Journal of Heredity 2012:103(2):230–239
doi:10.1093/jhered/esr132
Advance Access publication January 20, 2012

© The American Genetic Association. 2012. All rights reserved.
For permissions, please email: journals.permissions@oup.com.

The Genetics of Cuticular Hydrocarbon Profiles in the Fruit Fly *Drosophila simulans*

Manmohan D. Sharma, Christopher Mitchell, John Hunt, Tom Tregenza, and David J. Hosken

From the Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Cornwall Campus, Penryn TR10 9 EZ, UK.

Address correspondence to David J. Hosken at the address above, or e-mail: d.j.hosken@exeter.ac.uk.

Abstract

Female mate choice is one mechanism of sexual selection and, provided there is adequate genetic variation in the male traits that are the target of this selection, they will evolve via female choice. Cuticular hydrocarbons (CHCs) are important in *Drosophila* mate choice, but relatively little is known about the underlying genetic architecture of CHC profiles in *Drosophila simulans*. Here, we used gas chromatography–mass spectrometry to investigate patterns of genetic variation in the CHC profiles of male and female *D. simulans* using isofemale lines. We found substantial genetic variation for CHC profiles and individual CHC components, and individual CHCs were frequently strongly genetically correlated, with a tendency for negative covariance between long- and short-chain CHCs in males. Intersexual genetic covariances were often weak and frequently differed in sign. These findings are novel and significant, highlighting the previously unexplored genetic architecture of CHCs in *D. simulans* and suggest that this architecture may facilitate sex-specific CHC evolution.

Key words: *genetic variation, heritability, isolines, sexual dimorphism*

Female preference for certain males has been documented in many taxa (reviewed in Andersson 1994 and e.g., Ryan 1983; Moore AJ and Moore PJ 1988; Wilkinson and Reillo 1994). Females base their assessment of male attractiveness on many different characters and frequently assess multiple traits before choosing their preferred mate (Jennions and Petrie 1997; Johnstone 1997; Kodric-Brown and Nicoletto 2001; Candolin 2003; Patricelli et al. 2003; Hebets and Papaj 2005). Although morphological or acoustic characters have been the major focus of sexual selection studies (Andersson 1994), chemicals signals provided by insect cuticular hydrocarbons (CHCs) have also been the subject of intensive investigation (e.g., Chenoweth and Blows 2003; Blows et al. 2004; Hine et al. 2004).

CHCs are found on the exoskeleton of insects. While they are subject to natural selection, influencing desiccation resistance (Toolson 1982; Lockey 1988; Rouault et al. 2004), cold tolerance (Ohtsu et al. 1998), and starvation resistance (Hoffmann et al. 2001), they are often also key sexual signals (for a review, see Howard and Blomquist 2005). Although the role of CHCs as sexual signals is frequently poorly understood (Johansson et al. 2005; Johansson and Jones 2007), studies have highlighted their importance in mating (e.g., Ming and Lewis 2010; Grinsted et al. 2011; see reviews in Ferveur 2005; Wicker-Thomas 2007; Gomes et al. 2008; Blomquist and Bagnères 2010) and as mate recognition cues, CHCs are expected to be sexually dimorphic either qualitatively or quantitatively. Recent studies have confirmed this as CHCs are highly sexually dimorphic in many species, with many of the individual components being sex specific (Thomas and Simmons 2008), as one would expect with secondary sexual traits (Andersson 1994). For example, crickets often have distinct male and female CHC profiles (Warthen and Uebel 1980; Tregenza and Wedell 1997; Mullen et al. 2007). Additionally, even when a CHC occurs in both sexes, the quantities produced by males and females can differ substantially (Thomas and Simmons 2008). Such differences have been reported in mosquitoes, ticks, and fireflies (Estrada-Peña et al. 1996; Anyanwu et al. 2000; Caputo et al. 2005; South et al. 2008), and CHCs are also reported to evolve rapidly and are sexually dimorphic in grasshoppers and crickets (Neems and Butlin 1995; Buckley et al. 2003; Mullen et al. 2007; Thomas and Simmons 2008).

When compared with many other insects, *Drosophila* have a small number of CHCs (<60 vs. >100; Howard 1993; Howard and Blomquist 2005; Everaerts et al. 2010), and there is considerable divergence in the chain length, the number or position of double bonds, and sexual dimorphism across this group (for a review, see Ferveur 2005). Courtship in *Drosophila* is complex, involving a range

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org
Downloaded from http://jhered.oxfordjournals.org/ at University of Exeter Library on August 8, 2012

American Genetic Association

The Journal of Heredity

www.jhered.oxfordjournals.org

of visual, acoustic, gustatory, and chemical cues (Ewing 1983; O'Dell 2006; Stoop and Arthur 2009), and CHCs play an important role in courtship (Savarit et al. 1999). Furthermore, specific CHCs have been linked to courtship behaviors. For example, 5-Tricosene inhibits male courtship (Ferveur and Sureau 1996; Greenspan and Ferveur 2000), and 7-Tricosene not only inhibits male courtship (Jallon 1984; Ferveur and Sureau 1996; Lacaille et al. 2007) but also enhances female receptivity (Grillet et al. 2006). Additionally, 7-Pentacosene and 9-Pentacosene act synergistically to stimulate copulation attempts (Ferveur and Sureau 1996; Siwicki et al. 2005), and 7-Pentacosene also stimulates male courtship along with 7,11-dienes (Ferveur 1997). Despite such direct functional associations being noted for individual CHCs, it is important to note that only focusing on individual components may not provide complete understanding of CHC function given the complex multidimensional nature of CHC profiles and an absence of a full understanding of the genetic architecture of CHCs.

Recent studies have reported substantial genetic variation in *Drosophila* CHCs (e.g., Foley et al. 2007), whereas others find that the majority of this genetic variation is not available for selection due to its orientation relative to the direction of sexual selection (e.g., Blows et al. 2004; Hine et al. 2004; Van Homrigh et al. 2007). More recently, McGuigan and Blows (2009) investigated standing genetic variance underlying high and low fitness *Drosophila bunnanda* phenotypes and found substantial genetic variation in low but not in high fitness males. However, most studies exploring genetic variation and sexual dimorphism in *Drosophila* CHCs are restricted to the Hawaiian radiation (Alves et al. 2010), *D. melanogaster* (e.g., Antony and Jallon 1982; Jallon 1984; Foley et al. 2007), *D. serrata* (Chenoweth and Blows 2003; Hine et al. 2004), or *D. virilis* (Bartelt et al. 1986). In contrast, *D. simulans* CHCs remain relatively unexplored in the sexual selection literature, in spite of recent focus on sexual selection in this species (e.g., Taylor et al. 2007, 2009, 2010; Hosken et al. 2008; Sharma et al. 2010, 2011).

Here, we use 6 isofemale lines that have been employed in previous sexual selection investigations to investigate the genetic architecture of CHC profiles in *D. simulans*. Sharma et al. (2010) have shown that these isolines harbor significant genetic variation for male attractiveness, implying there is genetic variation for CHCs in these lines, whereas Taylor et al. (2007) have shown that male attractiveness is heritable. Additionally, CHCs are under sexual selection (Sharma et al. 2011), and quantitative sexual dimorphism has been reported in *D. simulans* CHC peaks (Ferveur and Cobb 2010).

Materials and Methods

Fly Stocks

We used 6 *Drosophila simulans* isolines, randomly chosen from 20 supplied by the Centre for Environmental Stress and Adaptation Research, La Trobe University, Australia. These isolines were collected in 2004 and have been maintained in multiple vials at a density >50 pairs per vial since then. To test if using 6 isolines captured most of the population variation (for any given trait), we ran a simulation assuming normally distributed (with a mean of 16 and standard deviation of 5) trait values in the source population with individuals (lines) being randomly sampled from this distribution. We ran 1000 iterations drawing 6 random samples from this normal distribution each time. We then compared the means of these 1000 samples and found that 80% of values fell within the range of 16 ± 6.35, suggesting that the 6 isolines we used here captured about 80% of the range of the underlying population.

All flies were reared on "*Drosophila* quick mix medium" (supplied by Blades Biological, Edenbridge, Kent, UK) at 25 °C and a 12:12 h light:dark cycle. Food was provided in excess so that differential larval competition was minimized. Subsequent housing conditions followed this regime unless stated otherwise. We collected 8 virgin males and females within 8 h of eclosion from each isoline and housed them individually to prevent social interactions from altering CHC profiles (e.g., Kent et al. 2008; Krupp et al. 2008; Thomas and Simmons 2011). Assuming that visual stimuli could potentially trigger CHC profile changes, we isolated housing vials with translucent polypropylene partitions that allow light passage but blur images sufficiently to prevent recognition. Individuals were processed for CHC extraction when they were 3 days old as adult CHC profiles are completely developed by this time (Antony and Jallon 1981).

Hydrocarbon Extraction

To quantify CHCs, individual flies were soaked in 50 µl Hexane containing 100 ng of Pentadecane as an internal standard. Pentadecane has widely been used as an internal standard, and it was also our choice because it is an inert hydrocarbon and its retention time did not overlap with any of the other hydrocarbons in our samples. After 4 min of soaking, vials were vortexed for 60 s to maximize extraction. A 1 µl sample of each fly extract was then injected into a gas chromatograph–mass spectrometer (GC-MS) (Agilent 7890A GC coupled with an Agilent 5975B MS) operating in pulsed splitless mode and fitted with a DB-1ms column (340 °C: 30 m × 250 µm × 0.25 µm) (J&W 122-0132 by J&W Scientific, Folsom, CA) using Helium as a carrier gas. Extract separation parameters were initially optimized and we chose to use a column temperature profile in which the analysis began at a temperature of 70 °C for 1 min and then rose by 20 °C/min to 240 °C followed by a 4 °C/min rise to 320 °C. The inlet and the transfer line from the GC to the MS were set at 250 °C. Chromatograms were acquired and analyzed using MSD Chemstation software version E.02.00.493 (Agilent, Foster City, CA).

We analyzed extracts derived from 96 flies (8 individual males and females from each of the 6 isolines), along with Pentadecane control standards that were loaded at the start and end of each run to check for contamination. CHC peaks

Downloaded from http://jhered.oxfordjournals.org/ at University of Exeter Library on August 8, 2012

were labeled by peak number, which corresponded to their retention times on the GC (see Figure 1a,b; Table 1). In total, 18 unique CHC peaks were identified and the areas under these peaks were quantified and expressed as proportional values after dividing by the Pentadecane standard (peak 1). Use of the internal standard in calculating proportions eliminates the problems of unit-sum-constraints faced when proportions are calculated relative to the sum of all peaks. To ensure multivariate normality, we $\log_{10}$ transformed our data prior to analysis.

Statistical Analysis

Drosophila hydrocarbons are known to show variation in the overall CHC composition or "blend," and also in the absolute amounts of individual components (Luyten 1982;

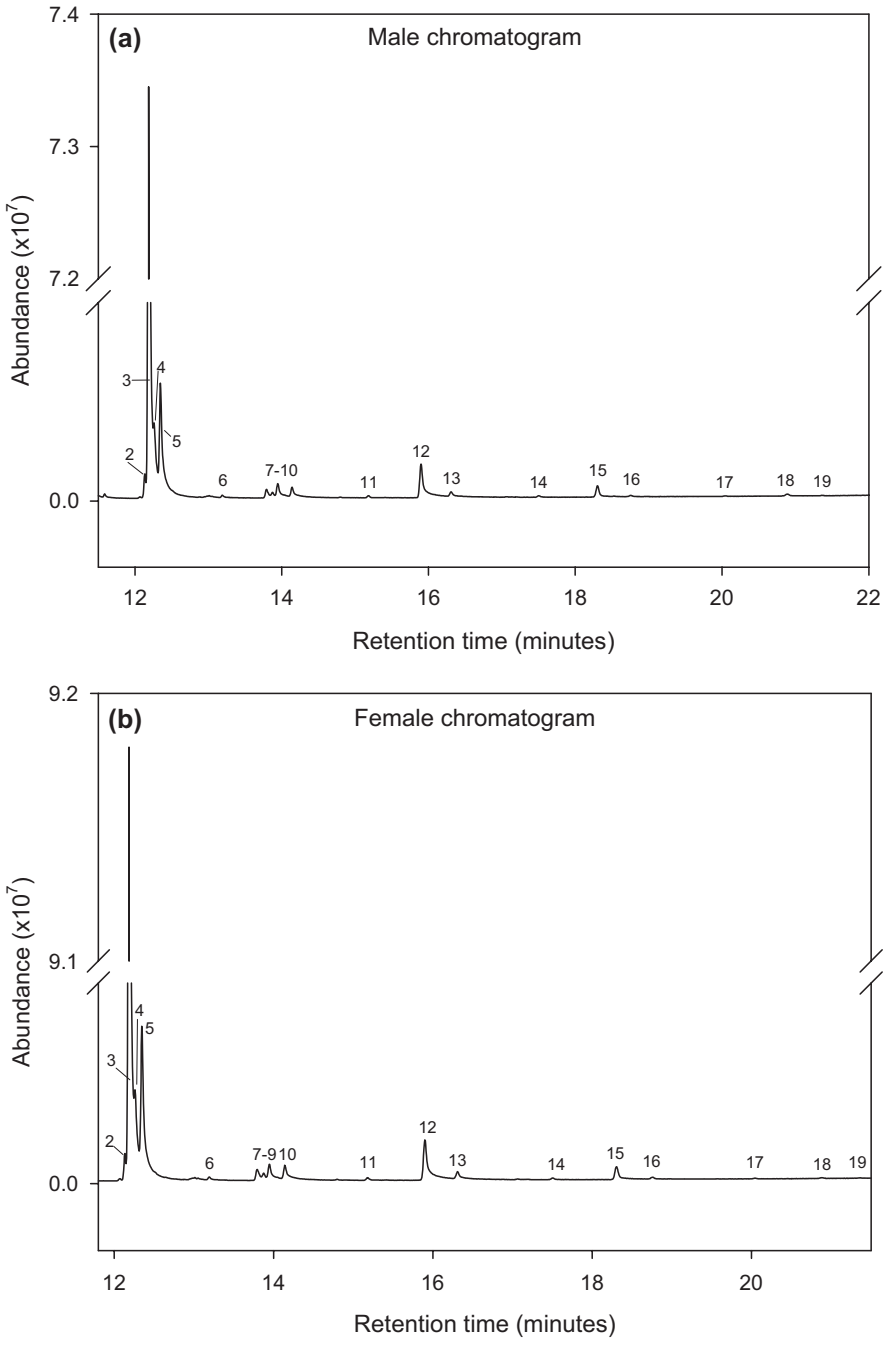

Figure 1. Typical GC profile for male (**a**) and female (**b**) *Drosophila simulans*. The *x* axis shows the retention time and the *y* axis the response from the ionization detector. Peak numbers are indicated (2–19; for details, see Table 1). Note that peak 1 (the standard) has been left out to improve visibility of other peaks.

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org
Downloaded from http://jhered.oxfordjournals.org/ at University of Exeter Library on August 8, 2012

Table 1 Mean relative contribution of the 18 CHC compounds identified on *Drosophila simulans* and their retention times (RT), names, formula, and molecular weights (MW)

					Male		Female	
Name	Peak	RT	Formula	MW	Mean	SE	Mean	SE
Pentadecane ISTD	CHC1	7.54	C15H32	212	—	—	—	—
9-Tricosene[a]	CHC2	12.13	C23H46	322	0.099	0.012	0.092	0.006
7-Tricosene	CHC3	12.19	C23H46	322	4.101	0.394	4.608	0.269
5-Tricosene[a]	CHC4	12.26	C23H46	322	0.483	0.038	0.534	0.025
Tricosane	CHC5	12.35	C23H48	324	1.080	0.084	1.181	0.061
Branched alkane	CHC6	13.20	C24H50	338	0.015	0.001	0.015	0.001
Branched alkane	CHC7	13.79	C25H52	352	0.074	0.011	0.061	0.006
9-Pentacosene[a]	CHC8	13.88	C25H50	350	0.035	0.004	0.030	0.002
7-Pentacosene[a]	CHC9	13.95	C25H50	350	0.100	0.010	0.099	0.006
5-Pentacosane[a]	CHC10	14.14	C25H52	352	0.085	0.009	0.098	0.009
Alkane	CHC11	15.19			0.012	0.001	0.013	0.001
Branched alkane	CHC12	15.91	C27H56	380	0.397	0.045	0.297	0.030
Heptacosane	CHC13	16.31	C27H56	380	0.044	0.011	0.046	0.005
Alkane	CHC14	17.50			0.010	0.001	0.011	0.001
Branched alkane	CHC15	18.30	C29H60	408	0.079	0.008	0.098	0.013
Alkane[b]	CHC16	18.76	C29H60[b]	408	0.010	0.001	0.012	0.001
Alkane[b]	CHC17	20.05	C30H62[b]	422	0.006	0.001	0.005	0.001
Alkane[b]	CHC18	20.88	C31H64[b]	436	0.011	0.003	0.008	0.001
Alkane[b]	CHC19	21.35	C31H64[b]	436	0.004	0.000	0.003	0.000

[a] Most likely candidates but require verification and thus these isomers of Tricosene and Pentacosene are not mentioned individually in the text.

[b] Trace levels therefore identification is tentative.

Jallon 1984; Ferveur and Jallon 1996). We therefore analyzed and interpreted our data in 2 ways, first by examining the overall CHC composition and then looking at individual components. We used principal component analysis (PCA) to reduce the dimensionality of the CHC profile because, otherwise, we would have too many dependent variables and too few degrees of freedom. As PCA summarizes a pattern of correlation among variables, it may be possible to interpret the resulting components in terms of a functional hypotheses (Moore 1997). Furthermore, PC scores can then be utilized in additional analysis. Note that PC scores describe different and independent aspects of underlying variation as the PCs are orthogonal to each other (Tabachnick and Fidell 1989).

All statistical analyses were performed with PASW (version 18) unless stated otherwise. PCA was performed and the PCs with eigenvalues greater than 1 (Norman and Streiner 2008) were then examined with a multivariate analysis of variance. Isoline and sex were included as main (fixed) effects in the model, and 3 PCs were entered as dependent variables.

We also estimated the heritability of CHCs for both males and females as well as the genetic correlations between CHCs both within and across the sexes. These were estimated in 2 ways. First, by using the PC scores to provide estimates for the CHC blends and then by using the individual $\log_{10}$ CHC proportions. In both instances, heritability was estimated as the coefficient of intraclass correlation (t) (Hoffmann and Parsons 1988; David et al. 2005) as:

$$t = \frac{V_b - V_w/n}{V_b + (n-1)V_w/n} = \frac{nV_b - V_w}{nV_b + (n-1)V_w}.$$

Here, n is the number of lines, and V_b and V_w are the between-line and within-line variance components, respectively, estimated directly from an ANOVA including line as the main effect. The standard error of the intraclass correlation (SE (t)) was calculated according to Becker (1984) as (here k is the number of individuals sampled within each line):

$$\mathrm{SE}(t) = \sqrt{\frac{2(1-t)^2[1+(k-1)t]^2}{k(k-1)(n-1)}}.$$

Genetic correlations (and their SEs) for CHC peaks within and between sexes were estimated using the jackknife method of Roff and Preziosi (1994). In short, this procedure first estimates the genetic correlation between 2 traits using mean estimates for each line (Via 1984; Gibert et al. 1998). A sequence of N pseudo-values is then computed by dropping each of the lines in turn and estimating the resulting correlations and using the formula:

$$S_{N,i} = Nr_N - (N-1)r_{N-1,i},$$

where $S_{N,i}$ is the i^{th} pseudo-value, r_N is the genetic correlation estimated using the means of all N inbred lines, and $r_{N-1,i}$ is the genetic correlation obtained by dropping the i^{th} inbred line alone (Roff and Preziosi 1994). The jacknife estimate of the genetic correlation (r_j) is then simply

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org
Downloaded from http://jhered.oxfordjournals.org/ at University of Exeter Library on August 8, 2012

American Genetic Association

The Journal of Heredity

www.jhered.oxfordjournals.org

the mean of the pseudo-values, and an estimate of the SE is given by:

$$\mathrm{SE} = \frac{\sum_{i=1}^{i=N} (S_{N,i} - r_j)^2}{N(N-1)}.$$

Using simulation models, Roff and Preziosi (1994) showed that this jacknife approach provides better genetic estimates than those based on conventional inbred line means when the number of inbred lines contained in the analysis is small (<20 lines), as occurs in our study. It is important to note that estimates of genetic (co)variance from inbred lines contain variance due to dominance and/or epistasis and therefore should be considered broad-sense estimates (Falconer and Mackay 1996).

We then examined the male and female genetic correlation matrices (obtained via the PC scores and those obtained from individual CHC components) for information concerning the relatedness of CHC traits (sensu Cheverud 1988), looking for highly (genetically) related PCs or CHC peaks, which are likely to coevolve (Lande 1980; Cheverud 1988). The overall integration of the male and female genetic correlation (r_G) matrices was assessed by using Mantel's randomization test (Mantel 1967) with ZT (Bonnet and Van De Peer 2002) and was based on 10 000 randomizations. Here, the observed matrix correlation is compared with an empirically derived distribution of matrix correlations and the proportion of randomly permuted matrix correlations exceeding the observed one gives an estimate of the probability of obtaining a matrix correlation greater than the observed one by chance. If the probability is low ($P < 0.05$), then the matrices are more similar than by chance alone. Note that a significant association would indicate that the intrasexual genetic correlations vary in similar directions and not that the values of any elements are identical in magnitude.

Additionally, we calculated the average absolute values of the correlations in each matrix along with the average disparity between the 2 matrices. In brief, we summed the absolute values of all off-diagonal correlations and divided by the number of correlation pairs to arrive at the average absolute value of correlation.

$$\bar{X} = \frac{\sum |r_{i,j}|}{n} \quad \text{for } i \neq j,$$

where $r_{i,j}$ refers to the correlation between characters i and j, and n is the number of correlation pairs. The average disparity between corresponding male and female genetic correlation matrices was determined by averaging the absolute values of differences between correlation pairs.

$$D = \frac{\sum |r_{\mathrm{MG},i,j} - r_{\mathrm{FG},i,j}|}{n} \quad \text{for } i \neq j,$$

where $r_{\mathrm{MG},i,j}$ and $r_{\mathrm{FG},i,j}$ refer to male and female genetic correlations between characters i and j, and n is the total number of correlation pairs (Willis et al. 1991; Roff 1995; Waitt and Levin 1998). D indicates the overall difference in the magnitude of association between the matrices, whereas the average absolute correlation values indicate the overall size of correlation within each matrix.

Results

Analysis of mass spectra and the retention times allowed us to distinguish 18 CHC peaks, most of which have previously been identified in *Drosophila* (e.g., Everaerts et al. 2010). Some peaks (see footnote of Table 1) were eluted in trace levels, and their identification is tentative. All 18 CHC peaks were shared by male and female *D. simulans* (see Figure 1a,b). No qualitative differences were found between isolines or sexes (i.e., no sex-specific CHC components were detected). However, we did find evidence of quantitative differences both between isolines and sexes (i.e., the same CHC components were expressed to different degrees in the sexes; Table 1).

PCA of the individual CHC components returned 3 PCs that had eigenvalues greater than 1, and these collectively explained 81% of the variance in CHC composition (Supplementary Table 1). Correlations between the individual CHC components and the derived PC scores (factor loadings in Supplementary Table 1) were used to examine the CHC components that contributed the most to each PC. All factor loadings greater than 0.3 were interpreted as biologically important (Tabachnick and Fidell 1989). PC1 was weighted positively by peaks 2–13 (Tricosene, Tricosane, branched alkanes, Pentacosene, Pentacosane, and Heptacosane) and negatively by peak 18 (alkane). PC2 was weighted negatively by peak 4 and positively by peaks 6, 11, and 13–19 (Tricosene, branched alkane, alkane, Heptacosane, etc.) and PC3 positively by peaks 13, 15, and 18 and negatively by peak 19 (Heptacosane, branched alkane, and alkanes). Thus, PC1 largely described the content of shorter chain CHCs (plus a trade-off with a single longer chain component), whereas PC2 largely describes longer chain CHCs (and one trade-off with a Tricosene), and PC3 described trade-offs within the longer chained hydrocarbons.

Multivariate analysis of the PC scores using isoline and sex as fixed factors and the 3 PC scores as dependent variables indicated isoline (Wilk's $\lambda = 0.224$; $F_{15,227} = 10.87$; $P < 0.001$), sex (Wilk's $\lambda = 0.898$; $F_{3,82} = 3.096$; $P < 0.031$), and their interaction (Wilk's $\lambda = 0.475$; $F_{15,226.767} = 4.68$; $P < 0.001$) all significantly influenced the multivariate combination of PCs. Post hoc univariate analyses indicated that the isolines effect was driven by PC1 ($F_{5,84} = 8.47$; $P < 0.001$) and PC3 ($F_{5,84} = 15.08$; $P < 0.001$). The same PCs were also responsible for the sex (PC1, $F_{5,84} = 5.3$; $P < 0.02$; PC3, $F_{5,84} = 5.99$; $P < 0.02$) and the interaction effect (PC1, $F_{5,84} = 11.38$; $P < 0.001$; PC3, $F_{5,84} = 2.95$; $P < 0.02$; Figure 2a,b). PC2 was not significantly influenced by any factor or interaction (all $P > 0.05$).

Sexes differed in the heritability estimates (t) calculated for the overall CHC blends (based on PC scores), but both PC1 and PC3 were heritable in males and females, whereas PC2 was not heritable in either sex (Supplementary Table 2). Heritability estimates based on the individual CHC components show that peaks 11, 13, 14, 16, 17, and 19 were not heritable in males and peaks 6, 11, 14, 17, and 19

Downloaded from http://jhered.oxfordjournals.org/ at University of Exeter Library on August 8, 2012

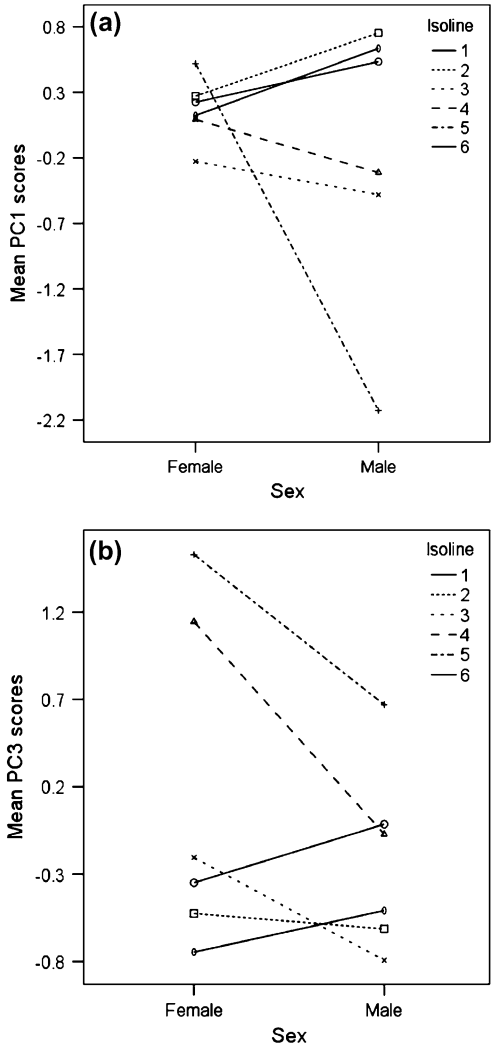

Figure 2. The isofemale line by sex interaction for principle components describing CHC blends. (**a**) Shows the isoline-by-sex interaction for PC1 and (**b**) the isoline-by-sex interaction for PC3.

were not heritable in females (Supplementary Table 3). Genetic correlations for individual hydrocarbon peaks within each sex indicated that the average correlation within the male matrix was 0.177 (Supplementary Table 4). The overall magnitude of genetic correlation, as measured by the average absolute value of correlations, had a value of 0.740 for males. For females, the genetic correlation matrix had an average correlation of 0.251 (Supplementary Table 4). In this case, the average absolute value of the correlation was 0.92.

Intersexual genetic correlations (estimated on PC scores) were both positive and negative in sign (Supplementary Table 5), and a similar trend was seen when genetic correlations were calculated for the individual peaks (Supplementary Table 6). However, many of the correlations for individual peaks were low (42% were less than 0.5), which indicates the sexual dimorphism for CHCs is in a relatively advanced stage (Lande 1980). Furthermore, most of the correlations above 0.5 were negative: In fact, 66% of all the intersexual correlations were negative. Mantel's test indicated that the matrix describing male CHCs significantly differed from the one that described females ($\rho < 0.001$; $P = 0.54$).

Although the average absolute value of correlation gives an indication of the overall strength of the individual correlations within a matrix, it can obscure the actual disparity between corresponding individual male and female genetic correlations. A large positive matrix correlation would indicate that male and female correlations vary in similar directions, but it does not provide any information to confirm if the magnitudes of individual correlations are identical. Considering matrix correlations together with average Disparity (*D*) estimates helps resolve this issue. The average disparity between male and female correlation matrices was 0.362, indicating that the individual elements of the male and female matrices are very different from each other.

Discussion

CHCs seem to play a major role in Dipteran mate choice and have been investigated thoroughly in several *Drosophila* species (e.g. Hine et al. 2004; Liimatainen and Jallon 2007; Chenoweth et al. 2008; Alves et al. 2010; Everaerts et al. 2010). Relatively, less attention has been paid to *D. simulans*, despite recent investigations of sexual selection in this species and the differences between it and its more thoroughly studied sister species *D. melanogaster* (Taylor et al. 2007; Hosken et al. 2008; Sharma et al. 2010). Here, we used 6 isolines to investigate the quantitative genetics of CHCs in male and female *D. simulans*. We found significant genetic variation for CHCs, including heritable variation for some individual CHC components. We also found significant (positive and negative) intra- and intersexual genetic correlations for CHCs in our isolines, with the genetic architecture of female and male CHCs differing markedly. Our results also confirm that CHC profiles are quantitatively sexually dimorphic in this species.

Overall CHC blends and many of the individual CHC components were significantly heritable, and it is this genetic variation that is needed if CHCs are to evolve. Nevertheless, heritability estimates from isofemale lines are best derived within 5 generations of line establishment from the wild to avoid overestimation of parameter values (Hoffmann and Parsons 1988). Therefore, the exact heritability values presented here should be treated with caution, even though our estimates are based on the intraclass correlation coefficient *t*, which provides more realistic heritability estimates compared with conventional means (David et al. 2005). Heritable variation in CHC profiles has been reported for a range of insects previously (e.g., Chapman et al. 1995; Thomas and Simmons 2009). Our analyses also revealed significant sex by isoline interactions. This indicates the presence of sex-specific genetic variation in CHCs, which is

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org
Downloaded from http://jhered.oxfordjournals.org/ at University of Exeter Library on August 8, 2012

American Genetic Association

The Journal of Heredity

www.jhered.oxfordjournals.org

reflected in the different heritabilities in the sexes. For example, peak 16 is significantly heritable in females but not males, whereas the converse is true of peak 6. Overall, our findings suggest that there is sufficient genetic variation present in this population for CHC profiles to evolve given appropriate selection. Consistent with this, CHC profiles evolve in a sex-specific manner when subject to natural selection and sexual selection (Sharma et al. 2011).

In addition to estimating straight heritabilities, we also calculated the broad-sense genetic variance–covariance matrix (**G**) for *D. simulans* CHCs (not the narrow-sense additive genetic variance–covariance matrices), both within and across the sexes, and as expected from the differences noted in the variance estimates, the sexes also differed in their covariances (see Supplementary Table 4). Cheverud (1984, 1988) suggested that **G** can be thought of as a measure of genetic constraints on evolution. Basically, the diagonal elements of **G** measure the short-term readiness of a character to respond to selection, and the off-diagonal elements measure how the evolution of one trait influences the coevolution of others.

Examination of the intrasexual **G** matrices indicates that many individual CHC peaks covary genetically with each other. CHC biosynthesis in *Drosophila* is considered to be a relatively simple system where genetic variants for CHC expression are expected to trade-off expression of one class of compounds for the others (Foley et al. 2007). This is consistent with the many negative genetic correlations we see between CHCs, especially in males between longer and shorter chain CHCs. These negative intramale genetic correlations suggest that male genotypes that produce more shorter chained CHCs tend to produce less longer chained CHCs. In one way, this could be interpreted as indicating that genotypes best suited under natural selection—for example, long-chain CHCs tend to be involved in desiccation resistance (Rouault et al. 2004)—are genotypes less likely to be favored under sexual selection where more volatile shorter chains are often more attractive (Ferveur and Cobb 2010). If this is so for *D. simulans*, the **G** we estimated for males may prevent the emergence of a genotype that could excel under both natural and sexual selection. This is consistent with classical interpretations of sexual trait evolution: Natural selection opposes sexual selection (Andersson 1994; Hosken and House 2011).

It is interesting, however, that comparison of **G** between the sexes indicates males and females do not face the same trade-offs as their intrasexual genetic architecture differs. As one example, females tend to have fewer strong negative correlations than males (10 vs. 28). If selection is ultimately responsible for the shape of **G**, this may reflect the fact that sexual selection is weaker on females, and they are typically viewed as being closer to naturally selected optima than males (Andersson 1994; and see Sharma et al. 2011). That is, it is probably easier to optimize **G** for 1 (naturally selected) task than for 2 tasks (sexual and natural selection). In any case, the male/female difference can clearly be seen by considering any single CHC (more or less). For example, although 7-Tricosene (peak 3) is a major constituent of both male and female CHC profiles in *D. simulans* (see Luyten 1982; Pechine et al. 1985; Ferveur and Jallon 1993), females express higher levels of it (Ferveur 1991; and see Table 1). If we compare the **G** matrix values for male peak 3 with those for female peak 3, it is obvious that the intrasexual correlations for each sex vary (see Supplementary Table 4). Strong intrasexual genetic correlations imply that the individual CHC peaks are not independent of each other, but it does not imply anything about the plasticity of such correlations, as environmental fluctuations may alter their magnitude or sign.

A shared genetic architecture may also constrain the independent evolution of the sexes. This constraint usually manifests as strong intersexual genetic correlations (r_{MF}: Lande 1980; Roff 1997). The magnitude of r_{MF} between homologous traits and the nature of selection on each sex could influence the evolution of sexual dimorphism (Lande 1980). We found many positive and negative r_{MF} for *D. simulans* CHCs, but the average magnitude of these correlations is weak, suggesting sexual dimorphism in CHCs is at an advanced stage (Lande 1980). Theoretically, the genetic architecture of traits under sexually antagonistic selection should evolve to minimize the genetic constraints on the independent evolution of the sexes, allowing the sexes to meet their sex-specific fitness optima (Lande 1980; Rhen 2000; Rice and Chippindale 2001; Badyaev 2002; but see Harano et al. 2010), and the intersexual covariances we find are largely consistent with this as most of them are below 50%. Nevertheless, the fact that we find correlations at all contrasts with findings from other *Drosophila* species (e.g., Chenoweth and Blows 2003) and with CHC expression studies on mutant *D. melanogaster* (Ferveur and Jallon 1993; Coyne et al. 1999; Dallerac et al. 2000; Wicker-Thomas and Jallon 2001; Fang et al. 2002), which indicate trait expression may be under independent genetic control in the sexes (also see Labeur et al. 2002; Ferveur 2005). However, in a recent meta-analysis, intersexual genetic correlations for homologous traits were predominantly large and positive (Poissant et al. 2010). Our results also contrast with this, for although we do find some covariance between the sexes, associations are for the most part negative. Additionally, **G** for males and females significantly differ, which may facilitate sex-specific evolution of CHC profiles even when individual CHCs show strong intersexual covariance, and there is evidence for this as *D. simulans* CHCs do evolve in a sex-specific manner (Sharma et al. 2011). However, our covariance estimates are based on broad-sense estimates of genetic variances because of the isoline approach we have employed, so additive covariances are likely to be even weaker, which may partly reconcile our findings with other *Drosophila* studies (e.g., Chenoweth and Blows 2003). It is important to note that genotype × environment interactions are also expected to influence r_{MF} estimates (Falconer and Mackay 1996; Lyons et al. 1994; Simons and Roff 1996). This means CHCs may evolve in different ways under different environmental conditions (e.g., Sharma et al. 2011), further underlining the importance of genotype × environment interactions in sexual selection (Ingleby et al. 2010).

Downloaded from http://jhered.oxfordjournals.org/ at University of Exeter Library on August 8, 2012

Our results are also consistent with previous work, suggesting that *D. simulans* is quantitatively sexually dimorphic in CHC profiles (Cobb and Jallon 1990; and see Ferveur and Jallon 1996). Sexual dimorphism is common in sexually selected characters, with sexes often differing in size, shape, and degree of sexual trait exaggeration (Darwin 1871; Andersson 1994). Examination of the male and female CHC chromatograms here reveals that all the peaks we detected are shared by the sexes but that sexes express different quantities for many of the shared peaks (Figure 1a,b) (and see Ferveur and Jallon 1993; Ferveur and Cobb 2010). Given the multivariate nature of overall CHC profiles, even small differences in CHC production can dramatically alter CHC blends and influence behavioral responses during mate choice. Sexually antagonistic selection, where traits shared by males and females have a sex-specific fitness optima (Rice and Chippindale 2001; Bonduriansky and Chenoweth 2009; Hosken et al. 2009), is considered to be an ultimate cause of sexual dimorphism. As stated above, we have some evidence for sex-specific CHC changes during experimental evolution, which is consistent with sex-specific fitness optima, but we currently do not know if this is due to sexually antagonistic selection in our experiments or sex differences in **G**.

Overall, our results indicate that *D. simulans* CHC sexual dimorphism is at an advanced stage, but there are still many significant intersexual genetic associations, many of them negative, and the genetic architecture of the CHCs differs between the sexes. Intersexual differences in the optimal CHC profiles are expected because the sexes invest differentially and differ in their reproductive roles, and hence, the direction of sexual and natural selection acting on specific traits should differ (Johnstone et al. 1996; Bonduriansky 2001). Consistent with all of this, there are sex differences in the CHC evolution (Sharma et al. 2011; also see Delcourt and Rundle 2011). What remains to be determined is precisely how selection actually acts on CHCs and if this varies across environments.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

Funding

Natural Environment Research Council (NE/G005303/1 to J.H. and D.J.H.); and the University Royal Society Fellowship (UF0762844 to J.H.).

References

Alves H, Rouault JD, Kondoh Y, Nakano Y, Yamamoto D, Kim YK, Jallon JM. 2010. Evolution of cuticular hydrocarbons of Hawaiian Drosophilidae. Behav Genet. 40:694–705.

Andersson M. 1994. Sexual selection. Princeton (NJ): Princeton University Press.

Antony C, Jallon JM. 1981. Evolution des hydrocarbures comportementalement actifs de *Drosophila melanogaster* au cours de la maturation sexuelle. C R Seances Acad Sci. 292:239–242.

Antony C, Jallon JM. 1982. The chemical basis for sex recognition in *Drosophila melanogaster*. J Insect Physiol. 28:873–880.

Anyanwu GI, Molyneux DH, Phillips A. 2000. Variation in cuticular hydrocarbons among strains of the *Anopheles gambiae* sensu stricto by analysis of cuticular hydrocarbons using gas liquid chromatography of larvae. Mem Inst Oswaldo Cruz. 95:295–300.

Badyaev AV. 2002. Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. Trends Ecol Evol. 17:369–378.

Bartelt RJ, Armold MT, Schaner AM, Jackson LL. 1986. Comparative analysis of cuticular hydrocarbons in the *Drosophila virilis* species group. Comp Biochem Physiol B. 83:731–742.

Becker W. 1984. Manual of quantitative genetics. Pullman (WA): Academic Enterprises.

Blomquist GJ, Bagnéres A-G. 2010. Insect hydrocarbons: biology, biochemistry, and chemical ecology. Cambridge (UK): Cambridge University Press.

Blows M, Chenoweth S, Hine E. 2004. Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. Am Nat. 163:329–340.

Bonduriansky R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol Rev Camb Philos Soc. 76:305–339.

Bonduriansky R, Chenoweth SF. 2009. Intralocus sexual conflict. Trends Ecol Evol. 24:280–288.

Bonnet E, Van De Peer Y. 2002. Zt: a software tool for simple and partial Mantel tests. J Stat Softw. 7:1–12.

Buckley SH, Tregenza T, Butlin RK. 2003. Transitions in cuticular composition across a hybrid zone: historical accident or environmental adaptation? Biol J Linn Soc. 78:193–201.

Candolin U. 2003. The use of multiple cues in mate choice. Biol Rev Camb Philos Soc. 78:575–595.

Caputo B, Dani FR, Horne GL, Petrarca V, Turillazzi S, Coluzzi M, Priestman AA, Torre Ad. 2005. Identification and composition of cuticular hydrocarbons of the major Afrotropical malaria vector *Anopheles gambiae* s.s. (Diptera: Culicidae): analysis of sexual dimorphism and age related changes. J Mass Spectrom. 40:1595–1604.

Chapman RF, Espelie KE, Sword GA. 1995. Use of cuticular lipids in grasshopper taxonomy: a study of variation in *Schistocerca shoshone* (Thomas). Biochem Syst Ecol. 23:383–398.

Chenoweth SF, Blows MW. 2003. Signal trait sexual dimorphism and mutual sexual selection in *Drosophila serrata*. Evolution. 57:2326–2334.

Chenoweth SF, Blows MW. 2008. Q_{ST} meets the G matrix: the dimensionality of adaptive divergence in multiple correlated quantitative traits. Evolution. 62:1437–1449.

Chenoweth SF, Rundle HD, Blows MW. 2008. Genetic constraints and the evolution of display trait sexual dimorphism by natural and sexual selection. Am Nat. 171:22–34.

Cheverud JM. 1984. Quantitative genetics and developmental constraints on evolution by selection. J Theor Biol. 110:155–171.

Cheverud JM. 1988. A comparison of genetic and phenotypic correlations. Evolution. 42:958–968.

Cobb M, Jallon J-M. 1990. Pheromones, mate recognition and courtship stimulation in the *Drosophila melanogaster* species sub-group. Anim Behav. 39:1058–1067.

Coyne JA, Wicker-Thomas C, Jallon JM. 1999. A gene responsible for a cuticular hydrocarbon polymorphism in *Drosophila melanogaster*. Genet Res. 73:189–203.

Dallerac R, Labeur C, Jallon JM, Knipple DC, Roelofs WL, Wicker-Thomas C. 2000. A δ9 desaturase gene with a different substrate specificity is responsible

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org
Downloaded from http://jhered.oxfordjournals.org/ at University of Exeter Library on August 8, 2012

for the cuticular diene hydrocarbon polymorphism in *Drosophila melanogaster*. Proc Natl Acad Sci U S A. 97:9449–9454.

Darwin C. 1871. The descent of man and selection in relation to sex. London: John Murray.

David JR, Gibert P, Legout H, Petavy G, Capy P, Moreteau B. 2005. Isofemale lines in *Drosophila*: an empirical approach to quantitative trait analysis in natural populations. Heredity. 94:3–12.

Delcourt M, Rundle HD. 2011. Condition dependence of a multicomponent sexual display trait in *Drosophila serrata*. Am Nat. 177:812–823.

Estrada-Peña A, Gray JS, Kahl O. 1996. Variability in cuticular hydrocarbons and phenotypic discrimination of *Ixodes ricinus* populations (Acarina: Ixodidae) from Europe. Exp Appl Acarol. 20:457–466.

Everaerts C, Farine JP, Cobb M, Ferveur JF. 2010. *Drosophila* cuticular hydrocarbons revisited: mating status alters cuticular profiles. Plos One. 5:e9607.

Ewing AW. 1983. Functional aspects of *Drosophila* courtship. Biol Rev Camb Philos Soc. 58:275–292.

Falconer DS, Mackay TFC. 1996. Introduction to quantitative genetics. New York: Longman.

Fang S, Takahashi A, Wu CI. 2002. A mutation in the promoter of desaturase 2 is correlated with sexual isolation between *Drosophila* behavioral races. Genetics. 162:781–784.

Ferveur JF. 1991. Genetic control of pheromones in *Drosophila simulans*. I. Ngbo, a locus on the second chromosome. Genetics. 128:293–301.

Ferveur JF. 1997. The pheromonal role of cuticular hydrocarbons in *Drosophila melanogaster*. Bioessays. 19:353–358.

Ferveur JF. 2005. Cuticular hydrocarbons: their evolution and roles in *Drosophila* pheromonal communication. Behav Genet. 35:279–295.

Ferveur JF, Cobb M. 2010. Behavioral and evolutionary roles of cuticular hydrocarbons in Diptera. In: Blomquist GJ, Bagnéres A-G, editors. Insect hydrocarbons: biology, biochemistry, and chemical ecology. Cambridge (UK): Cambridge University Press. p. 325–343.

Ferveur JF, Jallon JM. 1993. Genetic control of pheromones in *Drosophila simulans*. II. kete, a locus on the X chromosome. Genetics. 133:561–567.

Ferveur JF, Jallon JM. 1996. Genetic control of male cuticular hydrocarbons in *Drosophila melanogaster*. Genet Res. 67:211–218.

Ferveur JF, Sureau G. 1996. Simultaneous influence on male courtship of stimulatory and inhibitory pheromones produced by live sex-mosaic *Drosophila melanogaster*. Proc R Soc Lond B Biol Sci. 263:967–973.

Foley B, Chenoweth SF, Nuzhdin SV, Blows MW. 2007. Natural genetic variation in cuticular hydrocarbon expression in male and female *Drosophila melanogaster*. Genetics. 175:1465–1477.

Gibert P, Moreteau B, Moreteau JC, David JR. 1998. Genetic variability of quantitative traits in *Drosophila melanogaster* (fruit fly) natural populations: analysis of wild-living flies and of several laboratory generations. Heredity. 80:326–335.

Gomes CCG, Trigo JR, Eiras AE. 2008. Sex pheromone of the American warble fly, *Dermatobia hominis*: the role of cuticular hydrocarbons. J Chem Ecol. 34:636–646.

Greenspan RJ, Ferveur JF. 2000. Courtship in *Drosophila*. Annu Rev Genet. 34:205–232.

Grillet M, Dartevelle L, Ferveur JF. 2006. A *Drosophila* male pheromone affects female sexual receptivity. Proc R Soc Lond B Biol Sci. 273:315–323.

Grinsted L, Bilde T, d'Ettorre P. 2011. Cuticular hydrocarbons as potential kin recognition cues in a subsocial spider. Behav Ecol. 22:1187–1194.

Harano T, Okada K, Nakayama S, Miyatake T, Hosken DJ. 2010. Intralocus sexual conflict unresolved by sex-limited trait expression. Curr Biol. 20:2036–2039.

Hebets EA, Papaj DR. 2005. Complex signal function: developing a framework of testable hypotheses. Behav Ecol Sociobiol. 57:197–214.

Hine E, Chenoweth SF, Blows MW. 2004. Multivariate quantitative genetics and the lek paradox: genetic variance in male sexually selected traits of *Drosophila serrata* under field conditions. Evolution. 58:2754–2762.

Hoffmann AA, Hallas R, Sinclair C, Mitrovski P. 2001. Levels of variation in stress resistance in *Drosophila* among strains, local populations, and geographic regions: patterns for desiccation, starvation, cold resistance, and associated traits. Evolution. 55:1621–1630.

Hoffmann AA, Parsons PA. 1988. The analysis of quantitative variation in natural populations with isofemale strains. Genet Sel Evol. 20:87–98.

Hosken DJ, House CM. 2011. Sexual selection. Curr Biol. 21:R62–R65.

Hosken DJ, Stockley P, Tregenza T, Wedell N. 2009. Monogamy and the battle of the sexes. Annu Rev Entomol. 54:361–378.

Hosken DJ, Taylor ML, Hoyle K, Higgins S, Wedell N. 2008. Attractive males have greater success in sperm competition. Curr Biol. 18:R553–R554.

Howard RW. 1993. Cuticular hydrocarbons and chemical communication. In: Stanley-Samuelson DW, Nelson DR, editors. Insect lipids: chemistry, biochemistry and biology. Lincoln (NE): University of Nebraska Press. p. 179–226.

Howard RW, Blomquist GJ. 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Annu Rev Entomol. 50:371–393.

Ingleby FC, Hunt J, Hosken DJ. 2010. The role of genotype-by-environment interactions in sexual selection. J Evol Biol. 23:2031–2045.

Jallon JM. 1984. A few chemical words exchanged by *Drosophila* during courtship and mating. Behav Genet. 14:441–478.

Jennions MD, Petrie M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. Biol Rev Camb Philos Soc. 72:283–327.

Johansson BG, Jones TM. 2007. The role of chemical communication in mate choice. Biol Rev Camb Philos Soc. 82:265–289.

Johansson BG, Jones TM, Widemo F. 2005. Cost of pheromone production in a lekking *Drosophila*. Anim Behav. 69:851–858.

Johnstone RA. 1997. The tactics of mutual mate choice and competitive search. Behav Ecol Sociobiol. 40:51–59.

Johnstone RA, Reynolds JD, Deutsch JC. 1996. Mutual mate choice and sex differences in choosiness. Evolution. 50:1382–1391.

Kent C, Azanchi R, Smith B, Formosa A, Levine JD. 2008. Social context influences chemical communication in *D. melanogaster* males. Curr Biol. 18:1384–1389.

Kodric-Brown A, Nicoletto P. 2001. Female choice in the guppy (*Poecilia reticulata*): the interaction between male color and display. Behav Ecol Sociobiol. 50:346–351.

Krupp JJ, Kent C, Billeter JC, Azanchi R, So AKC, Schonfeld JA, Smith BP, Lucas C, Levine JD. 2008. Social experience modifies pheromone expression and mating behavior in male *Drosophila melanogaster*. Curr Biol. 18:1373–1383.

Labeur C, Dallerac R, Wicker-Thomas C. 2002. Involvement of desat1 gene in the control of *Drosophila melanogaster* pheromone biosynthesis. Genetica. 114:269–274.

Lacaille F, Hiroi M, Twele R, Inoshita T, Umemoto D, Maniere G, Marion-Poll F, Ozaki M, Francke W, Cobb M, et al. 2007. An inhibitory sex pheromone tastes bitter for *Drosophila* males. PLoS One. 2:e661.

Lande R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution. 34:292–305.

Liimatainen JO, Jallon JM. 2007. Genetic analysis of cuticular hydrocarbons and their effect on courtship in *Drosophila virilis* and *D. lummei*. Behav Genet. 37:713–725.

Lockey KH. 1988. Lipids of the insect cuticle: origin, composition and function. Comp Biochem Physiol B Biochem Mol Biol. 89:595–645.

Luyten I. 1982. Intraspecific and interspecific variations of the cuticular hydrocarbons in *Drosophila simulans* and related species. C R Seances Acad Sci. 295:733–736.

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org
Downloaded from http://jhered.oxfordjournals.org/ at University of Exeter Library on August 8, 2012

Lyons EE, Miller D, Meagher TR. 1994. Evolutionary dynamics of sex ratio and gender dimorphism in *Silene latifolia*: I. Environmental effects. J Hered. 85:196–203.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27:209–220.

McGuigan K, Blows MW. 2009. Asymmetry of genetic variation in fitness-related traits: apparent stabilizing selection on G_{max}. Evolution. 63:2838–2847.

Ming QL, Lewis SM. 2010. Mate recognition and sex differences in cuticular hydrocarbons of the diurnal firefly *Ellychnia corrusca* (Coleoptera: Lampyridae). Ann Entomol Soc Am. 103:128–133.

Moore AJ. 1997. The evolution of social signals: morphological, functional, and genetic integration of the sex pheromone in *Nauphoeta cinerea*. Evolution. 51:1920–1928.

Moore AJ, Moore PJ. 1988. Female strategy during mate choice: threshold assessment. Evolution. 42:387–391.

Mullen SP, Mendelson TC, Schal C, Shaw KL. 2007. Rapid evolution of cuticular hydrocarbons in a species radiation of acoustically diverse Hawaiian crickets (Gryllidae: Trigonidiinae: Laupala). Evolution. 61:223–231.

Neems RM, Butlin RK. 1995. Divergence in cuticular hydrocarbons between parapatric subspecies of the meadow grasshopper, *Chorthippus parallelus* (Orthoptera, Acrididae). Biol J Linn Soc. 54:139–149.

Norman G, Streiner D. 2008. Biostatistics: the bare essentials. Shelton (CT): People's Medical Publishing House USA Ltd.

O'Dell K. 2006. The voyeurs' guide to *Drosophila* courtship. J Neurogenet. 20:195.

Ohtsu T, Kimura MT, Katagiri C. 1998. How *Drosophila* species acquire cold tolerance: qualitative changes of phospholipids. Eur J Biochem. 252:608–611.

Patricelli GL, Uy JA, Borgia G. 2003. Multiple male traits interact: attractive bower decorations facilitate attractive behavioural displays in satin bowerbirds. Proc R Soc Lond B Biol Sci. 270:2389–2395.

Pechine JM, Perez F, Antony C, Jallon JM. 1985. A further characterization of *Drosophila* cuticular monoenes using a mass spectrometry method to localize double bonds in complex mixtures. Anal Biochem. 145:177–182.

Poissant J, Wilson AJ, Coltman DW. 2010. Sex-specific genetic variance and the evolution of sexual dimorphism: a systematic review of cross-sex genetic correlations. Evolution. 64:97–107.

Rhen T. 2000. Sex-limited mutations and the evolution of sexual dimorphism. Evolution. 54:37–43.

Rice WR, Chippindale AK. 2001. Intersexual ontogenetic conflict. J Evol Biol. 14:685–693.

Roff DA. 1995. The estimation of genetic correlations from phenotypic correlations: a test of Cheverud's conjecture. Heredity. 74:481–490.

Roff DA. 1997. Evolutionary quantitative genetics. New York: Chapman & Hall.

Roff DA, Preziosi R. 1994. The estimation of the genetic correlation: the use of the jacknife. Heredity. 73:544–548.

Rouault JD, Marican C, Wicker-Thomas C, Jallon JM. 2004. Relations between cuticular hydrocarbon (HC) polymorphism, resistance against desiccation and breeding temperature; a model for HC evolution in *D. melanogaster* and *D. simulans*. Genetica. 120:195–212.

Ryan MJ. 1983. Sexual selection and communication in a neotropical frog, *Physalaemus pustulosus*. Evolution. 37:261–272.

Savarit F, Sureau G, Cobb M, Ferveur JF. 1999. Genetic elimination of known pheromones reveals the fundamental chemical bases of mating and isolation in *Drosophila*. Proc Natl Acad Sci U S A. 96:9015–9020.

Sharma MD, Hunt J, Hosken DJ. Forthcoming 2011. Antagonistic responses to natural and sexual selection and the sex-specific evolution of cuticular hydrocarbons in *Drosophila simulans*. Evolution. doi:10.1111/j.1558-5646.2011.01468.x

Sharma MD, Tregenza T, Hosken DJ. 2010. Female mate preferences in *Drosophila simulans*: evolution and costs. J Evol Biol. 23:1672–1679.

Simons AM, Roff DA. 1996. The effect of a variable environment on the genetic correlation structure in a field cricket. Evolution. 50:267–275.

Siwicki KK, Riccio P, Ladewski L, Marcillac F, Dartevelle L, Cross SA, Ferveur JF. 2005. The role of cuticular pheromones in courtship conditioning of *Drosophila* males. Learn Mem. 12:636–645.

South A, LeVan K, Leombruni L, Orians CM, Lewis SM. 2008. Examining the role of cuticular hydrocarbons in firefly species recognition. Ethology. 114:916–924.

Stoop R, Arthur BI. 2009. An expressive body language underlies *Drosophila* courtship behavior. In Dana SK, Roy PK, Kurths J editors. Complex Dynamics in Physiological Systems: From Heart to Brain. Kolkata (India): Springer. p. 215–227.

Tabachnick B, Fidell L. 1989. Using multivariate statistics. New York: Harper Collins.

Taylor ML, Sharma MD, Hosken DJ. 2009. Sexual selection in flies: a comparison of *Drosophila simulans* and *D. melanogaster*. Anim Biol. 59: 391–402.

Taylor ML, Wedell N, Hosken DJ. 2007. The heritability of attractiveness. Curr Biol. 17:R959–R960.

Taylor ML, Wedell N, Hosken DJ. 2010. Attractive males do not sire superior daughters. Evol Ecol. 24:195–205.

Thomas ML, Simmons LW. 2008. Sexual dimorphism in cuticular hydrocarbons of the Australian field cricket *Teleogryllus oceanicus* (Orthoptera: Gryllidae). J Insect Physiol. 54:1081–1089.

Thomas ML, Simmons LW. 2009. Sexual selection on cuticular hydrocarbons in the Australian field cricket, *Teleogryllus oceanicus*. BMC Evol Biol. 9:162.

Thomas ML, Simmons LW. 2011. Short-term phenotypic plasticity in long-chain cuticular hydrocarbons. Proc R Soc Lond B Biol Sci. 278: 3123–3128.

Toolson EC. 1982. Effects of rearing temperature on cuticle permeability and epicuticular lipid composition in *Drosophila pseudoobscura*. J Exp Zool. 222:249–253.

Tregenza T, Wedell N. 1997. Definitive evidence for cuticular pheromones in a cricket. Anim Behav. 54:979–984.

Van Homrigh A, Higgie M, McGuigan K, Blows MW. 2007. The depletion of genetic variance by sexual selection. Curr Biol. 17:528–532.

Via S. 1984. The quantitative genetics of polyphagy in an insect herbivore. II. Genetic correlations in larval performance within and among host plants. Evolution. 38:896–905.

Waitt DE, Levin DA. 1998. Genetic and phenotypic correlations in plants: a botanical test of Cheverud's conjecture. Heredity. 80:310–319.

Warthen JD Jr, Uebel EC. 1980. Comparison of the unsaturated cuticular hydrocarbons of male and female house crickets, *Acheta domesticus* (L.) (Orthoptera: Gryllidae). Insect Biochem. 10:435–439.

Wicker-Thomas C. 2007. Pheromonal communication involved in courtship behavior in Diptera. J Insect Physiol. 53:1089–1100.

Wicker-Thomas C, Jallon JM. 2001. Control of female pheromones in *Drosophila melanogaster* by homeotic genes. Genet Res. 78:235–242.

Wilkinson GS, Reillo PR. 1994. Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. Proc R Soc Lond B Biol Sci. 255:1–6.

Willis JH, Coyne JA, Kirkpatrick M. 1991. Can one predict the evolution of quantitative characters without genetics? Evolution. 45:441–444.

Received May 23, 2011; Revised October 19, 2011; Accepted October 19, 2011

Corresponding Editor: L. Lacey Knowles

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org
Downloaded from http://jhered.oxfordjournals.org/ at University of Exeter Library on August 8, 2012