JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12740

Female choice for male cuticular hydrocarbon profile in decorated crickets is not based on similarity to their own profile

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Keywords:

cuticular hydrocarbon;
genetic compatibility;
Gryllodes sigillatus;
mate choice;
sexual selection.

Abstract

Indirect genetic benefits derived from female mate choice comprise additive (good genes) and nonadditive genetic benefits (genetic compatibility). Although good genes can be revealed by condition-dependent display traits, the mechanism by which compatibility alleles are detected is unclear because evaluation of the genetic similarity of a prospective mate requires the female to assess the genotype of the male and compare it to her own. Cuticular hydrocarbons (CHCs), lipids coating the exoskeleton of most insects, influence female mate choice in a number of species and offer a way for females to assess genetic similarity of prospective mates. Here, we determine whether female mate choice in decorated crickets is based on male CHCs and whether it is influenced by females' own CHC profiles. We used multivariate selection analysis to estimate the strength and form of selection acting on male CHCs through female mate choice, and employed different measures of multivariate dissimilarity to determine whether a female's preference for male CHCs is based on similarity to her own CHC profile. Female mating preferences were significantly influenced by CHC profiles of males. Male CHC attractiveness was not, however, contingent on the CHC profile of the choosing female, as certain male CHC phenotypes were equally attractive to most females, evidenced by significant linear and stabilizing selection gradients. These results suggest that additive genetic benefits, rather than nonadditive genetic benefits, accrue to female mate choice, in support of earlier work showing that CHC expression of males, but not females, is condition dependent.

Introduction

Good genes models and models focusing on genetic compatibility describe different kinds of indirect genetic benefits that can drive mate choice evolution (e.g. Andersson, 1994; Zeh & Zeh, 1996, 1997; Tregenza & Wedell, 2000; Mays & Hill, 2004; Neff & Pitcher, 2005; Puurtinen *et al.*, 2009; Hunt & Sakaluk, 2014). In good genes models, females use an absolute criterion to choose their mates, and males of high genetic quality, as signalled by elaborate display traits, are universally attractive to females. In contrast, according to the genetic compatibility models, females base their choice on a relative criterion, and male attractiveness depends on their own genotype: females prefer males that are genetically dissimilar (Mays & Hill, 2004; Kempenaers, 2007). The choice for good genes generates offspring that inherit better-than-average alleles, whereas the choice for compatibility results in offspring that have an optimal combination of maternal and paternal genes, and who benefit from heterozygosity at some or many loci (Tregenza & Wedell, 2000; Kempenaers, 2007; Charlesworth & Willis, 2009; Puurtinen *et al.*,

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2009). Although it is assumed that good genes are revealed by condition-dependent display traits (e.g. Rowe & Houle, 1996; Hunt *et al.*, 2004), the mechanism by which compatibility genes are detected is more puzzling.

Any evaluation of the genetic similarity of a prospective mate would first require a female to assess the genotype of the male, and then to compare it with her own. Of course, precopulatory evaluation of males might not be required at all if some form of post-copulatory mechanism favoured dissimilar males, as when sperm from more distantly related mates are more likely to fertilize a female's eggs (Olsson *et al.*, 1996; Zeh & Zeh, 1997; Gasparini & Pilastro, 2011). However, a growing body of evidence from a wide array of taxa, including humans (Wedekind *et al.*, 1995), mice (Penn, 2002), lizards (Olsson *et al.*, 2003) and sticklebacks (Aeschlimann *et al.*, 2003), suggests that a precopulatory assessment of genetic compatibility often is mediated by olfactory cues. In the vertebrate examples cited above, for example, females appear to assess genetic similarity at the major histocompatibility complex (MHC), a key gene complex in vertebrate immune function, using MHC-associated odours. In insects too, there is the potential for olfactory cues to reveal underlying genotype. Cuticular hydrocarbons (CHCs), the lipid substances embedded in the exoskeleton of most insects, often exhibit substantial phenotypic and genetic variation (e.g. Foley *et al.*, 2007; Thomas & Simmons, 2008; Van Zweden *et al.*, 2010) for mates to assess genetic similarity. Indeed, cockroaches have been shown to prefer nonsiblings as mating partners, and this inbreeding avoidance seems to be mediated by CHCs (Lihoreau & Rivault, 2008). In spotted cucumber beetles (Ali & Tallamy, 2010) and field crickets, *Teleogryllus oceanicus* (Thomas & Simmons, 2011), females preferentially mate with partners sharing a more dissimilar CHC profile. Moreover, similarity in CHC profiles between mating pairs was positively correlated with genetic similarity in *T. oceanicus*, suggesting that an individual's CHC profile maps closely to their underlying genotype (i.e. a high odour-genes covariance, Thomas & Simmons, 2011).

In general, CHCs have been shown to be the target of sexual selection and to affect male attractiveness in a number of insect species (Steiger & Stökl, 2014). A number of studies have revealed significant multivariate directional and stabilizing selection acting on CHCs (Chenoweth & Blows, 2005; Thomas & Simmons, 2009; Steiger *et al.*, 2013; Ingleby *et al.*, 2014), a strong indication that females use an absolute criterion to choose their mates and that CHCs are indicators of genetic quality rather than compatibility. Preferences for good genes and preferences for compatibility, however, are not necessarily mutually exclusive processes (Colegrave *et al.*, 2002; Roberts & Gosling, 2003; Mays & Hill, 2004; Puurtinen *et al.*, 2009).

At the most fundamental level, this arises because a given allele can contribute to additive genetic variation (i.e. good genes variation), to non-additive genetic variation (i.e. compatibility genes variation), or to both, depending on its frequency in the population (Puurtinen *et al.* 2009). This means that both mechanisms of mate choice can operate simultaneously in a population, as has been demonstrated empirically in mice (Roberts & Gosling, 2003). The decision to mate with the highest quality or most compatible male in the population will then, in theory, depend on the cost of incompatibility and the availability of mechanism(s) that facilitate post-copulatory female choice (e.g. sperm selection; Colegrave *et al.* 2002). A further level of complexity in the study of mate choice for male CHCs is the rapid plastic response of this trait to social interactions (Petfield *et al.* 2003, Krupp *et al.* 2008). For example, male *Drosophila serrata* adjust their CHC profile to match their female partner's profile within minutes of interacting (but without mating) and the extent of this plasticity is comparable to the change in CHCs that occurs by physical transfer during mating (Petfield *et al.* 2003). Similar rapid and plastic responses have also been observed in the CHC profiles of male *D. melanogaster* in response to the social environment (i.e. uniform or mixed-genotype social groups, Krupp *et al.* 2008), as well as the abiotic environment (i.e. light and time of day, Kent *et al.* 2007). The full extent of this rapid plasticity in CHC expression in other species, however, is poorly understood.

Decorated crickets, *Gryllodes sigillatus*, represent an ideal model system with which to measure sexual selection acting on male CHCs, and to test the hypothesis that females rely on CHCs to assess males in terms of genetic compatibility. Females mate frequently throughout their lifetime (Sakaluk *et al.*, 2002), but prefer novel males over previous mates (Ivy *et al.*, 2005). Females mating polyandrously produce more offspring surviving to adulthood compared with those mating monogamously or only a single time (Ivy & Sakaluk, 2005), suggesting that indirect genetic benefits are important to female fitness, whereas material benefits play a subordinate role. Females recognize and discriminate against previous mating partners by imbuing males with their own unique CHC cues during copulation (Ivy *et al.*, 2005; Weddle *et al.*, 2013), which renders males' profiles more similar to those of their mates. Experimental perfuming of males with female CHCs revealed a female aversion towards males bearing chemical cues similar to their own (Weddle *et al.*, 2013). These results suggest that females rely on a chemosensory self-referencing mechanism in which they use their own CHC cues to discriminate against previous mates. In fact, a recent study found unequivocal evidence of such a mechanism based on 'online processing', in which a female relies on her own current CHC phenotype to evaluate the CHC profiles of males, thereby facilitating avoidance of previ-

ous mates (Capodeanu-Nägler *et al.*, 2014). Experimental alteration of a female's CHC profile induced females to make predictable recognition errors (Capodeanu-Nägler *et al.*, 2014).

As CHCs of *G. sigillatus* are highly variable and exhibit significant genetic variation (Weddle *et al.*, 2012, 2013), they appear to offer a reliable indicator of an individual's underlying genotype. Consequently, we might predict that a female would employ the chemosensory self-referencing described above not only to detect her own CHC signature on a previous mate, but to evaluate a male's genetic similarity to avoid inbreeding and thereby enhance offspring fitness via genetic compatibility. Indeed, a quantitative genetic study using a full diallel cross among inbred lines revealed that females can, in fact, potentially gain genetic benefits for their offspring by mating with genetically compatible males (Ivy, 2007). However, this same study showed that females also secure indirect fitness benefits through paternally derived genes, supporting the good genes hypothesis (Ivy, 2007). Whether one or the other form of genetic benefit is promoted by a precopulatory mate choice based on CHCs is currently unknown.

Here, we determine whether female decorated crickets prefer certain males over others based on males' CHC profiles and whether any such preference is related to their own CHC profiles. We first use a multivariate selection analysis (Lande & Arnold, 1983) to estimate the strength and form of linear and nonlinear sexual selection acting on male CHCs through female mate choice. Then, we use a number of different measures of multivariate dissimilarity (the Bray–Curtis, Canberra and Euclidean indices) to determine whether a female's preference for male CHCs is based on similarity to her own CHC profile. We predicted that the mating preferences of females would be influenced by male CHCs, as has been documented in a range of other cricket species (Thomas & Simmons, 2009; Steiger *et al.*, 2013). If these preferences are contingent, in part, on the avoidance of genetic incompatibility, we further predicted that females would prefer males with CHC profiles that were dissimilar to their own. In contrast, if female preferences are predicated on a 'good genes' assessment, we predicted that any preference for male CHCs would be unrelated to females' own CHC profiles.

Material and methods

Experimental animals

Crickets used in this study were descendants from approximately 500 adult animals collected in Las Cruces, New Mexico, in 2001 that were used to initiate a laboratory population maintained at approximately 5000 panmictically breeding individuals (Ivy *et al.*, 2005). Since its initiation, the colony has consistently produced at least 150 new adults per week, and has not experienced any genetic bottlenecks. Crickets were kept in 55-L plastic containers and maintained in an environmental chamber on a 14 : 10 light : dark cycle at 28 °C. Animals were provided with Flukers® cricket chow (Fluker Farms, Baton Rouge, LA, USA) *ad libitum*, water supplied in 40-mL plastic tissue culture flasks plugged with cotton rolls, and egg cartons to provide shelter and to increase surface area for rearing nymphs. Moistened peat moss presented in small petri dishes was provided as an oviposition substrate and served as an additional source of water.

Experimental animals were removed from culture as fourth-instar nymphs and housed separately in same-sex groups until eclosion to adulthood. Newly eclosed crickets were collected daily and housed in individual containers (5 × 5 × 5 cm) where they were provided with water (5-mL test tubes plugged with cotton wool) and food (Flukers® cricket chow) *ad libitum*, and the containers cleaned weekly. This ensured that crickets were virgin during our mating trials and that there was no transfer of CHC between individuals. All crickets were maintained in these containers for 10–14 days until use in mating trials.

Mating trials

Female choice trials were performed as 'no-choice' trials involving a single male, using mating success as a measure of female mate choice. This protocol has been validated as a powerful method of assessing female mating preferences in other cricket species (e.g. Shackleton *et al.*, 2005) and has been successfully employed in previous work on *G. sigillatus* (Ivy & Sakaluk, 2007). Experimental crickets were 7–10 days old, and both males and females were used only once. Males used in trials were virgins, and all females had mated a single time prior to the trial. Females were mated prior to trials to ensure that they were choosing to mate with a male based on their CHC profile rather than simply to receive sperm from the male. Using virgin females in mating trials cannot separate between these two alternatives and may seriously underestimate the importance of female choice and its effect on the evolutionary process (e.g. Zeh & Zeh, 2007). A male was first placed in a clear plastic arena (30 × 18.5 × 11.5 cm), illuminated by red light and maintained at 30 °C, and allowed to acclimate for a minimum of 60 sec before the female was introduced. The male was given 20 min to initiate courtship after the female had been introduced into the arena. If the male failed to do so in this time period, he was excluded from the study, as it was deemed that the female had no way to assess his quality. A mating was recorded as successful if the male transferred a spermatophore in this 20-min period.

Table 1 Principal component (PC) analysis of male CHCs in *Gryllodes sigillatus*. PCs with eigenvalues exceeding 1 were considered biologically important and retained for multivariate selection analysis. For each PC, factor loadings exceeding |0.30| were interpreted as biologically important (in bold).

	PC1	PC2	PC3	PC4
Eigenvalue	6.420	5.872	1.554	1.064
% variance	35.664	32.623	8.631	5.909
Loadings				
7-MeC_{33}	**0.938**	0.049	−0.158	−0.085
5-MeC_{33}	**0.876**	0.253	−0.226	−0.021
3-MeC_{33}	**0.843**	0.114	−0.291	0.039
3,7-diMeC_{33}	**0.879**	0.098	−0.076	0.008
7-C_{35}ene	**0.543**	0.026	−0.237	**0.609**
3,13-diMeC_{36}	**0.398**	0.268	−0.141	**0.467**
5,9-diMeC_{36}	**0.453**	**0.728**	−0.275	−0.030
5,9-C_{37}diene	**0.321**	**0.832**	**0.354**	0.089
3,9-C_{37}diene	**0.383**	**0.649**	**0.563**	0.162
9,31-C_{37}diene	**0.317**	**0.895**	0.011	−0.192
7,31-C_{37}diene	**0.361**	**0.791**	0.232	−0.103
9,31-C_{38}diene	**0.756**	−0.167	−0.049	**−0.449**
Alkatriene ($C_{39}H_{74}$)	**0.592**	**−0.329**	**0.636**	−0.007
Alkatriene ($C_{39}H_{74}$)	**0.433**	**−0.653**	**0.513**	0.145
9,31-C_{39}diene	**0.703**	**−0.516**	−0.022	**−0.352**
7,31-C_{39}diene	**0.457**	**−0.761**	0.187	0.017
Alkatriene ($C_{41}H_{78}$)	**0.399**	**−0.772**	−0.007	0.188
9,31-C_{41}diene	**0.421**	**−0.797**	−0.185	0.019

Chemical analysis of cuticular hydrocarbons

Experimental crickets were freeze-killed at −80 °C for 10 min immediately after mating trials. CHCs were then extracted by whole-body immersion in 2 mL of hexane (Fisher Scientific, UK, H303-4) for 10 min. The extract was transferred to a 2-mL screwcap Target DP vial with Teflon/rubber septa and stored at 4 °C until further analysis. Prior to analysis, the hexane solvent in all samples was evaporated in a fume hood under N_2 gas. CHCs were then re-suspended in 1 mL of hexane containing 100 ppm dodecane as an internal standard, and this extract was used to quantify the CHC profile of each cricket.

The CHC profile of *G. sigillatus* consists of 18 individual CHC components, ranging in chain length from C_{33} to C_{41}, and consisting of a mixture of branched alkanes, alkenes, alkadienes and alkatrienes (Weddle *et al.*, 2013). Previously, we have used a combination of mass spectrometric characterization and dimethyl disulphide derivatives (DMDS) to identify 15 of the 18 CHC compounds, the exceptions being the position of the double bonds in three alkatrienes (see Table 1) that could not be located due to low abundance of DMDS derivatives (Weddle *et al.*, 2013). We have also shown that CHC profiles in *G. sigillatus* are sexually dimorphic and that this dimorphism is 'quantitative' rather than 'qualitative'; that is, males and females have the same CHC compounds, but differ in the relative amount of these compounds. Following the protocols outlined in Weddle *et al.* (2013), we quantified the CHC profiles of male and female *G. sigillatus* using gas chromatography–mass spectrometry (GC-MS). CHC extracts were run on an Agilent Technologies 7890A gas chromatograph (GC) coupled to an Agilent 5975B inert mass spectrometer (MS). A 1-μL volume of each sample was injected using an Agilent G6500 CTC PAL autosampler chilled to 5 °C onto a DB5-HT column (30 m × 0.25 mm ID × 0.1 μm film thickness). The optimal temperature programme for maximum separation of CHCs was as follows: hold at 100 °C for one minute, ramp from 100 °C to 350 °C at 7.5 °C min^{-1}, and hold at 350 °C for 4 min with a flow rate of 1.0 mL min^{-1} for the helium carrier gas (total run time per sample = 38.33 min). The area under each CHC peak was quantified using MSD Chemstation software (version E.02.00.493; Agilent Technologies, Cheshire, UK) with ions 55 and 57 set as the target ions for unsaturated and saturated compounds, respectively.

Prior to statistical analysis, the area under each CHC peak was divided by the area of the internal standard (dodecane) to control for drift in the sensitivity of the GCMS over time. Thus, in contrast to CHC studies that divide the area under each CHC peak by the total area of all CHC peaks (e.g. Thomas & Simmons, 2009), our dataset is not compositional in structure (i.e. the proportion of CHCs does not sum to 100%) and therefore is not subject to unit-sum constraint (Aitchison 1986). This proportion was then $\log_{10}$ transformed to ensure normality of each CHC peak in our dataset (Weddle *et al.*, 2012, 2013). This is not to be confused with a log contrast transformation that is used to remove unit-sum constraint in a compositional dataset (Aitchison 1986).

Statistical analysis

In total, 456 mating trials were performed for which both behavioural and GC-MS data were available for both crickets in a pair.

Multivariate selection analysis of male CHCs

Due to the large number of CHCs being examined (Table 1), we extracted principal components (PCs) based on the correlation matrix and retained PCs with eigenvalues exceeding 1 for multivariate selection analysis (Tabachnick & Fidell, 2001). In total, 4 PCs were retained for further analysis based on this criterion. We interpret factor loadings exceeding |0.30| as biologically important (Tabachnick & Fidell, 2001).

We used standard multivariate selection analysis (Lande & Arnold, 1983) to evaluate the strength and form of linear and nonlinear selection acting on male CHC profiles. An absolute fitness score was assigned to each male in our experiment, with one being assigned

to males that successfully obtained a mating and zero being assigned to males that were unsuccessful following courtship. Following Lande & Arnold (1983), this absolute fitness score was transformed to relative fitness by dividing by the mean absolute fitness of the population. To estimate the standardized linear selection gradients (β), a first-order linear multiple regression model was fitted using the four PCs describing male CHC composition as the predictor variables, and relative fitness as the response variable (Lande & Arnold, 1983). We used a second-order quadratic multiple regression model that included all linear, quadratic, and cross-product terms to estimate the matrix of nonlinear selection gradients (γ) that describes the curvature of the fitness surface. Quadratic regression coefficients are known to be underestimated by a factor of 0.5 using standard multiple regression analysis, so we doubled the quadratic selection gradients derived from this model (Stinchcombe *et al.*, 2008). As relative fitness does not conform to a normal distribution, we used a resampling procedure to assess the significance of our standardized selection gradients (Mitchell-Olds & Shaw, 1987). We randomly shuffled relative fitness scores across males in our data set to obtain a null distribution for each selection gradient where there is no relationship between PCs and relative fitness. We used a Monte Carlo simulation to determine the number of times (out of 9999 iterations) that each gradient pseudo-estimate was equal to or less than the original estimated gradient, and this was used to calculate a two-tailed probability value for each selection gradient in the model following the protocol outlined in Manly (1997). We conducted separate randomization tests for the linear multiple regression model and the full quadratic model.

As the strength of nonlinear selection gradients can be underestimated by interpreting the size and significance of individual $\boldsymbol{\gamma}$ coefficients (Blows & Brooks, 2003), we explored the extent of nonlinear selection acting on male CHCs by conducting a canonical analysis of the $\boldsymbol{\gamma}$ matrix to locate major eigenvectors of the fitness surface (Phillips & Arnold, 1989). We used the permutation procedure outlined in Reynolds *et al.* (2010) to determine the strength and significance of nonlinear selection operating along the eigenvectors of $\boldsymbol{\gamma}$, as the conventional 'double regression' method (DR, Bisgaard & Ankenman, 1996) that is commonly used is known to inflate the type 1 error rate. This procedure, however, does not estimate the strength of linear selection operating along the eigenvectors of $\boldsymbol{\gamma}$. We therefore used the DR method to estimate the strength of linear selection along these eigenvectors, as this method is unlikely to inflate type 1 error (R. J. Reynolds pers. comm.). The strength of linear selection along each eigenvector ($\mathbf{m}_i$) is given by theta ($\boldsymbol{\theta}_i$), whereas the strength of nonlinear selection is given by their eigenvalue ($\boldsymbol{\lambda}_i$).

We used thin-plate splines (Green & Silverman, 1994) to visualize the major eigenvectors from the fitness surface extracted from the canonical rotation of the γ. We used the *Tps* function in the FIELDS package in R (version 2.13.0, www.r-project.org) to fit the thin-plate splines, and visualized splines as a contour map using the value of smoothing parameter (λ) that minimized the generalized cross-validation score (Green & Silverman, 1994).

Multivariate dissimilarity in the CHC profile of males and females in a mating pair

To determine whether female preference for male CHC profiles is based on similarity (or dissimilarity) to their own CHC profile, we estimated three different multivariate dissimilarity indices for each mating pair in our experiment: Bray–Curtis index, Canberra index and Euclidean index (Quinn & Keough, 2002). Although these indices all measure multivariate dissimilarity based on continuous variables, they do so in different ways. The Bray–Curtis index (d_{BC}) was estimated as:

$$d_{BC} = \frac{\sum_{k=1}^{n} |x_{ik} - x_{jk}|}{\sum_{k=1}^{n} (x_{ik} + x_{jk})} \quad (1)$$

where x_{ik} and x_{jk} represent the kth CHC component in males and females, respectively. d_{BC} ranges between zero (completely similar CHC profiles in the sexes) and one (completely dissimilar CHC profiles in the sexes) and is heavily influenced by high CHC abundances in the data set because these are more likely to be different between the sexes (Quinn & Keough, 2002). The Canberra Index (d_C) was estimated as:

$$d_C = \frac{1}{k} \sum_{k=1}^{n} \frac{|x_{ik} - x_{jk}|}{(x_{ik} + x_{jk})} \quad (2)$$

where k is the number of CHC components in males and females combined minus the number of CHC components they share (in our case, $k = 18$; Wolda, 1981). Standardizing by k ensures d_C has an upper limit of one when males and females have completely dissimilar CHC profiles. d_C is less influenced by high CHC abundances in the data set compared to d_{BC} (Quinn & Keough, 2002). The Euclidean Index (d_E) was estimated as:

$$d_E = \sqrt{\sum_{k=1}^{n} (x_{ik} - x_{jk})^2} \quad (3)$$

and therefore represents a simple geometric measurement of the distance between two objects in multidimensional space. d_E is only bounded by zero when males and females have exactly the same CHC profiles and has no upper limit (Quinn & Keough, 2002).

Table 2 The vector of standardized linear selection gradients ($\boldsymbol{\beta}$) and the matrix of standardized quadratic and correlational gradients ($\boldsymbol{\gamma}$) for male CHCs in *Gryllodes sigillatus*. Randomization tests: $^{**}P < 0.01$; $^{***}P < 0.001$.

		γ			
	β	PC1	PC2	PC3	PC4
PC1	0.006	−0.004			
PC2	−0.036	−0.020	0.020		
PC3	−0.013	0.029	0.007	−0.124**	
PC4	−0.088**	0.026	0.006	0.003	−0.150***

Our multivariate dissimilarity indices were highly correlated (all correlation coefficients exceeded 0.73). We therefore examined differences in our dissimilarity indices using a multivariate analysis of variance (MANOVA) including mating success as a fixed effect (i.e. successful or not) and the three dissimilarity measures as the response variables. This overall multivariate model was followed by univariate ANOVAS to determine how the individual response variables contributed to any overall multivariate effect. Prior to analysis, we $\log_{10}$ transformed our dissimilarity indices to ensure normality. However, we present raw indices in our figures for ease of interpretation.

Results

Multivariate selection analysis on male CHCs

To reduce the dimensionality of our CHC data set, we used PC analysis. PC analysis of the 18 individual CHC components yielded 4 PCs with eigenvalues exceeding 1, which collectively explain 82.83% of the total variation in male CHC expression (Table 1). PC1 accounts for 35.66% of the variance in male CHC expression and is positively loaded to each CHC component. Consequently, this vector represents the absolute amount of CHCs possessed by males (Table 1). PC2 explains a further 32.62% of the total variation in male CHC expression and is positively loaded to shorter-chained CHCs (less than C_{37}) and negatively loaded to longer-chained CHCs (greater than C_{37}). This vector therefore represents the trade-off between long- and short-chained CHCs (Table 1). PC3 explains a further 8.63% of the total variation in male CHC expression and is positively loaded to four CHCs: 5,9-C_{37}diene, 3,9-C_{37}diene and two unidentified alkatrienes ($C_{39}H_{74}$) (Table 1). This vector therefore represents the absolute amount of these four specific CHCs possessed by males. PC4 explains the remaining 5.91% of the total variation in male CHC expression and is positively loaded to two CHCs (7-C_{35}ene and 3,13-diMeC_{36}) and negatively loaded to two CHCs (9,31-C_{38}diene and 9,31-C_{39}diene) (Table 1). This vector therefore represents the trade-off between these specific CHCs.

To determine if females prefer males based on the above CHC vectors we used multivariate selection analysis. Standardized linear and nonlinear selection gradients are presented in Table 2. There was significant linear sexual selection favouring lower values of PC4 (higher levels of 9,31-C_{38}diene and 9,31-C_{39}diene), as well as significant stabilizing selection operating on PC3 and PC4 (Table 2). Canonical analysis of the γ matrix resulted in two eigenvectors ($\mathbf{m_3}$ and $\mathbf{m_4}$) with significant nonlinear sexual selection, and in both cases, the associated eigenvalues were negative, indicative of multivariate stabilizing selection (Table 3, Figure 1). The dominant eigenvector of stabilizing selection ($\mathbf{m_4}$) is largely positively weighted to PC4, whereas $\mathbf{m_3}$ is largely positively weighted to PC3 (Table 3). There was

Table 3 The **M** matrix of eigenvectors from the canonical analysis of the γ for male CHCs in *Gryllodes sigillatus*. The linear ($\boldsymbol{\theta_i}$) and quadratic ($\boldsymbol{\lambda_i}$) gradients of selection along each eigenvector are given in the last two columns. The quadratic selection gradient ($\boldsymbol{\lambda_i}$) of each eigenvector ($\mathbf{m_i}$) is equivalent to the eigenvalue. Significance tests: $^{**}P < 0.001$; $^{***}P < 0.0001$.

	M				Selection	
	PC1	PC2	PC3	PC4	$\boldsymbol{\theta_i}$	$\boldsymbol{\lambda_i}$
$\mathbf{m_1}$	0.555	−0.828	0.067	0.054	0.020	0.031
$\mathbf{m_2}$	0.783	0.554	0.227	0.171	−0.008	−0.005
$\mathbf{m_3}$	−0.195	−0.065	0.962	−0.178	−0.031	−0.132***
$\mathbf{m_4}$	0.054	0.171	−0.178	0.968	−0.096**	−0.155**

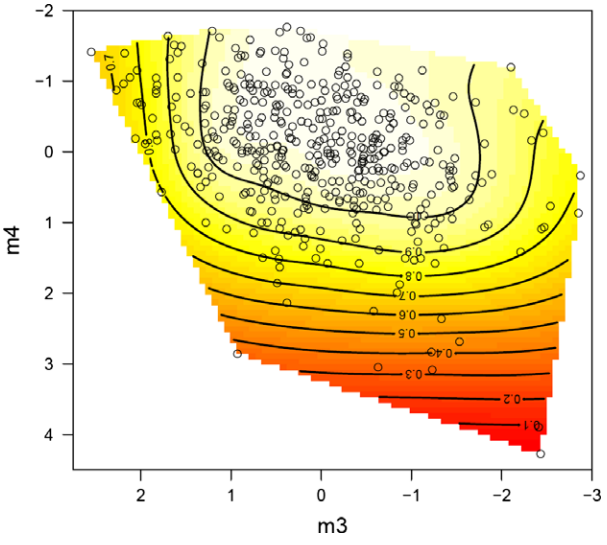

Fig. 1 Thin-plate spline contour-view visualization of the fitness surface on the two axes of nonlinear selection, $\mathbf{m_3}$ and $\mathbf{m_4}$. The open symbols represent individual data points for each male in our experiment. Colours represent the relative mating success of males, with white representing the highest relative fitness and red representing the lowest relative fitness. A smoothing parameter of $\lambda = 0.07$ was used to create the thin-plate spline.

Table 4 Multivariate analysis of variance (MANOVA) examining the effect of mating success (successful vs unsuccessful) on dissimilarity indices for male and female CHC profiles in *G. sigillatus*.

	MANOVA		
Source	Pillai's trace	$F_{3,452}$	P
Mating success	0.007	0.988	0.398
	Univariate ANOVAS		
	$F_{1,454}$	P	
Canberra index	2.554	0.111	
Bray–Curtis index	1.974	0.161	
Euclidean index	0.777	0.379	

also significant linear selection favouring low values of $\mathbf{m_4}$, which equates to reduced values of PC4 (or higher amounts of 9,31-C_{38}diene and 9,31-C_{39}diene) (Table 3).

Multivariate dissimilarity indices

To determine if female mate choice for male CHCs is dependent on their own CHC profile, we estimated three different multivariate dissimilarity indices (Bray-Curtis, Euclidean and Canberra indices). MANOVA revealed little effect of mating status (successful or unsuccessful) on our multivariate measures of CHC dissimilarity (Table 4). This finding was confirmed with univariate ANOVAS on each individual index of dissimilarity (Table 4, Figure 2). Our results, therefore, suggest that although there is a clear female mate choice for male CHCs, it is not based on dissimilarity (or similarity) to their own CHC profile.

Discussion

Our results show that female mating preferences in decorated crickets are based, in part, on the CHC profiles of prospective mates. Male CHC attractiveness was not, however, contingent on the CHC profile of the choosing female. This contrasts with work on the field cricket, *Teleogryllus oceanicus*, where females preferentially mate with males exhibiting dissimilar CHC profiles. Instead, female *G. sigillatus* appear to use an absolute criterion in their choice, as certain male CHC phenotypes were equally attractive to most females, as evidenced by significant linear and stabilizing selection gradients. Significant linear and/or nonlinear selection on CHCs has also been observed in a number of other insect species (Steiger & Stökl, 2014). Although linear selection favouring extreme elaboration of male sexual traits has often been regarded as the predominant mode of sexual selection, this view can be attributed partly to

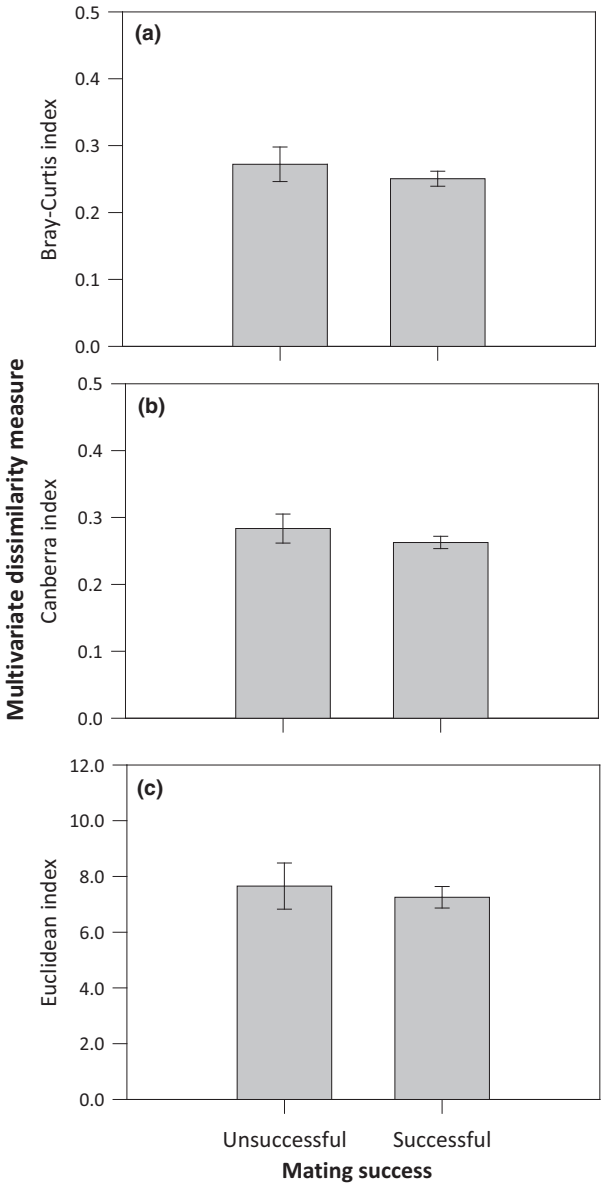

Fig. 2 Mean (±SE) cuticular hydrocarbon dissimilarity of successful and unsuccessful mating pairs using the (a) Bray–Curtis, (b) Canberra and (c) Euclidean indices. For each dissimilarity index, smaller values indicate more similar CHC profiles in males and females whereas larger values represent more dissimilar CHC profiles.

the fact that linear selection is the easiest form of selection to detect statistically (Blows & Brooks, 2003; Hunt *et al.*, 2009). More recent studies designed explicitly to detect both linear and nonlinear selection on male sexual traits have found significant nonlinear selection. This is true for both visual (e.g. Punzalan *et al.*, 2008) and acoustic traits (e.g. Brooks *et al.*, 2005; Bentsen *et al.*, 2006; Gerhardt & Brooks, 2009; Oh & Shaw,

2013; Ower *et al.*, 2013), as well as for CHCs (e.g. Chenoweth & Blows, 2005; Steiger *et al.*, 2013; Ingleby *et al.*, 2014). Our current study contributes to an emerging consensus that female mate choice can exert a complex pattern of sexual selection on male sexual traits (reviewed in Hunt & Sakaluk, 2014).

We found that the target of sexual selection was not the overall abundance of CHCs, but rather the relative abundance of specific CHCs. Furthermore, stabilizing selection on male CHCs was found to be stronger than directional selection. Interestingly, the only other two studies that have evaluated multivariate sexual selection on cricket CHCs also found significant stabilizing selection on male CHC profiles (Thomas & Simmons, 2009; Steiger *et al.*, 2013). Similarly, studies in *Drosophila* species have also documented multivariate stabilizing selection acting on CHCs (Chenoweth & Blows, 2005; Ingleby *et al.*, 2014). Our results revealed two major axes ($\mathbf{m_3}$ and $\mathbf{m_4}$) with significant stabilizing selection, with significant negative linear selection also existing along the dominant axis of sexual selection ($\mathbf{m_4}$). The pattern of sexual selection acting on $\mathbf{m_4}$ exists because the stabilizing selection acting on this axis is not symmetrical as was observed acting along $\mathbf{m_3}$ (Fig. 1). A similar pattern of selection was observed along the two dominant axes of sexual selection on acoustic calls ($\mathbf{m_4}$ and $\mathbf{m_5}$) in the field cricket, *Teleogryllus commodus* (Brooks *et al.*, 2005). In our study, this pattern of selection means that although directional selection favoured an increase in two alkadienes (9,31-C_{38}diene and 9,31-C_{39}diene) relative to 7-C_{35}ene and 3,13-diMeC_{36}, stabilizing selection favoured a more intermediate level of this trade-off between these specific CHCs. One possible explanation for this result is that there may be significant variation in female preferences within our study population, whereby different females may exercise directional mating preferences on the same CHC trade-off but in opposing directions, with selection in one direction (i.e. towards more 9,31-C_{38}diene and 9,31-C_{39}diene) being slightly stronger than in the opposite direction (Chenoweth & Blows, 2005). The net result would be the observed pattern, with strong stabilizing selection coupled to slightly weaker linear selection. There is no obvious reason, however, why females within our study population should show such contrasting mating preferences and further work is needed to determine whether such a pattern of mate choice actually exists.

Traditionally, species recognition and sensory tuning have been thought to influence mating preferences leading to stabilizing selection gradients (Butlin *et al.*, 1985; Ryan & Wilczynski, 1988; Ryan *et al.*, 1992). If mating with heterospecifics or individuals with very dissimilar genotypes (Mendelson & Shaw, 2012) leads to the production of offspring with reduced fitness or no offspring at all due to incompatibility, then females would be expected to prefer traits that are more typical of their own species, and show a preference for intermediate trait values around the population mean (Butlin *et al.*, 1985; Pfennig, 1998; Safi *et al.*, 2006; Shaw & Lesnick, 2009). On a proximate level, such unimodal preference functions arise when the receivers' sensory systems are optimally tuned to detect signals in a particular range. Such fine-tuning in females' sensory organs has been found in acoustic systems (Ryan & Wilczynski, 1988; Ryan *et al.*, 1992) and long-distance pheromone communication (Klun *et al.*, 1973; Baker *et al.*, 1998), but with respect to the perception of complex CHC profiles, there are currently no direct tests of this.

In general, little is known about how insects perceive and process the information encoded in complex CHC profiles. Traditionally, it was assumed that only a small subset of CHCs in the overall profile have a communicative function (e.g. Carlson *et al.*, 1978; Ginzel *et al.*, 2006), with individual CHCs perceived by taste sensilla (Lacaille *et al.*, 2007). However, it is becoming increasingly clear that complex CHC profiles can play a pivotal role in mate choice and nestmate recognition and that the entire CHC profile might actually be the unit of assessment (Ozaki *et al.*, 2005; Kühbandner *et al.*, 2013). For example, in the parasitic wasp, *Lariophagus distinguendus*, 3-MeC_{27} is a key component of the sex pheromone (Kühbandner *et al.*, 2012). However, it only elicits a response when presented in combination with a chemical background of other CHCs occurring on the cuticle of the wasp (Kühbandner *et al.*, 2012). Moreover, in a companion study, Kühbandner *et al.* (2013) were able to demonstrate that when increasing the absolute amount of some minor components of the natural CHC profile of this species, attractiveness decreased substantially indicating that the ratio of CHC components is decisive and that the entire profile is processed. Gustatory sensilla are typically innervated by only a small number of receptor neurons and might, therefore, not be suitable for the perception of more complex CHC profiles (Ozaki & Wada-Katsumata, 2010). However, in the ant, *Camponotus japonicus,* a specialized olfactory sensillum housing about 130 receptor neurons has been identified (Ozaki *et al.*, 2005). This sensillum is capable of discriminating complex colony-specific CHC profiles from nestmates and non-nestmates (Ozaki *et al.*, 2005). It is conceivable that such chemosensory sensilla, innervated by many receptor neurons, are tuned to a specific CHC pattern leading to a unimodal mating preference and hence, to the observed stabilizing selection gradients.

Previous studies have shown that female decorated crickets are capable of chemosensory self-referencing based on their own CHC profile (Ivy *et al.*, 2005; Weddle *et al.*, 2013; Capodeanu-Nägler *et al.*, 2014). During copulation, CHCs are transferred between mating partners rendering a male's profile more similar to the respective female (Weddle *et al.*, 2013). Females

avoid mating with such males bearing chemical cues similar to their own (Weddle *et al.*, 2013). On a sensory level, it has been suggested that females' receptor neurons might be desensitized to their own CHC profile, and only respond to profiles that are dissimilar (Capodeanu-Nägler *et al.*, 2014). In the current study, we predicted that females would adopt a more general rule, and mate only with males that activate receptor neurons above a specific threshold that translates to a preference for more dissimilar male profiles. Such a mechanism would allow, for example, the avoidance of inbreeding. However, we did not find any support for this prediction, as the likelihood of mating was not influenced by the CHC similarity of the respective pair. However, our results strongly corroborate those of a previous study, in which females were found to show no precopulatory discrimination between unrelated versus related (half-sib or full-sib) males (Weddle *et al.*, 2013). This raises the question as to how, on a proximate level, a female is able to use a self-referencing mechanism to avoid mating with males bearing CHCs similar to her own, while retaining the ability to make non-self-referencing mating decisions. One possible clue is that although males and females share the same CHC components, the pattern shows a clear sex-specific difference in their relative abundance (Weddle *et al.*, 2012). Even though a brother will, on average, be genetically and phenotypically more similar than an unrelated male, the CHC pattern will still be different from the female's own profile (or a male's profile imbued with her own CHCs) and therefore elicit a greater response of receptor neurons. In other words, the female might always engage in self-referencing, but the processed CHC profile has to be very similar to her own to result in a female aversion to mate.

The proximate mechanism detailed above does not, of course, explain why females have not evolved a more restricted behavioural response threshold such that female mating is only elicited when males are very dissimilar to avoid inbreeding. There are at least two possible reasons for this. First, inbreeding might not necessarily involve high costs. Although fitness usually decreases with genetic similarity of the parents, positive relationships between genetic similarity and reproductive success have been detected in, for example, Peron's tree frogs (Sherman *et al.*, 2008) and *Ambrosia* beetles (Peer & Taborsky, 2005). Second, it might not be adaptive to increase the response threshold as females might then exclude all mated males because they bear a female-like CHC pattern, and therefore, a more similar pattern. Ivy *et al.* (2005) showed that females specifically avoid mating with their previous partner, but do not generally avoid mated males. Such an individual recognition mechanism is only possible if the threshold is more relaxed and only very similar CHC patterns fail to evoke female mounting. Furthermore, the absence of a precopulatory mechanism of inbreeding avoidance does not preclude post-copulatory mechanisms that operate in this context. Female decorated crickets secure indirect genetic benefits by mating polyandrously (Ivy & Sakaluk, 2005), and some of these benefits might accrue because the receipt of a diverse mixture of sperm enables females to bias their paternity towards males that are genetically compatible. In fact, because the precise combination of genes in each zygote is affected by random segregation and crossing over during meiosis, precopulatory mate choice will not result in predictable offspring fitness. A post-copulatory choice based on the haploid genotype of sperm would be a more reliable mechanism to secure the benefits of compatible alleles (Puurtinen *et al.*, 2009).

In conclusion, our data indicate that female crickets prefer certain males over others based on the males' CHC profile, but that this preference is not related to their own profile. This suggests that the female preference in *G. sigillatus* is a mechanism to secure additive genetic benefits (i.e. good genes) rather than nonadditive genetic benefits (i.e. compatibility genes). Indeed, the possibility that CHCs might serve as a reliable indicator of the quality of males is supported by earlier work on this species. Weddle *et al.* (2012) found that the CHCs of males are condition dependent (i.e. genotype-by-diet interactions significantly influence CHC expression) and show a high degree of environmental determination, whereas the same is not true for CHC expression in females. This raises the possibility that male CHC expression may have evolved heightened condition dependence due to costs of trait expression (Gosden & Chenoweth, 2011) and might, therefore, be a reliable indicator of male quality. Further experiments are required, however, to determine whether females receive any genetic benefits by preferentially mating with males possessing a certain CHC profile.

In this regard, it would have been ideal to assess other independent measures of quality for males in our experiment (e.g. calling effort, sperm quality and lifespan) but unfortunately this was not tenable for logistic reasons. We do know from our previous experiments on this species (Weddle *et al.* 2012) that body size is typically highly correlated with the total amount of CHCs produced by a male which is represented by PC1. In our current study, however, PC1 was not found to influence female mate choice decisions (Tables 2 & 3). Our future studies also need to give greater consideration to the effects of non-genetic factors on CHC expression and whether this influences how CHCs evolve through sexual selection in *G. sigillatus*. In *Drosophila melanogaster*, both diet and gut microbiota have an important effect on female preference and male CHC expression, with at least the effects of the latter being readily transmitted across generations (Sharon *et al.* 2010). Consequently, it is likely that non-genetic inheritance models of sexual selection, such as the "environmental induction" model of Bonduriansky & Day

(2012), may prove to be more relevant to the evolution of CHCs in *G. sigillatus* than traditional "good-genes" models of sexual selection.

Acknowledgments

This work was supported by a grant from the National Science Foundation to S.K.S., and a University Royal Society Fellowship and a Royal Society Equipment Grant to J.H. A.C. was supported by the ERASMUS programme KOOR/BEST. S.S. was supported by a Feodor Lynen Fellowship from the Alexander von Humboldt Foundation and Illinois State University.

References

Aeschlimann, P.B., Häberli, M.A., Reusch, T.B.H., Boehm, T. & Milinski, M. 2003. Female sticklebacks *Gasterosteus aculeatus* use self-reference to optimize MHC allele number during mate selection. *Behav. Ecol. Sociobiol.* **54**: 119–126.

Ali, J.G. & Tallamy, D.W. 2010. Female spotted cucumber beetles use own cuticular hydrocarbon signature to choose immunocompatible mates. *Anim. Behav.* **80**: 9–12.

Andersson, M.B. 1994. *Sexual Selection*. Princeton University Press, Princeton.

Aitchison, J. 1986. *The Statistical Analysis of Compositional Data*. The Blackburn Press, New Jersey.

Baker, T.C., Fadamiro, H.Y. & Cosse, A.A. 1998. Moth uses fine tuning for odour resolution. *Nature* **393**: 530.

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

Bisgaard, S. & Ankenman, B. 1996. Standard errors for the eigenvalues in second-order response surface models. *Technometrics* **38**: 238–246.

Blows, M.W. & Brooks, R. 2003. Measuring nonlinear selection. *Am. Nat.* **162**: 815–820.

Bonduriansky, R. & Day, T. 2012. Nongenetic inheritance and the evolution of costly female preference. *J. Evol. Biol.* **26**: 76–87.

Brooks, R., Hunt, J., Blows, M.W., Smith, M.J., Bussiére, L.F. & Jennions, M.D. 2005. Experimental evidence for multivariate stabilizing sexual selection. *Evolution* **59**: 871–880.

Butlin, R.K., Hewitt, G.M. & Webb, S.F. 1985. Sexual selection for intermediate optimum in *Chorthippus brunneus* (Orthoptera: Acrididae). *Anim. Behav.* **33**: 1281–1292.

Capodeanu-Nägler, A., Rapkin, J., Sakaluk, S.K., Hunt, J. & Steiger, S. 2014. Self-recognition in crickets via on-line processing. *Curr. Biol.* **24**: R1117–R1118.

Carlson, D., Langley, P. & Huyton, P. 1978. Sex pheromone of the tsetse fly: isolation, identification, and synthesis of contact aphrodisiacs. *Science* **201**: 750–753.

Charlesworth, D. & Willis, J.H. 2009. The genetics of inbreeding depression. *Nat. Rev. Genet.* **10**: 783–796.

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

Colegrave, N., Kotiaho, J.S. & Tomkins, J.L. 2002. Mate choice or polyandry: reconciling genetic compatibility and good genes sexual selection. *Evol. Ecol. Res.* **4**: 911–917.

Foley, B., Chenoweth, S.F., Nuzhdin, S.V. & Blows, M.W. 2007. Natural genetic variation in cuticular hydrocarbon expression in male and female *Drosophila melanogaster*. *Genetics* **175**: 1465–1477.

Gasparini, C. & Pilastro, A. 2011. Cryptic female preference for genetically unrelated males is mediated by ovarian fluid in the guppy. *Proc. R. Soc. B: Biol. Sci.* **278**: 2495–2501.

Gerhardt, H.C. & Brooks, R. 2009. Experimental analysis of multivariate female choice in gray treefrogs (*Hyla versicolor*): evidence for directional and stabilizing selection. *Evolution* **63**: 2504–2512.

Ginzel, M., Moreira, J., Ray, A., Millar, J. & Hanks, L. 2006. (*Z*)-9-Nonacosene—Major component of the contact sex pheromone of the beetle *Megacyllene caryae*. *J. Chem. Ecol.* **32**: 435–451.

Gosden, T.P. & Chenoweth, S.F. 2011. On the evolution of heightened condition dependence of male sexual displays. *J. Evol. Biol.* **24**: 685–692.

Green, P. & Silverman, B. 1994. *Nonparametric Regression and Generalised Linear Models*. Chapman and Hall, London, UK.

Hunt, J. & Sakaluk, S.K. 2014. Mate choice. In: *The Evolution of Insect Mating Systems* (D.M. Shuker & L.W. Simmons eds), pp. 129–158. Oxford University Press, Oxford, UK.

Hunt, J., Bussière, L.F., Jennions, M.D. & Brooks, R. 2004. What is genetic quality? *Trends Ecol. Evol.* **19**: 329–333.

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

Ingleby, F.C., Hosken, D.J., Flowers, K., Hawkes, M.F., Lane, S.M., Rapkin, J. *et al.* 2014. Environmental heterogeneity, multivariate sexual selection and genetic constraints on cuticular hydrocarbons in *Drosophila simulans*. *J. Evol. Biol.* **27**: 700–713.

Ivy, T.M. 2007. Good genes, genetic compatibility and the evolution of polyandry: use of the diallel cross to address competing hypotheses. *J. Evol. Biol.* **20**: 479–487.

Ivy, T.M. & Sakaluk, S.K. 2005. Polyandry promotes enhanced offspring survival in decorated crickets. *Evolution* **59**: 152–159.

Ivy, T.M. & Sakaluk, S.K. 2007. Sequential mate choice in decorated crickets: females use a fixed internal threshold in pre- and postcopulatory choice. *Anim. Behav.* **74**: 1065–1072.

Ivy, T.M., Weddle, C.B. & Sakaluk, S.K. 2005. Females use self-referent cues to avoid mating with previous mates. *Proc. R. Soc. B: Biol. Sci.* **272**: 2475–2478.

Kent, C., Azanchi, R., Smith, B., Chu, A. & Levile, J. 2007. A model-based analysis of chemical and temporal patterns of cuticular hydrocarbons in male *Drosophila melanogaster*. *PLoS ONE* **2**: e962.

Kempenaers, B. 2007. Mate choice and genetic quality: a review of the heterozygosity theory. In: *Advances in the Study of Behavior* (H.J. Brockmann, T.J. Roper, M. Naguib, K.E. Wynne-Edwards, C. Barnard, M. John, eds), pp. 189–278. Academic Press, London, UK.

Klun, J.A., Chapman, O.L., Mattes, K.C., Wojtkowski, P.W., Beroza, M. & Sonnet, P.E. 1973. Insect sex pheromones: minor amount of opposite geometrical isomer critical to attraction. *Science* **181**: 661–663.

Krupp, J.J., Kent, C., Billeter, J.-C., Azanchi, R., So, A.K.-C., Schonfeld, J.A., Smith, B.P., Lucas, C. & Levine, J.D. 2008. Social experience modifies pheromone expression and mat-

ing behaviour in male *Drosophila melanogaster*. *Curr. Biol.* **18**: 1373–1383.

Kühbandner, S., Sperling, S., Mori, K. & Ruther, J. 2012. Deciphering the signature of cuticular lipids with contact sex pheromone function in a parasitic wasp. *J. Exp. Biol.* **215**: 2471–2478.

Kühbandner, S., Bello, J.E., Mori, K., Millar, J.G. & Ruther, J. 2013. Elucidating structure-bioactivity relationships of methyl-branched alkanes in the contact sex pheromone of the parasitic wasp *Lariophagus distinguendus*. *Insects* **4**: 743–760.

Lacaille, F., Hiroi, M., Twele, R., Inoshita, T., Umemoto, D., Manière, G. *et al.* 2007. An inhibitory sex pheromone tastes bitter for *Drosophila* males. *PLoS ONE* **2**: e661.

Lande, R. & Arnold, S. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.

Lihoreau, M. & Rivault, C. 2008. Kin recognition via cuticular hydrocarbons shapes cockroach social life. *Behav. Ecol.* **20**: 46–53.

Manly, B.F.J. 1997. *Randomization, Bootstrap and Monte Carlo Methods in Biology*. Chapman and Hall, London, UK.

Mays, H.L. & Hill, G.E. 2004. Choosing mates: good genes versus genes that are a good fit. *Trends Ecol. Evol.* **19**: 554–559.

Mendelson, T.C. & Shaw, K.L. 2012. The (mis)concept of species recognition. *Trends Ecol. Evol.* **27**: 421–427.

Mitchell-Olds, T. & Shaw, R. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* **41**: 1149–1161.

Neff, B.D. & Pitcher, T.E. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Mol. Ecol.* **14**: 19–38.

Oh, K.P. & Shaw, K.L. 2013. Multivariate sexual selection in a rapidly evolving speciation phenotype. *Proc. R. Soc. B: Biol. Sci.* **280**: 2013042.

Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelstrom, H. 1996. Sperm selection by females. *Nature* **383**: 585.

Olsson, M., Madsen, T., Nordby, J., Wapstra, E., Ujvari, B. & Wittsell, H. 2003. Major histocompatibility complex and mate choice in sand lizards. *Proc. R. Soc. B: Biol. Sci.* **270**: S254–S256.

Ower, G.D., Judge, K.A., Steiger, S., Caron, K.J., Smith, R.A., Hunt, J. *et al.* 2013. Multivariate sexual selection on male song structure in wild populations of sagebrush crickets, *Cyphoderris strepitans* (Orthoptera: Haglidae). *Ecol. Evol.* **3**: 3590–3603.

Ozaki, M. & Wada-Katsumata, A. 2010. Perception and olfaction of cuticular compounds. In: *Insect Hydrocarbons; Biology, Biochemistry, and Chemical Ecology* (G. Blomquist, A.-G. Bagnères, eds), pp. 207–221. Cambridge University Press, Cambridge.

Ozaki, M., Wada-Katsumata, A., Fujikawa, K., Iwasaki, M., Yokohari, F., Satoji, Y. *et al.* 2005. Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. *Science* **309**: 311–314.

Peer, K. & Taborsky, M. 2005. Outbreeding depression, but no inbreeding depression in haplodiploid ambrosia beetles with regular sibling mating. *Evolution* **59**: 317–323.

Penn, D.J. 2002. The scent of genetic compatibility: sexual selection and the major histocompatibility complex. *Ethology* **108**: 1–21.

Petfield, D., Chenoweth, S.F., Rundle, H.D. & Blows, M.W. 2003. Genetic variance in female condition predicts indirect genetic variance in male sexual displays. *Proc. Natl. Acad. Sci.* **102**: 6045–6050.

Pfennig, K.S. 1998. The evolution of mate choice and the potential for conflict between species and mate–quality recognition. *Proc. R. Soc. B: Biol. Sci.* **265**: 1743–1748.

Phillips, P. & Arnold, S. 1989. Visualizing multivariate selection. *Evolution* **43**: 1209–1222.

Punzalan, D., Rodd, F.H. & Rowe, L. 2008. Contemporary sexual selection on sexually dimorphic traits in the ambush bug *Phymata americana*. *Behav. Ecol.* **19**: 860–870.

Puurtinen, M., Ketola, T. & Kotiaho, J.S. 2009. The good-genes and compatible-genes benefits of mate choice. *Am. Nat.* **174**: 741–752.

Quinn, G.P. & Keough, M.J. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge, UK.

Reynolds, R.J., Childers, D.K. & Pajewski, N.M. 2010. The distribution and hypothesis testing of eigenvalues from the canonical analysis of the gamma matrix of quadratic and correlational selection gradients. *Evolution* **64**: 1076–1085.

Roberts, S.C. & Gosling, L.M. 2003. Genetic similarity and quality interact in mate choice decisions by female mice. *Nat. Genet.* **35**: 103–106.

Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. B: Biol. Sci.* **263**: 1415–1421.

Ryan, M.J. & Wilczynski, W. 1988. Coevolution of sender and receiver: effect on local mate preference in cricket frogs. *Science* **240**: 1786–1788.

Ryan, M.J., Perrill, S.A. & Wilczynski, W. 1992. Auditory tuning and call frequency predict population-based mating preferences in the cricket frog, *Acris crepitans*. *Am. Nat.* **139**: 1370–1383.

Safi, K., Heinzle, J. & Reinhold, K. 2006. Species recognition influences female mate preferences in the Common European Grasshopper (*Chorthippus biguttulus* Linnaeus, 1758). *Ethology* **112**: 1225–1230.

Sakaluk, S.K., Schaus, J.M., Eggert, A.-K., Snedden, W.A. & Brady, P.L. 2002. Polyandry and fitness of offspring reared under varying nutritional stress in decorated crickets. *Evolution* **56**: 1999–2007.

Shackleton, M.A., Jennions, M.D. & Hunt, J. 2005. Fighting success and attractiveness as predictors of male mating success in the black field cricket, *Teleogryllus commodus*: the effectiveness of no-choice tests. *Behav. Ecol. Sociobiol.* **58**: 1–8.

Sharon, G., Segal, D., Ringo, J.M., Hefetz, A., Zilber-Rosenberg, I. & Rosenberg, E. 2010. Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **107**: 20051–20056.

Shaw, K.L. & Lesnick, S.C. 2009. Genomic linkage of male song and female acoustic preference QTL underlying a rapid species radiation. *Proc. Natl. Acad. Sci.* **106**: 9737–9742.

Sherman, C.D.H., Wapstra, E., Uller, T. & Olsson, M. 2008. Males with high genetic similarity to females sire more offspring in sperm competition in Peron's tree frog *Litoria peronii*. *Proc. R. Soc. B: Biol. Sci.* **275**: 971–978.

Steiger, S. & Stökl, J. 2014. The role of sexual selection in the evolution of chemical signals in insects. *Insects* **5**: 423–438.

Steiger, S., Ower, G.D., Stökl, J., Mitchell, C., Hunt, J. & Sakaluk, S.K. 2013. Sexual selection on cuticular hydrocarbons of male sagebrush crickets in the wild. *Proc. R. Soc. B: Biol. Sci.* **280**: 20132353.

Stinchcombe, J.R., Agrawal, A.F., Hohenlohe, P.A., Arnold, S.J. & Blows, M.W. 2008. Estimating nonlinear selection

gradients using quadratic regression coefficients: double or nothing? *Evolution* **62**: 2435–2440.

Tabachnick, B.G. & Fidell, L.S. 2001. *Using Multivariate Statistics*. Allyn & Bacon, Boston.

Thomas, M. & Simmons, L. 2008. Cuticular hydrocarbons are heritable in the cricket *Teleogryllus oceanicus*. *J. Evol. Biol.* **21**: 801–806.

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

Thomas, M.L. & Simmons, L.W. 2011. Crickets detect the genetic similarity of mating partners via cuticular hydrocarbons. *J. Evol. Biol.* **24**: 1793–1800.

Tregenza, T. & Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage: Invited Review. *Mol. Ecol.* **9**: 1013–1027.

Van Zweden, J.S., Brask, J.B., Christensen, J.H., Boomsma, J.J., Linksvayer, T.A. & D'Ettorre, P. 2010. Blending of heritable recognition cues among ant nestmates creates distinct colony gestalt odours but prevents within-colony nepotism. *J. Evol. Biol.* **23**: 1498–1508.

Weddle, C.B., Mitchell, C., Bay, S.K., Sakaluk, S.K. & Hunt, J. 2012. Sex-specific genotype-by-environment interactions for cuticular hydrocarbon expression in decorated crickets, *Gryllodes sigillatus*: implications for the evolution of signal reliability. *J. Evol. Biol.* **25**: 2112–2125.

Weddle, C.B., Steiger, S., Hamaker, C.G., Ower, G.D., Mitchell, C., Sakaluk, S.K. *et al.* 2013. Cuticular hydrocarbons as a basis for chemosensory self-referencing in crickets: a potentially universal mechanism facilitating polyandry in insects. *Ecol. Lett.* **16**: 346–353.

Wedekind, C., Seebeck, T., Bettens, F. & Paepke, A.J. 1995. MHC-dependent mate preferences in humans. *Proc. R. Soc. B: Biol. Sci.* **260**: 245–249.

Wolda, H. 1981. Similarity indices, sample size and diversity. *Oecologia* **50**: 296–302.

Zeh, J.A. & Zeh, D.W. 1996. The evolution of polyandry I: Intragenomic conflict and genetic incompatibility. *Proc. R. Soc. B: Biol. Sci.* **263**: 1711–1717.

Zeh, J.A. & Zeh, D.W. 1997. The evolution of polyandry II: post-copulatory defences against genetic incompatibility. *Proc. R. Soc. B: Biol. Sci.* **264**: 69–75.

Zeh, J.A. & Zeh, D.W. 2007. Mate choice by non-virgin females contributes to reproductive isolation between populations of the harlequin beetle-riding pseudoscorpion. *Ethology* **113**: 1202–1211.

Data deposited at Dryad: doi: 10.5061/dryad.c0t7b

Received 10 June 2015; revised 14 August 2015; accepted 18 August 2015