JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12429

Between-sex genetic covariance constrains the evolution of sexual dimorphism in *Drosophila melanogaster*

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Keywords:

B matrix;
cuticular hydrocarbons;
G matrix;
intralocus sexual conflict;
sexually antagonistic selection.

Abstract

Males and females share much of their genome, and as a result, intralocus sexual conflict is generated when selection on a shared trait differs between the sexes. This conflict can be partially or entirely resolved via the evolution of sex-specific genetic variation that allows each sex to approach, or possibly achieve, its optimum phenotype, thereby generating sexual dimorphism. However, shared genetic variation between the sexes can impose constraints on the independent expression of a shared trait in males and females, hindering the evolution of sexual dimorphism. Here, we examine genetic constraints on the evolution of sexual dimorphism in *Drosophila melanogaster* cuticular hydrocarbon (CHC) expression. We use the extended **G** matrix, which includes the between-sex genetic covariances that constitute the **B** matrix, to compare genetic constraints on two sets of CHC traits that differ in the extent of their sexual dimorphism. We find significant genetic constraints on the evolution of further dimorphism in the least dimorphic traits, but no such constraints for the most dimorphic traits. We also show that the genetic constraints on the least dimorphic CHCs are asymmetrical between the sexes. Our results suggest that there is evidence both for resolved and ongoing sexual conflict in *D. melanogaster* CHC profiles.

Introduction

Despite much of the genome being shared between the sexes, sexually dimorphic expression of shared traits is common across many species (Andersson, 1994; Fairbairn *et al.*, 2007). Sexual dimorphism is often thought to be associated with intralocus sexual conflict, where disruptive selection on a shared trait between the sexes (i.e. sexually antagonistic selection) displaces one or both sexes from their fitness optima (Cox & Calsbeek, 2009). Sexually antagonistic selection should drive divergence in trait expression between the sexes, potentially allowing males and females to more closely achieve their sex-specific phenotypic optima (Bonduriansky & Chenoweth, 2009).

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The degree to which sexual dimorphism can evolve, and by extension the degree to which sexual conflict may be resolved, depends not only on sex-specific selection, but also on genetic constraints arising from a genome that is largely shared between the sexes (Lande, 1980, 1987). The quantitative genetic techniques for quantifying this shared genetic architecture between the sexes were developed over 30 years ago (Lande, 1980). Since then, many studies have used univariate intersexual genetic correlations, r_{mf} (Lande, 1980; Lynch & Walsh, 1998), and measures of selection, to quantify ongoing conflict. In particular, sexual conflict is normally inferred where there is a strong, positive intersexual genetic correlation combined with divergent selection between the sexes (e.g. Merila *et al.*, 1997, 1998; Delph *et al.*, 2004; Long & Rice, 2007; Poissant *et al.*, 2010).

However, while simpler to measure and interpret, a univariate approach overlooks genetic covariances between suites of traits that can only be examined from a multivariate perspective. This may be an important issue because selection is widely viewed as a multivariate process that is unlikely to act on traits individually

(Hansen & Houle, 2008; Blows & Walsh, 2009), and the genetic basis of many quantitative traits is often shared to varying degrees. Although a few recent studies have adopted the more complex approach outlined by Lande (1980), insufficient attention has been given to multivariate studies of the quantitative genetics of sexual conflict. This was highlighted recently in a review by Wyman *et al.* (2013), who emphasized the importance of between-sex and between-trait genetic covariances especially in the context of studies of sexual dimorphism.

Lande's (1980) approach is based on the following partitioning of the **G** matrix:

$$\mathbf{G}_{\mathrm{mf}} = \begin{pmatrix} \mathbf{G}_{\mathrm{m}} & \mathbf{B} \\ \mathbf{B}^{\mathrm{T}} & \mathbf{G}_{\mathrm{f}} \end{pmatrix} \quad (1)$$

where the submatrices $\mathbf{G_m}$ and $\mathbf{G_f}$ describe the genetic (co)variance for a given set of traits expressed within males and females, respectively, and **B** (and the transposed submatrix, $\mathbf{B}^{\mathrm{T}}$) represents the genetic covariance between the sexes for these shared traits. Unlike $\mathbf{G_m}$ and $\mathbf{G_f}$, **B** is not expressed in any subset of individuals. However, **B** is important because if there is a lot of genetic variation in **B,** then the potential for males and females to evolve independently for these traits is low (Lande, 1980). High positive between-sex genetic covariation will hinder divergence between the sexes, slowing the evolution of sexual dimorphism and limiting the potential for sexual conflict to be resolved. This constraint can be measured by calculating the predicted response to selection, using data on sex-specific selection and $\mathbf{G_{mf}}$, and comparing this prediction with the actual direction of selection (Blows & Walsh, 2009). If the two are aligned, then there is no constraint, but if they are rotated away from one another, there is a constraint on the evolution of sexual dimorphism. To date, very few studies have considered genetic constraints on the evolution of sexual dimorphism contained within the **B** matrix (e.g. Lewis *et al.*, 2011; Gosden *et al.*, 2012; Stearns *et al.*, 2012). Each of these studies has identified significant genetic constraints, suggesting that the **B** matrix may often harbour important variation that can strongly affect the evolution of sexual dimorphism reviewed by Wyman *et al.*, (2013).

Drosophila cuticular hydrocarbons (CHCs) and their derivative are an ideal set of traits to study in this context. CHCs are waxy long-chain carbon compounds produced on the adult cuticle of both males and females, and previous research suggests that these traits may be targets of sex-specific selection. For example, CHCs form a waterproof layer on the cuticle that helps protect the insect from desiccation, creating natural selection on CHCs that can vary with temperature and humidity (Gibbs *et al.*, 1998; Frentiu & Chenoweth, 2010; Kwan & Rundle, 2010). Female *Drosophila* are generally larger and produce more CHCs than males, and the relationship between CHC profile and desiccation resistance is stronger in female *D. melanogaster* than in males, suggesting that selection on waterproofing might be sex-specific (Foley & Telonis-Scott, 2011). In addition, the pheromonal roles of CHCs have been extensively studied in several *Drosophila* species, encompassing studies of species recognition, male and female mate choice, and male–male sexual competition (Coyne *et al.*, 1994; Savarit & Ferveur, 2002; Byrne & Rice, 2006; Grillet *et al.*, 2006; Rundle *et al.*, 2008, 2009; Billeter *et al.*, 2009; Bretman *et al.*, 2011; Ingleby *et al.*, 2013a). Sex-specific sexual selection on CHCs is also therefore highly likely. As a suite of traits, CHCs have been shown to exhibit considerable genetic variation (e.g. Hine *et al.*, 2004; Ingleby *et al.*, 2013b), to respond rapidly to altered selection (e.g. Higgie *et al.*, 2000; Bedhomme *et al.*, 2011) and to exhibit extensive qualitative and quantitative sexual dimorphism (e.g. Chenoweth & Blows, 2003; Foley *et al.*, 2007; Chenoweth *et al.*, 2008).

Here, we examine genetic constraints on the evolution of sexual dimorphism in *D. melanogaster* CHC profiles via estimation of the between-sex genetic covariances that comprise the **B** matrix. This multivariate approach to the study of genetic constraints has been relatively neglected compared with the wealth of univariate studies employing r_{mf} (although see Lewis *et al.*, 2011; Gosden *et al.*, 2012; Stearns *et al.*, 2012). We extend past work by applying, for the first time in this context, a Bayesian approach to Lande's (1980) calculations and quantify sex-specific selection and genetic constraints on the direction and magnitude of CHC evolution. Our analyses focus on a biologically relevant comparison between two sets of CHCs that exhibit qualitatively different degrees of sexual dimorphism. Highly dimorphic CHCs might have been subject to past sexually antagonistic selection which is now resolved, whereas a lesser extent of dimorphism could mean either that these traits are not under sexual conflict, or that they are under conflict but there are genetic constraints preventing the evolution of further dimorphism. We find significant genetic constraints within the **B** matrix for the least dimorphic CHCs but not for the most dimorphic CHCs. We consider the implications of this for the evolution of sexual dimorphism and sexual conflict resolution in *D. melanogaster* CHCs.

Materials and Methods

Hemiclonal lines and experimental setup

The flies used in this study represent hemiclonal haplotypes that were sampled from the $\mathrm{LH_M}$ base population of *D. melanogaster*. This population has been maintained in the laboratory for approximately 500 generations as a large, outbred stock with overlapping generations. For full details of how the hemiclones for this study were

set up, maintained and expressed as either males or females, see Innocenti & Morrow (2010). Within each hemiclonal line, each fly shares a genomic haplotype, which is expressed along with a random haplotype from the LH_M base population. Variation across hemiclonal lines is therefore equivalent to additive genetic variation, although some epistatic interactions remain (see Rice *et al.*, 2005 for details).

The CHC data used here were collected from a subset of the hemiclonal lines described in Innocenti and Morrow (2010), and the fitness data were taken from this previous study, using only data from the relevant lines. In total, CHC and fitness data for females were collected for 36 hemiclonal lines, and male data were collected for 29 of these lines. This subset of lines was chosen on the basis of fitness phenotypes: approximately one-third had divergent fitness between the sexes with high female and low male fitness, one-third had low female and high male fitness, and one-third had intermediate fitness for both sexes. Nonrandom sampling of the lines in this way meant we included a wide range of fitness variation among the selected lines to help to quantify selection. The extent to which this results in a nonrandom sampling of CHC genetic variance is difficult to quantify but likely to be mild.

The fitness assays are described in full in Innocenti and Morrow (2010) and were designed to replicate the competitive conditions experienced by adults in the stock population. Briefly, male and female lifetime reproductive success was assayed in competitive assays using competitor flies from the LH_M population marked with bw^- eye colour. For males, five wild-type hemiclonal males were housed in a vial with 10 competitor bw^- males and 15 virgin bw^- females for two days. The females were then transferred to individual vials and allowed to oviposit for 18 h, and the adult progeny from these vials were scored for eye colour. Six replicates were carried out for each line, and relative male fitness for each line was calculated by averaging across replicates the proportion of offspring sired by hemiclonal males (bw^+/bw^-) divided by the maximum proportion across all hemiclonal lines. For females, the analogous assay involved five hemiclonal females housed with 10 competitor bw^- females and 15 bw^- males for two days, before the focal hemiclonal females were transferred to individual vials and allowed to oviposit. Four replicates were carried out per line. Relative female fitness was calculated by averaging across replicates the mean number of progeny per vial divided by the maximum fecundity across all lines. Note that absolute relative fitness values will differ slightly from Innocenti and Morrow (2010) as relative fitness was recalculated here for only the subset of hemiclonal lines used.

CHC extraction, gas chromatography and peak integration followed the protocol described in detail in Kwan and Rundle (2010). CHCs were extracted from individual flies via a three-minute immersion in hexane, then vortexed for one minute, after which the fly was discarded. The resulting samples were analysed on an Agilent 6890N gas chromatograph using the parameters given in Kwan and Rundle (2010). Multivariate outliers were identified by Mahalanobis distances and removed, leaving 1013 females and 792 males in the final analyses. We integrated 20 CHCs (Fig. 1) that were expressed by both males and females (as determined via shared retention times and comparison with previously published profiles in Foley *et al.*, 2007 and Everaerts *et al.*, 2010), excluding all sex-limited CHCs as our focus was on shared traits that could potentially be under sexual conflict. To correct for technical error in estimating absolute concentrations, the integrated values of each CHC were expressed as proportions of the total concentration of all CHCs for each individual. The resulting compositional data have a unit-sum constraint that prevents the use of standard statistical methods (Aitchison, 1986). To transform this compositional data from the simplex to the usual real space, we employed a centred log-ratio (CLR) transformation on the proportional peak areas (Aitchison, 1986; Pawlowsky-Glahn & Buccianti, 2011), as follows:

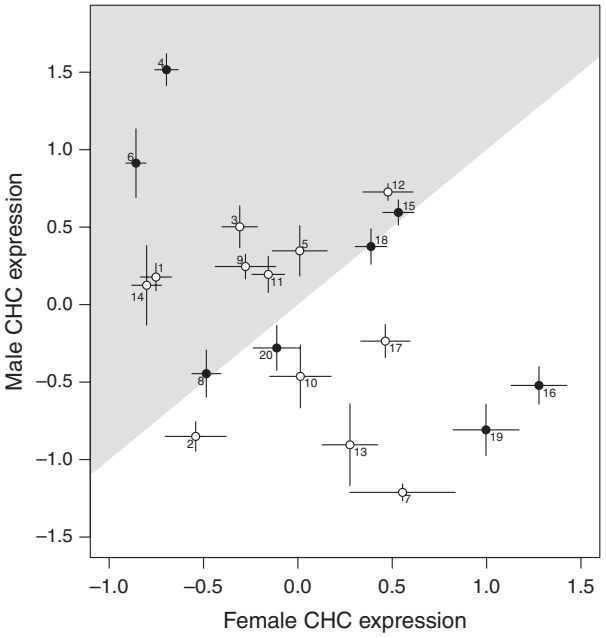

Fig. 1 Male expression plotted against female expression for each of the 20 shared CHC traits. Data were plotted after CLR transformation but prior to standardization, as sex-specific means with associated standard deviation. CHCs in the shaded area of the graph are male-biased in expression; female-biased CHCs are in the unshaded area. Closed circles indicate the least and most sexually dimorphic CHCs; open circles indicate CHCs with intermediate dimorphism. CHC numbers refer to the order of the CHC peaks on the chromatograph.

$$\text{trait}_n = \ln \frac{\text{prop}(\text{CHC}_n)}{\left(\prod_{n=1}^{k} \text{prop}(\text{CHC}_n)\right)^{\frac{1}{k}}} \quad (2)$$

where the divisor is the geometric mean of the proportional area of all k CHCs and the numerator is the proportion area of the n^{th} CHC. We favoured this transformation over the log-ratio (or log-contrast, as it is sometimes referred to) transformation that has been more commonly used in past CHC studies in *Drosophila* (e.g. Hine *et al.*, 2004; Rundle *et al.*, 2008) because the log-ratio transformation is not isometric and model fitting results (e.g. r^2 values) can depend on the arbitrary choice of denominator. The CLR transformation avoids this issue, but has the disadvantage of the resulting data being collinear (a zero-sum constraint), meaning that analyses that rely on full rank data matrices cannot be applied without first employing a dimensionality reduction technique such as principal components analysis. In our case, however, we are interested in only a subset of the CLR-transformed CHCs and this is therefore not an issue. Qualitatively similar results from the analyses below were obtained if we employed a log-ratio transformation.

Statistical analyses

We assessed the extent of sexual dimorphism for each trait using the difference between mean male and female expression. Male and female expression of each shared CHC is shown in Fig. 1, which also identifies the four least and four most sexually dimorphic CHCs. Phenotypic means and variances for each of these traits are given in Table 1. Our quantitative genetic analyses were conducted separately on these two subsets of traits because of statistical difficulties arising from the estimation of the 210 parameters that constitute the full $\mathbf{G_{mf}}$ matrix when all 20 CHCs are included within a single model. The four least and the four most sexually dimorphic CHCs are biologically relevant subsets of these traits with respect to questions concerning genetic constraints and the evolution of sexual dimorphism.

Prior to further analysis, CLR-transformed CHC scores were standardized to have a mean of zero and a unit variance (following Lande & Arnold, 1983). All analyses were carried out using R v.2.15.2 (http://www.R-project.org) and employed a Bayesian approach to Lande's (1980) analyses, thereby allowing us to directly calculate a credible interval for each genetic estimate from the posterior distribution.

First, multiple linear regression was used to estimate the linear selection gradients (β) for each sex independently (Lande & Arnold, 1983). These models used the MCMCglmm package v2.18 (Hadfield, 2010) with mean male and female relative fitness and mean male and female CHC score for each hemiclonal line. We used line means for the selection analyses as fitness and CHC data were collected from different individuals and during different experiments, and so although the lines were the same across data sets, the number of individuals and the time point differed. By extracting β from a posterior

Table 1 Phenotypic mean (μ) and variance (σ) for the 8 CLR-transformed CHC traits prior to any standardization. For each trait, data are given for (a) overall mean and variance; (b) female data only; and (c) male data only. The sex-specific posterior mean and credible interval for each trait are shown in (d). Nonoverlapping intervals show significant sexual dimorphism and are highlighted in bold. (e) The univariate intersexual genetic correlation for each trait, r_{mf}, calculated from separate univariate models of each trait.

	(a)		(b)		(c)		(d)		(e)
CHC	μ	σ	μ_{f}	σ_{f}	μ_{m}	σ_{m}	Female	Male	r_{mf}
Least dimorphic									
8	−0.468	0.016	−0.485	0.023	−0.445	0.006	**−0.149**	**0.160**	0.237
							−0.382–0.048	**0.098–0.324**	−0.148–0.569
15	0.560	0.008	0.533	0.007	0.595	0.007	**−0.304**	**0.382**	0.168
							−0.512 to −0.089	**0.092–0.668**	−0.223–0.521
18	0.382	0.010	0.388	0.013	0.375	0.007	0.071	−0.084	0.211
							−0.211–0.358	−0.326–0.156	−0.150–0.535
20	−0.186	0.026	−0.112	0.021	−0.280	0.016	**0.466**	**−0.563**	0.289
							0.231–0.702	**−0.740 to −0.380**	−0.075–0.594
Most dimorphic									
4	0.274	1.214	−0.696	0.011	1.516	0.004	**−0.884**	**1.128**	0.038
							−0.924 to −0.844	**1.087–1.171**	−0.308–0.381
6	−0.081	0.802	−0.858	0.049	0.913	0.003	**−0.870**	**1.111**	−0.050
							−0.923 to −0.816	**1.065–1.153**	−0.395–0.303
16	0.488	0.815	1.278	0.015	−0.521	0.022	**0.877**	**−1.122**	0.078
							0.827–0.924	**−1.181 to −1.066**	−0.270–0.409
19	0.205	0.832	0.997	0.027	−0.808	0.031	**0.869**	**−1.113**	−0.097
							0.807–0.926	**−1.180 to −1.045**	−0.438–0.256

distribution, we were able to directly calculate the 95% credible interval (95% CI) around each selection gradient. To test for overall differences between linear selection on males and females, we compared two MCMCglmm models of relative fitness with an approach analogous to a sequential model building procedure. The first model had sex and each CHC trait as predictors, and the second model included interactions between sex and each CHC to model sex-specific selection gradients. The difference in DIC between these models was used to quantify which model was the best fit.

We also tested for sex-specific disruptive, stabilizing and correlational selection on each set of CHCs by constructing multiple regression models that included squared and product terms of each trait combination as well as the linear terms. Although there was some evidence of weakly disruptive selection on females for two of the least dimorphic CHCs, inclusion of the nonlinear terms worsened the model fit for both the least and most dimorphic sets of CHCs, and so we focused on linear selection alone for further analyses.

The genetic variance–covariance matrix, **G**, for each set of four traits, was extracted from the posterior distribution of a multivariate mixed model in MCMCglmm which partitioned CHC variation between sex and line using individual CHC data for replicate males and females within each line. Each model had the respective set of four CHC traits as a multivariate response and sex as a fixed effect. An 8 × 8 matrix specified the variance structure of the random effects, allowing for group-level (hemiclonal line) cross-sex and cross-trait variances and covariances. Residual variance was partitioned between two 4 × 4 matrices that specified cross-trait variances and covariances within each sex. Models were tested with two different priors: one where the degree of belief parameter, nu, was set at 5 for each prior element, and one where nu = 0.5. The models were quantitatively sensitive to the prior specification due to a large covariance matrix being estimated from a relatively small number of lines, but qualitatively the overall conclusions were similar with each prior. We present the results from models where nu = 0.5 as we had no *a priori* expectations about the parameter values. The separate within-sex ($\mathbf{G_m}$ and $\mathbf{G_f}$) and between-sex (**B**) matrices were extracted from this full model. As above, we calculated 95% CI around each estimate directly from the posterior distribution of the model.

For comparison to the multivariate analyses, we also calculated the univariate intersexual genetic correlation, r_{mf}, for each trait (following Lynch & Walsh, 1998). Each genetic correlation was calculated using covariance estimates from a univariate MCMCglmm model for each trait independently. Calculations were based on the posterior distribution of the models, meaning that we generated a distribution of estimates for each calculation, giving us a point estimate and associated 95% CI.

We then performed the following calculations to examine sex-specific selection, responses to selection and multivariate genetic constraints, following Lande (1980). Again, all of the following calculations used the posterior distribution of the above models and we extracted the point estimate and 95% CI from the posterior distribution for each calculation.

First, we compared the direction of overall linear selection on each sex by calculating the angle, θ, between the linear selection vectors for each sex:

$$\theta = \cos^{-1}\left(\frac{\beta_f \bullet \beta_m}{\|\beta_f\|\|\beta_m\|}\right) \quad (3)$$

where β_f and β_m are the vectors of linear selection on females and males, respectively. The strength of linear selection on each sex was compared using the overall magnitude of each vector (i.e. $\|\beta_m\|$ and $\|\beta_f\|$).

Second, the predicted response to selection for each sex individually ($\Delta\bar{z}_m$ and $\Delta\bar{z}_f$) was calculated using their respective sex-specific components of **G** ($\mathbf{G_m}$ and $\mathbf{G_f}$) and the multivariate breeder's equation: $\Delta\bar{z} = \frac{1}{2}G\beta$ (Lande, 1980). The magnitude of the response to selection for each sex was calculated as $\|\Delta\bar{z}_m\|$ and $\|\Delta\bar{z}_f\|$. The factor of ½ is based on the assumption that there is equal maternal and paternal contribution to the offspring autosomal traits (Lande, 1980).

Next, the calculations for the predicted response to selection were repeated using the full **G** matrix, $\mathbf{G_{mf}}$ (1), following Lande (1980):

$$\begin{pmatrix}\Delta\bar{z}_{mB}\\ \Delta\bar{z}_{fB}\end{pmatrix} = \frac{1}{2}\begin{pmatrix}\mathbf{G}_m & \mathbf{B}\\ \mathbf{B}^T & \mathbf{G}_f\end{pmatrix}\begin{pmatrix}\beta_m\\ \beta_f\end{pmatrix} \quad (4)$$

where $\Delta\bar{z}_{mB}$ and $\Delta\bar{z}_{fB}$ represent the predicted response to selection of each sex given the shared genetic variation within **B**, and $\|\Delta\bar{z}_{mB}\|$ and $\|\Delta\bar{z}_{fB}\|$ give the magnitude of these selection responses.

Equation (3) was then used to make additional comparisons by substituting the relevant vectors of interest. For each sex individually, we calculated the angle between the vector of linear selection (β) and the vector of the predicted response to selection to quantify the multivariate genetic constraint (Blows & Walsh, 2009). We first calculated this angle using $\Delta\bar{z}$, giving the constraint imposed by sex-specific **G** ($\mathbf{G_m}$ and $\mathbf{G_f}$) and then repeated these calculations with $\Delta\bar{z}_B$, giving the constraint imposed by the full **G** matrix, $\mathbf{G_{mf}}$. We also estimated the angle between $\Delta\bar{z}$ and $\Delta\bar{z}_B$ for each sex individually, which determined whether the strength of the genetic constraint is altered by inclusion of the between-sex genetic covariances in **B**. Finally, the extent to which the direction of the response to selection differs between sexes was calculated as the angle between $\Delta\bar{z}_m$ and $\Delta\bar{z}_f$, and this was calculated both for $\Delta\bar{z}$ and $\Delta\bar{z}_B$.

Results

Sexual dimorphism

We found evidence for significant sexual dimorphism for both sets of CHCs studied. For the least dimorphic CHCs, although the mean male and female trait values were closely matched (Fig. 1 and Table 1), there was still evidence of significant sexual dimorphism for three of the four CHCs, as the posterior sex-specific credible intervals for these traits did not overlap (Table 1D). The subset of most dimorphic CHCs exhibited strongly significant dimorphism for all four traits (Fig. 1 and Table 1).

Directional selection

For both subsets of CHCs, there was some evidence of significant sex differences in directional selection. For the least dimorphic CHCs, the ΔDIC between models with and without sex-specific selection terms was greater than 100, indicating strong overall sex differences in selection (i.e. sexually antagonistic selection). Whereas the magnitude of selection on the least dimorphic CHCs did not differ significantly between the sexes, the directional selection gradients differed in sign between the sexes for each of these CHCs individually, although the credible intervals for two of these overlapped (Table 2A). Consistent with this, the angle between β_f and β_m was wide (156.90° [151.10–165.10]).

The linear selection gradients for the most dimorphic CHCs were highly variable, and as such, there were no significant differences between sexes for the direction of selection on each CHC individually, nor did the overall magnitude of the selection vectors differ between the sexes (Table 2B). However, comparison of models with and without sex-specific selection terms provided some evidence that differences in linear selection between the sexes were important (ΔDIC = 7.49), albeit to a lesser extent than for the least dimorphic traits. In addition, the angle between the overall sex-specific linear selection vectors for these traits was wide (94.99° [64.73–125.00]), although significantly narrower than the angle between β_f and β_m for the least dimorphic traits (156.90° [151.10–165.10]).

Genetic constraints – least dimorphic CHCs

There was considerable genetic (co)variance for these CHC traits in each sex (Table 3). We found that the predicted response to selection was strongly divergent between the sexes when calculated using sex-specific **G** (i.e. angle between $\Delta\bar{z}_m$and $\Delta\bar{z}_f$ = 139.40° [128.10–52.80]; Fig. 2a) and that this angle was similar to the angle between the vectors of directional selection on each sex (above). However, the angle between the

Table 2 Directional selection gradients on each centred log-ratio CHC for females (β_f) and males (β_m), and the predicted response to selection for each sex calculated without **B** ($\Delta\bar{z}_f$ and $\Delta\bar{z}_m$) and with **B** ($\Delta\bar{z}_{fB}$ and $\Delta\bar{z}_{mB}$). The magnitude of each vector is given in the shaded boxes. Each estimate is shown with 95% CI. (A) Least dimorphic CHCs (top half of table) were modelled separately from (B) most dimorphic CHCs (bottom half of table). Significant differences between males and females (i.e. the 95% CI do not overlap between sexes) are shown in bold. CHC numbers refer to the order of the CHC peaks on the chromatograph, as shown on Fig. 1.

CHC	β_f	β_m	$\Delta\bar{z}_f$	$\Delta\bar{z}_m$	$\Delta\bar{z}_{fB}$	$\Delta\bar{z}_{mB}$
A. Least dimorphic						
8	−0.228	0.132	−0.015	0.027	0.020	0.027
	−0.460 to −0.020	−0.114–0.380	−0.049–0.019	0.005–0.050	−0.015–0.056	0.003–0.050
15	−0.135	0.103	**−0.043**	**0.034**	−0.072	−0.042
	−0.426–0.177	−0.065–0.275	**−0.072 to −0.014**	**0.003–0.064**	−0.103–0.042	−0.075 to −0.010
18	**−0.226**	**0.364**	**0.051**	**−0.021**	−0.039	−0.044
	−0.541–0.092	**0.134–0.605**	**0.009–0.093**	**−0.046 to −0.003**	−0.081 to −0.003	−0.071 to −0.016
20	**0.627**	**−0.745**	**0.157**	**−0.089**	0.063	−0.053
	0.365–0.882	**−0.966 to −0.521**	**0.126–0.187**	**−0.103 to −0.074**	0.032–0.095	−0.072 to −0.035
Magnitude	0.756	0.866	**0.194**	**0.121**	0.144	0.107
	0.650–0.847	0.780–0.946	**0.161–0.220**	**0.100–0.136**	0.113–0.169	0.073–0.134
B. Most dimorphic						
4	−0.688	0.142	**−0.012**	**0.002**	**−0.012**	**0.001**
	−0.959 to −0.421	−0.861–1.160	**−0.013 to −0.011**	**−0.002–0.006**	**−0.013 to −0.010**	**−0.003–0.005**
6	0.216	0.409	0.007	0.006	0.006	0.006
	−0.146–0.588	−0.484–1.271	0.005–0.009	0.002–0.010	0.004–0.009	0.002–0.009
16	−0.127	0.030	−0.002	0.001	−0.002	0.001
	−0.554 to −0.333	−0.360–0.417	−0.005–0.001	−0.003–0.003	−0.005–0.001	−0.003–0.003
19	0.292	0.016	**0.008**	**0.001**	0.009	0.004
	0.005–0.564	−0.331–0.378	**0.005–0.010**	**−0.002–0.004**	0.006–0.011	−0.001–0.007
Magnitude	0.851	0.773	0.017	0.012	0.017	0.012
	0.756–0.937	0.520–0.943	0.015–0.019	0.008–0.014	0.015–0.019	0.009–0.015

Table 3 $\mathbf{G_{mf}}$ matrix for least dimorphic CLR-transformed CHCs. Female and male **G** matrices ($\mathbf{G_f}$ and $\mathbf{G_m}$) are shaded (upper left and lower right submatrices, respectively). The unshaded submatrices show the between-sex genetic covariances comprising the **B** matrix. Variances are in bold along the diagonal, and covariances are in plain text on the off-diagonals. Intervals represent 95% CI around each estimate.

		Female				Male			
$\mathbf{G_{mf}}$		8	15	18	20	8	15	18	20
Female	8	**0.504**	0.046	−0.029	0.160	**0.100**	−0.015	−0.100	−0.079
		0.281–0.874	−0.136–0.246	−0.290–0.217	−0.021–0.406	**−0.062–0.300**	−0.257–0.227	−0.319–0.093	−0.250–0.062
	15	0.046	**0.397**	0.338	0.155	0.004	**0.099**	0.049	0.078
		−0.136–0.246	**0.225–0.680**	0.140–0.655	−0.004–0.373	−0.157–0.165	**−0.111–0.344**	−0.135–0.248	−0.053–0.237
	18	−0.029	0.338	**0.722**	0.401	−0.032	−0.071	**0.125**	0.166
		−0.290–0.217	0.140–0.655	**0.416–1.233**	0.183–0.753	−0.241–0.164	−0.367–0.202	**−0.091–0.392**	0.008–0.383
	20	0.160	0.155	0.401	**0.485**	0.025	−0.130	−0.016	**0.104**
		−0.021–0.406	−0.004–0.373	0.183–0.753	**0.279–0.825**	−0.138–0.190	−0.386–0.080	−0.209–0.173	**−0.025–0.268**
Male	8	**0.100**	0.004	−0.032	0.025	**0.308**	−0.221	−0.109	−0.066
		−0.062–0.300	−0.157–0.165	−0.241–0.164	−0.138–0.190	**0.165–0.557**	−0.490 to −0.051	−0.306–0.044	−0.210–0.047
	15	−0.015	**0.099**	−0.071	−0.130	−0.221	**0.612**	0.141	0.070
		−0.257–0.227	**−0.111–0.344**	−0.367–0.202	−0.386–0.080	−0.490 to −0.051	**0.332–1.112**	−0.072–0.420	−0.093–0.268
	18	−0.100	0.049	**0.125**	−0.016	−0.109	0.141	**0.427**	0.237
		−0.319–0.093	−0.135–0.248	**−0.091–0.392**	−0.209–0.173	−0.306–0.044	−0.072–0.420	**0.226–0.780**	0.104–0.468
	20	−0.079	0.078	0.166	**0.104**	−0.066	0.070	0.237	**0.233**
		−0.250–0.062	−0.053–0.237	0.008–0.383	**−0.025–0.268**	−0.210–0.047	−0.093–0.268	0.104–0.468	**0.122–0.422**

response to selection of each sex was significantly reduced when calculated with the full **G** matrix, $\mathbf{G_{mf}}$ (angle between $\Delta\bar{z}_{mB}$ and $\Delta\bar{z}_{fB}$ = 78.36 [60.22–93.35]; Fig. 2b), indicating that the **B** matrix imposed a significant genetic constraint on the independent evolution of these CHCs between sexes. Examining each CHC individually gave a similar picture. The response to selection had the opposite sign between the sexes for all four CHCs when calculated without **B** (although the difference between sexes was nonsignificant for one trait, see Table 2A); but when **B** was included, the male and female responses to selection were not significantly different for any of the least dimorphic CHCs (Table 2A). In terms of overall magnitude, the predicted response to selection (without **B**) was significantly greater in females than in males, but when the between-sex genetic covariances in **B** were included in the calculation, the magnitude of the female response was reduced and the difference between sexes was no longer significant (Table 2A).

As the inclusion of **B** changed the extent to which the sexes were predicted to diverge, both in terms of direction and magnitude, the sexes were analysed separately to see whether the genetic constraints were sex specific. For males, we found that the **B** matrix imposed a significant constraint on the evolution of these CHCs, as the angle between β_m and $\Delta\bar{z}_{mB}$ was significantly wider than that between β_m and $\Delta\bar{z}_m$ (Table 4A). To quantify the extent to which genetic variation in **B** biased the male response to selection, we also calculated the angle between $\Delta\bar{z}_m$ and $\Delta\bar{z}_{mB}$, which was quite wide (46.55° [33.91–56.68]).

Table 4 Genetic constraints (the angle between the predicted response to selection, $\Delta\bar{z}$ or $\Delta\bar{z}_B$, and the vector of linear selection, β) for (A) the least dimorphic CHCs and (B) the most dimorphic CHCs.

	Alignment with β	
	A: Least dimorphic	B: Most dimorphic
Females		
$\Delta\bar{z}_f$	37.04	15.30
	31.30–43.38	11.82–18.70
$\Delta\bar{z}_{fB}$	47.09	16.70
	39.79–54.48	13.36–19.66
Males		
$\Delta\bar{z}_m$	41.03	13.96
	37.20–46.01	9.63–18.26
$\Delta\bar{z}_{mB}$	66.80	24.20
	61.50–73.48	16.47–31.60

For females, we found also that the genetic constraint increased with inclusion of **B** (the angle between β_f and $\Delta\bar{z}_f$ was smaller than the angle between β_f and $\Delta\bar{z}_{fB}$; Table 4A), but this increase was nonsignificant. **B** does not appear to impose a significant constraint on the direction of the female response to selection, although it should be noted that the angle between the female response to selection with and without **B** (angle between $\Delta\bar{z}_f$ and $\Delta\bar{z}_{fB}$ = 43.24° [33.31–53.37]) was not significantly smaller than that for males (above).

In summary, for these least dimorphic CHCs, there was evidence that between-sex shared genetic variation in the **B** matrix imposed significant constraints on CHC

evolution. This constraint was accounted for by a significant change in the male direction of CHC evolution, with some evidence of a reduced magnitude of the female response to selection (Fig. 2a and b). This asymmetry of the effects on males and females was reflected in the asymmetry of the **B** matrix itself: the matrix correlation between the upper and lower triangles of the **B** matrix was low and not significantly different from zero (0.33 [−0.20–0.71]).

Genetic constraints – most dimorphic CHCs

For the most dimorphic CHCs, genetic (co)variances were generally at least an order of magnitude lower than that of the least dimorphic traits (compare Tables 3 and 5) and in many cases not significantly different from zero (Table 5). Genetic variance in the **B** submatrix was particularly low for the most dimorphic CHCs (Table 5), suggesting that although these traits are expressed in both males and females, there is very little shared genetic variation underlying the extreme dimorphism. The angle between the predicted responses to selection in each sex was therefore unchanged by incorporating the **B** matrix, showing that **B** did not impose a genetic constraint on the potential for the sexes to evolve in different directions (angle between $\Delta\bar{z}_m$ and $\Delta\bar{z}_f = 88.85^\circ$ [61.89–114.30], and angle between $\Delta\bar{z}_{mB}$ and $\Delta\bar{z}_{fB} = 84.05^\circ$ [58.14–108.10]; Fig. 2c and d). In fact, both with and without **B**, the angle between the sexes predicted responses to selection was similar to the angle between the sex-specific selection vectors on these traits (above). For each CHC individually, the response to selection differed between sexes for 2 of 4 traits when calculated without **B** (Table 2B), and inclusion of **B** only altered this for one of these traits (and only marginally, see Table 2B), again indicating very little influence of **B**.

Consistent with this, the **B** matrix did not have any effect on the sex-specific genetic constraints for these traits. Genetic constraints on both females and males were weak and, for each sex, there was no significant difference between constraints calculated with or without **B** (Table 4B). The angle between the predicted response to selection with and without **B** was very small for females (angle between $\Delta\bar{z}_f$ and $\Delta\bar{z}_{fB} = 5.93^\circ$ [3.13–7.79]) and for males (angle between $\Delta\bar{z}_m$ and $\Delta\bar{z}_{mB} = 11.14^\circ$ [7.05–13.99]).

There was also no evidence that **B** imposed any constraint on the magnitude of the predicted response to selection for these traits. The magnitudes of the responses to selection were small and were not significantly different between the sexes, nor with inclusion of **B** (Table 2B). Furthermore, the magnitude of the response to selection for these highly dimorphic traits was about an order of magnitude smaller than that of the least dimorphic traits (compare Table 2A and 2B), concordant with the extent of genetic variation measured for these sets of traits.

In summary, for the most dimorphic traits, shared genetic variation between sexes was low, and as a result, there was no evidence of any genetic constraint imposed by the **B** matrix on either the direction or magnitude of male or female CHC evolution (Fig. 2c and d). There was also less evidence of asymmetry of **B**

Table 5 $\mathbf{G_{mf}}$ matrix for most dimorphic CLR-transformed CHCs. Female and male **G** matrices ($\mathbf{G_f}$ and $\mathbf{G_m}$) are shaded (upper left and lower right submatrices, respectively). The unshaded submatrices show the between-sex genetic covariances comprising the **B** matrix. Variances are in bold along the diagonal and covariances are in plain text on the off-diagonals. Intervals represent 95% CI around each estimate.

		Female				Male			
$\mathbf{G_{mf}}$		4	6	16	19	4	6	16	19
Female	4	**0.015**	−0.004	0.001	−0.002	**0.001**	0.001	−0.002	−0.004
		0.009–0.025	−0.013–0.002	−0.008–0.007	−0.011–0.006	**−0.005–0.007**	−0.005–0.007	−0.010–0.006	−0.014–0.005
	6	−0.004	**0.025**	−0.009	−0.009	−0.001	**−0.001**	−0.001	0.008
		−0.013–0.002	**0.015–0.043**	−0.021–0.001	−0.023–0.001	−0.009–0.006	**−0.010–0.006**	−0.011–0.010	−0.004–0.021
	16	0.001	−0.009	**0.022**	0.008	0.001	0.001	**0.002**	−0.005
		−0.008–0.007	−0.021–0.001	**0.014–0.037**	−0.001–0.021	−0.007–0.008	−0.007–0.007	**−0.007–0.012**	−0.018–0.005
	19	−0.002	−0.009	0.008	**0.033**	0.001	0.002	−0.003	**−0.004**
		−0.011–0.006	−0.023–0.001	−0.001–0.021	**0.020–0.056**	−0.008–0.010	−0.007–0.011	−0.015–0.009	**−0.019–0.009**
Male	4	**0.001**	−0.001	0.001	0.001	**0.013**	0.001	−0.001	−0.003
		−0.005–0.007	−0.009–0.006	−0.007–0.008	−0.008–0.010	**0.007–0.024**	−0.006–0.007	−0.009–0.008	−0.014–0.006
	6	0.001	**−0.001**	0.001	0.002	0.001	**0.014**	−0.002	0.001
		−0.005–0.007	**−0.010–0.006**	−0.007–0.007	−0.007–0.011	−0.006–0.007	**0.008–0.025**	−0.010–0.006	−0.008–0.011
	16	−0.002	−0.001	**0.002**	−0.003	−0.001	−0.002	**0.024**	0.001
		−0.010–0.006	−0.011–0.010	**−0.007–0.012**	−0.015–0.009	−0.009–0.008	−0.010–0.006	**0.013–0.043**	−0.012–0.013
	19	−0.004	0.008	−0.005	**−0.004**	−0.003	0.001	0.001	**0.033**
		−0.014–0.005	−0.004–0.021	−0.018–0.005	**−0.019–0.009**	−0.014–0.006	−0.008–0.011	−0.012–0.013	**0.018–0.060**

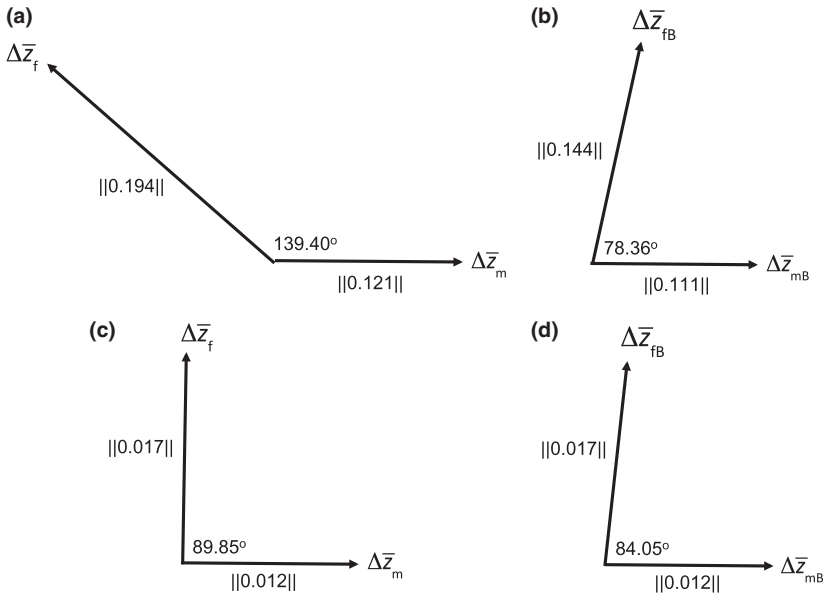

Fig. 2 The difference between the male and female response to selection for (a) the least dimorphic CHCs without **B**; (b) the least dimorphic CHCs with **B** included; (c) the most dimorphic CHCs without **B**; and (d) the most dimorphic CHCs with **B** included. For each pair of vectors, the angle between them is shown in degrees. Note that due to the magnitude of the response to selection of the most dimorphic CHCs being considerably smaller than for the least dimorphic CHCs, (c) and (d) are drawn on a larger scale than (a) and (b).

for the most dimorphic traits as the matrix correlation of the upper and lower triangles of the matrix was significantly higher than 0 (0.68 [0.28–0.88]).

Discussion

Sexually antagonistic selection occurs when the fitness optima for shared traits differ between the sexes, generating intralocus sexual conflict that promotes the evolutionary divergence of males and females. The evolution of sexual dimorphism has the potential to alleviate such conflict, but the extent to which this can evolve depends on the genetics underlying trait expression, and in particular shared genetic variation between the sexes (Lande, 1980). Here, we have shown that the oft-overlooked between-sex genetic covariance, as captured in the **B** matrix, causes genetic constraints on the evolution of sexual dimorphism in some *D. melanogaster* CHCs. We found that **B** imposed a significant constraint on the independent evolution of the least dimorphic CHCs between males and females, but not on the most dimorphic CHCs. This general pattern was also observed when we examined the difference in response to selection between the sexes for each of the eight CHCs individually. In addition, we found asymmetry in the way in which **B** biased the response to selection for the least dimorphic CHCs: the direction of the response to selection was constrained in males, whereas the magnitude of the response was reduced in females (although this was nonsignificant). In other words, including **B** in the genetic constraint calculations for the least dimorphic CHCs caused male and female responses to selection to become more closely aligned, and this appeared to be driven mostly by a change in direction for males (rotated further away from the direction of selection on males) and a slightly reduced magnitude of the female response. Underlying this asymmetry of genetic constraints was strong asymmetry in the **B** matrix for the least dimorphic CHCs. Identifying asymmetry between the sexes in this way is a particular strength of multivariate studies of genetic constraints, and another aspect which can easily be overlooked in univariate studies employing r_{mf} (Wyman *et al.*, 2013).

Multivariate studies of the role of genetic constraints arising from the **B** matrix are rare, although three recent studies have addressed this: Lewis *et al.* (2011) looked at life-history traits in a moth, *Plodia interpunctella*; Gosden *et al.* (2012) examined CHCs in *D. serrata*; and Stearns *et al.* (2012) carried out a study of several fitness-related traits in humans. Each found some evidence of asymmetry between the sexes in the constraints captured by the **B** matrix, suggesting that there is the potential for sexual conflict to influence trait evolution disproportionately between sexes.

Interestingly, the study on CHCs in *D. serrata* found constraints on the direction of the response in females and the magnitude of the response in males (Gosden *et al.*, 2012), the opposite of our results here for the least dimorphic CHCs in *D. melanogaster*. The results of these studies may differ because they used different measures of relative fitness. In particular, Gosden *et al.* (2012) focused on precopulatory sexual selection through mating success, whereas we measured fitness through overall reproductive success, which was designed to encompass pre- and post-copulatory sexual selection, as well as other forms of natural selection. Furthermore, the results could differ due to stronger selection on female CHCs in *D. melanogaster* than

D. serrata. Asymmetry of selection between the sexes is likely to translate to asymmetry in the sex-specific constraints (Lande, 1987), and asymmetry between male and female genetic constraints in **B** in *D. serrata* was attributed to asymmetry in the **B** matrix combined with strong directional selection on male CHCs (Gosden *et al.*, 2012; see also Rundle & Chenoweth, 2011). On the other hand, in *D. melanogaster*, we find that directional selection on females is likely to be stronger than selection on males. Selection on *D. melanogaster* CHCs has not been formalized in a multivariate selection analysis in any previous studies. Our results suggest that four of eight of the individual CHCs examined here were under significant selection in females (i.e. with a selection gradient significantly different from zero), compared with only two in males. Also, the median absolute linear selection gradient was higher in females (0.23) than in males (0.14), and in comparison with the median absolute linear selection gradient of 0.16 calculated in a large-scale meta-analysis by Kingsolver *et al.* (2001), suggests particularly strong directional selection on female CHCs in *D. melanogaster*. This particularly strong directional selection on female CHCs, combined with asymmetry in **B** for these traits, might explain why the inclusion of **B** in the genetic constraint calculations caused the male response to selection to rotate away from the direction of selection.

We might also expect results to differ between species and traits depending on the extent of sexual dimorphism observed. For instance, a high level of dimorphism suggests that genetic mechanisms have evolved to allow independent trait expression across the sexes. This could result from a history of sexually antagonistic selection, which would favour the evolution of such mechanisms to alleviate sexual conflict. This process might also contribute to depletion of genetic variation in the **B** matrix, because shared genetic variation between the sexes should decrease as trait expression becomes more independent in males and females. In general, CHC profiles are highly dimorphic in *D. melanogaster*, and in this study, not only did we find significant sexual dimorphism in even the least dimorphic CHCs, but at the other end of the scale, there were several sex-specific CHCs, as found previously in *D. melanogaster* (e.g. Foley *et al.*, 2007; Everaerts *et al.*, 2010). This overall high level of dimorphism might indicate widespread conflict resolution in this species for this suite of traits. However, to interpret sexual dimorphism in terms of sexual conflict and conflict resolution, consideration of both dimorphism and sex-specific selection is necessary (Bonduriansky & Chenoweth, 2009; Cox & Calsbeek, 2009; Pennell & Morrow, 2013). We address this here by comparing sex-specific selection, expression and genetic variation between subsets of the most and least dimorphic of the shared CHCs.

Our results for the most dimorphic CHCs were consistent with past sexual conflict that has been largely resolved. There was some evidence of sex differences in selection on these traits, but there was very little genetic variation, and the components of the **B** matrix in particular were close to zero. As such, predicted responses to selection were weak and the **B** matrix did not appear to bias responses in either sex. There was also little evidence of genetic constraints either with or without **B**. Taken together, these results suggest that the genetic architecture of these most dimorphic CHCs has evolved to allow independent trait expression between the sexes, and the depletion of genetic variation in the $\mathbf{G_f}$ and $\mathbf{G_m}$ matrices could be a remnant of previously strong sexually antagonistic selection. Consistent with these results, a recent manipulative evolution experiment also found evidence of resolved sexual conflict in *D. melanogaster* CHCs (Bedhomme *et al.*, 2011).

For the least dimorphic traits, on the other hand, there was an order of magnitude higher (co)variance for many elements of the **B** matrix, and we found that this between-sex genetic variation translated to significant genetic constraints on the independent evolution of these CHCs between the sexes. Further, there was evidence of strongly divergent selection between the sexes on these traits. Therefore, although these traits are significantly sexually dimorphic, this dimorphism does not match the extent to which the sex-specific fitness optima differ, suggesting unresolved sexual conflict on the least dimorphic CHCs (Cox & Calsbeek, 2009). This result highlights the importance of considering both dimorphism and selection when examining sexual conflict (Bonduriansky & Chenoweth, 2009; Cox & Calsbeek, 2009; Pennell & Morrow, 2013), as we find evidence of unresolved sexual conflict on a set of traits that are significantly sexually dimorphic.

This result contrasts with that of a recent male-limited experimental evolution study on *D. melanogaster* CHCs, which found little evidence of unresolved sexual conflict (Bedhomme *et al.*, 2011). Male-limited experimental evolution releases males from the constraint of opposing female selection such that shared traits should shift towards male optima in both sexes. Interestingly, the asymmetry we identified here between the sexes in terms of both selection and shared genetic variation might mean that the results of male- and female-limited experimental evolution would differ. Possibly, strong directional selection gradients for females could indicate that the trait values of the shared CHCs are closer to the male optima than the female optima. If this is the case, female-limited evolution might produce a bigger response than male-limited evolution for both sexes. Indeed, given the stronger selection on female CHCs, the resolution of any ongoing conflict on the least dimorphic CHCs will have to involve the breakdown of **B** and the subsequent evolution of increased sexual dimorphism through a greater change in females than in males (Lande, 1980). As an additional complication,

it is difficult to predict how **B** might change over evolutionary time, and how this will affect the genetic constraints it produces. It is thought that **B** should generally be less stable than $\mathbf{G_m}$ or $\mathbf{G_f}$, and there is some evidence of this in garter snakes (Barker *et al.*, 2010) and *D. serrata* (Gosden & Chenoweth, 2014). As such, caution is needed when comparing results from experiments using a quantitative genetic approach with those employing experimental evolution, and further research using both approaches will be important to improve our understanding of the evolution of the **B** matrix.

In a more general sense, it is clear from our study that a univariate approach to assessing genetic constraints between the sexes (using the intersexual genetic correlation r_{mf}) would have produced misleading results. For each of the eight CHCs we examined, the univariate intersexual genetic correlations were weak (see Table 1), and generally a lot lower than r_{mf} calculated for CHCs in *D. serrata* (Chenoweth & Blows, 2003; Chenoweth *et al.*, 2008; Gosden *et al.*, 2012), some *D. simulans* CHCs (Ingleby *et al.*, 2013b), and even across a broad range of species and types of trait as revealed in a meta-analysis by Poissant *et al.* (2010). Based on r_{mf} alone, one might have come to the conclusion that these low correlations, combined with the sex-specific patterns of selection, would allow the sexes to diverge and evolve dimorphism for these traits. Whereas this conclusion is consistent with our results for the most dimorphic CHCs, the considerable multivariate genetic constraints within the **B** matrix for the least dimorphic traits show that there is important variation within **B** that would have been overlooked from a univariate perspective (Lande, 1980). As trait expression and selection are unlikely to ever be completely independent of other traits, it seems that a multivariate approach will be needed, as has been shown here in *D. melanogaster* and in *D. serrata* previously (Gosden *et al.*, 2012).

In conclusion, our results show that inclusion of the **B** matrix in studies of sexual dimorphism and intralocus sexual conflict can uncover potentially important between-sex genetic variation. To interpret patterns of sexual dimorphism in terms of sexual conflict and conflict resolution, it is clear that this shared genetic variation should not be overlooked. Further research will need to develop our understanding of the **B** matrix, and in particular how **B** evolves, in order to clarify how between-sex genetic variation might affect the evolution of sexual dimorphism and the resolution of sexual conflict.

Acknowledgments

The authors are grateful to J. Barber, M. Delcourt, A. Groulx, C. Robillard and J. Sztepanacz for help with gas chromatography, and M.L. Head and J.D. Hadfield for constructive comments on an earlier version of the manuscript. We thank V. Pawlowsky-Glahn and J.J. Egozcue for helpful discussions concerning the transformation of compositional data. This research was supported by funding from the O.E. and Edla Johansson's Scientific Foundation, the Swedish Research Council (Grants 2009-331 & 2011-3701), the European Research Council (Grant Number 280632) and a University Research Fellowship from the Royal Society to EHM; and funding from the Canada Foundation for Innovation to HDR.

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Data deposited at Dryad: doi:10.5061/dryad.k0v17

Received 10 October 2013; revised 29 April 2014; accepted 5 May 2014