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Genotype-by-environment interactions for cuticular hydrocarbon expression in *Drosophila simulans*

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Abstract

Genotype-by-environment interactions (G × Es) describe genetic variation for phenotypic plasticity. Recent interest in the role of these interactions in sexual selection has identified G × Es across a diverse range of species and sexual traits. Additionally, theoretical work predicts that G × Es in sexual traits could help to maintain genetic variation, but could also disrupt the reliability of these traits as signals of mate quality. However, empirical tests of these theoretical predictions are scarce. We reared iso-female lines of *Drosophila simulans* across two axes of environmental variation (diet and temperature) in a fully factorial design and tested for G × Es in the expression of cuticular hydrocarbons (CHCs), a multivariate sexual trait in this species. We find sex-specific environmental, genetic and G × E effects on CHC expression, with G × Es for diet in both male and female CHC profile and a G × E for temperature in females. We also find some evidence for ecological crossover in these G × Es, and by quantifying variance components, genetic correlations and heritabilities, we show the potential for these G × Es to help maintain genetic variation and cause sexual signal unreliability in *D. simulans* CHC profiles.

Introduction

Genotype-by-environment interactions (G × Es) represent changes in the relative performance of different genotypes in alternate environments (Lynch & Walsh, 1998). G × Es are often interpreted as genetic variation for phenotypic plasticity, such that the direction and extent of plasticity in trait expression across environments differs between genotypes. These interactions have been extensively studied in agricultural research (Falconer, 1952; Kang & Gauch, 1996) and in evolutionary genetics (Via & Lande, 1985, 1987), but research on G × Es in the specific context of sexual selection has only more recently received research attention (see Greenfield & Rodríguez, 2004; Bussière *et al.*, 2008; Ingleby *et al.*, 2010).

Recent mathematical models have begun to explore the potential consequences of G × Es in sexual selection (Kokko & Heubel, 2008; Higginson & Reader, 2009). Broadly, the results of these models suggest that G × Es could help to maintain genetic variation in sexual traits (Kokko & Heubel, 2008); providing a solution to the lek paradox, which states that strong directional selection from female choice should deplete the genetic variance in male sexual traits which is necessary to maintain female choice for indirect benefits (Kirkpatrick & Ryan, 1991). This result is consistent with previous theory developed on G × Es in evolutionary genetics more generally (Via & Lande, 1987). In addition, a second prediction made from the models suggests that G × Es could disrupt the information content, and hence reliability, of sexual traits as signals or displays of mate quality (Higginson & Reader, 2009). The concept of signal reliability hinges upon a predictable relationship between phenotype and underlying genotype, and theory has demonstrated that G × Es for trait expression could weaken or completely remove any such relationship (Higginson & Reader, 2009). These theoretical outcomes highlight the influence that G × Es could have on the evolutionary dynamics of sexually selected traits. For example, mate choice based on some sexual

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signal can evolve through indirect benefits (offspring viability or attractiveness). This depends on there being genetic variation in sexual traits, as well as sexual signals being reliable indicators of the underlying genotype of a potential mate (Zahavi, 1975; Grafen, 1990). If G × Es in sexual trait expression cause signals to become unreliable, mating preferences will be costly and should be selected against. Understanding how sexual selection operates in nature therefore depends on understanding the role of G × Es in sexual selection and requires an empirical evaluation of existing theory.

To date, empirical work on G × Es in sexual selection has focussed on identifying whether or not there are G × Es for sexual trait expression. It is clear from this body of research that G × Es are widespread across a variety of sexual traits in a range of species [e.g. male eyespan in stalk-eyed flies (David *et al.*, 2000); male acoustic signalling (Danielson-François *et al.*, 2006) and female preference (Rodríguez & Greenfield, 2003) in waxmoths; dominance in male bank voles (Mills *et al.*, 2007); sperm length (Morrow *et al.*, 2008) and female preference in flies (Narraway *et al.*, 2010); male pigmentation in sticklebacks (Lewandowski & Boughman, 2008) and guppies (Grether, 2000); and genital morphology in treehoppers (Rodríguez & Al-Wathiqui, 2011)]. However, there are some inherent difficulties in relating such empirical work to current theory on G × Es in sexual selection. In particular, theory has defined 'strong' G × Es as ones which have ecological crossover of reaction norms and therefore involve a change in the ranked order of genotypes across environments, whereas 'weak' G × Es are those which do not have ecological crossover and represent only a change in the scale of genetic variation across environments (see Fig. 1). This distinction is explicit in both Kokko & Heubel's (2008) model, which assumes ecological crossover of G × E reaction norms, and Higginson & Reader's (2009) model, which tests interactions with no crossover. Although the classification of 'strong' and 'weak' G × Es is interesting and potentially useful, defining the strength of G × Es in this way makes interpretation in an empirical context difficult, as it is likely that the presence or absence of crossover in an empirical G × E will not be absolute. Rather, G × E variance will result from a combination of changes in the scale of variation across environments, as well as crossover. Therefore, whilst it is possible to test for significant ecological crossover of reaction norms in a particular experiment, interpreting this as the sole indicator of interaction strength could potentially be misleading (Ingleby *et al.*, 2010). Furthermore, with or without crossover, if a G × E only explains a small proportion of the overall phenotypic variation, it is unlikely to be very significant in evolutionary terms. Therefore, whilst the quantification of the degree of reaction norm crossover will be useful, estimation of variance components, cross-environment genetic correlations and trait heritabilities are also likely to be revealing. Both Falconer (1952) and Via & Lande (1985) noted that one trait expressed in multiple environments can be thought of as multiple genetically correlated traits, and in this way, the genetic correlation describes the extent to which the phenotypic expression of the trait in different environments has the same genetic basis. In other words, the stronger the genetic correlation across environments, the weaker the G × E effect on the expression of that trait (Falconer, 1952; Via & Lande, 1985). In the extreme, a genetic correlation of 1 indicates that trait values do not change across environments, and this strong correlation limits the potential for the trait to evolve independently under different conditions.

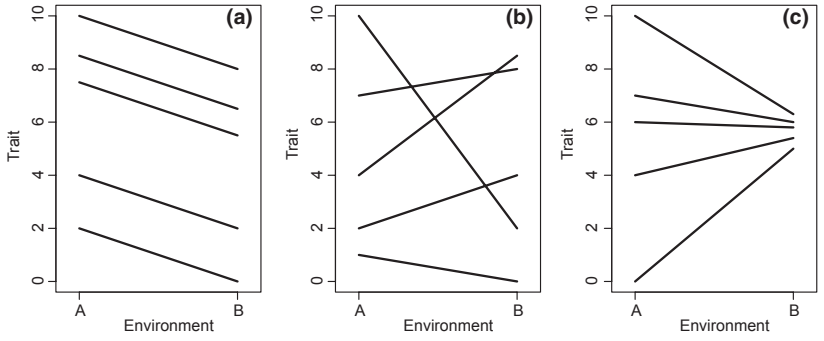

Fig. 1 Reaction norms showing some measure of trait expression (*y*-axis) across two hypothetical environments (*x*-axis). Each line represents a different genotype. (a) Phenotypic plasticity between environments but no G × E interaction, as each genotype responds to the environmental variation in the same way. (b) G × E interaction with ecological crossover of reaction norms. The direction and extent of phenotypic plasticity varies between genotypes and results in crossover in this example, where the ranked order of genotypes changes between environments. (c) G × E interaction without ecological crossover. Again, the direction and extent of phenotypic plasticity differs between genotypes, but here, reactions norms do not cross, and the main effect of the interaction is that the scale of genetic variation between genotypes changes between environments. 299 × 141 mm (72 × 72 DPI).

In this study, we test for G × Es in the expression of cuticular hydrocarbons (CHCs) in *Drosophila simulans*. Insect CHCs can function as chemical signals, and shorter-chained, more volatile CHCs are especially implicated in this (Ferveur, 2005). The role of CHCs in chemical signalling has been studied in several contexts. This includes signalling between conspecific males to assess levels of male–male competition in crickets (Thomas & Simmons, 2009), species recognition (Singer, 1998; Blows, 2002; Ferveur, 2005) and intraspecific mating preferences in a wide range of insect species, including bees (Vereecken *et al.*, 2007) and crickets (Ivy *et al.*, 2005), as well as a number of *Drosophila* species (Cobb & Ferveur, 1995; Blows, 2002; Wicker-Thomas, 2007). These studies suggest that CHC profiles are likely to be subject to sexual selection.

It has also been argued that insect CHCs originally evolved as a chemical barrier to help prevent water loss (Ferveur, 2005), and as such, it is likely that CHC profiles are subject to natural selection as well as sexual selection. Indeed, evidence from experimental evolution in *Drosophila* has demonstrated that CHC profile evolves through both natural and sexual selection (*D. serrata*, Rundle *et al.*, 2009; and *D. simulans*, Sharma *et al.*, 2012a), and as expected from these evolutionary responses, CHCs are heritable (*D. serrata*, Hine *et al.*, 2004; and *D. simulans*, Sharma *et al.*, 2012b). However, natural and sexual selection might favour different CHC profiles. It appears that long-chained CHCs form a more stable and protective layer than short-chained CHCs, and studies have shown that long-chained CHCs provide desiccation resistance in *D. melanogaster* (Savarit & Ferveur, 2002; Foley & Telonis-Scott, 2011), *D. mojavensis* (Gibbs *et al.*, 1998) and *D. serrata* (Frentiu & Chenoweth, 2010), and investment in long-chained CHCs evolves in response to desiccation stress in *D. melanogaster* (Kwan & Rundle, 2010). The role of sexual selection on CHC profile has been most thoroughly studied in *D. serrata*, where female preferences for male CHC profiles have been examined in detail (Chenoweth & Blows, 2005; Chenoweth *et al.*, 2008; Rundle *et al.*, 2008), and these preferences appear to exert directional selection on male CHC profile (Chenoweth & Blows, 2005). Given that production of CHCs is thought to be costly (Blows, 2002; Ferveur, 2005), it is also likely that the different CHC functions will be traded against each another, making these hydrocarbons a particularly interesting multivariate sexual trait on which to focus.

In *D. simulans*, previous work has demonstrated that there are no direct benefits or direct costs to mate choice (Taylor *et al.*, 2008, 2010) but that male attractiveness is heritable (Taylor *et al.*, 2007). Research has also shown that individual CHCs and overall CHC profiles are heritable in *D. simulans* (Sharma *et al.*, 2012b). Specific CHCs and overall CHC profiles also influence male-mating success (Ferveur & Cobb, 2010; Berry *et al.* in preparation; Ingleby *et al.*, in prep.). *Drosophila simulans* CHC profiles are therefore subject to sexual selection (Sharma *et al.*, 2012a). Furthermore, both dietary (Berry *et al.* in preparation; Ingleby *et al.*, in prep.) and temperature (Sharma *et al.*, 2012a; Ingleby *et al.*, 2012) effects on *D. simulans* CHCs have been identified. Given this evidence for both genetic and environmental variation in *D. simulans* CHC profile, it is likely that G × Es in CHC expression will be important.

Here, we used a quantitative genetic design to estimate the importance of G × Es for male and female CHC expression in *D. simulans*. Flies from a total of 60 iso-female lines were reared on different diets as larvae and then exposed to two temperature regimes post-eclosion in a fully factorial design, enabling the examination of any possible synergy between these environmental variables, as well as their individual effects. We estimated cross-environment genetic correlations and trait heritabilities for CHC expression, variance components for each of the G × E interactions and the degree of ecological crossover between environments to interpret the biological significance of any G × Es and their potential consequences for the operation of sexual selection in this species.

Materials and methods

Isolines and maintenance

Approximately 100 female *D. simulans* were collected from Greece in April 2010 and used to found iso-female lines (henceforth referred to as isolines) in the laboratory ($N = 65$). Within each isoline, approximately 25 male and 25 female offspring were used to found each generation. This process of inbreeding was repeated for 19 generations prior to this experiment, and therefore, lines can to some extent be considered distinct genotypes. Isolines were maintained on a cornmeal-based diet (supplied by Applied Scientific, Loughborough, UK; made from 1 L deionized water boiled with 90 g cornmeal, 80 g brown sugar, 25 g yeast, 12 g agar and 2 g methyl paraben) at 25 °C on a 10 : 14 h light–dark cycle.

Environmental manipulations

We used male and female flies from a total of 60 isolines. The experimental set-up used for each individual isoline in our quantitative genetic design is shown in Fig. 2. At 3–4 days post-eclosion, adult flies from each isoline were established in small (40 mL) vials with either 8 mL standard cornmeal diet (diet A) or 8 mL of a novel diet (diet B; made from 1 L of deionized water boiled with 102 g brown sugar, 72 g oatbran, 24 g yeast, 12 g agar and 2 g methyl paraben). These diets were chosen purely to create environmental variation rather than as a manipulation of diet quality. We

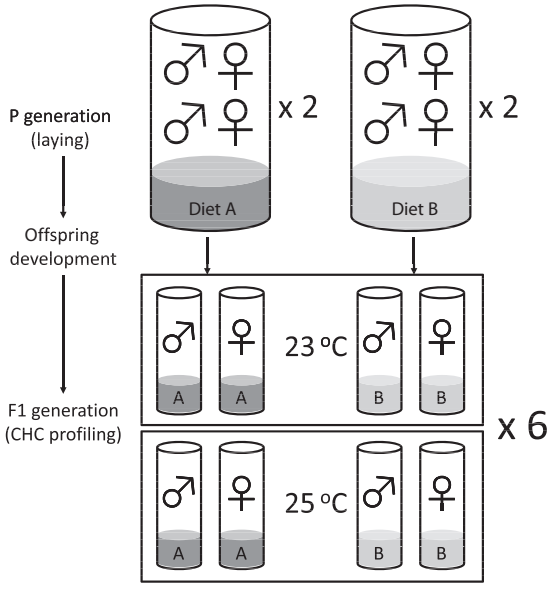

Fig. 2 Experimental setup for each individual isoline ($N = 60$). Isoline adults (P generation) were set up in laying vials of either diet A or diet B. There were two replicate vials of each diet for each isoline, and each vial had two males and two females. These adults were given a laying period of 3 days before being removed, and the vials were incubated at 25°C on a 10 : 14 light : dark cycle during offspring development. Ten days after laying, peak offspring eclosions occurred and male and female virgin offspring were collected from each diet (F1 generation). Offspring were housed individually on the same diet as development and assigned to either 23°C or 25°C post-eclosion temperature, creating a 2 × 2 factorial design of four environmental treatments. From each isoline, cuticular hydrocarbons **(**CHC) expression was analysed for six males and six females from each of the four treatments. 189 × 185 mm (96 × 96 DPI).

set up two replicate vials per isoline × environment combination, with two males and two females in each vial. These flies were given 3 days in which to lay in these vials before adults were removed, and the vials were then incubated at 25 °C on a 10 : 14 h light–dark cycle during offspring development. Peak offspring eclosions occurred 10 days after laying, at which point male and female virgin offspring were collected from each replicate vial for each isoline × environment combination. Each virgin was transferred into a small (5 mL) individual glass vial containing 1 mL of the same diet on which they had developed, and flies were then split equally between two post-eclosion temperatures (23 °C and 25 °C) in which they were incubated for 3 days. This created four environments, one from each combination of diet and post-eclosion temperatures in a 2 × 2 factorial design. In total, we reared six males and six females in each of these four environments from each isoline ($N = 2880$; Fig. 2). After 3 days in the post-eclosion temperature treatments, each fly was transferred into a glass auto-sampler vial (Chromacol, UK) using an aspirator and stored at −80 °C prior to CHC analysis.

Cuticular hydrocarbon extractions

Cuticular hydrocarbons extractions were carried out in sets of 100 samples per day and randomized throughout by diet, post-eclosion temperature, sex and isoline. CHC extraction was carried out by soaking each individual fly in 50 μL of HPLC-grade hexane, with 10 ppm pentadecane as an internal standard, for 5 min, using a vortex for the duration of the final minute to agitate the solution and maximize CHC extraction. The fly was then removed from the vial using metal forceps which had been cleaned in hexane between each sample.

From each hydrocarbon sample, 2 μL was injected into a GC-FID (Agilent 7890, Agilent Technologies, Berkshire, UK) 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 operated at 70 °C for 1 min and then increased at 20 °C min^{-1} to 180 °C, then 4 °C min^{-1} to 220 °C and finally 15 °C min^{-1} to 320 °C, where it was held for 2 min. Column flow was set at 1.2 mL min^{-1}. The FID detector heaters were set at 300 °C. The H_2 flow was 20 mL min^{-1}, and the air flow was 200 mL min^{-1}. Nitrogen was used to make up the column flow to 30 mL min^{-1}. 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 (Agilent, version B.04.02.SP1).

Statistical analysis

Data reduction using principal components analysis

We quantified expression of 22 different hydrocarbons. 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) to reduce the dimensionality of the data. CHC expression in *D. simulans* is quantitatively sexually dimorphic (Sharma *et al.*, 2012b; present study), but the same CHCs are produced by males and females. We were therefore able to identify and quantify expression of the same CHCs for males and females. We carried out PCA on the complete data set with male and female data combined, in order to obtain the same principal components (PCs) for males and females and allow examination of sex-specific patterns of CHC expression. Whilst not all of the CHC variation described by these vectors will be subject to sexual selection, expression of individual CHCs in *D. simulans* is strongly genetically correlated (Sharma *et al.*, 2012b), and so we chose to

examine multivariate combinations of CHC expression in order to assess the evolutionary significance of G × Es on CHC profile as a whole. PCs were extracted using the correlation matrix. We identified multivariate outliers based on Mahalanobis distances and removed these from the data, leaving 2429 individuals in subsequent analyses. Three orthogonal vectors with eigenvalues > 1 were extracted using PCA which together explained just over 70% of the total variation in CHC expression (Table 1). We interpret factor loadings for each CHC peak to these eigenvectors of more than 0.25 as biologically significant (Tabachnick & Fidell, 1989). For the models, we scaled the PC scores by the standard deviation of each PC to measure each PC on a comparable scale. We used variance standardization as PCs are already mean-centred.

Model fit and evaluation

We used Bayesian inferences implemented by the MCMCGLMM package (v.2.12; Hadfield, 2010) in R (v.2.13.0) to test multivariate generalized linear mixed effects models for the three PCs describing the variation in male and female CHCs in *D. simulans*. We included environmental terms (i.e. diet and post-eclosion temperature) as fixed effects and genetic and G × E terms as random effects. We ran chains for 200 000 iterations with a burn-in of 10 000 and a thinning interval of 30.

In each model, we used a relatively uninformative prior ($v = 0.02$ for both fixed and random effects), which means that models were fitted with very little *a priori* information about the expected parameter estimates. We tested all models with a more informative prior ($v = 2$) and found that our results were robust to changes in prior specification. However, the results presented here used the relatively uninformative prior distribution for all models.

For the random effects terms, we used the 'idh' variance structure, which fits a unique variance for each PC whilst assuming the random effect covariance between PCs is zero. We also ran models using unstructured variances (with the 'us' variance structure in MCMCGLMM), where all variances and covariances between PCs are estimated, and found that the results were similar. We used the 'idh' variance structure in the model results presented here as we were estimating environment-specific genetic variances, and so the use of 'idh' meant fewer estimated parameters and therefore reduced the need for highly informative priors.

We used preliminary analyses to test for the presence of sexual dimorphism in CHC expression. Firstly, we formed a full model with sex, diet, post-eclosion temperature and the interaction between environmental variables as fixed effects and isoline and the interactions between isoline, diet and post-eclosion temperature as random effects. We then used the deviance information criterion (DIC; see Spiegelhalter *et al.*, 2002) to compare this full model with and without sex as a fixed effect. A lower DIC estimate indicates a better-approximating model, and the DIC of the model with sex as a fixed effect was far lower than that of the model without (ΔDIC = 1769.87). Comparison of these preliminary models therefore gave very high support for sexual dimorphism in CHC expression. We also used between-sex genetic correlations for each PC (calculated from a simplified model with no G × Es) to examine overall differences between male and female CHC expression. As these sex differences were large, we ran separate models for each sex. For each sex, we tested a set of six plausible models (see Table 2). These models are multivariate models which include all three PCs of CHC expression, but the inclusion of a 'trait' term in each model allows us to examine effects on individual PCs (details below). We estimated the support for each of these models using the model DIC and also by calculating an approximate posterior probability for each model. This calculation takes into account the DIC of each model tested and for each provides a probability that can be used to identify the best approximating model out of the set being tested.

Table 1 Principal component analysis for cuticular hydrocarbons (CHC) expression in both sexes. Three principal components with eigenvalues > 1 were extracted for further analyses, explaining just over 70% of the total variation in CHC profile. Biological significance of each component was interpreted from factor loadings > 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
Eigenvalue	9.731	3.933	1.979
% variance	44.232	17.875	8.997
Loadings			
Octadecadiene	**0.680**	**−0.252**	0.029
Docosene	**0.403**	−0.039	**−0.314**
Docosane	**0.836**	0.102	**−0.328**
Branched alkane*	**0.714**	**−0.427**	−0.179
7-Tricosene	**0.845**	**−0.271**	0.138
Tricosene	**0.685**	**−0.286**	−0.138
Tricosane	**0.723**	−0.110	−0.025
Branched alkane*	**0.729**	**−0.430**	−0.205
Branched alkane*	**0.797**	−0.206	**−0.388**
Branched alkane*	**0.725**	**−0.405**	−0.075
Tetracosane	**0.700**	**0.613**	**−0.284**
Pentacosadiene	**0.651**	**0.459**	−0.265
Alkene*	**0.591**	0.029	**0.588**
Pentacosene	**0.537**	**0.258**	**0.400**
Pentacosane	**0.752**	**0.595**	0.017
Branched alkane*	**0.776**	0.071	**−0.258**
Hexacosane	**0.536**	**0.787**	−0.208
Heptacosane	**0.736**	**0.344**	−0.040
Branched alkane*	**0.500**	0.206	**0.589**
Alkane*	**0.540**	**0.800**	0.122
Alkane*	**0.417**	0.178	**0.592**
Alkane*	**0.486**	**0.807**	−0.147

Table 2 Summary of the set of six models tested. Male and female data were modelled separately. The model with the highest support for each sex is in boldface, chosen using the DIC and supported by the approximate posterior probability.

	Model rationale	Model formula* [fixed effects (random effects)]	Males [DIC (posterior model probability)]	Females [DIC (posterior model probability)]
(a)	No genetic component	Ed + Et + Ed : Et	15254.58 (< 0.0001)	14105.49 (< 0.0001)
(b)	Genetic component but no G × E interactions	Ed + Et + Ed : Et + (G)	14977.33 (0.003)	13913.21 (0.005)
(c)	G × E for post-eclosion temperature manipulation only	Ed + Et + Ed : Et + (G + G : Et)	14978.71 (0.001)	13906.52 (0.13)
(d)	G × E for dietary manipulation only	**Ed + Et + Ed : Et + (G + G : Ed)**	**14966.68 (0.53)**	13910.55 (0.02)
(e)	G × Es for both environmental manipulations	**Ed + Et + Ed : Et + (G + G : Et + G : Ed)**	14968.10 (0.25)	**13903.59 (0.58)**
(f)	Both G × Es plus G × ExE	Ed + Et + Ed : Et + (G + G : Et + G : Ed + G : Et : Ed)	14968.50 (0.21)	13905.16 (0.26)

DIC, deviance information criterion.
*Ed = diet; Et = post-eclosion temperature; G = isoline.

From the posterior distribution of the best model for each sex, we calculated the effect of the fixed environmental factors on CHC expression. From the posterior distribution of these models, we partitioned variance into genetic and G × E components (following Lynch & Walsh, 1998) and predicted mean trait value for each isoline in each environment from the posterior distribution (as an approximate equivalent to Best Linear Unbiased Predictors; BLUPs). We used these scores to plot reaction norms for each G × E term in the best model for each sex.

Model interpretation

We ran a separate model for each possible G × E (isoline × diet and isoline × temperature separately) in each sex (leaving fixed effects unchanged). We used these simpler models instead of the best model for each sex for ease of interpretation of the variance–covariance matrix. Following Lynch & Walsh (1998), we calculated cross-environment genetic correlations and heritabilities (with ± 95% credible intervals around each estimate) both between and within environments for each PC. These calculations were carried out directly from the posterior distribution of the variance–covariance matrix for each model, as opposed to using point estimates, therefore accounting for uncertainty around these estimates (Hadfield *et al.*, 2010). For the between-environment genetic correlations, we interpreted an estimate that deviated significantly from 1 (i.e. the credible interval did not overlap 1) as evidence for ecological crossover, because a correlation of 1 would have indicated a perfect correlation with no crossover.

In addition, we calculated the proportion of crossover within each G × E we identified, in order to quantify the crossover which can be seen in the reaction norms. This was calculated (following Danielson-François *et al.*, 2006) as the number of pairwise comparisons of isolines which had intersecting reaction norms, divided by the total number of possible pairwise comparisons.

The heritabilities represent broad-sense rather than narrow-sense estimates due to our use of isolines (David *et al.*, 2005). For our diet and temperature manipulations, we made a *post hoc* comparison of between-environment heritability estimates with the mean within-environment heritability estimates using a paired *t*-test, to show if heritability differed significantly between and within environments. These *t*-tests do not account for the uncertainty around the heritability point estimates.

Results

Principal components analysis

From the PCA, we extracted three PCs with eigenvalues exceeding 1 (Table 1). PC1 describes the absolute quantity of CHCs produced, as each of the 22 CHCs measured exhibited a positive loading > 0.25. For PC2, 14 of the 22 hydrocarbons have a loading > 0.25, with short-chained hydrocarbons negatively-loaded and long-chained hydrocarbons positively-loaded. We interpret PC2 as a trade-off between production of long and short-chained CHCs. For PC3, only nine of the 22 CHCs have loadings over 0.25, with a mixture of negative (mostly short-chained CHCs like docosene and docosane) and positive loadings (mostly long-chained CHCs such as pentacosene and a heavy alkene and branched alkane). This eigenvector therefore appears to describe a similar trade-off between these specific CHCs.

Model selection

The set of six models tested with male and female data are summarized in Table 2, along with the DIC estimate and approximate posterior probability associated with

each model as a measure of statistical support. We have identified model (d) as the best model for the male data, which includes an isoline × diet interaction and model (e) as the best model for the female data, which includes both isoline × diet and isoline × post-eclosion temperature interactions. Neither of these models includes the three-way interaction (isoline × diet × temperature). We present the results of each of these models [model (d) for males and model (e) for females] here in more detail. However, it is worth noting that whilst these models have the best support from the DIC and posterior probability, other models for each sex also have modest statistical support, namely models (e) and (f) for males and model (f) for females. Most importantly though, it is clear from all these models that G × E components of CHC expression are important in both sexes.

We modelled male and female CHC expression separately as preliminary analyses suggested high levels of sexual dimorphism (see Methods). These sex differences were highlighted throughout the process of model selection for each sex and resulted in a different best model for male and female CHC expression (Table 1). Furthermore, the between-sex genetic correlation for each PC demonstrated that the genetic correlation of PC1 between sexes was quite high ($r = 0.917$; 95% credible interval: 0.723–0.993), was slightly lower for PC3 ($r = 0.619$; 95% CI: 0.372–0.807) and very weak for PC2 ($r = 0.302$; 95% CI: −0.433 to 0.855), indicating an advanced stage of sexual dimorphism for PC2 and PC3 (Lande, 1980).

Environmental effects

The fixed effects of post-eclosion temperature, diet and the interaction between these two environmental variables were found in the best model for both male and female CHC expression (Table 2). There was a trend for both males (Fig. 3) and females (Fig. 4) to produce more CHCs overall (PC1) on the standard diet (diet A), although this effect was stronger in females as there was no overlap of credible intervals between diets.

Expression of PC2 and PC3 are dramatically different in males and females: males consistently have negative scores for PC2 and PC3 across all environments, whereas females consistently have positive scores. For males in the higher post-eclosion temperature, PC2 and PC3 have higher scores than at the lower temperature (Fig. 3), indicating decreased investment in short-chained CHCs when the temperature is raised. This effect is clearest for PC3, and the difference in PC3 score between temperatures appears slightly larger on diet B (Fig. 3). In females, credible intervals for PC2 and PC3 scores are large and overlap widely across all environments (Fig. 4), and as such environmental effects appear to be much weaker for PC2 and PC3 than for PC1.

Genetic and G × E effects

As shown in Table 2, the best models for males and females both included a genetic (isoline) component of

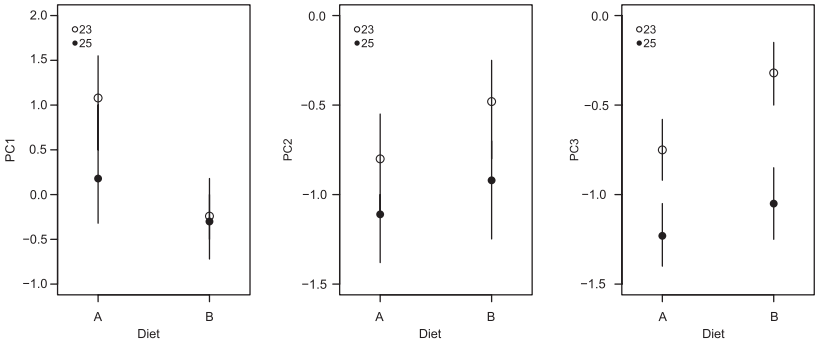

Fig. 3 Overall posterior mean ± 95% credible interval from the best model for male cuticular hydrocarbons (CHC) expression, showing the effect of diet (A or B; *x*-axis) and post-eclosion temperature (23 or 25°C; see legend) on male PC1, PC2 and PC3 scores (left to right). 211 × 105 mm (96 × 96 DPI).

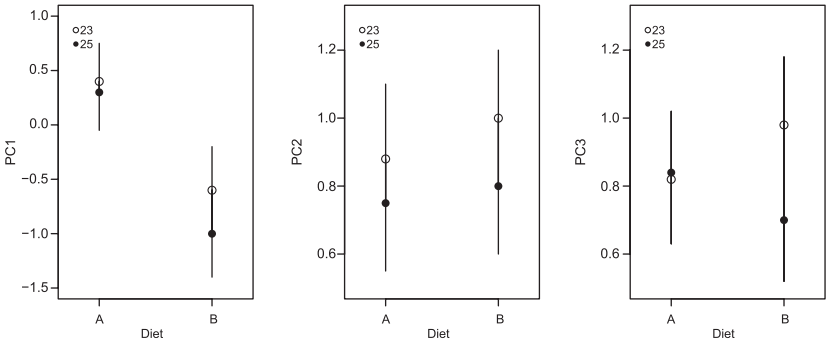

Fig. 4 Overall posterior mean ± 95% credible interval from the best model for female cuticular hydrocarbons (CHC) expression, showing the effect of diet (A or B; *x*-axis) and post-eclosion temperature (23C or 25°C; see legend) on female PC1, PC2 and PC3 scores (left to right). 211 × 105 mm (96 × 96 DPI).

CHC expression, although the genetic variance in PC2 was very low for both sexes (Table 3). However, male and female CHC expression differed in terms of which G × E effects were important.

In males, only the isoline × diet interaction appears in the best model. The variance in this interaction was quite high for male PC1 compared to the other male PCs (Table 3). The isoline × diet interaction for each male PC is shown in the reaction norms in Fig. 5a–c, where a large reduction in the extent of genetic variation in PC2 from the standard diet (diet A) to the novel diet (diet B) can be seen. Genetic correlation of each male PC across diets (Table 4) gives some evidence for ecological crossover, as the credible interval in each

Table 3 Posterior mean of genetic (Isoline) and G × E (Isoline × diet for males; Isoline × diet and Isoline × temperature for females) variance components (with 95% credible interval) for PC1, PC2 and PC3 for each sex. Components are calculated from the best model for each sex.

	Males		Females		
	Isoline	Isoline × diet	Isoline	Isoline × diet	Isoline × temperature
PC1	0.947 (0.491–1.604)	0.256 (0.071–0.549)	0.466 (0.183–0.878)	0.106 (0.014–0.276)	0.055 (0.005–0.173)
PC2	0.078 (0.022–0.171)	0.025 (0.007–0.106)	0.054 (0.014–0.119)	0.028 (0.005–0.068)	0.024 (0.003–0.069)
PC3	0.266 (0.165–0.407)	0.036 (0.012–0.071)	0.236 (0.144–0.369)	0.019 (0.003–0.047)	0.027 (0.006–0.062)

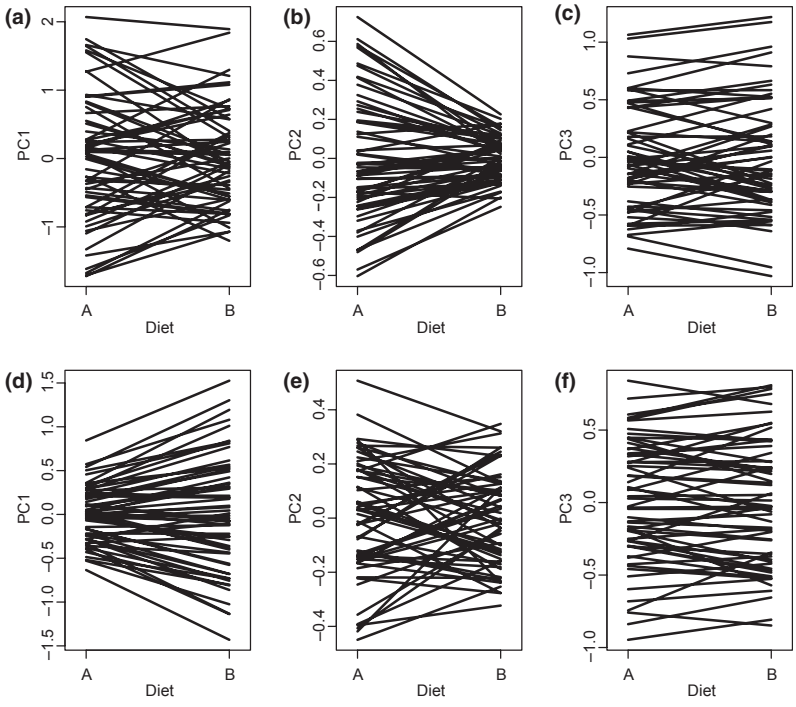

Fig. 5 Reaction norms for the isoline × diet interaction in male cuticular hydrocarbons (CHC) expression for (a) PC1, (b) PC2 and (c) PC3; and for female CHC expression for (d) PC1, (e) PC2 and (f) PC3. Each point represents the posterior mean for a given isoline in each environment, as an approximate equivalent to BLUPS, calculated from the posterior distributions of the best models for male and female CHC expression. Calculation of the proportion of crossover in male reaction norms showed 25% in PC1, 22% in PC2 and 19% in PC3; and in female reaction norms showed 12% in PC1, 35% in PC2 and 12% in PC3.

Table 4 Cross-environment genetic correlations (with 95% credible interval) for each male and female PC calculated from the posterior distribution of separate models for each possible G × E (fixed effects unchanged), using variance-scaled PC scores.

	Males		Females	
	Diet	Temperature	Diet	Temperature
PC1	0.562 (0.133 to 0.872)	0.662 (0.256 to 0.921)	0.446 (−0.065 to 0.794)	0.489 (−0.008 to 0.818)
PC2	0.307 (−0.512 to 0.877)	−0.262 (−0.767 to 0.383)	0.228 (−0.296 to 0.662)	0.209 (−0.317 to 0.660)
PC3	0.778 (0.551 to 0.928)	0.937 (0.835 to 0.988)	0.765 (0.533 to 0.912)	0.741 (0.505 to 0.896)

case does not overlap 1, showing a weakened genetic correlation across environments. However, the genetic correlation for PC3 was high, showing that male PC3 scores were still strongly correlated across diets, and crossover is therefore unlikely to be of high importance. The proportion of crossover calculated within each interaction was 25% in PC1, 22% in PC2 and 19% in PC3.

The best approximating model for female CHC expression included both isoline × diet and isoline × temperature terms. The isoline × diet interaction explained substantial variance in PC1 (Table 3), and an increase in genetic variation for PC1 from the standard diet (diet A) to the novel diet (diet B) is evident from the reaction norm (Fig. 5d–f). Table 4 shows that there is evidence for some ecological crossover of female reaction norms across diets. The genetic correlation across diets for female PC1 and PC2 was low, whilst genetic correlation across diets for female PC3 was high, and so crossover is likely to be more important in PC1 and PC2 than in PC3. The proportion of crossover calculated for each PC suggests a higher extent of crossover in PC2, and this can be visualized in the reaction norms presented in Fig. 5d–f (PC1: 12%; PC2: 35%; PC3: 12%).

The female isoline × temperature interaction accounted for a low level of variance in each PC (Table 3). Reaction norms for this interaction are shown in Fig. 6. Genetic correlation of female PCs across temperatures again gives some evidence for crossover, as each interval estimate is lower than 1, although the correlation of PC3 across temperatures is high, indicating less crossover within this interaction (Table 4). The proportion of crossover calculated in the isoline × temperature interaction was 23% in PC1, 25% in PC2 and 16% in PC3.

Across males and females, the interval estimated for all cross-environment genetic correlations does not overlap 1 (Table 4), indicating a weakened correlation of trait expression between environments and providing evidence for significant ecological crossover. However, this effect is generally very small in both male and

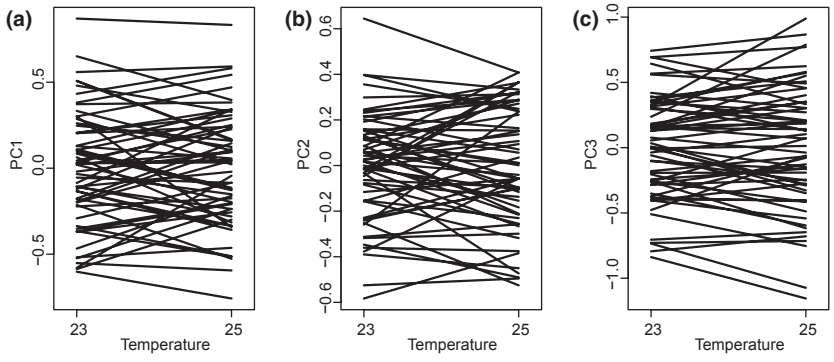

Fig. 6 Reaction norms for the isoline × post-eclosion temperature interaction in female cuticular hydrocarbons (CHC) expression for (a) PC1, (b) PC2 and (c) PC3. Each point represents the posterior mean for a given isoline in each environment, as a Bayesian equivalent to BLUPS, calculated from the posterior distribution of the best model for female CHC expression. Calculation of the proportion of crossover showed 23% in PC1, 25% in PC2 and 16% in PC3. There was no isoline × temperature interaction in the best model for male CHC expression.

Table 5 Heritability (with 95% credible interval) of each male PC, as calculated from the posterior distribution of separate models for each possible G × E (fixed effects unchanged), using PC scores scaled by standard deviation. Within-environment heritability is unshaded and between-environment heritability is shaded.

Environment	PC1	PC2	PC3
Temperature			
Within 23 °C	0.254 (0.110–0.462)	0.152 (0.060 to 0.299)	0.535 (0.309–0.871)
Between temperatures	0.158 (0.048–0.300)	−0.029 (−0.103 to 0.039)*	0.527 (0.321–0.827)
Within 25 °C	0.233 (0.099–0.420)	0.081 (0.022 to 0.181)	0.598 (0.353–0.961)
Diet			
Within diet A	0.344 (0.165–0.594)	0.095 (0.032 to 0.198)	0.584 (0.335–0.950)
Between diets	0.145 (0.028–0.294)	0.017 (−0.027 to 0.068)	0.500 (0.275–0.816)
Within diet B	0.198 (0.081–0.374)	0.035 (0.006 to 0.092)	0.715 (0.424–1.144)

*Interpreted as $H^2 = 0$ as the credible interval overlaps 0.

Table 6 Heritability (with 95% credible interval) of each female PC, as calculated from the posterior distribution of separate models for each possible G × E (fixed effects unchanged), using PC scores scaled by standard deviation. Within-environment heritability is unshaded and between-environment heritability is shaded.

Environment	PC1	PC2	PC3
Temperature			
Within 23 °C	0.176 (0.070 to 0.344)	0.117 (0.051 to 0.223)	0.449 (0.252–0.732)
Between temperatures	0.081 (−0.001 to 0.190)	0.027 (−0.038 to 0.102)	0.391 (0.204–0.648)
Within 25 °C	0.155 (0.059 to 0.310)	0.141 (0.054 to 0.285)	0.624 (0.355–1.016)
Diet			
Within diet A	0.115 (0.043 to 0.234)	0.138 (0.056 to 0.268)	0.506 (0.284–0.840)
Between diets	0.077 (−0.010 to 0.185)	0.027 (−0.032 to 0.099)	0.392 (0.205–0.657)
Within diet B	0.256 (0.112 to 0.461)	0.101 (0.042 to 0.198)	0.524 (0.296–0.850)

female PC3 across diets and temperatures. We also calculated heritabilities within and between environments for each PC for males (Table 5) and females (Table 6). Overall, PC2 exhibited low heritability estimates in both sexes ($0 < H^2 < 0.152$). There was more heritable genetic variation for PC1 ($0.077 < H^2 < 0.344$), and the heritability of PC3 was quite high ($0.391 < H^2 < 0.715$). For both sexes, heritability estimates are consistently lower between different environments than within the same environment. Paired *t*-tests showed that within-diet heritability was significantly higher than between-diet heritability ($t = 7.56$; $n = 6$; $P = 0.0006$). The same pattern, although slightly weaker, was found across the temperature manipulation, as heritability ($t = 6.05$; $n = 6$; $P = 0.002$) was higher within the same temperature than between different temperatures.

Discussion

Genotype-by-environment interactions (G × Es) for sexually selected traits have received increasing attention in recent years (Greenfield & Rodríguez, 2004; Bussière *et al.*, 2008; Ingleby *et al.*, 2010), and theory predicts they could help to maintain genetic variation in traits subject to strong sexual selection (Kokko & Heubel, 2008), whilst also having the potential to disrupt the reliability of sexual traits as signals of underlying mate quality (Higginson & Reader, 2009). Here, we measured CHC expression of male and female *D. simulans* from isolines reared across two axes of abiotic environmental variation (diet and post-eclosion temperature). Our results show that there are G × Es for diet in both male and female CHC expression and a G × E for temperature in female CHC expression. We also find some evidence for ecological crossover in each of the G × Es identified. We quantify each interaction using variance components, genetic correlations and heritabilities, and examine the potential implications for the operation of sexual selection in this species.

By using a PCA approach to analyse CHC expression, it is possible that not all the variation we measured is necessarily subject to sexual selection. In fact, these PC vectors probably encompass naturally and sexually selected variation in CHC profile, given the biological interpretation of the vectors, and the fact that these PCs are significantly aligned with PCs 1–3 in Sharma *et al.* (2012a), which evolved through natural and sexual selection. We also have direct evidence from another study that these PCs are subject to sexual selection (unpublished data). An alternative analysis could have focussed on the expression of the few individual CHCs which have been explicitly linked to sexual selection in *D. simulans*. For example, we measured expression of 7-tricosene, octadecadiene and pentacosadiene, each of which has been strongly implicated in *D. simulans* courtship and mating behaviour (Ferveur & Cobb, 2010). Each of these CHCs was significantly loaded onto PCs 1 and 2, and furthermore, analysis shows significant G × Es in the expression of these individual components (analysis not shown). However, not all CHC components have been individually assessed in a sexual selection context, and expression of individual CHCs in *D. simulans* is highly genetically correlated (Sharma *et al.*, 2012b), and so sexual selection on particular CHCs will drive the evolution of overall CHC profile through direct selection as well as correlated responses. Therefore, PCA was deemed more appropriate, and by using PCA to examine multivariate combinations of CHCs for which at least some of the variation will be subject to sexual selection, we aimed to assess the consequences of G × Es on the evolution of CHC profile as a whole.

Our experimental design also enabled us to test for a G × E × E interaction (between isoline, diet and temperature), which would have indicated synergy between the different G × E effects studied here. However, this three-way interaction was not important in either sex. Studies of synergy between environmental variables have previously given mixed results, and so further research on the role of interactions between environmental factors has been encouraged (Sih *et al.*, 2004). The lack of G × E × E interaction in our study indicates a very low level of genetic variation for the interaction between diet and temperature, and it is

therefore unlikely that this interaction will have a significant effect on the evolution of CHC profile in *D. simulans*.

Isoline × diet and dietary effects on CHC expression

In both males and females, we found an isoline × diet interaction which shows genetic variation for diet-dependent aspects of CHC expression. In particular, there is high variance in this interaction for PC1, which describes the overall production of CHCs. Given the extensive research documenting the condition dependence of sexual traits (Rowe & Houle, 1996), it is not surprising that the resources accumulated through diet affect overall investment in CHC production, although our data do not explicitly provide evidence for condition dependence. Gosden & Chenoweth (2011) found that dietary manipulation revealed condition dependence of male CHC expression in *D. serrata*, but they found no evidence for genetic variation underlying this diet-mediated plasticity. We find that male and female *D. simulans* in our study generally produce more CHCs on diet A than diet B, but the isoline × diet interaction in both sexes reveals that there is genetic variation underlying patterns of resource allocation across diets. The capture of genetic variance in condition-dependent traits is an idea which has been discussed previously as a mechanism to maintain genetic variation in sexual traits (Rowe & Houle, 1996; Tomkins *et al.*, 2004; Kokko & Heubel, 2008). We find weakened cross-environment genetic correlations for heritable aspects of male and female CHC profile between diets, and so our results are at least consistent with the concept that the response to selection can differ between dietary environments and genetic variation could be maintained across diets.

The potential for G × E to maintain genetic variation in sexually selected aspects of male CHC expression is of course dependent on whether there is G × E for female preference for male CHCs and if the reaction norms for preference and signal perfectly match across environments (Greenfield & Rodríguez, 2004). Without data on female preference G × E, we are unable to provide a definitive test for this. However, the G × E for diet in female CHC expression could also have evolutionary significance, as there is some evidence for male mate choice in *D. melanogaster* (Byrne & Rice, 2006) and *D. serrata*, where males prefer specific female CHC profiles (Chenoweth & Blows, 2005), such that G × Es in female CHC profile could influence the evolution of these female signals and the associated male mating preferences. However, experimental evolution in *D. simulans* suggests that the influence of sexual selection on female CHC profile might be weak compared to that of natural selection (Sharma *et al.*, 2012a); hence, the evolutionary significance of this G × E in female CHC expression is unclear. We do, however, find quite a high between-sex genetic correlation for PC1, and so evolution along PC1 in females might correlate quite strongly with that of PC1 in males.

Post-eclosion temperature and isoline × temperature effects on CHC expression

Whilst there is a clear overall temperature component to male CHC expression, we only find an isoline × temperature interaction in female CHCs. This G × E indicates that the effect of temperature on female CHC expression differs between genotypes; however, the overall effect of temperature on females is not as strong as in males. The low variance in the female isoline × temperature interaction probably reflects, in part, the low overall variance in female CHC expression between temperatures. Female *D. simulans* generally have larger body size than males, and so the surface area to volume ratio is lower in females, and this might therefore explain the weaker response to temperature variation in females. Furthermore, if the main function of short-chained CHCs is to act as sexual signals to allow males to attract females, then selection for female investment in short-chained CHCs might be weak, and females might invest more in long-chained, protective CHCs (Foley & Telonis-Scott, 2011). In agreement with this, we find that female PC2 and PC3 scores are consistently positive across both temperatures, indicating a bias towards production of long-chained CHCs regardless of temperature.

The lack of G × E for post-eclosion temperature in male CHC expression indicates that differences between temperatures are relatively consistent across genotypes. The effect of temperature on male CHCs is strong and can be seen clearly in the trade-off described by PC2 and PC3. Long-chained CHCs are likely to be naturally selected for desiccation resistance, and risk of desiccation will be elevated at higher temperatures (Savarit & Ferveur, 2002; Kwan & Rundle, 2010; Foley & Telonis-Scott, 2011). Consistent with this, we find that at lower temperatures, the decreased risk of desiccation appears to allow males of all genotypes to invest less in long-chained CHCs and therefore allocate more resources towards producing smaller, more volatile CHCs which could improve male attractiveness. Male attractiveness might therefore be affected by post-eclosion temperature, and we might expect female preferences to differ across a temperature gradient, although this remains to be established.

However, although there was no isoline × temperature interaction for male CHC expression in the best model, this interaction was important in two other male models with some statistical support, albeit more limited. This perhaps explains why we find a similar pattern in heritability and genetic correlation of male CHC expression across both temperature and diet

manipulations, although there is some evidence from the comparison of within- and between-environment heritability estimates that this pattern is weaker across temperatures than across diets. Given the large body of evidence for strong temperature-dependent selection on the trade-off between long- and short-chained CHCs in *Drosophila* (e.g. Gibbs *et al.*, 1998; Ferveur, 2005; Frentiu & Chenoweth, 2010), it is possible that the optimal male response in PC2 and PC3 to temperature variation has become canalized between genotypes. Alternatively, selection on this trade-off might simply have eroded genetic variation for plasticity in male CHC profile across temperatures (Roff & Fairbairn, 2006). This explanation is unconvincing for PC3, where estimates of genetic variance are high, but may be the case for PC2, and therefore, evolution along PC2 could be constrained by the low heritable genetic variation in this vector.

Quantifying G × E effects

Theory has distinguished between G × E interactions with or without ecological crossover as strong or weak interactions, respectively (Kokko & Heubel, 2008; Higginson & Reader, 2009). However, in empirical studies, this definition of interaction strength is difficult to apply. In part, this is because the influence an interaction has on trait expression and evolution will not only depend on changes in the rank order of genotypes between environments, but also on the extent of genetic variation present. Whilst we show here that there is evidence for ecological crossover of reaction norms in each of the G × Es we identify, we also find that the extent of this crossover varies, such that each G × E appears to result from a combination of ecological crossover and a change in the scale of variation across environments. This is likely to be the case in most empirical studies of G × Es, and so relating these results to theoretical work which is based on an absolute presence or absence of crossover is difficult.

Here, we calculate cross-environment trait heritability and genetic correlations in an attempt to quantify the effect of each G × E and predict its evolutionary significance. The application of cross-environment genetic correlations to the study of G × Es in evolutionary genetics was demonstrated over 25 years ago by Via & Lande (1985), but has not been used to a great extent in the recent spate of studies on G × Es in sexual traits (but see Jia *et al.*, 2000 and Rodríguez & Al-Wathiqui, 2011). With G × Es, heritability and genetic correlation will be weakened across different environments, and this is the overall pattern we find here. Furthermore, if crossover represents a strong interaction, we would expect the extent to which these estimates are weakened between different environments to be larger where there appears to be more crossover. However, this trend is not consistent within the genetic correlations and proportion of crossover we calculate from our data. Reaction norms and crossover are useful to examine phenotypic effects of G × Es, but as we show here, trait heritability and genetic correlation are also important when interpreting results in terms of sexual selection and trait evolution.

There is evidence from a range of insect species (Ivy *et al.*, 2005; Vereecken *et al.*, 2007; Wicker-Thomas, 2007), including *Drosophila* (Hine *et al.*, 2002; Sharma *et al.*, 2012a), that CHCs have evolved as a means by which females might assess male attractiveness. In *D. simulans*, we have found heritable genetic variation and isoline × diet interactions for aspects of male CHC expression that are likely to be sexually selected. For CHC profiles to be reliable signals of the underlying genetic quality of a male, there must be a predictable relationship between phenotype and the benefits of mating with a particular individual. Theoretical work has made the prediction that when there are G × Es in the expression of a sexual signal, the genotype–phenotype relationship can be disrupted by environmental change and environmental stress (Greenfield & Rodríguez, 2004; Higginson & Reader, 2009). This prediction is supported here by very low trait heritability between environments. Previously, heritability estimates have been used in this way to infer potential signal unreliability through environmental stress in the bank vole, *Clethrionomys glareolus* (Mills *et al.*, 2007). We studied what we believe to be an unstressful range of environmental variation and also find potential for CHC sexual signal reliability to be disrupted in *D. simulans*.

A large body of research has addressed the potential problem of the maintenance of genetic variation in sexual traits which are subject to directional selection through mating preferences (Kirkpatrick & Ryan, 1991; Rowe & Houle, 1996; Radwan, 2008). Recent theoretical work has proposed that G × Es could help to maintain genetic variation in sexual traits in heterogeneous environments (Greenfield & Rodríguez, 2004; Kokko & Heubel, 2008). In this way, although G × Es in sexual traits could disrupt sexual selection by potentially making sexual signals unreliable, they could also facilitate sexual selection by maintaining the genetic variation in sexual traits that is necessary for indirect benefits to accrue through mate choice (Kokko & Heubel, 2008). Indeed, there is already evidence from the waxmoth, *Achroia grisella*, which suggests that G × Es in male acoustic sexual signals contribute towards the maintenance of genetic variation (Jia *et al.*, 2000). We show here that G × Es have the potential to maintain genetic variation in sexually selected components of male CHC profile across the diets examined here. Theoretical work highlights the importance of G × Es with crossover in the maintenance of genetic variation (Kokko & Heubel, 2008), but in our data we find that G × Es which vary in the extent of crossover have the potential to maintain genetic variation in sexually selected components of male CHC profile. Our results illustrate the useful-

ness in an evolutionary context of considering estimates of genetic variation alongside phenotypic measurement of G × Es.

In conclusion, across the range of environmental variation studied here, we find G × Es in components of *D. simulans* CHC profiles, which are likely to function as sexual traits. Our data demonstrate G × Es for diet in male and female CHCs and a G × E for temperature for females. We find some evidence for ecological crossover in these G × Es and show that G × Es in this system cause weakened cross-environment genetic correlations and heritabilities. Therefore, as predicted by theory, G × Es in *D. simulans* CHC profiles have the potential to contribute to the maintenance of genetic variation, as well as the potential to disrupt the reliability of sexual signals. These are fundamental concepts in sexual selection research, and so further work will therefore be necessary to test the consequences of these G × Es on sexual selection, and particularly on the evolution of mating preferences.

Acknowledgments

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