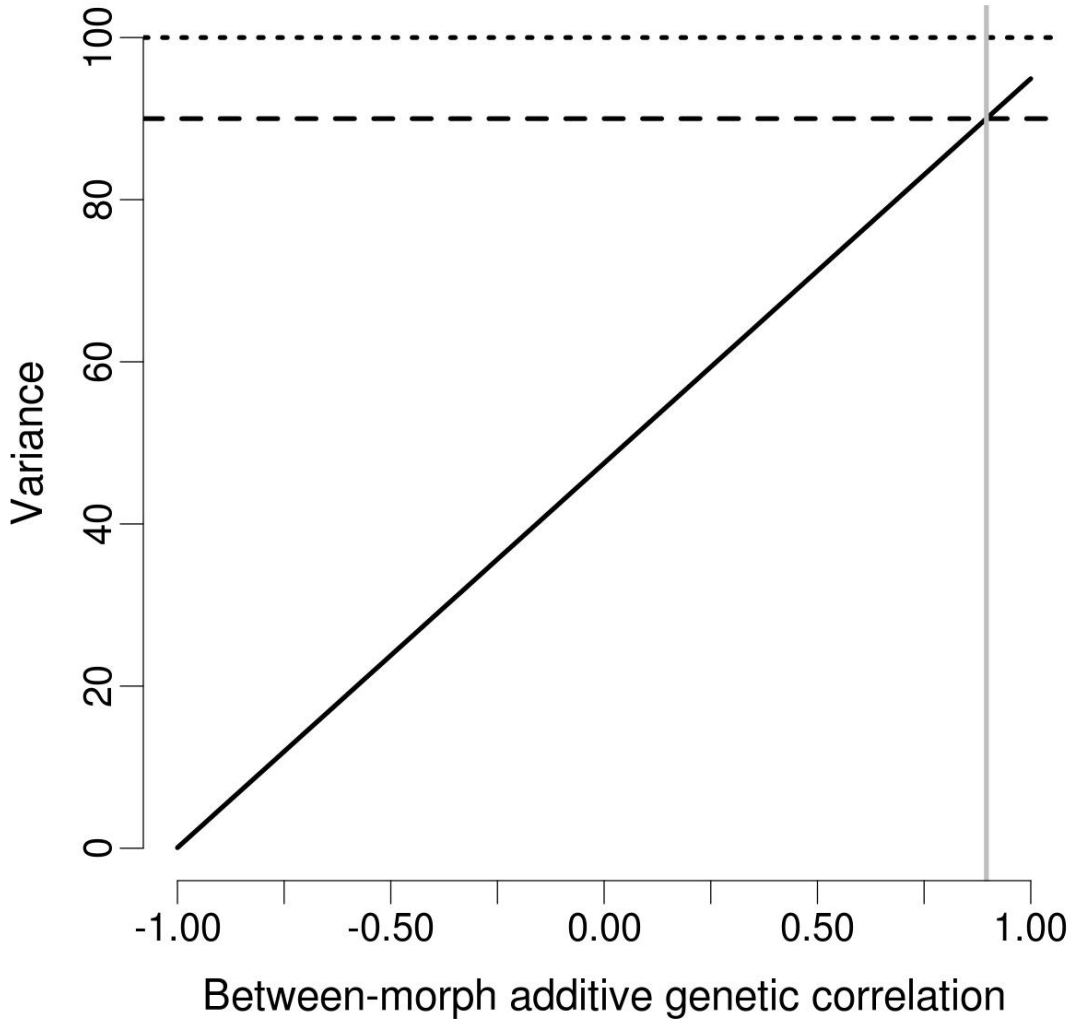

Figure 1.2. The predicted average variance (solid-black, diagonal line) when morph M1 variance equals 100 (dotted-black, horizontal line) and morph M2 variance equals 90 (dashed-black, horizontal line) using equation 1 from the text. The average variance will be less than either of the two variances whenever the between-morph additive genetic correlation is less than approximately 0.89 (vertical-grey line; see equation 2 in text). The variance on the y-axis can either be the additive genetic variance, offspring-parent covariance, sire variance, or dam variance.

CHAPTER 2

nadiv: an R package to create relatedness matrices for estimating non-additive genetic variances in animal models

Abstract

The Non-Additive InVerses (**nadiv**) R software package contains functions to create and use non-additive genetic relationship matrices in the animal model of quantitative genetics. This paper discusses the concepts relevant to non-additive genetic effects and introduces the package. **nadiv** includes functions to create the inverse of the dominance and epistatic relatedness matrices from a pedigree, which are required for estimating these genetic variances in an animal model. The paper focuses on three widely used software programs in ecology and evolutionary biology (ASReml, MCMCglmm, and WOMBAT) and how **nadiv** can be used in conjunction with each. Simple tutorials are provided in Appendix A.

Introduction

A major advance for the study of quantitative trait evolution in wild populations was precipitated by the adoption of the 'animal model', a mixed effects model with a long and proven history in the animal breeding sciences (Henderson, 1984; Lynch & Walsh, 1998; Kruuk, 2004). Using the similarity among relatives to elucidate the underlying genetic basis of phenotypic variation at the population level, the method: 1) enables researchers to control (or study in and of themselves) confounding factors due to environmental or other non-heritable sources of similarity between relatives, 2) simultaneously utilizes additional relationships beyond parent-offspring or half- and full-siblings in the estimation of genetic parameters, thereby increasing the types of populations and organisms able to be studied, and 3) is unbiased to selection within a population (Lynch & Walsh, 1998; Kruuk, 2004). Response variables in animal models may be univariate, multivariate, Gaussian or non-Gaussian. Further, solutions to the animal model may be obtained using Likelihood or Bayesian approaches (further information in Appendix A, *Relatedness matrices in the animal model* section and detailed descriptions of the animal model can be found in Lynch & Walsh, 1998; Sorensen & Gianola, 2002; Kruuk, 2004; Mrode, 2005).

The phenotypic variance of a quantitative trait can be broken down into additive genetic, non-additive genetic, and environmental sources of variation. The non-additive genetic variance can be further subdivided into dominance and epistatic variances. The additive, dominance, and epistatic genetic variances are proportional to the probability that individuals share alleles identical by descent at the same locus, for both alleles at the same locus, or for alleles at different loci, respectively. If one knows all the relationships in a population (*i.e.*, the pedigree) then the above genetic variances can be estimated in an animal model.

Non-additive genetic variances are seldom, if ever, estimated in ecological and evolutionary analyses (but see, Crnokrak & Roff, 1995; Waldmann, 2001), although the fields of

animal and plant breeding have been estimating these genetic variances for over two decades (*e.g.*, Hoeschele, 1991; Templeman & Burnside, 1991). This could be, in part, because non-additive genetic effects are assumed to be of little importance in predicting the evolutionary trajectory of moderately sized wild populations (Fisher, 1958). Also, studies of wild organisms typically have low numbers of individuals in a population, especially compared to the millions often handled in animal breeding. This is problematic, because datasets with too few individuals preclude the inclusion of too many random effects in an animal model (Kruuk, 2004) and have been shown to be problematic for the estimation of dominance variance (Misztal, 1997). However, if dominance genetic effects are present, but not included in an animal model, they can potentially bias the prediction of the additive genetic effects as well as the estimate of additive genetic variance (Lynch & Walsh, 1998; Ovaskainen *et al*. 2008; Waldmann *et al*. 2008; but see Misztal *et al*. 1997). Additionally, non-additive effects are of central interest to a number of evolutionary hypotheses, for example: dominance and epistasis are expected to contribute substantially to variation in fitness (Wright, 1929; Haldane, 1932; Fisher, 1958; Crnokrak & Roff, 1995; Merilä & Sheldon 1999), non-additive variance may determine the extent to which additive genetic variance increases after bottlenecks (Cockerham & Tachida, 1988; Goodnight, 1988; Willis & Orr, 1993; Barton & Turelli, 2004), epistasis can shape additive genetic effects and variances during processes such as mutation and selection (Gavrilets, 1993; Hermisson *et al.* 2003; Carter *et al.* 2005) which has consequences for the evolution of sex and recombination (Charlesworth, 1990), epistasis plays an integral part in speciation through the evolution of Dobzhansky-Muller incompatabilities (Crow & Kimura, 1970; Orr, 1995; Welch, 2004), the sign of genetic correlations between fitness-related traits may depend on the amount of dominance variance (Curtsinger *et al*. 1994; Roff, 1997; Merilä & Sheldon 1999), dominance potentially causes inbreeding depression or heterosis (Roff, 1997) especially in small populations of

conservation concern (Waldmann *et al*. 2008), and sex-linked dominance effects may play a role in the evolution of sexually dimorphic traits (Fairbairn & Roff, 2006).

Aside from being unable to obtain meaningful estimates of non-additive variances as a result of the overall size of a population (see "Sampling Covariances and Confidence Intervals" below), the next challenge to including dominance and epistasis in animal models is constructing the non-additive genetic relationship matrices (*i.e.*, dominance matrix **D** and the three digenic epistatic matrices: additive by additive **AA**, additive by dominance **AD**, and the dominance by dominance **DD** - where the additive genetic relationship matrix is represented by **A** and boldfaced, upper-case letters indicate a matrix). A further challenge is to obtain the inverses of these matrices, which is what is required to solve the system of equations in the animal model. Although the process of constructing the necessary matrix inverses has been worked out (*e.g.*, Hoeschele & VanRaden, 1991), only the creation of the additive inverse matrix has been incorporated into software used by most ecologists and evolutionary biologists: ASReml (Gilmour *et al*. 2009), **`MCMCglmm`** (Hadfield, 2010), and WOMBAT (Meyer, 2007). This paper gives an overview of the software package **`nadiv`** (Non-Additive InVerses), implemented in the widely used statistical program R (R Development Core Team, 2011), which can be used to construct dominance and epistatic genetic relatedness matrices and their inverses. The inverses can subsequently be used in a variety of animal model software programs for univariate or multivariate analyses of quantitative traits. Below, examples briefly demonstrate the main functions using **`nadiv`**'s simulated dataset `warcolak`.

Dominance relatedness matrix construction: `makeD()`

The relatedness in dominance genetic effects between individuals *i* and *j*, or coefficient of fraternity (Δ_{ij}), can be approximated by:

$$\Delta_{ij} = (\theta_{km}\theta_{ln} + \theta_{kn}\theta_{lm}) / 4 \quad (1)$$

(pp. 140-141 in Lynch & Walsh, 1998) where *k* and *l* represent the dam and sire of *i*, *m* and *n* the dam and sire of *j*, and θ is the additive genetic relatedness between individuals noted in the subscripts (elements in **A**). For a list of coefficients of fraternity between common types of relatives, I refer the reader to Lynch & Walsh (table 24.1 on p. 721, 1998) or tables 4 and 5 from Fairbairn & Roff (2006). Equation 1 assumes no inbreeding and ignores dominance connections through grandparents, both for the sake of computational tractability (Ovaskainen *et al.*, 2008). All pairwise Δ_{ij} in a population can be approximated using the `makeD`() function of **`nadiv`**, assuming no inbreeding. Accounting for the presence of inbreeding in the relatedness matrix adds a great deal of complexity to the estimation of dominance in an animal model (Smith & Mäki-Tanila, 1990). Despite the potential for inbreeding to alter the estimates of Δ_{ij}, de Boer & van Arendonk (1992) showed an unbiased impact on the estimates of random effects in an animal model when inbreeding is moderately low and included as a fixed effect in the model.

Similar to algorithms which construct the additive genetic relatedness matrix (or its inverse), `makeD()` requires a pedigree as the main input. The pedigree must contain three columns, ordered ID, Dam, Sire, and the rows are ordered such that all parents occur in the ID column before their offspring (if not, see `fixPedigree()` in **`pedantics`**; Morrissey & Wilson, 2010). All unknown parents (*e.g.*, the base population), should be indicated with "NA", "0", or "*":

```
id dam sire
 1  NA   NA
 2  NA   NA
 3   2    1
 4  NA    1
```

The output of `makeD()` is a list of objects, from which the inverse of the dominance relatedness matrix can be extracted in two forms, depending upon the program in which it is intended to be used. First, the output `Dinv` is the inverse of the sparse matrix **D** and can be included in an animal model using **`MCMCglmm`**, as demonstrated below (see the MCMCglmm tutorial in the Appendix A for more details)**:**

```
> warcolak.ped <- warcolak[, c(1:3)]
> Dinv <- makeD(warcolak.ped)$Dinv
> warcolak$IDD <- warcolak$ID
> model.MCMC <- MCMCglmm(phenotype ~ 1,
+   random = ~ID + IDD, data = warcolak,
+   ginverse=list(ID = Ainv, IDD = Dinv))
```

The object `listDinv` is the second form by which the inverse of the dominance relatedness matrix is returned from `makeD()`. It is formatted so as to facilitate inclusion in either ASReml or the ASReml-R package. This object is in the form of ASReml's general inverse list (also referred to as a g-inverse or giv; Gilmour *et al*., 2009), which contains the non-zero elements of the lower triangle of a sparse matrix, in row order. This can be used to include dominance as a random effect in the `asreml()` function in R (more details in Appendix A)**:**

```
> ginvD <- makeD(warcolak.ped)$listDinv
> model.asr <- asreml(phenotype ~ 1,
+     random = ~ped(ID) + giv(IDD), data = warcolak,
+     ginverse = list(ID = ginvA, IDD = ginvD))
```

The `listDinv` object can also be written to a text file for inclusion in analyses using the standalone ASReml program (Appendix A). Further, this format is very similar to what WOMBAT requires, however, the first two columns must instead be ordered "column" and then "row" (the opposite order of `listDinv`) and the log determinant of **D** must also be provided. The first two columns of the list can easily be switched in R before saving the inverse to a file. The log determinant is returned as the object `logDet` in `makeD()` (Appendix A).

Dominance relatedness matrix construction: `makeDsim()`

Ovaskainen *et al.* (2008) elegantly explain how eqn. 1 yields an approximation of Δ_{ij} and demonstrate a more accurate method, especially for complex pedigrees, to obtain estimates of **D** through iteration. Briefly, their method explicitly traces alleles through a pedigree, thereby incorporating effects of inbreeding and alternative routes by which alleles can be shared (two processes left out of eqn. 1). By repeatedly implementing this method, an estimate of the coefficient of fraternity (*i.e.*, the probability two individuals share both alleles identical by descent) is produced and standard errors (diminishing in magnitude with an increase in number of iterations) for the estimates in the **D** matrix can be calculated. The difference between the coefficients of fraternity derived from this method versus eqn. 1 are explained in Ovaskainen *et al*. (2008, particularly Figure 2.1C). The function `makeDsim()` implements this method as described in the appendix to Ovaskainen *et al*. R code, such as `makeDsim(warcolak.ped, N = 10000, calcSE = TRUE)`, will construct the **D** inverse in matrix and list formats for use in an animal model. The resulting output can then be supplied to **`MCMCglmm`**, **`asreml`**, ASReml, or WOMBAT as described above and indicated in Appendix A. The argument `N =` in `makeDsim` supplies the number of iterations and thereby influences the standard error of each entry in **D**.

Epistatic relatedness matrix construction

In addition to the dominance matrix, the three digenic epistatic relationship matrices (**AA**, **AD**, and **DD**) can be constructed using the functions `makeAA()` and `makeDomEpi()` (for example coefficients of relatedness due to digenic epistasis, see p. 145 of Lynch & Walsh, 1998). The latter of these two functions can construct and invert **D**, **AD**, and **DD**, all at once to save

computing time. The results returned by both of these functions can be passed to **MCMCglmm**, **asreml**, ASReml, and WOMBAT in the exact same way as previously discussed for `makeD()`.

Sampling Covariances and Confidence Intervals

One difficulty when estimating non-additive genetic variances is that the covariance between relatives due to non-additive genetic effects is highly confounded with other sources of similarities between relatives (*e.g.*, full siblings also display phenotypic similarities due to shared additive, maternal, and environmental effects). The sampling (co) variances for all random effects in an animal model can be informative for determining the extent to which random effects are confounded. These (co) variances of the variance estimates are derived from the 'Average Information' matrix in animal models that utilize the Average Information algorithm (Gilmour *et al.*, 1995) to obtain the Residual Maximum Likelihood (REML) parameter estimates. The function `aiFun()` extracts the sampling (co) variances from the Average Information matrix in **asreml**, allowing researchers to evaluate the precision and extent to which variance components are correlated with one another:

```
> aiFun(model = model.asr, Dimnames = c("Va", "Vd", "Ve"))
```

Further, Appendix A demonstrates how a vector of these (co) variances can be obtained from the standalone ASReml or WOMBAT programs and used in R. The sampling (co) variances are organized into a matrix with the sampling (co) variances of each variance component as the diagonal and below-diagonal elements and correlations as the above-diagonal elements. **MCMCglmm** uses a Bayesian approach to fitting models, not REML, but similar evaluations can be obtained by inspecting the posterior distributions and autocorrelation for variance components (Appendix A).

Determining the extent to which variance components are confounded with one another can also be achieved after an **asreml** analysis by examining the profile likelihood surface of each component using `proLik()`:

```
> profile.add <- proLik(model.asr, component =
"ped(ID)!ped")
```

A profile likelihood is a representation of the model log likelihood when projected onto the parameter space for one particular parameter (or subset of parameters; Meyer, 2008). The change in the model log likelihood (calculated as a likelihood ratio test statistic) can then be estimated along a range of values for a particular parameter, producing a profile likelihood surface. When graphically depicted, using `plot.proLik(profile.add)`, the profile likelihood surface of each variance component in an animal model (Figure 2.1) can be visually inspected to yield insights into the ability of the pedigree structure to produce precise and unconfounded variance component estimates (Meyer, 2008). An additional utility of profile likelihoods is that they can be used to determine confidence intervals for the variance components estimated in a mixed model. This is often a more appropriate method than using the standard errors (or sampling variances from the Average Information matrix; Meyer, 2008). Approximate 1- α upper and lower confidence limits can be obtained when using the `proLik()` function, for example by: `profile.add$UCL` and `profile.add$LCL`, respectively. The accuracy of the approximated confidence limits can be set with the `threshold` argument.

Additional functions

A few other functions are included in **nadiv** and may be useful to others working with pedigrees and sparse matrices (matrices containing mostly zeroes) in R. Notably, `makeA()` constructs the additive genetic relatedness matrix. `sm2list()` converts a sparse matrix (see the **Matrix**

package) to a list consisting of three columns ("row", "column", and value – the last being labeled by the user) that contain all non-zero, lower triangle elements of a matrix in row order. Finally, double first cousins are an informative relationship for estimating many types of genetic variance (*e.g.*, Fairbairn & Roff, 2006). The function `findDFC()` determines the number of unique pairs of double first cousins present in a pedigree.

Space, Speed, and Saving

Constructing the inverse of **D** can require a large amount of computer memory and time for large, complex pedigrees. Although some modified methods to address these constraints exist (*e.g.*, Hoeschele & vanRaden, 1991; Schaeffer, 2003), the functions contained in **`nadiv`** can be executed in a timely manner for the size and complexity of pedigrees usually studied in ecology and evolutionary biology (< 10,000 individuals), even on personal computers. Additionally, automatic parallelization of the processing is available for many of the functions in **`nadiv`** (the default is always to use a single processor), which can often result in dramatic time savings (Appendix A). Not all computer architectures will allow users to take advantage of this capability in R, so I refer those interested to the package documentation of **`nadiv`** for more consideration. Because creating **D** every session is time prohibitive for large populations, it is advisable to save non-additive inverse matrices to a hard drive. The R functions `save()` and `load()` are useful to store and retrieve, respectively, because they preserve the R attributes that are required by the animal model programs in R (*i.e.*, **`MCMCglmm`** and **`asreml`**).

More information about the functions in **`nadiv`** can be obtained from the package documentation (see the Comprehensive R Archive Network website: http://cran.r-project.org/web/packages/nadiv/index.html). For a more thorough treatment of how to use the functions in **`nadiv`**, please see Appendix A tutorials.

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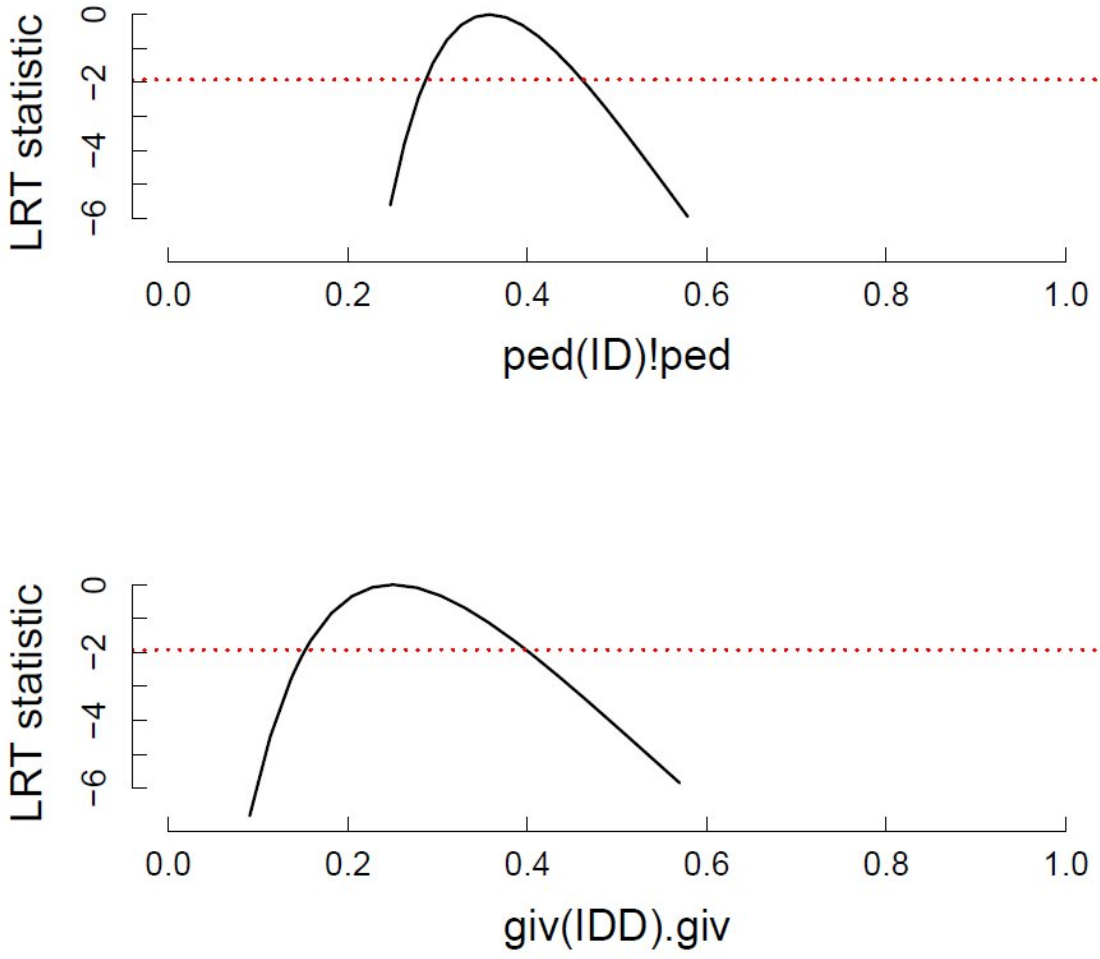

Figure 2.1. Log profile likelihoods for the additive (top) and dominance (bottom) genetic variance components estimated from the `warcolak` dataset. Plots were generated using the **`nadiv`** function `proLik()` to obtain each profile from an animal model fitted using the software ASReml-R and subsequently graphed using `plot.proLik()`. The 95% confidence interval limits for each variance estimate are indicated where the horizontal dashed line (corresponding to the log Likelihood Ratio Test statistic = $-1/2\chi^2_{0.05,1}$) intersects the profile. X-axis labels correspond to the ASReml-R model terms.

CHAPTER 3

Sex chromosome linked genetic variation affects estimates of additive genetic (co)variation.

Abstract

Evolutionary theory predicts that genetic variation for traits undergoing sexually antagonistic selection will accumulate on the sex chromosomes, reducing the magnitude of intralocus sexual conflict. The inheritance pattern of sex chromosomes creates major differences in the contribution of additive variance to phenotypic resemblance between relatives for the sex chromosomes versus the autosomes and yet very few empirical studies explicitly separate these two sources of variance. We use simulations to show how failure to include sex-linked additive (co)variances in analytical models affects the accuracy and precision of additive genetic (co)variance estimates. We compare results from simple (half-sib) to more complex (double first cousin) breeding designs and between two analytical methods (observed variance components and animal models). Our results show that when sex-linked variance is included in an analytical model, only the animal model analyses of the double first cousin pedigree yield both unbiased and precise estimates of sex-specific additive genetic (co)variances. When sex-linked variance was not included, estimates of sex-specific autosomal additive variances were biased (often >100%). Further, analytical models that did not estimate sex-linked (co)variances yielded biased estimates of the sex-linked between-sex additive genetic correlations. We interpret our results with respect to how they impact our identification of signatures of intralocus sexual conflict.

Introduction

Partitioning phenotypic variation into the constituent genetic and non-genetic sources of variation is the central paradigm in evolutionary quantitative genetics (Roff 2006). The component of phenotypic variation most studied to date is additive genetic variance, which summarizes the variance among individuals in the sum of each individual's average genetic effects for a trait (i.e., the breeding values; Falconer 1989). Estimates of additive genetic variance enable us to address two classes of questions in evolutionary biology (Turelli 1988; Houle 1992). The first class is concerned with predicting the evolutionary responses to artificial selection (reviewed in Hill and Caballero 1992) or natural selection (Kruuk et al. 2008) using the breeders' equation (Lush 1937; Lande 1979) or the "Secondary Theorem of Natural Selection" (Robertson 1966, 1968; Price 1970). The second class of questions is concerned with identifying the forces that shape additive genetic variance in a population (Turelli 1988). They specifically address how selection, mutation and drift change additive genetic variance (e.g., Reeve 2000; Jones 2003), how trait types differ in their relative amounts of additive variance (e.g., Mousseau and Roff, 1987; Roff and Mousseau 1987; Houle 1992), and the consequences of shared genetic variation for the correlated evolution of sets of traits (Lande 1979, 1980a), leading to quantitative genetic predictions regarding the divergence among populations (Steppan et al. 2002).

Estimates of additive genetic variance typically only focus on autosomal models of additive genetic variance; neglecting potential contributions from the sex chromosomes in species with chromosomal sex-determination. Most standard quantitative genetic textbooks (e.g., Falconer 1989; Roff 1997; Lynch and Walsh 1998) restrict their focus to the autosomes when discussing the covariance between relatives due to the expected contribution of additive genetic effects. Covariance between relatives due to additive genetic effects on the sex chromosomes is treated, at best, as an extra or complicating detail. Therefore, sex chromosome linked genetic

effects are treated as special cases and not incorporated into general methodologies for estimating additive genetic variance from laboratory breeding designs and wild populations. This is unfortunate, because evolutionary biology is replete with theory predicting genes to accumulate on the sex chromosomes, for example when they: facilitate the evolution of sexual dimorphisms or the resolution of intralocus sexual conflict (Fisher 1958; Charlesworth and Charlesworth 1980; Rice 1984; Fairbairn and Roff 2006; but see Charlesworth et al. 1987; Fry 2010), are involved in the evolution of female preferences and attractive male traits (Lindholm and Breden 2002; Kirkpatrick and Hall 2004), or play a role in prezygotic isolation leading to species differences and reinforcement of mating preferences (Charlesworth et al. 1987; Coyne and Orr 1989; Ritchie and Phillips 1998; Hall and Kirkpatrick 2006). Despite a large body of theory predicting the accumulation of additive genetic variance on the sex chromosomes, the impact of sex-chromosomal additive genetic variance on sex-specific additive variances and between-sex additive genetic correlations is currently unknown. In particular, sex-linked, sexually antagonistic genetic variance can play a major role in structuring between-sex additive genetic correlations and quantifying this influence may reveal key insights into the processes facilitating a resolution of intralocus sexual conflict.

For traits that differ between the sexes (e.g., sexual dimorphisms in secondary sexual characteristics), alleles with opposing effects in females and males evolve in response to sexually antagonistic selection and contribute disproportionately to genetic variance in fitness (Charlesworth and Hughes 1999; Connallon and Clark 2012, 2013), a pattern seen in many empirical studies of plants and animals identifying sexual antagonism (e.g., Chippindale et al. 2001; Fedorka and Mousseau 2004; Brommer et al. 2007; Foerster et al. 2007; Cox and Calsbeek 2010; Innocenti and Morrow 2010; Delph et al. 2011; Lewis et al. 2011; Mokkonen et al. 2011). This leads to a negative between-sex genetic correlation for fitness, which is considered a

signature of intralocus sexual conflict (Chippindale et al. 2001). However, this does not necessarily require a negative autosomal between-sex additive genetic correlation. Indeed, Rice's (1984) model prediction and subsequent empirical work (Chippindale et al. 2001; Gibson et al. 2002; Pischedda and Chippindale 2006) point to a sex chromosomal location of the intralocus sexual conflict that would result in a negative sex-linked between-sex additive genetic correlation for fitness. However, to date many attempts to quantify between-sex additive genetic correlations have not explicitly partitioned this correlation into separate autosomal and sex-chromosomal contributions (e.g., Calsbeek and Sinervo 2004; Fedorka and Mousseau 2004; Brommer et al. 2007; Foerster et al. 2007; Delcourt et al. 2009).

Explicitly accounting for the contribution of sex-linked (co)variance to genome-wide additive genetic (co)variances is necessary because the inheritance pattern for sex chromosomes produces a different expected covariance in additive genetic effects between many types of relatives, as compared to that of the autosomes (e.g., tables 4 and 5 in Fairbairn and Roff 2006). As a simple example, consider male and female full-siblings in a system of XX/XY chromosomal sex-determination (the same will apply in ZZ/ZW, but the results will be reversed between the sexes). The contribution of genome-wide additive genetic variance to the expected phenotypic covariance between full-brothers is ½ the autosomal plus the Y-linked and ½ the X-linked additive genetic variances. For full-sisters the expected covariance is due to ½ the autosomal plus ¾ the X-linked additive variances. Consequently, the genome-wide additive variance is greater than the quantity estimated from either full-brothers or full-sisters if only autosomal contributions are assumed. For example, an assumption of an autosomal basis to all phenotypic resemblance between full-sisters will only account for ½, and not ¾, of the X-linked variance. Although genetic variation on the sex-specific sex chromosome (Y or W), may be important in some species (e.g., Rice 1996; Charlesworth and Charlesworth 2000; Jobling and Tyler-Smith 2000;

Lindholm and Breden 2002; Kirkpatrick and Hall 2004; Otto et al. 2011), across taxa with chromosomal sex determination systems the Y and W chromosomes are often absent or degenerate. In the present analyses we restrict our focus to variance on the sex chromosome that both sexes share (X or Z) and only consider X- or Z-linkage when using the term "sex-linked" (note, the effect of dosage compensation is considered elsewhere).

The difference in copy number for the sex chromosome that both sexes share, causes the variance in sex-linked additive genetic effects to differ between the sexes by a factor of two (Bulmer 1980; Fernando and Grossman 1990; Lynch and Walsh 1998). This last point is a long-standing (Bohidar 1964; James 1973) but underappreciated relationship which further alters the expected contribution of additive genetic effects to the phenotypic covariance between relatives beyond that discussed above. The copy number difference between the sexes also reduces the maximum range of the between-sex additive genetic correlation on the sex chromosomes. This reduction is because sex-linked additive allelic effects make different contributions to the phenotypic variance in males or females. Whereas the maximum range for correlated genetic variation on the autosomes is -1 to +1, the expectation for correlated variance on the shared sex chromosome (X or Z) is only from $-1/\sqrt{2}$ ($\approx$-0.71) to $+1/\sqrt{2}$ ($\approx$0.71) (see *Animal models* in Methods below).

The potential effects of sex-linked additive variance on estimates of additive genetic (co)variation made under assumptions of autosomal inheritance should not be dismissed as simply a methodological concern in quantitative genetic analyses. The departures from the autosomal model caused by sex-linkage are, in and of themselves, important properties to consider when determining the location of sexually antagonistic genetic variation or elucidating the role of genetic variation in population responses to selection. Further, many commonly studied organisms (e.g., *Drosophila* and *Silene*) have relatively large sex chromosomes (Cowley

et al. 1986; Grabowska-Joachimiak and Joachimiak 2002), which might be associated with high levels of sex-linked genetic variance (Fairbairn and Roff 2006). Table 3.1 contains a survey of the (few) studies to our knowledge which have sought to directly quantify the relative amounts of additive genetic variation on autosomes versus the sex chromosomes. These often report appreciable amounts of sex-linked variance: across studies, the means (medians) of the proportion of genome-wide additive genetic variance that can be attributed to sex-linked additive genetic variance range from 2.7% (0%) to 50.7% (41.6%) (Table 3.1).

To a first approximation, the effects of un-modeled sex-linked variance on additive genetic variance estimates have been previously considered. It has been recognized that sex-linked variance causes heritability estimates derived from different methods to differ (e.g., sire on daughter versus sire on son estimates where sires and sons do not share an X chromosome, but sires and daughters do) and, therefore, the importance of considering sex-linkage has been assessed via these comparisons (Sheridan et al. 1968; Cowley et al. 1986; Cowley and Atchley 1988; and reviewed in Beilharz 1963). More recent analyses have incorporated estimates of sex-linked (co)variances to explicitly account for the contribution of these (co)variances to genome-wide additive genetic (co)variances (Chenoweth and Blows 2003; Mezey and Houle 2005; Chenoweth et al. 2008). However, to date, the general effect of the existence of sex-linked variance on estimates of additive genetic variance has not been explored.

Here we ask the question, do models that assume a fully autosomal basis for covariance between relatives yield biased estimates of sex-specific additive genetic variances and between-sex additive genetic correlations when sex-linked additive genetic variation is present? We begin by presenting the statistical genetic theory underlying sex-linked additive genetic variance with a particular focus on the common biological assumptions made when implementing mixed effect statistical models. We then undertake simulations to construct phenotypes with a known

quantitative genetic architecture (consisting of autosomal additive, sex-linked additive, and residual variances) for subsequent analysis with two widely used variance partitioning methods. We find that sex-specific additive genetic variance estimates can be biased when sex-linked additive variance is not explicitly considered in quantitative genetic analyses. The magnitude of the bias will depend on both the amount of sex-linked variance and the pedigree structure of each analysis. We also find that estimates of the between-sex genetic correlation will be biased when models only utilize autosomal expectations.

Methods

The model of sex-linked additive genetic variation

The statistical theory behind the estimation of additive genetic variation on the sex chromosomes has a long history (Hogben 1932; Bohidar 1964; James 1973; Fernando and Grossman 1990), as does variation in the interpretation of the underlying assumptions of this theory (Becker 1967; Sheridan et al. 1968; Dickerson 1969; Kempthorne 1969; Pirchner 1969; James 1973; Grossman and Eisen 1989; Kent et al. 2005a). To clarify the latter, we briefly present the underlying theory and assumptions germane to analyzing sex-linked variation.

The basic model of sex chromosomal additive variance assumes that additive allelic effects are equal in the two sexes for all loci (i.e., no genotype-by-sex interaction on the sex chromosomes), and that the population is in gametic equilibrium (Fernando and Grossman 1990). Therefore, the model assumes no mechanism of global sex chromosome dosage compensation [Muller 1931; Lucchesi 1978; we note that dosage compensation will not qualitatively change the results and conclusions below (Wolak et al., unpublished manuscript)]. This model also assumes that the un-shared sex chromosome (i.e., the Y or W) is devoid of any alleles affecting the trait of interest (Fernando and Grossman 1990), although genetic variation on the Y or W chromosome

can be estimated independently (e.g., Postma et al. 2011). A final assumption is that alleles influencing the trait are not exchanged between the two different sex chromosomes (i.e., there is no recombination between the X and Y or Z and W during meiosis), such as observed in the pseudoautosomal regions of sex chromosomes (Otto 2011).

Under the assumptions stated above, the sex-linked additive genetic value at a locus for a trait in the homogametic sex (g_{CC}) and heterogametic sex (g_C) is:

$$g_{CC}=\alpha_{\mathbf{p\text{-}hom}}+\alpha_{\mathbf{p\text{-}het}} \quad (1)$$

$$g_C=\alpha_{\mathbf{p\text{-}hom}} \quad (2)$$

(Fernando and Grossman 1990) where α is defined as the average additive genetic effect of an allele at a sex-linked locus. The subscripts "p-hom" and "p-het" distinguish between the alleles contributed by the homogametic and heterogametic parent, respectively. From equations 1 and 2, the relationship between genotypic values *s*, or the sum of genetic values for all sex-linked loci, of the two sexes is $s_{\mathbf{hom}}=2s_{\mathbf{het}}$ ($s_{hom}=\sum g_{CC,i}$ and $s_{het}=\sum g_{C,i}$). Therefore, the mean genotypic value of the homogametic sex individuals is twice that of the heterogametic sex individuals in a population. For a single locus, the variance in allelic effects is defined as $Var(\alpha)=Var(\alpha_{\mathbf{p\text{-}hom}})=Var(\alpha_{\mathbf{p\text{-}het}})$. Following from equations 1 and 2, the (co)variances in additive genetic values summed across all sex-linked loci in the homogametic and heterogametic sexes are:

$$Var(s_{\mathbf{hom}})=2\sum Var(\alpha) \quad (3)$$

$$Var(s_{\mathbf{het}})=\sum Var(\alpha) \quad (4)$$

$$Cov(s_{\mathbf{hom}},s_{\mathbf{het}})=\sum Cov(\alpha,\alpha_{\mathbf{p\text{-}het}})+Cov(\alpha,\alpha_{\mathbf{p\text{-}hom}})=2\sum Var(\alpha) \quad (5)$$

(Fernando and Grossman 1990). Note that we have left out the dominance contributions to genetic variance for simplicity (see ch. 24 Lynch and Walsh 1998).

Under the above assumptions, the expected sex-linked additive genetic variance for non-inbred homogametic sex individuals in a population ($\sigma^2_{s\text{-}hom}$) is twice that of the non-inbred heterogametic sex individuals in the population ($\sigma^2_{s\text{-}het}$). Because $\sigma^2_{s\text{-}hom}$ can be expressed as a linear function of $\sigma^2_{s\text{-}het}$ (i.e., $\sigma^2_{s\text{-}hom}=2\sigma^2_{s\text{-}het}$), the expectations for additive genetic allelic effects of the heterogametic sex can be expressed in terms of $\sigma^2_{s\text{-}hom}$ (Bulmer 1980). Similarly, the covariance between sexes can be expressed as a linear function of $\sigma^2_{s\text{-}het}$ (see *Animal models* below for more details). This is done to simplify analytical models used to estimate sex-linked additive genetic.

Simulation approach

We used an individual-based variance components simulation approach (ch. 4 Roff 2010) to uncover the bias in estimates of additive genetic (co)variances arising from the failure to include sex-linked additive variance in the underlying models. We refer to the two sexes as either the homogametic (e.g., XX or ZZ) or heterogametic (e.g., XO or ZW) sex. Homogametic and heterogametic phenotypes were modeled as:

$$y_{hom}=\mu+a_{hom}+s_{hom}+e \quad (6)$$

$$y_{het}=\mu+a_{het}+s_{het}+e \quad (7)$$

where an individual phenotype y is a linear combination of a population mean μ (=0) and random effects representing sex-specific autosomal additive genetic effects (a) sex-specific sex-linked additive genetic effects (s) and residual effects (e). The genetic and residual effects were

simulated as Gaussian distributions of random effects following the methods in Van Vleck (1994; details in Appendix B1).

Residual and autosomal additive genetic variances for males and females were constant in all simulations ($\sigma^2_{e\text{-}hom}=\sigma^2_{e\text{-}het}=\sigma^2_{a\text{-}hom}=\sigma^2_{a\text{-}het}=50$) and the autosomal between-sex additive genetic correlation was always one ($\sigma_{a\text{-}hom,het}=50$). We chose seven values for the sex-linked additive genetic variance, expressed as the proportion of phenotypic variance attributed to sex-linked additive variance in the homogametic sex ($h_s^2=\sigma^2_{s\text{-}hom}/\sigma^2_P$). Note that when the sex-linked variance in the heterogametic sex is expressed as a function of the sex-linked variance in the homogametic sex (see above), the phenotypic variances of the heterogametic sex is less than the homogametic sex. The range of h_s^2 values simulated (Table 3.2) encompasses the range of sex-linked additive variance estimates reported to date (Table 3.1). The range of h_s^2 values was simulated for each of three different values of the sex-linked between-sex additive genetic correlations, which span the entire parameter space ($1/\sqrt{2}\approx0.71$, 0, and $-1/\sqrt{2}\approx-0.71$).

The change in additive (i.e. autosomal plus sex-linked) genetic variance and between-sex correlation reflects the influence of sex-linked additive variance on each estimate. The high genomic heritability (autosomal+sex-linked; range: 0.5-0.71) for simulated traits was chosen to avoid model convergence difficulties when employing the restricted maximum likelihood (REML) techniques in the analytical models described below (see A*nalytical models*). This typically occurs when too many parameters occupy the area close to the boundaries of parameter space (i.e., variance components near zero when they are restricted to be positive).

Pedigrees considered

The extent to which autosomal expectations of covariances between relatives do not properly model sex-linked additive covariances depends on the types of relationships contained in the

pedigree of a particular study design or population. To determine the impact of pedigree structure on the bias introduced by sex-linked additive variance, we chose to focus on two laboratory breeding designs in our simulations: the paternal half-sib and the double first cousin design (Fairbairn and Roff 2006). The half-sib (HS) pedigree was composed of 60 sires, each mated to 10 dams, resulting in 10 offspring per dam-family (five of each sex). Although the pedigree included sires and dams, phenotypes were only simulated for the offspring generation. We did this to mimic half-sib laboratory breeding designs and analyses which typically only utilize phenotypic information from the offspring generation. Thus, the HS pedigree consisted of 6,660 individuals, but phenotypic information was only incorporated for 6,000 individuals (50.5% individuals of the homogametic sex).

The double first cousin (DFC) pedigree consisted of 50 breeding units of the form described in Fairbairn and Roff (2006) and depicted in figure 3.1. This breeding design creates full- and half-sib relationships among offspring within a "mating group", first and double first cousins between offspring among "mating groups", and grandparent-grandoffspring, parent-offspring, and uncle/aunt-nephew/niece relationships between generations (Figure 3.1). A single unit of this DFC design yields 120 individuals from three generations. Since the original specification of the design includes phenotypic records on all individuals (Fairbairn and Roff 2006), we simulated phenotypes for all individuals in the pedigree. Both pedigree size and the number of individuals with phenotypic records equal 6,000 in our simulations of the DFC design (53.3% individuals of the homogametic sex).

While extremely common in use, the HS design contains fewer types of relatives in general as well as fewer types of relatives informative for disentangling sex-linked sources of additive genetic variance from other contributions to phenotypic variance. The DFC modifies the paternal half-sib HS design to create sets of single and double first cousin relationships between

families. When all three generations in the DFC design are included, the great variety of relationships increases the power to separate sex-linked additive variance from other contributions to phenotypic variance in laboratory breeding designs (Fairbairn and Roff 2006; Meyer 2008). We focus on the HS and DFC designs, because they represent pedigree structures that contain either very few or many different types of pair-wise relationships between individuals, respectively. We expect the variety of pair-wise relationships for most other pedigrees (e.g., wild population pedigrees) to fall somewhere in between the HS and DFC designs.

Analytical models

In addition to comparing two pedigree structures, we contrast two analytical models. For half-sib experiments in general, empiricists can analyze phenotypic data using either (1) a nested mixed effects linear model (e.g., sire and dam variance components model; Cowley et al. 1986) or (2) the mixed effects 'animal model' (Henderson 1973; Lynch and Walsh 1998; Kruuk 2004). Therefore, we investigated both analytical approaches with the HS simulations. However, for multigenerational pedigrees, such as the DFC design, only the animal model is applicable. We adopted a bivariate approach, which models females and males as separate "traits" (Mrode 2005), thus enabling us to obtain sex-specific genetic variance and between-sex genetic correlation estimates. Our approach for both analytical models was to analyze the simulated data by first modeling both autosomal and sex-linked sources of additive genetic variance. These "informed" models assess our ability to obtain unbiased estimates of additive genetic variance from the analytical models as well as the two pedigree designs. We then conducted a "naïve" model, where only autosomal patterns of inheritance were included in the statistical model. Given that all data contained sex-linked variance (Table 3.2), we used the naïve model to elucidate the bias

present in estimates of additive genetic (co)variances for females and males when implementing the analytical mixed effects models and to highlight the difference in bias between the HS and DFC pedigree designs.

Nested mixed effects model of observed variance components

We used a bivariate nested mixed effects linear model (e.g., Cowley et al. 1986; Cowley and Atchley 1988; Chenoweth and Blows 2003) to decompose phenotypic variance in the HS breeding design into the observed sire (σ^2_{sire}), dam (σ^2_D), and within-family (σ^2_W) variances for each sex and the between-sex sire, dam, and within-family covariances. Observed variance component estimates from the nested linear model were obtained using restricted maximum likelihood (REML) in R (v2.15.0, R Development Core Team 2012) using the **asreml** package (v3.0 Butler et al. 2009). The observed components of variation are expressed in terms of the genetic (or causal) variance components under an assumed genetic model using an appropriate design matrix (following Chenoweth et al. 2008; see Appendix B1). A bivariate model, where the observed variance components are estimated for each sex, is necessary because the contribution of sex-linked variance to the observed variance components differs between the homogametic and heterogametic sexes. This model produces estimates of the sex-linked variance separately for the homogametic ($V_{s\text{-}hom}$) and heterogametic ($V_{s\text{-}het}$) sexes. This is contrary to the animal model which estimates the heterogametic sex-linked variance as a linear function of the homogametic sex-linked variance (see below).

Animal models

We used the mixed effects animal model (Henderson 1973; Lynch and Walsh 1998; Kruuk 2004), which directly estimates genetic and non-genetic variance components to analyze data for both

the HS and DFC designs. As noted in the *Pedigrees considered* section, animal model analyses were conducted on phenotypic data from all individuals in the DFC design, but only the offspring generation of the HS design. However, the full pedigree information was used from each design.

We used the "makeS()" function from the **nadiv** package (v2.11) in R to construct the inverse of the sex-linked additive genetic relatedness matrix according to the algorithm developed by Fernando and Grossman (1990). This matrix was used with the **asreml** package for R (v3.0 Butler 2009) to solve the animal model using restricted maximum likelihood. We used a bivariate animal model to estimate the autosomal additive genetic, sex-linked additive genetic, and residual variances in each sex separately. Detailed statistical descriptions of both the naïve and the informed animal models can be found in the Appendix B1.

Sex-linked variance of the heterogametic sex ($\sigma^2_{s\text{-}het}$) was estimated as a linear function of the homogametic sex-linked variance ($\sigma^2_{s\text{-}hom}$). For the heterogametic sex (only one copy of the shared sex chromosome X or Z), an animal model estimates the sex-linked additive genetic variance for a hypothetical set of relatives in the base population expressed as a linear function of individuals that are diploid for the shared sex chromosome. We will represent the variance in these hypothetical homogametic base population individuals of the heterogametic sex as $\sigma^2_{s\text{-}het(hom)}$ (therefore, $\sigma^2_{s\text{-}hom}=\sigma^2_{s\text{-}het(hom)}=2\sigma^2_{s\text{-}het}$). The covariance between the homogametic sex and the heterogametic sex, expressed as a linear function of the homogametic sex, is $\sigma_{s\text{-}hom,het(hom)}$. Thus, the maximum (minimum) sex-linked between-sex additive genetic correlation reported by animal model software is one (minus one) when $\sigma^2_{s\text{-}hom}=\sigma^2_{s\text{-}het(hom)}=\sigma_{s\text{-}hom,het(hom)}$. When this correlation $[\sigma_{s\text{-}hom,het(hom)}/\sqrt{(\sigma^2_{s\text{-}hom}\sigma^2_{s\text{-}het(hom)})}]$ is instead expressed as the raw (co)variances in sex-linked additive genetic effects, in other words when the sex-linked variance in the heterogametic sex is not expressed as the variance in hypothetical diploid individuals, but rather as that of the individuals of the heterogametic sex and likewise for the sex-linked between-sex covariance [i.e.,

$0.5\sigma_{s\text{-}hom,het(hom)}/\sqrt{(\sigma^2_{s\text{-}hom}0.5\sigma^2_{s\text{-}het(hom)})}=\sigma_{s\text{-}hom,het}/\sqrt{(\sigma^2_{s\text{-}hom}\sigma^2_{s\text{-}het})}$], the maximum (minimum) sex-linked between-sex correlation is $1/\sqrt{2}\approx 0.71$ ($-1/\sqrt{2}\approx -0.71$). We use V_i and COV_i to denote REML estimated parameter variances and covariances of the *i*th effect, as opposed to the true values in the data set, as set by the simulations (for which we have used the general notation σ^2_i and σ_i). Notably, estimates of the sex-linked (co)variance components are expressed as a function of $V_{s\text{-}hom}$ (i.e., $V_{s\text{-}hom}$, $V_{s\text{-}het(hom)}$, and $COV_{s\text{-}hom,het(hom)}$).

Fitting a bivariate model (see equations B1.4 and B1.5 in Appendix B1) necessitates a separate residual variance to be estimated for each sex (but no residual covariance between the sexes; Mrode 2005). Failing to do so could reduce the power to detect sex-linked variance as well as bias estimates of other variance components (Kent et al. 2005ab). The resulting parameter estimates from the bivariate animal model are $V_{s\text{-}hom}$ representing the homogametic sex base population individuals and $V_{s\text{-}het(hom)}$ representing the hypothetical heterogametic sex base population individuals that have two copies of the shared sex chromosome. The estimated variance in sex-linked additive genetic effects for the heterogametic sex ($V_{s\text{-}het}$) is therefore $\frac{1}{2}V_{s\text{-}het(hom)}$ (where $V_{s\text{-}het(hom)}$ is the variance parameter estimate from the animal model) and similarly the estimated covariance in sex-linked additive genetic effects between the homozygotic and heterozygotic sexes ($COV_{s\text{-}hom,het}$) equals $\frac{1}{2}COV_{s\text{-}hom,het(hom)}$ (where $COV_{s\text{-}hom,het(hom)}$ is the covariance parameter estimate from the animal model).

Descriptor statistics

For both pedigree structures, we used the techniques described above to simulate phenotypes based on each possible parameter combination (Table 2). For every pedigree structure and parameter combination, 5,000 sets of phenotypes were simulated and analyzed using the mixed models ("naïve" and "informed" and observed variance components models and animal models).

From these models, estimates of the autosomal and (from the informed model) sex-linked additive genetic and residual (co)variances were obtained. We report the proportion bias in additive genetic variance estimates, for the homogametic and heterogametic sexes separately, as: $(V_a - \sigma^2_a)/\sigma^2_a$, where V_a is the autosomal additive genetic variance estimated by the mixed model and σ^2_a is the variance of the simulated autosomal additive genetic effects. The additive genetic variance bias estimates were sorted from smallest to largest and the 125th and 4,875th values were used as the lower and upper limits, respectively, encompassing the 95% quantile of estimates.

The between-sex additive genetic correlation was estimated from the naïve model as:

$$r_{\mathbf{a-hom,het}} = COV_{\mathbf{a-hom,het}} / \sqrt{V_{\mathbf{a-hom}} \times V_{\mathbf{a-het}}} \quad (8)$$

and from the informed model, which incorporates both autosomal and sex-linked additive genetic effects, was calculated as:

$$r_{\mathbf{g-hom,het}} = \left(COV_{\mathbf{a-hom,het}} + COV_{\mathbf{s-hom,het}}\right) / \sqrt{\left(V_{\mathbf{a-hom}} + V_{\mathbf{s-hom}}\right) \times \left(V_{\mathbf{a-het}} + V_{\mathbf{s-het}}\right)} \quad (9)$$

We will refer to the between-sex additive genetic correlation from the naïve model (equation 8) as the autosomal between-sex genetic correlation and from the informed model (equation 9) as the genomic between-sex genetic correlation. Note, equation 9 uses the sex-linked between-sex covariance $COV_{\mathbf{hom,het}}$, however an animal model returns a different sex-linked between-sex covariance estimate ($COV_{\mathbf{hom,het(hom)}}$=twice the expected value of $\sigma_{\mathbf{hom,het}}$; see *Model of sex-linked additive genetic variation* and *Animal models*). Again, estimates were sorted in increasing order

and the 95% quantile was calculated. All simulations and analyses were performed in the R statistical software (v2.15.0, R Development Core Team, 2012).

Results

"Informed" models: sex-specific additive variance

Autosomal additive variance components estimates from the observed variance components informed model of the HS pedigrees tend to be accurate (mean bias close to zero), but not precise (large 95% quantiles; Figure 3.2A-C). Particularly, the precision in the homogametic sex is worse than that of the heterogametic sex, as observed by the larger 95% quantiles. Sex-linked additive variance estimates for the heterogametic sex are overestimated when the simulated sex-linked variance is low (h_s^2<0.2; Appendix B2 Figure B2.1A-C). Animal models of the HS design are unable to separate autosomal from sex-linked additive variance in the homogametic sex when the sex-linked between-sex genetic correlation is less than the maximum possible parameter value (<0.71; 2nd and 3rd columns in Figure 3.2). This is seen in figure 3.2 by the increase in mean bias as h_s^2 increases (Figure 3.2E,F; as well as the 100% underestimation of sex-linked additive variance in Appendix B2 Figure B2.2B,C). Biases in autosomal additive variance estimates from the DFC pedigree design analyzed with informed animal models are zero for all parameter combinations (Figure 3.2G-I). The animal model analyses are also sensitive to the starting values of the model parameters. When we re-ran the HS models with starting values close to the simulated parameter values we obtained different estimates of autosomal, sex-linked, and residual variances (results not shown). However, we find that the log-likelihoods of these models were almost identical (<0.1% difference) to models where no starting values were intentionally assigned by us (ASReml assigns default starting values of 0.05 times the phenotypic variance for

all variances and values of 0.1 for all correlations). This implies that these parameter estimates have large confidence intervals as a result of relatively flat profile likelihood surfaces.

"Informed" models: between-sex genetic correlations

The estimate of the genomic between-sex additive genetic correlation is unbiased when the HS pedigree is analyzed using the informed observed variance components models (Figure 3.3A-C). However, the animal model produces underestimates whenever the simulated between-sex correlation is less than the maximum value of approximately 0.71 (Figure 3.3D-F). This departure from the expected value is most likely driven by the observed bias in the sex-specific additive variance estimates (Figure 3.2E,F). Estimates of the genomic between-sex additive genetic correlation using an informed animal model and the DFC pedigree match the predicted values for the genomic between-sex correlation (dashed grey line; Figure 3.3G-I).

Overall, these results indicate that accurate and precise separation of autosomal and sex-linked sources of additive genetic (co)variance is best accomplished with a complex pedigree structure (e.g., DFC), however, we note that the observed variance components analysis of the HS pedigree does produce unbiased (albeit imprecise) estimates.

"Naïve" models: sex-specific additive variance

For both pedigrees and analytical models, sex-specific estimates of autosomal additive variance from naïve models are upward biased, with bias increasing as the sex-linked variance increases (h_s^2; Figure 3.4). For a given sex-linked variance (h_s^2) and sex-linked between-sex correlation, the bias in sex-specific autosomal additive variance is greatest in the HS pedigree analyzed with a naïve animal model (Figure 3.4D-F) and lowest for the DFC pedigree analyzed with a naïve

animal model (Figure 3.4G-I). Further, biases in the homogametic sex show a faster increase with increasing sex-specific additive variance (h_s^2) than do biases in the heterogametic sex.

Quantifying the bias in estimates of residual variance (the only other variance component in the naïve models) further indicates to which component(s) un-modeled sex-linked additive genetic variance is assigned when sex-linkage is ignored in naïve models (see Appendix B3 Figure B3.1). Overall these results indicate that, regardless of the method of analysis or pedigree, naïve models will often yield estimates of autosomal additive variance that are neither accurate estimates of the true autosomal additive variance nor accurate estimates of the genome-wide additive (autosomal+sex-linked) variance. However, the autosomal additive variance estimates for the heterogametic sex in a HS pedigree analyzed with naïve observed variance components are the exception; estimates of autosomal additive variance from these naïve models equal the genome-wide additive variance (see Appendix B3 Figure B3.1, top row).

"Naïve" models: between-sex genetic correlations

The naïve observed variance components models failed to produce between-sex additive genetic correlations within the acceptable parameter space of correlations (from -1 to 1) when the between-sex additive genetic covariance and sex-specific additive variance estimates were used. This extreme bias is attributed to a failure of the model to produce reasonable estimates of the observed covariance components to which sex-linkage contributes (i.e., dam and within-family covariance). Instead, we calculated the between-sex additive genetic correlation from the observed sire (co)variances:

$$r_{\mathbf{a-hom,het}} = COV_{\mathbf{sire-hom,het}} / \sqrt{V_{\mathbf{sire-hom}} \times V_{\mathbf{sire-het}}} \qquad (10)$$

This method does not suffer the extreme bias mentioned above, because sex-linked covariance does not contribute to the sire covariance.

In both pedigrees and for both analytical methods, estimates of the autosomal between-sex genetic correlations ($r_{\mathbf{a\text{-}hom,het}}$) from naïve models are not significantly different (as judged by the limits of the 95% quantiles) from the expected genomic between-sex genetic correlations ($r_{\mathbf{g\text{-}hom,het}}$) when the sex-linked between-sex genetic correlation is zero (Figure 3.5B,E,H). However, when the sex-linked between-sex genetic correlation is 0.71, the autosomal between-sex genetic correlation ($r_{\mathbf{a\text{-}hom,het}}$) underestimates the expected genomic between-sex genetic correlations ($r_{\mathbf{g\text{-}hom,het}}$) (Figure 3.5A,D,G). For the DFC animal models and HS observed variance components models this bias increases as the sex-linked additive variance increases (Figure 3.5A,G), but this is not evident for animal model analyses of the HS pedigree, where the overall bias is not very large (Figure 3.5D). When the sex-linked between-sex genetic correlation is -0.71, the observed variance components analysis of the HS pedigree and the animal model analyses of the DFC pedigree increasingly overestimate the genomic between-sex additive genetic correlation as the amount of sex-linked additive variance increases (Figure 3.5C,I). Conversely, the autosomal between-sex correlation underestimates the genomic between-sex correlation when the animal model is used to analyze the HS pedigree (Figure 3.5F). It is noteworthy that all naïve models under-estimate the autosomal between-sex genetic correlation (i.e., horizontal dashed line in Figure 3.5) for all parameter combinations, analytical models, and pedigree designs when sex-linked variance is present. In other words, analyses modeling only autosomal additive genetic effects produce underestimates of the true autosomal between-sex correlations, but these estimates better reflect the genomic (autosomal plus sex-linked) between-sex correlation in additive genetic effects.

Discussion

We investigated how sex-linked additive genetic variance affects estimates of genetic (co)variances modeled under autosomal expectations of the covariance between relatives. By simulating populations with known contributions of genetic variance components to phenotypic variance, we are able to show that bias occurs and that the magnitude of bias in sex-specific additive variances depends on the variety of relationships in the pedigree used, the method of statistical analysis, and the magnitude of sex-linked additive (co)variance. We also find that estimates of the between-sex genetic correlation will be biased whenever the sex-linked between-sex correlation is non-zero and sex-linked additive variance is present.

When sex-linked variance is explicitly modeled in an analysis, as in our "informed" models, the animal model analysis of the DFC pedigree and the observed variance components model analysis of the HS pedigree resulted in unbiased estimates of the sex-specific additive variances and between-sex additive correlations. However, the informed animal model analyses of the HS design did overestimate autosomal additive variance in the homogametic sex. Although specifying starting values in the HS animal model which were close to the simulated values reduced the bias in the informed models, this approach is impractical as true values are not known when analyzing empirical data. Further, the model fit with these altered starting values, as judged by the maximum of the log-likelihood function, provides no useful information to discern which set of variance parameter estimates better fit the data. In other words, parameter estimates are accompanied by very large confidence intervals. We interpret this as a failure of the animal model to correctly partition the phenotypic variance when very little information is provided by the pedigree to separate autosomal and sex-linked additive variance.

Although the observed variance components method produced mean biases near zero, the range of the 95% quantiles is quite large. This indicates that the analytical method has very low

statistical power, despite the extremely large experimental design (6,000 phenotypes gathered from 10 offspring in each full-sib family of a nested half-sib design with 60 sires and 10 dams mated to each sire). In an alternative approach, Mezey and Houle (2005) incorporate information from parent-offspring phenotypic covariances into the design matrix. This would help to ameliorate the low power stemming from the limited number of different relationships informative for estimating sex-linked variance in a paternal half-sib design, although mostly for the female estimates (see Appendix B4). Male estimates are little improved by the addition of mean male offspring on sire covariance estimates because the number of these relationships (e.g., 60 in our study) is typically much lower than the number of mean female offspring on dam pairs (e.g., 600 in our study) in a paternal half-sib design (Appendix B4).

Our results indicate that ignoring sex-linked additive genetic (co)variances may affect predictions of evolutionary responses to selection or conclusions regarding the effects of evolutionary forces on additive genetic variation. The impacts of ignoring sex-linked (co)variances will depend, in part, upon the number of different types of relationships among individuals in a study population. The HS and DFC pedigrees contain very few and many different types of relationships, respectively, useful in disentangling the different sources of phenotypic variance and thus represent two extremes in a range of possible population structures. These extremes highlight a major consequence for applying additive genetic variance estimates to inferences regarding evolutionary theory when sex-linked (co)variances have been ignored.

Using additive genetic variance estimates to draw conclusions about evolutionary processes necessitates consideration of which estimated component of variance in a statistical model contains the additive genetic variance in sex-linked genetic effects when sex-linkage is ignored (i.e., a naïve model). The three generation DFC pedigree contains many different types of relationships that enabled the naïve animal model analyses to estimate autosomal additive

variance with the least inflation from the sex-linked variance. When not included in the animal model, much of the sex-linked additive genetic variance is contained in the estimates of the residual variance. For pedigrees containing a sufficient variety of relationships, estimates of additive variance from naïve models will thus reflect the evolutionary response to selection caused mostly by autosomal additive variance and leaving out any sex-linked variance from consideration. Similarly, detailing the changes in additive genetic variance as a consequence of selection, mutation, or drift will again mostly reflect changes in the autosomal additive variance. However, two caveats deserve further discussion.

First, estimates of autosomal additive variance were still inflated by sex-linked additive variance in the naïve animal models of the DFC pedigree. Therefore, inferences about evolutionary processes drawn from these estimates may be inaccurate with respect to both considerations of only autosomal additive variance and to the genome-wide (autosomal + sex-linked) additive variance. Secondly, we only modeled simulated traits with simple genetic architectures. It is unclear how naïve models will partition sex-linked additive variance in the presence of the additional sources of phenotypic variance often encountered in empirical studies (e.g., measurement error, common environment effects, non-additive/indirect genetic effects, etc.). Conversely, estimates of additive genetic variance from the HS design (both naïve analytical models) attributed all sex-linked additive variance to autosomal additive variance. Therefore, half-sib experiments can still approximate the genome-wide additive variance even when excluding sex-linked additive effects from the model. However, un-modeled sex-linked variance will reduce the precision of the additive variance estimates and, in some cases, cause the models to attribute too much variance to additive genetic effects (see Appendix B3). Further, the predicted response to selection caused by sex-linked genes is more complex than that of autosomal genes (see Griffing 1965; Lande 1980b). Therefore, even though naïve analyses of HS

pedigrees tend to approximate the genome-wide additive variance, it is inappropriate to use this quantity to predict evolutionary responses to selection in a breeders' equation.

In organisms or laboratory systems where only simple pedigree structures are readily produced (e.g., half-sib design), the hampered ability to precisely separate autosomal from sex-linked additive variance will prevent researchers from testing specific predictions regarding the genomic location (autosomal vs. sex-linked) of additive genetic variance (*sensu* Fairbairn and Roff 2006). Pedigree structures similar to the complexity of the DFC design are best suited to empirically evaluate these predictions. Where data from wild populations are used (for example, long-term studies of pedigreed populations), an outstanding question is whether or not the animal model will have enough statistical power to estimate sex-linked additive genetic variance. So far, we are only aware of three studies that have attempted this in un-manipulated populations (humans: Pan et al. 2007; Kosova et al. 2010; passerine birds: Husby et al. 2013). Although these authors do report simple pedigree statistics, they do not include the numbers of first cousins (single and double) which Fairbairn and Roff (2006) identified as being particularly useful for separating sex-linked additive genetic variance from other sources of phenotypic variance. Additionally, no formal analysis quantifying the statistical power to estimate sex-linked additive variances in wild population pedigrees has been completed. Such necessary power analyses can follow the *ad hoc* approaches of Thériault et al. (2007) and DiBattista et al. (2009). However, a comprehensive approach using simulated populations (e.g., Clément et al. 2001; Kruuk and Hadfield 2007; Morrissey and Wilson 2010) may prove more useful than any one collection of *ad hoc* case studies. Until a general power analysis has been completed, the sampling (co)variances of the variance estimates are the only means of exploring the influence of confounding sources of variation on the sex-linked additive variance estimates.

Above, our results indicate medium to high levels of sex-linked variance bias estimates of sex-specific additive variances and between-sex additive genetic correlations in a manner that depends on sex, pedigree design, and method of analysis. However, to date only a few studies have controlled for the difference in covariances between relatives for autosomal versus sex-linked genes when estimating additive genetic variance (e.g., Chenoweth and Blows 2003; Mezey and Houle 2005). In general, we have too few empirical estimates of sex-linked variance to assess the impacts of ignoring the differences between autosomal and sex-linked contributions to additive genetic variance. Above, we show the conditions under which sex-specific estimates of genome-wide additive (autosomal+sex-linked) variances are biased. Bias occurs when sex-linked additive variance is large and excluded from the analysis and the study designs are particularly powerful for separating autosomal and sex-linked sources of additive genetic variance. Conversely, in simple designs such as the HS pedigree, overall bias in genome-wide additive genetic variance is lower than in more complex study designs. However, this is accompanied by the inability to uncover the biologically interesting components of additive variance, because HS pedigrees are unable to precisely separate autosomal from sex-linked additive variance. Further, our study shows that the potential for bias in estimates of additive genetic (co)variances, specifically the between-sex additive genetic correlation, can impact our ability to properly address fundamental questions such as the evolution of sexually antagonistic genetic variation.

Alleles with opposing effects in females and males evolve in response to sexually antagonistic selection and create a negative between-sex genetic correlation for fitness. Evidence for the overrepresentation of sexually antagonistic genes on the sex chromosomes remains equivocal (Curtsinger 1980; Reinhold 1998; Gibson et al. 2002; Parisi et al. 2003; Fitzpatrick 2004; Bonduriansky 2007; Long and Rice 2007; Mank 2009; Innocenti and Morrow 2010) and our results indicate that studies which seek to use between-sex additive genetic correlations as

evidence of sexually antagonistic fitness variation (e.g., Chippindale et al. 2001) need to consider the potential impact of sex-linked variance on estimates of between-sex correlations. In cases where most of the segregating additive genetic variation in fitness is sexually antagonistic and restricted to the sex chromosomes (e.g., Gibson et al. 2002), we expect the sex-linked between-sex correlation to be less than or equal to zero and autosomal between-sex correlations close to unity. Indeed, Chenoweth et al. (2008) found that autosomal between-sex additive genetic correlations in *Drosophila serrata* tended to be positive and close to unity for the sexually dimorphic cuticular hydrocarbons investigated. However, when the authors combined the sex-linked between-sex additive genetic correlations (often negative) with the autosomal correlations yielded very low genomic between-sex additive genetic correlations. Our results above demonstrate this finding over a range of parameter combinations. Further, we have shown that analyses that model all additive variance as autosomal lead to biased estimates of between-sex additive genetic correlations and potential misinterpretation of empirical estimates for the identification of sexually antagonistic genetic variation.

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Table 3.1. Empirical estimates of sex-linked additive genetic variance (X- or Z-linked). The symbols V_S, V_A, and V_P represent empirical estimates of sex-linked additive genetic, autosomal additive genetic, and phenotypic variances, respectively. These studies investigated morphological, behavioral, physiological, and life history traits, but the majority of traits are morphological.

Class	Organism	Number of estimates	V_S / V_P mean (median)	$V_S/(V_S + V_A)$ mean (median)	Reference
Insecta					
	Drosophila melanogaster				
		4	0.015 (0)	0.094 (0)	Sheridan et al. 1968
		26	0.135 (0.140)	0.265 (0.260)	Cowley et al. 1986
		30	0.110 (0.110)	0.202 (0.217)	Cowley and Atchley 1988
		50	0.069 (0.065)	0.130 (0.108)	Mezey and Houle 2005
	Bombyx sp.				
		2	0.219 (0.219)	0.371 (0.371)	Zhu and Weir 1996
Aves					
	Ficedula albicollis				
		8	0.073 (0.025)	0.156 (0.061)	Husby et al. 2012
	Taeniopygia guttata				
		9	0.021 (0)	0.156 (0)	Husby et al. 2012
Mammalia					
	Mus musculus				
		2	0.108 (0.108)	0.099 (0.099)	Zhu and Weir 1996
	domestic pig				
		2	0.001 (0.001)	0.027 (0.027)	Wittenburg et al. 2011
	Papio hamadryas				
		16	0.031 (0)	0.044 (0)	Willmore et al. 2009
	Homo sapiens				
		6	0.115 (0.085)	0.507 (0.416)	Kosova et al. 2010
		30	0.121 (0)	0.239 (0)	Pan et al. 2007

Table 3.2. Parameter values used to simulate phenotypes. Autosomal additive genetic (σ^2_a) and residual (σ^2_e) variances were held constant for both sexes. Sex-linked additive variance is expressed as different proportions of the total phenotypic variance (h_s^2) in the homogametic sex.

			Homogametic sex	**Heterogametic sex**
h_s^2	σ^2_a	σ^2_e	σ^2_s	σ^2_s
0.0005	50	50	0.05	0.025
0.05	50	50	5.5	2.75
0.11	50	50	12.5	6.25
0.18	50	50	21.5	10.75
0.25	50	50	33.35	16.675
0.33	50	50	50	25
0.43	50	50	75	37.5

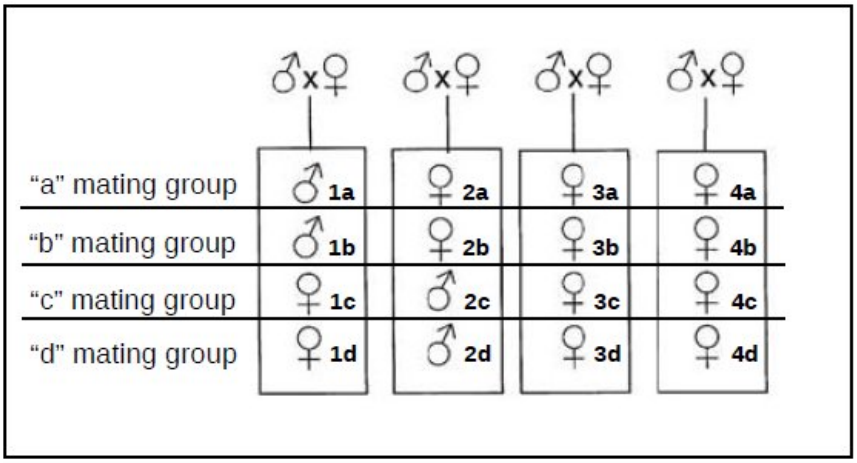

Figure 3.1. One unit of the double first cousin (DFC) breeding design of Fairbairn and Roff (2006). Eight unrelated individuals of the grandparent generation form four mating pairs (top row of male and female symbols). Each mating pair contributes four full-siblings to the parental generation (each vertical rectangle encloses full-sibs of the parental generation sharing the same numerical label: "1", "2", "3", or "4"). From the parental generation, every individual in a full-sib family is assigned to a different "mating group" (lowercase letters) and is mated to an individual from one of the three other full-sib families in their mating group (different rectangles within the same mating group letter), as in a half-sib breeding design. Each mating between parental generation individuals creates eight offspring in the F1 generation (four female and four male; not depicted). Individuals in the F1 generation are full- and half-sibs within a "mating group". Relationships between F1 offspring from different mating groups are first and double first cousins.

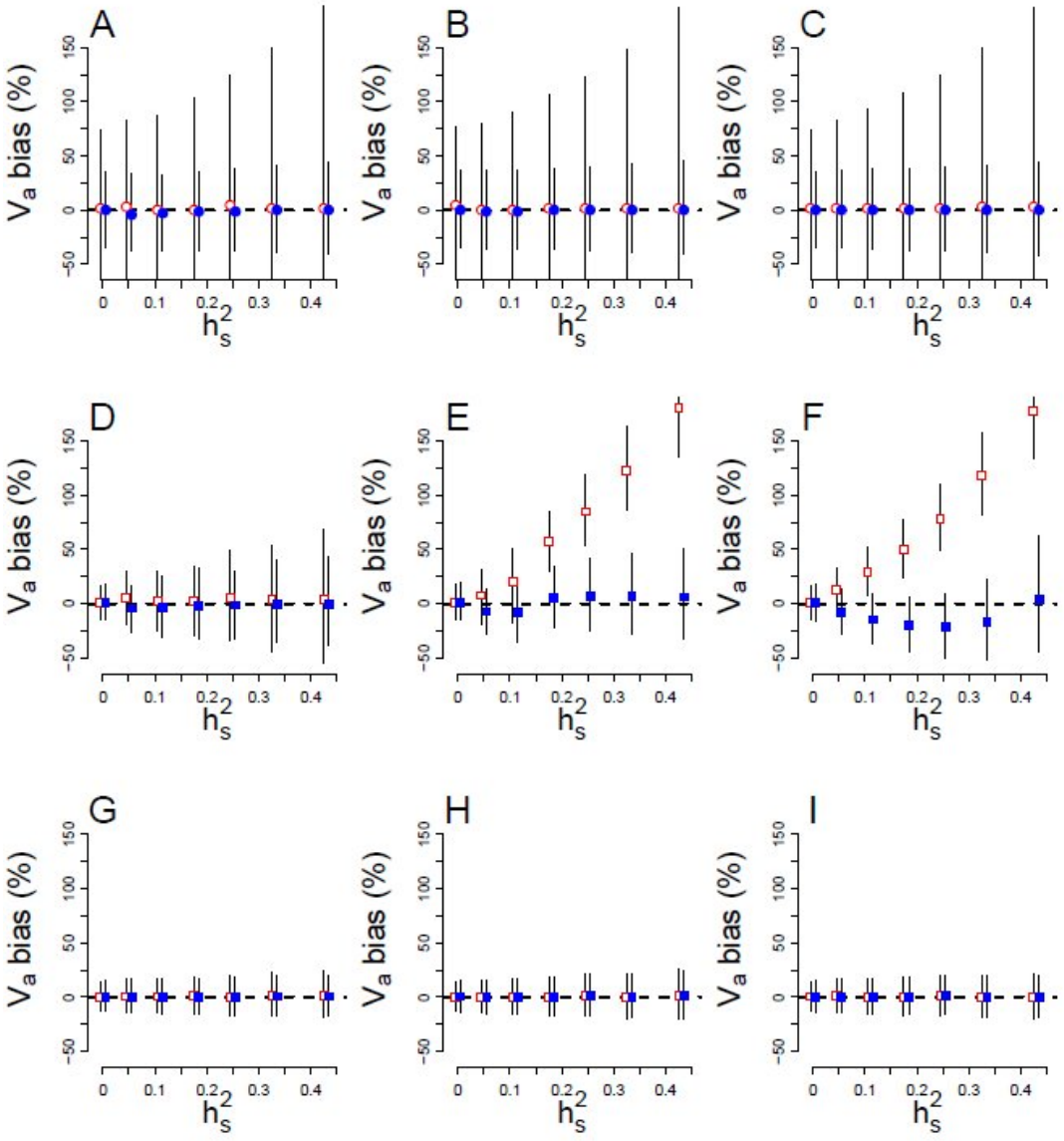

Figure 3.2. Analyses implementing "informed" observed variance components models (circles) of half-sib pedigrees (A-C) or animal models (boxes) of half-sib pedigrees (D-F) and DFC pedigrees (G-I). The sex-linked between-sex additive genetic correlation is approximately 0.71 (first column, maximum parameter value), 0 (second column), or -0.71 (third column, minimum parameter value). Each panel depicts the average percent bias in autosomal additive variance estimates in the homogametic sex (open symbols) and the heterogametic sex (closed symbols) as a function of the proportion of phenotypic variance comprised of sex-linked variance (h_s^2) and when the sex-linked between-sex additive genetic correlation is approximately 0.71 (first column, maximum possible parameter value), 0 (second column), and -0.71 (third column, minimum possible parameter value). In all panels, bars indicate the extent of the 95% quantile of estimates. Note in panels A-C, the 95% quantiles are symmetrical and lower limits of the homogametic sex have been cut off to preserve space; also in panels E and F the upper limit of the 95% quantiles for the homogametic sex extend above the plotting region for the largest h_s^2.

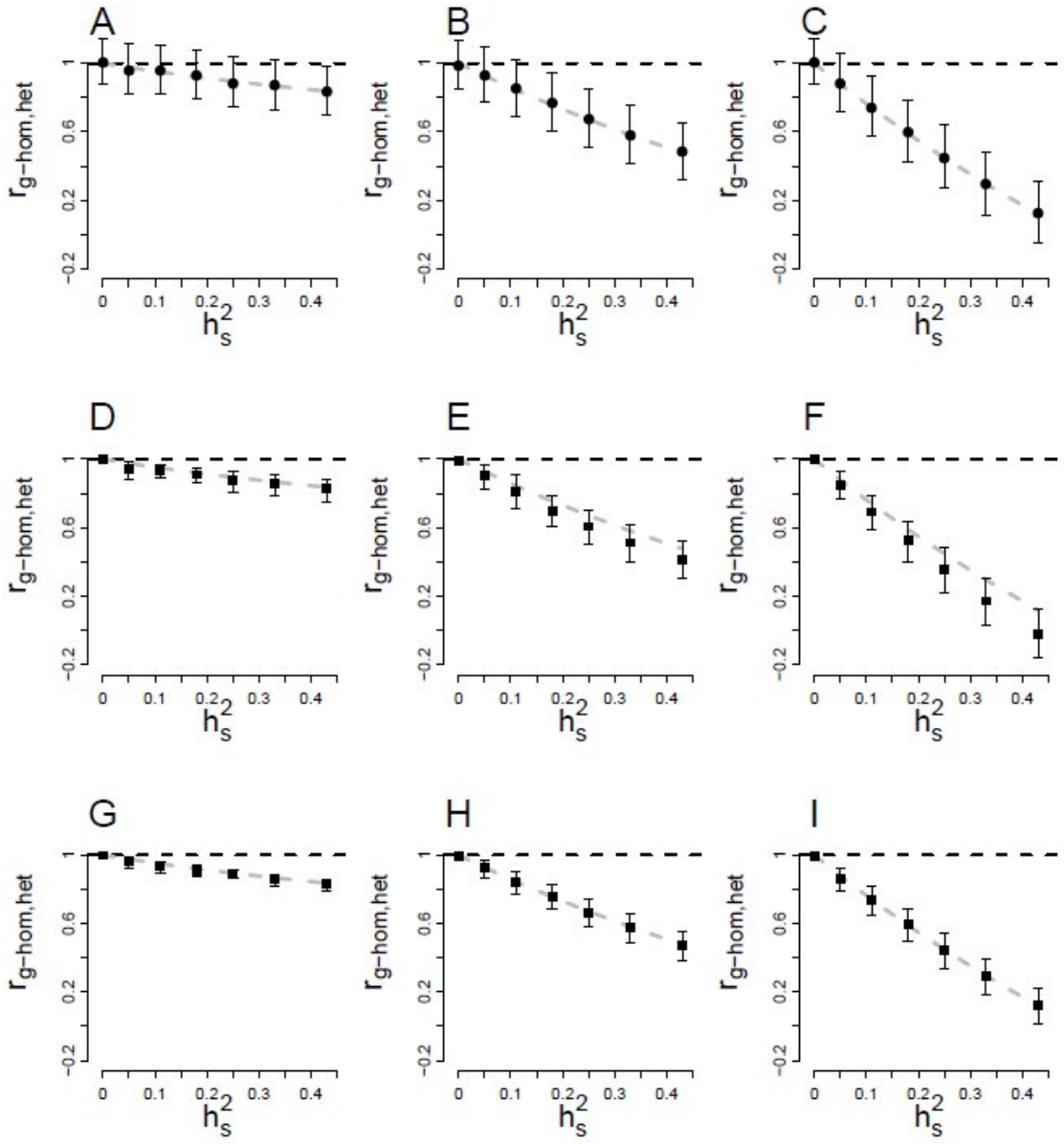

Figure 3.3. Analyses implementing "informed" observed variance components models (circles) of half-sib pedigrees (A-C) or animal models (boxes) of half-sib pedigrees (D-F) and the DFC pedigrees (G-I). Each panel depicts the genomic (autosomal+sex-linked) between-sex additive genetic correlation ($r_{g\text{-}hom,het}$) when the sex-linked between-sex additive genetic correlation is approximately 0.71 (first column, maximum possible parameter value), 0 (second column), and -0.71 (third column, minimum possible parameter value) (note that from equation (9), estimates of $r_{g\text{-}hom,het}$ are a function of all the genetic (co)variance parameters). The horizontal black line indicates the simulated autosomal between-sex additive genetic correlation of one and the diagonal grey line indicates the expected genomic between-sex additive genetic correlation. In all panels, bars indicate the extent of the 95% quantile of estimates.

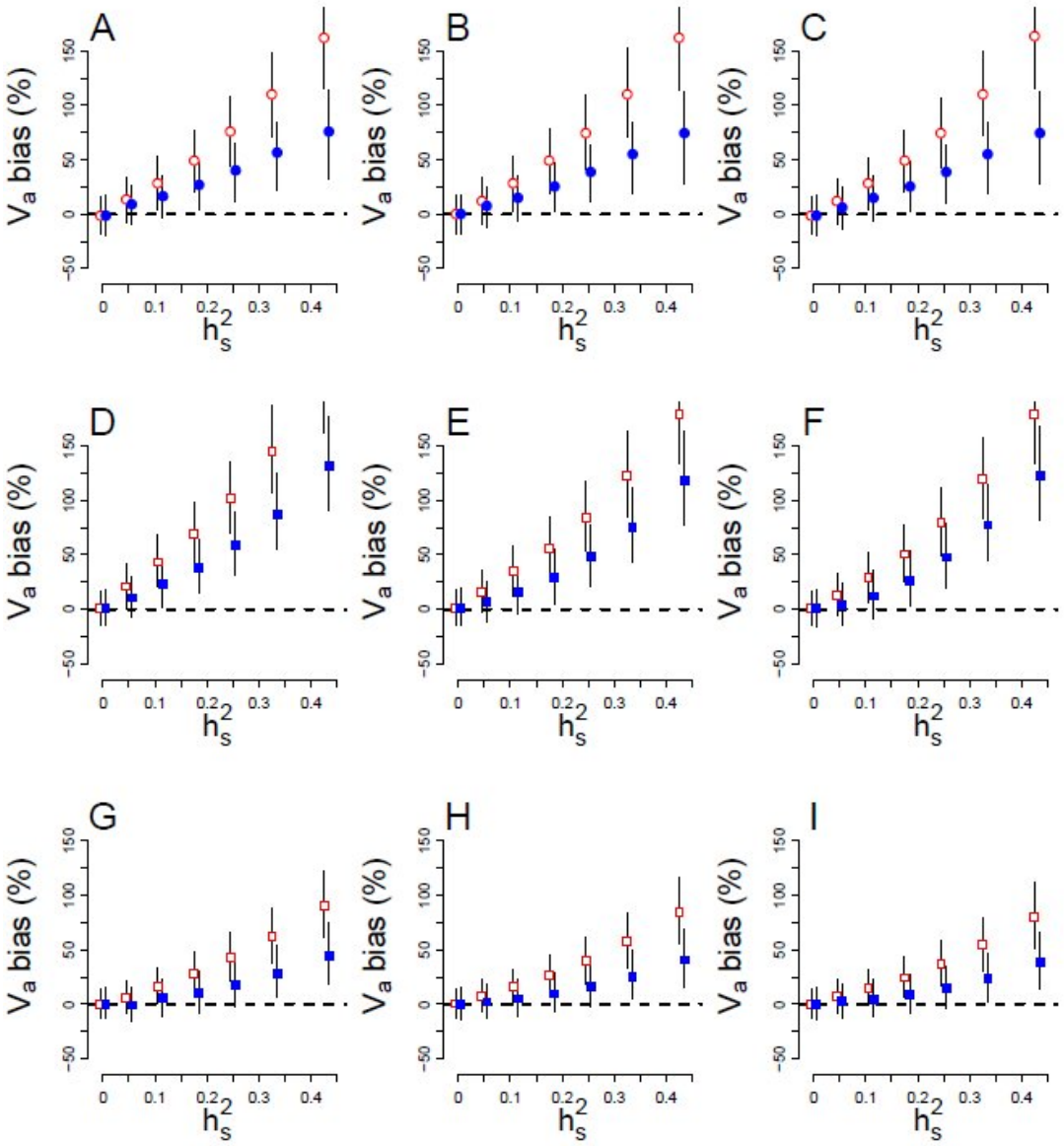

Figure 3.4. Analyses implementing "naïve" observed variance components models of half-sib pedigrees (A-C); circles or animal models (boxes) of half-sib pedigrees (D-F) and the DFC pedigrees (G-I); the sex-linked between-sex additive genetic correlation is approximately 0.71 (first column, maximum possible parameter value), 0 (second column), and -0.71 (third column, minimum possible parameter value). Each panel depicts the average percent bias in autosomal additive variance estimates as a function of the proportion of phenotypic variance comprised of sex-linked variance (h_s^2). Panel arrangement, symbols, and lines as in figure 3.2. Note that the point for the most extreme value of the sex-linked variance (h_s^2) in panel D lies above the plotted region to conserve space (only the arrow depicting the extent of the lower confidence limit is visible).

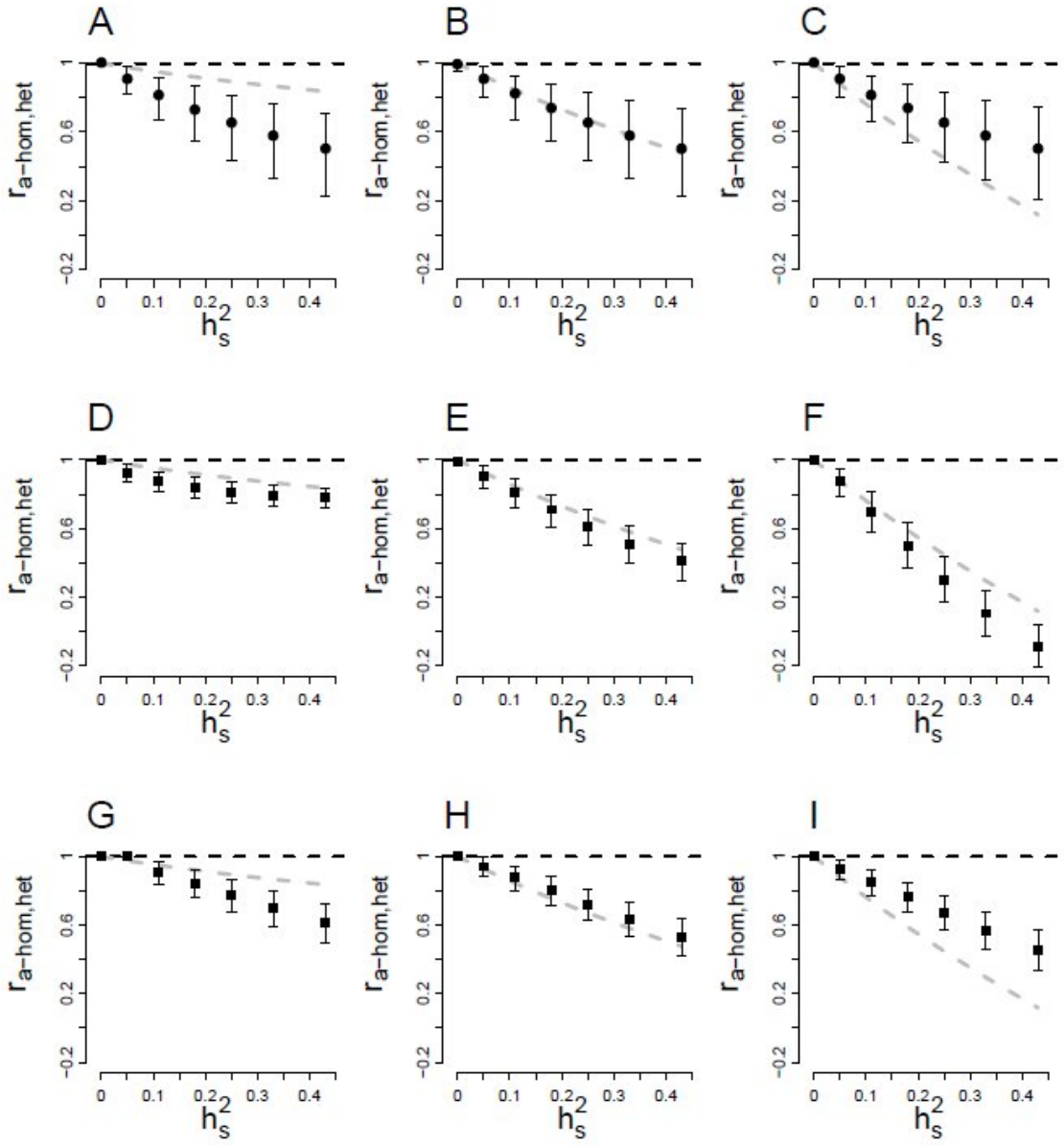

Figure 3.5. Analyses implementing "naïve" observed variance components models (circles) of half-sib pedigrees (A-C) or animal models (boxes) of half-sib pedigrees (D-F) and the DFC pedigrees (G-I). Each panel depicts the autosomal between-sex additive genetic correlation ($r_{a-hom,het}$). Panel arrangement, symbols, and lines as in figure 3.3.

CHAPTER 4

Sex chromosomal additive genetic (co)variation under alternative models of dosage compensation: Estimating sex-linked variance when the form of dosage compensation is unknown

Abstract

Sex-linked additive genetic variance is predicted to reduce genetic sexual conflict and facilitate the evolution of sexual dimorphisms. The animal model has allowed quantitative geneticists to address a greater number of hypotheses in a wider range of experimental systems and populations than previously, but detection of sex-linked variance remains problematic. The current method to disentangle autosomal and sex-linked sources of additive genetic variance in animal models makes the untenable assumption of no global sex chromosomal dosage compensation. Here, we develop a genetic model of sex-linked genetic effects and derive general expressions to calculate the sex-linked genotypic covariances between pairs of individuals under all known mechanisms of global sex chromosomal dosage compensation. We then use these expressions to construct sex-linked relatedness matrices for subsequent use in animal models and demonstrate how to parameterize algorithms to directly obtain the inverse of sex-linked relatedness matrices. We address the differences among estimates of sex-linked variance when different assumptions about dosage compensation are made and discuss the process of estimating sex-linked additive (co)variances when the status of dosage compensation is unknown for a particular study organism. Finally, we discuss how to use the animal model to estimate sex-specific sex-linked additive genetic variance and between-sex genetic correlations affected by sex-biased gene expression.

Introduction

As a consequence of divergent reproductive roles between the sexes, female and male organisms have different optimal phenotypes which we recognize as dimorphisms in primary and secondary sexual traits (Darwin 1874; Fairbairn et al. 2007; Fairbairn 2013). The differences between sexes are presumed to reflect sex-specific evolutionary responses toward different fitness optima (Hedrick and Temeles 1989; Fairbairn et al. 2007). However, correlations between the sexes in the expression of shared alleles constrain the evolution of sexual dimorphism (Lande 1980; Reeve and Fairbairn 2001), creating genetic conflict between the sexes (Parker 1979; Arnqvist and Rowe 2005; Bonduriansky and Chenoweth 2009). Thus, evolutionary biologists are faced with explaining how sexual dimorphisms evolve in the face of genetic constraint resulting from the sexes sharing the same genome (Lande 1980, 1987; Reeve and Fairbairn 1996, 1999, 2001; Fairbairn 1997; Badyaev 2002; Fairbairn and Roff 2006).

Evolutionary theory predicts that genes with sex-specific fitness effects will accumulate on or be linked to the sex chromosomes in organisms with chromosomal sex determination (i.e., sex-linkage; Fisher 1931; Charlesworth and Charlesworth 1980; Rice 1984; Charlesworth et al. 1987, but see Fry 2010). However, few theoretical models that predict sex-linkage of sexually antagonistic variation consider mechanisms of global sex chromosome dosage compensation. We follow Muller et al. (1931) and Lucchesi (1978) to operationally define dosage compensation as epigenetic effects that tend to equalize the sex-linked gene expression between the homogametic and heterogametic sex (note this differs from Mank et al. 2011). Global sex chromosomal dosage compensation mechanisms alter the expression level of an entire sex chromosome and can be grouped into the following four classes according to the sex in which the mechanism operates and its effect on the expression levels of the sex chromosomes (Table 4.1): (*i*) doubling of expression on the single shared sex chromosome in the heterogametic sex (HEDO), (*ii*) random, with respect

to parent of origin, inactivation of one sex chromosome in the homogametic sex (HORI), (*iii*) halved expression levels for each sex chromosome in the homogametic sex (HOHA), and (*iv*) inactivation of the paternal sex chromosome in the homogametic sex (HOPI). Depending on the specific form of dosage compensation, selection is predicted to either favor or disfavor sex-linkage of sexually antagonistic alleles (Charlesworth et al. 1987; Rogers et al. 2003; Carrel and Willard 2005; Fairbairn and Roff 2006; Turner 2006; Mank et al. 2010).

In part, the predicted accumulation of sex-linked sexually antagonistic alleles differs among forms of dosage compensation because of the way dosage compensation affects the distribution of sex-linked genetic effects. When global dosage compensation occurs, each mechanism equalizes the average phenotypic effects of sex-linked allele(s) in one sex as compared to the other sex. However, the forms of dosage compensation differentially affect the variance in sex-linked allelic effects. In the simple case where no mechanism of global dosage compensation (NGDC) occurs, the variance in sex-linked additive genetic effects in the homogametic sex is expected to be twice that in the heterogametic sex (James 1973; Bulmer 1980; Lynch and Walsh 1998; Kent et al. 2005). However, the HEDO, HORI, and HOHA forms of dosage compensation cause the variance in sex-linked additive genetic effects in the heterogametic sex to be twice that in the homogametic sex (the opposite to NGDC; James 1973; Bulmer 1980; Lynch and Walsh 1998; Kent et al. 2005) and the HOPI pattern of dosage compensation causes the variance in sex-linked effects to be equal between the two sexes (Bulmer 1980; Lynch and Walsh 1998). Consequently, different forms of dosage compensation have different expectations for the covariance in sex-linked additive genetic effects among relatives in a population. This presents a major obstacle for empiricists seeking to quantify the contribution of sex-linked genetic variation to evolutionary responses in non-model organisms. Further, if the presence or form of dosage compensation is unknown, but the expected variance in

sex-linked genetic effects depends on the form of dosage compensation, can we still obtain accurate estimates of sex-linked additive genetic variance in a study population?

To obtain a comprehensive picture of the prevalence and quantities of additive genetic variance located on the sex chromosomes, particularly when dosage compensation occurs, necessitates the development of a statistical method flexible enough to accommodate the estimation of sex-linked variance under all forms of dosage compensation. To date the mixed effect statistical model, known as the 'animal model' (Henderson 1973) in livestock breeding programs, represents the best modeling approach to estimate sex-linked additive variances by its incorporation of the sex-linked additive genetic relatedness matrix inverse ($\mathbf{S}^{-1}$; e.g., Fernando and Grossman 1990). However, the previous theory used to directly construct $\mathbf{S}^{-1}$ (based on a single locus genetic model) assumes the absence of global sex chromosomal dosage compensation (Fernando and Grossman 1990). When dosage compensation mechanisms do occur, they alter the expected covariances between relatives in sex-linked allelic effects for one or the other sex (James 1973; Bulmer 1980; Lynch and Walsh 1998; Kent et al. 2005). Consequently, this affects the sex-linked relatedness matrix used in an animal model ($\mathbf{S}^{-1}$), depending on which form of dosage compensation underlies sex-linked gene expression in an organism.

Further, because genetic models for the construction of the sex-linked relatedness matrix inverses under various forms of dosage compensation are not available, we do not know how our estimates will be affected by using the wrong form of the sex-linked relationship matrix in organisms for which we do not know the presence or type of sex chromosomal dosage compensation (e.g., all organisms *not* in Table 4.1). Fernando and Grossman (1990) comment that instead of using animal models to estimate sex-linked variances given the data and assuming a certain form of dosage compensation (or none at all), instead animal models and likelihood

based model comparison techniques could potentially be used to determine which form of dosage compensation best fits the data. However, currently no methods exist for the direct creation of the sex-linked relatedness matrix inverse for any form of dosage compensation and thus this conjecture has yet to be tested.

In this paper, we create a general framework to describe the quantitative genetic properties of sex-linked additive variance under different mechanisms of global sex chromosome dosage compensation. We begin by deriving expectations of the population genotypic mean and variance at a single sex-linked locus for each form of dosage compensation in table 4.1. From these expectations we formulate expressions in terms of coefficients of dosage compensation to describe the expected covariance between relatives due to sex-linked additive genetic effects. Next, we develop the necessary formulae to apply rules that allow us to directly construct the inverse of the sex-linked relatedness matrix, under all forms of dosage compensation, for use in animal models. We then test Fernando and Grossman's (1990) conjecture that likelihood based model comparisons could be used to test for the presence and form of dosage compensation underlying sex-linked additive variances contributing to the phenotypic variance. Results from these tests are then used to help inform empiricists on how to proceed with an animal model analysis even when the presence or specific form of dosage compensation is not known for their particular study organism.

Genetic model

First, we create a genetic model with a single variable to describe the effect of dosage compensation on the genotypic mean and variance of both sexes. Previously, single locus models of sex-linked loci have been constructed (e.g., James 1973; Fernando and Grossman 1990; Kent et al. 2005). However, these models are too specific and can only describe one or two forms of

dosage compensation at a time. Our model enables us to use one framework that is later used to formulate the covariance between relatives due to sex-linked genetic effects under all forms of sex chromosomal dosage compensation in table 4.1. For simplicity, we assume the population is in gametic equilibrium, sex-linked non-additive effects do not affect the trait of interest, and recombination does not occur between the X and Y or Z and W during meiosis (such as observed in the pseudoautosomal regions of sex chromosomes in some organisms; Otto et al. 2011). We also begin with an assumption that the genotypic value of a sex-linked additive allele is equal in the two sexes (i.e., no locus-specific, sex-biased gene expression, but this condition is relaxed later).

Population genotypic mean and variance

Assume a sex-linked locus in the homogametic sex has alleles A_1 and A_2, where the genotypic value for the A_1 homozygote is $+a$ and the genotypic value for the A_2 homozygote is $-a$. In the heterogametic sex, the genotypic value for the A_1 genotype is $+0.5a$ and for the A_2 genotype it is $-0.5a$. Let p represent the frequency of A_1 alleles and λ_i represent the average expression level of an allele inherited from the homogametic sex parent ($\lambda_{p\text{-}hom}$) or heterogametic sex parent ($\lambda_{p\text{-}het}$). The quantity λ_i is determined for each allele at a locus as the product of the probability of expression times the relative level of expression, with the normal expression level of an allele equaling one. The genotypic values and frequencies of the possible genotypes are shown in table 4.2. For non-inbred individuals, the mean genotypic values in a population for the homogametic ($\bar{g}_{hom}$) and heterogametic ($\bar{g}_{het}$) sex are:

$$\bar{g}_{hom} = 0.5a(\lambda_{p\text{-}hom}+\lambda_{p\text{-}het})p^2 + 0.5a(\lambda_{p\text{-}hom}-\lambda_{p\text{-}het})pq + 0.5a(-\lambda_{p\text{-}hom}+\lambda_{p\text{-}het})pq - 0.5a(\lambda_{p\text{-}hom}+\lambda_{p\text{-}het})q^2 \quad (1)$$

$$\bar{g}_{het} = 0.5a\lambda_{p\text{-}hom}p - 0.5a\lambda_{p\text{-}hom}q \quad (2)$$

and the genotypic variances for the homogametic and heterogametic sex are:

$$V(g_{hom}) = p^2[0.5a(\lambda_{p\text{-}hom}+\lambda_{p\text{-}het})-\bar{g}_{hom}]^2 + pq[0.5a(\lambda_{p\text{-}hom}-\lambda_{p\text{-}het})-\bar{g}_{hom}]^2 + pq[0.5a(-\lambda_{p\text{-}hom}+\lambda_{p\text{-}het})-\bar{g}_{hom}]^2 + q^2[-0.5a(\lambda_{p\text{-}hom}+\lambda_{p\text{-}het})-\bar{g}_{hom}]^2 \quad (3)$$

$$V(g_{het}) = p[0.5a\lambda_{p\text{-}hom} - \bar{g}_{het}]^2 + q[-0.5a\lambda_{p\text{-}hom} - \bar{g}_{het}]^2 \quad (4)$$

Equations 1-4 simplify to the general expressions shown in table 4.3.

Substitution of λ_i values specific to each mechanism of dosage compensation into the general expressions of the population-wide genotypic mean and variance yields the mean and variance for each form of dosage compensation in table 4.3. Here, we assume an allele has the same effect in one sex as the other to facilitate between-sex comparisons of the genotypic means and variances (condition relaxed below; see *Discussion*). When global dosage compensation does not occur (NGDC: $\lambda_{p\text{-}hom}=\lambda_{p\text{-}het}=1$), the expected genotypic mean and variance in the homogametic sex [$a(p\text{-}q)$ and $2pqa^2$] equal the expectations for an autosomal locus with no dominance (p. 129, eqn. 8.5 Falconer 1989). When the gene at a sex-linked locus in the heterogametic sex is up-regulated to twice the expression level of the gene dose (HEDO) the value $\lambda_{p\text{-}hom}=2$. For random inactivation of one whole sex chromosome in the homogametic sex (HORI) and halved expression on each sex chromosome in the homogametic sex (HOHA), average allele expression levels for the two sex chromosomes equal one-half ($\lambda_{p\text{-}hom}=\lambda_{p\text{-}het}=0.5$). Note that for the HORI and HOHA mechanisms, $\lambda_{p\text{-}hom}=\lambda_{p\text{-}het}=0.5$ for different reasons. For

HORI, the probability of expression in the population for an allele at a given sex-linked locus equals one half for both alleles and the relative expression level of each allele is one. However, for HOHA, the probability of expression equals one for each allele at a sex-linked locus, but the relative expression level of each allele equals one-half. Finally, if the paternal sex chromosome is always inactivated in the homogametic sex, the average expression levels of the two sex-linked alleles are $\lambda_{p\text{-}hom}=1$ and $\lambda_{p\text{-}het}=0$.

For polygenic traits, the sex-linked additive genetic variance for non-inbred individuals of the homogametic sex ($\sigma^2_{S\text{-}hom}$) and for the heterogametic sex ($\sigma^2_{S\text{-}het}$) is obtained by summation over all sex-linked loci (assuming no linkage disequilibrium or epistasis; Falconer 1989). For convenience (e.g., Bulmer 1980; Kent et al. 2005), the sex-linked variance in the heterogametic sex is expressed as a linear function of the sex-linked variance in the homogametic sex (e.g., last row of table 4.3). When global dosage compensation does not occur (NGDC) the sex-linked additive genetic variance in the homogametic sex is twice the sex-linked variance in the heterogametic sex. However, under the HEDO, HORI, and HOHA forms of dosage compensation, the sex-linked additive genetic variance in the homogametic sex remains the same, but the sex-linked variance in the heterogametic sex is instead twice that of the homogametic sex. In the HOPI model, the sex-linked variances of the two sexes are equal.

Silencing of one X-chromosome during HORI in human females, or lyonization, may be incomplete, whereby around 10% of loci are expressed on both X-chromosomes in some individuals (Carrel and Willard 2005; but see Johnston et al. 2008). We note that our use of λ_i in equations 1-4 can account for such cases of incomplete inactivation. Recall, the HORI model assumes the relative expression level of an allele is always one and across a population the average probability of expression equals one-half. Under the assumption that X-inactivation in the HORI mechanism is complete, $\lambda_{p\text{-}hom}=\lambda_{p\text{-}het}=0.5$. However, at the individual loci where X-

inactivation is absent both the relative expression level and probability of expression equal one ($\lambda_{p\text{-}hom}=\lambda_{p\text{-}het}=1$). With incomplete X-inactivation, summing across all sex-linked loci yields the population mean and variance in a similar manner as above. Looking across all sex-linked loci contributing to a polygenic trait, the genotypic mean and variance will be a mixture of the means and variances for the NGDC and HORI mechanisms (Table 4.3). For example, assume a trait is controlled by 10 sex-linked loci, allelic expression is equal between the sexes, and one locus escapes X-inactivation in the female (homogametic sex). The male (heterogametic sex) mean and variance remain unchanged from those developed in the HORI model and the female mean is:

$$\overline{G}_{\text{hom}} = \sum_{l=1}^{9} 0.5a(p-q) + \sum_{l=10}^{10} a(p-q) \tag{5}$$

and the variance is:

$$V(G_{\text{hom}}) = \sum_{l=1}^{9} 0.5pqa^2 + \sum_{l=10}^{10} 2pqa^2 \tag{6}$$

Consequently, the coefficients for the sex-linked additive genetic variance in the heterogametic sex expressed as a function of the sex-linked variance will change (in the example above it would change from $2\sigma^2_{S\text{-}hom}$ to approximately $1.54\sigma^2_{S\text{-}hom}$). This deviation from the expected relationship between sex-linked variances in the two sexes can be thought of as a genotype-by-sex interaction and modeled as such.

Genotypic covariance between pairs of individuals

Here, we use the phenotypic similarity between relatives to quantify the contribution of sex-linked additive genetic variance to phenotypic variance in a population, using expressions of the genotypic covariance between pairs of individuals. Expressions of genotypic covariances are based on the expectations of variance developed above and can be shown to scale up from a single locus to an entire genotype additively, as presented above (and see Falconer 1989; Lynch and Walsh 1998; Kent et al. 2005).

Derivations of the genotypic covariance between pairs of individuals for the NGDC and HORI models of dosage compensation have been covered elsewhere (Bohidar 1964; James 1973; Grossman and Eisen 1989; Fernando and Grossman 1990; Kent et al. 2005), so we will only summarize the points salient to extending these methods for the HEDO, HOHA, and HOPI models of dosage compensation. Assuming many loci contribute to trait variation in a population, the contribution of sex-linked additive genetic variation to the phenotypic resemblance between individuals *i* and *j* is expressed by the equation:

$$C(g_i, g_j) = I_{i,j} \times \psi_{i,j} \times L_i \times L_j \times \sigma^2_{S\text{-}hom} \quad (7)$$

(Kent et al. 2005). Here the genotypic covariance $C(g_i, g_j)$ is a function of the relatedness between the two individuals ($I_{i,j} \times \psi_{i,j}$) multiplied by the contribution of allelic effects in each individual relative to the sex-linked variance ($L_i \times L_j$) and the homogametic sex-linked variance ($\sigma^2_{S\text{-}hom}$). The terms L_i and L_j are coefficients of dosage compensation (named *lyonization coefficients* in mammals; Kent et al. 2005) which scale the allelic contributions to phenotypic similarity in terms of sex-linked variance in the homogametic sex. This allows the effect of an allele in one sex to be compared to the effect of that same allele if it were to be expressed in the

other sex. $I_{i,j}$ accounts for the different number of sex chromosomes in the homogametic sex compared to the heterogametic sex (two vs. one, respectively) when calculating the relatedness. $\psi_{i,j}$ is the kinship coefficient, or probability of sharing one (P_1) and two (P_2) alleles identical-by-descent (IBD; see p.43, table 3.6 Bulmer 1980 for examples in common relationships for sex-linked loci). The quantities $I_{i,j}$ and $\psi_{i,j}$ are invariant with respect to both the probability of an allele being expressed at a locus and the level of expression of that allele due to dosage compensation. Therefore, $I_{i,j}$ and $\psi_{i,j}$ are the same for all models of dosage compensation (Table 4.4). This implies that the portion of phenotypic resemblance between two individuals attributable to the sex-linked additive genotypic covariance varies among the mechanisms of dosage compensation by the differences in the product $L_i \times L_j$. When the sex-linked additive genetic variance is expressed in terms of the sex-linked variance in the homogametic sex, the genotypic covariance between two relatives of the homogametic sex remains the same in all models.

Equation 7 can be written in matrix notation, to encompass all pairs of individuals in a population at once, as $\mathbf{S}\sigma^2_{S\text{-}hom}$ (where upper-case letters in bold-faced type represent a matrix. In **S**, each element $s_{i,j}$ is the genotypic covariance between individuals in the *i*th row and the *j*th column [$C(g_i, g_j)$].

Mixed model for the prediction of genetic effects

Mixed effect statistical models can be used to partition phenotypic variation in a population into genetic and environmental sources of variation. The variance partitioning approach assumes that random effects (e.g., autosomal and sex-linked additive genetic effects) describe individual deviations from the population mean caused by different sources of variation (Lynch and Walsh 1998). Mixed effect models can be used to estimate autosomal and sex-linked additive genetic

sources of variance from expressions of covariances among individuals. Using matrices containing all pairwise genotypic covariances between individuals sharing sex-linked alleles, developed above, we can obtain estimates of sex-linked additive genetic variance under any form of dosage compensation. Consequently, we can use these mixed effect models to test Fernando and Grossman's (1990) conjecture that the presence and form of dosage compensation can be assessed using likelihood based comparisons between mixed effect statistical models that each describe sex-linked additive genetic variation under a different form of dosage compensation.

A simple description of an individual's phenotype for a polygenic trait, y_i, is:

$$y_i = \mu + \alpha_{A\text{-}i} + \alpha_{S\text{-}i} + e_i \quad (8)$$

Here, μ is the phenotypic mean in a population, $\alpha_{A\text{-}i}$ is the phenotypic deviation caused by the autosomal additive genotype of individual *i* (autosomal breeding value), $\alpha_{S\text{-}i}$ is the phenotypic deviation caused by the sex-linked additive genotype of individual *i* (sex-linked breeding value), and e_i represents the phenotypic deviation caused by residual effects experienced by individual *i* (e.g., non-additive genetic or environmental sources of variation). For a population with both phenotypic measures and information indicating every individual's parents (a pedigree), statistical predictions for the autosomal breeding values, sex-linked breeding values, and environmental deviations are obtained using a linear mixed effects model known as the animal model (Henderson 1973; Lynch and Walsh 1998). A univariate animal model, for a trait with phenotypic values determined as in equation 8, takes on the matrix form:

$$\mathbf{y} = \mathbf{X}\mu + \mathbf{Z}_A\boldsymbol{\alpha}_A + \mathbf{Z}_S\boldsymbol{\alpha}_S + \mathbf{e} \quad (9)$$

In the simple case where all *n* individuals in a population are measured once for phenotype *y*, then equation 9 describes the *n*x1 vector of phenotypes, **y**, as a function of the population mean μ, plus the *n*x1 vector of autosomal breeding values for each individual in $\boldsymbol{\alpha}_A$ (where lower-case characters in bold-faced type indicate vectors), plus the *n*x1 vector of sex-linked breeding values for each individual in $\boldsymbol{\alpha}_S$, and the *n*x1 vector of residual deviations in vector **e**. The matrices **X**, $\mathbf{Z_A}$, and $\mathbf{Z_S}$ are *n*x1, *n*x*n*, and *n*x*n* incidence matrices relating the fixed and random effects to each observation in **y**. The random effects ($\boldsymbol{\alpha}_A$, $\boldsymbol{\alpha}_S$, and **e**) are assumed to follow independent Gaussian distributions. These are expressed as functions of the mean and variance of the distributions by: $\boldsymbol{\alpha}_A \sim N(0, \mathbf{A}\sigma^2_A)$, $\boldsymbol{\alpha}_S \sim N(0, \mathbf{S}\sigma^2_{S\text{-hom}})$, and $\mathbf{e} \sim N(0, \mathbf{I}\sigma^2_E)$. Here, **I** is an identity matrix (*n*x*n* with 1s along the diagonal) reflecting the assumption that the residual deviations are uncorrelated among individuals.

Estimates of variance components in an animal model ($\hat{\sigma}^2_A$, $\hat{\hat{\sigma}}^2_{S\text{-hom}}$, and $\hat{\sigma}^2_E$) are obtained from the mixed model equations:

$$\begin{bmatrix} \mathbf{X^T X} & \mathbf{X^T Z}_A & \mathbf{X^T Z}_S \\ \mathbf{Z}_A^{\mathbf{T}}\mathbf{X} & \mathbf{Z}_A^{\mathbf{T}}\mathbf{Z}_A + \dfrac{\hat{\sigma}^2_E}{\hat{\sigma}^2_A}\mathbf{A^{-1}} & \mathbf{Z}_A^{\mathbf{T}}\mathbf{Z}_S \\ \mathbf{Z}_S^{\mathbf{T}}\mathbf{X} & \mathbf{Z}_S^{\mathbf{T}}\mathbf{Z}_A & \mathbf{Z}_S^{\mathbf{T}}\mathbf{Z}_S + \dfrac{\hat{\sigma}^2_E}{\hat{\sigma}^2_{S-\text{hom}}}\mathbf{S^{-1}} \end{bmatrix} \begin{bmatrix} \hat{\beta} \\ \hat{\alpha}_A \\ \hat{\alpha}_S \end{bmatrix} = \begin{bmatrix} \mathbf{X^T y} \\ \mathbf{Z}_A^{\mathbf{T}}\mathbf{y} \\ \mathbf{Z}_S^{\mathbf{T}}\mathbf{y} \end{bmatrix} \quad (10)$$

(Henderson 1973; Fernando and Grossman 1990). As can be seen in equation (10), solving this system of linear equations requires the inverses of the autosomal and sex-linked additive genetic covariance matrices ($\mathbf{A^{-1}}$ and $\mathbf{S^{-1}}$). Algorithms have been developed for constructing $\mathbf{A^{-1}}$ directly, bypassing the computationally intensive process of preliminary construction of **A** followed by

matrix inversion (e.g., Henderson 1976; Meuwissen & Luo 1992). Fernando and Grossman (1990) developed an algorithm to directly construct $\mathbf{S}^{-1}$, assuming no global dosage compensation mechanism (NGDC). However, no algorithms have been adapted for the direct construction of $\mathbf{S}^{-1}$ under any model of dosage compensation.

Direct computation of $\boldsymbol{S}^{-1}$ with dosage compensation

The sex-linked additive genetic value for individual *i* ($\alpha_{S\text{-}i}$) can be described by a linear equation which includes the contributions of the parental sex-linked breeding values to an individual plus a deviation caused by Mendelian sampling of parental genotypes during gametogenesis (Fernando and Grossman 1990). Table 4.5 presents these equations for both sexes under the four forms of dosage compensation and the case without dosage compensation. Considering all individuals in a population at once, a vector of sex-linked breeding values is predicted by:

$$\boldsymbol{\alpha}_S = \mathbf{P}\boldsymbol{\alpha}_{S\text{-}p} + \boldsymbol{\varepsilon} \qquad (11)$$

(Quaas 1988; Fernando and Grossman 1990). In equation 11 the matrix **P** relates progeny to parents, $\boldsymbol{\alpha}_{S\text{-}p}$ is the vector of parental sex-linked breeding values, and $\boldsymbol{\varepsilon}$ is the vector of deviations caused by Mendelian sampling. The non-zero elements in row *i* of **P**, for individual *i*, are either 1 or 0.5 in the columns representing the dam or sire of individual *i*. Whether the element in **P** for a parent of *i* is 1 or 0.5 depends on the specific form of dosage compensation and which parent is the homogametic sex. The elements of **P** under all models of dosage compensation are shown in table 4.5 as the coefficients of the parental sex-linked breeding values. If both parents are unknown for an individual, all elements of **P** are zero in the row corresponding to that individual (except the diagonal which is always one). For example, under the NGDC model, an individual

of the homogametic sex will have an entry of 0.5 in the column corresponding to its homogametic parent and a one in the column corresponding to its heterogametic parent. For an individual of the heterogametic sex, it will only have a 0.5 in the column corresponding to its homogametic parent.

From equation 11, the covariance matrix of $\boldsymbol{\alpha}_S$ (or sex-linked relatedness matrix **S**) is obtained by taking the variance of both sides of equation 9:

$$V(\boldsymbol{\alpha}_S) = \mathbf{S}\sigma^2_{S\text{-hom}} = (\mathbf{I} - \mathbf{P})^{-1}\,\mathbf{V}\,(\mathbf{I} - \mathbf{P}`)^{-1}\,\sigma^2_{S\text{-hom}} \qquad (12)$$

(Quaas 1988; Fernando and Grossman 1990). Here, **P`** denotes the matrix transpose of **P** and $(\mathbf{I} - \mathbf{P})^{-1}$ represents a matrix that contains each individual's expected contribution of sex-linked genes from all of its ancestors. The matrix **V** is the covariance in Mendelian sampling deviations ($\boldsymbol{\varepsilon}$) for all members of the population. However, all off-diagonal elements (covariance between individuals in their Mendelian sampling deviations) can be shown to equal zero (Fernando and Grossman 1990). The diagonal elements of **V** are the Mendelian sampling variances for each individual, which are functions of the inbreeding coefficients at a sex-linked locus.

Taking the inverse of both sides to equation 12 yields the inverse of the sex-linked relationship matrix:

$$\mathbf{S}^{-1} = (\mathbf{I} - \mathbf{P})\,\mathbf{V}^{-1}\,(\mathbf{I} - \mathbf{P}`), \qquad (13)$$

which can be constructed according to simple rules (Henderson 1976; Fernando and Grossman 1990). The algorithm specific to the NGDC model is presented in Fernando and Grossman (1990). Construction of **P** under alternative models of dosage compensation is a straightforward

matter of substituting 0s, 0.5s, and 1s depending on the specific form of dosage compensation (Table 4.5). However, completion of the right hand side of equation 13 requires the variance in Mendelian sampling deviations to be calculated specifically for each model of dosage compensation.

*Computation of the diagonal elements of **V***

Fernando and Grossman (1990) specify the values along the diagonal of the Mendelian sampling variance matrix (**V**) for a model with no global dosage compensation (NGDC). Here, we derive the values for the four dosage compensation models considered above. The work below closely follows the derivation for the autosomal case presented by Mrode (pp27-28 ch. 2.2, 2005) and Quaas (1988). Therefore, after setting up a general equation that applies to sex-chromosomal variation under any form of dosage compensation, we only list the final expressions for each case (Table 4.6). Finally, note that **V** is constructed below, whereas $\mathbf{V}^{-1}$ is the necessary quantity for the direct construction of $\mathbf{S}^{-1}$. However, element *i* along the diagonal of $\mathbf{V}^{-1}$ is simply the reciprocal of element *i* along the diagonal of **V** ($v_i^{-1} = 1/v_i$).

For an individual *i*, with both the homogametic parent *hom* and heterogametic parent *het* known in the population, a general formula for the *i*th diagonal element of **V** is:

$$v_{i,i}=(I_{i,i}\times\psi_{i,i}\times L_i^2)-P_{i,hom}^2\times(I_{hom,hom}\times\psi_{hom,hom}\times L_{hom}^2)-P_{i,het}^2\times(I_{het,het}\times\psi_{het,het}\times L_{het}^2)$$
$$-2\times P_{i.hom}\times P_{i,het}\times(I_{hom,het}\times\psi_{hom,het}\times L_{hom}\times L_{het}) \qquad (14)$$

Here, L_i is the coefficient of dosage compensation appropriate to the sex of *i*, $P_{i,hom}$ and $P_{i,het}$ are the elements in the *i*th row and either *hom* or *het* column of the matrix **P** (or the coefficient of the appropriate parent from table 4.5), and L_i, L_{hom}, and L_{het} are the coefficients of dosage

compensation for individual *i*, the homogametic parent, and heterogametic parent, respectively. The first term in equation 14 is the variance for the sex-linked breeding value of individual *i*. The second and third terms in equation 14 represent the variances of the homogametic and heterogametic parents' sex-linked breeding values, respectively, each multiplied by the square of the corresponding coefficient from the **P**-matrix. The final term is $2P_{i,hom}P_{i,het}$ times the covariance in sex-linked breeding values between the homogametic and heterogametic parents of individual *i*. Equation 14 is derived from re-arranging equation 11 to express the Mendelian sampling deviation of individual *i* as the difference between the sex-linked breeding value for individual *i* and its parent's average sex-linked breeding value and subsequently taking the variance of both sides. Note the heterogametic sex parent does not contribute a shared sex chromosome (X or Z) to offspring of the heterogametic sex. Therefore, the third and fourth terms of equation 14 drop out of the equation for heterogametic sex individuals.

For an individual *i* of the homogametic sex, if the homogametic parent is unknown, but the heterogametic parent is known, then the third and fourth terms drop out of equation 14 to yield:

$$v_{i,i}=(I_{i,i}\times\psi_{i,i}\times L_i^2) - P_{i,hom}^2\times(I_{hom,hom}\times\psi_{hom,hom}\times L_{hom}^2) \quad (15)$$

Whereas, if the heterogametic parent is unknown, but the homogametic parent is known, then the second and fourth terms of equation 14 drop out and the diagonal element of **V** is:

$$v_{i,i}=(I_{i,i}\times\psi_{i,i}\times L_i^2) - P_{i,het}^2\times(I_{het,het}\times\psi_{het,het}\times L_{het}^2) \quad (16)$$

If both parents are unknown, $v_{i,i}=(I_{i,i}\times\psi_{i,i}\times L_i^2)$. For individual *i* of the heterogametic sex, if the homogametic parent is not known, $v_{i,i}=(I_{i,i}\times\psi_{i,i}\times L_i^2)$. Substitution of I, L, ψ, and P from a given model of dosage compensation into the above equations gives the specific values in table 4.6. Note the HEDO, HORI, and HOHA forms of dosage compensation have equivalent values in tables 4.4 and 4.5 and therefore produce the same values for the expected Mendelian sampling variance (Table 4.6).

The algorithm for the direct construction of $\mathbf{S}^{-1}$ under the various models of dosage compensation is coded in the R language for statistical computing (R Development Core team, 2012) and is freely available in the R package **nadiv** (v2.12, Wolak 2012) or by contacting the first author. Output from the **nadiv** function `makeS` can be used in conjunction with animal model software, such as **MCMCglmm** (Hadfield, 2010), ASReml (Gilmour et al. 2009; Butler 2009), or WOMBAT (Meyer 2007; for specific details about interfacing **nadiv** output with these animal model software programs, see Wolak 2012) to estimate sex-linked additive genetic (co)variances.

What to do if the mechanism of global dosage compensation is unknown?

Choosing the appropriate model of sex chromosome dosage compensation to include in an animal model is a problem for most empirical systems. The presence or specific type of global dosage compensation is only known in a few organisms (Table 4.1). Some general patterns do emerge which can be used to guide the empiricist (e.g., birds and lepidopterans seem not to have a global sex chromosome dosage compensation mechanism). However, evidence regarding the mechanism of dosage compensation is very spotty both across major taxa (reptiles are noticeably absent) as well as within taxa (only one species of beetle!). Further, different mechanisms of dosage compensation have been found in related species within taxa (e.g., within orthopterans

Acheta domesticus shows evidence of HEDO whereas results from *Gryllotalpa fossor* suggest a HORI mechanism). Therefore, it is unreliable to use related taxa as an informative guide.

Despite this, comparisons among the sex-linked relatedness matrices that arose from our model above suggest minor consequences for using a biologically incorrect model of dosage compensation in an animal model analysis. This somewhat non-intuitive result can be understood by comparing the different sex-linked relatedness matrices as they affect the distribution of sex-linked breeding values in a population. Above we noted that, for a specific population, the sex-linked breeding values for a single trait follow an approximately Gaussian distribution with a mean of zero and (co)variance of $\mathbf{S}\sigma^2_{\text{S-hom}}$. Differences between sex-linked relationship matrices under the various models of dosage compensation only differ from one another at elements representing heterogametic-heterogametic pairs or opposite sex pairs (entries for the homogametic-homogametic sex pairs are consistent from mechanism to mechanism; see Table 4.4). The covariance between relatives from a relationship involving an individual of the heterogametic sex will only differ from one model of dosage compensation to the other by the coefficients of dosage compensation. Therefore, the relationship matrices (and similarly for the inverse of the relationship matrix used in the animal model) are interchangeable between models of dosage compensation in the sense that element-wise multiplication (i.e., a Hadamard product) of a relationship matrix by a matrix of constants (representing the difference in the coefficients of dosage compensation) will translate between relationship matrices for the different models of dosage compensation.

We illustrate this with a simple example of a full-sib family where the pedigree is ordered sire, dam, son, and daughter. For simplicity, we compare only the NGDC and HORI matrices and specify males as the heterogametic sex. Assuming a sex-linked additive genetic variance ($\sigma^2_{\text{S-hom}}$) of 50, the distribution of sex-linked breeding are:

$$\mathbf{S}_{\text{NGDC}}\,\sigma^2_{\text{S-hom}} = \begin{bmatrix} 25 & & & \\ & 50 & & \\ & 25 & 25 & \\ 25 & 25 & 12.5 & 50 \end{bmatrix}, \quad \mathbf{S}_{\text{HORI}}\,\sigma^2_{\text{S-hom}} = \begin{bmatrix} 100 & & & \\ & 50 & & \\ & 50 & 100 & \\ 50 & 25 & 25 & 50 \end{bmatrix}$$

(For simplicity, we show only elements on or below the diagonal). From these matrices, the above relationships between sex-linked variances in the two sexes can be seen. For NGDC, the sex-linked variance for non-inbred males (heterogametic sex) is one half (diagonal elements equal to 25) that of non-inbred females (homogametic sex; diagonal elements equal to 50). However, in the HORI model, male sex-linked variance is twice that (100) of the female variance (50). Also note the covariance between males and females is one-half the sex-linked variance in the NGDC model (e.g., sire-daughter covariance=25), whereas it equals the sex-linked variance in the HORI model (e.g., sire-daughter covariance=50). These results are the simple application of different coefficients of dosage compensation in table 4.4. The combined effect is that the $\mathbf{S}_{\text{HORI}}$ matrix is the element-wise product of the $\mathbf{S}_{\text{NGDC}}$ matrix with a scalar matrix, whose elements are the element-by-element ratio of HORI coefficients of dosage compensation to NGDC coefficients of dosage compensation:

$$\mathbf{S}_{\text{NGDC}}\,\sigma^2_{\text{S-hom}} \,\#\, \mathbf{S}_{\text{scalar}} = \begin{bmatrix} 25 & & & \\ & 50 & & \\ & 25 & 25 & \\ 25 & 25 & 12.5 & 50 \end{bmatrix} \# \begin{bmatrix} 4 & & & \\ & 1 & & \\ & 2 & 4 & \\ 2 & 1 & 2 & 1 \end{bmatrix} = \mathbf{S}_{\text{HORI}}\,\sigma^2_{\text{S-hom}}$$

Here the # symbol denotes the element-wise (or Hadamard) product of two matrices. The opposite calculation involves using a matrix with the reciprocal values of $\mathbf{S}_{scalar}$.

Because the sex-linked relatedness matrices under different forms of dosage compensation (and for the purposes of solving the mixed model equations, the inverses of these matrices) only differ from one another by a scalar amount, in an ideal experiment animal models which use any of the dosage compensation relatedness matrices will produce equal maximum log likelihoods upon convergence. This occurs because the difference between relationship matrices, or more basically the distribution of sex-linked breeding values in a population, is a constant amount from one form of dosage compensation to another (or to NGDC). This constant amount does not affect the calculations concerned with maximizing the log-likelihood of the models. Therefore, Fernando and Grossman's (1990) conjecture is not supported since likelihood ratio tests between animal models cannot be used to determine the form of dosage compensation underlying the phenotypic distribution of a trait.

Discussion

Above, we derive a general model to express the genotypic covariance between relatives under all possible alternative mechanisms of global sex chromosomal dosage compensation. We also extended the algorithms used to construct sex-linked relatedness matrices for use in the animal model to include these alternative models of dosage compensation and supply R code for this in the **`nadiv`** package. Thus, we have developed both the theory and the tools necessary to estimate sex-linked additive genetic variance from pedigrees under any of the known forms of global sex chromosome dosage compensation. Unfortunately, the animal model cannot be used to detect one form of dosage compensation over another as originally suggested (Fernando and Grossman 1990). However, since the differences between the alternative forms of dosage

compensation only serve to scale the sex-linked variance by a multiple of two, empiricists can estimate the variance in sex-linked breeding values without bias even when the underlying mechanism of global sex chromosome dosage compensation is unknown.

Above (Table 4.3), we assumed that an allele has the same genotypic value in both sexes and show that under NGDC the mean genotypic value of the homogametic sex is expected to be twice the mean in the heterogametic sex. However, in any of the above models there exist no *a priori* reasons to expect alleles at a sex-linked locus to have equal effects in the sexes. When alleles have sex-specific effects (i.e., genotypic values $a_{female} \neq a_{male}$) the relationship between the sex-linked variances in the homogametic and heterogametic sexes will deviate from those amounts described above (Table 4.3). The effect of an allele in the heterogametic sex can be expressed as a function of the effect of the same allele in the homogametic sex times the between-sex covariance in allelic effects. The between-sex covariance in sex-linked effects can be expressed in terms of the genotypic sex-linked variance. Thus, we can easily extend the above formulas to incorporate sex-bias in gene expression into estimates of sex-linked additive (co)variances using the animal model.

Dealing with genotype-by-sex interactions on the sex chromosomes in animal models uses the same approach to the way in which animal models handle genotype-by-sex interactions on the autosomes. A single trait can instead be thought of as two traits (the trait as expressed in the homogametic sex and the trait as expressed in the heterogametic sex) and estimates of the sex-linked breeding values for a trait in the homogametic sex can be estimated for individuals of the heterogametic sex and vice versa. The result is that one individual has two breeding values for a given phenotype; one value expressing the additive effect of its genotype (i.e., breeding value) when the genotype is expressed in the homogametic sex and another value expressing the breeding value when the individual's genotype is expressed in the heterogametic sex. The

covariance between the two breeding values across the entire population is the sex-linked between-sex additive genetic covariance.

In an animal model, this is analogous to fitting a bivariate model where trait one is the phenotype of interest measured on females and trait two is the phenotype of interest measured on males (Mrode 2005). The sex-specific sex-linked variances and between-sex covariance estimates from an animal model, all expressed as functions of the homogametic sex, can be transformed to reflect the actual variance in sex-linked breeding values for the heterogametic sex trait (i.e., divide the variance estimate in the heterogametic sex by two for NGDC or multiply by two for HEDO/HORI/HOHA) or the covariance between the two sexes (i.e., again divide the covariance estimate by two for NGDC or multiply by two for HEDO/HORI/HOHA). Since the variances are equal in the HOPI model, no transformation is necessary.

Sex-biased gene expression indicates locus-specific genotype-by-sex interactions producing different effects of an allele depending on the sex in which the allele is being expressed. Global dosage compensation is not the same as sex-biased gene expression (Mank 2009; Mank and Ellegren 2009b). The former constitutes a chromosome-wide mechanism to equalize the effect of an allele on the single shared sex chromosome in the heterogametic sex with the two copies of the allele in the homogametic sex. Genotype-by-sex interactions are an explanation for the existence of sexual dimorphism despite the sexes sharing the same genome (Mank 2009) and will decrease the between-sex additive genetic correlation on the sex chromosomes. We note that the models developed above readily allow the incorporation of genotype-by-sex interactions, regardless of the form of global sex chromosome dosage compensation. This further enables researchers to empirically evaluate the genetic architecture underlying sexually dimorphic traits, with particular attention on sex-linked additive genetic (co)variances.

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Table 4.1. Evidence for the occurrence of global sex chromosome dosage compensation mechanisms in animals and plants. Taxonomic information from Myers et al. (2013).

Class	Order	Family	Organism	Sex chromosome system	Pattern of dosage compensation[†]	Reference
	Caryophyllales	Caryophyllaceae	*Silene latifolia*	XX/XY	NGDC	Muyle et al. 2012
Craniata	Galliformes	Phasianidae	*Gallus gallus* (Domestic chicken)	ZZ/ZW	NGDC	Kuroiwa et al. 2002; Itoh et al. 2007; Ellegren et al. 2007; Mank and Ellegren 2009a; Itoh et al. 2010; but see McQueen et al. 2001
Craniata	Gasterosteiformes	Gasterosteidae	*Gasterosteus aculeatus*	XX/XY	NGDC	Leder et al. 2010
Craniata	Passeriformes	Corvidae	*Corvus corone*	ZZ/ZW	NGDC	Wolf and Bryk 2011
Craniata (Infraclass: Eutheria)			(Placental mammals)	XX/XY	HORI	Lyon 1962; Johnston et al. 2008; Livernois et al. 2012
Craniata (Infraclass: Metatheria)			(Marsupial mammals)	XX/XY	HOPI	Cooper 1990
Craniata (Subclass: Prototheria)	Monotremata	Ornithorhynchidae	*Ornithorhynchus anatinus*	XXXXXXXXXX/ XYXYXYXYXY	NGDC	Deakin et al. 2008
Insecta	Coleoptera	Tenebrionidae	*Tribolium castaneum*	XX/XY	HEDO	Prince et al. 2010
Insecta	Diptera	Drosophilidae	*Drosophila*	XX/XY	HEDO	Lucchesi 1978; Vicoso and Bachtrog 2009
Insecta	Diptera	Sciaridae	*Sciara ocellaris*	XX/X0	HEDO	da Cunha et al. 1994
Insecta	Lepidoptera	Bombycidae	*Bombyx mori* (silkworm)	ZZ/ZW	NGDC	Arunkumar 2009; Zha et al. 2009; but see Walters and Hardcastle 2011

Insecta	Lepidoptera	Nymphalidae	*Heliconius erato*	ZZ/ZW	NGDC	Johnson and Turner 1979
Insecta	Lepidoptera	Nymphalidae	*Heliconius melpomene*	ZZ/ZW	NGDC	Johnson and Turner 1979
Insecta	Lepidoptera	Pyralidae	*Plodia interpunctella*	ZZ/ZW	NGDC	Harrison et al. 2012
Insecta	Orthoptera	Gryllidae	*Acheta domesticus*	XX/XY	HEDO	Rao and Ali 1982
Insecta	Orthoptera	Gryllotalpidae	*Gryllotalpa fossor*	XX/X0	HORI	Rao and Padmaja 1992
Secernentea	Rhabditida	Rhabditidae	*Caenorhabditis elegans*	XX/X0	HOHA	Vicoso and Bachtrog 2009; Meyer 2010
Trematoda	Strigeatida	Schistosomatidae	*Schistosoma mansoni*	ZZ/ZW	NGDC	Vicoso and Bachtrog 2011

†NGDC=no global doasge compensation, HEDO=doubling of expression in the heterogametic sex, HORI=random (with respect to parent of origin) inactivation of one allele in the homogametic sex, HOHA=halved expression for each allele in the homogametic sex, and HOPI=inactivation of the paternal allele in the homogametic sex

Table 4.2. Properties of a bi-allelic, sex-linked locus in a population. The average expression level of an allele (λ_i) enables all mechanisms of global dosage compensation to be modeled.

	Homogametic Sex			
Genotype	A_1A_1	A_1A_2	A_2A_1	A_2A_2
Frequency	p^2	pq	pq	q^2
Genotypic Value	$0.5a(\lambda_{p\text{-}hom} + \lambda_{p\text{-}het})$	$0.5a\lambda_{p\text{-}hom} - 0.5a\lambda_{p\text{-}het}$	$-0.5a\lambda_{p\text{-}hom} + 0.5a\lambda_{p\text{-}het}$	$-0.5a(\lambda_{p\text{-}hom} + \lambda_{p\text{-}het})$
	Heterogametic Sex			
Genotype	A_1			A_2
Frequency	p			q
Genotypic Value	$b\lambda_{p\text{-}hom}$			$-b\lambda_{p\text{-}hom}$

Table 4.3. Population-wide, sex-linked genotypic means and variances when dosage compensation is absent (NGDC) and under different forms of dosage compensation†.

		Form of Dosage Compensation				
	General expressions	NGDC	HEDO	HORI	HOHA	HOPI
Homogametic Sex						
mean=	$0.5a(\lambda_{p\text{-}hom} + \lambda_{p\text{-}het})(p\text{-}q)$	$a(p\text{-}q)$	$a(p\text{-}q)$	$0.5a(p\text{-}q)$	$0.5a(p\text{-}q)$	$0.5a(p\text{-}q)$
V(ghom)=	$pqa^2[2(\lambda_{p\text{-}hom}+\lambda_{p\text{-}het})^2pq+(p^2+q^2)(\lambda^2_{p\text{-}hom}+\lambda^2_{p\text{-}het})-4pq\lambda_{p\text{-}hom}\lambda_{p\text{-}het}]$	$2pqa^2$	$2pqa^2$	$0.5pqa^2$	$0.5pqa^2$	pqa^2
Heterogametic Sex						
mean=	$0.5a\lambda_{p\text{-}hom}(p\text{-}q)$	$0.5a(p\text{-}q)$‡	$a(p\text{-}q)$‡	$0.5a(p\text{-}q)$‡	$0.5a(p\text{-}q)$‡	$0.5a(p\text{-}q)$‡
V(ghet)=	$\lambda^2_{p\text{-}hom}pqa^2$	pqa^2‡	$4pqa^2$‡	pqa^2‡	pqa^2‡	pqa^2‡
Sex-linked additive genetic variance of the heterogametic sex ($\sigma^2_{S\text{-}het}$) in terms of the homogametic sex ($\sigma^2_{S\text{-}hom}$)		$0.5\sigma^2_{S\text{-}hom}$	$2\sigma^2_{S\text{-}hom}$	$2\sigma^2_{S\text{-}hom}$	$2\sigma^2_{S\text{-}hom}$	$1\sigma^2_{S\text{-}hom}$

†HEDO=doubling of expression in the heterogametic sex, HORI=random (with respect to parent of origin) inactivation of one allele in the homogametic sex, HOHA=halved expression for each allele in the homogametic sex, and HOPI=inactivation of the paternal allele in the homogametic sex

‡assuming the effect of an allele is the same in both sexes

Table 4.4. Components of the sex-linked genotypic covariance between two individuals, *i* and *j* when dosage compensation is absent (NGDC) and under different forms of dosage compensation†.

			Form of Dosage Compensation				
			NGDC	HEDO	HORI	HOHA	HOPI
	$I_{i,j}$	$\psi_{i,j}$‡	$L_{i,j}$	$L_{i,j}$	$L_{i,j}$	$L_{i,j}$	$L_{i,j}$
homogametic - homogametic	2	$P_1/4 + P_2/2$	1	1	1	1	1
heterogametic - heterogametic	1	P_1	0.5	2	2	2	1
homogametic - heterogametic	$\sqrt{2}$	$P_1/2$	$1/\sqrt{2}$	$\sqrt{2}$	$\sqrt{2}$	$\sqrt{2}$	1

†Forms of Dosage Compensation as in Table 4.3

‡P_1 and P_2 are the probabilities of 1 and 2 pairs of identical genes at a locus, respectively

Table 4.5. Sex-linked additive genetic breeding values as a function of parental breeding values and Mendelian sampling when dosage compensation is absent (NGDC) and under different forms of dosage compensation†.

	NGDC	HEDO	HORI	HOHA	HOPI
$\alpha_{S\text{-}hom,i}$=	$0.5\alpha_{S\text{-}hom,p} + \alpha_{S\text{-}het,p} + \varepsilon_i$	$0.5\alpha_{S\text{-}hom,p} + 0.5\alpha_{S\text{-}het,p} + \varepsilon_i$	$0.5\alpha_{S\text{-}hom,p} + 0.5\alpha_{S\text{-}het,p} + \varepsilon_i$	$0.5\alpha_{S\text{-}hom,p} + 0.5\alpha_{S\text{-}het,p} + \varepsilon_i$	$0.5\alpha_{S\text{-}hom,p} + \varepsilon_i$
$\alpha_{S\text{-}het,i}$=	$0.5\alpha_{S\text{-}hom,p} + \varepsilon_i$	$\alpha_{S\text{-}hom,p} + \varepsilon_i$	$\alpha_{S\text{-}hom,p} + \varepsilon_i$	$\alpha_{S\text{-}hom,p} + \varepsilon_i$	$0.5\alpha_{S\text{-}hom,p} + \varepsilon_i$

†Forms of Dosage Compensation as in Table 4.3.

Table 4.6. Equations to calculate the Mendelian sampling variance in sex-linked genetic effects for a given individual under the assumption of no global dosage compensation (NGDC) and four different forms of dosage compensation†.

	Form of Dosage Compensation		
	NGDC	HEDO / HORI / HOHA	HOPI
both parents known			
homogametic sex	$0.25(1 - F_{p\text{-}hom})$	$0.25(1 - F_{p\text{-}hom})$	$0.25(3\text{-}F_{p\text{-}hom})$
heterogametic sex	$0.25(1 - F_{p\text{-}hom})$	$1 - F_{p\text{-}hom}$	$0.25(3\text{-}F_{p\text{-}hom})$
only homogametic sex parent unknown			
homogametic sex	0.5	0.5	1
heterogametic sex	0.5	2	1
only heterogametic sex parent unknown			
homogametic sex	$0.25(3\text{-}F_{p\text{-}hom})$	$0.25(3\text{-}F_{p\text{-}hom})$	$0.25(3\text{-}F_{p\text{-}hom})$
both parents unknown			
homogametic sex	1	1	1
heterogametic sex	0.5	2	1

†Forms of Dosage Compensation as in Table 4.3.

CHAPTER 5

Sex differences in epistatic effects covary with the magnitude of sexual dimorphism in population crosses of water striders (*Aquarius remigis*).

Abstract

Phenotypic differences between females and males are widely observed in organisms with separate sexes. How these sexual dimorphisms evolve despite the sexes sharing a majority of their genome remains an unresolved issue in evolutionary biology as theoretical models often disagree over the genetic mechanisms that are predicted to facilitate the evolution of sexual dimorphism. Here we present results from population crosses in the water strider *Aquarius remigis* that were conducted to assess the empirical support for previous theoretical predictions. We improve on previous model selection and parameter estimation protocols used with joint-scaling tests by introducing Akaike Information Criterion based multimodel inference when estimating the genetic effects responsible for the phenotypic differences among the 10 lines in each of our two population crosses. Our results show significant differences between the sexes in the magnitude of composite genetic effects. Further, between-sex differences in the three digenic epistatic effects and dominance maternal genetic effects are strongly associated with the magnitude of sexual dimorphism across a range of morphological traits. As sexual dimorphism increased so too did the difference between female and male estimates of these four composite genetic effects. Our results provide support for the prediction of sex-specific non-additive genetic effects facilitating the evolution of sexually dimorphic traits and have particular relevance in light of the previously noted role of epistasis and sexual conflict in the process of speciation.

Introduction

Female and male organisms often have different phenotypes that we recognize as dimorphisms in primary and secondary sexual traits (Darwin 1874; Ghiselin 1974; Hedrick and Temeles 1989; Fairbairn 1997; Badyaev and Hill 2003; Fairbairn et al. 2007; Fairbairn 2013). Sexual dimorphisms evolve and are maintained by selection favoring sex-specific optimal phenotypes (*op. cit.* and Meagher 1992; Roff 1997; Blanckenhorn 2005). However, correlations between the sexes in the expression of shared alleles impede the evolution of sexual dimorphism (Lande 1980; Reeve and Fairbairn 2001), creating genetic conflict between the sexes (Parker 1979; Arnqvist and Rowe 2005; Bonduriansky and Chenoweth 2009). Because females and males share the majority of their genomes, genetic correlations for homologous traits between the sexes are widespread and generally high (>0.8, Roff 1997; Lynch and Walsh 1998; Poissant et al. 2010). Therefore, a major focus in evolutionary biology is the identification of genetic mechanisms that allow sexual dimorphisms to evolve in spite of shared genomes and strong genetic constraints on the independent evolution of the two sexes.

Selection that acts in an opposing manner in females and males, called sexually antagonistic selection, is common due to the divergent reproductive roles between the sexes (Bonduriansky 2007; Cox and Calsbeek 2009; Innocenti and Morrow 2010). When such selection acts at a given locus, it gives rise to intralocus sexual conflict (Rice 1992; Chippindale et al. 2001; Bonduriansky and Chenoweth 2009). Intralocus conflict can be resolved by genetic mechanisms that restrict allelic expression to one sex, such as sex-linkage, sex specific non-additive genetic effects, sex-limited gene expression, genomic imprinting, and condition dependence (Fisher 1958; Kidwell et al. 1977; Rice 1984; Rhen 2000; Rice and Chippindale 2001; Rice and Chippindale 2002; Day and Bonduriansky 2004; Bedhomme and Chippindale 2007; Bonduriansky 2007; Bonduriansky and Chenoweth 2009; Fry 2010).

Evolutionary theory predicts that alleles with sex-specific fitness effects will be located on or translocated to the sex chromosomes, where these occur (i.e., sex-linkage; Fisher 1958; Charlesworth and Charlesworth 1980; Rice 1984; Rice and Chippindale 2001; Charlesworth 2002; Gibson et al. 2002). In organisms with XX/XY or XX/XO chromosomal sex determination systems, X-linked recessive alleles are always expressed in males and crossing over only occurs between sex chromosomes in females (note that throughout we restrict discussion to XX/XY or XO systems, however the following arguments and results also apply to female heterogametic, ZZ/ZW, systems by reversing the sexes from the X-linked case presented). When experiencing sexually antagonistic selection, a rare recessive allele located on the X chromosome and beneficial to males, but deleterious in females, is exposed to selection in males. However, it is hidden from selection disfavoring the male beneficial allele in all but the small fraction of females with the homozygous recessive genotype. A similar situation can be shown for the spread of a rare dominant allele beneficial in females, but deleterious in males. Additionally, loci with sexually antagonistic alleles are predicted to accumulate on the X chromosome at a higher rate than autosomes (Charlesworth et al. 1987), because of the way the interaction of effective population size and variance in reproductive success differs between the autosomes and sex chromosomes (Caballero 1995; Charlesworth 2001; Laporte and Charlesworth 2002; Vicoso and Charlesworth 2009). Altogether, these properties lead to the prediction that alleles with sexually antagonistic fitness effects should be overrepresented on the X chromosome (Kidwell et al. 1977; Curtsinger 1980; Rice 1984; Hedrick and Parker 1997).

However, it remains undetermined whether or not sexually antagonistic genes are in fact overabundant on X chromosomes (Curtsinger 1980; Reinhold 1998; Gibson et al. 2002; Parisi et al. 2003; Fitzpatrick 2004; Bonduriansky 2007; Long and Rice 2007; Mank 2009; Innocenti and Morrow 2010) and to date only a few empirical studies support this prediction (Chippindale et al.

2001; Gibson et al. 2002; Pischedda and Chippindale 2006; Foerster et al. 2007; Connallon and Jakubowski 2009). Further, the growing body of literature investigating epigenetic inheritance patterns and sexual dimorphism in organisms without chromosomal sex determination (e.g., some fish and reptiles) suggests that autosomes are a plausible location for sexually antagonistic variation (Mank 2009). Finally, the prediction of X-linkage from Rice's (1984) model is sensitive to an assumption of equal dominance in each sex and a more recent model predicts that genes with sex-specific dominance effects on fitness will more often accumulate on the autosomes when experiencing sexually antagonistic selection (Fry 2010).

Though the predictions from theoretical models are useful for identifying potential genetic mechanisms mitigating intralocus sexual conflict, most models to date (e.g., Kidwell et al. 1977; Rice 1984; Fry 2010) have been based on single-locus genetics and hence do not adequately predict the complexities of the genetic architectures underlying the polygenic bases for sexually dimorphic traits (Mank 2009; Connallon and Clark 2010). Empirical study of the properties of genetic architectures that facilitate the evolution of sexual dimorphism has focused on (*i*) quantifying differences between the sexes at the level of gene expression and how sex-specific selection shapes the genome (e.g., Gibson et al. 2004; Ellegren and Parsch 2007; Mank 2009), (*ii*) separating the autosomal versus sex-linked contribution of additive genetic variance to population-wide variation and how these are associated with sexual dimorphism (e.g., Fairbairn and Roff 2006; Poissant et al. 2010; Husby et al. 2012), and (*iii*) contrasting the allelic effects and gene frequencies between populations or inbred lines to locate mechanisms underlying sexually dimorphic traits (e.g., Reinhold 1994, 1998; Wolfenbarger and Wilkinson 2001). The line cross approach (i.e., method *iii*) is particularly advantageous because it can be used to identify the location (autosomal vs. sex-chromosomal) and characterize the mode (e.g., additive, dominant, epistatic, etc.) of genetic effects contributing to sexually dimorphic traits, while simultaneously

characterizing the phenotypic impact of these genetic effects. The latter point is an advantage of the line cross method over gene expression studies (i.e., method *i*). Line crosses quantify the net effect of gene action leading to phenotypic differences between populations. Thus, line cross analyses may not provide as much insight into the within-population gene action and evolutionary potential (e.g., genetic variation) as population variance partitioning methods (i.e., method *ii*). However, line cross analyses are more suitable than variance partitioning methods for studying the function and importance of epistasis in evolutionary dynamics. This is evidenced by a robust body of empirical work studying the role of epistasis in natural populations through line crosses (e.g., Hard et al. 1992; Lair et al. 1997; Armbruster et al. 1998; Fenster and Galloway 2000; Fox et al. 2004) as opposed to the variance partitioning approach. The latter has largely ignored epistasis because of the inherent difficulty in obtaining estimates and the fact that variance partitioning is overall a less informative approach to studying non-additive aspects of genetic architectures, such as distinguishing between directional and non-directional epistasis (Hansen in press).

Past line cross analyses have been able to identify many of the genetic mechanisms proposed to explain the evolution of sexually dimorphic traits. Sex-differences in heterosis have long been observed in crosses between lines or populations (e.g., Cox 1960; Stonaker 1963; White et al. 1970) and a few line cross analyses find evidence for sex-specific patterns of non-additive genetic effects (e.g., Fox et al. 2004, 2011). Also, large effects of X-linked genes are commonly found in line crosses (e.g., Grula and Taylor 1980; Barbato 1991; Hagger and Stranzinger 1992; Reinhold 1994, 1998, 2002; Wolfenbarger and Wilkinson 2001) and are primarily associated with sexually selected traits (Reinhold 1999; Wolfenbarger and Wilkinson 2001). Despite the accumulating evidence from line cross analyses for both X-linkage and sex-

specific genetic architectures, the extent to which the presence and magnitude of these effects are associated with the magnitude of sexual dimorphism has yet to be investigated.

We use line crosses between populations of the water strider *Aquarius remigis* to test the prediction that sex-specific selection will lead to the evolution of sex-linked and/or sex-specific non-additive genetic effects. Our experimental design allows us to quantify the autosomal and X-linked sources of additive and non-additive genetic effects in each sex across a range of traits varying in both the direction and magnitude of sexual dimorphism. We present the results of two different population crosses, with 10 genetic lines in each experimental cross. To quantify the genetic architecture responsible for phenotypic differences among these lines, we demonstrate a novel application of multimodel inference to improve upon previous protocols for analyzing population crosses. Using this robust methodology, we show that the contribution of non-additive and X-linked genetic effects to observed differences among lines differs between the sexes. Finally, we show that the magnitude of differences between the sexes in epistatic genetic effects correlates positively with the magnitude of sexual dimorphism as predicted.

Materials and methods

Study organism

The water strider *Aquarius remigis* is a common and widespread semi-aquatic insect throughout North America, inhabiting the surfaces of streams and small rivers (Scudder 1971; Calabrese 1979; Polhemus and Chapman 1979; Preziosi and Fairbairn 1992; Gallant et al. 1993). Adults range from 12-16mm in total length (Fairbairn 2005) and take about 35 days to develop from egg laying to final molt when reared at 25° C. Most populations consist primarily of wingless, non-flying individuals and hence there is very little gene flow between populations on different streams (Zera 1981; Preziosi and Fairbairn 1992). Winged individuals and dispersal between

streams are more common in Californian populations (Kaitala and Dingle 1992; Fairbairn and King 2009), but allozyme studies nevertheless indicate significant genetic differentiation of Californian populations among different watersheds (Preziosi and Fairbairn 1992). Adults from geographically distinct populations interbreed readily in the laboratory (e.g., Gallant and Fairbairn 1997). As is typical of water striders (Hemiptera: Gerridae), *A. remigis* has an XX/XO system of chromosomal sex determination with 10 pairs of autosomes, an additional pair of X chromosomes in females, and a single X chromosome in males (the haploid autosomal number ranges from 9 to 11 in other gerrid species; Andersen 1982; Spence and Madison 1986; Kiseliova and Fairbairn, unpublished data).

Sexual dimorphism and sex-specific selection on morphological traits in the wild, throughout the adult lifespan of *A. remigis*, have been detailed extensively over the past three decades (e.g., Preziosi and Fairbairn 2000; Ferguson and Fairbairn 2000; Fairbairn 2007). Across their range, females average about 8% larger than males in total length but the magnitude and direction of sexual dimorphism varies greatly among different body components: genital components and the width of the front-femur are larger in males, female abdomens are larger than male abdomens, and thorax and leg components tend to not differ between the sexes. The most extreme dimorphisms are in genital length (2.5 to 3 times longer in males) and abdomen length (60% to 70% longer in females). Previous studies have found significant heritabilities for all body components in both sexes and significant genetic correlations among traits, both within and between the sexes (Preziosi and Roff 1998; Fairbairn 2007). Estimates of selection in the wild indicate that the magnitude and direction of these sexual dimorphisms reflect a balance between natural and sexual selection. Both sexes experience net stabilizing selection on total length (Preziosi and Fairbairn 2000). In females, selection on total length is driven by fecundity selection, favoring the evolution of longer abdomens, balanced by longevity selection favoring

the evolution of shorter total length (Preziosi and Fairbairn 1997, 2000). Males experience sexual selection for longer genitalia opposed by weaker sexual and longevity selection for small somatic size; resulting in the observed net stabilizing selection on total length (Preziosi and Fairbairn 1996; Ferguson and Fairbairn 2000; Preziosi and Fairbairn 2000; Bertin and Fairbairn 2005). Further, males also experience weak sexual selection favoring smaller mid-femur lengths (Preziosi and Roff 1998; Preziosi and Fairbairn 2000).

Collection, breeding design, and rearing protocols

We collected *A. remigis* from three populations in southern California to use in population line crosses. For the first experiment in the spring of 2010, we collected adults and nymphs from Prisoners' Stream on Santa Cruz Island (SCI; latitude=33.997529°N, longitude=119.715192°W; n=32 females, 45 males, and 80 nymphs) and from Rattlesnake Creek near Santa Barbara (RSC; latitude=34.459683°N, longitude=119.692105°W; n=119 females, 81 males, and 272 nymphs), approximately 50 km north of SCI. The second experiment was initiated in the spring of 2011 by collecting individuals from the same SCI location (n=90 females, 75 males, and 126 nymphs) and from a stream running through Los Laureles Canyon near Lake Cachuma in southern California (LLC; latitude=34.521504°N, longitude=119.839927°W; n=22 females, 37 males, and 123 nymphs), approximately 59km north-northwest of SCI and approximately 15km northwest of RSC. These populations were chosen to maximize the contrast in body size between the populations within each experiment. Water striders from SCI were used in both crosses and are among the largest in North America, whereas, RSC and LLC are similar in size and are among the smallest populations in North America (see Appendix table A1 in Fairbairn 2005). Because of the similarity in size between the RSC and LLC populations, we could not use line cross analyses to compare the genetic basis of morphological differences between these populations.

Individuals were collected from the wild and placed in laboratory stream tanks (140cm long x 45cm wide x 10cm deep) at room temperature (range 18-26°C) with a light:dark cycle of 14:10 hours. Females, males, and juveniles of each population were kept in separate tanks at approximately equal densities and were given Styrofoam cups as resting spots and a place for female oviposition. The juvenile tanks were checked daily for newly eclosed adults and any adults that were discovered were moved to a new tank containing individuals of the same sex and population. Allowing nymphs to eclose as adults in the laboratory ensured we had virgin females to use for mating to a male from a different population. After all newly eclosed females had been adults for at least seven days, we set up mating pairs in small (35cm long x 20cm wide x 7cm deep) cages located inside environmental growth chambers. The proportion of winged versus non-winged individuals used to create mating pairs reflected the distribution of wing morphologies found in each of the wild populations. We created 10 distinct genetic lines from each population cross in each experiment (Table 5.1).

The small rearing cages were filled with de-ionized water and arranged as blocks in growth chambers that were set to maintain a constant 25°C and 14:10hour light cycle. The different blocks in the growth chambers correspond to different shelves. Cages were ordered in a randomly assigned sequence according to the cross type. Mating pairs housed in the small rearing cages were given half of a Styrofoam cup and a disk of foam as resting and oviposition sites. Mating pairs were kept in the rearing cages until at least 20 eggs had developed eyespots. The presence and development of eggs was checked daily and pairs that had not produced any eggs were replaced. Once nymphs hatched, additional Styrofoam strips were added as resting locations. Upon eclosion as adults, individuals were uniquely marked and placed in laboratory stream tanks (separately for each sex and line). Individuals from the first generation of lab-reared crosses were later used to create the second generation using the same protocol as above. These

mating pairs were chosen to ensure even representation from each family within the first generation lines. Mating pairs were then assigned at random within lines, except for the F2 and F2r lines where extra care had to be taken to ensure no sibling matings (note, sibling mating is impossible in the backcross lines). Adults from the second generation were preserved one day post-eclosion in 70% ethanol.

Adults in stream tanks were given approximately half of a cricket (either adult *Gryllus firmus* or 6wk. old *Acheta domestica*) per day (6days/wk.) and supplemented with adult *Drosophila melanogaster*. Nymphs reared in the growth chamber were fed daily in excess of that required for maximum longevity (Blanckenhorn et al. 1995). This consisted of approximately 1-3 *Drosophila* per day for first through third instar nymphs. Beginning with the third instar, nymphs were given half of a 4wk. old *A. domestica* in addition to *Drosophila*.

Morphological traits were measured from digital photos of each individual, obtained using a Spot Insight 3.2.0 color camera attached to a Leica Wild M3c dissecting microscope. Using the digitizing software SigmaScan Pro 5.0, we took linear measurements of thorax length (Lthorax), abdomen length (Labd), total length (Ltotal; note that this measure includes Lthorax, Labd, Lgenital, and Lpyg which are also included in our analyses), and abdomen width (Wabd). We also measured length of the front femur (Lff), width of front-femur (Wff), length of mid-femur (Lmf), and length of hind-femur (Lhf). We measured the three legs on the right-hand side of the body in ventral view. If these legs were missing, deformed, or the landmarks were unclear, we instead measured the leg on the left-hand side of the body. We also measured total genital length (Lgenital), length of segment 8 (Lseg8), male pygophore length (Lpyg; sex-limited trait that is a component of Lgenital), length of segment 7 along the margin of the body (Lseg7mar), and width between the connexival spines (Wspine). Pictures were taken at 12.5x magnification for all somatic and leg components and at 20x magnification for the genital components. To

remove the confounding effects of wing morphology on the size of body components (Fairbairn 1992), only wingless individuals were used in the analyses (84% of striders reared were wingless).

X-linkage analyses

Comparison of the two female reciprocal hybrid lines, produced in the first generation of crossing (i.e., F1 and F1r) was used to assess the influence of maternal effects on phenotypic differences. If maternal effects are not present, then comparison of the first generation male reciprocal hybrids can be used to assess the extent to which X-linked effects differ between the two parent populations (Carson and Lande 1984; Nunney 1996). Male F1 and F1r lines both inherit a haploid set of autosomes from each population, but only a single X chromosome inherited from their maternal population. If maternal effects or X-linkage contribute to the difference between these lines, the direction of divergence between trait averages of each reciprocal line should be in the direction of each line's maternal population. Therefore, to test these hypotheses we used one-tailed tests. To control for the probability of incorrectly rejecting one or more true null hypotheses as a consequence of conducting multiple tests for the presence of X-linkage and/or maternal effects, a sequential Bonferroni adjustment (Holm 1979; Rice 1989) was applied to the resulting p-values from the female and male t-tests.

Line cross genetic analyses

We used joint-scaling tests to estimate the composite genetic effects explaining the differences between line means. Cavalli (1952) and Hayman (1960b) first suggested the joint scaling test as a method to test the significance of how well certain genetic models fit the observed line means. Joint-scaling tests use a least-squares regression of line means produced by a line cross breeding

design to estimate the net or composite genetic effects (the sum of the allelic effects across all loci on the genotypic value) responsible for the differences in phenotype observed between two populations (Hayman 1958, 1960a; Lynch and Walsh 1998 ch. 9). The matrix equation of the linear regression model is:

$$\bar{\mathbf{z}} = \mathbf{Ma} + \mathbf{e} \qquad (1)$$

where $\bar{\mathbf{z}}$ is a 10x1 vector containing our 10 line means, **M** is a matrix of coefficients that specify the contributions of composite genetic effects to the deviation of each line from the F_2 reference population, **a** is the *p*x1 vector of *p* composite genetic effects, and **e** is the 10x1 vector of residual errors (vector of deviations between observed and predicted line means). The matrix **M** contains all ones in the first column to estimate an intercept of the regression and an additional column for each of the following composite genetic effects: additive genetic [*a*], dominance genetic [*d*], three digenic epistatic ([*axa*], [*axd*], and [*dxd*]), maternal additive genetic [m_a], maternal dominance genetic [m_d], cytoplasmic genetic [*c*], additive genetic X-linked [X_a], and dominance genetic X-linked [X_d]. We used an **M** matrix with the F_2 line mean as the reference population in our analyses (see Appendix C1 Table C1.1). The F_2 has the advantage of being in Hardy-Weinberg and gametic phase equilibrium for genes derived within a population as well as those genes derived between populations (Lynch and Walsh 1998 p.206). Alternatively, a hypothetical population of F_∞ offspring could be used for the reference population (e.g., Kearsey and Pooni 1996). The choice of one particular reference population over another is trivial, as they yield similar results that can be translated between the two parameterizations (Basford and De Lacy 1979; Roff and Emerson 2006). Further, we used an **M** matrix specific to each sex for separate female and male regressions (e.g., Polak and Starmer 2001).

Typically, model fitting begins with a simple additive only genetic model and proceeds sequentially to include more genetic effects (e.g., dominance, epistasis, X-linkage, etc.) to improve the fit of the expectation from a particular genetic model to the observed data. The least-squares estimates of **a** are obtained by weighting the regression by the matrix **V** (the diagonal elements are equal to the squared standard errors of the line means) to account for among line differences in the accuracy with which line means have been estimated. Model fit is then assessed using a χ^2-distributed, weighted residual sum-of-squares test statistic:

$$RSS_W = \sum_{i=1}^{10} \frac{e_i^2}{v_{ii}} \qquad (2)$$

(Lynch and Walsh 1998, chapter 9). In equation 2, e_i is the ith element of **e** and v_{ii} is the ith diagonal element of **V**. Next, hierarchical genetic models are either compared to one another using a likelihood-ratio test or the most parsimonious genetic model is selected using Akaike's Information Criterion (AIC; e.g., Bieri and Kawecki 2003; Fox et al. 2004). However, for linear regression models in general, and particularly for joint-scaling tests, the magnitude of a parameter estimate is often dependent upon the other parameters included in the model (Kearsey and Pooni 1996, p.32-33; Bieri and Kawecki 2003; Burnham and Anderson 2002, chapter 1). Therefore, the first approach, which compares hierarchical genetic models to one another via likelihood-ratio testing, yields biased estimates of composite genetic effects once epistatic, maternal, or sex-linked genetic effects are present but not in the model (Kearsey and Pooni 1996). Further, there is no statistical theory supporting the use of likelihood-ratio tests to conduct hypothesis testing as a basis for model selection (Burnham and Anderson 2002, p. 42). Employing AIC in joint-scaling tests was advocated by Bieri and Kawecki (2003) to select the most parsimonious model

[defined by Bieri and Kawecki (2003) as "the model that made the best compromise between the amount of variance explained and the number of parameters"]. Past implementations of this approach have only used the parameter estimates from the most parsimonious model (identified with AIC model selection) in analyses. However, this ignores both model selection bias and model selection uncertainty (Burnham and Anderson 2002, pp. 43-47). For example, parameter estimates of composite genetic effects that contribute very little to the difference between two populations in a cross, but are retained in the most parsimonious model, are biased upwards and their sampling variances are biased downwards. This results in increased type I error rates when employing the typical *post hoc* tests for the statistical significance of each parameter estimate in the AIC selected best model (e.g., Bieri and Kawecki 2003; Fox et al. 2004). Instead, we used multimodel inference to obtain model-averaged parameter estimates for each composite genetic effect considered in this study and estimates of unconditional sampling variances over the entire set of candidate models (Burnham and Anderson 2002 ch. 4). Since multimodel inference has not previously been used in conjunction with joint-scaling tests, we describe our approach below.

First, we *a priori* chose a set of candidate models specific to each sex. Female and male candidate model sets differed because in males the X-linked dominance genetic effect [X_d] is un-defined and the X-linked additive genetic effect [X_a] is completely confounded with the additive maternal genetic effect [m_a] for the 10 lines we created in our crosses. Therefore, in the male models we chose only the [m_a] composite genetic effect, but note that the quantity estimated by this parameter represents the net effect of both additive maternal and X-linked genetic effects. If all possible combinations of the eight genetic parameters for males or the 10 genetic parameters for females were considered, we would have a total of 256 (2^8) or 1024 (2^{10}) unique models for males and females, respectively (e.g., Bieri and Kawecki 2003; Fox et al. 2004, 2011). To reduce the number of candidate models, models either included or excluded the three digenic epistatic

effects ([*axa*], [*axd*], and [*dxd*]) together as a group, the two maternal genetic effects ([m_a] and [m_d]) together as a group, and the two X-linked genetic effects ([X_a] and [X_d] for females only) as a group (e.g., Bieri and Kawecki 2003; Fox et al. 2004; Fox et al. 2011). We also included the composite additive genetic effect [*a*] in all models. Further, we limited the total number of model parameters being estimated (*p* genetic parameters plus the intercept) to one less than the total number of line means observed to assess the fit of even the most highly parameterized models (ch. 9, Lynch and Walsh 1998). These conditions resulted in 16 candidate model sets for males and 32 for females.

Each model was fitted to the observed line means for each sex and each experiment separately. The weighted residual sums-of-squares were calculated (equation 2) and parameter estimates ($\hat{\mathbf{a}}$) and sampling variances were obtained for each composite genetic effect in a particular model (Lynch and Walsh 1998, chapter 9). The AIC is calculated as:

$$\text{AIC} = -2\ln(L) + 2K \tag{3}$$

where *L* is the likelihood of the model given the data and *K* is the total number of parameters estimated in the regression. For weighted least-squares regression, this equation can be re-written as:

$$\text{AIC} = n\ln(RSS_W) + 2\text{K} \tag{4}$$

(see Appendix C5 for a derivation of the weighted least-squares regression log-likelihood and calculation of *K* for these models).

Using the AIC values of all models in the candidate set, we calculated the AIC differences for the *m*th model as $\Delta_m = AIC_m - AIC_{min}$ (Burnham and Anderson 2002, pp. 70-71). Here AIC_{min} is the model with the minimum AIC value for a particular trait in a given sex for a given experiment. From these, we calculated the Akaike weights as:

$$w_m = \frac{\exp(-\frac{1}{2}\Delta_m)}{\sum_{r=1}^{R}\exp(-\frac{1}{2}\Delta_r)} \tag{5}$$

(Burnham and Anderson 2002, p. 75). In equation 5 the Akaike weight, or relative likelihood of the model given the data, is simply the likelihood of model *m* given the data relative to the sum of likelihoods across all *R* models in the candidate set. The Akaike weights from every model in the candidate set (p. 152 Burnham and Anderson 2002) were then used to calculate a model averaged estimate for each composite genetic parameter. The parameter estimate from each model is weighted by the relative likelihood of that model given the data:

$$\hat{\bar{\theta}} = \sum_{m=1}^{R} w_m \hat{\theta}_m \tag{6}$$

(Burnham and Anderson 2002, p. 150), where $\hat{\bar{\theta}}$ is the model averaged parameter estimate (MAPE) of a given composite genetic effect and $\hat{\theta}_m$ is the parameter estimate of that composite genetic effect in the *m*th model. If a parameter does not occur in a given model, then $\hat{\theta}_m = 0$. We obtained an estimate of the unconditional standard error for a model averaged parameter estimate as:

$$\widehat{SE}(\hat{\bar{\theta}}) = \sum_{m=1}^{R} w_m \sqrt{\widehat{\text{var}}(\hat{\theta}_m) + (\hat{\theta}_m - \hat{\bar{\theta}})^2} \quad (7)$$

(Burnham and Anderson 2002, p. 162), where $\widehat{\text{var}}(\hat{\theta}_m)$ is the squared standard error of the parameter estimate from the *m*th model. The MAPE (equation 6) and its standard error (equation 7) are unconditional with respect to any one model, but they are still conditional on the chosen set of candidate models.

Joint-scaling tests were conducted separately for the sexes within each experiment. Across experiments, however, the same candidate set of models were used for each sex. When comparing the MAPEs between sexes, we added the female additive X-linked MAPE (X_a) to the female additive maternal genetic MAPE (m_a). This is the equivalent to the quantity estimated by the male maternal genetic MAPE, since we could not statistically separate male additive maternal genetic effects from male additive X-linked genetic effects in our experiments. To correct for differences in mean phenotype between sexes and experiments, we divided the MAPEs and their standard errors by the phenotypic difference between the means of parental lines for a given sex (Fox et al. 2011). This standardized the MAPEs (and standard errors) and converted them into expressions describing the proportion of phenotypic difference between the parental lines that can be attributed to the estimate of each composite genetic effect (i.e., MAPE). To test for differences in MAPEs between sexes and experiments, we calculated Wald chi-squared statistics for each MAPE:

$$\chi_1^2 = \frac{(\hat{\bar{\theta}}_1 - \hat{\bar{\theta}}_2)^2}{[\widehat{SE}(\hat{\bar{\theta}}_1)^2 + \widehat{SE}(\hat{\bar{\theta}}_2)^2]} \quad (8)$$

(Fox et al. 2004, 2011). In equation 6, the subscripts 1 and 2 refer to a particular experiment and sex combination.

We conducted linear regressions of the female and male MAPE of each composite genetic effect on the sexual dimorphism index of the traits studied to test the prediction that differences between the sexes in composite genetic effects should covary with the magnitude of sexual dimorphism observed in the parent populations. The sexual dimorphism index (SDI) is calculated as: (mean size of the larger sex / mean size of the smaller sex) – 1 (Lovich and Gibbons 1992; Fairbairn et al. 2007). By convention, the SDI is arbitrarily assigned a negative value when males are the larger sex and a positive value when females are the larger sex. The SDI only differs between parent populations in abdomen length, front-femur length, and spine width. Although statistically significant, these differences in SDI are relatively minor (abdomen length: SDIs in experiment one=0.640 vs. 0.622 and experiment two=0.633 vs. 0.606, front-femur length: SDIs in experiment one=0.005 vs. -0.032 and experiment two=-0.017 vs -0.041, and spine width: SDIs in experiment one=-0.058 vs. -0.129 and experiment two=-0.053 vs. -0.149). We therefore used the average SDI of the two parent populations. Specifically, we used the absolute value of the SDI in our regressions with MAPEs, because we are interested in how the composite genetic effects covary with the magnitude, and not the direction, of sexual dimorphism.

We conducted a separate regression analysis for each of the eight composite genetic effects estimated in both sexes. A single regression analysis modeled each trait's MAPE of one type of composite genetic effect as a function of the categorical variable sex, the covariate SDI, and the interaction between sex and SDI. We weighted the regression by the unconditional sampling error of the MAPEs ($\widehat{SE}(\hat{\theta})$) to incorporate differences in precision among these estimates into the linear models. We first used the initial regression model to compare the female

and male slopes of the relationship between the MAPEs and SDI. If the slopes of females and males did not differ significantly from one another ($p>0.05$ for the interaction term between sex and SDI in the model), we instead modeled the relationship between the MAPEs and SDI fitting a common slope for both sexes (i.e., conducting a second model with sex and SDI as main effects, but without the interaction between sex and SDI).

All analyses were performed on both un-transformed and log-transformed measurement data. However, results from the transformed data were quantitatively similar and qualitatively the same as results from analyses on the un-transformed data. Therefore, we only report the results from analyses on un-transformed data. Additionally, to remove any confounding effects of rearing cage and/or among family differences within lines, we used cage means for all analyses. This approach has the added benefit of creating more normally distributed data upon which to conduct our parametric statistical tests. All analyses were performed using R version 2.15.0 (R Development Core Team. 2012).

Results

The line cross breeding design produced a combined total of 3,535 water striders from the two experiments. The number of individuals of each sex reared from the line cross as well as the number of cage means used for each line in the analyses are shown in table 5.1.

Maternal effects and X-linkage: First generation reciprocal crosses

Across all traits, our comparisons of the first generation reciprocal hybrid female lines provided little evidence of maternal genetic effects (Table 5.2). Only mid-femur length in experiment one and the leg traits and two genital traits in experiment two showed evidence of a significant difference between the reciprocal hybrid lines of females. Of these, only the difference for front-

femur width (Wff) remained significant at the experiment-wide critical value after a sequential Bonferroni correction.

In experiment one, none of the differences between the two male reciprocal hybrids in the first generation were significant (at either and individual p-value threshold of 0.05 or after sequential Bonferroni correction; Table 5.2). In the second experiment, front-femur width and segment 8 length showed significant differences between the lines at the significance level for individual tests ($p<0.05$), however these differences were no longer significant after controlling for multiple tests using the sequential Bonferroni correction. Therefore, we conclude that we find no evidence for X-linkage of the male morphological traits.

Many of the differences between the means of the male F1 line (P1 dam x P2 sire) and the F1r line (P2 dam x P1 sire) were in the opposite direction to that expected if maternal genetic effects and or X-linked genetic effects were present (Figures 5.1, 5.2, and 5.3). If the differences between parent populations were due to autosomal additive genetic effects only, we would expect the male F1 and F1r line means to be equal. Given that animals from Santa Cruz Island (SCI=P2) are larger than those from the two mainland populations (RSC[Exp. 1], LLC[Exp. 2]=P1), if maternal or X-linked genetic effects differed between the parent populations, then the F1 line mean would be less than the average of the two parent populations and the F1r would be greater than the average of the two parent populations. For example, male F1 and F1r line means for thorax length in figure 5.1 are expected to fall on the dashed line under a purely autosomal additive genetic model. If maternal or X-linked genetic effects are present, then the F1 (RSC x SCI) line mean would be expected to more closely resemble the maternal population (P1=RSC x RSC) phenotype and fall below the dashed line. Similarly, the F1r (SCI x RSC) line mean would be expected above the dashed line. The opposite pattern can be seen for 13 out of 16 traits in experiment one and six out of 16 traits in experiment two (also see figures 5.1-5.3) where the F1

line mean is greater than the F1r line mean. Overall, these results indicate that we found no support for X-linkage or maternal genetic effects contributing to the differences between first generation hybrid lines.

Joint-scaling tests

An additive only model was not sufficient to explain the differences between populations for either female or male traits in both experiments (see for example, Appendix C2 Tables C2.2, C2.5, C2.8, C2.11). This can also be seen in figures 5.1-5.3 where most of the line means do not occur along the line denoting the additive expectation (i.e., the dashed line). Out of all the different genetic models in our candidate set, the models that included non-additive and maternal genetic effects as well as X-linked effects displayed the lowest AIC values (Table 5.3 and see Appendix C2 for all AIC values, AIC differences, and Akaike weights). Analyses of the female line means allowed for separate estimation of the additive maternal genetic effects (m_a) from the additive X-linked effects (X_a). Often, combinations of maternal and X-linked effects were included in the AIC best model. When extending the range of models considered from just the AIC best model to those models with AIC values within two of the best AIC value ($\Delta AIC<2$; considered a guideline for identifying models fitting the data with equal support as the best model; Burnham and Anderson 2002), seven traits (out of 12) in each experiment had a model with both X-linked and maternal genetic effects in the extended set of best models (see Appendix C2).

The magnitudes of model averaged parameter estimates (MAPEs), standardized by the difference in parent population line means (i.e., P2 minus P1), indicate large contributions of non-additive and maternal or X-linked composite genetic effects to the differences between the parent population line means in both sexes and experiments (see Appendix C3.1). However, the

contribution of composite genetic effects to differences between parent population line means differed for females and males (Figure 5.4). Wald test statistics indicate that some non-additive MAPEs did differ significantly between the sexes (Table 5.4). Only three composite genetic effect MAPEs in experiment one differed between the sexes, whereas six differed between the sexes in experiment two (Figure 5.4). The MAPEs for dominance-by-dominance epistasis (*dxd*) in segment 8 length differed between the sexes in both experiments. Only the experiment two difference between the sexes in MAPEs for *dxd* in segment 8 length remained significant at the sequential Bonferroni adjusted critical value. However, the sequential Bonferroni adjustment is probably overly conservative when applied to MAPEs (for a given trait, MAPEs for all composite genetic effects are highly interdependent).

We tested for an association between the magnitude of sexual dimorphism and each composite genetic effect's MAPE in both sexes. The SDI varied greatly among the 12 traits studied (Table 5.5); ranging from genital length being 191% to 202% larger in males to abdomen length being 61% to 64% larger in females. Increased sexual dimorphism was associated with more negative values of the *dxd* composite genetic effects in experiment one and more positive values of the *d* effects in experiment two, but this relationship did not differ between the sexes (Table 5.6). This indicates that the net effects of dominant (*d*) and dominance-by-dominance epistatic (*dxd*) alleles are in the direction of the populations with larger water striders (P2) and smaller water striders (P1, either RSC or LLC), respectively. Further, with greater magnitudes of sexual dimorphism, the net dominance or dominance-by-dominance epistatic effects of alleles had a larger contribution to phenotypic differences among lines.

In the first experiment, the relationship between sexual dimorphism and the additive-by-additive (*axa*) and additive-by-dominance (*axd*) epistatic genetic effects differed between the sexes (Table 5.6, Figure 5.5). For both of these composite genetic effects, 9 (out of 13) *axa* and

10 *axd* MAPEs in females were within one standard error from zero and did not change with the level of sexual dimorphism across traits (see Appendix C3.1). However, male MAPEs of both additive-by-additive and additive-by-dominance epistasis became more negative as the sexual dimorphism of the traits increased (Figure 5.5), as indicated by a negative slope in males that is significantly different from the female slope (Table 5.6). In the second experiment, these components neither differed between sexes nor did they show any significant relationship with sexual dimorphism (Table 5.6). However, in the second experiment the magnitude of both dominance-by-dominance epistasis (*dxd*) and dominance maternal genetic effects (m_d) showed a relationship with the magnitude of sexual dimorphism and this differed significantly between the sexes (Table 5.6). Again, the female MAPEs of these parameters did not show any relationship with the degree of sexual dimorphism across traits. However, the estimates of dominance-by-dominance (*dxd*) and dominance maternal genetic effects (m_d) in male traits were more negative as sexual dimorphism increased (Figure 5.5).

Discussion

We conducted crosses between natural populations of the water strider, *A. remigis*, to test for the presence of X-linked genetic effects and to quantify the genetic effects responsible for variation in the magnitude of sexual dimorphism across a range of morphological traits. We found 17 estimates (across all traits) of non-additive composite genetic effects that differed in magnitude between females and males. Further, our results suggest that increased sexual dimorphism is associated with increased negative epistatic (*axa*, *axd*, and *dxd*) and maternal dominance genetic effects (m_d) in males, but not females.

Unfortunately, our experimental design did not allow a direct comparison between our two methods of assessing the contribution of X-linked effects to male phenotypes: (*i*) tests using

male F1 hybrid lines to assess the contribution of X-linked effects and (*ii*) joint scaling tests using the 10 genetic lines to quantify the phenotypic effects of X-linked genes in males. The comparison of first generation reciprocals can only test for X-linkage in males (assuming no maternal effects). In the joint scaling tests on the lines created in our experiments we could only estimate one parameter to summarize the net effect of both additive X-linked and additive maternal genetic effects in male traits. If X-linked and maternal genetic effects are opposite in sign, they might cancel each other out to produce no net effect. Because of this, joint-scaling tests are prone to type II errors (Bradshaw and Holzapfel 2000). However, the use of the joint-scaling test allows for the independent detection of X-linked effects on differences in female phenotypes among all 10 lines.

The evidence from all of our analyes indicates that our results are equivocal with respect to confirming the prediction of X-linked genetic effects in the genetic architecture of sexually dimorphic traits. Our data show no indication that genetic effects on the X chromosome are sufficient to explain differences between the first generation reciprocal hybrid male lines. When studying the genetic effects responsible for line differences among all 10 lines in the two experiments, X-linked composite genetic effects were important components of the models identified by the joint-scaling tests in female traits. However, we saw no difference between sexually dimorphic and non-dimorphic traits in the importance of X-linked effects. Instead, differences between the sexes in the magnitude of non-additive genetic effects were correlated with the degree of sexual dimorphism across traits. Thus, our results demonstrate that sex-specific non-additive effects better explain the sex differences in genetic architecture underlying sexual dimorphism than do X-linked genetic effects. Therefore, of the two main predictions from evolutionary theory that have been proposed to explain the genetic architecture facilitating the

evolution of sexual dimorphisms, our results are most consistent with the prediction of sex-specific non-additive genetic effects.

Alternatively, X-by-autosomal epistatic interactions, where the effect of an autosomal gene depends on the genotype at both X chromosomal and autosomal loci, could also be an important part of the genetic architecture for sexually dimorphic traits. Our analyses did not include X-by-autosomal epistatic effects and only quantified the phenotypic effects of X chromosomal loci with additive and dominance genetic effects. However, sex-linked modification of sex-specific autosomal expression has been observed for size in Portuguese Water Dogs (Chase et al. 2005) and may function to reduce intralocus sexual conflict (Bonduriansky and Chenoweth 2009). The sex-specific epistatic effects we detected could also be caused by genomic imprinting. For example, Hager et al. (2008) observed quantitative trait loci that were differentially imprinted, based on sex. Modifier loci that are differentially silenced in the two sexes and inherited from two different populations would also show an epistatic effect dependent both on sex and population of origin. However, the genetic model applied in our joint-scaling tests is unable to disentangle such complex interactions and thus might instead attribute such effects to the digenic epistatic effects we did include (*axa*, *axd*, and *dxd*). Additionally, the difference we observed between the sexes in maternal dominance genetic effects (m_d) could be representing a parent-of-origin imprinting effect on dominant alleles. Parent-of-origin imprinting effects lead to differences in the genotypic value of reciprocal heterozygotes (Spencer 2002, 2009). Thus, if these effects are not explicitly included in the analyses, we would expect to see a difference between the sexes in the dominance genetic effects attributed to the maternal population (because we did not include a paternal effect) and not the progeny themselves (Spencer 2002, 2009). If X-by-autosomal epistasis and sex-specific genomic imprinting are present in sexually dimorphic traits, this implies that parts of the existing genetic machinery

responsible for the basic cascade of events leading to sexual differentiation may have been adopted and evolved to facilitate the expression of sexually dimorphic phenotypes (Cline and Meyer 1996).

Although results from both experiments support the conclusion that between-sex differences in non-additive effects correlate with the magnitude of sexual dimorphism, we did not observe any overlap between the two experiments in the particular genetic effects demonstrating this relationship. A difference between the two experiments is not unexpected and is consistent with the hypothesis that polygenic traits that respond to selection often display multiple genetic solutions among lines/populations (Falconer 1989; Endler et al. 2001; Garland and Rose 2009). Thus, although a similar phenotype is observed (i.e., sexual dimorphism) in all three populations used above, this does not imply that allele frequencies with similar effects or even at the same loci will change in response to selection or even be present. We could not directly compare the two populations characterized by relatively small body size (RSC and LLC) using the line cross analyses conducted here. However, given that both were crossed to SCI, the differences in the genetic effects detected in the two sets of crosses provide indirect evidence that the genetic architectures of RSC and LLC differ from each other. Support for this conclusion can be gleaned from a comparison of model averaged parameter estimates within-sexes, but between experiments (similar to comparing the MAPEs between sexes, but within-years: see Appendix C4). Statistical comparison of the genetic effects between years suggests that there are subtle differences in the genetic architectures estimated from the two experiments, particularly in traits expected to be under strong selection (e.g., male genital length and male segment 8 length; Appendix C4 Table C4.1). Inter-annual variation in the SCI population might also be causing the difference in our results for the two experiments but we do not think this is the case because the results of a pilot experiment using RSC and SCI conducted a year earlier yielded similar results to experiment one

in the current study (i.e., no year-by-line interaction and similar evidence for X-linkage and or maternal effects; see Materials and Methods *X-linkage analyses* and Appendix C4 Table C4.2).

It is noteworthy that three out of the four genetic differences between the sexes associated with the magnitude of sexual dimorphism are attributed to epistasis. This implies that non-additive genetic interactions contributing to water strider morphological traits are likely to play a central role in past and/or future responses to selection (Hansen *in press*). Given that epistasis is thought to play a significant role in the evolution of postzygotic reproductive isolation (Orr 1995; Orr and Turelli 2001; Johnson 2000; Gavrilets 1993, 2004; Coyne and Orr 2004; Fierst and Hansen 2010; Bank et al. 2012), the epistatic associated with sexual dimorphism may also be instrumental in the larger processes of sexual selection and sexual conflict that drive speciation in the *remigis* clade of water striders (Lande 1981; Gallant et al. 1993; Gallant and Fairbairn 1996, 1997; Parker and Partridge 1998; Rice 1998; Dieckmann and Doebeli 1999; Arnqvist et al. 2000).

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Table 5.1. Counts of the number of individuals or number of cages in each genetic line. Lines can be categorized as: the original parental populations (P), the first filial generation (F1), the second filial generation (F2), or a backcross between the first filial generation and the lines created directly from the original parental population (B=PxF). All but the P1 and P2 lines can be further subdivided as being a reciprocal (r) or not, which denotes the difference between dam and sire identity in the cross of two lines.

	Experiment 1				**Experiment 2**			
	Female		**Male**		**Female**		**Male**	
Line (dam x sire)	No. cage means	No. individs	No. cage means	No. individs	No. cage means	No. individs	No. cage means	No. individs
P1[a]	19	92	18	101	28	100	31	91
B1a (P1 x F1)	21	73	23	85	30	121	31	94
B1ra (F1 x P1)	15	58	21	81	32	159	31	139
F1 (P1 x P2)	16	92	19	187	25	76	27	86
F2 (F1 x F1)	16	61	14	60	27	113	28	87
F2r (F1r x F1r)	18	77	19	80	33	125	32	103
F1r (P2 x P1)	10	103	7	72	20	65	19	60
B2rb (F1r x P2)	20	88	20	88	30	98	32	93
B2b (P2 x F1r)	14	36	20	53	22	71	25	71
P2 (SCI x SCI)	9	47	12	130	23	69	19	50

a: in experiment one dam and sire were from RSC and in experiment two dam and sire were from LLC

Table 5.2. Results from t-tests to assess the null hypothesis that first generation reciprocals (F1 and F1r) do not differ from one another. The ‡ symbol indicates the unadjusted p-value is significant at a critical level after sequential Bonferroni adjustment, where the significance threshold for the *i*th p-value is: α_i=0.05/(1+50-i) (Rice 1989). Trait abbreviations described in the Materials and Methods section.

	Experiment 1									
	Female					**Male**				
	F1 (P1xP2)	F1r (P2xP1)	t	df	p	F1 (P1xP2)	F1r (P2xP1)	t	df	p
Lthorax	6.768	6.751	0.310	23.59	0.62	6.536	6.299	2.897	7.94	0.99
Labd	6.863	6.896	-0.580	23.72	0.28	4.138	4.055	1.604	7.39	0.92
Ltotal	16.127	16.111	0.153	22.51	0.56	15.028	14.625	2.342	7.03	0.97
Wabd	2.906	2.941	-0.955	12.62	0.18	2.489	2.453	1.136	12.19	0.86
Lff	4.422	4.414	0.179	15.89	0.57	4.478	4.494	-0.247	8.07	0.41
Wff	0.568	0.578	-1.242	14.25	0.12	0.722	0.706	1.412	10.55	0.91
Lmf	9.564	9.765	-1.978	13.56	0.034	10.130	9.899	1.531	7.53	0.92
Lhf	9.021	9.092	-0.822	16.05	0.21	9.560	9.298	1.729	7.23	0.94
Lgenital	0.923	0.916	0.386	16.71	0.65	2.875	2.781	2.673	12.60	0.99
Lseg8	0.664	0.662	0.085	15.50	0.53	1.338	1.293	2.915	13.40	0.99
Lpyg	--	--	--	--	--	1.363	1.348	0.734	12.06	0.76
Lseg7mar	1.716	1.733	-0.980	22.22	0.17	1.964	1.869	2.400	7.87	0.98
Wspine	1.564	1.540	0.784	15.66	0.78	1.656	1.675	-0.478	10.88	0.32
	Experiment 2									
	Female					**Male**				
	F1 (P1xP2)	F1r (P2xP1)	t	df	p	F1 (P1xP2)	F1r (P2xP1)	t	df	p
Lthorax	6.804	6.753	1.137	41.60	0.87	6.536	6.490	0.859	40.59	0.8
Labd	6.865	6.927	-0.921	42.57	0.18	4.180	4.180	-0.014	43.73	0.49

Ltotal	16.174	16.255	-0.818	40.28	0.21	15.113	15.091	0.195	28.44	0.58
Wabd	2.882	2.914	-1.165	30.80	0.13	2.491	2.524	-1.398	39.07	0.085
Lff	4.422	4.497	-1.886	37.43	0.034	4.528	4.592	-1.570	31.11	0.063
Wff	0.540	0.573	-4.280	41.61	<0.001‡	0.701	0.721	-2.049	33.57	0.024
Lmf	9.585	9.764	-1.877	40.55	0.034	9.968	10.047	-0.690	29.56	0.25
Lhf	8.948	9.182	-2.296	42.95	0.013	9.475	9.551	-0.701	31.87	0.24
Lgenital	0.929	0.977	-2.295	39.80	0.014	2.801	2.840	-1.026	28.26	0.16
Lseg8	0.643	0.683	-1.893	31.75	0.034	1.249	1.283	-1.705	29.58	0.049
Lpyg	--	--	--	--	--	1.382	1.374	0.585	40.57	0.72
Lseg7mar	1.733	1.725	0.387	43.00	0.65	1.984	1.944	1.737	38.11	0.95
Wspine	1.497	1.559	-2.897	42.93	0.003	1.637	1.655	-0.469	41.56	0.32

Table 5.3. The best model, as indicated by the lowest AIC value, out of the candidate model set for each trait. The letters A, D, and E denote the autosomal additive, dominance, and digenic epistatic (*axa*, *axd*, and *dxd*) composite genetic effects to distinguish them from other genetic effects that contain similar letters.

	Experiment 1		**Experiment 2**	
	Female	**Male**	**Female**	**Male**
Lthorax	$ADEX_aX_d$	ADEc	$ADEX_aX_d$	ADEc
Labd	$ADEX_ac$	$ADEm_am_dc$	ADm_am_dc	$ADEm_am_dc$
Ltotal	$ADEX_am_ac$	AEm_am_dc	$AEX_aX_dm_am_d$	$ADEm_am_dc$
Wabd	$ADEX_am_ac$	ADEc	$ADEX_am_ac$	AD
Lff	$ADEm_am_dc$	$ADEm_am_dc$	$ADEm_am_dc$	$ADEm_am_dc$
Wff	$ADEX_am_ac$	$ADEm_am_dc$	$ADEX_am_ac$	ADE
Lmf	$ADEm_am_dc$	Am_am_dc	$ADEX_ac$	$ADEm_am_dc$
Lhf	$ADEm_am_dc$	AEm_am_d	$ADEX_am_ac$	AEamdmc
Lgenital	$ADEm_am_dc$	AEm_am_dc	$ADEX_aX_d$	$ADEm_am_dc$
Lseg8	$AEX_aX_dm_am_d$	$ADEm_am_d$	$ADEX_am_ac$	$ADEm_am_dc$
Lpyg	--	$ADEm_am_d$	--	$ADEm_am_dc$
Lseg7mar	$ADEX_am_ac$	$ADEm_am_dc$	$ADEm_am_d$	$ADEm_am_d$
Wspine	$ADEm_am_dc$	AEm_am_dc	$ADEX_am_ac$	$ADEm_am_dc$

Table 5.4. Wald test statistics for the differences between female and male MAPEs in each of the two experiments. Probabilities are indicated with asterisks (***<0.001<**<0.01<*<0.05) and are calculated from a chi-squared distribution with one degree of freedom. The ‡ symbol indicates the test is significant at a critical level after sequential Bonferroni adjustment, where the significance threshold for the *i*th p-value is: α_i=0.05/(1+192-i) (Rice 1989).

	Experiment 1							
	a	**d**	**axa**	**axd**	**dxd**	**m_a**	**m_d**	**c**
Lthorax	0.0796	0.677	0.000587	0.147	0.889	0.661	0.342	1.26
Labd	0.296	0.0651	1.29	1.06	1.03	0.00153	1.4	0.96
Ltotal	0.0202	0.0804	3.11	4.22*	1.86	0.103	2.71	1.07
Wabd	0.0551	0.149	2.89	2.42	0.496	2.17	0.28	5.68*
Lff	0.193	0.128	0.284	0.293	1.05	0.528	0.000619	0.00388
Wff	0.789	0.505	2.36E-06	0.0329	2.2	1.42	0.81	0.684
Lmf	0.765	2.1	3.03	0.148	0.867	0.655	0.594	0.13
Lhf	0.671	0.817	0.202	0.212	0.926	0.987	0.0769	0.414
Lgenital	0.406	0.569	1.21	2.92	0.519	0.0204	0.000504	1.27
Lseg8	0.000357	2.73	0.438	4.45*	9.99**	0.000854	3.48	0.047
Lseg7mar	0.303	0.0837	0.00648	0.752	0.409	3.78E-05	0.0106	1.46
Wspine	0.003	0.321	0.0729	2.17	0.723	2.47	0.23	2.9
	Experiment 2							
	a	**d**	**axa**	**axd**	**dxd**	**m_a**	**m_d**	**c**
Lthorax	3.13	0.581	0.543	0.0696	0.82	7.71**	0.219	1.08
Labd	1.34	0.832	0.00919	0.00363	3.94*	0.753	5.39*	0.421
Ltotal	1.73	4.09*	0.22	0.00356	7.53**	1.25	0.746	1.09
Wabd	2.67	4.52*	5.16*	2.02	3.72	2.97	0.325	1.15
Lff	0.00223	0.391	0.688	1.35	0.0808	1.46	1.28	1.91
Wff	0.098	1.78	2.76	0.511	1.13	1.13	1.3	5.56*
Lmf	1.15	0.936	1.29	0.00539	0.0333	1.52	2.7	0.0599
Lhf	2.16	0.184	0.626	0.7	0.0465	2.14	1.14	0.0999
Lgenital	0.187	2.31	0.0914	0.162	5.13*	0.134	11.4***	0.0138
Lseg8	0.577	12.2***	0.687	0.26	37.8***‡	0.207	6.44*	0.0169
Lseg7mar	1.61	0.954	2.51	1.57	0.469	3.13	0.16	0.00102
Wspine	0.00492	1.29	0.0747	2.07	1.71	0.421	1.45	1.21

Table 5.5. The sexual dimorphism index [(larger sex / smaller sex) – 1) for the parent population lines in experiment one and two. Positive values indicate females are the larger sex, whereas a negative value indicates that males are the larger sex.

	Experiment 1		Experiment 2	
	SCI	**RSC**	**SCI**	**LLC**
Lthorax	0.0435	0.0246	0.0345	0.0312
Labd	0.6414	0.6234	0.6390	0.6088
Ltotal	0.0709	0.0595	0.0677	0.0593
Wabd	0.2012	0.1528	0.1455	0.1291
Lff	0.0017	-0.0349	-0.0134	-0.0415
Wff	-0.2389	-0.3031	-0.2706	-0.2663
Lmf	-0.0282	-0.0555	-0.0421	-0.0629
Lhf	-0.0478	-0.0674	-0.0470	-0.0807
Lgenital	-2.0212	-1.9875	-1.9054	-1.9368
Lseg8	-0.9815	-0.9948	-0.9056	-0.9828
Lseg7mar	-0.1344	-0.1288	-0.1444	-0.1532
Wspine	-0.0542	-0.1391	-0.0508	-0.1458

Table 5.6. Comparison of female and male regressions. The estimates of each composite genetic effect were regressed on the magnitude of sexual dimorphism (absolute values of SDI). Asterisks (*) indicate the p-value (***<0.001<**<0.01<*<0.05) for tests of the null hypotheses: (*i*) female and male slopes do not differ from one another, (*ii*) the difference between female and male intercepts is zero, (*iii*) the slope common to both sexes is zero (Combined) or if the slopes differed between sexes the Female slope is reported (male slope obtained by subtracting difference between slopes from the Female estimate), or (*iv*) the coefficient of determination (R^2) from the best fitting regression (slopes either different or combined for the two sexes) equals zero. Parentheses indicate 1 standard error.

	Experiment 1				
	Difference between intercepts	**Slope**			**R^2**
		Combined	**Female**	**Female - Male**	
a	-0.0319 (0.0330)	-0.0703 (0.0352)			0.231
d	-0.106 (0.262)	0.160 (0.205)			0.0339
axa	--	--	0.439 (0.279)	-0.774 (0.312)*	0.301
axd	--	--	0.132 (0.150)	-0.582 (0.176)**	0.701***
dxd	-0.0641 (0.245978)	-0.690 (0.260)*			0.255*
m_a	0.0284 (0.0125)*	0.0393 (0.0211)			0.373**
m_d	-0.0231 (0.0172)	1.69E-08 (7.30E-08)			0.0811
c	0.0090 (0.0136)	0.000978 (0.0146)			0.0213
	Experiment 2				
	Difference between intercepts	**Slope**			**R^2**
		Combined	**Female**	**Female - Male**	
a	-0.0550 (0.030)	-0.0194 (0.03673)			0.152
d	0.231 (0.2105)	1.48 (0.462)**			0.381**
axa	-0.0501 (0.117)	0.0866 (0.166)			0.0237
axd	0.0484 (0.0744)	-0.147 (0.095)			0.13
dxd	--	--	-0.369 (0.488)	-1.54 (0.714)*	0.424*
m_a	0.00837 (0.00727)	-0.0136 (0.0123)			0.0904
m_d	--	--	-1.47E-08 (2.81E-08)	-0.160 (0.0539)**	0.449**
c	-0.0172 (0.0135)	-0.00236 (0.00352)			0.0931

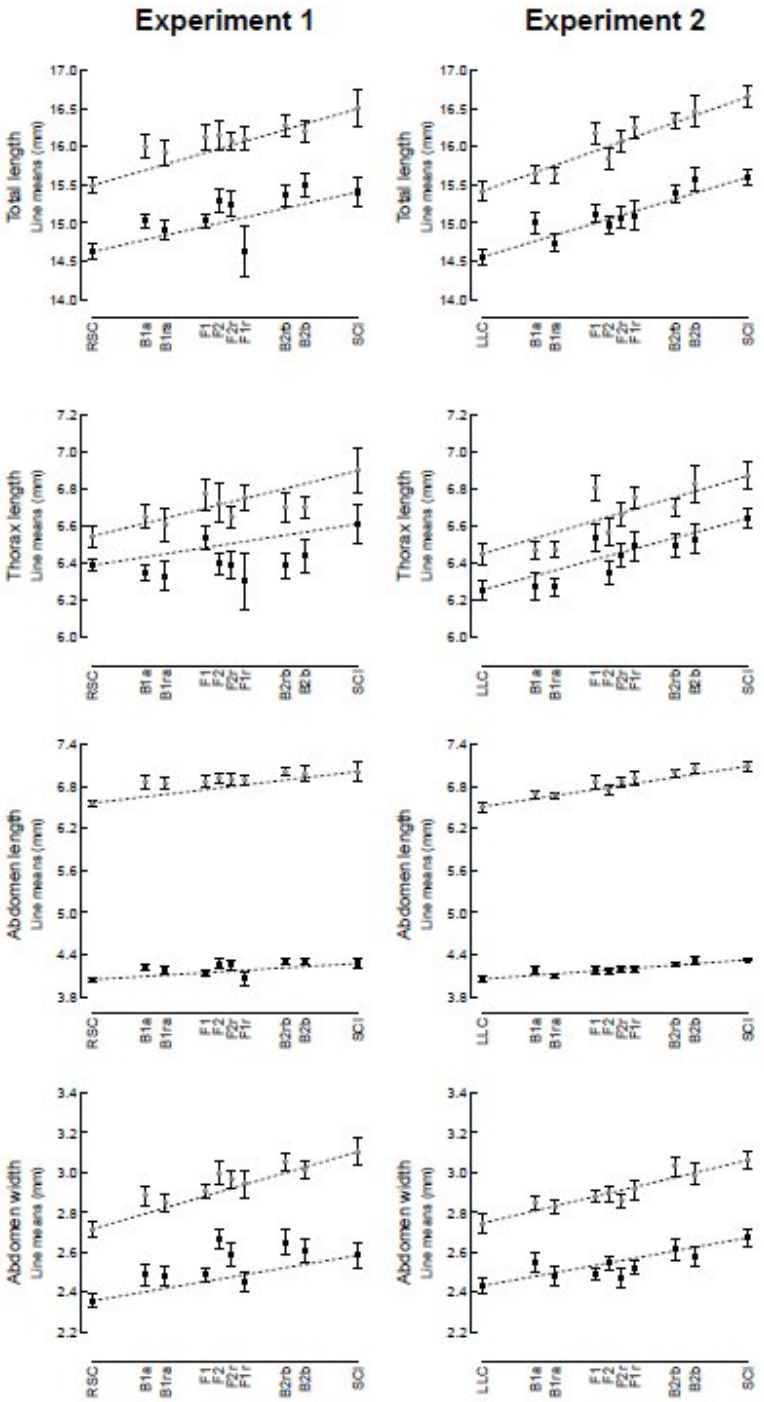


Figure 5.1. Observed line means (±2SE) of somatic traits for the population cross in experiment one (left column) and experiment two (right column). Each plot depicts female (open circles) and male (filled squares) line means. Line means are arranged from left to right along the x-axis to reflect the approximate proportion of P2 (SCI x SCI) genes each line contains. The dashed line indicates the additive expectation.

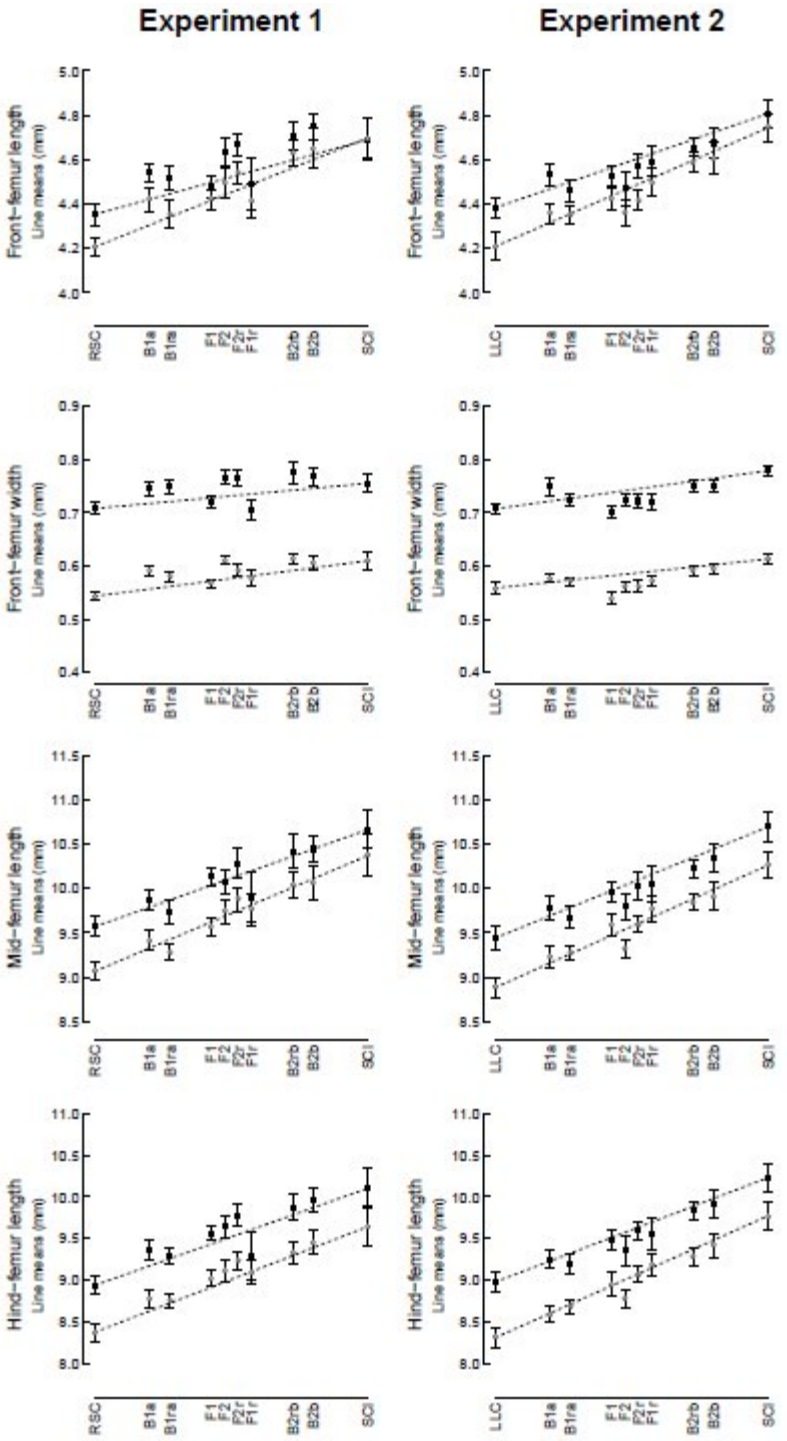


Figure 5.2. Observed line means (±2SE) of leg traits for the population cross in experiment one (left column) and experiment two (right column). Arrangement, symbols, and lines are the same as in figure 5.1.

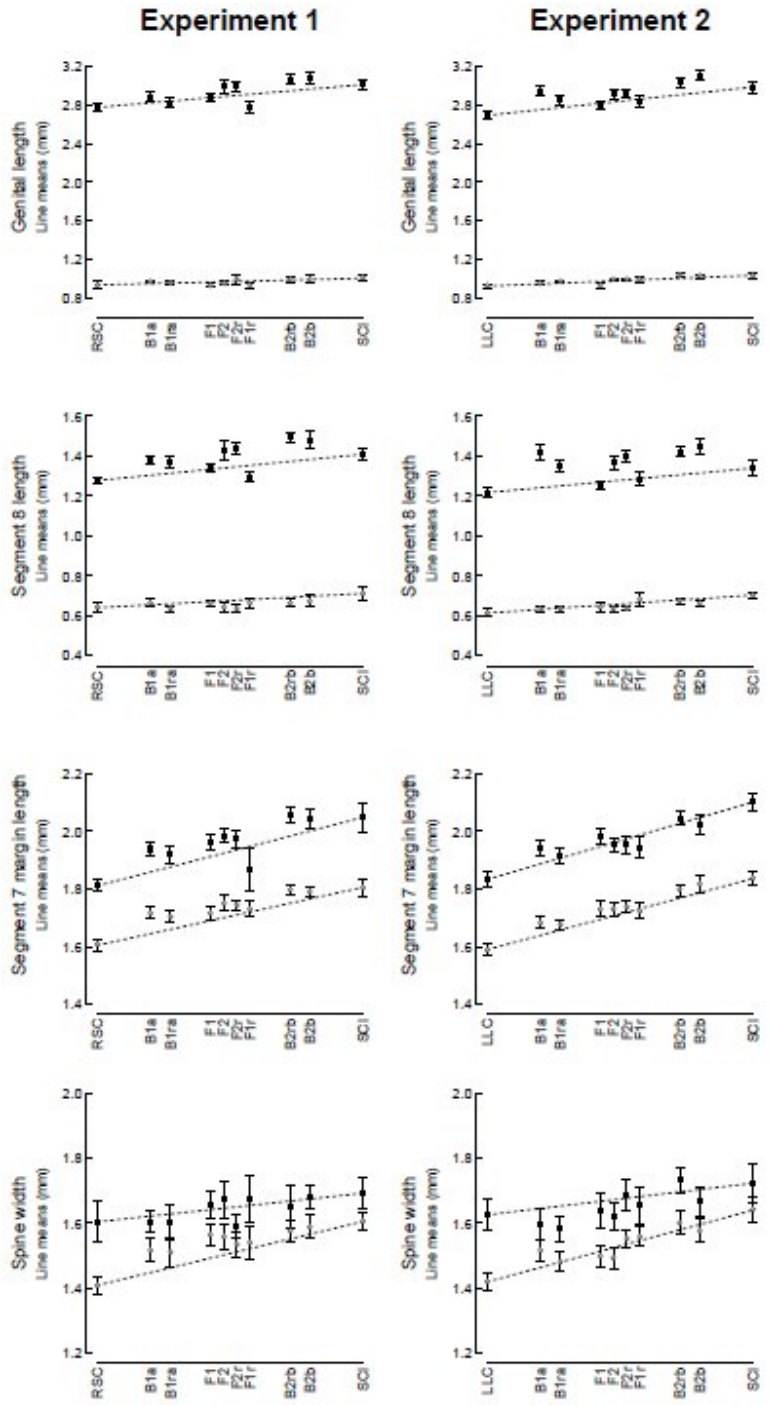


Figure 5.3. Observed line means (±2SE) of genital traits for the population cross in experiment one (left column) and experiment two (right column). Arrangement, symbols, and lines are the same as in figure 5.1.

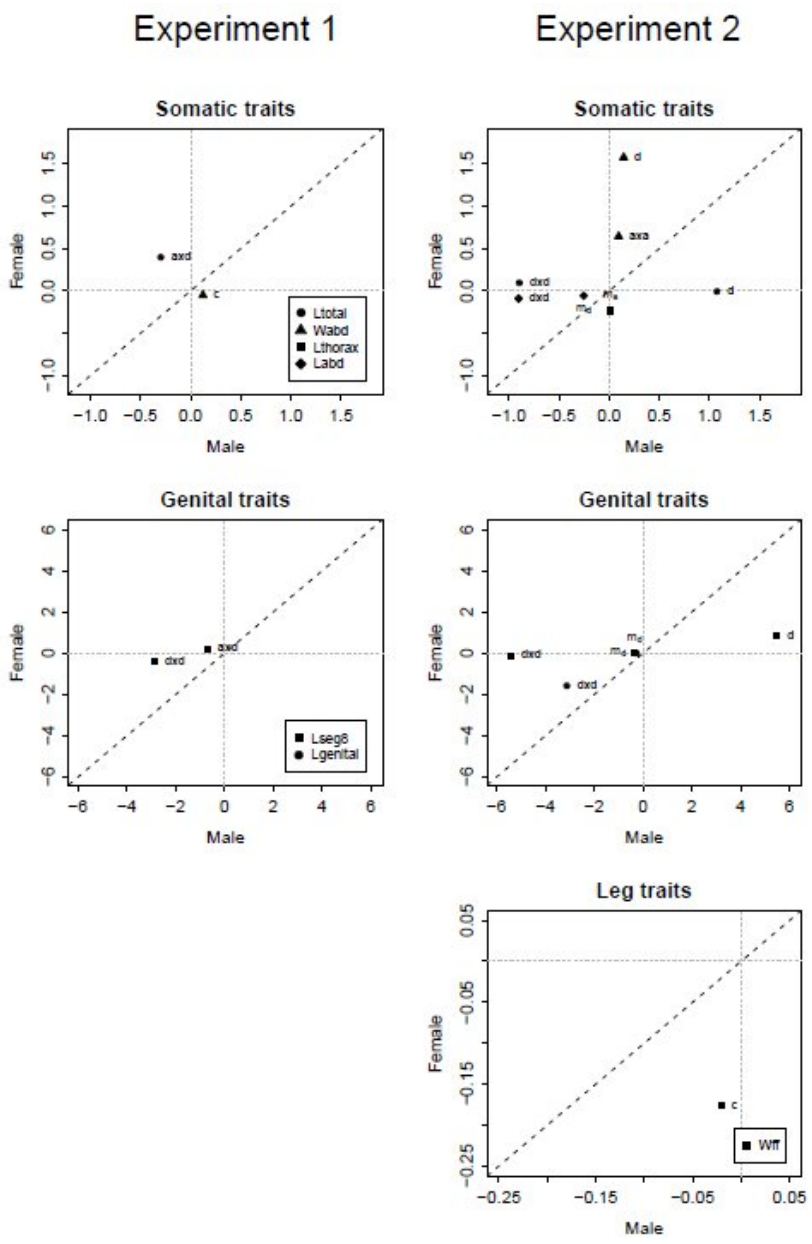


Figure 5.4. Differences between the female and male model averaged parameter estimates (MAPEs) that are significantly greater than zero (Wald chi-squared tests, see main text). MAPEs are divided by the difference between the two parent population lines (P2 minus P1). Equal effects in the two sexes fall on the black, dashed line running diagonally through each plot. The horizontal and vertical grey dashed lines indicate where female and male MAPEs, respectively, do not contribute to the observed difference between the parental lines in that particular sex. Letters next to points indicate the composite genetic effect that each point represents. Legends in the panels found in the left column reflect symbols used for a particular subset of traits in both experiment one (left column) and experiment two (right column). Note, no MAPEs differed significantly between the sexes for leg traits in experiment one.

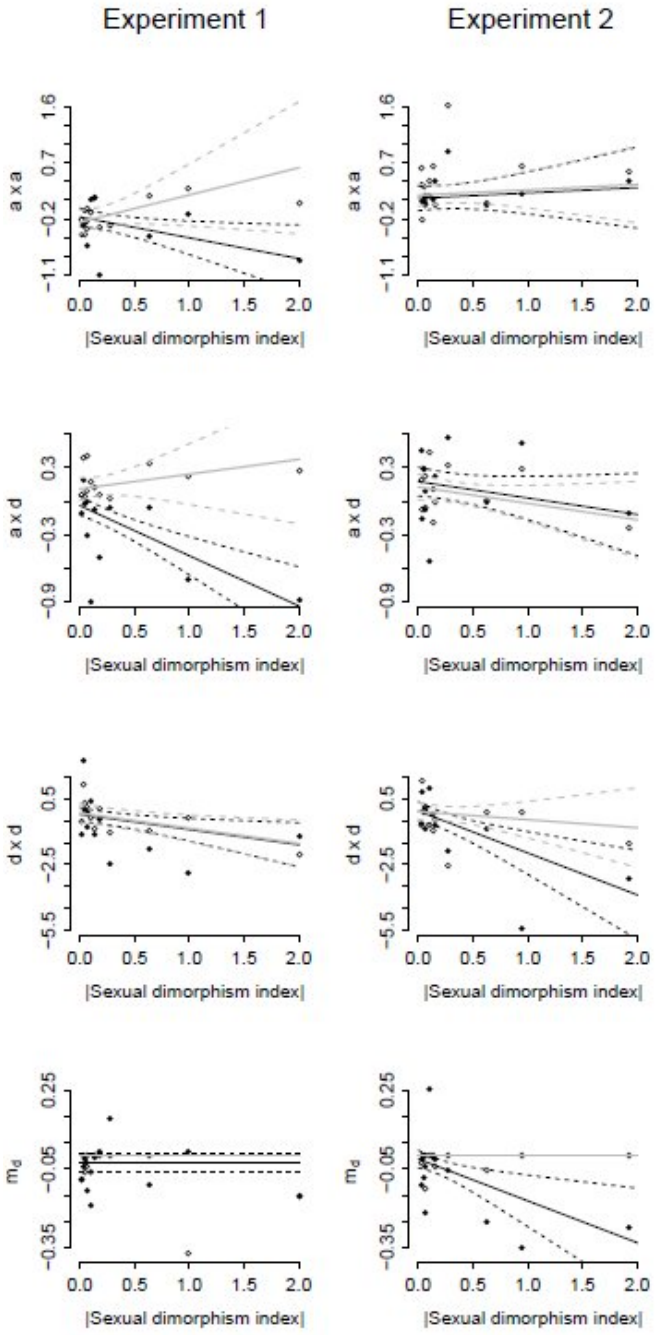


Figure 5.5. Regressions of composite genetic effect estimates on the magnitude of sexual dimorphism across traits. Points indicate female (open circles) or male (closed, black circles) model averaged parameter estimates for a given absolute value of the sexual dimorphism index for each trait. Regression lines (solid) are accompanied by their 95% confidence limits (dashed lines) for females (grey) and males (black) in either experiment one (left column) or experiment two (right column).

CONCLUSION TO THE DISSERTATION

As a consequence of their disparate roles in reproduction, the two sexes often have markedly different phenotypes. However, in spite of the prevalence of sexual dimorphism, we have little knowledge of the genetic architecture underlying these differences. In my first chapter I have shown that ignoring these differences at the genetic level can lead to biased estimates of additive genetic variances. Similarly, in my third chapter I have shown that bias arises in estimates of additive genetic variance when additive genetic variance located on the sex chromosomes is ignored. These biases can greatly affect conclusions regarding many evolutionary processes such as responses to selection and genetic drift. Of particular note is the bias that is seen in estimates of between-sex additive genetic correlations when sex-linkage is left out of an analysis. Often a negative between-sex additive genetic correlation is sought as an indicator of genetic sexual conflict. My results suggest that analyses that do not explicitly account for sex-linked additive genetic variance could be making erroneous conclusions regarding the presence of genetic sexual conflict. For example, the interesting dynamic between the sex chromosomal versus autosomal location of sexually antagonistic variation will be completely missed and consequently many between-sex additive genetic correlations will be biased upwards.

In my second and fourth chapters I expand the available methods for estimating genetic variance contributing to phenotypic variation. Specifically, the development of the non-additive and sex-linked relatedness matrices as options in the quantitative geneticist's toolkit will allow sex-specific non–additive variance and/or sex-linked additive genetic variance to be quantified. With estimates in hand, quantitative geneticists will be able to evaluate the roles these components of the genetic architecture play in the evolution of sexual dimorphism. Finally, evidence from the population crosses conducted in *Aquarius remigis* contributes empirical

evidence for the first time supporting the role of sex-specific non-additive genetic effects in the evolution of sexual dimorphism.

There are a few obvious avenues of future research following from the results obtained in the analytical, simulation, and empirical studies presented in my dissertation along with the development of quantitative genetic methodologies. Specifically, it remains to be tested whether or not sex-specific non-additive genetic variances are key components of phenotypic variation in sexually dimorphic traits. Further, only a handful of studies have quantified the amount of sex-linked additive genetic variance contributing to sexually dimorphic trait variation. Particularly, investigation into the difference between autosomal and sex-linked between-sex additive genetic correlations deserves much more attention. Careful separation of these two quantities in a range of populations, each over a range of traits varying in sexual dimorphism, is necessary to fully understand the extent to which shared additive genetic variation between the sexes constrains the evolution of sexual dimorphism.

APPENDIX A: Supporting Information for Chapter 2

A1 Relatedness matrices in the animal model

A brief description of a linear mixed model will be formulated below (following Lynch & Walsh (1998) *Genetics and Analysis of Quantitative Traits*) with an aim toward demonstrating how the inverse of a relatedness matrix is incorporated into an animal model. Beginning with a univariate model of Gaussian observations, where there is only one observation per individual and one random effect (in this case the additive genetic effect), the model can be specified as:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{e} \qquad \text{(A1.1)}$$

where **y** is a n x 1 vector of phenotypes, **β** a p x 1 vector of fixed effects, **u** a n x 1 vector of random effects (breeding values here), **e** is a n x 1 vector of residual deviations, and **X** and **Z** are n x p and n x q incidence matrices relating the fixed and random effects, respectively, to each observation. The expected means for the components in this model are E[**u**] = E[**e**] = 0 and therefore E[**y**] = **Xβ**. The variance of **y** is var(**y**) = $\mathbf{V} = \mathbf{ZGZ}^{\mathbf{T}} + \mathbf{R}$, where **G** and **R** are the q x q and n x n variance-covariance matrices for the additive and residual effects, respectively. Since the covariance among additive effects is the same as the additive genetic covariance between relatives, $\mathbf{G} = \sigma^2_A\mathbf{A}$, where **A** is the additive genetic relationships matrix. The residual deviations are assumed uncorrelated, therefore $\mathbf{R} = \sigma^2_E\mathbf{I}$, where **I** is an identity matrix (n x n with 1s along the diagonal).

Finding solutions for the fixed and random effects (**β** and **u**) and variance components (σ^2_A and σ^2_E) in s.1 is thoroughly explained elsewhere (*e.g.*, Lynch & Walsh, 1998; Sorensen & Gianola 2002), so I will spare the details here. The relevant point is that the following mixed model equations for the animal model:

$$\begin{pmatrix} n & \mathbf{1}^T \\ \mathbf{1} & \mathbf{I} + \lambda \mathbf{A}^{-1} \end{pmatrix} \begin{pmatrix} \hat{\mu} \\ \hat{\mathbf{u}} \end{pmatrix} = \begin{pmatrix} \sum^n y_i \\ \mathbf{y} \end{pmatrix} \tag{A1.2}$$

where $\lambda = \sigma^2_E/\sigma^2_A$, μ is the overall mean, and **1** is a *n* x 1 vector of 1s, require $\mathbf{A^{-1}}$ to obtain the solution (utilizing either Likelihood or Bayesian methods to solve the equations).

The same can be shown for the case of more than one random effect. Now the univariate model is specified:

$$\mathbf{y = X\beta + Z_1 u_1 + Z_2 u_2 + e} \tag{A1.3}$$

such that $\mathbf{Z_1 u_1}$ and $\mathbf{G_1}$ are the same as **Zu** and **G** in equation s.1. $\mathbf{Z_2}$ is the incidence matrix relating the random effects in $\mathbf{u_2}$, which in this example will represent dominance genetic effects. Here, the covariance matrix $\mathbf{G_2} = \sigma^2_D\mathbf{D}$, where **D** represents the dominance genetic relatedness matrix, has the inverse $\mathbf{G_2^{-1}} = \sigma^{-2}_D\mathbf{D^{-1}}$. The mixed model equations, incorporating the estimation of another random effect (*e.g.*, dominance genetic effects in this example) are:

$$\begin{pmatrix} \mathbf{X}^T\mathbf{X} & \mathbf{X}^T & \mathbf{X}^T \\ \mathbf{X} & \mathbf{I} + \lambda_A \mathbf{A}^{-1} & \mathbf{I} \\ \mathbf{X} & \mathbf{I} & \mathbf{I} + \lambda_D \mathbf{D}^{-1} \end{pmatrix} \begin{pmatrix} \hat{\beta} \\ \hat{a} \\ \hat{d} \end{pmatrix} = \begin{pmatrix} \mathbf{X}^T\mathbf{y} \\ \mathbf{y} \\ \mathbf{y} \end{pmatrix} \tag{A1.4}$$

where λ_A is λ above and $\lambda_D = \sigma^2_E/\sigma^2_D$. Finding the solutions requires $\mathbf{A^{-1}}$ and $\mathbf{D^{-1}}$. Extending to the multivariate case, it is easy to see how the mixed model equations for a multivariate model would rely on $\mathbf{A^{-1}}$ and $\mathbf{D^{-1}}$, but I will refer the interested reader to Lynch & Walsh (1998) for further details.

In the above general linear model (eqn. s.1), Gaussian observations (**y**) are assumed to be multivariate normal such that $\mathbf{y} \sim \text{MVN}(\mathbf{X\beta}, \mathbf{V})$. For the purpose of demonstrating how the relationship matrices are incorporated into the animal model, non-Gaussian data are treated in a similar way for a generalized linear model. However, the observations are a function of latent variables, which correspond to the terms on the right hand side of equation s.1, which are related through an appropriate predictor function. Thus, the latent variables represented by **l** (lower-case "L" and not a "one") can be expressed in terms of fixed and random effects as:

$$\mathbf{l} = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{e} \qquad \text{(A1.5)}$$

Thus, for animal models of non-Gaussian response variables, solving equation A1.5 necessitates the same mixed model equations as in equation A1.2.

A2 **nadiv**

The inverse of relatedness matrices for dominance and epistatic genetic effects can be created using `makeD()`, `makeDsim()`, `makeAA()`, and/or `makeDomEpi()`. Using these functions in R and manipulating their output is extremely similar from one function to the next, so only one [*i.e.*, `makeD()`] is provided in the examples below. For more information on the other functions, see the help documentation accompanying the **nadiv** package. The `warcolak` dataset used below is a simulated, three-generation design without inbreeding (see the help pages for this dataset in **nadiv** and the breeding design described in Fairbairn, D.J. & Roff, D.A. (2006) *Heredity*, **97**:319-328). Because there is absolutely no inbreeding in this population, I do not consider it when specifying the animal model. However, when applying these models to other populations, which may have inbreeding, it is necessary to fit the inbreeding coefficients as a covariate in the model. An explanation of this can be found in Walsh and Lynch's draft of Chapter 15 for their new book (Volume 2: Evolution and Selection of Quantitative Traits. http://nitro.biosci.arizona.edu/zbook/NewVolume_2/newvol2.html)

Below, examples are used to indicate the steps necessary to estimate additive and dominance genetic variance using **MCMCglmm**, **asreml**, ASReml (standalone), and WOMBAT. More complete tutorials covering the general use of each program can be found in the supplementary material for Wilson *et al*. (2010) *Journal of Animal Ecology*, **79:**13-26. What is shown below is meant to demonstrate the use of functions in **nadiv**, and not how to analyze data using animal models. When applying similar models to other datasets, the practitioner should ensure that the models are properly adapted to suit the demands of the analysis and system being modeled.

Parallel processing

A note about ways to improve the speed of **nadiv** functions. Many of the functions have the built in option to utilize parallel processing across a single computer when carrying out various computations. This implements functions from the **multicore** package in R. However, **multicore** is not useable under all operating systems – it seems to be supported for Linux and Mac but it is unclear how well (if at all) this package will work under Windows – and so the defaults in **nadiv** have been set to not use multiple processors. However, enabling this only requires setting `parallel = TRUE` in the arguments for functions that have this capability. See the help documentation for each function for further details.

Some considerations are necessary to ensure the greatest amount of time saving possible. Due to overhead incurred when setting up the multiple processors to run in parallel, it might actually be slower to use parallelization if the function is to be used on a small dataset. Additionally, RAM will need to be allocated for each processor engaged in executing the R functions. For some very large datasets this might necessitate using less than the maximum number of processors (or else the computer will run out of RAM and either quit with an error or use "swap" space – *e.g.* the much slower hard drive). Specifying the number of parallel R processes to create can be achieved by setting a numerical value for the `ncores` argument. By default, this argument is set to use all processors available. The ASReml-R Tutorial below contains some comments about instances when to use parallel processing as well as some potential trade-offs to its use in **nadiv**.

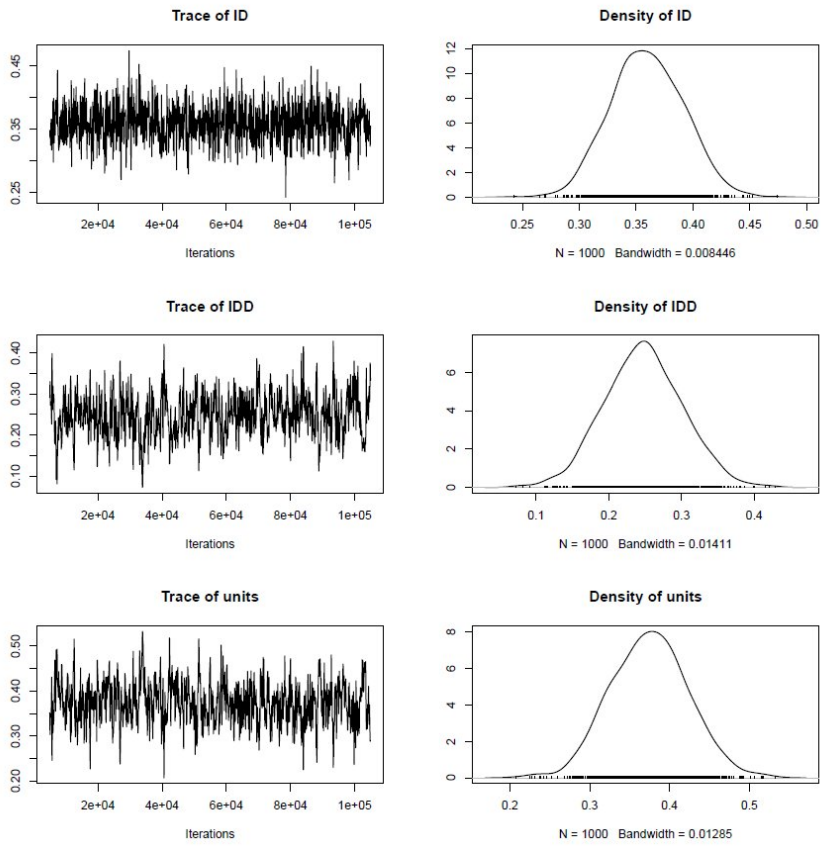
A3 MCMCglmm Tutorial

```
> library(nadiv)
> library(MCMCglmm)

> #Additive and dominance example

> priorADE <- list(G = list(G1 = list(V =1,  nu = 0.002),
+  G2 = list(V = 1, nu = 0.002)),
+  R = list(V = 1, nu = 0.002))

> Ainv <- inverseA(warcolak[, 1:3])$Ainv
> Dinv <- makeD(warcolak[, 1:3])$Dinv
> warcolak$IDD <- warcolak$ID

> warcolak.MCMC <- MCMCglmm(trait1 ~ 1, random = ~ID + IDD,
+  ginverse = list(ID = Ainv, IDD = Dinv), data = warcolak,
+  prior = priorADE,
+  nitt = 105000, thin = 100, burnin = 5000,
+  verbose = TRUE)
```

The above example is for the simple purpose of demonstrating the proper use of `makeD()` and how to include it in the `ginverse` list of a call to `MCMCglmm()`. Including any of the epistatic genetic effects into the model is very similar, both in the use of the function to obtain the inverse of the relationship matrix and the inclusion into a **`MCMCglmm`** model. However, for each random effect that depends upon the individual identity (*e.g.*, ID), a separate identifying column might be necessary (*e.g.*, for above, "IDD" is a copy of "ID" for referencing the dominance effect). Including an extra copy of the ID column is also used in the ASReml examples below. Additionally, an accompanying entry in the `ginverse` list, linking the name of the random effect in the model to the correct generalized inverse, is necessary.

We can see how well the MCMCglmm model was able to separate the three variance estimates, by plotting the posterior distributions:

```
> plot(warcolak.MCMC$VCV)
```



In addition to inspecting the autocorrelation visually (from the traces above), we can check for autocorrelation a bit more quantitatively with:

```
> autocorr(warcolak.MCMC$VCV)
, , ID

                    ID          IDD       units
Lag 0     1.000000000 -0.26494432 -0.07278217
Lag 100  -0.000012508 -0.10539016  0.09549293
Lag 500   0.036393095 -0.04985777  0.04914678
Lag 1000 -0.066606835 -0.04590237  0.05578282
Lag 5000 -0.079375908  0.05365031 -0.02371619

, , IDD

                   ID          IDD       units
Lag 0    -0.26494432  1.00000000 -0.89076965
```

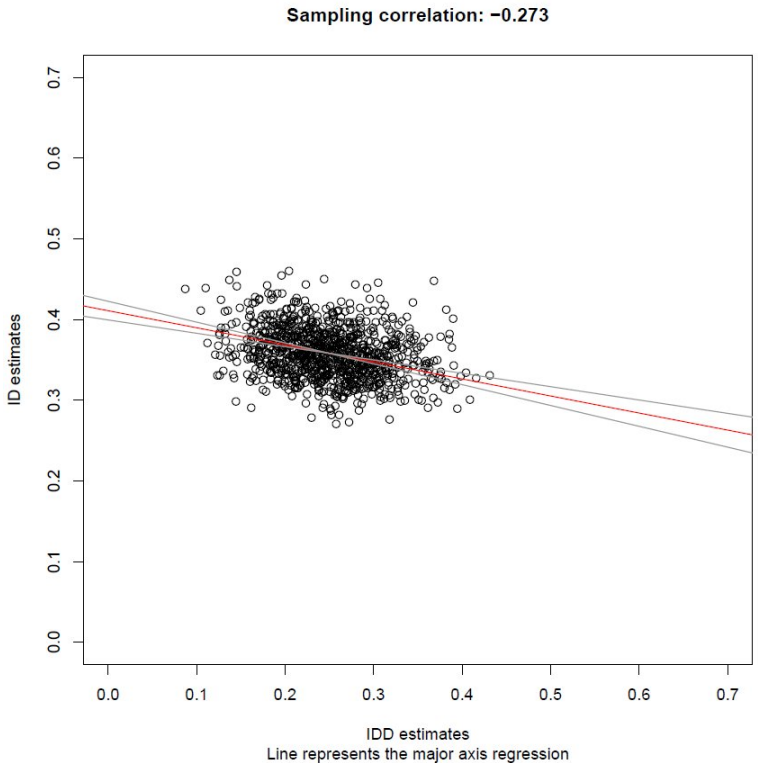
```
Lag 100  -0.08938836  0.57207016 -0.54355028
Lag 500  -0.01558564  0.11492239 -0.10663915
Lag 1000  0.02792617 -0.01921861  0.02510860
Lag 5000 -0.02093646 -0.02934648  0.04967262

, , units

                    ID          IDD       units
Lag 0    -0.072782169 -0.890769649  1.00000000
Lag 100   0.094689577 -0.537598269  0.51085097
Lag 500  -0.002208612 -0.088331598  0.07303680
Lag 1000 -0.011661740  0.026924969 -0.03826952
Lag 5000  0.049615606  0.009750489 -0.04206244
```

There appears to be some autocorrelation, particularly between the dominance and residual variances (*i.e.*, "IDD" and "units"), and it would be best to re-run the model after altering the number of iterations (*i.e.*, `nitt`), burnin (*i.e.*, `burnin`), and sampling interval (*i.e.*, `thin`) parameters in `MCMCglmm()`.

We can also estimate the sampling (co)variance between two terms, for example the additive and dominance variances ("ID" and "IDD"). To do this along with an accompanying plot of the Model II regression:

```
> library(lmodel2)
> ID.est <- warcolak.MCMC$VCV[,
"ID"][1:dim(warcolak.MCMC$VCV)[1]]
> IDD.est <- warcolak.MCMC$VCV[,
"IDD"][1:dim(warcolak.MCMC$VCV)[1]]
> mareg <- lmodel2(ID.est~IDD.est)
> x11(w = 8, h = 8)
> plot(mareg, method = "MA", xlim = c(0,0.7), ylim = c(0,0.7),
+     xlab = "IDD estimates", ylab = "ID estimates",
+     main = paste("Sampling correlation: ", round(mareg$r, 3),
sep =""),
+     sub = "Line represents the major axis regression")
```

yields the following graph.



Further, we can obtain the modes of each variance component's posterior distribution along with the 95% Highest Posterior Density (HPD) intervals:

```
> posterior.mode(warcolak.MCMC$VCV)
+        ID       IDD     units
+ 0.3585311 0.2488164 0.3994836

> summary(warcolak.MCMC)$Gcovariances
+     post.mean  l-95% CI  u-95% CI  eff.samp
+ ID  0.3596518 0.3022913 0.4210762 1000.0000
+ IDD 0.2475114 0.1494159 0.3520785  242.4259

> summary(warcolak.MCMC)$Rcovariances
+       post.mean  l-95% CI  u-95% CI eff.samp
+ units 0.3747735 0.2859493 0.4696829 287.8212
```

Finally, another way to assess the significance of the dominance term is to compare the Deviance Information Criterion (DIC) between the full model and one where the dominance is not included.

```
> #Additive only model
> priorAE <- list(G = list(G1 = list(V = 1,  nu = 0.002)),
+   R = list(V = 1,  nu = 0.002))

> warcolak.MCMC2 <- MCMCglmm(trait1 ~ 1, random = ~ID,
+   ginverse = list(ID = Ainv), data = warcolak,
+   prior = priorAE,
+   nitt = 102000, thin = 100, burnin = 2000,
+   verbose = TRUE)
```

Show the DICs for the full additive and dominance model and then the additive only model:

```
> warcolak.MCMC$DIC
+ [1] 12878.99
> warcolak.MCMC2$DIC
+ [1] 14058.49
```

A4 ASReml-R Tutorial

```
> library(nadiv)
> library(asreml)

> ginvA <- asreml.Ainverse(warcolak[ , c(1,3,2)])$ginv
```

Note, the above ASReml function wants the columns for the pedigree ordered "ID", "Sire", "Dam" – hence "warcolak[, c(1,3,2)]". This is a different order than both **`nadiv`** and **`MCMCglmm`** use ("ID", "Dam", "Sire").

```
> ginvD <- makeD(warcolak[ ,1:3])$listDinv
```

We can compare the creation of the dominance relatedness matrix and its inverse for both options to the parallel argument. First, the time for the non-parallel operation:

```
> system.time(Dout <- makeD(warcolak[, 1:3], parallel = FALSE))
starting to make D....done
   user  system elapsed
  5.610   2.340   7.959
```

The "elapsed" column indicates the total time taken to execute the command. Now the time for the parallel operation:

```
> library(multicore)
> system.time(Dout2 <- makeD(warcolak[,1:3], parallel = TRUE,
ncores = 8))
starting to make D....done
   user  system elapsed
  5.780   2.910   7.968
```

On a Dell laptop with an Intel® Core™ i7-2820QM CPU (2.3Ghz – 8 cores available), 8GiB of system memory, and using a Linux 64-bit operation system, we see there is no real advantage to utilizing the parallel option. However, the `warcolak` pedigree is quite simple and the

dominance relatedness matrix rather sparse (as well as the additive relationship matrix). Therefore, on a larger or more related pedigree the parallel option might end up saving much more time. However, caution should be taken when using this feature for very large pedigrees with many related individuals. The amount of RAM used in the above parallel version of `makeD()` was a few times greater that of the non-parallel execution. If the memory requirements are high, the number of processors able to be used may be limited by the total RAM available and not the total number of processors of the computer.

```
> warcolak$IDD <- warcolak$ID

> warcolak.asr <- asreml(trait1 ~ 1,
+     random = ~ ped(ID) + giv(IDD),
+     ginverse = list(ID = ginvA, IDD = ginvD),
+     data = warcolak)

asreml(): 3.0.1 Library: 3.01gl IA32  Run: Mon Feb 13 12:21:38
2012

     LogLik         S2      DF
  -2457.8362      0.7690  5399  12:21:39
  -2412.4444      0.6773  5399  12:21:39
  -2368.0852      0.5622  5399  12:21:39
  -2340.5521      0.4413  5399  12:21:39
  -2336.0457      0.3872  5399  12:21:39
  -2335.8565      0.3735  5399  12:21:39
  -2335.8560      0.3726  5399  12:21:39
  -2335.8560      0.3726  5399  12:21:39

Finished on: Mon Feb 13 12:21:39 2012
LogLikelihood Converged

> summary(warcolak.asr)$varcomp
                 gamma component  std.error   z.ratio constraint
ped(ID)!ped  0.9601333 0.3577059 0.03155830 11.334763   Positive
giv(IDD).giv 0.6717242 0.2502566 0.05324035  4.700507   Positive
R!variance   1.0000000 0.3725586 0.04904087  7.596901   Positive
```

We can see that the variance component estimates are very similar to what **MCMCglmm** reported as the posterior distribution modes. If we wanted to examine the sampling covariances among the variance components, we could inspect the inverse of the Average Information matrix:

```
> aiFun(model = warcolak.asr, Dimnames = c("Va", "Vd", "Ve"),
inverse = TRUE)
         Va       Vd       Ve
Va  0.02644  0.72012 -0.85731
Vd  0.02642  0.05090 -0.95985
Ve -0.00684 -0.01062  0.00241
```

where the sampling correlations are printed above the diagonal and the covariances are below the diagonal. The sampling variances (along the diagonal) could be used to obtain approximate 95% Confidence Intervals (CIs).

```
> aiCI(warcolak.asr, Dimnames = c("Va", "Vd", "Ve"))

         UCL  estimate       LCL
Va 0.4196273 0.3577059 0.2957845
Vd 0.3546491 0.2502566 0.1458642
Ve 0.4687767 0.3725586 0.2763405
```

However, this method makes an assumption about the normality of the likelihood surface which often does not hold (Meyer, K. (2008) *Heredity*, **101**:212-221.). A better way to construct CIs would be to use the log profile likelihood for each component. Here again, I will compare the `proLik` function with and without parallel processing enabled. First, without:

```
> system.time(profileA <- proLik(full.model = warcolak.asr,
+    component = "ped(ID)!ped", negative = FALSE, nsample.units =
4,
+    nse = 3.5))

   user  system elapsed
 29.590   1.840  31.467
```

And now setting `parallel = TRUE`:

```
> system.time(profileA2 <- proLik(full.model = warcolak.asr,
+    component = "ped(ID)!ped", negative = FALSE, nsample.units =
4,
+    nse = 3.5, parallel = TRUE, ncores = 8))

   user  system elapsed
 52.410  13.640   9.784
```

Here, utilizing the parallel processing saves a lot of time, even for this very simple model. It is easy to see how this option could create huge time savings for animal models which take over a minute to converge for one set of parameter values. The additive variance estimate's CI limits are:

```
> profileA$UCL
[1] 0.4610137
> profileA$LCL
[1] 0.2862408
```

And the dominance variance profile likelihood and CI limits are:

```
> profileD <- proLik(full.model = warcolak.asr,
+    component = "giv(IDD).giv", negative = FALSE,
+    nsample.units = 4, nse = 3, parallel = TRUE)

> profileD$UCL
[1] 0.398427
> profileD$LCL
[1] 0.1525705
```

Note the discrepancy, particularly in the upper confidence limit (UCL), when comparing the confidence intervals estimated from the profile likelihoods to those derived from the Average Information matrix (*i.e.*, `aiCI()` above). When plotted,

```
> par(mfrow = c(2,1))
>   plot.proLik(profileA, xlim = c(0, 1))
>   plot.proLik(profileD, xlim = c(0, 1))
```

these profile likelihoods yield a graph similar to figure 2.1 in the main text. Here we can see the asymmetry of the profile likelihood which the confidence intervals estimated from the Average Information matrix do not reflect.

The following illustrates the simulation approach to estimating the dominance relatedness matrix (Ovaskainen *et al.* (2008) *Proceedings of the Royal Society B*, **275**: 669-678.):

```
> system.time(Dsim <- makeDsim(warcolak[, 1:3], N = 10000,
+    invertD = TRUE, calcSE = TRUE))
> ginvDsim <- Dsim$listDsiminv
starting to make D....done
making Dsim ....done
    user   system  elapsed
3142.580    5.370 3151.608
```

We can use this D-inverse in the `ginverse` argument of the `asreml()` call exactly the same as demonstrated above when using `makeD()`.

Finally, additional random effects representing non-additive genetic variances can be added to multivariate animal models in the same way as any other random effect. The same procedures as above can be used to construct the necessary dominance (or epistatic) relatedness matrix inverse. As in any multivariate animal model, additional specifications are necessary in the model to indicate the G and R structure, as shown here for a bivariate model:

```
> warcolak.asr2 <- asreml(fixed = cbind(trait1, trait2) ~ trait,
     random = ~ us(trait):ped(ID) + us(trait):giv(IDD),
     ginverse = list(ID = ginvA, IDD = ginvD),
     rcov = ~ units:us(trait),
     data = warcolak)
```

```
> summary(warcolak.asr2)$varcomp
                                          gamma   component  std.error
z.ratio  constraint
trait:ped(ID)!trait.trait1:trait1   0.35780564  0.35780564 0.03155212
11.3401461    Positive
trait:ped(ID)!trait.trait2:trait1  -0.01214159 -0.01214159 0.02328397
-0.5214568    Positive
trait:ped(ID)!trait.trait2:trait2   0.42928030  0.42928030 0.03431675
12.5093514    Positive
trait:giv(IDD)!trait.trait1:trait1  0.24926719  0.24926719 0.053206061
4.6849396     Positive
trait:giv(IDD)!trait.trait2:trait1  0.03381735  0.03381735 0.037714452
0.8966683     Positive
trait:giv(IDD)!trait.trait2:trait2  0.27888802  0.27888802 0.053445162
5.2182092     Positive
R!variance                          1.00000000  1.00000000         NA
NA        Fixed
R!trait.trait1:trait1               0.37340260  0.37340260 0.04902295
7.6168942     Positive
R!trait.trait2:trait1              -0.01894427 -0.01894427 0.03486782
-0.5433166    Positive
R!trait.trait2:trait2               0.31388399  0.31388399 0.04958689
6.3299792     Positive
```

A5 ASReml (standalone) Tutorial

In R, run the following commands to obtain a text file with the generalized inverse of **D**:

```
> library(nadiv)
> ginvD <- makeD(warcolak[ ,1:3])$listDinv
> write.table(ginvD, "ASwarcolak.giv", col.names = FALSE,
      row.names = FALSE)
```

Now, make sure the files "ASwarcolak.dat" and "ASwarcolak.ped" from the Supporting Information have been downloaded. In the ASReml program, create and run the following "warcolak.as" file and run the job:

```
Univariate Additive and Dominance example
      ID !P
      Sire
      Dam
      IDD
      sex 2
      trait1
      trait2
ASwarcolak.ped
ASwarcolak.giv
ASwarcolak.dat !SCORE
trait1 ~ mu !r ID giv(IDD,1)
```

Note: adding the `!SCORE` qualifier writes the Average Information matrix to the file "ASwarcolak.AIM", in this example (generically named "*basename*.AIM"). This can then be used to inspect the sampling (co) variances of the random effects in R as:

```
> asreml.out <- scan("<your-path-here>/ASwarcolak.aim")
> asreml.out
[1]   289.2033   272.2135   505.9344  2023.8973  3007.5787
19447.7270
```

```
> aiFun(AI.vec = asreml.out, inverse = FALSE, Dimnames = c("Va",
"Vd", "Ve"))
         Va       Vd       Ve
Va  0.02643  0.72011 -0.85730
Vd  0.02641  0.05089 -0.95985
Ve -0.00684 -0.01062  0.00240
```

Alternatively, and perhaps more simply, the ".vvp" file produces the same results (*e.g.*, "ASwarcolak.vvp") and can be viewed in the ASReml program.

A6 WOMBAT Tutorial

In R, run the following commands to obtain a text file with the generalized inverse of **D**:

```
> library(nadiv)
> Dout <- makeD(warcolak[,1:3])
> Dinv <- Dout$listDinv[, c(2,1,3)]
```

The line directly above is because WOMBAT wants the generalized inverse columns ordered "column", "row", "inverse". Now save this generalized inverse to the hard drive:

```
> write.table(Dinv, "dominance.gin", col.names = FALSE,
      row.names = FALSE)
```

and obtain the log of the determinant of D:

```
> Dout$logDet
[1] -27.30874
```

The next step is to copy the log of the determinant (-27.30874) and paste it into the file "dominance.gin" as the first line. The first 5 lines of "dominance.gin" should look like:

```
-27.30874
1 1 1
2 2 1
3 3 1
4 4 1
```

Place the generalized inverse of **D** ("dominance.gin") in the same directory as the parameter file. From the Supporting Information, download "WOMBATdata.d" and "WOMBATped.d" and place these in the directory one above from where the "dominance.gin" and parameter file are located. Next construct the following as a parameter file ("wombat.par") and run it from the command line (*i.e.*, type: `wombat wombat.par`):

```
COM Univariate analysis of simulated warcolak dataset in nadiv
PED ../WOMBATped.d
DAT ../WOMBATdata.d
  animal
  sire
  dam
  dominance 5400
  record
END DAT
ANALYSIS UNI
MODEL
  RAN animal NRM
  RAN dominance GIN
  TRA record
END MODEL
VAR animal 1
0.1
VAR dominance 1
0.1
VAR error 1
0.1
```

Note, for simplification, the file "WOMBATdata.d" does not contain the columns "sex" or trait1" from the `warcolak` dataset. Also, the Average Information matrix can be obtained from the "AvInfoCovs" output file. Note two points, however. First, these are the elements of the Average Information matrix and not its inverse (which the latter is necessary for obtaining the sampling (co) variances). This can be handled by setting the `aiFun()` argument `inverse` equal to FALSE. Secondly, this Average Information matrix is for the parameters estimated by WOMBAT, which is generally the leading columns of the Cholesky factors of the covariance matrix estimated and not the parameters on their original scale (as is the case from ASReml). These, however, could be read into R. For example:

```
> wombat.ai <- read.table("AvInfoCovs", skip = TRUE)
```

where the argument, `skip = TRUE`, is necessary because the file "AvInfoCovs" contains the log likelihood, of the iteration generating the Average Information matrix, as the first line.

APPENDIX B: Supporting Information for Chapter 3

B1 Simulation and analysis details and design matrix for observed variance components model

Simulation details

The autosomal additive, sex-linked additive, and residual effects were each random draws from multivariate normal distributions, where *a* is a random draw from the distribution N(0, $\mathbf{G}_a \otimes \mathbf{A}$), *s* is a random draw from the distribution N(0, $\mathbf{G}_s \otimes \mathbf{S}$), and *e* is a random draw from the distribution N(0, **R**), following methods described by Van Vleck (1994). Here, $\otimes$ denotes the direct product between two matrices and

$$\mathbf{G}_a = \begin{bmatrix} \sigma^2_{a-hom} & \sigma_{a-hom,het} \\ \sigma_{a-het,hom} & \sigma^2_{a-het} \end{bmatrix} \quad \text{(B1.1)}$$

and

$$\mathbf{G}_s = \begin{bmatrix} \sigma^2_{s-hom} & \sigma_{s-hom,het} \\ \sigma_{s-het,hom} & \sigma^2_{s-het} \end{bmatrix} \quad \text{(B1.2)}$$

represent the additive genetic covariance matrices for autosomal and sex-linked effects, respectively. The matrices **A** and **S** are the autosomal and sex-linked additive genetic relationship matrices which are constructed based upon the algorithms of Meuwissen and Luo (1992. Computing inbreeding coefficients in large populations. Genet. Sel. Evol. 24:305-313) and Fernando and Grossman (1990), respectively, for each pedigree type (see *Pedigrees considered* in main text) using the **nadiv** (v2.11; Wolak 2012) package in R (R Development Core Team, 2012). The homogametic and heterogametic residual covariance matrix is:

$$\mathbf{R} = \begin{bmatrix} \sigma^2_{\text{e–hom}} & \mathbf{0} \\ \mathbf{0} & \sigma^2_{\text{e–het}} \end{bmatrix} \quad \text{(B1.3)}$$

Thus, the residual effects have equal variances for each sex ($\sigma^2_{\text{e-hom}}=\sigma^2_{\text{e-het}}$), but are completely uncorrelated ($\sigma_{\text{e-hom,het}}=0$)

Analysis details

The naïve animal model implemented is specified as:

$$\begin{bmatrix} \mathbf{y}_{\text{hom}} \\ \mathbf{y}_{\text{het}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}_{\text{hom}} & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_{\text{het}} \end{bmatrix} \begin{bmatrix} \beta_{\text{hom}} \\ \beta_{\text{het}} \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_{\text{a–hom}} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_{\text{a–het}} \end{bmatrix} \begin{bmatrix} \mathbf{a}_{\text{hom}} \\ \mathbf{a}_{\text{het}} \end{bmatrix} + \begin{bmatrix} \mathbf{e}_{\text{hom}} \\ \mathbf{e}_{\text{het}} \end{bmatrix}$$

(B1.4)

and the informed animal model as:

$$\begin{bmatrix} \mathbf{y}_{\text{hom}} \\ \mathbf{y}_{\text{het}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}_{\text{hom}} & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_{\text{het}} \end{bmatrix} \begin{bmatrix} \beta_{\text{hom}} \\ \beta_{\text{het}} \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_{\text{a–hom}} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_{\text{a–het}} \end{bmatrix} \begin{bmatrix} \mathbf{a}_{\text{hom}} \\ \mathbf{a}_{\text{het}} \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_{\text{s–hom}} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_{\text{s–het}} \end{bmatrix} \begin{bmatrix} \mathbf{s}_{\text{hom}} \\ \mathbf{s}_{\text{het}} \end{bmatrix} + \begin{bmatrix} \mathbf{e}_{\text{hom}} \\ \mathbf{e}_{\text{het}} \end{bmatrix}$$

(B1.5)

Here $\mathbf{X}$, $\mathbf{Z}_{\mathbf{a}}$, and $\mathbf{Z}_{\mathbf{s}}$, are design matrices relating fixed ($\mathbf{X}$) or random ($\mathbf{Z}_{\mathbf{a}}$, $\mathbf{Z}_{\mathbf{s}}$) effects to the appropriate record in $\mathbf{y}_{\text{hom}}$ or $\mathbf{y}_{\text{het}}$. The vectors $\boldsymbol{\beta}$, $\mathbf{a}$, $\mathbf{s}$, and $\mathbf{e}$ contain the sex-specific fixed effects, autosomal additive genetic effects, sex-linked additive genetic effects, and residual effects, respectively. The random effects $\mathbf{a}$, $\mathbf{s}$, and $\mathbf{e}$ are assumed to be multivariate normally distributed

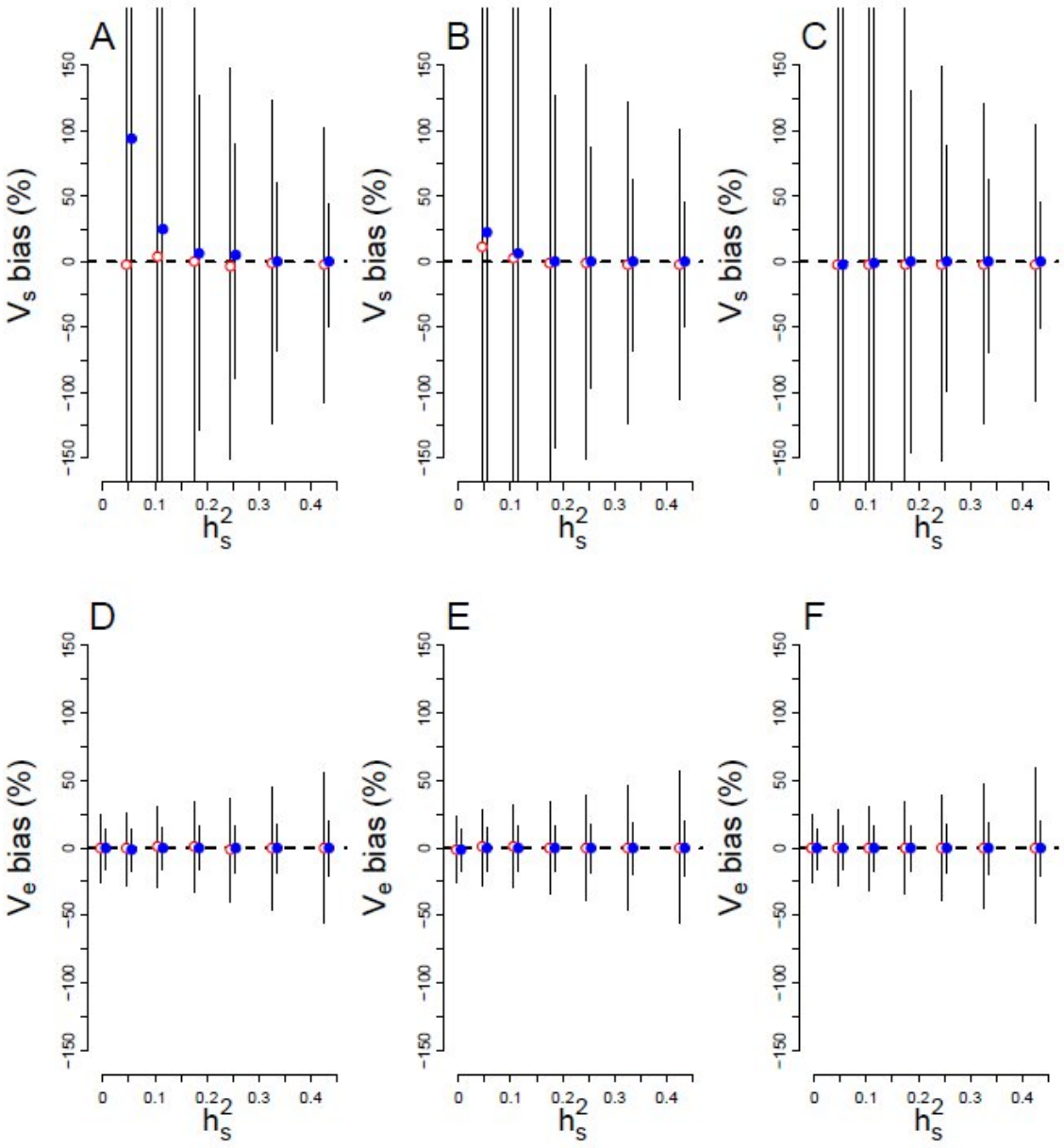
following: $\mathbf{a} \sim N(0, \mathbf{G}_a \otimes \mathbf{A})$, $\mathbf{s} \sim N(0, \mathbf{G}_s \otimes \mathbf{S})$, $\mathbf{e} \sim N(0, \mathbf{R})$. Again, **A** and **S** are the autosomal and sex-linked relationship matrices and $\mathbf{G}_a$, $\mathbf{G}_s$, and **R** take the same form as in equations B1.1-B1.3, respectively. However, the (co)variances in $\mathbf{G}_a$, $\mathbf{G}_s$, and **R** are now the REML estimates obtained when solving the mixed model equations of the animal model (Patterson and Thompson. 1971. Recovery of inter-block information when block sizes are unequal. Biometrika. 58:545-554; Lynch and Walsh 1998).

Design matrix

Adapted from Chenoweth et al. 2008. See main text for symbol definitions.

Observed components	Genetic or causal components								
	$\sigma^2_{a\text{-}hom}$	$\sigma^2_{a\text{-}het}$	$\sigma^2_{s\text{-}hom}$	$\sigma^2_{s\text{-}het}$	$\sigma^2_{e\text{-}hom}$	$\sigma^2_{e\text{-}het}$	$\sigma_{a\text{-}hom,het}$	$\sigma_{s\text{-}hom,het}$	$\sigma_{e\text{-}hom,het}$
Homogametic Sex									
sire variance	0.25	0	0.5	0	0	0	0	0	0
dam variance	0.25	0	0.25	0	0	0	0	0	0
within-family variance	0.5	0	0.25	0	1	0	0	0	0
Heterogametic Sex									
sire variance	0	0.25	0	0	0	0	0	0	0
dam variance	0	0.25	0	0.5	0	0	0	0	0
within-family variance	0	0.5	0	0.5	0	1	0	0	0
Between-sex									
sire covariance	0	0	0	0	0	0	0.25	0	0
dam covariance	0	0	0	0	0	0	0.25	0.5	0
within-family covariance	0	0	0	0	0	0	0.5	0.5	1

B2 Biases in sex-linked and residual variance estimates from "informed" models



F

igure B2.1. Analyses of the HS pedigree when implementing "informed" observed variance components models (circles). The panels depict average percent bias in estimates of sex-linked (top row) additive and residual (bottom row) variances in the homogametic sex (open, red symbols) and the heterogametic sex (closed, blue symbols) as a function of the proportion of phenotypic variance comprised of sex-linked variance (h_s^2) and when the sex-linked between-sex additive genetic correlation is approximately (A) 0.71, (B) 0, and (C) -0.71. In all panels, bars indicate the extent of the 95% quantile of estimates. Note for the smallest value of h_s^2, the sex-linked additive genetic variances were not estimable. Also, the error bars for the three smallest values of h_s^2 depicted extend beyond the range of the plotting region.

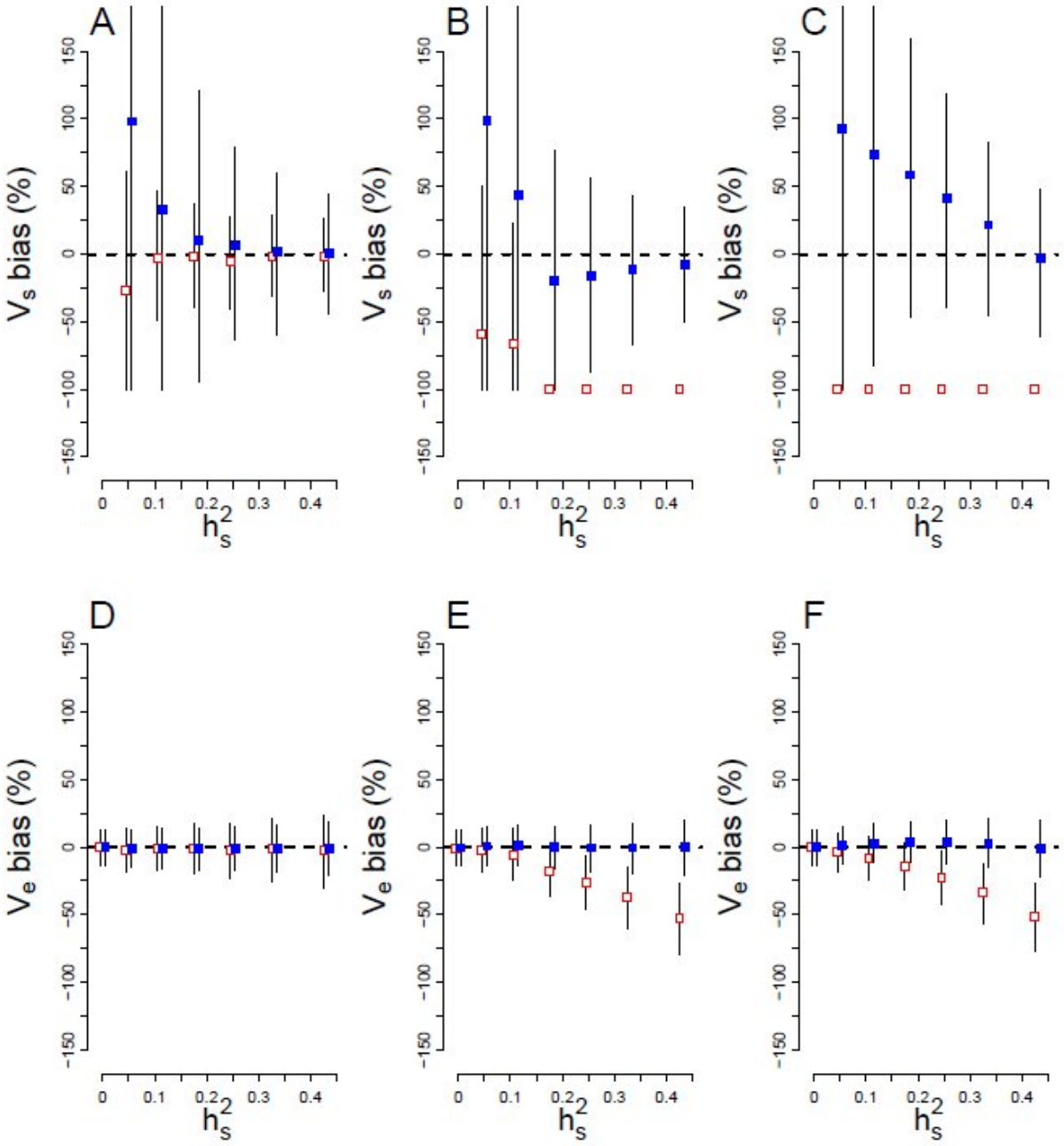


Figure B2.2. Analyses of the HS pedigree when implementing "informed" animal models (boxes). Panel arrangement, symbols, and lines as in figure B2.1. Note in panels (B) and (C) models estimating sex-linked variance for the homogametic sex converged on the boundary of the parameter space (i.e., zero). Specifying different starting values improved the model behavior, but those results are not shown (see text for a discussion).

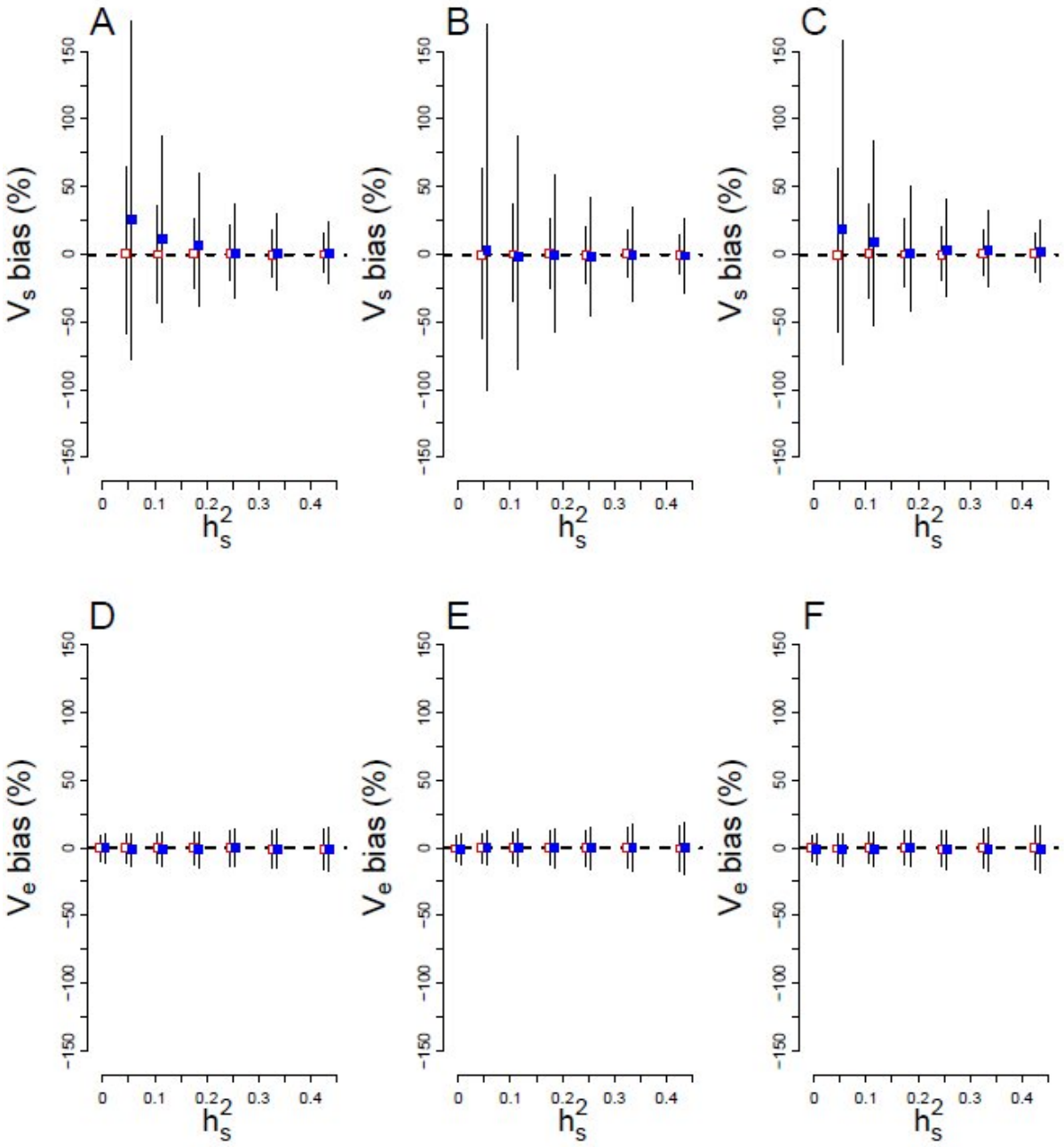


Figure B2.3. Analyses of the DFC pedigree when implementing "informed" animal models (boxes). Panel arrangement, symbols, and lines as in figure B2.1.

B3 Biases in residual variance estimates from "naïve" models

The HS pedigree analyzed with a naïve observed variance component model attributes sex-linked variance in both sexes to the autosomal additive variance estimate. However, this model tends to attribute too much variance to the autosomal additive variance in the homogametic sex, resulting in an underestimate of the residual variance at higher levels of simulated sex-linked variance (h_s^2) (Figure B3.1A-C). A similar result is observed for the HS pedigree analyzed with a naïve animal model, however the tendency to over-assign variance to the autosomal additive variance component occurs in both sexes when sex-linked variance (h_s^2) is higher (Figure B3.1D-F). The residual variance is instead biased upward when the DFC pedigree is analyzed with a naïve animal model (Figure B3.1G-I).

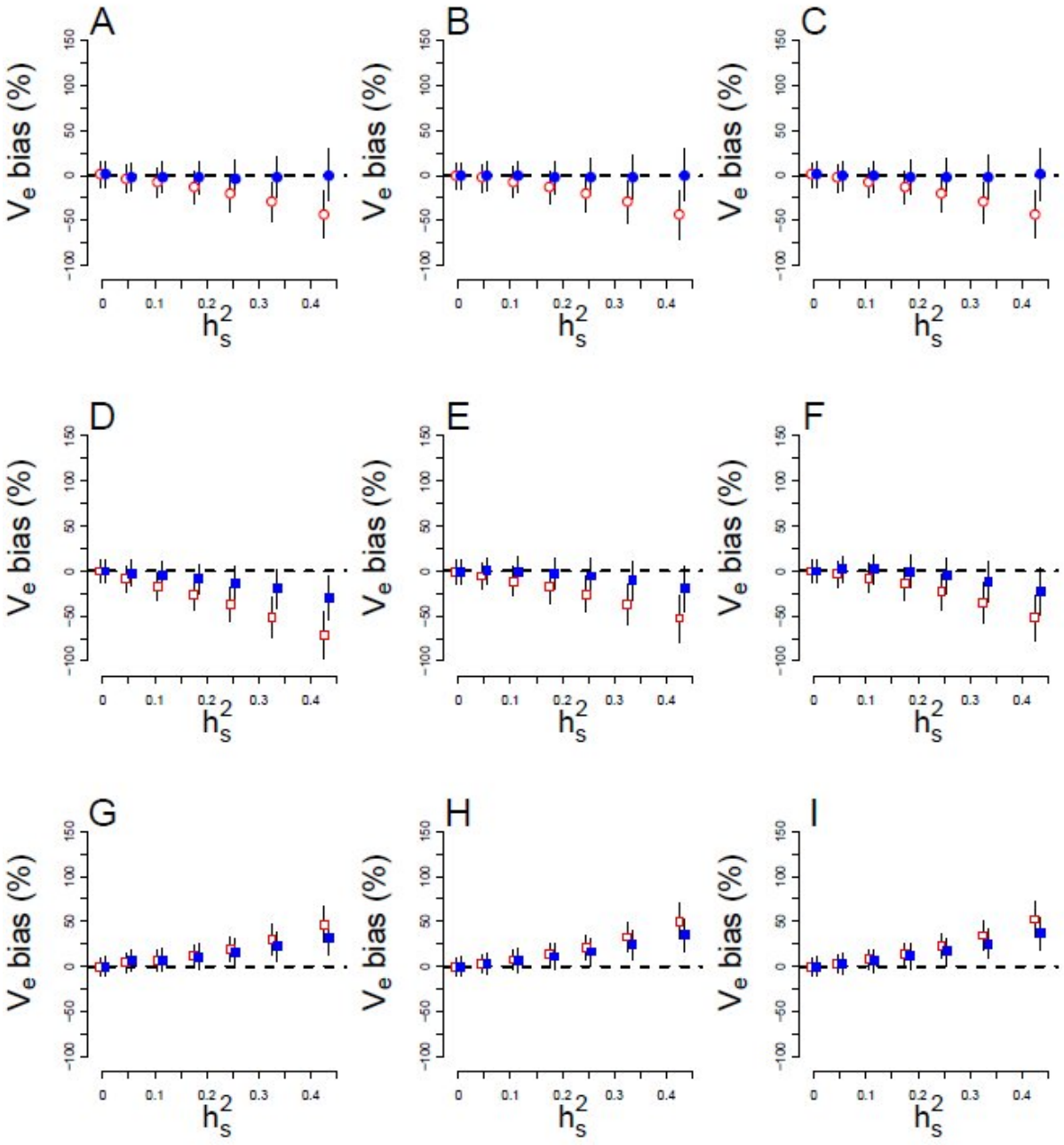


Figure B3.1 Analyses of: the HS pedigree when implementing "naïve" observed variance components models (circles; A-C) and "naïve" animal models (squares; D-F), and the DFC pedigree when implementing "naïve" animal models (boxes, G-I). The panels depict average percent bias in residual variance estimates in the homogametic sex (open, red symbols) and the heterogametic sex (closed, blue symbols) as a function of the proportion of phenotypic variance comprised of sex-linked variance (h_s^2) and when the sex-linked between-sex additive genetic correlation is approximately 0.71 (first column), 0 (second column), and -0.71 (third column). In all panels, bars indicate the extent of the 95% quantile of estimates.

B4 Half-sib analyses with parent phenotypes

Below is the design matrix used in observed variance components analyses of the HS data with offspring-parent covariances included. Adapted from Chenoweth et al. (2008) and Mezey and Houle (2005). Below, dams/daughters were assumed to be the homogametic sex and sires/sons the heterogametic sex.

Observed components	Genetic or causal components								
	$\sigma^2_{a\text{-}hom}$	$\sigma^2_{a\text{-}het}$	$\sigma^2_{s\text{-}hom}$	$\sigma^2_{s\text{-}het}$	$\sigma^2_{e\text{-}hom}$	$\sigma^2_{e\text{-}het}$	$\sigma_{a\text{-}hom,het}$	$\sigma_{s\text{-}hom,het}$	$\sigma_{e\text{-}hom,het}$
Dam on mid-daughter covariance	0.5	0	0.5	0	0	0	0	0	0
Sire on mid-son covariance	0	0.5	0	0	0	0	0	0	0
Homogametic Sex									
sire variance	0.25	0	0.5	0	0	0	0	0	0
dam variance	0.25	0	0.25	0	0	0	0	0	0
within-family variance	0.5	0	0.25	0	1	0	0	0	0
Heterogametic Sex									
sire variance	0	0.25	0	0	0	0	0	0	0
dam variance	0	0.25	0	0.5	0	0	0	0	0
within-family variance	0	0.5	0	0.5	0	1	0	0	0
Between-sex									
sire covariance	0	0	0	0	0	0	0.25	0	0
dam covariance	0	0	0	0	0	0	0.25	0.5	0
within-family covariance	0	0	0	0	0	0	0.5	0.5	1

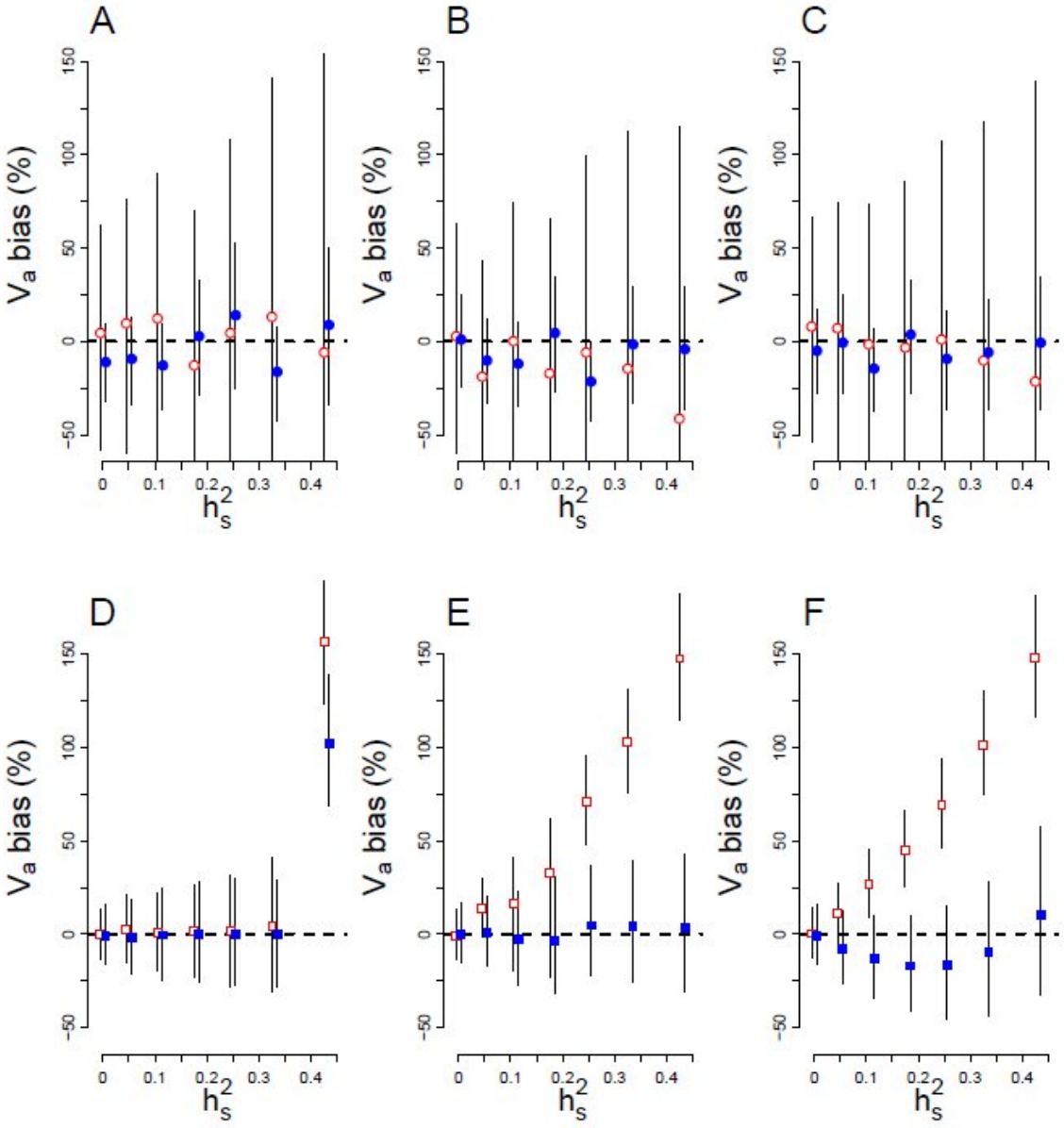
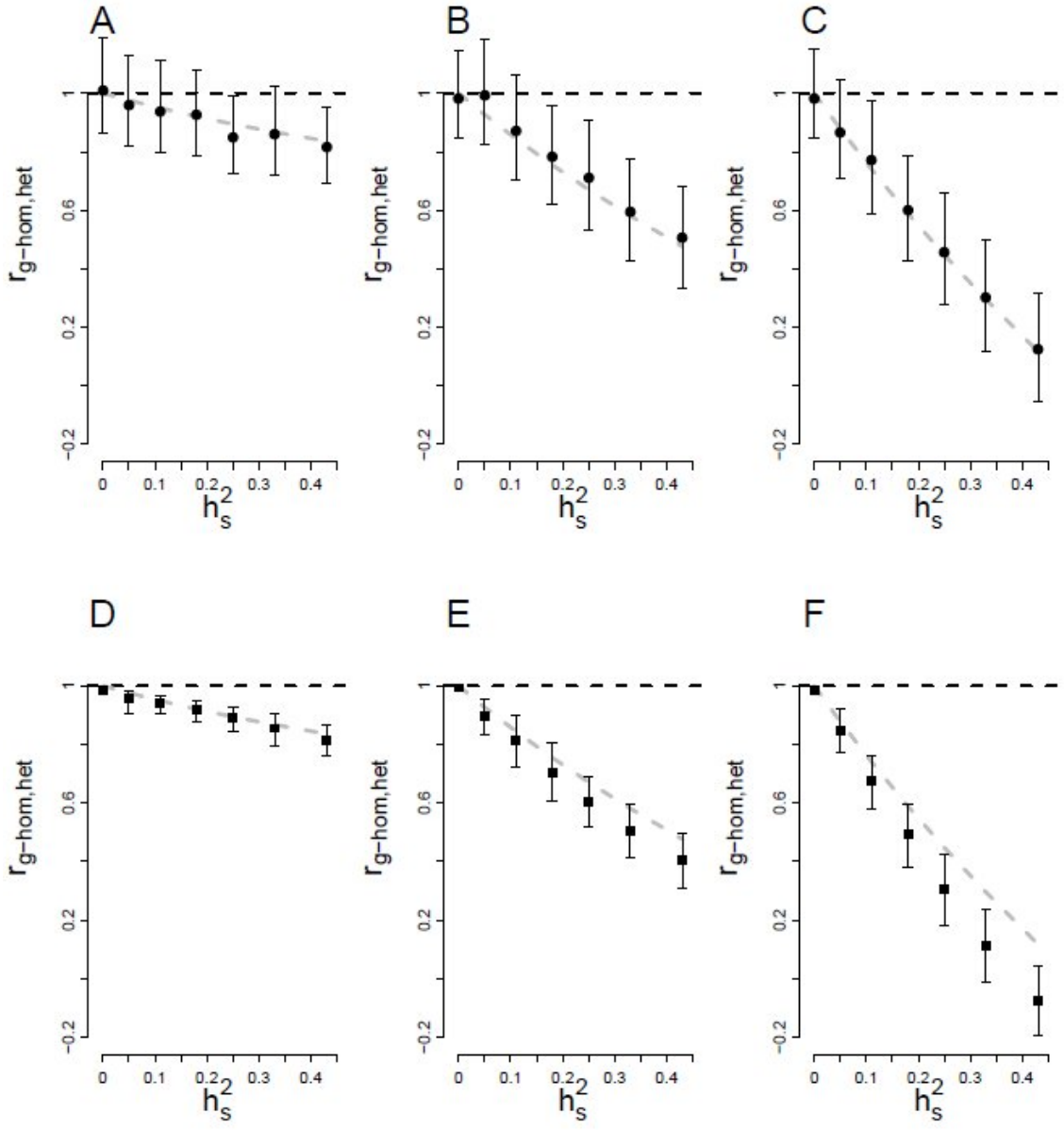


Figure B4.1. Analyses of the HS pedigree when implementing "informed" observed variance components models (circles, A-C) or "informed" animal models (squares, D-F) with parent phenotypic information included in the analyses. The panels depict average percent bias in autosomal additive variance estimates in the homogametic sex (open, red symbols) and the heterogametic sex (closed, blue symbols) as a function of the proportion of phenotypic variance comprised of sex-linked variance (h_s^2) when the sex-linked between-sex additive genetic correlation is approximately 0.71 (first column), 0 (second column), or -0.71 (third column). In all panels, bars indicate the extent of the 95% quantile of estimates.



F

igure B4.2. Analyses of the HS pedigree when implementing "informed" observed variance components models (circles, A-C) or "informed" animal models (squares, D-F) with parent phenotypic information included in the analyses. The panels depict the genomic (autosomal and sex-linked) between-sex additive genetic correlation ($r_{g\text{-}hom,het}$) when the sex-linked between-sex additive genetic correlation is approximately 0.71 (first column), 0 (second column), or -0.71 (third column). The horizontal black line indicates the simulated autosomal between-sex additive genetic correlation of one and the diagonal grey line indicates the expected genomic between-sex additive genetic correlation. In all panels, bars indicate the extent of the 95% quantile of estimates.

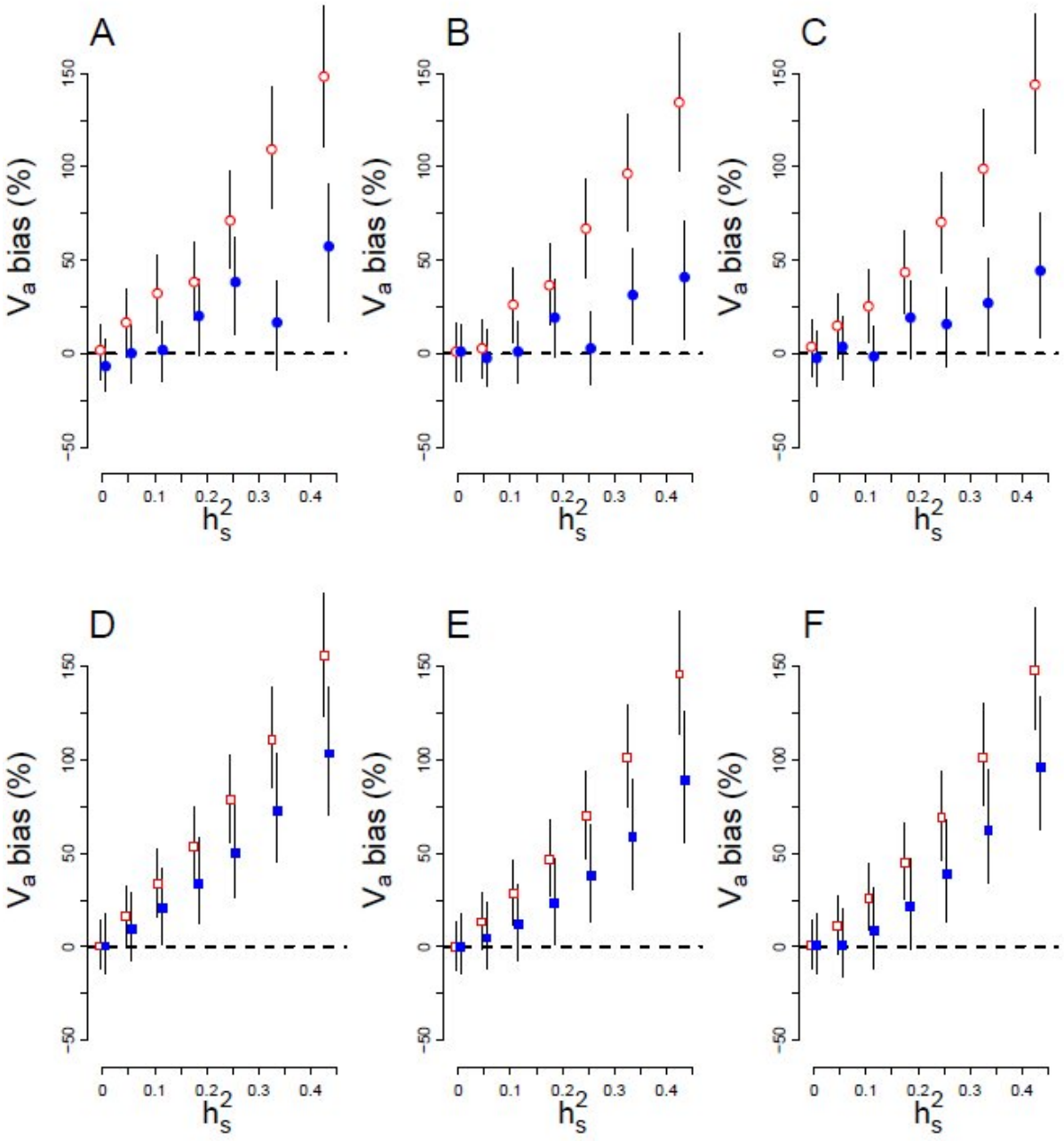


Figure B4.3. Analyses of the HS pedigree when implementing "naïve" observed variance components models (circles, A-C) or "naïve" animal models (squares, D-F) with parent phenotypic information included in the analyses. The panels depict average percent bias in autosomal additive variance estimates. Panel arrangement, symbols, and lines as in figure B4.1.

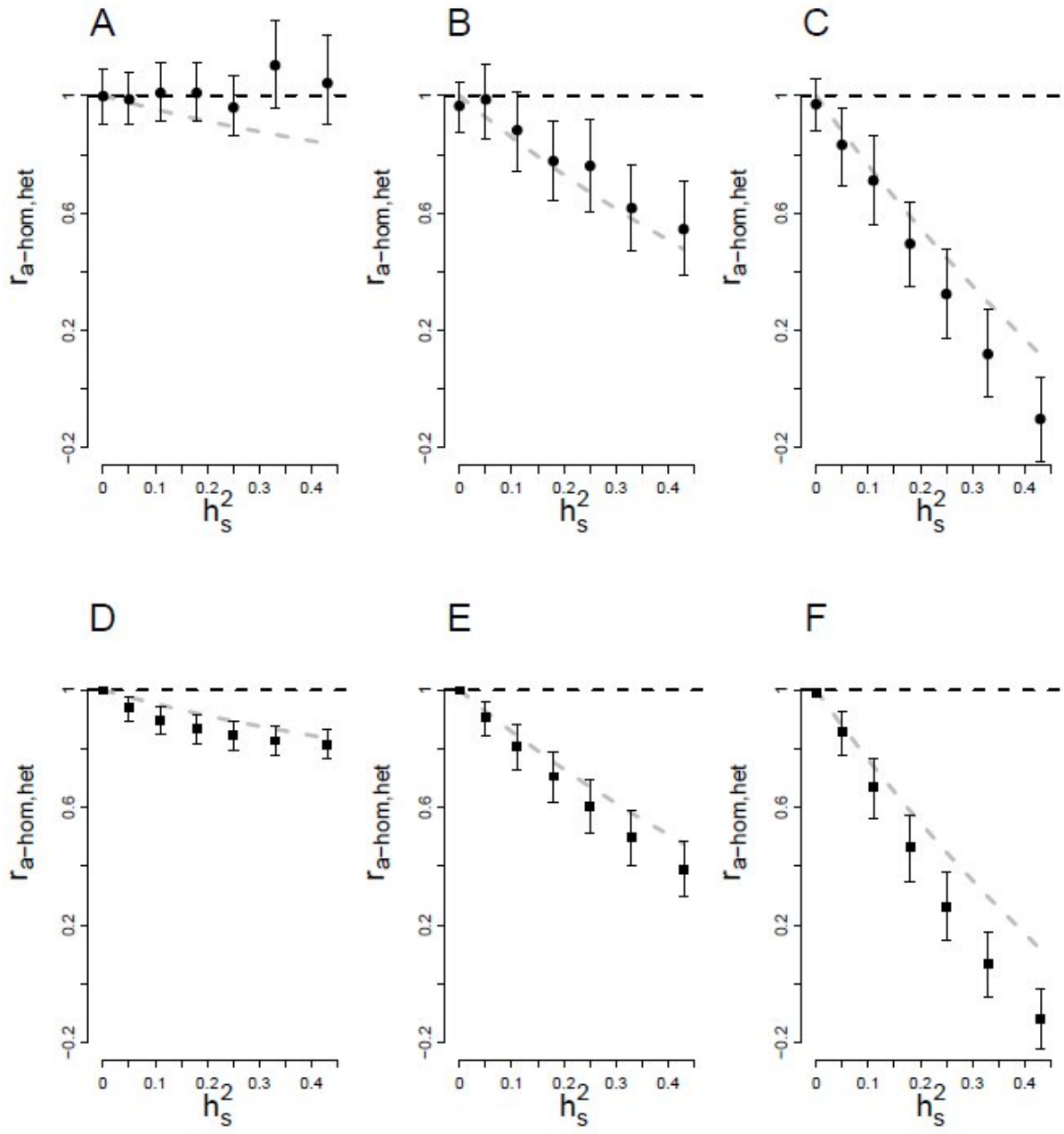


Figure B4.4. Analyses of the HS pedigree when implementing "naïve" observed variance components models (circles, A-C) or "naïve" animal models (squares, D-F) with parent phenotypic information included in the analyses. The panels depict the autosomal between-sex additive genetic correlation ($r_{a\text{-}hom,het}$). Panel arrangement, symbols, and lines as in figure B4.2. Note, unlike in the text the between-sex additive genetic correlation was estimated from the design matrix and not from the sire (co)variance components (see *Results*).

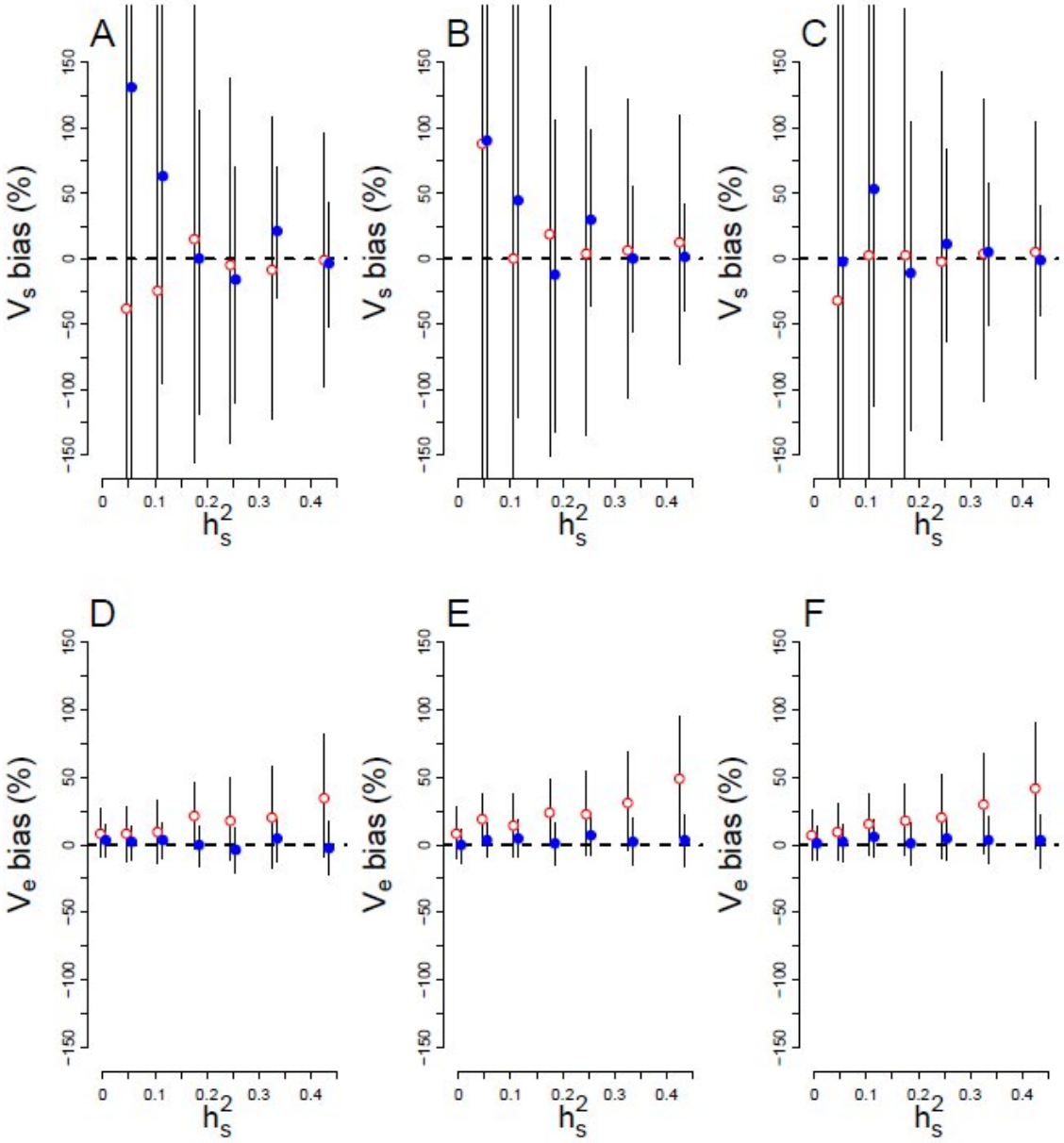


Figure B4.5. Analyses of the HS pedigree when implementing "informed" observed variance components models (circles) with parent phenotypic information included in the analyses. The top panels (A-C) depict the average percent bias in sex-linked additive variance estimates and the bottom panels (D-F) depict the average percent bias in residual variance estimates when the sex-linked between-sex additive genetic correlation is approximately 0.71 (first column), 0 (second column), or -0.71 (third column). Symbols, and lines as in figure B4.1. Note for the smallest value of h_s^2, the sex-linked additive genetic variances were not estimable. Also, the error bars for the three smallest values of h_s^2 depicted extend beyond the range of the plotting region.

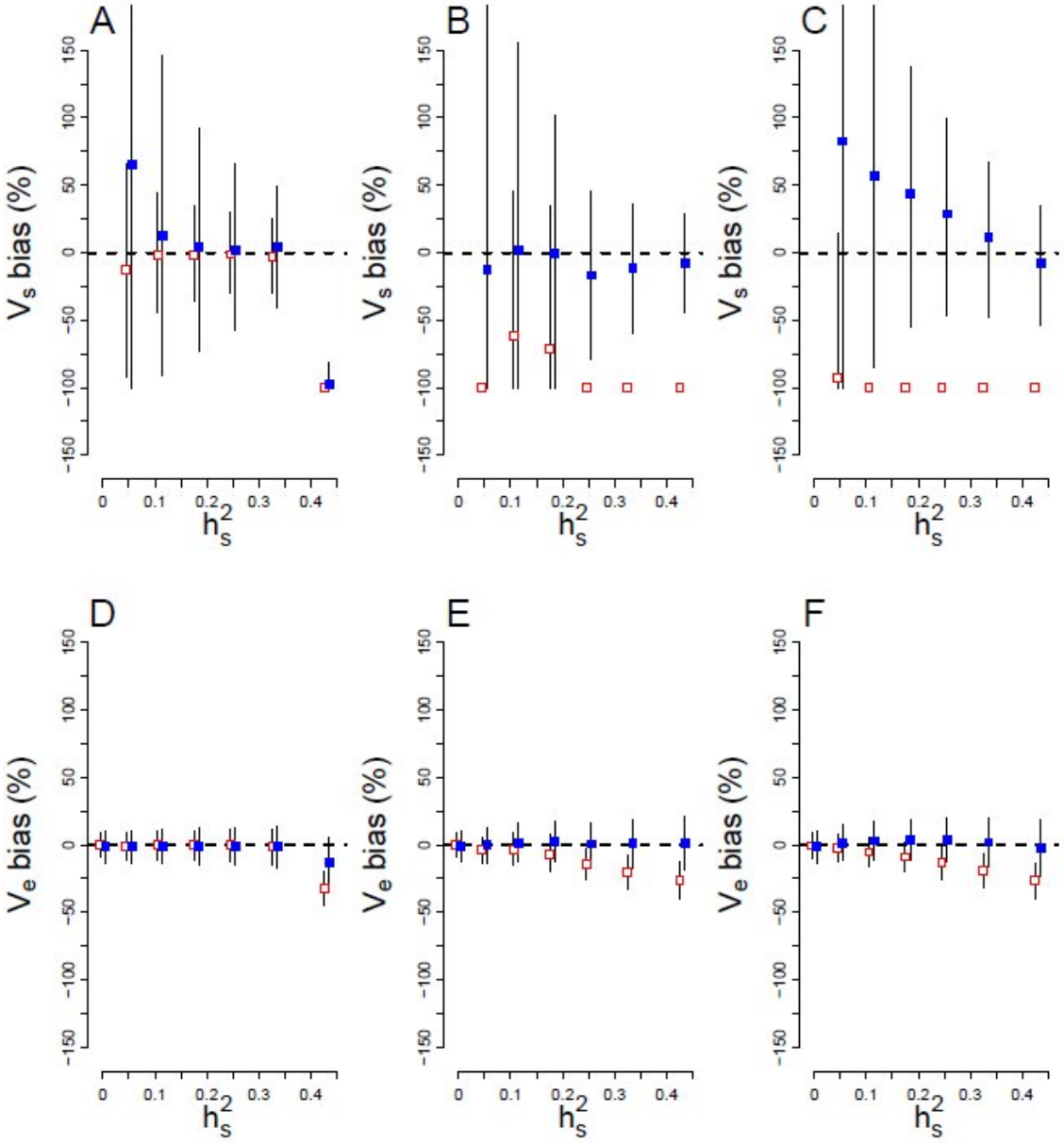


Figure B4.6. Analyses of the HS pedigree when implementing "informed" animal models (squares) with parent phenotypic information included in the analyses. The top panels (A-C) depict the average percent bias in sex-linked additive variance estimates and the bottom panels (D-F) depict the average percent bias in residual variance estimates when the sex-linked between-sex additive genetic correlation is approximately 0.71 (first column), 0 (second column), or -0.71 (third column). Symbols and lines as in figure B4.1. Note some models estimating sex-linked variance for the homogametic sex converged on the boundary of the parameter space (i.e., zero). Specifying different starting values improved the model behavior, but those results are not shown (see text for a discussion).

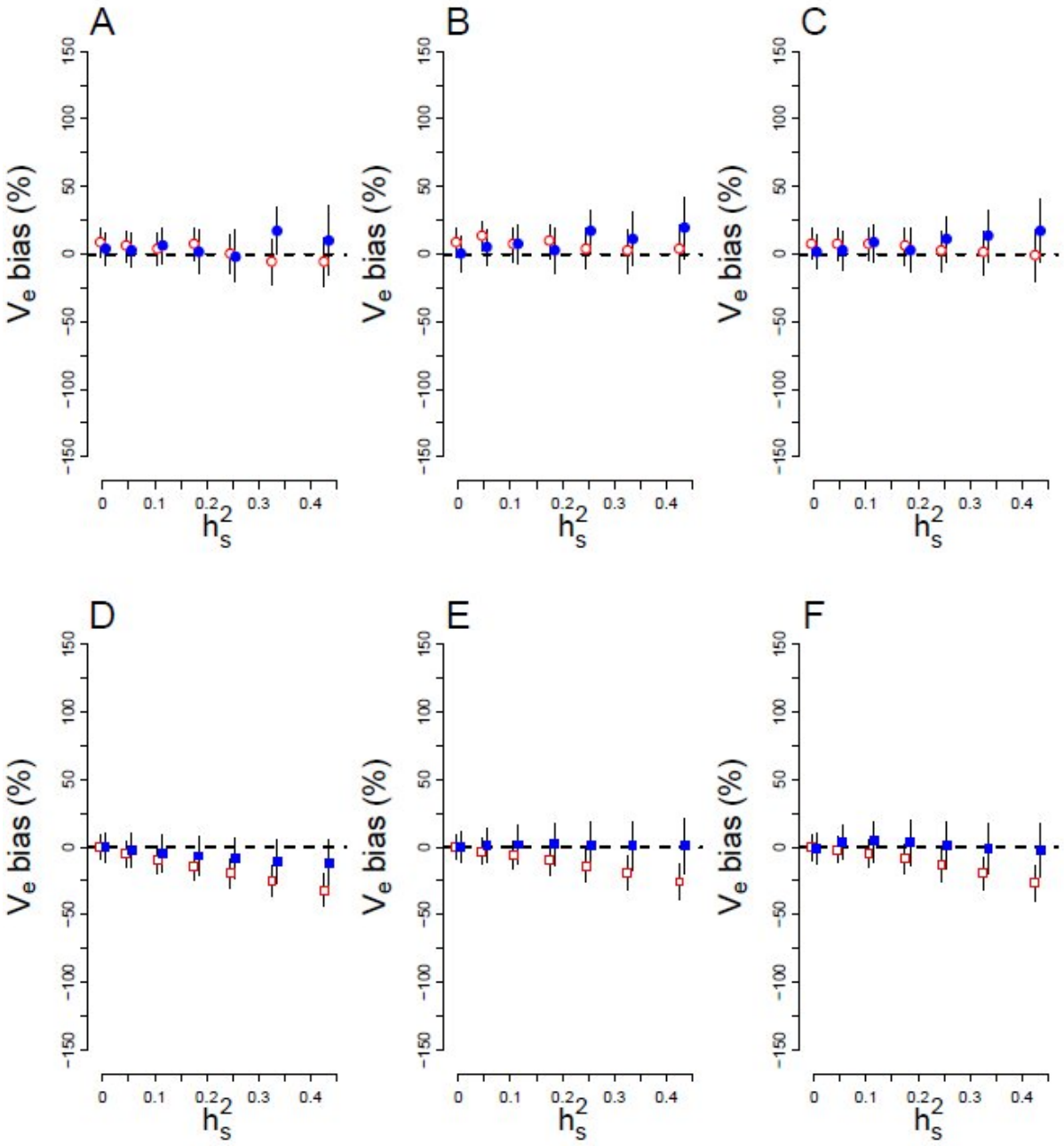


Figure B4.7. Analyses of the HS pedigree when implementing "naïve" observed variance components models (circles; panels A-C) or "naïve" animal models (squares; panesl D-F) with parent phenotypic information included in the analysis. The panels depict average percent bias in residual variance estimates when the sex-linked between-sex additive genetic correlation is approximately 0.71 (first column), 0 (second column), or -0.71 (third column). Symbols and lines as in figure B4.1.

APPENDIX C: Supporting Information for Chapter 5

C1 Joint scaling test coefficient matrices

Table C.1.1. The **M** matrix of coefficients used in the joint scaling tests (F_2 reference metric).

	Composite genetic effect										
Male	**m**	**a**	**d**	**axa**	**axd**	**dxd**	**m_a**	**m_d**	**c**	**X_a**	
P1	1	1	0	1	0	0	1	0	1	1	
B1a (P1xF1)	1	0.5	0.5	0.25	0.25	0.25	1	0	1	1	
B1ra (F1xP1)	1	0.5	0.5	0.25	0.25	0.25	0	1	1	0	
F1 (P1xP2)	1	0	1	0	0	1	1	0	1	1	
F2 (F1xF1)	1	0	0.5	0	0	0.25	0	1	1	0	
F2r (F1rxF1r)	1	0	0.5	0	0	0.25	0	1	-1	0	
F1r (P2xP1)	1	0	1	0	0	1	-1	0	-1	-1	
B2rb (F1rxP2)	1	-0.5	0.5	0.25	-0.25	0.25	0	1	-1	0	
B2b (P2xF1r)	1	-0.5	0.5	0.25	-0.25	0.25	-1	0	-1	-1	
P2	1	-1	0	1	0	0	-1	0	-1	-1	
Female	**m**	**a**	**d**	**axa**	**axd**	**dxd**	**m_a**	**m_d**	**c**	**X_a**	**X_d**
P1	1	1	0	1	0	0	1	0	1	1	0
B1a (P1xF1)	1	0.5	0.5	0.25	0.25	0.25	1	0	1	1	0
B1ra (F1xP1)	1	0.5	0.5	0.25	0.25	0.25	0	1	1	0.5	0.5
F1 (P1xP2)	1	0	1	0	0	1	1	0	1	0	0
F2 (F1xF1)	1	0	0.5	0	0	0.25	0	1	1	0.5	0.5
F2r (F1rxF1r)	1	0	0.5	0	0	0.25	0	1	-1	-0.5	0.5
F1r (P2xP1)	1	0	1	0	0	1	-1	0	-1	0	1
B2rb (F1rxP2)	1	-0.5	0.5	0.25	-0.25	0.25	0	1	-1	-0.5	0.5
B2b (P2xF1r)	1	-0.5	0.5	0.25	-0.25	0.25	-1	0	-1	-1	0
P2	1	-1	0	1	0	0	-1	0	-1	-1	0

C2 Model AIC statistics

Traits are abbreviated as follows: thorax length (Lthorax), abdomen length (Labd), total length (Ltotal), abdomen width (Wabd), front-femur length (Lff), front-femur width (Wff), mid-femur length (Lmf), hind-femur length (Lhf), genital length (Lgenital), segment 8 length (Lseg8), segment 7 margin length (Lseg7mar), and spine width (Wspine). Composite genetic effects are abbreviated as in the *Materials and Methods* of the main text, except autosomal additive, dominance and digenic epistatic effects are abbreviated as A, D, and E, respectively.

File: "C2FemaleExp1.xlsx"

Table C2.1. Female experiment 1 AIC values.

Table C2.2. Female experiment 1 AIC differences (ΔAIC).

Table C2.3. Female experiment 1 AIC weights (*w*).

File: "C2MaleExp1.xlsx"

Table C2.4. Male experiment 1 AIC values.

Table C2.5. Male experiment 1 AIC differences (ΔAIC).

Table C2.6. Male experiment 1 AIC weights (*w*).

File: "C2Female Exp2.xlsx"

Table C2.7. Female experiment 2 AIC values.

Table C2.8. Female 2011 experiment 2 differences (ΔAIC).

Table C2.9. Female 2011 experiment 2 weights (*w*).

File: "C2MaleExp2.xlsx"

Table C2.10. Male experiment 2 AIC values.

Table C2.11. Male experiment 2 AIC differences (ΔAIC).

Table C2.12. Male experiment 2 AIC weights (*w*).

C3 Model averaged parameter estimates

Table C3.1 (in file "C3MAPE.xlsx") contains the model averaged parameter estimates (MAPEs) for females and males. The MAPE for each composite genetic effect is expressed as the proportion of the difference between the two parental lines (P1 and P2) that is explained by each MAPE. Wald tests were conducted to test if each MAPE differed between females and males in each experiment.

Table C3.2 (in file "C3MAPE.xlsx") contains the female specific MAPEs. To compare female and male MAPEs, the sum of m_a and X_a in females was used to reflect the quantity reflected by the estimate of m_a in males. However, we could separately estimate m_a and X_a in females as well as X_d and therefore present them here.

C4 Among year variation in a population cross

We compared the model averaged estimates (MAPEs) of composite genetic effects between the two experiments, within each sex. We used the same Wald test statistic as in the main text where we compared the MAPEs between sexes, but within experiments. Results are presented in table C4.1 and suggest that the differences in genetic architectures observed in each experiment are mostly the same. However, there were a few traits for which the two experiments differed in the estimates of the composite genetic effects. This suggests differences between RSC and LLC (the two populations not crossed) in the type and strength of genetic effects underlying these traits.

Pilot experiment comparison

In the spring of 2009, we collected water striders from the same two streams as Experiment one in the main text to conduct a pilot experiment of the line cross between Santa Cruz Island (SCI) and Rattlesnake Creek (RSC). The sampling and rearing protocol were the same as described in the main text, except instead of seeding the rearing cages with eggs from mating pairs we created the lines in mass-mating tanks to produce eggs for each cross type. We collected eggs from the tanks and then placed these in rearing cages in the growth chamber. Consequently, in the pilot experiment individuals reared in cages were not necessarily related (individuals within a cage from Experiment one and two were either full- or half-siblings; they always had the same dam). We only produced the first generation of crosses: P1 (RSC x RSC), F1 (RSC x SCI), F1r (SCI x RSC), and P2 (SCI x SCI). Here, we compare line means from this pilot experiment in 2009 to Experiment one from the main text (conducted in 2010) for the four lines of the first lab reared generation only. Note that in 2009 we did not measure length of the segment 7 margin (Lseg7mar) or spine width (Wspine) and so cannot compare these between 2009 and 2010.

Overall, we observed a very similar pattern in the line means from one year to the next in this cross. We expressed both F1 (RSC x SCI) and F1r (SCI x RSC) cage means as deviations from the mean of the two parent populations. Thus, the deviations represent the distance from an additive only genetic model. Any departure from the additive expectation is presumed to reflect the composite genetic effects we quantified for experiments one and two in the main text. If there is no difference in the deviation from the additive expectation from one year to the next in each line, we conclude that the genetic effects underlying the two parent populations are the same from one year to the next. We tested for a significant year-by-line interaction using analysis of variance on the F1 and F1r cage means expressed as deviations from each year. The only significant interaction term in the ANOVA was for male thorax length (Lthorax: $p=0.044$). These results suggest that the pattern of line differences does not change from one year to the next and we conclude that the genetic architecture, as estimated from our sampled individuals from the RSC and SCI populations, is the same in 2009 as in 2010.

We also compared first generation reciprocal hybrid female lines to test for maternal genetic effects (see *X-linkage analyses* section of Materials and Methods). We found evidence of maternal effects contributing to the difference between reciprocal hybrid lines in Lhf, Lgenital, and Lseg8 (Table C4.2). However, these p-values were no longer significant at a Bonferroni-adjusted critical level when correcting for all tests (table-wide significance) or even just female tests (just the tests in the top half of the table). Similar to the results from Experiment one, none of the male comparisons were significant indicating no X-linkage for the traits non-significant in the female comparisons (Table C4.2). Therefore, when comparing the RSC and SCI population cross conducted in two separate years, we see very close agreement between the results for males, however, results for female genital and some leg traits do not agree between the years.

Table C4.1. Comparison of model averaged parameter estimates between years and within sex. P-values of the Wald chi-squared tests are presented below. Abbreviations are explained in the text.

	Female									
	a	**d**	**axa**	**axd**	**dxd**	**m_a**	**m_d**	**c**	**X_a**	**X_d**
Lthorax	p<0.05	0.891	0.902	0.193	0.858	0.849	0.719	0.657	p<0.01	0.498
Labd	0.282	0.184	0.698	0.196	0.062	0.945	0.146	0.432	0.398	0.55
Ltotal	0.094	0.755	0.809	0.099	0.614	0.756	0.118	0.809	p<0.05	0.306
Wabd	0.984	p<0.05	p<0.01	0.342	p<0.05	0.826	0.721	0.882	0.967	0.453
Lff	0.377	0.134	p<0.01	0.603	0.744	0.281	0.419	0.835	0.813	0.545
Wff	0.106	p<0.01	p<0.0001	0.387	p<0.05	0.233	0.741	0.128	0.741	0.579
Lmf	0.296	p<0.05	p<0.01	0.193	0.257	0.847	0.155	0.912	0.152	0.354
Lhf	0.178	0.181	p<0.05	0.448	0.676	0.599	0.287	0.5	0.228	0.549
Lgenital	0.973	0.859	0.571	0.441	0.709	0.202	0.432	0.399	0.521	p<0.05
Lseg8	0.607	0.705	0.419	0.884	0.68	0.338	p<0.05	p<0.05	0.362	0.685
Lseg7mar	0.366	0.315	0.645	0.511	0.224	0.724	0.206	0.517	0.109	0.332
Wspine	0.638	0.595	0.268	0.409	0.656	0.412	0.499	p<0.001	0.191	0.473
	Male									
	a	**d**	**axa**	**axd**	**dxd**	**m_a**	**m_d**	**c**		
Lthorax	0.483	0.122	0.61	0.468	0.164	0.452	0.703	0.131		
Labd	0.257	0.91	0.364	0.88	0.226	0.743	0.242	0.233		
Ltotal	0.295	0.065	p<0.05	0.45	0.791	0.823	0.39	0.107		
Wabd	0.206	0.333	p<0.01	0.06	0.671	0.881	0.473	p<0.05		
Lff	0.852	0.8	0.067	0.065	0.323	0.408	0.813	0.21		
Wff	0.337	0.786	0.104	0.185	0.527	0.314	0.233	0.515		
Lmf	0.315	0.517	0.446	0.073	0.792	0.349	0.308	0.636		
Lhf	0.351	0.58	0.07	0.559	0.57	0.66	0.741	0.12		
Lgenital	0.094	p<0.0001	p<0.01	p<0.05	p<0.001	0.115	0.357	0.423		
Lseg8	0.07	0.055	0.662	p<0.05	p<0.01	0.48	p<0.05	p<0.05		

Lseg7mar	0.57	0.726	0.406	0.184	0.269	0.8	0.902	0.312
Wspine	0.871	0.62	0.944	0.707	0.659	$p<0.01$	0.17	$p<0.001$

Table C4.2. One-tailed t-tests comparing first generation reciprocals. P-values are no longer significant after a sequential Bonferroni correction accounting for all comparisons (42) or just the number of comparisons for each sex (female=20, male=22).

	Female									
	2010					**2009**				
	F1 (P1xP2)	F1r (P2xP1)	t	df	p	F1 (P1xP2)	F1r (P2xP1)	t	df	p
Lthorax	6.768	6.751	0.310	23.59	0.62	6.713	6.770	-1.107	25.99	0.14
Labd	6.863	6.896	-0.580	23.72	0.28	6.872	6.864	0.173	25.72	0.57
Ltotal	16.127	16.111	0.153	22.51	0.56	16.050	16.159	-1.181	25.40	0.12
Wabd	2.906	2.941	-0.955	12.62	0.18	2.874	2.875	-0.021	25.98	0.49
Lff	4.422	4.414	0.179	15.89	0.57	4.429	4.462	-0.627	25.95	0.27
Wff	0.568	0.578	-1.242	14.25	0.12	0.525	0.518	0.862	25.59	0.8
Lmf	9.564	9.765	-1.978	13.56	0.034	9.642	9.733	-1.015	25.08	0.16
Lhf	9.021	9.092	-0.822	16.05	0.21	8.813	9.011	-2.004	23.57	0.028
Lgenital	0.923	0.916	0.386	16.71	0.65	0.925	0.954	-1.916	25.44	0.033
Lseg8	0.664	0.662	0.085	15.50	0.53	0.646	0.678	-2.825	25.24	0.0045
	Male									
	2010					**2009**				
	F1 (P1xP2)	F1r (P2xP1)	t	df	p	F1 (P1xP2)	F1r (P2xP1)	t	df	p
Lthorax	6.536	6.299	2.897	7.94	0.99	6.456	6.389	1.286	15.24	0.89
Labd	4.138	4.055	1.604	7.39	0.92	4.125	4.048	1.871	14.35	0.96
Ltotal	15.028	14.625	2.342	7.03	0.97	15.159	14.974	1.128	13.59	0.86
Wabd	2.489	2.453	1.136	12.19	0.86	2.517	2.428	1.512	11.57	0.92
Lff	4.478	4.494	-0.247	8.07	0.41	4.479	4.513	-0.747	13.49	0.23
Wff	0.722	0.706	1.412	10.55	0.91	0.672	0.652	1.415	16.29	0.91
Lmf	10.130	9.899	1.531	7.53	0.92	10.055	9.857	1.450	11.23	0.91
Lhf	9.560	9.298	1.729	7.23	0.94	9.385	9.391	-0.046	13.35	0.48
Lgenital	2.875	2.781	2.673	12.60	0.99	3.053	3.041	0.141	13.05	0.56
Lseg8	1.338	1.293	2.915	13.40	0.99	1.496	1.506	-0.165	13.54	0.44
Lpyg	1.363	1.348	0.734	12.06	0.76	1.437	1.414	0.657	14.99	0.74

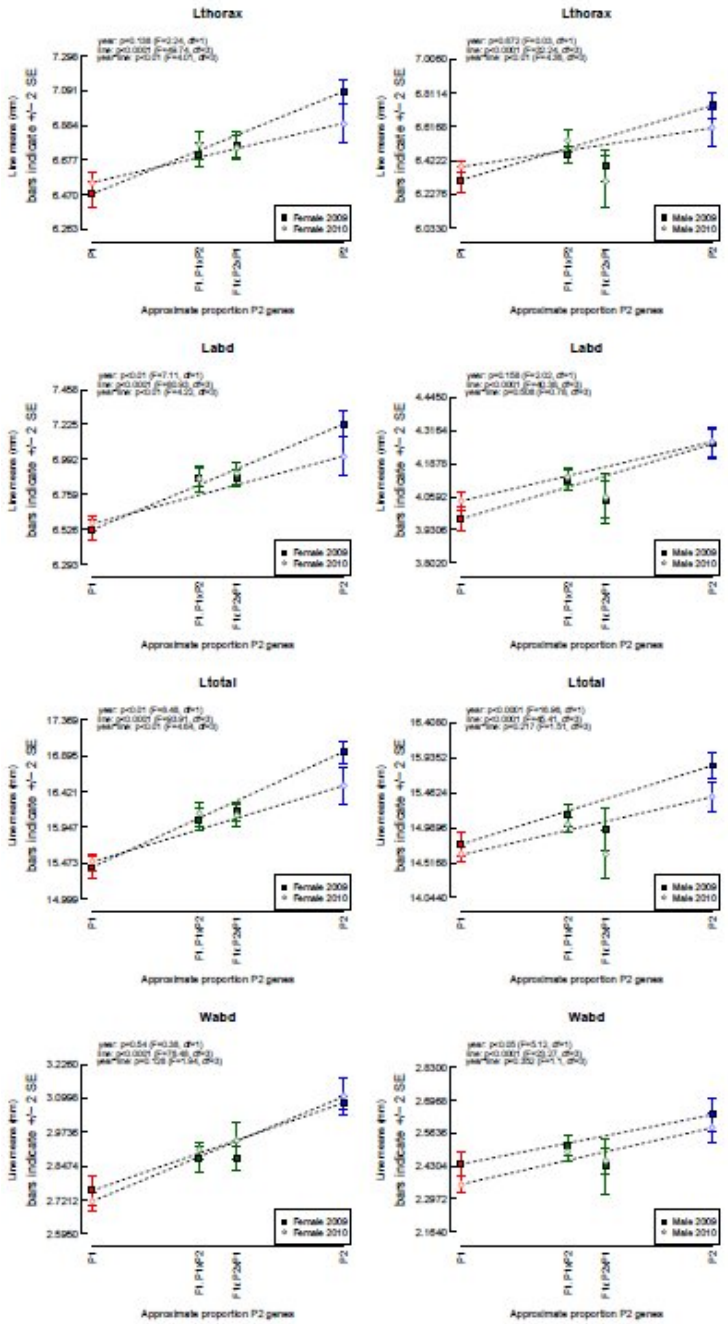


Figure C4.1. Observed line means (±2SE) of somatic traits for the population cross in experiment one in 2010 (open circles) and the pilot experiment in 2009 (filled boxes). Each plot depicts female (left column) or male (right column) line means. Line means are arranged from left to right along the x-axis to reflect the approximate proportion of P2 (SCI x SCI) genes each line contains. The dashed line indicates the additive expectation.

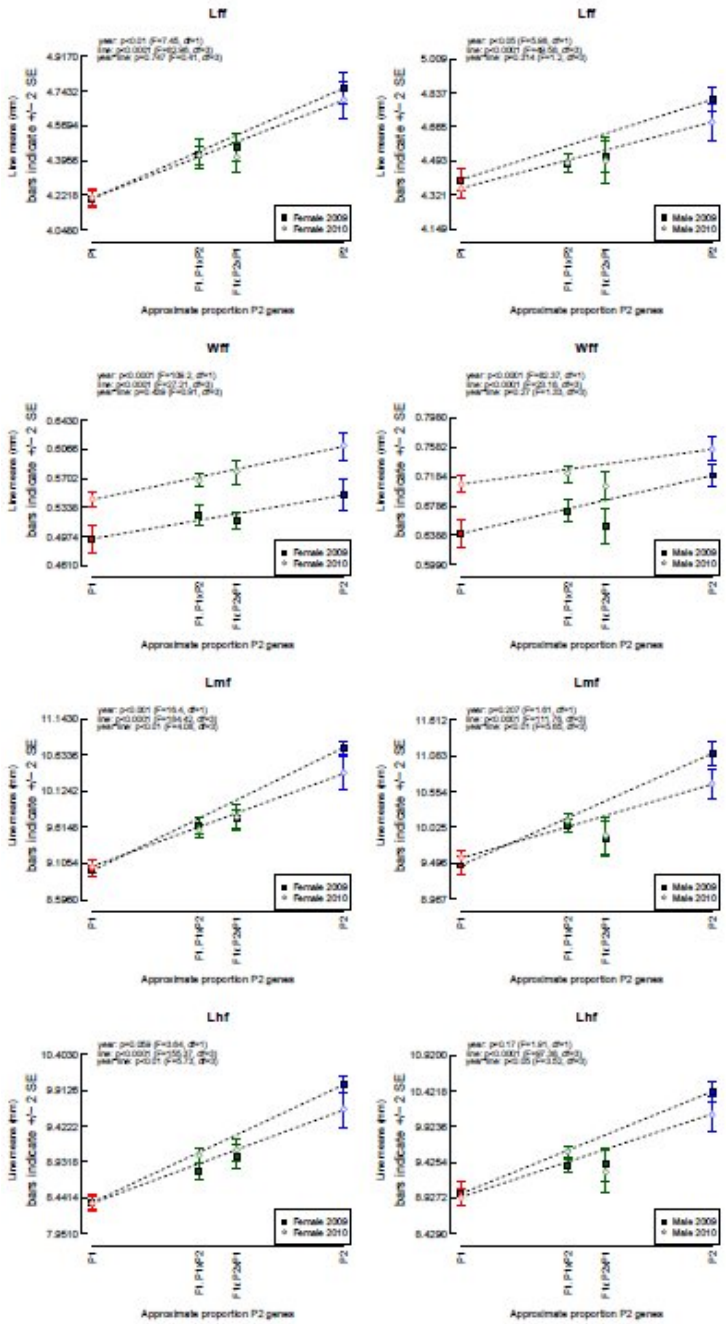


Figure C4.2. Observed line means (±2SE) of leg traits for the population crosses. Arrangement and symbols the same as in Figure C4.1.

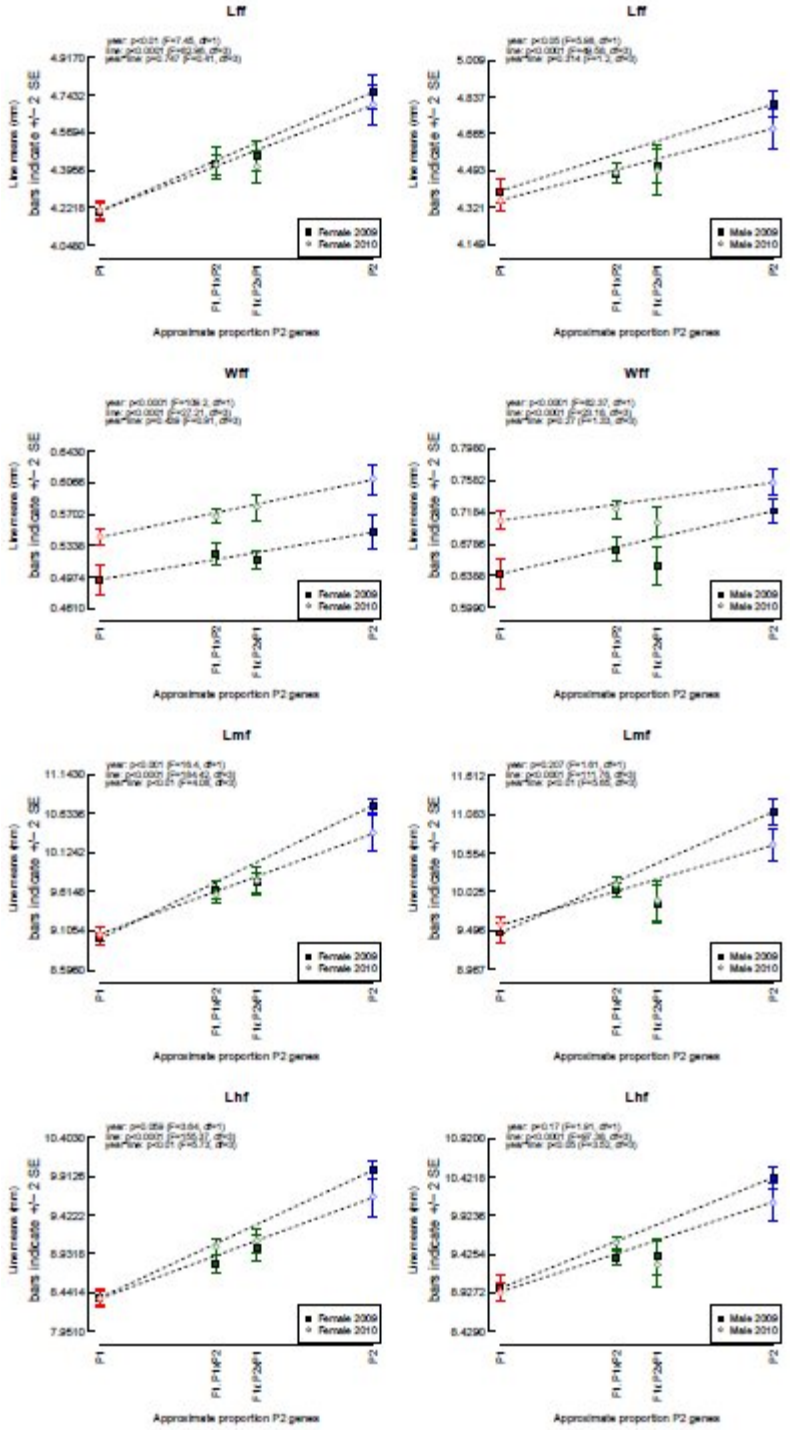


Figure C4.3. Observed line means (±2SE) of genital traits for the population crosses. Arrangement and symbols the same as in Figure C4.1.

C5 Derivation of a log-likelihood function and AIC from weighted least-squares regression
The natural logarithm of the likelihood function (L) of a weighted least-squares regression is:

$$\ln(L) = -\tfrac{1}{2}[n\ln(\hat{\sigma}^2_{e-ML}) + n + n\ln(2\pi) - \ln\left|\mathbf{V}^{-1}\right|] \quad \text{(C5.1)}$$

We assume the matrix $\mathbf{V^{-1}}$ contains only diagonal elements representing the squared standard error of the line means (the standard error of the line means are independent of one another). This enables the logarithm of $|\mathbf{V^{-1}}|$ (the determinant of $\mathbf{V^{-1}}$) to instead be expressed as a sum of logarithms of the diagonal elements of $\mathbf{V^{-1}}$. The maximum likelihood estimate of the residual variance ($\hat{\sigma}^2_{e-ML}$) is simply the RSS_W/n. With these simplifications, equation C5.1 reduces to:

$$\ln(L) = -\tfrac{1}{2}n\ln(RSS_W) + \tfrac{n}{2}\ln(n) - \tfrac{n}{2} - \tfrac{n}{2}\ln(2\pi) - \sum_{i=1}^{n}\ln(SE_i) \quad \text{(C5.2)}$$

where n is the number of lines in the experiment and SE_i is the standard error of the mean for the ith line. All but the first term in equation A2 are either an additive constant or will yield the same value for all models and therefore will take on a constant value in all models of the candidate set. Since the constant contributes the same value to the AIC of all models, it can be ignored. Thus, substitution of the first term in equation A2 into equation 3 from the text will yield the AIC of a weighted least-squares regression model in equation 4 above.

Note, the number of parameters estimated in the model, K, is equal to the number of genetic parameters (p) plus two. The two additional model parameters are the intercept and residual variance ($\hat{\sigma}^2_{e-ML}$) estimated in the regression (Burnham and Anderson 2002, p. 63). We note our equation 4 above differs from the AIC equation of Bieri and Kawecki (2003, their

equation 6). However, our AIC formula is equivalent to the least-squares regression AIC formula of Burnham and Anderson (2002, p. 63).