JOURNAL OF Evolutionary Biology
eseb European Society for Evolutionary Biology

doi: 10.1111/jeb.12045

Heritability of male attractiveness persists despite evidence for unreliable sexual signals in *Drosophila simulans*

F. C. INGLEBY, J. HUNT & D. J. HOSKEN

Centre for Ecology and Conservation, School of Biosciences, University of Exeter, Cornwall, Tremough, Penryn, UK

Keywords:

cuticular hydrocarbons;
G × Es;
genotype-by-environment interactions;
honest signalling;
sexual selection.

Abstract

Sexual signals can be used to attract mates, but to be honest indicators of signaller quality they need to convey information reliably. However, environmental variation and genotype-by-environment (G × E) interactions have the potential to compromise the reliability of sexual signals. Here, we test the reliability of cuticular hydrocarbons (CHCs) as signals of heritable aspects of male attractiveness in *Drosophila simulans*. We examined the heritability of male attractiveness and a measure of the difference between fathers' and sons' CHC profiles across dietary and temperature environments. Our results show that environmental heterogeneity disrupts the similarity of some components of father and son CHC profile. However, overall male attractiveness is heritable within and across environments, so that sire attractiveness is a good predictor of son attractiveness even with environmental heterogeneity. This suggests that although some male CHC signals are unreliable, attractive genotypes retain their attractiveness across environments on average.

Introduction

Mate choice (usually by females) can be based on sexual signals expressed in the opposite sex (usually males), and sexual signals may reflect the possible benefits of mating with that individual. Benefits of choice can be direct through resources provided to the female (Andersson, 1994; Møller & Jennions, 2001) or indirect through attractiveness or viability benefits inherited by the offspring (Lande, 1981; Kirkpatrick, 1982; Wedell & Tregenza, 1999; Head *et al.*, 2005; Taylor *et al.*, 2007). The evolution of preferences for sexual displays depends to some extent on signal reliability, because expressing preferences for signals that do not contain reliable information about benefits will be costly and should be selected against (Maynard Smith & Harper, 2003).

For sexual signals to reliably indicate benefits to females, we expect a positive correlation between benefits and signal attractiveness. This positive correlation has been found in several species with direct benefits of mate choice (see Andersson, 1994), although clear evidence from species with indirect benefits of mate choice is less common (e.g. Qvarnström *et al.*, 2006). It is also thought that information contained in sexual signals can vary between environments (Greenfield, 1994; Candolin, 2003). If male signal expression is dependent to some extent on the environment, then this environmental component could mean that the signal is a less reliable indicator of benefits across heterogeneous environments. In this way, sexual signals may become unreliable if the environmental conditions change or if males move between different environments (Greenfield & Rodríguez, 2004; Higginson & Reader, 2009; reviewed by Ingleby *et al.*, 2010). This potential for environmental heterogeneity to compromise the reliability of sexual signals could affect the evolution of mate preferences and the operation of sexual selection by female choice in heterogeneous environments.

In terms of direct benefits of mate choice, if a male advertises for mates in different environmental conditions from those experienced during development, then female assessment of the male could be based on a signal phenotype which does not reflect the ability of the male to provide direct benefits in the current

Correspondence: D. J. Hosken, Centre for Ecology and Conservation, School of Biosciences, University of Exeter, Cornwall Campus, Tremough, Penryn, TR10 9EZ, UK.
Tel.: +44 1326 371843; fax: +44 1326 371829;
e-mail: D.J.Hosken@exeter.ac.uk

environment. On the other hand, a reliable signal of indirect benefits of mate choice needs to contain information about the underlying genetic quality of a potential mate. If genotype performance differs between environments, then a male could develop an attractive signal in one environment, but the genes inherited by the offspring may not yield benefits if offspring experience different environmental conditions (Ingleby *et al.*, 2010).

Consequently, sexual signals of genetic quality could be unreliable when the genetic variance underlying sexual trait expression (V_G; the variance explained by genetic effects) changes between environments. Changes in genetic variation across heterogeneous environments, both within a generation and across evolutionary time, can be driven by environmental stressors or differential selection between environments (reviewed by Hoffmann & Merilä, 1999). In addition to these overall environmental effects, genotype-by-environment interactions (G × Es) can be associated with changes in genetic variation across environments. A G × E in trait expression will mean that individuals with identical genotypes can differ phenotypically when exposed to different environmental conditions and that the extent and direction of this plasticity differs between genotypes (Lynch & Walsh, 1998). G × Es can be interpreted as genetic variation for phenotypic plasticity and might therefore involve changes in genetic variation across environments, although this is not necessarily always the case. Recent theoretical work has demonstrated that G × Es can potentially disrupt sexual signal reliability in heterogeneous environments (Greenfield & Rodríguez, 2004; Kokko & Heubel, 2008; Higginson & Reader, 2009; reviewed by Ingleby *et al.*, 2010), and a few empirical studies also find this (Jia *et al.*, 2000; Mills *et al.*, 2007). Consequently, it appears that both environmental heterogeneity with and without G × Es in sexual signal expression can potentially decrease the reliability of a sexual signal for indirect benefits by disrupting the relationship between trait expression and underlying genetic quality. Indeed, some studies have shown G × E for a variety of fitness components (Mills *et al.*, 2007; Danielson-François *et al.*, 2009; Lewis *et al.*, 2012). Ultimately, the breakdown of the relationship between signal phenotype and underlying genotype has the potential to degrade the correlation between signal and preference, which is essential to many models of sexual selection for indirect benefits (Kokko *et al.*, 2006), although empirical work examining this idea is scarce.

In *Drosophila*, body size (Partridge *et al.*, 1987), courtship behaviour (Hall, 1994) and sex comb structure (Markow, 1996) have all been shown to contribute to male attractiveness. More recently, research on a number of species has found that cuticular hydrocarbons (CHCs) are an important determinant of male attractiveness and that short-chained, volatile CHCs can act as sexual pheromones to attract mates (Cobb & Ferveur, 1995; Blows, 2002; Wicker-Thomas, 2007). Experimental evolution has shown that CHC profiles evolve in response to sexual selection in *D. serrata* and *D. simulans* (Blows, 2002; Rundle *et al.*, 2009; Sharma *et al.*, 2012a). In *D. serrata,* female mate preference exerts strong directional selection on male CHC profiles (Chenoweth & Blows, 2005). There is also evidence for a strong environmental component of CHC expression in *Drosophila* species. In particular, temperature variation is thought to favour longer-chained CHCs that form a stable and protective chemical barrier to water loss, and in *D. melanogaster* (Savarit & Ferveur, 2002; Foley & Telonis-Scott, 2011), *D. mojavensis* (Gibbs *et al.*, 1998) and *D. serrata* (Frentiu & Chenoweth, 2010), studies have shown that long-chained CHCs can provide desiccation resistance.

Here, we present a quantitative genetic study of male attractiveness and CHC profile in *D. simulans* across a range of environmental variation. Research on our study population has indicated that there are temperature and diet effects on CHC expression and also that there are G × Es with the potential to disrupt signal reliability (Ingleby *et al.*, 2012). Previous work on *D. simulans* has demonstrated that there are no direct benefits or costs to mate choice (Taylor *et al.*, 2008, 2010; Sharma *et al.*, 2012b), but that male attractiveness is heritable (Taylor *et al.*, 2007; Hosken *et al.*, 2008). Thus, female mating preferences in this species could evolve for genetic benefits, as females who mate with attractive males will have attractive sons. As noted above, *D. simulans* CHCs are heritable and evolve through sexual selection (Sharma *et al.*, 2012a), and the influence of male CHCs on female mating preferences is widely documented in *D. simulans* and related *Drosophila* species (reviewed by Ferveur & Cobb, 2010).

Therefore, CHCs in *D. simulans* are an ideal trait on which to focus to examine how environmental variation and G × Es might affect sexual signalling. CHCs contribute to male attractiveness, and heritable male attractiveness could provide genetic benefits to female mate choice. However, if environmental variation and G × Es disrupt the heritability of male CHC profile, this could disrupt the inheritance of male attractiveness across environments and thus impact the operation of sexual selection. In other words, if moving across environments means males are no longer reliably signalling their attractiveness, females may mate without gaining the benefit of producing attractive sons.

We reared male *D. simulans* from forty-seven isofemale lines across a range of abiotic environmental variation (post-eclosion temperature and diet) for two generations. For both sires and sons, we quantified CHC expression and attractiveness. We measured the heritability of male attractiveness across environments, to assess cross-environment genetic benefits of female preference in this species. We then consider this alongside the expression of male CHC profile, a sexual signal

in *D. simulans*, across these environments. We also test the absolute difference between sire and son CHC profile and relate this to environmental and G × E components of CHC expression. Based on theoretical work, we make two predictions: (1) Environmental heterogeneity and G × Es for male CHC expression will make CHCs unreliable sexual signals of male attractiveness by disrupting the similarity of sire and son CHC profiles across different environments; and (2) Where sexual signals are unreliable, benefits of mating preferences will be compromised through reduced heritability of male attractiveness across environments and therefore reduced genetic benefits of mate choice across environments.

Methods

Isolines and maintenance

Approximately 100 female *D. simulans* were collected from Greece in April 2010 and used to found iso-female lines (isolines) in the laboratory ($N = 65$ as some isolines were lost during domestication). A random subset of 47 of these remaining isolines was used in this experiment. Within each isoline, 25 male and 25 female offspring were used to produce each new generation, and this process of inbreeding was repeated for 21 generations prior to this experiment, such that each isoline had been heavily inbred and can be considered as a distinct genotype (David *et al.*, 2005). The wild-collected flies were also used to set up an outbred population containing the same genetic variation as the isolines. The outbred population was maintained with overlapping generations at an approximate population size of 500 flies. All flies were maintained on a standard cornmeal-based diet (Applied Scientific, UK) at 25 °C on a 10:14-h light/dark cycle, unless stated otherwise.

Environmental manipulations

We carried out post-eclosion temperature and dietary manipulations as separate experiments but with equivalent experimental design (see Fig. 1). The environmental manipulations created a narrow range of environmental variation which *D. simulans* are likely to experience in the laboratory, as opposed to creating stressful or extreme environments. We set up the same set of 47 isolines for the post-eclosion temperature and dietary manipulations, but only 44 of these isolines survived on the novel diets used for the diet manipulation. Flies from the outbred population were reared on the standard cornmeal diet, to generate a stock of outbred flies from a standard environment for use in attractiveness assays (described below).

For each experiment, adult flies were taken from each isoline and used to set up two replicate laying vials (40 mL vials with 8 mL of medium) for each isoline/environment combination, with two males and two females in each. For the post-eclosion temperature manipulation, all flies laid on the cornmeal-based diet. For the dietary manipulation, we used two novel diets: a homemade oat-based medium (consisting of oatbran, sugar and yeast set in water and agar; diet A) and a soy-based medium (Genesee Scientific, USA; diet B). These diets were chosen purely to create dietary environmental variation rather than to manipulate diet quality. Flies were given 72 h in which to lay eggs in these vials before being removed, and the vials were then incubated at 25 °C during offspring development. During this 72-h laying period, flies from the outbred population laid in large vials (150 mL) with 30 mL of the standard cornmeal diet, and these vials were then kept in the same incubator during offspring development.

Peak offspring eclosion occurred after 11 days, and virgin flies were collected from both isoline and outbred population vials. Vials were cleared out between 7 am and 8 am. Newly eclosed virgin adults were collected between approximately 11 am and 1 pm, and again between 5 pm and 7 pm. Virgin females were collected from the outbred population vials and subsequently housed individually in small vials of the standard cornmeal diet (40 mL vial with 8 mL of medium). This created a stock of outbred females reared in a standard environment for use in attractiveness assays and avoided biasing assays through any co-evolved genetic effects within isolines. In this first experimental generation, both virgin males and females were collected from the isoline vials and transferred into small individual glass vials (5 mL with 1 mL of medium). For the dietary manipulation, flies were kept on the same food type as development at 25 °C. For the post-eclosion temperature manipulation, all flies were kept on the standard cornmeal diet but split evenly between two post-eclosion temperatures: 23 °C or 25 °C. Flies were given 72 h to mature in their allocated environment.

After 72 h, approximately six replicate males from each isoline/environment combination were used to measure male attractiveness. We assessed male attractiveness in assays between a focal (isoline) male and a standard (outbred population) female, where we made courtship observations and measured copulation latency (the time elapsed between placing a male and female together and the start of copulation) within a 3-h period. In *Drosophila*, females have control over acceptance or rejection of courting males (Speith, 1974; Markow, 1996), and so preferred, attractive males are able to achieve copulation more rapidly. Indeed, many previous studies have used copulation latency as a metric to assess overall female preference and male attractiveness (e.g. Speith, 1974; Kyriacou & Hall, 1986; Barth *et al.*, 1997; Ritchie *et al.*, 1999; Acebes *et al.*, 2003; Shackleton *et al.*, 2005; Taylor *et al.*, 2007; Hosken *et al.*, 2008; Narraway *et al.*, 2010; Sharma *et al.*, 2010), and copulation latency in *D. simulans* is determined by both male (attractiveness) and female (preference) effects

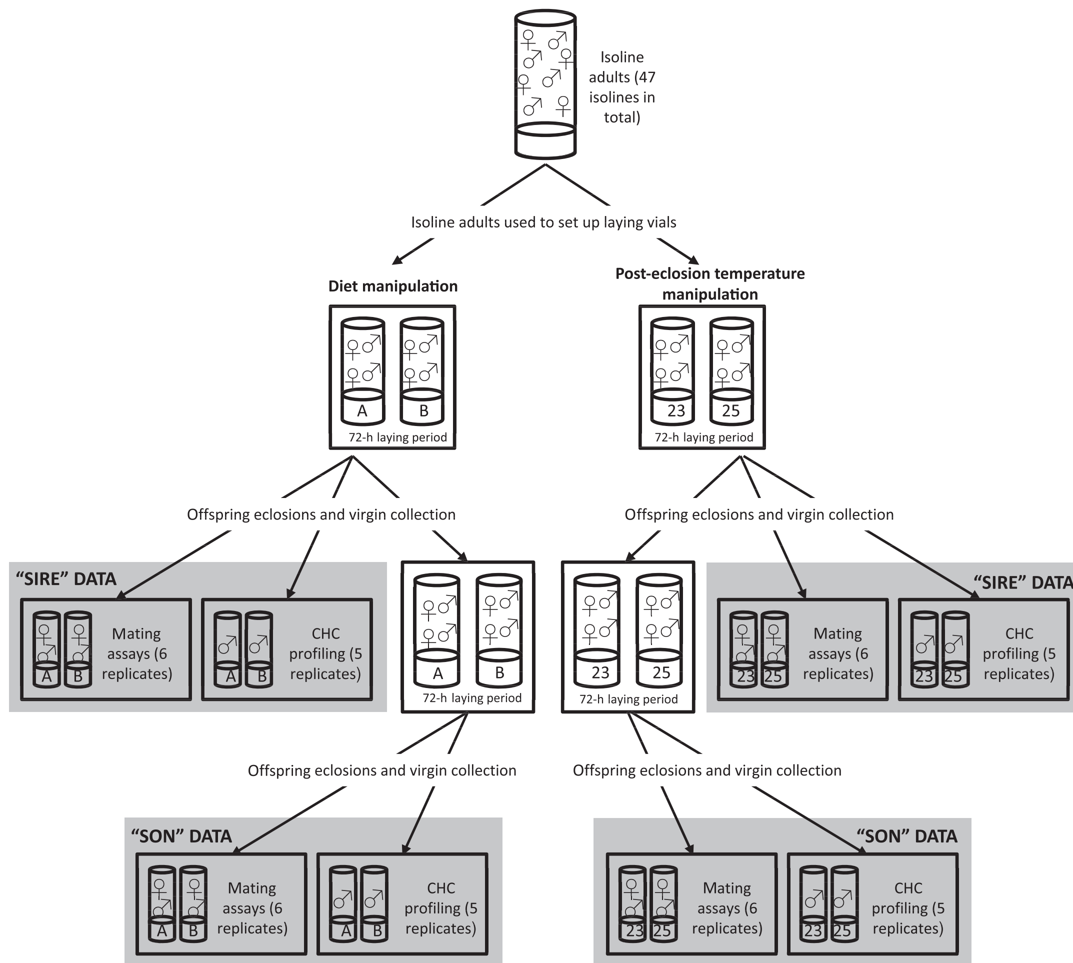

Fig. 1 Experimental design, showing the setup for a single isoline. Vials marked 'A' and 'B' denote flies which were reared on oat-based and soy-based diets, respectively. Vials marked '23' and '25' denote flies which were kept in 23 °C and 25 °C post-eclosion temperatures, respectively. Adult flies from each isoline were set up in laying vials as shown (with two replicate vials of each isoline/environment combination) and allowed a 72-h laying period. During this period, vials of the standard diet were also set up for laying in the outbred population. Offspring development took 11 days (until peak eclosions) at 25 °C. Virgin collection was carried out as described in text. Offspring were used in three ways. (1) Six replicate males from each isoline/environment combination were used in mating assays with outbred females, to assess male attractiveness (detail in text). (2) Five replicate males from each isoline/environment combination were used for CHC profiling. Mating assays and CHC analysis in this generation provided 'sire' data. (3) Isoline males and females from each environment were combined in fresh laying vials for 72 h (with two replicate vials of each isoline/environment combination as before). Again, laying vials were placed in the stock population during this period. Offspring development and virgin collection were carried out as in the previous generation. Mating assays and CHC profiling were carried out as before, providing 'son' data from this generation.

(Sharma *et al.*, 2010). Copulation latency is highly positively correlated with latency between first courtship and copulation (Taylor *et al.*, 2007), but is easier to accurately observe and record.

The results of these assays provided measures of 'sire' attractiveness. Five further replicate males from each isoline/environment combination were transferred into glass auto-sampler vials (supplied by Chromacol, UK) and frozen at −80 °C for storage prior to CHC profiling. This provided the 'sire' CHC data.

The remaining isoline males were combined with the isoline females (as above) to produce subsequent 'offspring', with two vials for each isoline/environment combination with two males and two females in each. Males and females from the same environments were housed together. As before, adults were given 72 h in which to mate and lay eggs before being removed, and again, large vials of standard cornmeal diet were set up in the outbred population during this 72-h period. All vials were then incubated at 25 °C during offspring development.

Peak eclosions for the 'offspring' generation occurred after 11 days, and virgin collection was carried out as described above. This time females were collected only

from the vials from the outbred population. These females were housed exactly as before and again used in attractiveness assays with isoline males. Males were collected from the isoline vials set-up in the previous generation. Male collection, housing and CHC and attractiveness measurements were carried out exactly as described for the sire generation. The CHC and attractiveness measurements in this second experimental generation provided 'son' data.

Cuticular hydrocarbon extractions

We used gas chromatography to analyse CHC profile of sires and sons ($N = 1820$). Hydrocarbon extractions were carried out in sets of 100 samples per day and randomized throughout by experiment, environment, isoline and generation. Hydrocarbon extraction involved soaking the fly in 50 μL of a solution of 10 ppm pentadecane in HPLC-grade hexane for 5 min, using a vortex for the duration of the final minute to agitate the solution and maximize extraction. The fly was then removed from the vial using forceps sterilized in hexane.

Two microlitre of each hydrocarbon sample was injected into a GC-FID (Agilent 7890) fitted with two injectors, and two DB-1 columns of 30 m × 0.25 mm internal diameter × 0.25 μm film thickness. We used hydrogen as a carrier gas. The inlet was set at 250 °C, and the injection was in pulsed splitless mode. Separation of the extract was optimized using a column profile, which began at 70 °C for 1 min and then increased at 20 °C per minute to 180 °C, then 4 °C per minute to 220 °C and finally 15 °C per minute to 320 °C, where it was held for 2 min. Column flow was set at 1.2 mL per minute. The FID detector heaters were set at 300 °C. The H_2 flow was 20 mL per minute, and the air flow was 200 mL per minute. Nitrogen was used to make up the column flow to 30 mL per minute. This protocol has been optimized previously for *D. simulans* (Sharma *et al.*, 2012a). Peak integration of hydrocarbon data was carried out using GC ChemStation software (version B.04.02.SP1).

Statistical analysis

Data handling

We used copulation latency to assess male attractiveness (see above). Only males that were observed courting were included in analyses. Of these males, individuals that did not mate during the 3-h assay ($N = 140$ in post-eclosion temperature experiment; $N = 133$ in diet experiment) were allocated a time of 3 h as a conservative estimate of copulation latency in terms of our experiment. Random variation between mating assays on different days was controlled for by standardizing copulation latency to the daily mean.

We measured the expression of 22 CHCs for each male. Prior to analysis, we calculated relative peak area by dividing each peak by the area of the internal standard peak (pentadecane) and then used a log transformation to normalize the data. We used principal components analysis (PCA) in SPSS (Chicago, IL, USA, v.19) to reduce the dimensionality of this data set. We used the correlation matrix of male CHC expression data from both the post-eclosion temperature and dietary manipulations together, so that we could extract the same principal components (PCs) of CHC expression across both manipulations. Multivariate outliers were identified by Mahalanobis distances and removed from the data set, leaving a slightly unbalanced experimental design, with a total of 1686 individuals across all isolines and environments used in the analysis. Five PCs with eigenvalues greater than 1 were extracted, which together explained approximately 82% of the total variation in CHC expression, and we interpret biological significance of these PCs from factor loadings exceeding 0.25 (Tabachnick & Fidell, 1989).

In another study and different data set, we had extracted 3 PCs from PCA and used these PCs in a selection analysis which demonstrated that all 3 PCs were subject to sexual selection (Ingleby *et al.* in prep). We tested the correlation between these three sexually selected PC vectors from the unpublished data set with PCs 1–3 extracted from the current study and found significant and strong correlations between each (PC1: $r = 0.521$, d.f. = 20, $P = 0.013$; PC2: $r = 0.636$, d.f. = 20, $P = 0.001$; and PC3: $r = 0.581$, d.f. = 20, $P = 0.004$). PCs 1–3 for both data sets therefore align well and as a result PCs 1–3 in the current study describe vectors of CHC expression, which are sexually selected (although it is unclear if PCs 4 and 5 are subject to sexual selection). To further verify this, we projected the CHC data set from the current study into the 3-dimensional multivariate space described by the 3 PCs from the unpublished data set, thereby obtaining PC scores for the individuals used in the current experiment along the exact same PC vectors that were shown to be sexually selected (the unpublished dataset). We repeated all subsequent analyses with both sets of PCs (the 5 PCs extracted from PCA in this dataset and the 3 PCs calculated by projecting the current data into the unpublished PCA space) and found that the results are nearly identical for both, further supporting our view that PCs 1–3 in both manuscripts are closely aligned. See Tables S1 and S2 and Figures S1 and S2 for the results of the analyses with the projected PCs. Here, we present the analyses with the 5 PCs extracted from PCA with the current data set, as these PCs will more fully represent the variation in CHC expression measured in the current experiment.

Environmental and G × E effects

Further analyses were carried out separately for data from the dietary and post-eclosion temperature manipulations, although analysis for each experiment was

equivalent. For each experiment, we tested for G × Es in sire and son attractiveness using a GLMM, assuming a normal distribution because the standardized copulation latency data were normally distributed. We tested for G × Es in sire and son CHC expression using a mixed-model MANOVA (Weerahandi, 2004) implemented in the PROC GLM procedure of SAS version 9.2, SAS Institute Inc., Cary, NC, USA. The five PCs describing CHC expression were included as the response variables (each PC was normally distributed). In each model, we specified generation, environment (either temperature or diet) and the generation × environment interaction as fixed effects, and isoline and all interactions including isoline (isoline × generation, isoline × environment and isoline × generation × environment) as random effects. We used the appropriate error structure and degrees of freedom for each term in the mixed-model MANOVA following the protocol outlined Appendix 1 of Zar (1999, page 474, Table A.3). We used univariate GLMMs with the same error structure to determine how each PC contributed to the overall significant multivariate effects detected in the mixed-model MANOVA. We interpreted both isoline × environment and isoline × environment × generation interactions as indicative of a G × E, although this effect might be inconsistent between generations in the case of an isoline × environment × generation interaction. The generation term effectively acts as a blocking term because it was impossible to assay sires and sons simultaneously.

Heritability of attractiveness

For these analyses, the mean standardized sire and son copulation latency for each isoline in each environment were used as estimates of male attractiveness.

As genetic benefits of female mate choice have previously been identified through heritability of male attractiveness in *D. simulans* (Taylor *et al.*, 2007), we tested heritability of male attractiveness across environments to assess genetic benefits of preference in this study. Heritability was estimated as double the regression coefficient (Falconer, 1989) from a linear regression (in R, Vienna, Austria, v.2.13.0) of sire and son isoline means. We did this for each of the four possible sire/son environmental combinations (henceforth referred to as 'treatments') in each manipulation. The four treatments were as follows for the post-eclosion temperature manipulation: (1) sire and son both in 23 °C; (2) sire and son both in 25 °C; (3) sire in 23 °C and son in 25 °C; and (4) sire in 25 °C and son in 23 °C; and for the dietary manipulation: (1) sire and son both on diet A; (2) sire and son both on diet B; (3) sire on diet A and son on diet B; and (4) sire on diet B and son on diet A. Regression of sire and son between different environments meant re-analysis of the same data used in the regression of sire and son within the same environment, and so all *P* values for heritability are Bonferroni-adjusted to correct for two tests. These heritability estimates represent broad-sense heritability (H^2, the proportion of total phenotypic variance, V_P, explained by the genetic variance, V_G) because we were using isolines (David *et al.*, 2005). Calculating heritability from inbred lines is likely to inflate heritability estimates (David *et al.*, 2005), but our aim here is not to quantify the absolute magnitude of heritability (which has been done previously in *D. simulans*, see Taylor *et al.*, 2007), but to make comparisons of heritability across environments.

Absolute difference between sire and son attractiveness and CHC expression

These analyses used the mean attractiveness scores as calculated above. Similarly, we calculated mean sire and son PC score (for each of the five PCs of CHC expression) for each isoline in each environment.

For each isoline, we estimated the absolute difference between sires and sons attractiveness and PC scores by calculating the difference between mean sire and son scores within each of the four treatments and representing this as an absolute value (= |x|). We used this measure to show how similar sire and son CHC expression was in each of the environmental treatments. This measure of absolute difference could be more informative than heritability estimates when the phenotypic mean is different between generations. The absolute differences were log-transformed to conform to a normal distribution and then a separate GLMM (in R v.2.13.0) for attractiveness, and for each PC was used to test for variation in these absolute differences between the four treatments, with treatment as a fixed effect and isoline as a random effect. Again, this involved using the data set once within environments and again between environments, and so the *P* values associated with these tests are also Bonferroni-adjusted to correct for two tests.

Results

Principal component analysis

We extracted five PCs with eigenvalues greater than 1 from the results of PCA (Table 1). All 22 peaks had positive loadings for PC1 (although the loading for octadecadiene was less than 0.25), and so this PC appears to represent the overall quantity of CHCs produced. We interpret both PC2 and PC4 as a trade-off between the production of long- and short-chained CHCs. PC2 is weighted in favour of long-chained CHCs, with positive loadings greater than 0.25 for several long-chained CHCs (pentacosane, hexacosane, heptacosane and some longer alkanes and branched alkanes), and negative loadings less than 0.25 for short-chained CHCs such as docosene and tricosene. PC4 is weighted in favour of shorter CHCs, although only 5 of the 22 peaks have loadings over 0.25 for PC4. Short-chained CHCs

Table 1 Principal component analysis for CHC expression in both post-eclosion temperature and dietary manipulations. Five principal components with eigenvalues greater than 1 were extracted, explaining 82% of the total variation in CHC expression. Biological significance of each component was interpreted from factor loadings exceeding 0.25 (in bold). CHCs are named where known; unnamed CHCs (asterisks) are described by basic chemical structure. CHCs are listed in order of increasing chain length.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	11.100	3.431	1.463	1.180	1.041
% variance	50.453	15.597	6.650	5.365	4.733
Loadings					
Octadecadiene	0.211	−0.180	**0.255**	**0.696**	**0.252**
Docosene	**0.611**	**−0.294**	0.233	**0.441**	0.031
Docosane	**0.762**	−0.218	**−0.527**	0.158	−0.076
Branched alkane*	**0.825**	**−0.329**	0.089	−0.077	−0.177
7-Tricosene	**0.886**	**−0.294**	0.134	0.084	−0.092
Tricosene	**0.715**	−0.225	**0.351**	0.061	0.034
Tricosane	**0.844**	−0.189	−0.195	0.231	0.025
Branched alkane*	**0.851**	−0.121	**−0.256**	−0.014	−0.208
Branched alkane*	**0.859**	−0.038	**0.352**	0.002	−0.155
Branched alkane*	**0.809**	−0.170	−0.240	−0.060	−0.192
Tetracosane	**0.834**	0.158	**0.437**	0.054	−0.107
Pentacosadiene	**0.575**	**−0.561**	−0.195	**−0.322**	0.172
Alkene*	**0.801**	0.231	**0.268**	−0.238	−0.176
Pentacosene	**0.849**	0.126	**0.363**	−0.120	−0.178
Pentacosane	**0.809**	**0.345**	−0.012	0.059	0.097
Branched alkane*	**0.646**	**−0.469**	0.037	**−0.264**	**0.403**
Hexacosane	**0.637**	**0.646**	−0.249	0.064	−0.057
Heptacosane	**0.664**	**0.330**	−0.008	−0.207	**0.481**
Branched alkane*	**0.534**	**0.471**	**0.369**	**−0.252**	−0.105
Alkane*	**0.544**	**0.763**	−0.074	0.187	0.006
Alkane*	**0.467**	**0.581**	−0.025	−0.020	**0.470**
Alkane*	**0.435**	**0.716**	−0.028	−0.075	**0.289**

(octadecadiene and docosene) are highly positively loaded, and long-chained CHCs (pentacosadiene and two branched alkanes) are negatively loaded. PC3 and PC5 are positively loaded for different long-chained CHCs (including pentacosene and a branched alkane in PC3, and heptacosane and some long-chained and branched alkanes in PC5), whereas loadings for short-chained hydrocarbons are mixed (PC3 is positively loaded for octadecadiene and heavily negatively loaded for docosane, and PC5 is positively loaded for octadecadiene). These vectors describe investment in long-chained CHCs with some trade-offs between other specific CHCs.

Attractiveness and CHC expression across post-eclosion temperatures

There were no environmental or G × E effects on overall male attractiveness across temperature (Table 2), but there was a genetic (isoline) component (Table 2). Consistent with this, the heritability of overall attractiveness

Table 2 Results from separate GLMMs for temperature and diet manipulations with male attractiveness as the response variable; environment (temp or diet), generation (gen) and environment × generation interaction as fixed effects; and isoline, isoline × generation, isoline × environment and isoline × environment × generation as random effects. Significance is highlighted in bold.

	F	d.f.	*P*
Temperature			
Isoline	**3.709**	**46,18**	**<0.001**
Temperature	0.003	1,46	0.955
Generation	0.052	1,47	0.821
Isoline × Gen	1.194	46,46	0.183
Isoline × Temp	0.946	46,46	0.576
Temp × Gen	0.950	1,47	0.330
Isoline × Temp × Gen	1.051	46,809	0.385
Diet			
Isoline	**2.810**	**43,19**	**< 0.001**
Diet	1.111	1,43	0.292
Generation	0.227	1,44	0.634
Isoline × Gen	0.534	43,43	0.994
Isoline × Diet	0.811	43,43	0.802
Diet × Gen	0.108	1,44	0.743
Isoline × Diet × Gen	0.785	43,867	0.838

was significant and repeatable across all temperature treatments (Table 3). This was also reflected in the absolute difference between sire and son attractiveness scores, which did not vary significantly across treatments (Fig. 2). Sire attractiveness is clearly a reliable predictor of son attractiveness across these temperatures.

Across post-eclosion temperatures, significant isoline and temperature effects revealed genetic and environmental components of male CHC expression (Table 4). We also found evidence for G × E across temperatures from isoline × temperature and isoline × temperature × generation interactions in overall CHC expression (Table 4). Examination of these effects for individual PCs shows that the isoline × temperature interaction is not significant for any PC individually (Table 4). The significant isoline × temperature × generation interaction for PC3 (Table 4), however, shows that there is a G × E, but that this effect is not consistent between generations. There is a strong effect of temperature on PC2, and to a slightly lesser extent on PC3 and PC4 (Table 4). The effect of temperature and isoline × temperature interaction on each PC is shown in the reactions norms in Fig. 3.

The absolute difference between sire and son PC2 ($F_{3,47} = 37.818$; $P < 0.001$; Fig. 2) and PC3 ($F_{3,47} = 11.072$; $P = 0.022$; Fig. 2) scores varied significantly between treatments. Whilst both these PCs had a significant temperature component, only PC3 had a significant G × E component (Table 4).

Table 3 Broad-sense heritability estimates (H^2 [SE]) of male attractiveness for each sire/son environment combination. All heritabilities are significant ($P < 0.05$) after Bonferroni correction.

Sire/son environment	H^2 (SE)
Temperature	
23/23	0.774 (0.128)
23/25	0.652 (0.147)
25/23	0.744 (0.115)
25/25	0.608 (0.135)
Diet	
A/A	0.944 (0.103)
A/B	0.832 (0.108)
B/A	0.686 (0.131)
B/B	0.602 (0.133)

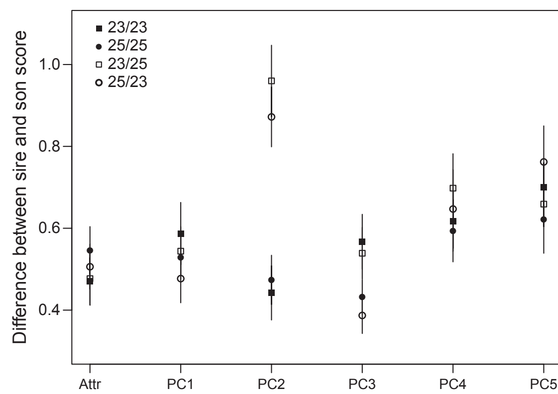

Fig. 2 Mean absolute difference (±SE) between isoline sire and son attractiveness (as standardised copulation latency) and PC scores (PC1–5) in each post-eclosion temperature treatment (see key for sire/son environment). Filled points represent constant environment treatments and open points represent changing environment treatments. Variation between treatments is significant in PC2 and PC3 (asterisks).

CHC expression and attractiveness across diets

Neither diet nor isoline × diet interaction had any significant effect on overall male attractiveness (Table 2), and attractiveness was largely determined by isoline (Table 2). Again, heritability of overall attractiveness was significant and consistent across diets (Table 3), and the absolute difference between sire and son attractiveness scores did not vary across diet treatments (Fig. 4). Sire attractiveness therefore reliably predicts son attractiveness across the diets studied here.

Male CHC expression across diets had a significant genetic (isoline) and dietary component (Table 5). Evidence for G × Es across diets is shown in the significant isoline × diet and isoline × diet × generation interactions in Table 5. There are G × Es for each PC individually: the isoline × diet interaction is significant for PC2 (Table 5), and the isoline × diet × generation

Table 4 Results from a mixed-model MANOVA with PCs 1–5 of CHC expression as response variables; temperature, generation and temperature × generation interaction as fixed effects; and isoline, isoline × temperature, isoline × generation and isoline × temperature × generation as random effects. Results of individual univariate GLMMs for each PC are given in the second section of the table. Significance is highlighted in bold. Error structure was specified following Zar (1999).

	Wilks' λ	F	d.f.	P
Overall MANOVA				
Generation	**0.678**	**3.99**	**5,42**	**0.005**
Temperature	**0.188**	**36.38**	**5,42**	**< 0.001**
Isoline	**0.228**	**4.65**	**230,3096**	**< 0.001**
Gen × Temp	**0.740**	**2.95**	**5,42**	**0.023**
Gen × Isoline	**0.583**	**1.54**	**230,3096**	**< 0.001**
Temp × Isoline	**0.646**	**1.23**	**230,3096**	**0.011**
Gen × Temp × Isoline	**0.635**	**1.29**	**230,3096**	**0.003**

	F	d.f.	P
Univariate GLMMs			
PC1			
Generation	0.57	1,47	0.454
Temperature	0.19	1,48	0.664
Isoline	**3.60**	**46,10**	**0.017**
Gen × Temp	0.32	1,48	0.572
Gen × Isoline	1.09	46,46	0.389
Temp × Isoline	0.67	46,46	0.912
Gen × Temp × Isoline	1.11	46,625	0.290
PC2			
Generation	0.000	1,48	0.964
Temperature	**132.78**	**1,47**	**< 0.001**
Isoline	**3.42**	**46,22**	**0.001**
Gen × Temp	**9.40**	**1,48**	**0.004**
Gen × Isoline	0.97	46,46	0.535
Temp × Isoline	1.40	46,46	0.131
Gen × Temp × Isoline	1.12	46,625	0.279
PC3			
Generation	**11.52**	**1,47**	**0.001**
Temperature	**7.03**	**1,48**	**0.011**
Isoline	**6.39**	**46,10**	**0.002**
Gen × Temp	0.78	1,47	0.381
Gen × Isoline	1.10	46,46	0.373
Temp × Isoline	0.67	46,46	0.913
Gen × Temp × Isoline	**1.74**	**46,625**	**0.002**
PC4			
Generation	0.43	1,47	0.517
Temperature	**48.47**	**1,47**	**< 0.001**
Isoline	1.77	46,21	0.079
Gen × Temp	1.71	1,47	0.197
Gen × Isoline	1.42	46,46	0.121
Temp × Isoline	0.91	46,46	0.626
Gen × Temp × Isoline	1.30	46,625	0.094
PC5			
Generation	**5.03**	**1,47**	**0.030**
Temperature	0.02	1,47	0.891
Isoline	1.51	46,34	0.107
Gen × Temp	0.99	1,48	0.325

Table 4 (*Continued*)

	F	d.f.	*P*
Gen × Isoline	**1.66**	**46,46**	**0.045**
Temp × Isoline	1.37	46,46	0.145
Gen × Temp × Isoline	1.14	46,625	0.252

interaction is significant for each PC except PC2, where it is marginally nonsignificant (Table 5). Overall dietary components of CHC expression are significant for PC1, PC3 and PC5 (Table 5). Reaction norms of CHC expression across diets are shown for each PC in Fig. 5.

The absolute difference between sire and son PC scores varies significantly between diet treatments for PC1 ($F_{3,44}$ = 20.458; P < 0.001; Fig. 4), PC3 ($F_{3,44}$ = 12.588; P = 0.012; Fig. 4) and PC5 ($F_{3,44}$ = 11.712; P = 0.016; Fig. 4), therefore showing variation only in PCs which have both E and G × E components (Table 5).

Discussion

Theory predicts that sexual signal reliability can be compromised by environmental heterogeneity and G × Es in sexual trait expression (Higginson & Reader, 2009). In *D. simulans*, female mating preferences are likely to evolve via genetic benefits through heritable male attractiveness (Taylor *et al.*, 2007; Hosken *et al.*, 2008), and CHCs are an important heritable component of male attractiveness in this species (Sharma *et al.*, 2012a). Here, we have found G × Es and strong environmental components to male CHC expression, even across the relatively narrow and un-stressful range of environmental variation studied here. PCs 1–3 are sexually selected vectors of CHC expression, and the evidence that differences in sire and son scores for PCs 1 and 3 vary across diets and PCs 2 and 3 vary across temperatures suggests that these aspects of CHC profile are unreliable indicators of male genetic quality across some of the environments studied here. In the light of this, we would also expect heritability of male attractiveness to vary across environments, but our results clearly demonstrate that heritability of male attractiveness is maintained consistently across all environments we examined. As total male attractiveness is heritable across environments, this shows that females can gain genetic benefits of mate choice across environments, and so sexual selection for genetic benefits can operate across the environments studied here, in spite of the evidence for unreliable male CHC signals.

These results might seem surprising given the evidence from a number of studies that CHCs contribute significantly to male attractiveness in many *Drosophila* species (e.g. Cobb & Ferveur, 1995; Blows, 2002; Wicker-Thomas, 2007; Ferveur & Cobb, 2010; Sharma *et al.*, 2012a). However, our measure of male attractiveness represents total attractiveness, and whilst CHCs influence mate choice, CHC profile is not the only determinant of total attractiveness. Indeed, we only find evidence for signal unreliability in certain sexually selected aspects of CHC profile here and not others, and so total attractiveness might be maintained across environments as a result of reliable aspects of CHC signals and by other sexual traits. In addition to CHCs, a number of traits are known to influence mating decisions in *Drosophila*, including body size (Partridge *et al.*, 1987), courtship behaviour (Hall, 1994) and sex comb structure (Markow, 1996). Multiple signals are thought to contain more information than a single trait and could compensate for any signal unreliability of particular sexual cues and environmental variation in signal expression (Candolin, 2003). For instance, if females assess males based on multiple sexual traits, then we might expect that even if one particular signal is an unreliable indicator of mate quality across some environments, other signals could be reliable. In this way, females will still be able to gauge overall male quality, and the overall outcome of female mate choice will be unaffected by unreliability of any particular sexual signal, as we find here. In fact, it is likely that selection will favour female preferences that focus on reliable aspects of male sexual signals. Preferences for signals

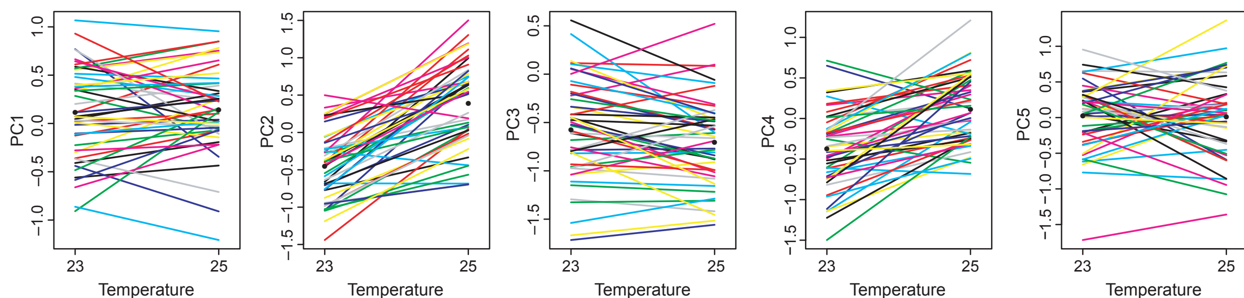

Fig. 3 Reaction norms for PCs 1–5 (left-right) of male CHC expression across post-eclosion temperatures. Each line represents an isoline. Points represent overall mean PC score across all isolines within each temperature.

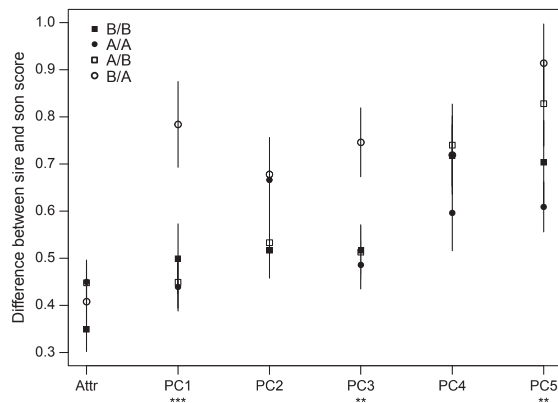

Fig. 4 Mean absolute difference (±SE) between isoline sire and son attractiveness (as standardised copulation latency) and PC scores (PC1–5) across dietary treatments (see key for sire/son environment). Filled points represent constant environment treatments and open points represent changing environment treatments. Variation between treatments is significant in PC1, PC3 and PC5 (asterisks).

that have G × E or strong environmental components may be selected against due to signal unreliability across heterogeneous environments, and these signals might evolve to be less important in mating decisions.

Male CHC expression across diets

In our dietary manipulation, we found variation in the absolute difference between sire and son PC1, PC3 and PC5 scores between diets. Overall, it appears that PC1 and PC3 represent CHC vectors that are unreliable sexual signals across diets, because we have reason to believe these PCs are under sexual selection. The other sexually selected vector, PC2, appears to be a more reliable aspect of CHC profile, because the absolute difference between sire and son PC2 scores was relatively consistent across treatments. Each sexually selected PC had a significant G × E component (isoline × diet in the case of PC2; isoline × diet × generation for PC1 and PC3), yet only PC1 and PC3 show evidence of signal unreliability across diets. It is possible that the G × E for PC2 does not involve changes in V_G across environments, and so it is less likely that the similarity between sire and son along this vector will be disrupted across environments. Alternatively, the consistency of sire and son similarity along PC2 across environments could reflect the lack of significant overall effect of diet on this vector. PC1 and PC3 each had significant diet and G × E components, suggesting that signal unreliability might be due to a combination of environmental and G × E effects.

PC1 described a vector representing variation in the overall quantity of CHCs produced, and PC3 appeared to describe investment in long-chained CHCs, with

Table 5 Results from a mixed-model MANOVA with PCs 1–5 of CHC expression as response variables; diet, generation and diet × generation interaction as fixed effects; and isoline, isoline × diet, isoline × generation and isoline × diet × generation as random effects. Results of individual univariate GLMMs for each PC are given in the second section of the table. Significance is highlighted in bold. Error structure was specified following Zar (1999).

	Wilks' λ	*F*	d.f.	*P*
Overall MANOVA				
Generation	**0.351**	**14.45**	**5,39**	**< 0.001**
Diet	**0.096**	**73.25**	**5,39**	**< 0.001**
Isoline	**0.166**	**6.97**	**215,3452**	**< 0.001**
Gen × Diet	**0.433**	**10.21**	**5,39**	**< 0.001**
Gen × Isoline	**0.572**	**1.91**	**215,3452**	**< 0.001**
Diet × Isoline	**0.484**	**2.52**	**215,3452**	**< 0.001**
Gen × Diet × Isoline	**0.548**	**2.07**	**215,3452**	**< 0.001**

	F	d.f.	*P*
Univariate GLMMs			
PC1			
Generation	**15.40**	**1,44**	**< 0.001**
Diet	**21.10**	**1,43**	**< 0.001**
Isoline	**3.55**	**43,13**	**0.008**
Gen × Diet	1.58	1,44	0.215
Gen × Isoline	0.56	43,43	0.969
Diet × Isoline	1.45	43,43	0.112
Gen × Diet × Isoline	**1.70**	**43,697**	**0.004**
PC2			
Generation	**6.82**	**1,44**	**0.012**
Diet	0.44	1,43	0.509
Isoline	**2.77**	**43,34**	**0.001**
Gen × Diet	**28.80**	**43,34**	**< 0.001**
Gen × Isoline	1.55	43,43	0.078
Diet × Isoline	**1.66**	**43,43**	**0.049**
Gen × Diet × Isoline	1.39	43,697	0.055
PC3			
Generation	1.57	1,44	0.218
Diet	**90.81**	**1,44**	**< 0.001**
Isoline	**6.10**	**43,4**	**0.049**
Gen × Diet	0.00	1,43	0.982
Gen × Isoline	0.74	43,43	0.838
Diet × Isoline	0.68	43,43	0.892
Gen × Diet × Isoline	**2.82**	**43,697**	**< 0.001**
PC4			
Generation	**28.43**	**1,44**	**< 0.001**
Diet	1.93	1,43	0.172
Isoline	**2.08**	**43,18**	**0.046**
Gen × Diet	3.79	1,43	0.058
Gen × Isoline	0.69	43,43	0.886
Diet × Isoline	1.64	43,43	0.055
Gen × Diet × Isoline	**2.31**	**43,697**	**< 0.001**
PC5			
Generation	0.67	1,43	0.419
Diet	**105.39**	**1,44**	**< 0.001**
Isoline	**1.98**	**43,25**	**0.036**
Gen × Diet	**18.16**	**1,44**	**< 0.001**
Gen × Isoline	**1.69**	**43,43**	**0.045**
Diet × Isoline	1.00	43,43	0.506
Gen × Diet × Isoline	**2.04**	**43,697**	**< 0.001**

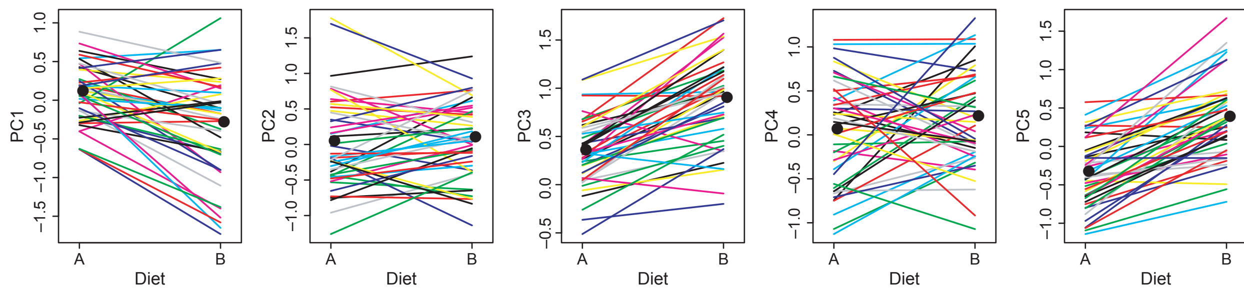

Fig. 5 Reaction norms for PCs 1–5 (left-right) of male CHC expression across diets. Each line represents an isoline. Points represent overall mean PC score across all isolines within each diet.

some specific trade-offs between other CHCs. The effect of diet on these vectors is consistent with the large body of research on condition dependence of sexual traits (Rowe & Houle, 1996) and the evidence that CHCs are costly to produce (Blows, 2002; Ferveur, 2005). Indeed, there is evidence from *D. serrata* of condition dependent expression of male CHCs, and this was revealed by a diet manipulation (Gosden & Chenoweth, 2011). There was no evidence of G × E in CHC expression across diets in *D. serrata* (Gosden & Chenoweth, 2011), but we find G × E across diets in *D. simulans*. It appears that this G × E contributes to the unreliability of some sexually selected CHC components across diet manipulation in the present study.

Male CHC expression across post-eclosion temperatures

In the post-eclosion temperature manipulation, the absolute difference between sire and son PC2 and PC3 scores varied between temperatures. If sire and son scores for these vectors are very different from one another, it suggests that these vectors of sire CHC profile are not good predictors of the corresponding son PC score, and so the CHC components described by these vectors are likely to be unreliable as sexual signals. There were both significant G × E and environmental components to PC3, and both are likely to contribute to signal unreliability. However, there was no evidence of any G × E effects for PC2, and so the extremely strong environmental component of expression could account for signal unreliability in this instance.

We interpreted the vector described by PC2 as a trade-off between long- and short-chained CHCs and PC3 as investment in long-chained CHCs with some trade-offs between other specific CHCs. Such trade-offs could be mediated through shared metabolic pathways for CHC expression (Ferveur, 2005), costs of CHC expression (Blows, 2002; Ferveur, 2005) or environmental effects. Short-chained CHCs are thought to function mainly as sexual pheromones, whereas long-chained CHCs are likely to have a protective role in preventing water loss through the cuticle (Ferveur, 2005; Wicker-Thomas, 2007). The strong influence of temperature on relative investment in long- and short-chained CHCs has been shown before, not only in our study system (Ingleby *et al.*, 2012), but also in *D. melanogaster* (Savarit & Ferveur, 2002; Foley & Telonis-Scott, 2011), *D. mojavensis* (Gibbs *et al.*, 1998) and *D. serrata* (Frentiu & Chenoweth, 2010). As such, it is likely that the trade-off described by PC2 will be under strong temperature-dependent selection and furthermore that a raised temperature could be stressful to the flies in terms of this trade-off in CHC investment. This would provide a mechanism through which environmental variation, even without G × Es, could disrupt the similarity of sire and son PC2 scores between temperatures (Hoffmann & Merilä, 1999).

Sexual signal reliability

We have shown that in *D. simulans*, a combination of G × E and environmental effects cause elements of CHC profiles to be unreliable sexual signals across diets and temperatures, providing valuable support for model predictions which have suggested that G × Es in sexual signals can cause signal unreliability across environments (Higginson & Reader, 2009). The role of G × Es in this process was less obvious across temperatures than across diets, however, and overall our results suggest that whilst G × Es may be important, environmental heterogeneity by itself could also lead to signal unreliability through changes in the genetic variance between environments.

Either way, it is clear from our results that some aspects of CHC profile cannot act as reliable indicators of male quality across different diets and temperatures. In spite of this, we show that the heritability of attractiveness across the same range of environmental variation is maintained. A recent study in the field cricket, *Gryllus lineaticeps*, found a similar result (Tolle & Wagner, 2010). In this species, males provide direct benefits to the females through seminal fluid substances that increase female fecundity. It was found that in spite of G × Es

rendering male acoustic signals unreliable across different diets, the quality of the direct benefits provided to females varied only with male genotype, with no G × E. Our results describe a similar phenomenon, but in a system where females receive only genetic benefits of mate choice. In addition, we demonstrate that whilst G × Es can disrupt signal reliability, it is not always necessary to invoke G × Es to explain unreliable signals, as environmental heterogeneity can disrupt signal reliability even without G × Es in signal expression.

Nonetheless, in both the cricket study and our own, sexual signal unreliability did not alter the overall outcome of female choice, such that females received benefits of mate choice in spite of unreliable sexual signals. It is therefore likely that multiple sexual signals, and possibly selection for females to pay less attention to more unreliable aspects of male attractiveness, could attribute for these results, as discussed above. However, it is also possible that genetic, environmental and G × E effects on female preference enable female mating decisions to track the environmental differences in male sexual signal expression and reliability. In this way, adaptive plasticity in female preferences might account for the heritability of male attractiveness across different environments. Environmental variation in female preferences has been found across a number of species, and some studies have shown genetic variation underlying female preferences (reviewed by Jennions & Petrie, 1997), but very little is known about G × E effects on mate preference (although see Rodríguez & Greenfield, 2003; Narraway *et al.*, 2010). Our study randomized these effects across treatments using females from a standard environmental and genetic background. However, further investigation of genetic variation in female preference across environments will be useful. This is because the genetic covariance between male signal and female preference is central to many models of sexual selection (Kirkpatrick & Ryan, 1991; Kokko *et al.*, 2006), but little is known about how environmental variation could influence this.

Across the diet and post-eclosion temperature variation studied here, we show that G × Es and environmental heterogeneity can disrupt the reliability of male CHC profiles to function as a signal of heritable aspects of male quality in *D. simulans*. However, we show that the heritability of total male attractiveness is maintained in spite of unreliable sexual signal components. Thus, we can see that mate choice for genetic benefits could operate in an ecological context with environmental variation. However, it is unknown whether this is mediated by multiple male sexual signals, or variation in female preferences, or if both signals and preference variation facilitate the accumulation of indirect benefits of mate choice. Further work should consider how female preferences might vary across environments and with G × Es, and also the consequences of unreliable sexual signals and environmental heterogeneity on the genetic covariance between male sexual traits and female mating preferences.

Acknowledgments

This work was funded by the ESF and NERC (DJH and JH), and a Royal Society Fellowship and Equipment grant (JH). The authors are grateful to Chris Mitchell for help with gas chromatography and Natasa Fytrou for collecting the flies used in this study. Luc Bussière and two anonymous reviewers provided helpful comments on the manuscript.

References

Acebes, A., Cobb, M. & Ferveur, J-F. 2003. Species-specific effects of single sensillum ablation on mating position in *Drosophila*. *J. Exp. Biol.* **206**:3095–3100.

Andersson, M. 1994. *Sexual Selection*. Princeton University Press, New Jersey, USA.

Barth, M., Hirsch, H.V.B. & Heisenberg, M. 1997. Rearing in different light regimes affects courtship behaviour in *Drosophila melanogaster*. *Anim. Behav.* **53**: 25–38.

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

Candolin, U. 2003. The use of multiple cues in mate choice. *Biol. Rev.* **78**: 575–595.

Chenoweth, S.F. & Blows, M.W. 2005. Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. *Am. Nat.* **165**: 281–289.

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

Danielson-François, A.M., Zhou, Y. & Greenfield, M.D. 2009. Indirect genetic effects and the lek paradox: inter-genotypic competition may strengthen genotype × environment interactions and conserve genetic variance. *Genetica* **136**: 27–36.

David, J.R., Gilbert, P., Legout, H., Pétavy, G., Capy, P. & Moreteau, B. 2005. Isofemale lines in *Drosophila*: an empirical approach to quantitative trait analysis in natural populations. *Heredity* **94**: 3–12.

Falconer, D.S. 1989. *Introduction to Quantitative Genetics*. Longman, London, UK.

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

Ferveur, J-F. & Cobb, M. 2010. Behavioral and evolutionary roles of cuticular hydrocarbons in Diptera. In: *Insect Hydrocarbons* (G.J. Blomquist & A.G. Bagnères, eds), pp. 325–343. Cambridge University Press, New York, USA.

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

Frentiu, F.D. & Chenoweth, S.F. 2010. Clines in cuticular hydrocarbons in two *Drosophila* species with independent population histories. *Evolution* **64**: 1784–1794.

Gibbs, A.G., Louie, A.K. & Ayala, J.A. 1998. Effects of temperature on cuticular lipids and water balance in a desert *Drosophila*: is thermal acclimation beneficial? *J. Exp. Biol.* **201**: 71–80.

Gosden, T.P. & Chenoweth, S.F. 2011. On the evolution of heightened condition dependence of male sexual displays. *J. Evol. Biol.* **24**: 685–692.
Greenfield, M.D. 1994. Cooperation and conflict in the evolution of signal interactions. *Annu. Rev. Ecology and Systematics* **25**: 97–126.
Greenfield, M.D. & Rodríguez, R.L. 2004. Genotype-environment interaction and the reliability of mating signals. *Anim. Behav.* **68**: 1461–1468.
Hall, J.C. 1994. The mating of a fly. *Science* **264**: 1702–1714.
Head, M.L., Hunt, J., Jennions, M.D. & Brooks, R. 2005. The indirect benefits of mating with attractive males outweigh the direct costs. *PLoS Biol.* **3**: e33.
Higginson, A.D. & Reader, T. 2009. Environmental heterogeneity, genotype-by-environment interactions and the reliability of sexual traits as indicators of male quality. *Proc. R. Soc. Lond. B* **276**: 1153–1159.
Hoffmann, A.A. & Merilä, J. 1999. Heritable variation and evolution under favourable and unfavourable conditions. *Trends Ecol. Evol.* **14**: 96–101.
Hosken, D.J., Taylor, M.L., Hoyle, K., Higgins, S. & Wedell, N. 2008. Attractive males have greater success in sperm competition. *Curr. Biol.* **18**: R553–R554.
Ingleby, F.C., Hunt, J. & Hosken, D.J. 2010. The role of genotype-by-environment interactions in sexual selection. *J. Evol. Biol.* **23**: 2031–2045.
Ingleby, F.C., Hosken, D.J., Flowers, K., Hawkes, M.F., Lane, S.M., Rapkin, J. *et al.* 2012. Genotype-by-environment interactions for cuticular hydrocarbon expression in *Drosophila simulans*. *J. Evol. Biol.*, doi: 10.1111/jeb.12030.
Jennions, M.D. & Petrie, M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biol. Rev.* **72**: 283–327.
Jia, F-Y., Greenfield, M.D. & Collins, R.D. 2000. Genetic variance of sexually selected traits in waxmoths: maintenance by genotype × environment interaction. *Evolution* **54**: 953–967.
Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. *Evolution* **36**: 1–12.
Kirkpatrick, M. & Ryan, M.J. 1991. The evolution of mating preferences and the paradox of the lek. *Nature* **350**: 33–38.
Kokko, H. & Heubel, K. 2008. Condition dependence, genotype-by-environment interactions and the lek paradox. *Genetica* **132**: 209–216.
Kokko, H., Jennions, M.D. & Brooks, R. 2006. Unifying and testing models of sexual selection. *Annu. Rev. Ecol. Evol. Syst.* **37**: 43–66.
Kyriacou, C.P. & Hall, J.C. 1986. Interspecific genetic control of courtship song production and reception in *Drosophila*. *Science* **232**: 494–497.
Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proc. Natl Acad. Science.* **78**: 3721–3725.
Lewis, S.M., Tigreros, N., Fedina, T. & Ming, Q.L. 2012. Genetic and nutritional effects on male traits and reproductive performance in *Tribolium* flour beetles. *J. Evol. Biol.* **25**: 438–451.
Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, MA.
Markow, T.A. 1996. Evolution of *Drosophila* mating systems. *Evol. Biol.* **29**: 73–106.
Maynard Smith, J. & Harper, D. 2003. *Animal Signals*. Oxford University Press, Oxford, UK.
Mills, S. C., Alatano, R. V., Koskela, E., Mappes, J., Mappes, T. & Oksanen, T. A. 2007. Signal reliability compromised by genotype-by-environment interaction and potential mechanisms for its preservation. *Evolution* **61**: 1748–1757.
Møller, A.P. & Jennions, M.D. 2001. How important are direct fitness benefits of sexual selection? *Naturwissenschaften* **88**: 401–415.
Narraway, C., Hunt, J., Wedell, N. & Hosken, D.J. 2010. Genotype-by-environment interactions for female preference. *J. Evol. Biol.* **23**: 2550–2557.
Partridge, L., Hoffmann, A. & Jones, J.S. 1987. Male size and mating success in *Drosophila melanogaster* and *D. pseudoobscura* under field conditions. *Anim. Behav.* **35**: 468–476.
Qvarnström, A., Brommer, J.E. & Gustafsson, L. 2006. Testing the genetics underlying co-evolution of mate choice and ornament in the wild. *Nature* **441**: 84–86.
Ritchie, M.G., Halsey, E.J. & Gleason, J.M. 1999. *Drosophila* song as a species-specific mating signal and the behavioural importance of Kyriacou and Hall cycles in *D. melanogaster*. *Anim. Behav.* **58**: 649–657.
Rodríguez, R.L. & Greenfield, M.D. 2003. Genetic variance and phenotypic plasticity in a component of female mate choice in an ultrasonic moth. *Evolution* **57**: 1304–1313.
Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B* **263**: 1415–1421.
Rundle, H.D., Chenoweth, S.F. & Blows, M.W. 2009. The diversification of mate preferences by natural and sexual selection. *J. Evol. Biol.* **22**: 1608–1615.
Savarit, F. & Ferveur, J.F. 2002. Temperature affects the ontogeny of sexually dimorphic cuticular hydrocarbons in *Drosophila melanogaster*. *J. Exp. Biol.* **205**: 3241–3249.
Shackleton, M.A., Jennions, M.D. & Hunt, J. 2005. Fighting success and attractiveness as predictors of male mating success in the black field cricket, *Teleogryllus commodus*: the effectiveness of no-choice tests. *Behav. Ecol. Sociobiol.* **58**: 1–8.
Sharma, M.D., Tregenza, T. & Hosken, D.J. 2010. Female mate preferences in *Drosophila simulans*: evolution and costs. *J. Evol. Biol.* **23**: 1672–1679.
Sharma, M.D., Hunt, J. & Hosken, D.J. 2012a. Antagonistic responses to natural and sexual selection and the sex-specific evolution of cuticular hydrocarbons in *Drosophila simulans*. *Evolution* **66**: 665–677.
Sharma, M.D., Griffin, R.M., Hollis, J., Tregenza, T. & Hosken, D.J. 2012b. Reinvestigating good genes benefits of mate choice in *Drosophila simulans*. *Biol. Linn. Soc.* **103**: 923–934.
Speith, H. 1974. Courtship behaviour in *Drosophila*. *Annu. Rev. Entomol.* **19**: 385–405.
Tabachnick, B. & Fidell, L. 1989. *Using Multivariate Statistics*. Harper Collins, New York, USA.
Taylor, M.L., Wedell, N. & Hosken, D.J. 2007. The heritability of attractiveness. *Curr. Biol.* **17**: R959–R960.
Taylor, M.L., Wedell, N. & Hosken, D.J. 2008. Sexual selection and female fitness in *Drosophila simulans*. *Behav. Ecol. Sociobiol.* **62**: 721–728.
Taylor, M.L., Wedell, N. & Hosken, D.J. 2010. Attractive males do not sire superior daughters. *Evol. Ecol.* **24**: 195–205.
Tolle, A.E. & Wagner, W.E. 2010. Costly signals in a field cricket can indicate high- or low-quality direct benefits depending upon the environment. *Evolution* **65**: 283–294.

Wedell, N. & Tregenza, T. 1999. Successful fathers sire successful sons. *Evolution* **53**: 620–625.
Weerahandi, S. 2004. *Generalized Inference in Repeated Measures*. Wiley, New Jersey, USA.
Wicker-Thomas, C. 2007. Pheromonal communication involved in courtship behavior in Diptera. *J. Insect Physiol.* **53**: 1089–1100.
Zar, J.H. 1999. *Biostatistical Analysis* (Fourth Edition). Prentice-Hall, New Jersey, USA.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Results from a mixed model MANOVA with PCs 1–3 of CHC expression (projected PC scores) as response variables; post-eclosion temperature, generation and temperature × generation interaction as fixed effects; and isoline, isoline × temperature, isoline × generation and isoline × temperature × generation as random effects.

Table S2 Results from a mixed model MANOVA with PCs 1–3 of CHC expression (projected PC scores) as response variables; diet, generation and diet × generation interaction as fixed effects; and isoline, isoline × diet, isoline × generation and isoline × diet × generation as random effects.

Figure S1 Mean absolute difference (±SE) between isoline sire and son attractiveness (as standardised copulation latency) and PC scores (PC1–3) across temperatures (see key for sire/son temperature).

Figure S2 Mean absolute difference (±SE) between isoline sire and son attractiveness (as standardised copulation latency) and PC scores (PC1–3) across diets (see key for sire/son diet).

Data deposited at Dryad: doi:10.5061/dryad.18h00

Received 23 July 2012; accepted 10 October 2012