doi:10.1111/j.1558-5646.2011.01468.x

# ANTAGONISTIC RESPONSES TO NATURAL AND SEXUAL SELECTION AND THE SEX-SPECIFIC EVOLUTION OF CUTICULAR HYDROCARBONS IN *DROSOPHILA SIMULANS*

**Manmohan D. Sharma,[1] John Hunt,[1] and David J. Hosken[1,2]**

[1]*Centre for Ecology & Conservation, School of Biosciences, University of Exeter, Cornwall Campus, Penryn TR10 9EZ, United Kingdom*

[2]*E-mail: d.j.hosken@exeter.ac.uk*

Received March 7, 2011
Accepted September 6, 2011
*Data Archived: Dryad: doi:10.5061/dryad.227f5pn9*

**Natural and sexual selection are classically thought to oppose one another, and although there is evidence for this, direct experimental demonstrations of this antagonism are largely lacking. Here, we assessed the effects of sexual and natural selection on the evolution of cuticular hydrocarbons (CHCs), a character subject to both modes of selection, in *Drosophila simulans*. Natural selection and sexual selection were manipulated in a fully factorial design, and after 27 generations of experimental evolution, the responses of male and female CHCs were assessed. The effects of natural and sexual selection differed greatly across the sexes. The responses of female CHCs were generally small, but CHCs evolved predominantly in the direction of natural selection. For males, profiles evolved via sexual and natural selection, as well as through the interaction between the two, with some male CHC components only evolving in the direction of natural selection when sexual selection was relaxed. These results indicate sex-specific responses to selection, and that sexual and natural selection act antagonistically for at least some combinations of CHCs.**

**KEY WORDS:** **Cuticular hydrocarbons, *Drosophila simulans*, experimental evolution, natural selection, sexual selection.**

Sexual selection is responsible for evolution of many conspicuous traits and behaviors, and is classically thought to be opposed by natural selection, at least once sexual traits have become sufficiently exaggerated (Lande 1981; Arnold 1983; Andersson 1994). This antagonism between sexual and natural selection is built into many evolutionary models of sexual trait evolution (Lande 1981; Pomiankowski et al. 1991; Mead and Arnold 2004), and is supported by some iconic studies. For example, sexual selection frequently favors louder and/or longer calls (e.g., Rand and Ryan 1981; Bentsen et al. 2006) because these call characteristics make males easier to detect which provides them with a mating advantage (Gwynne 2001). However, these same call characteristics can make signalers more conspicuous to nonintended receivers, and there are many examples of predators using sexual signals to locate signaling males (Endler 1980; Tuttle and Ryan 1981; Hosken et al. 1994; Zuk et al. 2006). Nevertheless, although the independent effects of sexual and natural selection on specific traits have been documented, there has been relatively little direct experimental investigation of the combined evolutionary effects of natural and sexual selection acting on male sexual traits (but see Blows 2002; Skroblin and Blows 2006; Hine et al. 2011). This is surprising because it is the interaction between these episodes of selection that will ultimately determine the net strength and form of selection operating on male sexual traits (Hunt et al. 2009).

In addition to the potential antagonism that may exist between sexual and natural selection, selection can also be sexually antagonistic, with males and females having different selective optima for shared traits (Rice and Chippindale 2001; Bonduriansky and Chenoweth 2009; Hosken et al. 2009). Sexually antagonistic selection is an ultimate cause of sexual dimorphism, and the widespread occurrence of sexual dimorphism suggests this is common, especially with regard to secondary sexual traits (Bonduriansky and Chenoweth 2009). Indeed, a recent review of the literature showed that sexually antagonistic selection was relatively common, particularly for shared traits that are subject to sexual selection (Cox and Calsbeek 2009). In non sex-role reversed species it is typically males that bear the elaborate sexual traits because they enhance male mating success. Females usually do not carry exaggerated sexual traits as they are typically under weaker directional sexual selection, and may therefore reside nearer to naturally selected optima, especially if trait development comes at a fecundity cost (Gwynne 2001). However, sexual differences in trait exaggeration will only occur when the genetic architecture of the shared traits permits dimorphism to evolve (Bonduriansky and Chenoweth 2009; Harano et al. 2010).

In many *Drosophila* species, cuticular hydrocarbons (CHCs) are important determinants of male attractiveness and hence mating success (Cobb and Ferveur 1995; Blows 2002; Wicker-Thomas 2007). CHCs are also subject to natural selection, being important in providing desiccation resistance for many insects, including *Drosophila* (Hadley 1981; Gibbs and Rajpurohit 2010). Typically, longer chained CHCs are more important in waterproofing, whereas shorter chained, more volatile CHCs are involved in sexual signalling over short distances, although longer chain CHCs can also act as contact pheromones (Hadley 1981; Wicker-Thomas 2007; Ferveur and Cobb 2010). However, although there is population variation and geographic clines in CHCs for many species (Ferveur et al. 1996; Ferveur 2005), the selection responsible for this variation in CHCs is not always well understood (Coyne and Elwyn 2006). One of the few exceptions to this generality is *Drosophila serrata*, where natural and sexual selection operating on CHCs has been extremely well studied. There is mutual mate choice for CHCs in this species, with the sexes expressing contrasting mating preferences (Chenoweth and Blows 2005). Females largely exert linear sexual selection on male CHCs, whereas males prefer intermediate female CHCs generating stabilizing sexual selection on female profiles (Chenoweth and Blows 2005). Work on populations along the east coast of Australia has shown that male CHCs have diverged across the geographic range of this species (Chenoweth et al. 2008; Chenoweth and Blows 2008; Frentiu and Chenoweth 2009) and that female choice of male CHCs also differs across these populations (Chenoweth et al. 2008; Rundle et al. 2008). However, this observed divergence in male CHCs is only weakly explained by the differences in sexual selection across populations (Chenoweth et al. 2010). Across these same populations, Frentiu and Chenoweth (2009) showed a significant association between variation in male CHCs and temperature variation, with more longer chained compounds being produced in populations experiencing warmer temperatures, suggesting that natural selection also plays an important role in CHC evolution in this species. Consistent with this, Blows (2002) used experimental evolution to show that although both natural and sexual selection independently influenced the evolution of CHCs, the greatest effect occured through the interaction of the two forms of selection. When natural and sexual selection operated together, male CHCs evolved to be more exaggerated compared with when sexual selection acted alone, whereas female CHCs evolved against the direction favored by natural selection in the presence of sexual selection (Blows 2002). More recently, Rundle et al. (2009) used the same experimental evolution approach to show that the interaction between natural and sexual selection also plays an important role in the evolution of female mating preferences for male CHCs in *D. serrata*.

In contrast to the comprehensive understanding of CHC evolution in *D. serrata*, knowledge of other *Drosophila* species is less complete. In *D. pseudoobscura*, the rate of evaporative water loss (EWL) is reduced in individuals with proportionally more long chained alkanes and alkadienes, particularly *n*-pentacosadiene (Toolson 1982; also see Foley and Telonis-Scott 2011). Likewise, in *D. mojavensis*, the relative abundance of short-chained alkadienes decreases with temperature (Markow and Toolson 1990) and in *D. affinis* an increase in temperature increased both the chain length of CHCs and altered the structural position of double bonds (Jackson 1996). In *D. melanogaster*, increasing the temperature from 20°C to 25°C increased the proportion of long chained CHCs (Savarit and Ferveur 2002), a pattern that is also observed in populations artificially selected for desiccation resistance (Gibbs et al. 1997; Kwan and Rundle 2010), as well as in natural populations distributed along a temperature gradient (Frentiu and Chenoweth 2009). Considerable research has also been conducted on the role of CHCs in *D. melanogaster* sexual recognition (Ferveur and Cobb 2010). One CHC component in particular, (*Z*)-7-Tricosene, inhibits courtship behavior in males, but females are more sexually receptive to males expressing more of this CHC component (Ferveur and Sureau 1996; Grillet et al. 2006). More recent work suggests that this pattern is likely to be considerably more complex (Krupp et al. 2008; Kent et al. 2007), but formal estimates of sexual selection on CHCs are still lacking for this species. Furthermore, although CHCs appear to play an important role in both natural and sexual selection in *D. melanogaster*, it is not known whether these episodes of selection interact to shape the evolution of CHCs in this or any other *Drosophila* species other than *D. serrata*.

Here, we use experimental evolution in replicate populations of *D. simulans* to assess the combined effects of natural and sexual selection on the evolution of male and female CHCs. Although these flies have previously been reported to be sexually monomorphic in their CHC profiles (males and females express the same CHCs) (Cobb and Ferveur 1995), they nevertheless display sexual dimorphism in the relative abundance of these shared CHCs (Sharma et al. 2011). We have found that CHCs are an important determinant of male attractiveness in *D. simulans* (Lisa Berry et al. unpubl. data) and that male attractiveness is itself heritable (Taylor et al. 2007). We have also shown that there is genetic variation for female mating preferences (Sharma et al. 2010) and found substantial genetic (co)variation for male CHCs (Sharma et al. 2011), suggesting the potential for CHCs to evolve via sexual selection. However, there has been no direct demonstration of the effect of sexual or natural selection on CHC evolution in *D. simulans*. Here, we show that both natural and sexual selection, as well as their interaction, play an important role in the evolution of CHCs in this species. Importantly, this occurs in a sex-specific manner, with evidence for antagonistic sexual and natural selection being especially pronounced for male CHC profiles.

## Materials and Methods

### DERIVATION OF FLY STOCKS

The flies used in this study were derived from 20 iso-female lines supplied by the Centre for Environmental Stress and Adaptation Research, La Trobe University, Australia. These were collected from a wild population at Tuncurry, Eastern Australia in March, 2004. Stock flies (the mixed isolines) 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, and had been maintained in large population cages (ca. 800–1000 flies/cage) with overlapping generations and free mate choice for ca. four years prior to the start of this investigation. We have previously shown that this stock harbors substantial genetic and phenotypic variation in all characters that have been investigated, including CHCs (e.g., Taylor et al. 2007; Hosken et al. 2008; Wright et al. 2008; Okada et al. 2011).

### EXPERIMENTAL MANIPULATION OF NATURAL AND SEXUAL SELECTION

Experimental populations of flies were propagated under relaxed and elevated sexual and natural selection in a fully factorial design (four populations per treatment combination = 16 populations in total). The standard rearing temperature of 25°C (to which flies had been exposed for more than four years) represented the relaxed natural selection treatment, and constant low-grade temperature stress (a 2°C elevation to 27°C) was used to generate the elevated natural selection treatment. This temperature elevation was chosen because 27°C is very close to the *D. simulans* sterility threshold. Temperature has also been shown to affect life-history traits and the ontogeny of CHCs in *Drosophila* (Murphy et al. 1983; Savarit and Ferveur 2002). Sexual selection was relaxed by housing flies as monogamous pairs, and was elevated by housing each female with four males. We had 60 females per population in the elevated sexual selection treatment and 64 females in the nonsexual selection treatment. This difference in female number was an attempt to equalize effective population size ($N_e$) as there were higher numbers of males present and the potential for polyandry in the elevated sexual selection treatment. However, because of female mating behavior (they are unlikely to mate with more than two males; Taylor et al. 2008b) and approximately 80% sperm displacement (e.g., Hosken et al. 2008), we calculated that an additional four pairs was sufficient to standardize $N_e$. Populations evolved under these experimental conditions for 27 generations before CHCs were measured.

Briefly, our selection protocol was as follows: flies were housed together for six days in "interaction vials" before being placed into new "egg-laying vials" where females were allowed to oviposit for two days. After two days of egg laying, adults were discarded and vials incubated until offspring emergence. Offspring emerging from these vials on the day of peak emergence (approximately nine days after egg laying) were pooled within populations and chosen at random to start subsequent generations (Fig. 1). This was done to standardize selection on development time across our experimental populations. Newly emerged flies were collected and housed by sex (within population) to ensure virginity before individuals were chosen to start each subsequent generation. Food was provided in excess during the experimental evolution so that differential larval competition (e.g., the potential for nonsib competition in the sexual selection lines) was minimized (>40 mL/vial maximizes offspring emergence rates, Michelle Taylor et al. unpubl. data).

After 27 generations of selection, a haphazardly chosen subset of flies from our selection populations was allowed to oviposit for 24 h and subsequently vials were incubated at 26°C irrespective of the temperature treatment they originally evolved under (25°C or 27°C) until offspring emergence. This standardizes the development temperature across all our treatments so that any subsequent differences in CHCs were not simply due to rearing temperature differences during development, which have been shown to alter *Drosophila* CHC profiles (Savarit and Ferveur 2002). Emerging virgin adults (30 males and 30 females from each population) were collected and sexed within 4 h of eclosion as *Drosophila* CHCs have been shown to be identical for both sexes for a short time (3–6 h) after emergence (Pechine et al. 1988). These flies were housed individually to avoid CHC changes

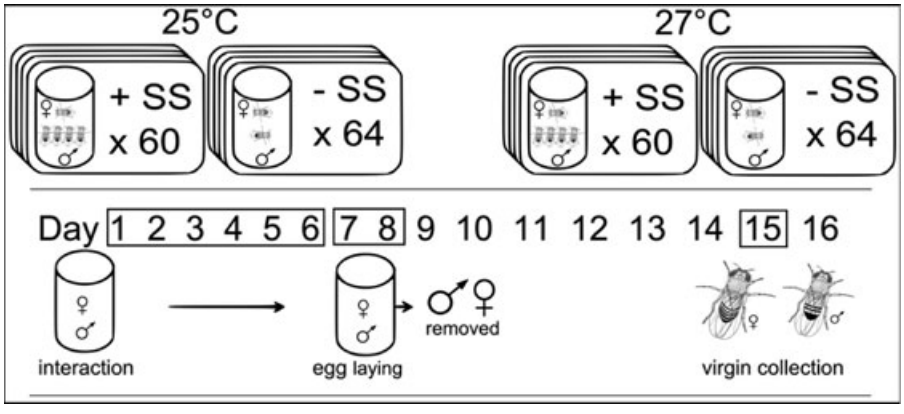


**Figure 1.** **The selection protocol used in our study. The relaxed natural selection regime populations were maintained at 25°C, which is the temperature flies have been housed at for >4 years. The enhanced natural selection populations were housed at 27°C, which is a novel, stressful temperature for these flies (above this temperature, males become sterile). In the enhanced sexual selection populations single females were housed with four males, and in the relaxed sexual selection populations, single females were housed with single males. Females and males were housed for six days in interaction vials before they were moved to laying vials for two days (days seven and eight). Adults were then discarded. Eggs from the egg-laying vial were allowed to develop and only individuals emerging from these vials were used to start subsequent generations (virgin collection on day 15). The design is fully factorial with four replicate populations per treatment combination. Selection followed these regimes for 27 generations then flies were reared at 26°C for one generation, and CHCs were assayed.**

due to social interactions (Petfield et al. 2005; Kent et al. 2008; Krupp et al. 2008). Visual stimuli are important in *Drosophila* courtship and may lead to individuals altering their CHC profiles. We therefore isolated glass vials visually using translucent plastic partitions that allowed light passage, but would make image recognition difficult. Individuals were processed for CHC extraction when they were three days old, as by this time adult CHC profiles are established (Antony and Jallon 1982; Schaner et al. 1989).

## CHC EXTRACTION AND GAS CHROMATOGRAPHY

To quantify male and female CHCs, individuals were transferred to 1-mL glass vials and soaked in 50 μl hexane containing an internal standard of pentadecane at a concentration of 10 ppm for 5 min. The vials were vortexed for the last 60 sec to maximize extraction. A 1-μl sample from each fly extract was then injected into a GCMS (Agilent 7890A GC coupled with an Agilent 5975B Mass Spectrometer) operating in pulsed split-less 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, 91 Blue Ravine Road, Folsom, CA) using helium as a carrier gas. Extract separation was optimized—we tested a range of ramp speeds before selecting the following as best—using 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 180°C followed by a 4°C/min rise to 220°C, and 15°C/min rise to 320°C where it was held for 2 min. The transfer line from the GC to the MS was set at 250°C. Chromatograms were acquired and analyzed using MSD Chemstation software version E.02.00.493 (Agilent, Foster City, CA). We identified individual CHC components based on retention times and mass spectrometry. The ratio of ions 55–57 was used to distinguish between alkanes and alkenes and molecular ions were used to determine the molecular weight of each CHC component.

CHCs were extracted and analyzed from 960 flies (30 individual males and females from each of the 16 populations) along with pentadecane control standards that were loaded at the start and end of each run to check for contamination of our samples. CHC peaks were labeled by peak number (1–25), which corresponded to their retention times on the GC (see Fig. 2, Table 1), and standardized values for each peak were calculated by dividing by the pentadecane internal standard. Data for each CHC peak were $\log_{10}$ transformed prior to statistical analysis to ensure normality.

## STATISTICAL ANALYSIS

Due to the large number of individual CHC components examined, we used principal component (PC) analysis to reduce the CHCs into a smaller number of dimensions. We extracted PCs across the entire dataset (based on the correlation matrix) that included both males and females to ensure that PCs were directly comparable across the sexes. We retained PCs with eigenvalues exceeding one for further analysis and interpret factor loadings for individual CHC components to each PC of 0.25 or above as biologically important (Tabachnick and Fidell 1989). We examined our data for multivariate outliers using Mahalanobis distances and a total of 12 datapoints (five males, seven females)

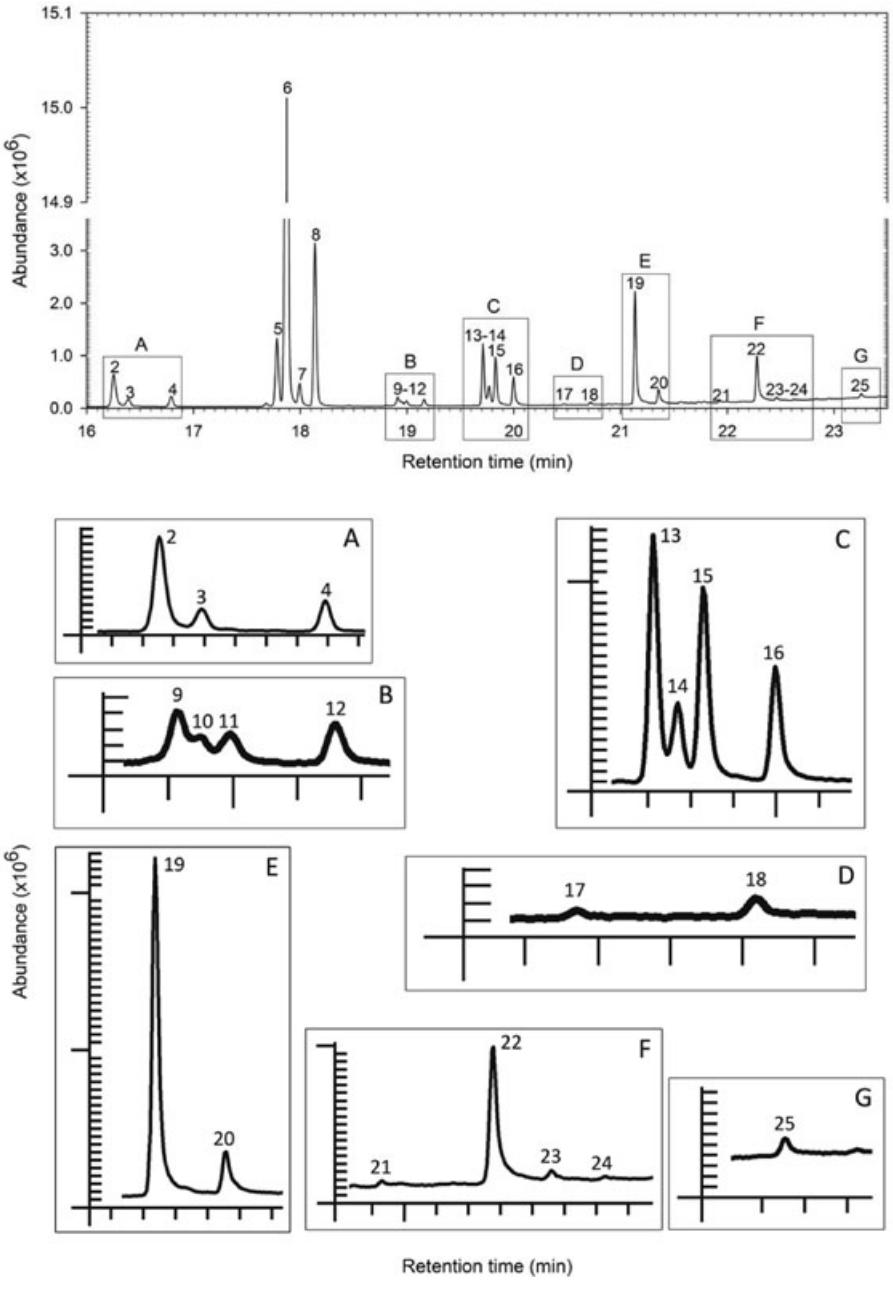


**Figure 2.** **A typical GC profile of a male *Drosophila simulans*. The *x*-axis shows the retention time (in minutes) and the *y*-axis the abundance of each peak, measured as the area under the peak. Note to improve the visualization of peaks 2–25, the chromatogram does not show the Pentadecane internal standard at a retention time of 7.489 min. Boxed regions (A–G) have been magnified for visual clarity (scale bar as per actual).**

were excluded from further analysis. Once these outliers were removed, we calculated mean PC scores for each sex in each of the replicate populations and all subsequent analyses were based on these mean values. Although the distribution of variances explained by each PC indicated pooling the sexes to generate PCs did not complicate our interpretation of eigenvectors (i.e., some vectors may represent differences between males and females), to check this, we assessed the correlations between each PC for males and females and there were no significant associations between PCs for either sex (data not shown). We started by analyzing this mean PC data using a multivariate analysis of variance (MANOVA) including our two experimental treatments (sexual selection and natural selection) and sex as fixed effects and PCs as the response variables. Having shown that the evolutionary responses of CHCs differed across the sexes (see below), we then conducted separate MANOVAs within each sex to investigate these differences. In each instance, we supported our MANOVA models with univariate ANOVAs to aid with interpreting the

**Table 1.** The identification of the 24 cuticular hydrocarbon compounds in male and female *D. simulans* and their relative contribution, expressed as the mean percentage (±SD) of the total abundance of all peaks. Retention time is in minutes and molecular weight in daltons.

| Peak no. | Retention time | Formula | Molecular weight | %(±SD) in males | %(±SD) in females | Name |
|---|---|---|---|---|---|---|
| 1 | 7.489 | $C_{15}H_{32}$ | 212 | | | Pentadecane ISTD |
| 2 | 16.253 | $C_{18}H_{38}$ | 250 | 0.65±0.46 | 0.09±0.21 | Octadecadiene |
| 3 | 16.391 | $C_{22}H_{44}$ | 308 | 0.50±0.12 | 0.42±0.12 | Docosene |
| 4 | 16.793 | $C_{22}H_{46}$ | 310 | 1.89±0.62 | 1.52±0.47 | Docosane |
| 5 | 17.783 | $C_{23}H_{48}$ | 324 | 3.66±0.71 | 2.73±0.71 | Branched alkane |
| 6 | 17.873 | $C_{23}H_{46}$ | 322 | 40.18±4.14 | 40.05±5.76 | 7-Tricosene |
| 7 | 17.990 | $C_{23}H_{46}$ | 322 | 1.50±0.34 | 1.43±0.41 | Tricosene |
| 8 | 18.138 | $C_{23}H_{48}$ | 324 | 22.43±3.21 | 22.44±3.20 | Tricosane |
| 9 | 18.917 | $C_{24}H_{50}$ | 338 | 0.39±0.13 | 0.29±0.10 | Branched alkane |
| 10 | 18.927 | $C_{24}H_{50}$ | 338 | 0.23±0.07 | 0.20±0.06 | Branched alkane |
| 11 | 18.996 | $C_{24}H_{50}$ | 338 | 0.29±0.08 | 0.24±0.08 | Branched alkane |
| 12 | 19.160 | $C_{24}H_{50}$ | 338 | 0.93±0.52 | 0.89±0.26 | Tetracosane |
| 13 | 19.711 | $C_{25}H_{48}$ | 348 | 1.78±0.92 | 1.00±0.57 | Pentacosadiene |
| 14 | 19.774 | $C_{25}H_{50}$ | 350 | 0.96±0.31 | 1.27±0.58 | Pentacosene |
| 15 | 19.827 | $C_{25}H_{50}$ | 350 | 2.38±0.50 | 2.52±0.68 | Pentacosene |
| 16 | 19.997 | $C_{25}H_{52}$ | 352 | 3.68±1.10 | 4.83±1.03 | Pentacosane |
| 17 | 20.468 | $C_{25}H_{52}$ | 352 | 0.14±0.05 | 0.11±0.05 | Branched alkane |
| 18 | 20.717 | $C_{26}H_{54}$ | 366 | 0.46±0.77 | 0.53±0.30 | Hexacosane |
| 19 | 21.135 | $C_{27}H_{56}$ | 380 | 10.08±3.76 | 6.31±2.91 | Heptacosane |
| 20 | 21.352 | $C_{27}H_{56}$ | 380 | 2.15±1.38 | 4.21±1.70 | Branched alkane |
| 21 | 21.930 | Unresolved | Unresolved | 0.33±0.50 | 0.38±0.24 | Alkane |
| 22 | 22.279 | $C_{29}H_{60}$ | 408 | 4.31±1.65 | 6.58±2.57 | Alkane |
| 23 | 22.459 | $C_{29}H_{60}$ | 408 | 0.60±0.49 | 1.10±0.64 | Alkane |
| 24 | 22.618 | Unresolved | Unresolved | 0.12±0.07 | 0.15±0.11 | Alkane |
| 25 | 23.253 | $C_{30}H_{62}$ | 422 | 0.36±0.19 | 0.67±0.97 | Alkane |

overall multivariate effect (Tabachnick and Fidell 1989). All analyses were conducted in JMP (version 8. SAS Institute Inc., Cary, NC, 1989–2010) and data are presented as mean ± 1 SE.

## Results

We obtained four significant PCs that collectively explained 77% of the variation in CHCs in male and female *D. simulans* (Table 2). The first principal component (PC1) described the total abundance of CHCs, with all individual CHC components loading positively to this dimension and 19 of 24 CHC components exceeding a loading of 0.25. PC2 largely described the trade-off between long- (positively loaded) and short-chained (negatively loaded) CHC components. Both PC3 and PC4 describe the trade-off between specific CHC components, although unlike PC2 there does not appear to be any obvious structural pattern to these trade-offs. PC3 is positively loaded by seven CHC components (Octadecadiene, Docosane, Tetracosane, Hexacosane, and three unidentified alkanes [peaks 9, 11, and 21]) and negatively loaded by three CHC components (Pentacosene and two unidentified alkanes [peaks 17 and 22]), whereas PC4 is positively loaded by five CHC components (Pentacosadiene, Hexacosane, Heptacosane, and two unidentified alkanes [peaks 17 and 21]) and negatively loaded by three CHC components (7-Tricosene, Tricosene, and an unidentified alkane [peak 25]).

A MANOVA indicated that natural selection, sexual selection, and sex all significantly affected the multivariate combination of PCs (Table 3). Additionally, there were significant interactions between sexual selection and sex, and between sex and sexual and natural selection (Table 3). Univariate post-hoc tests indicated that sex significantly contributed to variation in all PCs other than PC1, while other effects were largely confined to PC3 and PC4. The highly significant sex effect (alone and in the interactions) is consistent with sexual dimorphism and sexually dimorphic responses to selection, and hence to facilitate subsequent interpretation, we conducted separate analyses on males and females.

For males, MANOVA revealed significant effects of sexual selection, natural selection, and their interaction on the multivariate combination of PCs (Table 4, Fig. 3). Post-hoc ANOVAs indicated the multivariate interaction was entirely driven by evolution along PC4, as there were no other significant univariate interactions. For this PC, elevated natural selection favored

**Table 2.** Principal Component analysis for female and male CHCs, respectively. Principal components with an eigenvalue over 1 are retained for further analysis and factor loadings over |0.25| (in bold) are interpreted as biologically significant.

| | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| Eigenvalue | 9.459 | 5.547 | 1.972 | 1.545 |
| % variance | 39.414 | 23.111 | 8.216 | 6.437 |
| Loadings | | | | |
| Octadecadiene | 0.075 | **−0.718** | **0.406** | 0.225 |
| Docosene | **0.743** | **−0.287** | 0.102 | −0.219 |
| Docosane | **0.563** | −0.167 | **0.598** | −0.214 |
| Branched alkane | **0.867** | **−0.349** | −0.026 | 0.153 |
| 7-Tricosene | **0.870** | −0.165 | −0.170 | **−0.317** |
| Tricosene | **0.631** | **−0.264** | 0.007 | **−0.566** |
| Tricosane | **0.852** | 0.022 | −0.124 | −0.098 |
| Branched alkane | **0.708** | −0.175 | **0.501** | −0.042 |
| Branched alkane | **0.807** | −0.065 | 0.181 | −0.124 |
| Branched alkane | **0.716** | **−0.250** | **0.273** | −0.073 |
| Tetracosane | **0.585** | **−0.410** | **0.516** | 0.222 |
| Pentacosadiene | **0.720** | **−0.412** | −0.234 | **0.435** |
| Pentacosene | **0.821** | 0.190 | **−0.335** | 0.154 |
| Pentacosene | **0.870** | 0.090 | −0.118 | −0.094 |
| Pentacosane | **0.649** | **0.611** | −0.141 | 0.028 |
| Branched alkane | **0.595** | **0.329** | **−0.282** | **0.348** |
| Hexacosane | 0.178 | **0.749** | **0.381** | **0.352** |
| Heptacosane | **0.745** | **0.361** | −0.242 | **0.431** |
| Branched alkane | 0.229 | **0.897** | −0.013 | −0.157 |
| Alkane | 0.071 | **0.681** | **0.362** | **0.360** |
| Alkane | **0.631** | **0.566** | **−0.321** | 0.039 |
| Alkane | 0.159 | **0.869** | 0.074 | 0.013 |
| Alkane | **0.266** | **0.404** | −0.140 | −0.033 |
| Alkane | **0.285** | **0.735** | −0.160 | **−0.252** |

**Table 3.** Multivariate analysis of variance (MANOVA) examining the effect of sexual selection, natural selection, and sex on the evolution of CHCs in *D. simulans*. To aid the interpretation of the overall multivariate effect, we also provide univariate ANOVAs for each term in the multivariate model.

| | MANOVA | | |
|---|---|---|---|
| | Wilk's λ | $F_{4,21}$ | *P* value |
| Sexual selection (A) | 0.523 | 4.794 | 0.007 |
| Natural selection (B) | 0.408 | 7.611 | 0.001 |
| Sex (C) | 0.071 | 68.474 | 0.0001 |
| A×B | 0.653 | 2.784 | 0.053 |
| A×C | 0.544 | 4.395 | 0.010 |
| B×C | 0.809 | 1.243 | 0.323 |
| A×B×C | 0.440 | 6.694 | 0.001 |
| | Univariate ANOVAs | | |
| | | $F_{1,24}$ | *P* value |
| Sexual selection (A) | PC1 | 0.326 | 0.573 |
| | PC2 | 0.477 | 0.497 |
| | PC3 | 0.001 | 0.969 |
| | PC4 | 15.056 | 0.001 |
| Natural selection (B) | PC1 | 3.171 | 0.088 |
| | PC2 | 2.034 | 0.167 |
| | PC3 | 5.904 | 0.023 |
| | PC4 | 10.722 | 0.003 |
| Sex (C) | PC1 | 0.042 | 0.840 |
| | PC2 | 210.778 | 0.0001 |
| | PC3 | 36.244 | 0.0001 |
| | PC4 | 33.328 | 0.0001 |
| A×B | PC1 | 2.593 | 0.120 |
| | PC2 | 0.006 | 0.937 |
| | PC3 | 0.564 | 0.460 |
| | PC4 | 4.183 | 0.052 |
| A×C | PC1 | 1.765 | 0.196 |
| | PC2 | 0.439 | 0.514 |
| | PC3 | 6.931 | 0.015 |
| | PC4 | 2.020 | 0.168 |
| B×C | PC1 | 0.246 | 0.624 |
| | PC2 | 1.925 | 0.178 |
| | PC3 | 0.293 | 0.593 |
| | PC4 | 2.206 | 0.151 |
| A×B×C | PC1 | 0.010 | 0.920 |
| | PC2 | 0.106 | 0.747 |
| | PC3 | 0.003 | 0.955 |
| | PC4 | 24.169 | 0.0001 |

negative PC scores, whereas elevated sexual selection favored positive PC scores, and when both were elevated, sexual selection had the stronger effect, as under these conditions, PC4 scores were almost identical to those obtained when sexual selection was elevated but natural selection relaxed (Fig. 3D). The significant multivariate sexual selection effect was due to the effect of sexual selection on PC4 and to a lesser, but still statistically significant extent, PC3. For both PCs, sexual selection favored positive values, whereas when sexual selection was relaxed, lower scores evolved (Fig. 3C, D). The multivariate impact of natural selection was due to its effects on PC1 and PC2, and there were no other significant effects of natural selection. Here, elevated natural selection favored higher scores of both these PCs (Fig. 3A, B).

For females, CHC profiles were not greatly affected by our treatments (Table 4). MANOVA showed that only the interaction between sexual and natural selection affected the multivariate combination of PCs, and post-hoc tests indicated this was only due to the sexual–natural selection interaction influencing PC4. When sexual selection and natural selection were relaxed, this CHC combination evolved to negative PC scores. However, under relaxed natural selection and elevated sexual selection, positive PC4 values were favored, whereas the converse was true when

**Table 4. Multivariate Analysis of Variance (MANOVA) examining the effect of sexual selection, natural selection and their interaction on the CHC profile of male and female *D. simulans*. To aid the interpretation of the overall multivariate effect, we also provide univariate ANOVAs for each sex.**

| | MANOVA | | | | | |
|---|---|---|---|---|---|---|
| | Females | | | Males | | |
| | Wilks' λ | $F_{4,9}$ | $P$ value | Wilks' λ | $F_{4,9}$ | $P$ value |
| Sexual selection (A) | 0.573 | 1.679 | 0.238 | 0.199 | 9.035 | 0.003 |
| Natural selection (B) | 0.473 | 2.507 | 0.116 | 0.114 | 17.485 | 0.0001 |
| A×B | 0.286 | 5.617 | 0.015 | 0.373 | 3.781 | 0.045 |
| | Univariate ANOVAs | | | | | |
| | | $F_{1,12}$ | $P$ value | | $F_{1,12}$ | $P$ value |
| Sexual selection (A) | PC1 | 1.070 | 0.321 | PC1 | 0.918 | 0.357 |
| | PC2 | 0.664 | 0.431 | PC2 | 0.001 | 0.980 |
| | PC3 | 2.538 | 0.137 | PC3 | 5.289 | 0.040 |
| | PC4 | 1.920 | 0.191 | PC4 | 33.014 | 0.0001 |
| Natural selection (B) | PC1 | 0.489 | 0.498 | PC1 | 8.291 | 0.014 |
| | PC2 | 0.001 | 0.982 | PC2 | 6.357 | 0.027 |
| | PC3 | 1.345 | 0.269 | PC3 | 3.545 | 0.084 |
| | PC4 | 7.195 | 0.020 | PC4 | 3.761 | 0.076 |
| A×B | PC1 | 0.869 | 0.370 | PC1 | 3.639 | 0.081 |
| | PC2 | 0.060 | 0.811 | PC2 | 0.049 | 0.829 |
| | PC3 | 0.246 | 0.629 | PC3 | 0.357 | 0.561 |
| | PC4 | 15.391 | 0.002 | PC4 | 9.638 | 0.009 |

both sexual selection and natural selection were elevated (Fig. 4). This indicates that the actions of natural selection were most clearly seen along this PC.

## Discussion

Although natural and sexual selection have been implicated in the evolution of male sexual traits, and the accepted dogma is that natural selection counters sexual selection after sufficient exaggeration of male sexual traits, there have been very few experimental studies examining the joint effects of both modes of selection on trait evolution (Andersson 1994). Here, we use experimental evolution to assess the joint effects of both natural and sexual selection on the evolution of CHCs in *D. simulans*. We found that the responses of CHCs to both episodes of selection were very different for males and females and that natural and sexual selection interact to drive CHC evolution. Importantly, when sexual and natural selection are elevated, natural selection has a greater effect on female CHC evolution, but sexual selection has a greater effect on some aspects of male CHC profiles. Additionally, although many aspects of male CHC profiles evolved in a predictable fashion via sexual and/or natural selection, some male CHC combinations were only able to evolve in the naturally selected direction in the absence of sexual selection. In contrast, there was only limited evolution of female CHC profiles through the independent effects of natural and sexual selection, but there was a significant interaction between sexual and natural selection that influenced female CHC evolution.

Many aspects of male CHCs evolved during our study, and natural and sexual selection, together with their interaction, were implicated in this. Elevated natural selection saw males evolve an increase in their total CHC content (PC1) and increase their longer chain CHCs (PC2). This is presumably because of increased EWL at higher temperature (= the elevated natural selection treatment) (Gibbs and Rajpurohit 2010). Consistent with this explanation, the evolution of CHCs through natural selection has been documented in *Drosophila* previously, with increased total CHCs in a high-EWL environment (Kwan and Rundle 2010), and changes in specific CHCs such as Pentacosadiene that alter EWL (Toolson and Kuper-Simbrón 1989). Furthermore, sexual size dimorphism (males are smaller than females and thus have higher surface area to volume ratios) could at least partly explain why this evolution was only significant in males. It should also be noted that in the ancestral temperature treatment (relaxed natural selection), males were apparently producing more shorter chained CHCs than females (PC2) regardless of the sexual selection

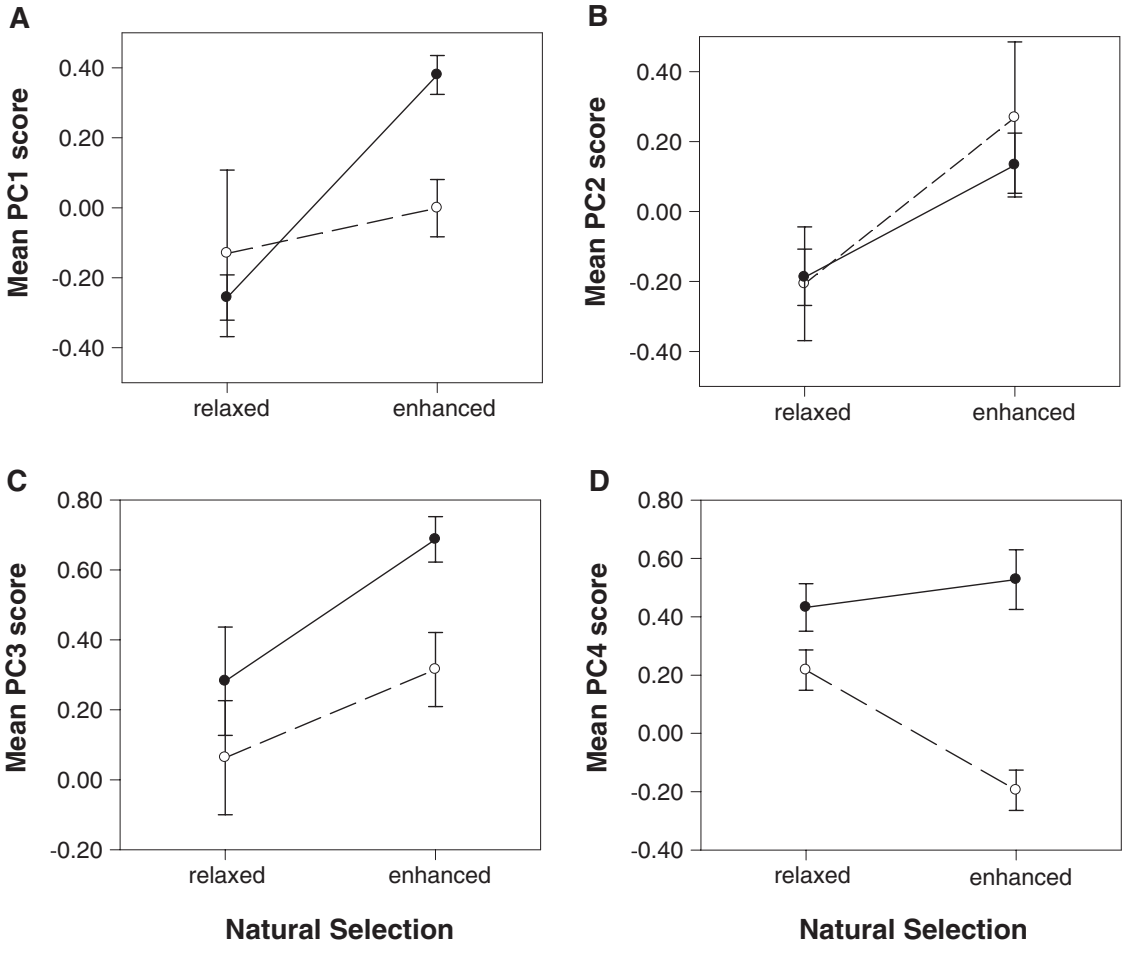


**Figure 3.** **The evolutionary response of male CHCs to natural and sexual selection. Figures A–D represent the mean (± SE) values for each of the four principal components (PCs), describing the variation in male CHC profiles. In each instance, closed symbols (•) with solid lines represent the enhanced sexual selection treatment, whereas open symbols (o) with dashed lines represent the relaxed sexual selection treatment.**

treatment. Some of these CHCs are apparently needed to stimulate female mating (e.g., 7-Tricosene; Ferveur and Cobb 2010) and the male-specific elevated-natural selection effect for PC2 may be (partly) because females are already sitting close to the naturally selected optima for elevated temperature (see Fig. 4B).

The interaction between sexual and natural selection influencing male CHC evolution (PC4) is particularly interesting. When we experimentally elevated natural selection by increasing temperature, male CHCs only evolved along PC4 when sexual selection was relaxed. When sexual selection co-occurred with elevated natural selection, populations did not evolve toward this naturally selected blend at all, and in fact the CHC profile described by PC4 is similar for sexual selection treatments in both the relaxed (ancestral) and elevated natural selection populations. Thus, elevated natural selection is only able to drive CHC evolution toward a new naturally selected peak in the absence of sexual selection, and sexual selection can be strong enough to overwhelm natural selection on some aspects of the male CHC profile. This finding is consistent with the conventional interpretation of sexual selection on male sexual traits (Andersson 1994) and additionally implies that sexual selection can be costly for males as it drives male traits from their naturally selected optima. Again, this is consistent with the standard interpretation of net selection on male sexual traits, and similar results have been reported for other *Drosophila* species. For example, sexual selection is not adaptive in *D. melanogaster* (Holland 2002), and in fact consistent with our findings, sexual selection opposes viability selection in this species (Wilkinson 1987). However, unlike *D. melanogaster*, there is no evidence for selection via sexual conflict in *D. simulans* (Taylor et al. 2008a, b), and hence the results we present are best explained by classical sexual selection theory. Interactions between natural and sexual selection also influence CHC evolution in other *Drosophila* (Blows 2002; and also see Tregenza et al. 2000), and like here, there is some evidence that male CHC components are costly, which is generally

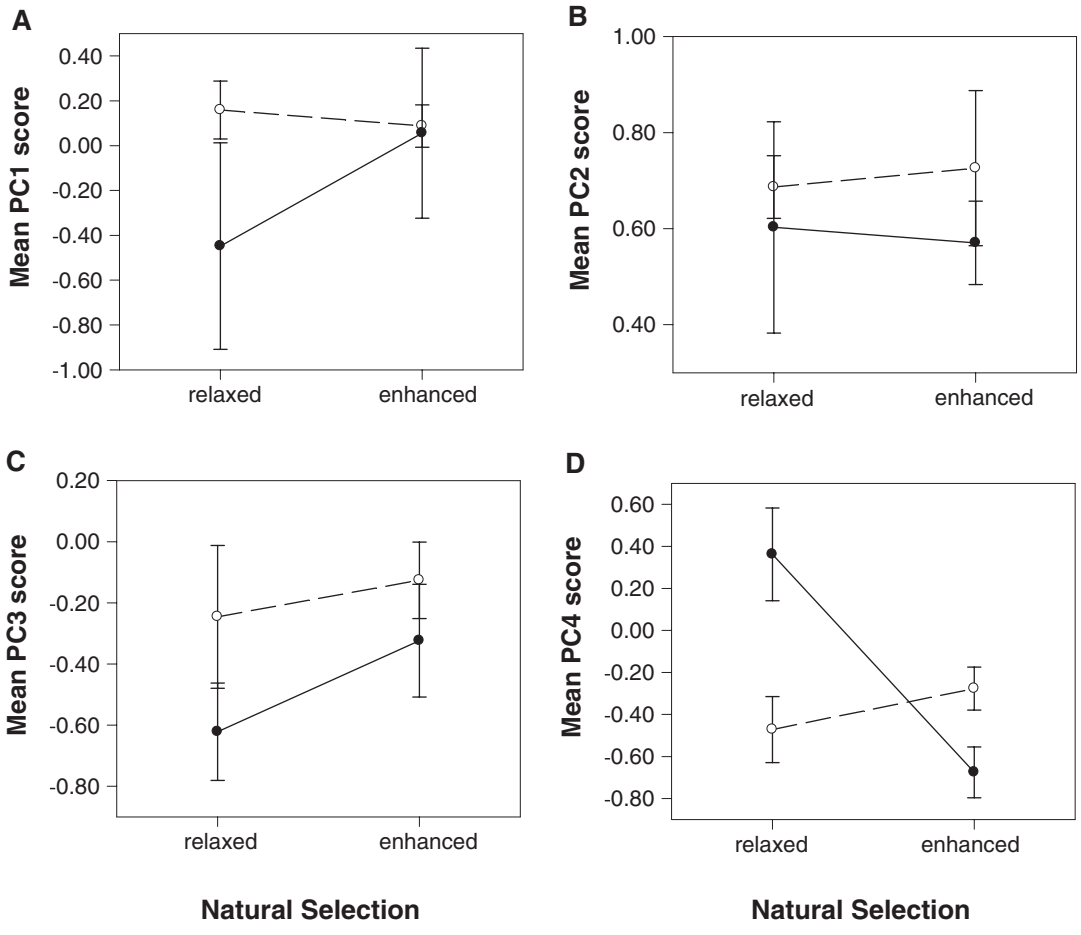


**Figure 4.** **The evolutionary response of female CHCs to natural and sexual selection. Figures A–D represent the mean (± SE) values for the four principal components (PCs) describing the variation in female CHC profiles. In each instance, closed symbols (•) with solid lines represent the enhanced sexual selection treatment, whereas open symbols (o) with dashed lines represent the relaxed sexual selection treatment.**

seen as a prerequisite for honest sexual-signalling (Zahavi 1975; Grafen 1990). Moreover, like here, sexual selection in isolation only acted on male, but not female CHC profiles (Blows 2002). However, under the relaxed, ancestral natural selection conditions, males from our sexual and no-sexual selection populations had similar CHC profiles along PC4. This indicates the male blends favored by sexual selection are not costly under relaxed natural selection (= ancestral conditions). One interpretation of this natural/sexual selection interaction is that given enough time and/or constant conditions,, sexual selection eventually hones in on naturally selected optima, but in the short term, the two types of selection do not align. This outcome is theoretically predicted when there is direct selection on female preference (Kirkpatrick 1985), although there is no evidence for this in *D. simulans* (Taylor et al. 2008a, b; Sharma et al. 2010). In any case, sexual selection is clearly not always adaptive (Wade 1987). Finally, the populations subjected to sexual selection appeared to be slightly divergent in their CHC profiles (at PC4) in the different environments (elevated/relaxed natural selection). Although far from conclusive, this slight difference could be indicative of some environment-specific sexual selection (Ingleby et al. 2010).

What is less clear is why some particular aspects of the PC4 evolved the way they did in males. To take a single example, Pentacosadiene has been implicated in water loss prevention in some *Drosophila* species (Toolson 1982), and hence more of it may have been expected with high natural selection. However, PC4 is loaded positively by a number of longer chained CHCs, including Pentacosadiene indicating more of this was expressed in males evolving with elevated sexual selection. This CHC has also been directly shown to influence mate choice in some *Drosophila* (Chenoweth and Blows 2003) and is implicated in sexual selection by our findings. Nonetheless, it is not entirely clear why levels of Pentacosadiene were not always higher when natural selection (temperature) was elevated (especially since this CHC was also negatively loaded with PC2).

In contrast to males, there was relatively limited female CHC evolution, although the significant interaction is revealing. When sexual selection was relaxed under the ancestral temperature regime (i.e., relaxed sexual and natural selection), female CHCs evolved toward a new PC4 profile, and this CHC combination was largely identical to the profile that evolved when natural selection was elevated. This indicates there is some sexual selection on female CHCs, directly or indirectly, but that elevated natural selection overwhelms this, and evolution of female CHCs was predominantly in the naturally selected direction. These findings are largely consistent with orthodox interpretation of the relative contributions of sexual and natural selection to female character evolution—natural selection is generally thought to shape female characters more than sexual selection. For example, females usually have no exaggerated secondary sexual characters because of (presumed) fecundity costs associated with developing and carrying them (Gwynne 2001). This was the only significant microevolutionary consequence of our experimental treatments for female CHC profiles, but largely mirrors work on the *D. serrata* species complex where interactions between natural and sexual selection influenced female CHC evolution in experimental populations (Blows 2002). Whether the sexual selection contribution to the interaction in our study was due to male mate preference for certain female CHC blends, or genetic correlations between male and female CHCs remains to be established. However, there are many significant intersexual genetic correlations for CHCs in our populations (Sharma et al. 2011), which contrasts with at least some other *Drosophila* (Chenoweth and Blows 2003). Additionally, the female CHC blend in the relaxed natural selection-enhanced sexual selection treatment is very similar to that of males in the same treatment, as were the blends in the relaxed sexual selection-enhanced natural selection treatment. So although male mate-choice causing female CHC evolution cannot be ruled out, especially because male preference for certain combination of female CHCs has been found in *D. serrata* (Chenoweth and Blows 2005), it seems likely that certain responses occur because of intersexual genetic correlations. It is also possible that the female profiles evolved in response to increases or reductions in male sexual harassment. Consistent with this, in the enhanced sexual selection-reduced natural selection treatment, females expressed less 7-Tricosene, a CHC that stimulates male courtship behavior (Jallon 1984; Ferveur and Cobb 2010). Furthermore, in all treatments other than the relaxed (ancestral) natural-enhanced sexual selection treatment, females expressed less Pentacosadiene, which may also reduce male sexual excitement (Ferveur 2005). However, in these same treatments, females also tended to express more Tricosene, which stimulates male courtship (Jallon 1984; Ferveur and Cobb 2010), and male harassment does not seem to lower female fitness in our founder population (Taylor et al. 2008b). Thus, specific female responses are somewhat enigmatic, which is at least consistent with claims that the functions of CHCs in reproduction, and to a lesser extent dessication resistance, are often more complicated than appreciated at times (Ferveur and Cobb 2010).

Although the reasons for some of the detailed CHC evolution documented are not entirely clear, male and female responses to selection on CHC profiles were unambiguously different. This may be because selection is sexually antagonistic for CHCs, as indicated by sexual dimorphism in CHC profiles, but this sex-specific evolution is also consistent with the genetic architecture (**G**) that exist for CHCs in these flies. Sexually, antagonistic selection has been documented many times in *Drosophila* (e.g., Rice and Chippindale 2001; Innocenti and Morrow 2010) and, while intersexual genetic correlations for CHCs in other flies are often inconsequential (Chenoweth and Blows 2003), we find many significant correlations across the sexes and the majority of these are negative (Sharma et al. 2011). Additionally, there are significant differences in the **G** matrix of female and male CHCs. It is therefore not surprising that female response to selection did not mirror males, although it is not exactly clear from our work whether **G** or selection is the major cause of this. However, the sex-specific changes in PC4 are arguably more consistent with sex-specific selection (the sexes had similar profiles in some treatments but not others), and similar results have been found in another study where male and female responses to sexual and natural selection also differed greatly (Blows 2002). Finally, CHC evolution can be constrained by a lack of genetic variation in the direction of selection (McGuigan et al. 2008), and as the present study employed experimental evolution where flies did the selecting, lack of adequately aligned genetic variation could also explain some of the sexual differences we report.

Overall, our findings indicate that both natural and sexual selection act on *D. simulans* CHCs in a sex-specific manner. Furthermore, microevolutionary responses indicate sexual and natural selection act antagonistically on at least some CHC components, so sexual selection is often not adaptive. These findings are largely consistent with conventional views of evolution through sexual selection (Andersson 1994; Gwynne 2001), although these have rarely been demonstrated experimentally. CHCs are only one component of male attractiveness, with variation in CHC profiles explaining about 10% of the variation in male mating success in our populations. Whether the CHC profile that makes a male attractive in one environment is the same that confers attractiveness in others remains to be investigated, but there is some indication that this may not be the case. Additionally, how other characters determining male attractiveness are affected by natural and sexual selection is worthy of additional work. Nevertheless, our findings suggest sexual selection shapes male CHC profiles to a greater extent than female profiles, which tended to evolve in the direction of natural selection. This is consistent with current orthodoxy

that sexual selection is typically stronger in males than females, and refutes recent claims that sexual selection theory is somehow flawed (see discussion in, e.g., Dall et al. 2006; Shuker 2010).

## ACKNOWLEDGMENTS

This work was supported by NERC (NE/G005303/1—DJH and JH) and The Royal Society (Royal Society Fellowship and Equipment grant—JH). We thank Dave Punzalan, Daphne Fairbairn, Ted Morrow, and three anonymous referees for helpful comments on the manuscript, Chris Mitchell for GCMS help and Tom Tregenza for discussion of the experimental design.

## LITERATURE CITED

Andersson, M. 1994. Sexual selection. Princeton Univ. Press, Princeton, NJ.

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

Arnold, S. J. 1983. Sexual selection: the interface of theory and empiricism. Pp. 67–107 *in* P. Bateson, ed. Mate choice. Cambridge Univ. Press, UK.

Bentsen, C. L., J. Hunt, M. D. Jennions, and R. Brooks. 2006. Complex multivariate sexual selection on male acoustic signalling in a wild population of *Teleogryllus commodus*. Am. Nat. 167:E102–E116.

Blows, M. W. 2002. Interaction between natural and sexual selection during the evolution of mate recognition. Proc. R. Soc. Lond. B 269:1113–1118.

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

Chenoweth, S. F., and M. W. Blows. 2003. Signal trait sexual dimorphism and mutual sexual selection in *Drosophila serrata*. Evolution 57:2326–2334.

———. 2005. Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. Am. Nat. 165:281–289.

———. 2008. QST meets the G matrix: the dimensionality of adaptive divergence in multiple correlated quantitative traits. Evolution 62:1437–1449.

Chenoweth, S. F., H. D. Rundle, and M. W. Blows. 2008. Genetic constraints and the evolution of display trait sexual dimorphism by natural and sexual selection. Am. Nat. 171:22–34.

———. 2010. Experimental evidence for the evolution of indirect genetic effects: changes in the interaction effect coefficient, Psi ($\Psi$), due to sexual selection. Evolution 64:1849–1856.

Cobb, M., and J. F. Ferveur. 1995. Evolution and genetic control of mate recognition and stimulation in *Drosophila*. Behav. Proc. 35:35–54.

Cox, R. M., and R. Calsbeek. 2009. Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. Am. Nat. 173:176–187.

Coyne, J. A., and S. Elwyn. 2006. Does the desaturase-2 locus in *Drosophila melanogaster* cause adaptation and sexual isolation? Evolution 60: 279–291.

Dall, S. R. X., J. M. McNamara, N. Wedell, and D. J. Hosken. 2006. Debating sexual selection and mating strategies. Science 31:689.

Endler, J. A. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34:76–91.

Ferveur, J. F., M. Cobb, H. Boukella, and J. M. Jallon. 1996. World-wide variation in *Drosophila melanogaster* sex pheromone: behavioural effects, genetic bases and potential evolutionary consequences. Genetica 97:73–80.

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

Ferveur, J. F., and M. Cobb. 2010. Behavioral and evolutionary roles of cuticular hydrocarbons in Diptera. Pp. 325–343 *in* G. J. Blomquist and A.-G. Bagnéres, eds. Insect hydrocarbons: biology, biochemistry, and chemical ecology. Cambridge Univ. Press, Cambridge.

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

Foley, B. R., and M. Telonis-Scott. 2011. Quantitative genetic analyses suggests causal association between cuticular hydrocarbon composition and dessiccation survival in *Drosophila melanogaster*. Heredity 106: 68–77.

Frentiu, F. D., and S. F. Chenoweth. 2009. Clines in cuticular hydrocarbons in two *Drosophila* species with independent population histories. Evolution 64:1784–1794.

Gibbs, A. G., A. K. Chippindale, and M. R. Rose. 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. J. Exp. Biol. 200:1821–1832.

Gibbs, A. G., and S. Rajpurohit. 2010. Cuticular lipids and water balance. Pp. 100–120 *in* G. J. Blomquist and A.-G. Bagnéres, eds. Insect hydrocarbons: biology, biochemistry, and chemical ecology. Cambridge Univ. Press, Cambridge.

Grafen, A. 1990. Biological signals as handicaps. J. Theor. Biol. 144:517–546.

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

Gwynne, D. 2001. Katydids and bush-crickets: reproductive behavior and evolution of the Tettigoniidae. Cornell Univ. Press, Ithaca.

Hadley, N. F. 1981. Cuticular lipids of terrestrial plants and arthropods: a comparison of their structure, composition and waterproofing functions. Biol. Rev. 56:23–47.

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

Hine, E., K. McGuigan, and M. W. Blows. 2011. Natural selection stops the evolution of male attractiveness. Proc. Natl. Acad. Sci. USA. 108:3659–3664.

Holland, B. 2002. Sexual selection fails to promote adaptation to a new environment. Evolution 56:721–730.

Hosken, D. J., W. Bailey, J. O'Shea, and J. Roberts. 1994. Localization of insect calls by the bat *Nyctophilus geoffroyi* (Chiroptera, Vespertilionidae)—a laboratory study. Aust. J. Zool. 42:177–184.

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

Hosken, D. J., M. L. Taylor, K. Hoyle, S. Higgins, and N. Wedell. 2008. Attractive males have greater success in sperm competition. Curr. Biol. 18:R553–R554.

Hunt, J., C. J. Breuker, J. A. Sadowski, and A. J. Moore. 2009. Male-male competition, female mate choice and their interaction: determining total sexual selection. J. Evol. Biol. 22:13–26.

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

Innocenti, P., and E. H. Morrow. 2010. The sexually antagonistic genes of *Drosophila melanogaster*. PLoS Biol. 8:e1000335, doi: 10.1371/journal.pbio.1000335.

Jackson, L. L. 1996. Involvement of cuticular hydrocarbons in the incomplete behavior isolation between *Drosophila affinis* and *Drosophila melanogaster*. Pp. 320. The 37th *Drosophila* Conference, San Diego.

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

Kent, C., R. Azanchi, B. Smith, A. Chu, and J. Levine. 2007. A model based analysis of chemical and temporal patterns of cuticular hydrocarbons in male *Drosophila melanogaster*. PLoS ONE 2:e962, doi: 10.1371/journal.pone.0000962.

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

Kirkpatrick, M. 1985. Evolution of female choice and male parental investment in polygynous species: the demise of the 'sexy son'. Am. Nat. 125:788–810.

Krupp, J. J., C. Kent, J. C. Billeter, R. Azanchi, A. K. C. So, J. A. Schonfeld, B. P. Smith, C. Lucas, and J. D. Levine. 2008. Social experience modifies pheromone expression and mating behavior in male *Drosophila melanogaster*. Curr. Biol. 18:1373–1383.

Kwan, L., and H. D. Rundle. 2010. Adaptation to desiccation fails to generate pre- and postmating isolation in replicate *Drosophila melanogaster* laboratory populations. Evolution 64:710–723.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. Proc. Natl. Acad. Sci. USA 78:3721–3725.

Markow, T. A., and E. C. Toolson. 1990. Temperature effects on epicuticular hydrocarbons and sexual isolation in *Drosophila mojavensis*. Pp. 315–331 *in* J. S. F. Barker, W. T. Starmer, and R. J. MacIntyre, eds. Ecological and evolutionary genetics f Drosophila. Plenum Press, New York.

McGuigan, K., A. Van Homrigh, and M. W. Blows. 2008. An evolutionary limit to male mating success. Evolution 62:1528–1537.

Mead, L. S., and S. J. Arnold. 2004. Quantitative genetic models of sexual selection. Trends Ecol. Evol. 19:264–271.

Murphy, P. A., J. T. Giesel, and M. N. Manlove. 1983. Temperature effects on life history variation in *Drosophila simulans*. Evolution 37:1181–1192.

Okada, K., J. D. Blount, M. D. Sharma, R. R. Snook, and D. J. Hosken. 2011. Male attractiveness, fertility and susceptibility to oxidative stress are influenced by inbreeding in *Drosophila simulans*. J. Evol. Biol. 24: 363–371.

Pechine, J. M., C. Antony, and J. M. Jallon. 1988. Precise characterization of cuticular compounds in young *Drosophila* by mass-spectrometry. J. Chem. Ecol. 14:1071–1085.

Petfield, D., S. F. Chenoweth, H. D. Rundle, and M. W. Blows. 2005. Genetic variance in female condition predicts indirect genetic variance in male sexual display traits. Proc. Natl. Acad. Sci. USA 102:6045–6050.

Pomiankowski, A., Y. Iwasa, and S. Nee. 1991. The evolution of costly mate preferences. I. Fisher and biased mutation. Evolution 45:1422–1430.

Rand, A. S., and M. J. Ryan. 1981. The adaptive significance of a complex vocal repertoire in a Neotropical frog. Z. Tierpsychol. 57:209–214.

Rice, W. R., and A. K. Chippindale. 2001. Intersexual ontogenetic conflict. J. Evol. Biol. 14:685–693.

Rundle, H. D., S. F. Chenoweth, and M. W. Blows. 2008. Comparing complex fitness surfaces: among-population variation in mutual sexual selection in *Drosophila serrata*. Am. Nat. 171:443–454.

Rundle, H. D., S. F. Chenoweth, and M. W. Blows. 2009. The diversification of mate preferences by natural and sexual selection. J. Evol. Biol. 22:1608–1615.

Savarit, F., and J. F. Ferveur. 2002. Temperature affects the ontogeny of sexually dimorphic cuticular hydrocarbons in *Drosophila melanogaster*. J. Exp. Biol. 205:3241–3249.

Schaner, A. M., P. D. Dixon, K. J. Graham, and L. L. Jackson. 1989. Components of the courtship-stimulating pheromone blend of young male *Drosophila melanogaster*: (Z)-13-tritriacontene and (Z)-11-tritriacontene. J. Insect Physiol. 35:341–345.

Shuker, D. M. 2010. Sexual selection: endless forms or tangled bank? Anim. Behav. 79: E11–E17.

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

Sharma, M. D., C. Mitchell, J. Hunt, T. Tregenza, and D. J. Hosken. 2011. The genetics of cuticular hydrocarbon profiles in the fruit fly Drosophila simulans. J. Hered. *In press*.

Skroblin, A., and M. W. Blows. 2006. Measuring natural and sexual selection on breeding values of male display traits in *Drosophila serrata*. J. Evol. Biol. 19:35–41.

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

Taylor, M. L., N. Wedell, and D. J. Hosken. 2007. The heritability of attractiveness. Curr. Biol. 17:R959–R960.

Taylor, M. L., N. Wedell, and D. J. Hosken. 2008a. Sexual selection and female fitness in *Drosophila simulans*. Behav. Ecol. Sociobiol. 62:721–728.

Taylor, M. L., C. Wigmore, D. J. Hodgson, N. Wedell, and D. J. Hosken. 2008b. Multiple mating increases female fitness in *Drosophila simulans*. Anim. Behav. 76:963–970.

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

Toolson, E. C., and R. Kuper-Simbrón. 1989. Laboratory evolution of epicuticular hydrocarbon composition and cuticular permeability in *Drosophila pseudoobscura*—effects on sexual dimorphism and thermal acclimation ability. Evolution 43:468–473.

Tregenza, T., S. H. Buckley, V. L. Pritchard, and R. K. Butlin. 2000. Inter- and intrapopulation effects of sex and age on epicuticular composition of meadow grasshopper, *Chorthippus parallelus*. J. Chem. Ecol. 26: 257–278.

Tuttle, M. D., and M. J. Ryan. 1981. Bat predation and the evolution of frog vocalizations in the neotropics. Science 214:677–678.

Wade, M. J. 1987. Measuring sexual selection. Pp. 197–207 *in* J. W. Bradbury and M. B. Andersson, eds. Sexual selection: testing the alternatives. Wiley, Chichester.

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

Wilkinson, G. S. 1987. Equilibrium analysis of sexual selection in *Drosophila melanogaster*. Evolution 41:11–21.

Wright, L. I., T. Tregenza, and D. J. Hosken. 2008. Inbreeding, inbreeding depression and extinction. Conserv. Genet. 9:833–843.

Zahavi, A. 1975. Mate selection: a selection for a handicap. J. Theor. Biol. 53:205–214.

Zuk, M., J. T. Rotenberry, and R. M. Tinghitella. 2006. Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets. Biol. Lett. 2:521–524.

Associate Editor: E. Morrow