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Environmental heterogeneity, multivariate sexual selection and genetic constraints on cuticular hydrocarbons in *Drosophila simulans*

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Abstract

Sexual selection is responsible for the evolution of many elaborate traits, but sexual trait evolution could be influenced by opposing natural selection as well as genetic constraints. As such, the evolution of sexual traits could depend heavily on the environment if trait expression and attractiveness vary between environments. Here, male *Drosophila simulans* were reared across a range of diets and temperatures, and we examined differences between these environments in terms of (i) the expression of male cuticular hydrocarbons (CHCs) and (ii) which male CHC profiles were most attractive to females. Temperature had a strong effect on male CHC expression, whereas the effect of diet was weaker. Male CHCs were subject to complex patterns of directional, quadratic and correlational sexual selection, and we found differences between environments in the combination of male CHCs that were most attractive to females, with clearer differences between diets than between temperatures. We also show that genetic covariance between environments is likely to cause a constraint on independent CHC evolution between environments. Our results demonstrate that even across the narrow range of environmental variation studied here, predicting the outcome of sexual selection can be extremely complicated, suggesting that studies ignoring multiple traits or environments may provide an over-simplified view of the evolution of sexual traits.

Introduction

Our understanding of sexual selection has deepened considerably over the past few decades (see Majerus, 1986; Andersson & Simmons, 2006; Hosken & House, 2011), and the potential for sexual selection to vary between environments has been highlighted in a number of different contexts. This includes the possibility of sexual selection driving ecological speciation and local adaptation (Ritchie, 2007; van Doorn *et al.*, 2009), the role of the environment in determining condition and the condition dependence of sexual traits (Rowe & Houle, 1996; Hunt *et al.*, 2004; Cotton *et al.*, 2006; Cornwallis & Uller, 2010), and most recently, the effect of genotype-by-environment interactions and their evolutionary consequences for sexual traits (Bussiere *et al.*, 2008; Kokko & Heubel, 2008; Higginson & Reader, 2009; Ingleby *et al.*, 2010).

When patterns of sexual selection vary between environments, environmental variation can prevent the depletion of genetic variation and therefore provide a resolution to the lek paradox (Kirkpatrick & Ryan, 1991; Hoffmann & Merila, 1999). This has been demonstrated both theoretically (Kokko & Heubel, 2008) and empirically (e.g. Jia *et al.*, 2000; Moller & Szep, 2005) and is central to our understanding of sexual trait evolution between environments as genetic variation is necessary for any trait to evolve. In addition, plasticity in sexual signals and mating preferences across environments can influence the

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coevolution of signal and preference (Greenfield & Rodríguez, 2004; Zhou *et al.*, 2011; Ingleby *et al.*, 2013a). This coevolution is of fundamental importance to some models of sexual selection (Lande, 1981), but the potential for it to vary between environments is poorly understood.

Cuticular hydrocarbons (CHC) have been shown to be strongly influenced by the environment and to be subject to sexual selection in a number of insect species. They are particularly well studied in species of cricket (e.g. Thomas & Simmons, 2009, 2011; Thomas *et al.*, 2011; Weddle *et al.*, 2012) and *Drosophila* (Ferveur, 2005). In *Drosophila*, long-chained or heavily branched CHCs are waxy and largely nonvolatile, creating a stable, protective barrier that helps prevent water loss through the cuticle (Ferveur, 2005). Studies have shown that *D. mojavensis* (Gibbs *et al.*, 1998), *D. melanogaster* (Savarit & Ferveur, 2002), *D. serrata* (Frentiu & Chenoweth, 2010) and *D. simulans* (Sharma *et al.*, 2012; Ingleby *et al.*, 2013b) produce more long-chained CHCs at higher temperatures. Similarly, desiccation stress exerts selection on *D. melanogaster* to invest more in CHC production, and particularly long-chained CHCs (Kwan & Rundle, 2009; Foley & Telonis-Scott, 2010).

In contrast, short-chained, more volatile CHC components are often used for chemical communication, functioning as short-range or contact pheromones. Specifically, studies have implicated 7-tricosene and various dienes in *Drosophila* courtship and mating behaviour (reviewed by Ferveur & Cobb, 2010), whereas in *D. serrata* methyl-branched alkanes play a key role in mating success (Chenoweth & Blows, 2005; Petfield *et al.*, 2005; Delcourt *et al.*, 2010). Consistent with this, experimental evolution studies with *D. serrata* (Blows, 2002; Chenoweth & Blows, 2005; Chenoweth *et al.*, 2008; Rundle *et al.*, 2009) and *D. simulans* (Sharma *et al.*, 2012) found that CHC profiles evolve in response to sexual (as well as natural) selection. There is also evidence that *Drosophila* CHCs are costly to produce (Blows, 2002; Ferveur, 2005), and accordingly, there is evidence of condition dependence of CHC profiles (Gosden & Chenoweth, 2011). As such, it is likely that trade-offs exist between different CHC components and their diverse functions.

Drosophila CHCs are therefore ideal for studying how environmental variation affects sexual trait evolution. Indeed, an experimental evolution study with *D. serrata* found an interaction between the effect of natural and sexual selection on CHC profile (Blows, 2002), suggesting that sexual selection on CHCs could differ between environments. However, variation in patterns of selection between environments does not necessarily give an accurate representation of how CHCs will evolve. The response to selection will also depend on genetic variation for CHC expression, and especially how much genetic variation there is in the direction of selection within each environment. A deficit of genetic variation in the direction of selection will impose a strong genetic constraint on CHC evolution, and predicted responses would differ substantially from those that only consider selection (Blows & Walsh, 2009). Furthermore, the genetic covariance in trait expression across environments, which can be quantified by expanding the genetic variance–covariance (**G**) matrix for a given set of traits across multiple environments (see below), can also impose a genetic constraint on the independent evolution of a given phenotypic trait in different environments (Via & Lande, 1985). That is, common genetic variation underlying trait expression across different environments could prevent traits from evolving in the direction predicted by selection within a single environment. For this reason, studies that characterize cross-environment patterns of sexual selection and genetic (co)variation for multiple traits provide insight into how both selection and genetic constraints contribute to trait evolution (Blows, 2007; Blows & Walsh, 2009).

Here, we examine how sexual selection through female mate choice acts on the CHC profiles of male *D. simulans* reared in a range of different laboratory environments, by measuring both the attractiveness and CHC profile of males from each environment and employing a standard multivariate selection analysis (Lande & Arnold, 1983). We quantify differences in male CHC profile between environments, especially between temperatures, and identify complex patterns of directional, quadratic and correlational sexual selection. We find evidence of variation between environments in terms of what type of male CHC components are most attractive to females. Furthermore, using estimates of the **G** matrix underlying male CHC expression (Ingleby *et al.*, 2013b), we are able to calculate genetic constraints on CHC evolution within each environment and compare these to genetic constraints calculated with inclusion of the between-environment genetic covariances. Together, these analyses describe selection and genetic constraints on male CHCs across environments and allow us to predict how CHC evolution might vary across heterogeneous environments.

Methods

Stock populations

Female *D. simulans* were collected from Greece in April 2010 and their offspring were used to set up a laboratory population. We also used females from this collection to set up inbred lines, for more detail see the section on genetic constraints below. This population was maintained at an approximate size of 500 individuals, with overlapping generations, for 8 months prior to this study. Flies were kept on a cornmeal-based diet (supplied by Applied Scientific, London, UK) at 25 °C.

Environmental manipulations

The experiment was carried out in 7 blocks, with each environmental treatment replicated in approximately equal numbers in each block. We reared male flies from the laboratory population in each of four different experimental environments. We used two different diets: the original cornmeal-based diet (diet 1; 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) and a novel diet (diet 2; made from 1 L deionized water boiled with 102 g brown sugar, 72 g oat bran, 24 g yeast, 12 g agar and 2 g methyl paraben). These diets were chosen purely to create variation in dietary environment rather than to manipulate diet quality *per se*. 100 vials of each of these two diets (40 mL vials with 8 mL of medium) were placed into the population cage for 24 h to allow egg laying, after which point they were removed and incubated at 25 °C on a 10 : 14 h light/dark cycle during offspring development. Peak eclosions occurred after 11 days, and virgin males were collected from each diet. $N = 200$ vials across both diets per block, and one male was collected from each vial, although some vials did not produce a male, and so numbers varied slightly between blocks. This eliminated effects of common rearing environment within each laying vial. Each male was transferred to an individual glass vial containing the same diet experienced during development. Males from each diet were then split equally between two post-eclosion temperatures, 23 °C and 25 °C, creating four treatments: diet 1 at 23 °C (treatment A); diet 2 at 23 °C (treatment B); diet 1 at 25 °C (treatment C); and diet 2 at 25 °C (treatment D). Treatment C closely replicated the standard laboratory environment experienced by the flies. During the 24-h laying period, large vials (150 mL vials with 30 mL of medium) of a potato-based diet (diet 3; supplied by Blades Biological, Kent, UK) were also added to the population cage, to rear virgin females that were used to assess male attractiveness in mating trials. All females were treated identically, in order to create a stock of virgin females from a common environment that was distinct from the experimental treatments used for males, and these females were used in mating trials. Vials were removed from the cage after 24 h and incubated at 25 °C on a 10 : 14 h light/dark cycle during offspring development. Peak eclosion from these vials occurred after 11 days, and virgin females were collected and transferred to individual 40 mL vials containing 8 mL of diet 3 and incubated at 25 °C.

Male attractiveness assays and CHC extraction

Male attractiveness was assessed in mating assays carried out at 3 days post-eclosion between a standard female and a male from one of the treatments. These assays were carried out in the standard environment (diet 3 at 25 °C), such that the environmental manipulation was confined to the male rearing environment, whereas the female rearing environment and assay environment were kept constant to minimize environmental variation in female preferences and focus on male variation. Each assay lasted 3 h during which courtship and mating behaviour were recorded. We measured attractiveness as a binary response – the male either mated or did not mate during the 3-h period. Males that were not observed courting a female at any point in the 3-h assay were excluded from the data set. *Drosophila* females have control over acceptance or rejection of courting males (Speith, 1974; Markow, 1996), and so males that courted and achieved a mating are likely to be more attractive than males that courted but did not mate. Indeed, many previous studies with *Drosophila* and other model insects have used no-choice mating assays to assess overall male attractiveness and female preference, where the choice is whether or not to mate with a given male (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). No-choice assays allow us to uncouple mate choice from male–male competition, which would be confounded in trials using multiple males. Furthermore, in *Drosophila*, studies using single and multimale assays produce qualitatively similar results (e.g. Avent *et al.*, 2008; Taylor *et al.*, 2008).

After mating had occurred, or after 3 h had elapsed in the case of males that did not mate, males and females were separated by aspiration. Females were discarded and males were frozen at −80 °C in individual glass autosampler vials (supplied by Chromacol, Hertfordshire, UK) prior to CHC extraction. Hydrocarbon extractions were carried out in sets of 100 samples per day and randomized throughout by treatment. Hydrocarbon extractions and analysis followed a protocol optimized previously for *D. simulans* (see details in Ingleby *et al.*, 2013b for full details).

Statistical analyses

Principal components analysis

The expression of 22 CHCs was quantified for each male. Log-contrast CHC scores were calculated relative to the size of an internal standard peak within each sample, which corresponded to a fixed 10 ppm concentration of pentadecane added prior to gas chromatography. This log-transformation normalized the data. We ran principal components analysis (PCA) to reduce the dimensionality of the data. PCs were extracted using the correlational matrix for a combined data set including (i) the CHC data from this experiment and (ii) the CHC data from a previous experiment examining the quantitative genetics of CHC expression across the same set of environments (Ingleby *et al.*, 2013b). PCA was

carried out on the combined CHC data to ensure that the derived PCs from selection data in this study and the genetic data (Ingleby *et al.*, 2013b) were equivalent and therefore could be used to estimate genetic constraints within and between environments (see below). We identified multivariate outliers based on Mahalanobis distances and excluded these from the data set, leaving 635 males in the final analysis. We extracted three orthogonal vectors with eigenvalues greater than 1, which cumulatively explained ca. 75% of the total variation in male CHC expression (Table 1). We used factor loadings greater than 0.25 to interpret the biological significance of these vectors (following Tabachnick & Fidell, 1989).

Environmental components of CHC expression

We tested for an environmental component of male CHC expression with a multivariate analysis of covariance (MANCOVA) implemented in SPSS (IBM version 20) as follows: PC1, PC2, PC3 ~ Diet × Temp + Block + ε, where diet, temperature and diet × temperature interaction were fixed effects, experimental block (1–7) was included as a covariate, and ε is the residual variance.

Table 1 Results of principal components analysis for male CHC expression. Biological significance of each component was interpreted from factor loadings > 0.25 (in bold) (Tabachnick & Fidell, 1989). 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			
Octadecadien	**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

Environmental variation in male CHC attractiveness

From the mating assays, males were scored as either 1 (mated) or 0 (unmated) during the 3-h assay. Preliminary analysis with a GLM tested for differences between male rearing environments in overall male attractiveness as follows: Y ~ Diet × Temp + Block + ε, where Y was a binomial 1 or 0 score for mating; diet, temperature and diet × temperature interaction were fixed effects; and block was a covariate.

Further analyses then examined variation in male CHC attractiveness. We quantified the strength and form of linear and nonlinear sexual selection on male CHCs in each environment using a standard multivariate selection analysis (Lande & Arnold, 1983). We calculated individual relative fitness within each environment by dividing individual fitness score by the mean fitness score for each treatment. PCs 1–3 had been calculated across all treatments, and so PC scores were also standardized within each environment. These calculations were carried out within each environment to allow comparisons of the strength and form of linear and nonlinear selection between environments. For each treatment, we fitted a linear regression to estimate $\boldsymbol{\beta}$, the vector of linear (directional) selection on each PC, as follows: relative fitness ~ PC1 + PC2 + PC3 + ε, using the standardized PCs. Next, we estimated the matrix of nonlinear selection, $\boldsymbol{\gamma}$, using a quadratic regression model which incorporated linear, quadratic and correlational selection terms for each PC in each treatment as follows: relative fitness ~ PC1 + PC2 + PC3 + $PC1^2$ + $PC2^2$ + $PC3^2$ + (PC1 × PC2) + (PC1 × PC3) + (PC2 × PC3) + ε. Quadratic regression coefficients were doubled as recommended by Stinchecombe *et al.* (2008). We performed a canonical analysis of $\boldsymbol{\gamma}$ to produce the **M** matrix, which identifies the major axes of nonlinear selection (vectors $\boldsymbol{m_1}$–$\boldsymbol{m_3}$ in this analysis), and used the R code provided by Reynolds *et al.* (2010) to estimate the strength of selection along the eigenvectors and calculate permutation *P* values for the eigenvalues. The vectors of nonlinear selection with the strongest selection gradients for each treatment were plotted using the 'vis.gam' function in the 'mgvc' package in R (v.2.13.0).

In order to test whether the overall strength and form of sexual selection differed between treatments, we used a sequential model building approach (Draper & John, 1988), which uses partial *F*-tests to compare models with and without terms of interest (see also Chenoweth & Blows, 2005). Pairwise comparisons of treatments were used to determine where these differences occurred. When significant differences between pairwise treatments were located, inspection of the individual interaction terms between the form of selection (i.e. linear, quadratic or correlational selection) and PC scores were used to determine which PC score (s) contributed to the overall difference (see Appendix

A of Chenoweth & Blows, 2005 for full details of this analysis).

Then, we tested for differences in the direction of linear selection by calculating the angle between pairwise combinations of the $\boldsymbol{\beta}$ vectors in each treatment. The angle between two vectors, ϕ_L, can be calculated as:

$$\phi_L = \cos^{-1}\left(\frac{a \cdot b}{\|a\|\|b\|}\right) \quad (1)$$

where $a = \beta$ in one treatment and $b = \beta$ in the treatment being compared, and $\|a\| = \sqrt{a \cdot a}$ and $\|b\| = \sqrt{b \cdot b}$. The subscript L refers to the direction of *linear* selection. $\phi_L = 0^o$ indicates the vectors are perfectly aligned and there is no difference between the direction of selection, whereas $\phi_L = 180°$ indicates the maximum possible difference in the direction of selection between environments, as selection vectors have meaningful directionality. To determine the significance of ϕ_L, we estimated the 95% credible interval (CI) of this angle using a novel Bayesian approach, implemented in R (v. 2.13.0) using the 'MCMCglmm' package (Hadfield, 2010). Bayesian inference was used to give a posterior distribution of $\boldsymbol{\beta}$ in each treatment, using the linear regression model specified above, and the angle with associated 95% CI between the vectors was calculated directly from this distribution (R code provided on request to FCI).

Genetic constraints and evolvability

In a previous study (Ingleby *et al.*, 2013b), we used *D. simulans* from inbred lines, or isolines, which were derived from the same genetic background as the outbred population in this study. We reared these flies across the same range of laboratory environments as used here and extracted the same 3 PCs of male CHC expression as for the selection data (as described above). From these data, we were therefore able to estimate the genetic variance–covariance matrix, **G**, for male CHC expression within each of these four environments, denoted $\mathbf{G_A}$–$\mathbf{G_D}$ here for treatments A–D. Note that these genetic estimates are broad-sense (V_G) rather than narrow-sense (V_A) estimates. **G** was estimated from the posterior distribution of a Bayesian multivariate mixed model using the 'MCMCglmm' package as follows: PC1, PC2, PC3 ~ Treatment + Isoline + ε, where treatment is a trait-specific fixed factor with levels A–D denoting treatments, isoline is a 12 × 12 matrix specifying trait- and environment-specific genetic variation, and ε is a 3 × 3 matrix specifying trait-specific residual variances. The full **G** matrix is shown in Table S1, with individual environment-specific **G** matrices highlighted. Using these **G** matrices along with the $\boldsymbol{\beta}$ vectors identified in this study, we calculated the predicted response of male CHC profile to sexual selection, $\Delta\bar{z}$, using the multivariate breeder's equation: $\Delta\bar{z} = \beta\mathbf{G}$ (Lande, 1979), and then tested for genetic constraints by measuring the alignment of $\boldsymbol{\beta}$ and $\Delta\bar{z}$ (Blows & Walsh, 2009) within each environment. Genetic constraint can be estimated as the angle, ϕ_W, between these two vectors using equation (1), adapted so $a = \beta$ and $b = \Delta\bar{z}$, with the subscript w referring to *within* environments. $\phi_W = 0^o$ indicates the vectors are perfectly aligned and there is no constraint; $\phi_W = 90°$ indicates the vectors are orthogonal and there could be a strong constraint. The significance of angle ϕ_W was estimated using the Bayesian approach described above, integrating over uncertainty in both **G** and β by using the posterior distribution for both (R code provided on request to FCI). We also examined evolvability, e, as an additional measure of the ability of a population to evolve in the direction of selection. To do so, we applied the same Bayesian approach outlined above to equation (1) from Hansen and Houle (2008), where $e = (\beta'\mathbf{G}\beta)/|\ \beta\ |^2$.

We then repeated these genetic constraint calculations with inclusion of between-environment genetic covariances, following Via and Lande (1985). These calculations use a **G** matrix expanded across multiple environments as:

$$\mathbf{G}_{\text{full}} = \begin{pmatrix} \mathbf{G}_{\text{A}} & \mathbf{G}_{\text{AB}} & \mathbf{G}_{\text{AC}} & \mathbf{G}_{\text{AD}} \\ \mathbf{G}_{\text{BA}} & \mathbf{G}_{\text{B}} & \mathbf{G}_{\text{BC}} & \mathbf{G}_{\text{BD}} \\ \mathbf{G}_{\text{CA}} & \mathbf{G}_{\text{CB}} & \mathbf{G}_{\text{C}} & \mathbf{G}_{\text{CD}} \\ \mathbf{G}_{\text{DA}} & \mathbf{G}_{\text{DB}} & \mathbf{G}_{\text{DC}} & \mathbf{G}_{\text{D}} \end{pmatrix} \quad (2)$$

where the diagonal consists of the individual **G** matrices for each treatment (A–D), and the off-diagonals contain submatrices with the between-environment genetic (co)variances for each trait. This between-environment genetic variation is not expressed in any individual, but can reveal constraints of shared genetic architecture between environments. $\mathbf{G}_{\text{full}}$ is shown in Table S1. The mean evolutionary response ($\Delta\bar{z}_A - \Delta\bar{z}_D$) of the PCs in each environment was calculated using the hard selection model described by Via and Lande (1985), which we expanded to include four environments as follows:

$$\begin{pmatrix} \Delta\bar{z}_A \\ \Delta\bar{z}_B \\ \Delta\bar{z}_C \\ \Delta\bar{z}_D \end{pmatrix} = \begin{pmatrix} \mathbf{G}_A & \mathbf{G}_{AB} & \mathbf{G}_{AC} & \mathbf{G}_{AD} \\ \mathbf{G}_{BA} & \mathbf{G}_B & \mathbf{G}_{BC} & \mathbf{G}_{BD} \\ \mathbf{G}_{CA} & \mathbf{G}_{CB} & \mathbf{G}_C & \mathbf{G}_{CD} \\ \mathbf{G}_{\text{DA}} & \mathbf{G}_{\text{DB}} & \mathbf{G}_{\text{DC}} & \mathbf{G}_{\text{D}} \end{pmatrix} \begin{pmatrix} [q_A \bar{W}_A/\bar{W}]_{\beta_A} \\ [q_B \bar{W}_B/\bar{W}]_{\beta_B} \\ [q_C \bar{W}_C/\bar{W}]_{\beta_C} \\ [q_D \bar{W}_D/\bar{W}]_{\beta_D} \end{pmatrix}. \quad (3)$$

where q_{A}–q_{D} are the proportions of individuals in each environment, $\bar{W}_A - \bar{W}_D$ are the mean fitness values within each individual environment, and $\bar{W}$ is the mean fitness across all environments. This model uses the mean fitness to weight the contribution of each environment to the overall population (Via & Lande, 1985). This therefore accounts for differential survival across our treatments, although as these differences were subtle, using the alternative soft selection model described in Via and Lande (1985), which does not weight by fitness in this way, gives qualitatively identical results

(results not shown). We used equation (1) and the methods described above to estimate the angle $\phi_{\mathbf{B}}$, where the subscript *B* refers to *between* environments, between $\boldsymbol{\beta}$ and $\Delta\bar{z}$ for each environment to examine whether genetic covariance between environments [as summarized in equation (2)] is likely to constrain the independent CHC evolution between environments. Similarly, we repeated the evolvability calculation as described above using the full **G** matrix as well.

Results

Principal components analysis

Principal component analysis resulted in 3 PCs that collectively explained 71.10% of the total variation in male CHC expression (Table 1). PC1 clearly represented overall investment in CHC production, as each peak was positively and highly loaded to this vector. For PC2, short-chained CHCs were generally negatively loaded, whereas long-chained CHCs positively loaded, and so we interpreted this vector as the balance between short- and long-chained CHCs, with individuals with high PC2 scores biasing production of long-chained CHCs over short-chained CHCs. The interpretation of PC3 was less clear as only 10 of the 22 peaks had loadings over 0.25, but there appeared to be a similar pattern to PC2, as 5 short-chained CHCs were negatively loaded and 4 long-chained CHCs were positively loaded. Seven of the 10 peaks significantly loaded on PC3 were ones that did not contribute significantly to PC2. We therefore interpreted PC3 as an additional vector of variation in the balance between short- and long-chained CHCs, although involving fewer CHCs.

Environmental variation in CHC expression

Temperature had a significant effect on overall male CHC profile, but there was no multivariate effect of either diet or temperature x diet interaction (Table 2). Individual analysis of each of the PCs showed that environmental components of CHC expression were concentrated on PC3, where temperature had a strong effect and there also was an effect of diet (Table 2; Fig. 1). Individuals on diet 2 generally had higher PC3 scores than individuals on diet 1, indicating these individuals invested more in long-chained CHCs than short-chained. On both diets, individuals from the higher temperature had consistently higher PC3 scores than individuals from the lower temperature (Fig. 1), showing that at higher temperature, males invested more in long-chained CHCs.

Environmental variation in male CHC attractiveness

There was a significant difference in the proportion of males mated between dietary treatments ($F_{1,644}$ = 7.598; P = 0.006) but not between temperatures ($F_{1,644}$ = 0.874; P = 0.350). There was no significant block effect ($F_{1,644}$ = 0.206; P = 0.650) or interaction between diet and temperature ($F_{1,644}$ = 0.129; P = 0.720). This analysis demonstrated that males reared in treatment C (diet 1 at 25 °C; which was closest to the environment in which these populations were laboratory-adapted) were the most attractive overall, as the highest proportion of them mated during the 3-h assay; whereas males from treatment B (diet 2 at 23 °C; the treatment which was least similar to the standard laboratory environment) were the least attractive overall (proportion of males mated in treatment A = 0.58 [85/146]; B = 0.48 [79/165]; C = 0.62 [100/161]; D = 0.51 [83/163]).

Table 2 Results of a multivariate analysis of covariance (MANCOVA with 3 PCs of male CHC expression as response variables, diet and temperature as factors and block as a covariate), followed by a breakdown of the component univariate ANCOVAS for each PC. Terms in bold are significant at $P < 0.05$.

	Pillai's trace	*F*	d.f.	*P*
Diet	0.007	1.477	3,368	0.220
Temperature	**0.059**	**13.053**	**3,368**	**<0.001**
Diet × temperature	0.001	0.312	3,368	0.816
Block	0.010	2.203	3,368	0.087
PC1	Diet	0.298	1,630	0.585
	Temperature	0.025	1,630	0.875
	Diet × temperature	0.225	1,630	0.635
	Block	1.179	1,630	0.278
PC2	Diet	0.008	1,630	0.928
	Temperature	0.355	1,630	0.552
	Diet × temperature	0.214	1,630	0.644
	Block	0.610	1,630	0.435
PC3	**Diet**	**3.785**	**1,630**	**0.042**
	Temperature	**33.634**	**1,630**	**<0.001**
	Diet × temperature	0.481	1,630	0.488
	Block	1.438	1,630	0.231

With respect to male CHC attractiveness, in treatment A (diet 1 at 23 °C), there was significant negative linear selection and significant disruptive selection on PC2 (Table 3), showing that males who invested heavily in short-chained CHCs (i.e. extreme negative PC2 scores) were most attractive. There was also significant stabilizing selection on PC1 and correlational selection between PCs 1 and 3 (Table 3), meaning that males from treatment A that produced an intermediate overall amount of CHCs (intermediate PC1 scores) were generally the most attractive.

These trends are reflected in the results of the canonical analysis, which identified significant disruptive and negative directional selection on vector $\boldsymbol{m_1}$, which was heavily loaded for PC2 in treatment A (Table 4). The canonical analysis also identified strong but nonsignificant stabilizing selection on vector $\boldsymbol{m_3}$, which was heavily loaded for PCs 1 and 3 (Table 4). This is clearly seen

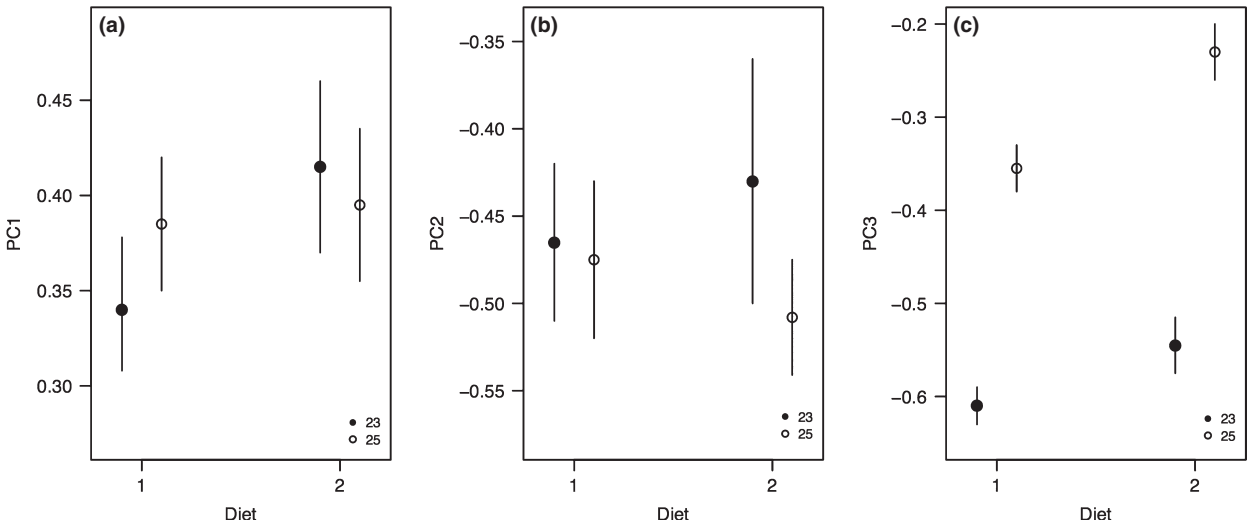

Fig. 1 Mean PC score (± SD) for (a) PC1; (b) PC2; and (c) PC3 across diets 1 and 2, with separate points for 23 °C (filled points) and 25 °C (open points) post-eclosion temperatures.

Table 3 Results of a standard selection analysis (Lande & Arnold, 1983) for sexual selection (through female preference) on 3 PCs of male *D. simulans* CHC expression, across each combination of dietary and temperature environments. The vector of standardized directional selection gradients is shown by $\boldsymbol{\beta}$, and the matrix of standardized quadratic (diagonal) and correlational (below diagonal) selection gradients is shown by $\boldsymbol{\gamma}$. Values in bold are significant ($P < 0.05$) after randomization tests.

		γ		
	β	PC1	PC2	PC3
A: Diet 1, 23 °C				
PC1	0.060	**−0.190**		
PC2	**−0.180**	0.099	**0.304**	
PC3	−0.014	**0.199**	−0.150	−0.156
B: Diet 2, 23 °C				
PC1	0.001	−0.032		
PC2	0.083	0.047	**−0.266**	
PC3	0.025	−0.001	0.013	0.242
C: Diet 1, 25 °C				
PC1	0.047	**−0.206**		
PC2	−0.097	−0.027	**0.156**	
PC3	**0.222**	**0.222**	**0.253**	0.090
D: Diet 2, 25 °C				
PC1	0.040	0.184		
PC2	−0.100	−0.035	**−0.244**	
PC3	**−0.233**	−0.096	0.059	0.140

Table 4 The **M** matrix containing estimates of the strength of linear ($\boldsymbol{\theta_i}$) and nonlinear ($\boldsymbol{\lambda_i}$) selection along eigenvectors m_1–m_3 in each treatment. Values in bold show significant selection at $P < 0.05$ as described in the text.

	M			Selection	
	PC1	PC2	PC3	θ_i	λ_i
A: Diet 1, 23 °C					
m_1	0.083	0.964	−0.252	**−0.165**	**0.352**
m_2	0.709	0.121	0.695	0.011	0.021
m_3	0.700	−0.237	−0.674	0.094	−0.416
B: Diet 2, 23 °C					
m_1	−0.001	0.025	0.999	0.027	0.243
m_2	0.981	0.191	−0.004	0.016	−0.023
m_3	−0.192	0.981	−0.025	0.081	−0.276
C: Diet 1, 25 °C					
m_1	0.224	0.679	0.699	**−0.145**	**0.406**
m_2	−0.580	0.669	−0.464	−0.033	0.004
m_3	0.783	0.302	−0.544	0.077	**−0.371**
D: Diet 2, 25 °C					
m_1	0.768	−0.124	−0.629	**0.190**	0.269
m_2	0.639	0.073	0.766	**−0.160**	0.065
m_3	0.049	0.990	−0.135	−0.065	−0.254

in the fitness surface in Fig. 2a, where fitness peaks are at extremes $\boldsymbol{m_1}$, and particularly the negative extreme, showing high relative fitness of males investing highly in short-chained CHCs. The fitness peaks also correspond to males with intermediate $\boldsymbol{m_3}$ scores, meaning that males with intermediate investment in overall CHC production had high relative fitness.

Attractiveness of male CHCs from treatment C (diet 1 at 25 °C) was very similar to those from treatment A (same diet at a different temperature). PC1 was subject to stabilizing selection, such that intermediate investment in overall amount of CHCs (intermediate PC1 scores) was most attractive (Table 3). Similarly to treatment A, there was also evidence in treatment C that the balance between long- and short-chained CHCs (PC2) was under disruptive selection (Table 3). However, in treatment C, there was also significant positive linear selection on PC3, suggesting that increased

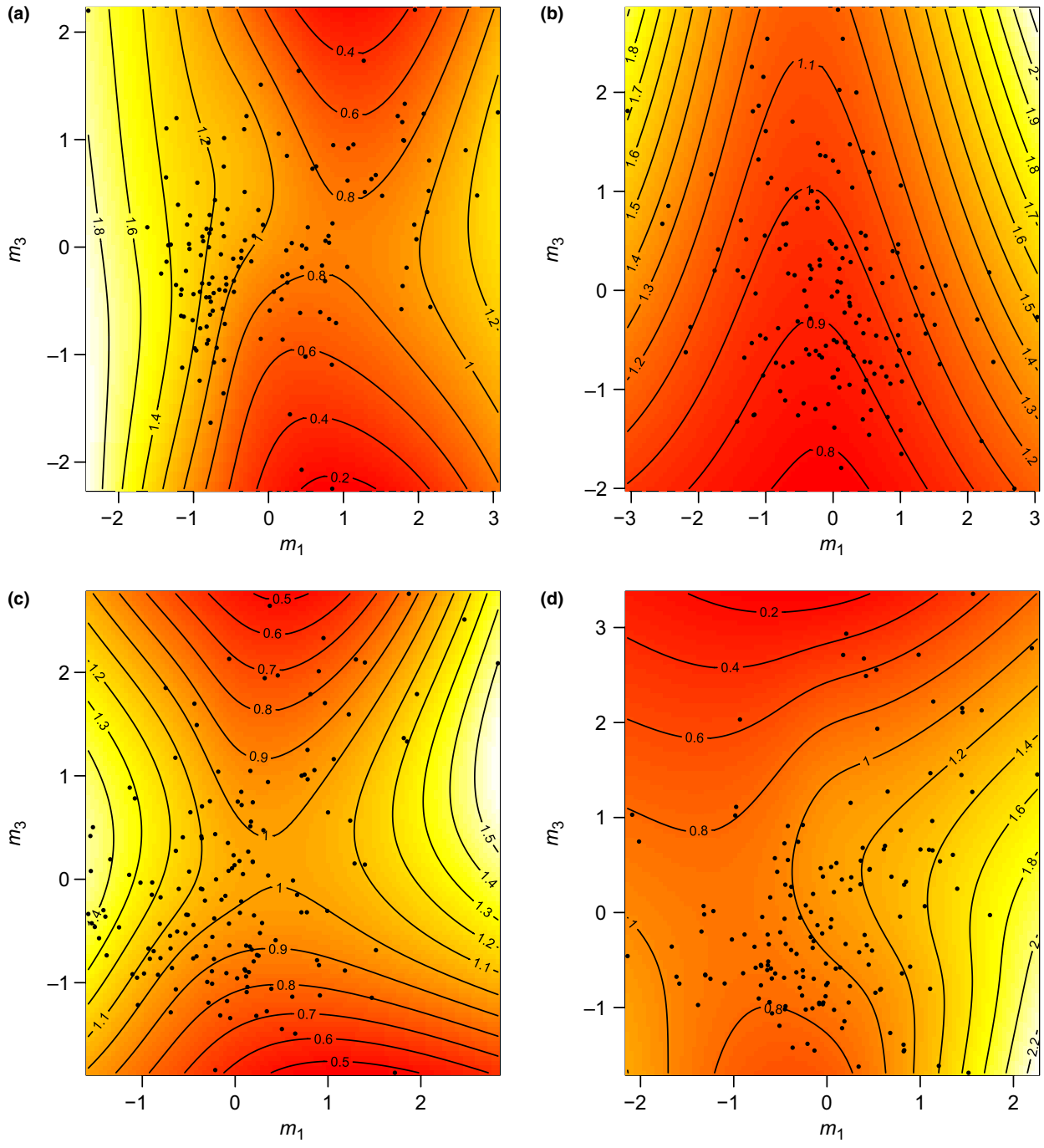

Fig. 2 Fitness surfaces plotted on the two major axes ($\boldsymbol{m_1}$ and $\boldsymbol{m_3}$) of nonlinear sexual selection on male CHCs for (a) diet 1 at 23 °C (treatment A); (b) diet 2 at 23 °C (treatment B); (c) diet 1 at 25 °C (treatment C); and (d) diet 2 at 25 °C (treatment D). Points represent individual males. Contours describe relative fitness within each environment. Pale yellow coloration indicates a peak in the fitness surface, and red indicates a trough.

investment in certain long-chained CHCs was attractive (Table 3).

The fitness surface for this environment (Fig. 2c) was comparable to the surface for treatment A (Fig. 2a). Fitness peaks were at extremes of $\boldsymbol{m_1}$, representing the significant disruptive selection on this vector. As $\boldsymbol{m_1}$ was heavily loaded for both PC2 and PC3 (Table 4), this indicates that males who invested heavily in short-chained

CHCs were most attractive, as found with treatment A. Vector $\boldsymbol{m_3}$ was under significant stabilizing selection and heavily loaded for PC1 (Table 4; Fig. 2c), again suggesting that males with intermediate overall investment in CHCs (intermediate PC1 scores) were most attractive. Patterns of sexual selection on males reared on diet 1 therefore varied little between temperatures.

In treatment B (diet 2 at 23 °C), only PC2 was under significant sexual selection (stabilizing selection; Table 3), suggesting that males with a balance of long- and short-chained CHCs were most attractive in this environment. Consistent with this, the canonical analysis identified vector $\boldsymbol{m_3}$, which was heavily loaded for PC2 and under stabilizing selection (Table 4), although nonsignificant. On the other hand, PC3 (which also represented a balance of particular long- and short-chained CHCs, and was loaded heavily onto vector $\boldsymbol{m_1}$) was under weakly disruptive selection (Table 3). The most attractive males in this environment therefore seemed to produce a very specific balance of long- and short-chained CHCs, although note also that males from this treatment were generally the least attractive of all the treatments (see above; proportion males mated in treatment $B = 0.48$) and most males sit in the trough of the fitness surface in Fig. 2b.

PC2 was under significant stabilizing selection in treatment D (diet 2 at 25 °C; Table 3) such that attractive males in this environment produced a balance of long- and short-chained CHCs, somewhat similar to treatment B (diet 2 at 23 °C). This is reflected in the results of the canonical analysis, which identified (nonsignificant) stabilizing selection on vector $\boldsymbol{m_3}$, which was heavily loaded for PC2 (Table 4). However, in contrast to the patterns of selection in other environments, vector $\boldsymbol{m_1}$ in treatment D (heavily loaded for PCs 1 and 3) showed significant positive directional and nonsignificant disruptive selection (Table 4), perhaps explaining the high peak of fitness at extreme positive $\boldsymbol{m_1}$ values in Fig. 2d. Attractive males in treatment D therefore invested heavily in CHCs overall, with a balance of short- and long-chained CHCs.

Overall, there were greater differences in patterns of selection between diets than between temperatures: selection in treatments A and C was very similar, and selection in treatments B and D had some similarities. These patterns of selection were largely consistent with the results of the sequential models that formally compared the strength of sexual selection between environments. Patterns of linear ($F_{3,619} = 4.453$, $P = 0.004$), quadratic ($F_{3,616} = 7.209$, $P < 0.001$) and correlational ($F_{3,604} = 4.312$, $P = 0.005$) sexual selection were all significantly different between treatments. From the individual interaction terms in the sequential model, we found that differences in linear sexual selection between treatments were driven by PC2 and PC3 (PC1: $F_{1,619} = 0.321$, $P = 0.571$; PC2: $F_{1,619} = 5.888$, $P = 0.016$; PC3: $F_{1,619} = 6.551$, $P = 0.011$); differences in quadratic sexual selection between treatments were attributable to PC2 (PC1: $F_{1,616} = 0.894$, $P = 0.444$; PC2: $F_{1,616} = 5.398$, $P = 0.001$; PC3: $F_{1,616} = 1.248$, $P = 0.292$); and differences in correlational selection were due to differences in selection on PC2 and PC3 between treatments (PC1: $F_{1,604} = 0.410$, $P = 0.746$; PC2: $F_{1,604} = 2.615$, $P = 0.041$; PC3: $F_{1,604} = 2.989$, $P = 0.031$). More generally, differences in sexual selection between environments seem to be attributable to differences in sexual selection on the balance between long- and short-chained CHCs (PCs 2 and 3) rather than on overall CHC investment (PC1).

In terms of the difference in the direction of linear selection between environments, the angles between $\boldsymbol{\beta}$ vectors were quite wide (Table 5), especially between treatments A and B (between diets at 23 °C), B and C (between diets and temperatures) and B and D (between temperatures on diet 2), although the 95% intervals overlap for all pairwise comparisons, indicating that the angles are not significantly different from each other.

Genetic constraints and evolvability

The genetic constraints calculated within each environment individually, ϕ_W, indicated that constraints on CHC evolution were weak and did not differ between environments (95% CI overlap in all comparisons; Table 6a). However, by accounting for between-environment covariances in these calculations to give $\phi_{\mathbf{B}}$, the genetic constraints were stronger, and the difference between ϕ_W and $\phi_{\mathbf{B}}$ was significant for treatments A and B (no overlap of CI between ϕ_W and $\phi_{\mathbf{B}}$ estimates for these treatments in Table 6). This suggests that between-environment genetic covariances could impose a significant genetic constraint in the 23 °C environments, but not in the 25 °C environments. Further, $\phi_{\mathbf{B}}$ was higher for the 23 °C environments than it was the 25 °C environments, although this comparison was only significant for treatment B (no overlap of CI between $\phi_{\mathbf{B}}$ estimates for treatment B with either treatment C or D in Table 6b). There was therefore some

Table 5 The angle, $\phi_{\mathbf{L}}$, between the vectors of *linear* selection ($\boldsymbol{\beta}$) for each pairwise comparison of treatments (A–D). This provides a measure of the difference in the direction of linear selection between each treatment, with 95% credible intervals shown below each angle estimate.

ϕ_L	A	B	C
B	104.70 76.54–136.00		
C	73.15 42.37–100.30	100.50 68.40–134.50	
D	76.76 48.09–102.40	100.80 70.41–133.40	58.54 30.53–79.23

Table 6 Genetic constraint on male CHC profile, estimated as $\phi_{\mathbf{W}}$ and ϕ_B, the angle between the vector of the predicted responses to sexual selection of each PC ($\Delta\bar{z}$) and the vector of linear selection gradients on each PC ($\boldsymbol{\beta}$), with 95% credible interval shown below each estimate. (a) Genetic constraint calculated *within* each environment and (b) calculated with inclusion of the *between*-environment genetic covariances.

Treatment	(a) ϕ_W [95% CI]	(b) ϕ_B [95% CI]
A (diet 1; 23 °C)	19.68	47.82
	12.50–26.69	28.79–60.04
B (diet 2; 23 °C)	29.99	82.34
	23.67–38.32	59.32–106.40
C (diet 1; 25 °C)	19.80	36.79
	15.15–24.58	18.61–46.33
D (diet 2; 25 °C)	20.82	26.02
	17.35–24.90	15.10–31.94

evidence that the genetic constraint on males reared at the lower temperature was higher than those reared at the higher temperature due to genetic covariance in CHC expression between environments.

By examining evolvability as well as the angle between $\boldsymbol{\beta}$ and $\Delta\bar{z}$, we found further support for this pattern of genetic constraints across environments. As can be seen in Table 7, evolvability is significantly reduced in each environment when calculated with inclusion of the between-environment genetic covariances compared with the within-environment estimates. Furthermore, evolvability is lower in the 23 °C treatments compared with the 25 °C treatments, although this difference is nonsignificant. These results provide further evidence for genetic constraints imposed by between-environment covariance, and that these constraints might be stronger at 23 °C than 25 °C.

Discussion

By quantifying sexual selection and the genetic architecture of male CHCs across a range of temperatures and diets, we have provided a comprehensive examination of how male CHC profile could evolve through sexual selection in *D. simulans* and how this could vary between environments. Here, we discuss how the environmental variation in male CHC expression, sexual selection through female choice and genetic architecture might be interpreted for a more detailed understanding of how CHCs evolve.

Table 7 Evolvability, e, of male CHC profile with 95% credible interval, calculated following Hansen and Houle (2008). (a) Evolvability *within* each environment, e_W, calculated using **G** from each treatment (A–D) individually and (b) evolvability, e_B, calculated with $\mathbf{G_{full}}$, therefore including *between*-environment genetic covariances.

Treatment	(a) e_W [95% CI]	(b) e_B [95% CI]
A (diet 1; 23 °C)	0.199	0.063
	0.164–0.231	0.035–0.089
B (diet 2; 23 °C)	0.189	0.020
	0.111–0.246	−0.029–0.060
C (diet 1; 25 °C)	0.243	0.090
	0.192–0.298	0.056–0.123
D (diet 2; 25 °C)	0.249	0.081
	0.189–0.298	0.053–0.103

Environmental variation in male CHC expression

The environmental manipulations successfully altered male CHC phenotypes between treatments, with a particularly strong effect seen between temperatures. Based on what is known about *Drosophila* CHC production, we expected to see a strong effect of temperature during the 3-day post-eclosion period, as this is when CHCs are being produced on the newly eclosed adult cuticle (Ferveur, 2005). Even a small increase in temperature, as shown here, should alter the risk of desiccation and therefore the relative costs of investing in short- versus long-chained CHCs. We found that males at higher temperatures invested more in long- than short-chained CHCs, shown by PC3. These results are therefore consistent with a previous study on this population (Ingleby *et al.*, 2013b) as well as numerous other studies documenting a similar effect of temperature on *Drosophila* CHCs (*D. mojavensis*, Gibbs *et al.*, 1998; *D. melanogaster,* Savarit & Ferveur, 2002; *D. serrata,* Frentiu & Chenoweth, 2010; *D. simulans*, Sharma *et al.*, 2012).

Phenotypic differences in male CHCs between diets were weaker, which might seem surprising given the results of previous studies which have identified fairly strong effects of diet on *Drosophila* CHC expression (e.g. Gosden & Chenoweth, 2011). However, in this population of *D. simulans*, we have found a strong G × E in male CHC expression between these diets (Ingleby *et al.*, 2013b), and so by only looking at population-level diet effects in the present study, we are likely to have overlooked genetic variation for plasticity across diets. Given the weak evidence here of overall dietary effects on male CHC phenotype, the differences between diets in terms of which males were attractive are striking. In addition to male CHCs, there are a number of male sexual traits that influence female mate choice in *Drosophila*, including courtship song and dance (Speith, 1974). It is possible that environmental effects on these and other traits not measured in our study account for some differences in male attractiveness between environments.

Environmental variation in male CHC attractiveness

We found that male CHC attractiveness clearly varied between the diets on which the males were reared, whereas between temperatures, differences in male

CHC attractiveness were subtler. Attractiveness varied between environments both in terms of the overall investment in CHCs (PC1) and the balance of production of long- and short-chained CHCs (PCs 2 and 3), although the results of the sequential models suggested that there was more evidence of differences in attractiveness of PCs 2 and 3 between environments.

Whereas on diet 1 attractive males invested heavily in short-chained CHCs with an intermediate investment in CHCs overall, on diet 2 attractive males were those which produced a balance of long- and short-chained CHCs. Differences in male CHC attractiveness between temperature treatments were less clear, although on diet 2 there was some evidence that at the higher temperature attractive males invested more in overall CHC production, whereas no such trend was found on diet 2 at the lower temperature. The only difference in male CHC attractiveness between temperatures on diet 1 was some evidence that attractive males at the higher temperature might increase investment in some long-chained CHCs (PC3). These trends make sense given the evidence that *Drosophila* invest more in CHCs, and particularly long-chained CHCs, at higher temperatures to protect from the increased risk of desiccation (e.g. Kwan & Rundle, 2009; Foley & Telonis-Scott, 2010). In general, however, it seems that our dietary manipulation had a more consistent effect on male CHC attractiveness than did the temperature manipulation.

We found more evidence of nonlinear than linear sexual selection on male CHCs. The absolute magnitude of linear selection gradients in this study was low: the median $\boldsymbol{\beta}$ gradient was 0.07, compared to that of 0.16 in a large meta-analysis of animals and plant selection studies, and that of 0.18 for linear selection specifically via mating success (Kingsolver *et al.*, 2001). Given that Kingsolver *et al.* (2001) found that sexual selection tended to be stronger than viability selection, it is perhaps surprising to measure such low directional selection gradients on sexual signals. However, the linear selection identified here is of a similar magnitude to that measured on average for male CHCs in the related *D. serrata* (median linear selection gradient of 0.05–0.09; Blows *et al.*, 2004; Chenoweth & Blows, 2005).

In contrast, the nonlinear selection gradients estimated in our study were strong. In fact, the median absolute gradient for disruptive/stabilizing selection in our study was 0.19, compared to 0.01–0.02 in studies of *D. serrata* (Blows *et al.*, 2004; Chenoweth & Blows, 2005) and 0.10 in the meta-analysis, although the median for nonlinear selection via mating success was stronger at 0.16 (Kingsolver *et al.*, 2001). These gradients are fairly evenly distributed around 0, suggesting that neither stabilizing nor disruptive selection is predominant in our study, similar to the results of Kingsolver *et al.* (2001), and so both these forms of selection could impact of male CHC evolution. Furthermore, from the patterns of nonlinear selection identified using both the standardized gradients and the canonical analysis, it seems that nonlinear sexual selection could drive male CHC evolution in opposing directions on the different diets. For instance, the balance between long- and short-chained CHCs was under disruptive selection on diet 1 but stabilizing selection on diet 2. Interestingly, although it might be expected that this balance between long- and short-chained CHCs would be under stabilizing selection at the higher temperature, where desiccation stress would favour the production of long-chained CHCs but sexual selection would favour the production of short-chained pheromonal CHCs, this was only true on one diet but not the other. Overall, it seems that nonlinear selection is likely to more strongly affect male CHC evolution than linear selection, contrary to the results of Kingsolver *et al.* (2001).

In this experiment, male rearing environment was manipulated, whereas the female rearing environment and the mating assay environment were kept constant. By doing so, the phenotypic distribution of male CHC profiles was altered between treatments, but there was very limited potential for female choice to be directly affected by environmental variation. This means that we quantified and compared fitness surfaces for male CHC attractiveness in each environment as opposed to measuring what females from different environments prefer. Although this allows us to focus on the effect of the environment on males, variation in female CHC profile between environments (shown in Ingleby *et al.*, 2013b) could also affect the outcome of a mating interaction. In addition, female preference itself might vary when the female environment is manipulated (Cotton *et al.*, 2006), which has been demonstrated in several species, including *D. serrata* (Rundle *et al.*, 2005) and crickets (Hunt *et al.*, 2005). In this population of *D. simulans*, we found G × E variation but little evidence of an overall environmental effect in female mate choice between temperatures (Ingleby *et al.*, 2013a). The importance of examining both male and female perspectives of a mating interaction across different environments is highlighted further by the few studies that have attempted to do so. For instance, in guppies, whereas male sexual signalling behaviour varied between different water flow environments, female preference was unaffected (Head *et al.*, 2010). The potential for environmental variation in female traits will undoubtedly complicate the picture yet further than we have explored here, and so in future work, it will be necessary to examine how the male and female sides of the mating interaction might vary across environments.

Genetic constraints on male CHC evolution

Whether or not male CHC profile evolves in response to selection will depend on the genetic architecture underlying CHC expression, and how this variation

aligns with the direction of selection (Blows & Walsh, 2009). Within each environment we studied, the genetic constraint on male CHC evolution was low, with the angle between the vector of linear selection and the vector of the predicted response to selection ranging between 19 and 30°. These results indicate much weaker genetic constraints on *D. simulans* CHC evolution than has been identified in other *Drosophila* species. For example, constraints of approximately 75° in *D. serrata* (Blows *et al.*, 2004) and 88° in *D. bunnanda* (Van Homrigh *et al.*, 2007) suggest there is far less potential for **G** to bias the evolution of male CHCs through sexual selection in *D. simulans*.

Previous studies have used experimental evolution to demonstrate that CHC profiles can evolve through sexual selection in both *D. serrata* (Blows, 2002; Chenoweth & Blows, 2005; Chenoweth *et al.*, 2008; Rundle *et al.*, 2009) and *D. simulans* (Sharma *et al.*, 2012). Importantly, an interaction between the effects of natural and sexual selection on CHCs (Blows, 2002) implies that variation in the physical environment might cause differences in patterns of sexual selection and the evolution of CHC profile, thus highlighting the importance of examining genetic constraints in different environments (Sgro & Hoffmann, 2004).

In the present study, it was clear from both measures of genetic constraint that between-environment genetic covariances underlying male CHC expression created a constraint on the independent evolution of male CHCs in different environments. Further, there was some evidence that this constraint was greater on male CHC evolution in the lower temperature than in the higher temperature. These results could, in part, be explained by the results of a previous study where a strong G × E was found in male CHC expression across these diets, but not across these temperatures (Ingleby *et al.*, 2013b). G × E across diets will generate the potential for the response to selection to differ between diets and genetic variation to be maintained. Between temperatures, on the other hand, there was very little evidence for G × E (Ingleby *et al.*, 2013b), nor for consistent differences in sexual selection (this study), but there was clearly a strong overall temperature effect on male CHC phenotype, not only in this population (Ingleby *et al.*, 2013b; this study) but also in *Drosophila* more generally (e.g. Savarit & Ferveur, 2002; Frentiu & Chenoweth, 2010). Persistent selection at the temperature these flies have evolved at in the laboratory could have depleted genetic variation in CHC plasticity between temperatures, creating the genetic constraint we find here when they are subject to a new temperature.

Similarly, other studies have shown that genetic constraints can be higher in novel or stressful environments than in benign environments or environments to which a population has adapted. In *D. serrata*, for instance, there was some evidence that **G** was more closely aligned with the direction of linear selection in laboratory conditions than field conditions (Hine *et al.*, 2004). In a wild population of Soay sheep, lower genetic correlations between traits in a favourable environment than in a stressful environment indicated that genetic constraints might be stronger in the stressful environment (Robinson *et al.*, 2009).

In summary, this study has shown that male *D. simulans* CHC profile is subject to sexual selection through female mate choice, but the predicted evolutionary response of male CHCs can differ substantially between environments. These results emphasize the importance of multivariate and cross-environment studies of sexual selection and reveal the potential for evolutionary trajectories of sexual traits to greatly differ between environments and populations.

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Supporting information

Additional Supporting Information may be found in the online version of this article:
Table S1 G matrix for PC1-PC3 calculated across all treatments (A–D), using data from Ingleby *et al.* (2013b).

Data deposited at Dryad: doi:10.5061/dryad.r06rn

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