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Sex-specific genotype-by-environment interactions for cuticular hydrocarbon expression in decorated crickets, *Gryllodes sigillatus*: implications for the evolution of signal reliability

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Abstract

Phenotypic traits that convey information about individual identity or quality are important in animal social interactions, and the degree to which such traits are influenced by environmental variation can have profound effects on the reliability of these cues. Using inbred genetic lines of the decorated cricket, *Gryllodes sigillatus*, we manipulated diet quality to test how the cuticular hydrocarbon (CHC) profiles of males and females respond across two different nutritional rearing environments. There were significant differences between lines in the CHC profiles of females, but the effect of diet was not quite statistically significant. There was no significant genotype-by-environment interaction (GEI), suggesting that environmental effects on phenotypic variation in female CHCs are independent of genotype. There was, however, a significant effect of GEI for males, with changes in both signal quantity and content, suggesting that environmental effects on phenotypic expression of male CHCs are dependent on genotype. The differential response of male and female CHC expression to variation in the nutritional environment suggests that these chemical cues may be under sex-specific selection for signal reliability. Female CHCs show the characteristics of reliable cues of identity: high genetic variability, low condition dependence and a high degree of genetic determination. This supports earlier work showing that female CHCs are used in self-recognition to identify previous mates and facilitate polyandry. In contrast, male CHCs show the characteristics of reliable cues of quality: condition dependence and a relatively higher degree of environmental determination. This suggests that male CHCs are likely to function as cues of underlying quality during mate choice and/or male dominance interactions.

Introduction

The ability of an organism to communicate effectively with other individuals is vital in many kinds of social interactions (Greenfield, 2002). The evolution of animal communication systems has led, therefore, to the evolution of complex visual, auditory and chemical cues that provide information about an individual's species, sex, genetic relatedness, mating status, social dominance, mate quality and individual identity (Mateo, 2004). However, environmental variation can often have profound effects on the expression of phenotypic traits underlying signal production and can, therefore, influence the reliability of signal content (Greenfield & Rodriguez, 2004; Higginson & Reader, 2009; Ingleby *et al.*, 2010). When variation in the environment (e.g. availability of resources, costs of resource acquisition) affects the ability of an organism to acquire the resources necessary to allocate towards the production or maintenance of traits that enhance fitness, these traits can evolve condition dependence (Rowe & Houle, 1996; Hunt *et al.*, 2004b). For condition-dependent traits, levels of expression are dependent upon the

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phenotypic condition of the organism. Phenotypic condition can be defined as the total amount of acquired resources that are available for allocation towards the production or maintenance of traits that enhance fitness and is determined by the combined effects of the somatic state, the epigenetic state and the genotype of the organism (Rowe & Houle, 1996; Hill, 2011). Individuals of higher phenotypic condition have a larger acquired pool of resources from which to draw for allocation towards different traits of fitness and are, therefore, better able to afford any costs associated with trait expression (Andersson, 1982; Rowe & Houle, 1996; Hunt *et al.*, 2004b).

Genotype-by-environment interactions (GEIs) occur when the relative phenotypic performance of different genotypes is affected by the environment in which they are expressed (Lynch & Walsh, 1998). Because the genotype of the signaller is an important component of phenotypic condition, there can be a large component of genetic variation in the ability of organisms to acquire resources, such that some genotypes may be better able to maintain optimal signal expression under relatively poor environmental conditions than others (Rowe & Houle, 1996; Hunt *et al.*, 2004b). GEIs are not necessary for the evolution of condition-dependent signals. However, if environmental effects on variation in trait expression are genotype specific, this indicates genetic variance for acquisition ability (phenotypic condition), such that some genotypes are relatively better at acquiring resources to allocate towards fitness-related traits such as sexual signals (Hunt *et al.*, 2004b). Recent theoretical and empirical work has focused on how GEIs can potentially affect the reliability of condition-dependent signals of mate quality (reviewed by Ingleby *et al.*, 2010). Signal reliability can be compromised by a reduction in genetic variance in signal expression and by increased environmental variation, where the information content of signals becomes degraded under harsh or stressful environmental conditions (Higginson & Reader, 2009).

Cues or signals of quality are intended to convey important information about the overall phenotypic and genetic constitution of the signaller, either as a potential mate or as a potential competitor, and should therefore be dependent on phenotypic condition because costs of expression are typically high (Zahavi, 1975, 1977; Andersson, 1982; Dale *et al.*, 2001). For sexually selected traits, handicap theory suggests that trait expression must be costly to provide a reliable indicator of male quality, because only high-quality males can afford the costs associated with expression (Zahavi, 1975, 1977). For traits that show both genetic and environmental components of variation, if condition-dependent expression of phenotypic traits provides reliable indicators of male phenotypic and genetic quality, females may gain direct or indirect benefits by using these condition-dependent cues for mate-choice decisions (Andersson, 1986). Therefore, condition-dependent cues that convey information about mate quality are more likely to evolve if they reliably reflect both the phenotypic and genotypic conditions of the male, as well as the potential phenotype of any resulting offspring that he might sire (Andersson, 1986).

Conversely, when there is a strong benefit to being recognized individually, signals or cues of individual identity should be highly variable within populations and independent of phenotypic condition, with a relatively high degree of genetic determination leading to individually distinctive cues (Dale *et al.*, 2001; Thom & Hurst, 2004; Tibbetts & Dale, 2007). Traits conveying reliable signals of identity are thought to be largely independent of phenotypic condition because they have relatively low costs of expression (Dale *et al.*, 2001). If costs of expression are low, individuals across environments should be able to acquire sufficient resources to allocate towards the production or maintenance of optimal levels of trait expression for reliable signal content, regardless of genetic background.

In insects, chemical communication is an important mode of communication between individuals, and it is often facilitated by cuticular hydrocarbons (CHCs; Howard & Blomquist, 1982, 2005). CHCs are lipid compounds that are present on the surface of the insect epicuticle, preventing desiccation and serving as a barrier to microorganisms (Lockey, 1988). Besides providing these basic physiological functions, these compounds have been demonstrated to function as recognition cues in a variety of insect species, including crickets, facilitating species recognition, kin recognition and sex recognition (Tregenza & Wedell, 1997; Nagamoto *et al.*, 2005; Ryan & Sakaluk, 2009). Several features of CHCs combine to make them excellent candidates as recognition cues used to distinguish between individuals: chemical stability, low volatility (due to long carbon chains), and a diversity of structures allowing for significant variability in lipid composition. It is perhaps not surprising therefore that CHCs have been widely established as the chemical basis for kin recognition in social insects, with a significant genetic component to the cues used to determine relatedness among nestmates (Howard *et al.*, 1982; Howard, 1993; Dani *et al.*, 2001).

Environment has been shown to be an important source of within-population variance in CHCs for many insect species (Rundle *et al.*, 2005). Indeed, there is ample evidence that dietary hydrocarbons are incorporated into the cuticular lipids of many insects from the food they consume (Blomquist & Jackson, 1973). For example, the CHC profile of the myrmecophilous salticid spider, *Cosmophasis bitaeniata*, closely resembles that of its host ant *Oecophylla smaragdina*. Elgar & Allan (2004) demonstrated that the spider acquires the CHCs by eating the host ant larvae and that variation in the

CHC profiles of the spider depends upon the colony of origin of the ant larvae prey, rather than the parentage of the spider. Additionally, Steiger *et al.* (2007) found that nutritional conditions influence the CHC profiles of the burying beetle, *Nicrophorus vespilloides*, and suggested that diet may provide the precursors for unsaturated hydrocarbons used in breeding partner recognition. Additional changes in female CHCs during breeding bouts appear to be linked with carrion consumption early in the breeding cycle (Steiger *et al.*, 2008).

The decorated cricket, *Gryllodes sigillatus*, occurs worldwide in both tropical and subtropical regions, and its occurrence is often associated with human habitation (Thomas, 1985; Smith & Thomas, 1988). Females of this species gain genetic benefits by mating polyandrously through paternally derived genes that enhance offspring viability (i.e. good genes), the avoidance of genetic incompatibility or some combination of these benefits (Sakaluk *et al.*, 2002; Ivy & Sakaluk, 2005; Ivy, 2007). To maximize these benefits, females preferentially mate with novel mating partners over previous mates in mate-choice trials (Ivy *et al.*, 2005). We recently demonstrated that CHCs are the underlying chemical cues used by females to facilitate recognition and discrimination against previous mating partners (C.B. Weddle, S. Steiger, C.G. Hamaker, G.D. Ower, C. Mitchell, S.K. Sakaluk & J. Hunt, unpublished data). Females 'tag' males with their own unique CHCs during mating and are later able to recognize previous mates in subsequent encounters through chemosensory self-referencing (C.B. Weddle, S. Steiger, C.G. Hamaker, G.D. Ower, C. Mitchell, S.K. Sakaluk & J. Hunt, unpublished data). Female CHCs show high additive genetic variation, indicating that they can provide phenotypically unique chemical cues for individual recognition of 'self.' Moreover, female CHCs have been shown to be physically transferred to males during mating as detected by SPME (solid-phase microextraction) fibres. Lastly, experimental application of female CHCs to males results in a female mating bias away from males bearing chemical cues that are similar to their own (C.B. Weddle, S. Steiger, C.G. Hamaker, G.D. Ower, C. Mitchell, S.K. Sakaluk & J. Hunt, unpublished data). Self-referent phenotype matching does not necessarily require any specialized cognitive abilities on the part of females, because the phenotype of 'self' is always available for reference and can be referred to during any interaction with another individual as a template for recognition (Hauber & Sherman, 2001). However, the extent to which variation in CHCs of this species is determined by environmental factors, such as diet, remains unclear.

As CHCs play an important role in chemosensory self-referencing of female decorated crickets, it is possible that changes in the nutritional environment an individual female experiences could affect the efficacy of such a mechanism (Haplin, 1980). Indeed, nestmate recognition can be altered in eusocial insects if sisters from the same colony are separated and reared on different diets (Liang & Silverman, 2000; Silverman & Liang, 2001; Richard *et al.*, 2004). When sisters are subsequently reintroduced, they are more aggressive towards nestmates that are reared on different diets than they are towards those reared on the same diet, despite their genetic similarity. Additionally, the authors demonstrated that the chemical differences in CHC components of individuals in the two diet treatments were due to differences in CHCs of the insect prey items each group consumed (Liang & Silverman, 2000; Silverman & Liang, 2001). Because we know that female *G. sigillatus* use CHCs as cues of individual identity to facilitate self-recognition (C.B. Weddle, S. Steiger, C.G. Hamaker, G.D. Ower, C. Mitchell, S.K. Sakaluk & J. Hunt, unpublished data), prevailing signalling theory would predict that variation in female CHCs should be independent of phenotypic condition and therefore not affected by nutritional environment (Dale *et al.*, 2001).

Male CHCs in *G. sigillatus* do not appear to provide cues of individual identity to females during mating interactions, because in mate-choice trials, females do not show behavioural discrimination between the inbred brother of a male with whom she has recently mated and a novel male of equal age and mating status (Ivy *et al.*, 2005). However, evidence from a recent multivariate selection analysis has shown that female precopulatory mate choice exerts strong sexual selection on male CHCs, with females favouring males that produce a greater overall quantity of CHCs, as well as an intermediate mixture of short- and long-chained CHCs (J. Hunt, K. Jensen, C. Mitchell, S. Steiger, S.N. Gershman & S.K. Sakaluk, unpublished data). There is no evidence at present that male CHCs in *G. sigillatus* are condition dependent or whether these chemical compounds are used by females as cues to signal male quality. However, evidence from other insect species confirms that male CHCs can be used as condition-dependent cues of quality in female mate choice. For example, studies of *Drosophila serrata* suggest that certain male CHCs are under sexual selection by female choice (Hine *et al.*, 2002; Chenoweth & Blows, 2003, 2005; Blows *et al.*, 2004; Petfield *et al.*, 2005) and thus have evolved towards condition dependence in their expression, which has been shown in both field (Hine *et al.*, 2004) and laboratory studies (Gosden & Chenoweth, 2011). Experimental alteration of laboratory resource environments can also cause changes in the CHC profiles of males, leading to population divergence in female mating preferences, an important evolutionary component of reproductive isolation (Rundle *et al.*, 2005). In the cricket, *Teleogryllus oceanicus*, the chemosensory information required to illicit a mounting response in female crickets during mating interactions

indicates a role for male CHCs in female mate choice (Balakrishnan & Pollack, 1997), and male CHCs have been shown to be under sexual selection (Thomas & Simmons, 2009a). Additionally, the CHCs of male *T. oceanicus* show phenotypic plasticity due to the acoustic environment (Thomas *et al.*, 2011), where in the absence of singing conspecific males, individual males show an increase in the relative concentration of certain CHCs shown to be attractive to females (Thomas & Simmons, 2009a). GEIs for male CHCs would suggest that these cues play a role as a condition-dependent sexual signal of genetic quality (Hunt *et al.*, 2004a,b). If male *G. sigillatus* do indeed use CHCs as cues to signal quality, we predict that male CHC profiles should show the evolutionary characteristics inherent to signals of quality, including condition dependence and high additive genetic variance.

Here, we manipulated the nutritional rearing environment of decorated crickets, *G. sigillatus*, from different inbred genetic lines to determine how the CHC profiles of different male and female genotypes respond in alternate environments. Given that female *G. sigillatus* use CHCs as cues of individual identity to facilitate self-recognition (C.B. Weddle, S. Steiger, C.G. Hamaker, G.D. Ower, C. Mitchell, S.K. Sakaluk & J. Hunt, unpublished data), we hypothesized that variation in female expression of CHCs under varying nutritional environments is independent of phenotypic condition, such than any small effects due to nutritional rearing environment should be independent of genotype. Because cues of identity are thought to have relatively low costs of expression (Dale *et al.*, 2001), females should be able to acquire sufficient resources to allocate towards the production or maintenance of optimal levels of CHC expression for reliable signal content. Therefore, we predicted that variation in female CHC expression would not show significant GEIs. In contrast, because we have evidence that male *G. sigillatus* CHCs are under sexual selection by female choice (J. Hunt, K. Jensen, C. Mitchell, S. Steiger, S.N. Gershman & S.K. Sakaluk, unpublished data), this suggests that these cues may function as cues of male mate quality during mating interactions. We hypothesized that variation in male expression of CHCs under varying nutritional environments is condition dependent, such that any effects due to diet should be dependent on the genotype of the male. Male genotypes of lower overall genetic quality may be unable to acquire sufficient resources to produce and maintain optimal levels of trait expression in low-quality environments relative to other genotypes. We predicted, therefore, that variation in male CHC expression would show significant GEIs. Here, we show that the CHCs of both males and females vary depending on the nutritional rearing environment, but that GEIs are sex specific, indicating differential selection pressures on the CHCs of males and females of this species.

Materials and methods

Study animals

Animals used in this study originated from approximately 500 adult *G. sigillatus* collected from Las Cruces, New Mexico, in 2001 that were used to establish a large panmictic laboratory population maintained at around 5000 individuals. From this population, nine inbred lines were created by subjecting randomly selected individuals to 23 generations of full-sib (brother–sister) mating, followed by three generations of panmixis within each line (Ivy, 2007). According to Green (1981), after 20 generations of brother–sister inbreeding, a line is commonly accepted as 'inbred,' with the probability of homozygosity at a given locus calculated at approximately 0.98. All crickets were housed in 25-L plastic storage containers in a constant temperature chamber maintained at 30 ± 1 °C on a 14-h:10-h light/dark cycle. Crickets were provided with food (Go Cat® Senior pellets, Purina, Croydon, UK) and water *ad libitum*, as well as egg cartons for substrate and shelter. Moistened cotton wool in Petri dishes (10 cm diameter) was provided as an oviposition substrate.

Manipulation of nutritional environment

To determine the response of male and female CHCs across two different nutritional environments, we manipulated the condition of animals by feeding both sexes with either a high-quality diet or a low-quality diet from hatching until adulthood. The high-quality diet consisted of a dry weight mixture of 100% Go Cat® Senior pellets (32% protein, 49% carbohydrates, 10% fat, 7% ash and 2% fibre), whereas the low-quality diet comprised 50% Go Cat® Senior pellets and 50% Farmland® oatmeal (17% protein, 55% carbohydrates, 7% fat and 21% fibre). Thus, our high- and low-quality diets differ in both nutritional composition and overall energy content. Both cat pellets and oatmeal were ground in a food processor and sieved prior to mixing. Newly hatched nymphs were fed this mixture as a powder until 3 weeks of age, after which the same diet was presented in pellet form. Pellets were created by adding water to the diet mixture and drying in a Plexiglas drying oven at 30 °C for 24 h.

We isolated 100 nymphs per line (total $n = 900$ individuals) within 24 h of hatching. Half of the nymphs from each line were then randomly assigned to the high-quality diet treatment, and the remaining half were assigned to the low-quality treatment. Nymphs were reared separately in individual plastic containers (5 cm × 5 cm × 5 cm) and provided with their prescribed diet treatment (as outlined above), water *ad libitum* and a piece of egg carton for shelter. All crickets were maintained in a constant temperature room set at

28 ± 1 °C and a 14-h:10-h light/dark cycle until 7 days after eclosion as adults.

Chemical analyses of CHC extracts

Seven days after adult eclosion, crickets were freeze-killed at −80 °C for 10 min. CHCs were extracted by whole-body immersion in 2 mL of hexane (Fisher H303-4, Fisher Scientific, Loughborough, UK) for 10 min. The extract was transferred to a 2-mL screw cap Target DP vial with Teflon/rubber septa and stored at 4 °C until further analysis. Prior to analysis with gas chromatography, the hexane solvent in all samples was evaporated in a fume hood, and CHCs were resuspended in 1 mL of hexane containing 100 ppm dodecane as an internal standard. The use of an internal standard allowed standardization of both retention times and peak areas across samples.

We injected 1 μL of the extracted CHC sample into a GC-MS (Agilent 7890A Gas Chromatograph coupled with an Agilent 5975B Mass Spectrometer and an Agilent CTC PAL Autosampler chilled to 5 °C, Agilent Technologies, Cheshire, UK) fitted with a DB1-MS column (30 m × 0.25 mm ID × 0.1 μm film thickness) using helium as the carrier gas. We started by running 12 CHC samples (six males and six females) extracted from crickets from the outbred population from which our inbred lines were derived, on a slow temperature profile to ensure that all CHC peaks were detected. The slow temperature profile began at 50 °C for 1 min, then increasing at 5 °C per minute to 350 °C before being held at 350 °C for 4 min (total run time = 65 min). The inlet and MS transfer line were set at 325 and 300 °C, respectively, and the injection was run in the pulsed splitless mode. We identified peaks using the NIST library in MSD Chemstation software (Agilent Technologies, version E.02.00.493), and to aid this process, we also ran a straight-chain alkane standard that contained all alkanes from C_7 to C_{40}. As not all CHC compounds appeared in the NIST library, they were identified according to their molecular weight, mass spectrum and retention time compared with these standards. We used ion 57 as the target ion to quantify the abundance of each CHC compound. Once peaks had been identified, the same 12 CHC samples were run using a faster temperature profile, which started at 50 °C for 0.5 min, rising at 20 °C per minute to 320 °C, then 7.5–350 °C where it was held for 3 min (total run time = 21 min). No CHC compounds were missing when we used this faster method, nor did the relative abundance of specific CHC compounds differ. Consequently, all CHC samples were run using this faster method.

To analyse differences in CHC profiles across genetic lines, we used a multivariate approach modified after Dietmann *et al.* (2005) and Herzner *et al.* (2006). The standardized peak areas at each retention time were $\log_{10}$ transformed to meet the assumptions of parametric analyses. To examine sexual dimorphism in *G. sigillatus* CHCs, we used discriminant function analysis (DFA) using the CANDISC procedure in SAS. We examined the strength of the relationships between the CHC peaks and the discriminant function by interpreting factor loadings > |0.20| as contributing significantly to variation between males and females. We used the DISCRIM procedure in SAS (SAS Institute, Cary, NC, USA, version 9.2) to determine the extent to which the CHC profiles of individuals could correctly predict their sex. The proportion of individuals misclassified was estimated using Lachenbruch's jackknife procedure (CROSSVALIDATE option in SAS), in which each observation is classified based on the discriminant function derived from analysis of the remaining n-1 observations (Stevens, 2002; SAS Institute Inc., 2006).

We used principal component analysis (PCA) via the FACTOR procedure in SAS to determine the CHC components that contributed to most of the observed variation due to diet for each sex and to reduce the number of independent variables used in subsequent analyses. We extracted principal components (PC) for each sex separately. We retained PCs with eigenvalues exceeding one for further analysis and interpreted factor loadings for individual CHC components to each PC of |0.25| or higher as biologically important (Tabachnick & Fidell, 1998). The PC scores derived from the PCAs were subsequently analysed for each sex by mixed-model multivariate analyses of variance (MANOVA) using the GLM procedure in SAS to test for effects of diet (fixed effect), sex (fixed effect), genotype (random effect) and all possible interactions. We specified appropriate error terms for the denominators of all F tests and follow-up tests for fixed and random factors according to Zar (1999).

While significant GEIs are indicated by nonzero reaction norms resulting in a nonparallel change in trait expression across environments, ecological crossover is a special case of GEI indicated by intersection of reaction norms (nonzero) across genotypes and environments. (Greenfield & Rodriguez, 2004). Strong GEIs can generate ecological crossover, in which different genotypes are superior under different environmental conditions resulting in a rank order change of genotypes across environments (Greenfield & Rodriguez, 2004). Therefore, we tested for ecological crossover in PCs that showed significant GEIs by calculating the rank order correlation of genotypes across environments to determine whether the rank order of CHC expression of different genotypes change with nutritional environments. Each genetic line was assigned a rank from 1 to 9 based on the mean phenotypic response of CHCs in each environment. We randomly shuffled the rank order of CHCs across genotypes in the high-quality diet using a randomization test in POPTOOLS (version 3.0, Hood, 2011) to obtain an expected

distribution for no correlation between genotypic rank and environment. The probability values for each test were calculated as the number of iterations (of 10 000) in which the pseudocorrelations generated by the randomly shuffled data were greater than or equal to the actual correlation value (Narraway *et al.*, 2010). Strong correlations would indicate that the genotypic rank order of the phenotypic response of CHCs in one environment is a good predictor of genotypic rank order in the other environment, whereas a weak correlation would suggest that genotypic ranks show ecological crossover and are therefore poor predictors of genotypic rank order across nutritional environments (Greenfield & Rodriguez, 2004; Hunt *et al.*, 2004b).

Genetic analyses of CHCs

We calculated the heritability of CHC components for both males and females for both nutritional environments. The heritability of each CHC compound was calculated as the intraclass correlation from an ANOVA on inbred lines using the protocol established by David *et al.* (2005). We also calculated genetic correlations for both sexes across nutritional environments, as well as correlations for genotype and diet between the sexes. Genetic correlations and standard errors were estimated using the jackknife procedure of Roff & Preziosi (1994). This method estimates the genetic correlation between two traits (in this case, CHC peaks) by using the inbred line means for each trait. Then, a sequence of N (nine genetic lines) pseudovalues is calculated by dropping each of the lines in turn and estimating the resulting correlations using the formula:

$$S_{N,i} = Nr_N - (N-1)r_{N-1,i},$$

where $S_{N,i}$ is the ith pseudovalue, r_N is the genetic correlation estimated using the means of all N inbred lines and $r_{N\text{-}1,i}$ is the genetic correlation obtained by dropping the ith inbred line alone (Roff & Preziosi, 1994). The jackknife genetic correlation (r_j) is then given as the mean of the pseudovalues and an estimate of standard error (SE) is given by:

$$SE = \frac{\sum_{i=1}^{i=N} (S_{N,i} - r_j)^2}{N(N-1)}.$$

It is important to note that these estimates of genetic (co)variance should be considered broad-sense estimates, as they contain variance due to dominance and/or epistasis (Falconer & Mackay, 1996). Heritabilities and genetic correlations were considered statistically significant if the value divided by the standard error was > 1.96, allowing rejection of the null hypothesis of no correlation with a two-tailed t-distribution and infinite degrees of freedom.

Table 1 Discriminant function analysis (DFA) illustrating sexual dimorphism in the 15 cuticular hydrocarbons (CHCs) of *Gryllodes sigillatus*. A single discriminant function (eigenvalue = 1.966, Wilks' Lambda = 0.337, d.f. = 15, $P = 0.0001$) explained 100% of the variance in CHCs across the sexes. This function correctly classified 90.09% of females and 96.59% of males based on their CHCs. We consider CHC peaks with factor loadings in excess of 0.20 (shown in bold) as contributing significantly to this classification.

Peak	Chemical formula	Description	DF1
1	$C_{33}H_{68}$	Branched alkane #1	0.063
2	$C_{33}H_{68}$	Branched alkane #2	0.184
3	$C_{33}H_{68}$	Branched alkane #3	**0.275**
4	$C_{34}H_{70}$	Branched alkane #4	0.024
5	$C_{35}H_{70}$	Alkene #1	**0.220**
6	$C_{36}H_{74}$	Branched alkane #5	−0.046
7	$C_{36}H_{74}$	Branched alkane #6	0.068
8	$C_{37}H_{74}$	Alkene #2	**−0.217**
9	$C_{38}H_{76}$	Alkene #4	0.024
10	$C_{39}H_{76}$	Alkadiene #3	0.091
11	$C_{39}H_{76}$	Alkadiene #4	0.131
12	$C_{39}H_{78}$	Alkene #5	0.120
13	$C_{39}H_{78}$	Alkene #6	0.174
14	$C_{41}H_{80}$	Alkadiene #5	**0.245**
15	$C_{41}H_{82}$	Alkene #7	**0.239**

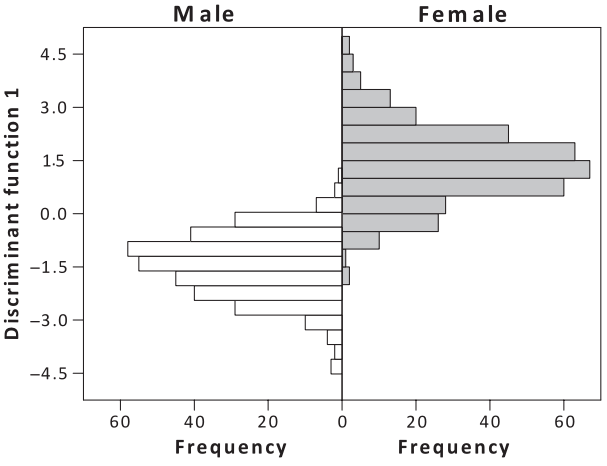

Fig. 1 Histogram showing the dimorphism in cuticular hydrocarbon (CHC) profiles of male (white bars) and female (grey bars) *Gryllodes sigillatus*.

Results

Chemical analyses of CHC extracts

We obtained hydrocarbon extracts from 345 females and 326 males from the nine genetic lines with approximate equal representation across dietary environments. Gas chromatography detected 15 hydrocarbon peaks that had previously been identified for this species (C.B. Weddle, S. Steiger, C.G. Hamaker, G.D. Ower,

Table 2 Principal component (PC) analysis of the 15 cuticular hydrocarbons (CHCs) of male and female *Gryllodes sigillatus*. PC analysis was conducted separately for each sex. In each instance, we consider PCs with eigenvalue exceeding one as biologically important and retain these for further analysis. In both sexes, a total of 3 PCs were derived explaining a total of 80.569% and 85.218% of the variation in female and male CHCs, respectively. For each PC, we consider factor loading exceeding 0.25 (shown in bold) as biologically important.

	Females			Males		
	PC1	PC2	PC3	PC1	PC2	PC3
Eigenvalue	8.711	2.202	1.172	8.776	3.001	1.005
% variance	58.075	14.682	7.812	58.510	20.009	6.699
Peak						
1	**0.953**	0.117	0.036	**0.952**	0.079	0.162
2	**0.876**	−0.026	0.015	**0.874**	0.007	**0.270**
3	**0.906**	−0.039	**0.250**	**0.918**	−0.010	**0.263**
4	**0.697**	−0.083	−0.133	**0.819**	0.072	0.001
5	**0.662**	**−0.430**	**0.281**	**0.654**	**−0.630**	0.050
6	**0.684**	−0.138	**0.539**	**0.744**	−0.210	**0.269**
7	**0.778**	−0.194	**0.409**	**0.659**	**−0.556**	**0.368**
8	**0.618**	**−0.673**	−0.221	**0.528**	**−0.770**	−0.181
9	**0.909**	−0.065	−0.098	**0.891**	−0.124	−0.114
10	**0.810**	−0.137	**−0.410**	**0.813**	−0.133	**−0.518**
11	**0.779**	0.030	**−0.443**	**0.811**	0.062	**−0.520**
12	**0.890**	0.112	−0.163	**0.898**	**0.258**	−0.151
13	**0.753**	**0.394**	0.141	**0.711**	**0.517**	−0.031
14	**0.426**	**0.863**	−0.023	**0.493**	**0.824**	0.073
15	**0.452**	**0.741**	0.052	**0.482**	**0.772**	0.110

C. Mitchell, S.K. Sakaluk & J. Hunt, unpublished data). The DFA for sexual dimorphism in the 15 CHCs of *G. sigillatus* showed significant variation due to sex (Wilks' Lambda = 0.337, d.f. = 15, $P = 0.0001$). The single discriminant function (eigenvalue = 1.966) correctly classified 90.09% of females and 96.59% of males based on their CHCs (Table 1). A histogram plot clearly shows the dimorphism in CHC profiles of males and females of this species (Fig. 1). Therefore, we ran separate analyses on males and females to examine the effects of diet on variation in CHCs.

The PCAs for variation in male and female CHCs due to diet each isolated three PC that explained 80.57% and 85.23% of the total variation in female and male CHCs, respectively (Table 2). The factor loadings for the PCs (Table 2) showed that for both sexes, all CHC peaks contributed significantly to the variation accounted for by PC1. This result is consistent with changes in the absolute relative abundance of CHCs due to nutritional rearing environment, with higher values of PC1 representing an increase in absolute quantity of CHCs due to diet. For females, PC2 was weighted negatively by lower molecular weight alkenes (#1 and #2) and weighted positively by high molecular weight molecules such as alkenes (#6 and #7) and alkadiene #5. A similar trend was seen for males, with PC2 being weighted negatively by lower molecular weight alkenes (#1 and #2) and branched alkane #6 and weighted positively by alkenes (#5, #6 and #7) and alkadiene #5. Therefore, for both sexes, higher values of PC2 represented a greater abundance of long-chained vs. short-chained hydrocarbons due to diet. Similarly, variation in PC3 for females was weighted positively by several lower molecular weight compounds consisting of branched alkanes (#3, #5 and #6) and alkene #1, and weighted negatively by slightly higher molecular weight unsaturated alkadienes (#3 and #4). For males, variation in PC3 was similar to that of females, being weighted positively by lower molecular weight branched alkanes (#2, #3, #5 and #6) and weighted negatively by higher molecular weight alkadienes (#3 and #4). Higher values of PC3 for both sexes indicate relatively higher quantities of branched alkanes relative to alkadienes due to diet. Therefore, patterns of observed variation in PC2 and PC3 indicate a trade-off between long- and short-chained CHCs due to differences in nutritional rearing environment.

The results of the mixed-model MANOVA for females showed a significant effect of genotype on variation in female CHCs, but no significant interaction between diet and genotype; the effect of diet fell just short of statistical significance (Table 3). Separate univariate mixed-model ANOVAs for each of the three PCs revealed that only PC1 showed significant variation in female CHCs due to diet (Table 3), with CHCs showing lower

Table 3 Mixed-model MANOVA showing the effects of diet, genotype (line) and their interaction on the three principal components (PCs) describing cuticular hydrocarbons in female *Gryllodes sigillatus*. Random effects (line, diet × line) were tested against mean squared error (MSE), whereas the fixed effect (diet) was tested against the mean square for diet × line (Zar, 1999). The MANOVA was followed by a series of univariate ANOVAs to determine which PCs contributed to the overall multivariate effect.

	MANOVA				
Model	Error term	Pillai's Trace	d.f.	*F*	*P*
Diet (A)	A × B	0.703	3,6	4.731	0.051
Line (B)	MSE	1.068	24,981	22.610	**0.0001**
A × B	MSE	0.078	24,981	1.101	0.334

Model	ANOVA PC	d.f.	*F*	*P*
Diet (A)	1	1,8.029	13.961	**0.006**
	2	1,8.047	0.780	0.401
	3	1,8.017	1.313	0.286
Line (B)	1	8,8	9.462	**0.0023**
	2	8,8	39.127	**0.0001**
	3	8,8	26.287	**0.0001**
A × B	1	8,327	0.971	0.460
	2	8,327	0.619	0.769
	3	8,327	1.652	0.109

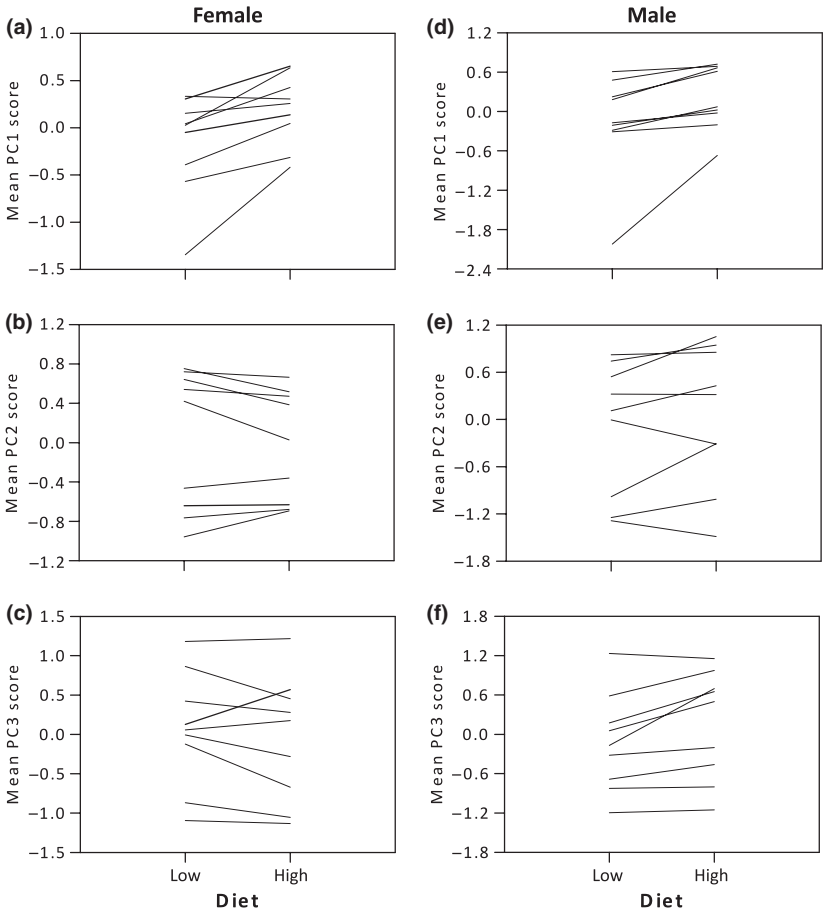

Fig. 2 Genotypic reaction norms for the three principal components (PCs) describing female (a–c) and male (d–f) cuticular hydrocarbons across the two alternate nutritional environments (high-quality diet or low-quality diet). Each reaction norm is based on the mean PC score for each genotype (inbred line) examined.

mean trait values in the low-quality diet relative to the high-quality diet. This indicates that females in the low-quality diet tended to have lower relative abundances of all CHC compounds compared with females in the high-quality diet. All three PCs showed significant effects of genotype on variation in female CHCs, a result which is consistent with our previous work (C.B. Weddle, S. Steiger, C.G. Hamaker, G.D. Ower, C. Mitchell, S.K. Sakaluk & J. Hunt, unpublished data). There were no significant interactions between female genotype and diet (GEI) for any of the three PCs (Table 3).

The genotypic reaction norms for females across diet treatments revealed that although most female genotypes showed nonzero reaction norms across environments, indicating at least some small amount of phenotypic plasticity in CHCs due to diet, very few significantly deviated from zero (Fig. 2). The general trend also showed that most female genotypes responded similarly across nutritional environments (Fig. 2). For PC1, there was a general decrease in relative abundance of female CHC compounds in the low-quality diet relative to the high-quality diet for all genetic lines (mean PC 1 ± SE: high = 0.192 ± 0.069, low = −0.166 ± 0.066, difference = 0.358, Fig. 2a) with the exception of line E. For PC2, most females showed slightly lower CHC trait values in the high-quality diet than in the low-quality diet (mean PC2 ± SE: high = −0.033 ± 0.049, low = 0.027 ± 0.047, difference = −0.060, Fig. 2b), indicating a higher relative abundance of long-chained vs. short-chained hydrocarbons in the low-quality diet. This trend was true of all genetic lines with the exception of lines A, B, H and I which showed the opposite response. Finally for PC3, female genotypic responses were generally slightly lower in the high-quality diet than in the low-quality diet (mean PC3 ± SE: high = −0.050 ± 0.071; low = 0.062 ± 0.0678; difference = −0.112; Fig. 2c), indicating relatively higher quantities of branched alkanes relative to alkadienes in the low-quality diet, with the exception of lines A, D and H which showed the opposite response.

The results of the mixed-model MANOVA for males showed no main effect of diet, but a significant main effect of genotype and a significant interaction between diet and genotype (Table 4). Separate univariate mixed-model ANOVAs for each of the three PCs revealed

Table 4 Mixed-model MANOVA showing the effects of diet, genotype (line) and their interaction on the three principal components (PCs) describing cuticular hydrocarbons in male *Gryllodes sigillatus*. Random effects (line, diet × line) were tested against mean squared error (MSE), whereas the fixed effect (diet) was tested against the mean square for diet × line (Zar, 1999). The MANOVA was followed by a series of univariate ANOVAs to determine which PCs contributed to the overall multivariate effect.

	MANOVA				
Model	Error term	Pillai's trace	d.f.	*F*	*P*
Diet (A)	A × B	0.531	3,6	2.273	0.181
Line (B)	MSE	1.552	24,924	41.256	**0.0001**
A × B	MSE	0.157	24,924	2.134	**0.0012**

	ANOVA			
Model	PC	d.f.	*F*	*P*
Diet (A)	1	1,8.033	8.213	**0.021**
	2	1,8.028	2.331	0.165
	3	1,8.041	7.755	0.024
Line (B)	1	8,8	9.874	**0.002**
	2	8,8	28.352	**0.0001**
	3	8,8	26.950	**0.0001**
A × B	1	8,308	2.218	**0.027**
	2	8,308	2.563	**0.010**
	3	8,308	1.771	0.082

significant main effects of diet for both PC1 and PC3, but not for PC2 (Table 4). All three PCs showed significant main effects of genotype on variation in male CHCs. There were significant interactions between diet and genotype for PC1 and PC2, but not for PC3 (Table 4).

The genotypic reaction norms for males across diet treatments tended to be higher than those for females (Fig. 2). For PC1, there was a general decrease in CHC components from the high- to the low-quality diet for males of all genetic lines, indicative of a decrease in overall quantity of CHCs (mean PC 1 ± SE: high = 0.210 ± 0.096; low = −0.167 ± 0.090; difference = 0.377; Fig. 2d). For PC2, there was also a general decrease in CHC components in high-quality diet relative to the low-quality diet (mean PC2 ± SE: high = 0.053 ± 0.078; low = −0.108 ± 0.072; difference = 0.161; Fig. 2e), indicative of a decrease in long-chained vs. short-chained hydrocarbons. This trend was observed for all genetic lines except A, G and I, which showed increases in the quantities of long-chained vs. short-chained hydrocarbons due to decreases in dietary quality. Finally for PC3, male phenotypic responses were, again, generally higher in the high-quality diet relative to the low-quality diet for all genotypes (mean PC3 ± SE: high = 0.150 ± 0.073; low = −0.128 ± 0.068; difference = 0.278; Fig. 2f), with the exception of line C. This indicates a decrease in the quantity of branched alkanes relative to alkadienes due to decreases in dietary quality.

Table 5 Heritability (h^2) and standard error estimates for each of the three principal components (PC) of variation in cuticular hydrocarbons (CHCs) from male and female *Gryllodes sigillatus* reared on high-quality or low-quality diets. Statistically significant estimates are shown in bold. Genetic variance in CHCs did not differ greatly across environments for either males or females.

		Females		Males	
Diet quality	PC	h^2	SE	h^2	SE
High	1	**0.800**	**0.080**	**0.869**	**0.057**
	2	**0.929**	**0.033**	**0.974**	**0.013**
	3	**0.952**	**0.023**	**0.964**	**0.018**
	Mean	0.893	0.046	0.935	0.029
Low	1	**0.808**	**0.078**	**0.935**	**0.030**
	2	**0.915**	**0.039**	**0.973**	**0.013**
	3	**0.959**	**0.020**	**0.956**	**0.021**
	Mean	0.894	0.046	0.955	0.022

Table 6 Genetic correlations (*rG*) and standard error estimates between nutritional environments for each of the three principal components (PCs) of variation in cuticular hydrocarbon from male and female *Gryllodes sigillatus*. Statistically significant estimates are shown in bold.

	Females		Males	
PC	*rG*	±SE	*rG*	±SE
1	**0.854**	**0.004**	**0.860**	**0.001**
2	**0.975**	**0.000**	**0.936**	**0.001**
3	**0.919**	**0.006**	**0.925**	**0.002**
Mean	0.916	0.003	0.907	0.001

Despite significant GEIs for male CHCs, we did not find any evidence of ecological crossover. For the two PCs that showed significant GEI, genotypic rank orders across environments were highly correlated (PC1: $r = 0.90$, $P = 0.0018$; PC2: $r = 0.90$, $P = 0.0022$). These results indicate that genotypic rank order for variation in male CHCs in the high-quality nutritional environment is a good predictor of genotypic rank order in the low-quality nutritional environment (Greenfield & Rodriguez, 2004; Hunt *et al.*, 2004b).

Genetic analyses of CHCs

The heritability estimates of the PCs describing variation in CHCs were uniformly high and statistically significant for both sexes in each environment (Table 5). For females, the average heritability (±1 SE) was the same for both nutritional environments (high: 0.893 ± 0.046; low: 0.894 ± 0.046; paired *t*-test: $t_2 = -0.1$, $P = 0.933$). For males, the average heritability was slightly lower in the high-quality diet than in the low-quality diet, but this difference was not statisti-

cally significant (high: 0.935 ± 0.029; low: 0.955 ± 0.022; paired *t*-test: $t_2 = -0.82$, $P = 0.498$). The high estimates of heritability for both sexes approaching 1.0 indicate that the majority of phenotypic variation in CHCs has a genetic basis, and should, therefore, be under relatively less environmental influence (David *et al.*, 2005).

There were significant genetic correlations for each sex across nutritional environments (Table 6). Average genetic correlations for both males and females across environments were highly, positively correlated (females: $r_G = 0.810 \pm 0.053$; males: $r_G = 0.907 \pm 0.001$).

Discussion

Our results show that the CHCs of the decorated cricket, *G. sigillatus*, vary depending on the nutritional rearing environment. Consistent with our predictions, GEI effects on variation in CHC expression were found to be sex specific, indicative of differential selection for signal reliability on the CHCs of males and females of this species. There was no significant GEI for females, suggesting that any effects of dietary rearing environment on female CHCs are not genotype specific and that the expression of female CHCs is independent of condition. In contrast, male CHC profiles did show significant GEI, with significant variation in CHC expression explained by genotype specific responses to nutritional rearing environment in terms of both absolute quantities of CHCs (PC1) as well as trade-offs between short- and long-chained hydrocarbons (PC2). These results indicate that the effects of diet on male CHCs are genotype specific and that variation in expression of male CHCs is, therefore, dependent on condition.

The effect of diet on female CHCs, although suggestive, was not quite statistically significant. However, even if this effect were real, its apparent influence on female CHC expression was only observed for PC1, which represents the absolute quantity of CHCs. Females in the high-quality diet tended to have higher relative abundances of CHCs compared with females in the low-quality diet, a result that is not altogether surprising given that a high-quality dietary rearing environment produces relatively larger crickets with more epicuticular surface area, and therefore higher overall quantities of CHC. However, we found no significant GEI for females, indicating that although female CHCs may show some degree of phenotypic plasticity with respect to signal strength (but not content), this effect is independent of female genotype. Female phenotypic expression of CHCs, therefore, appears to be independent of phenotypic condition, with a strong degree of genetic determination. These results are supported by the results of the genetic analyses as well. Female CHCs displayed equally high additive genetic variance in both environments, indicating that genotypic variation in female CHCs is not affected by nutritional environment. The estimates of covariance between environments were all high and positive. Given that many CHC compounds are known to share common biochemical pathways during insect lipid production, compounds of similar chemical nature are likely to share a genetic basis (Howard & Blomquist, 2005; Van Homrigh *et al.*, 2007). The substantial genetic covariance for CHCs across environments suggests that these compounds are likely to be inherited together, making them more reliable as cues for recognition of self (Falconer & Mackay, 1996), regardless of nutritional environment.

Consistent with our predictions, our results indicate that female CHCs do indeed show the characteristics of evolutionarily reliable cues for individual recognition: CHCs are highly variable within populations, show a high degree of genetic determination and are independent of environmental influence at the genotypic level (condition independent) (Dale *et al.*, 2001; Thom & Hurst, 2004; Tibbetts & Dale, 2007). Females appear to be able to acquire and allocate sufficient resources to produce and/or maintain optimal levels of CHC expression for reliable signal content, regardless of genetic background. Although females on the high-quality diet tended to exhibit higher quantities of CHCs relative to those on the low-quality diet, the other PCs of variation representing trade-offs in short- vs. long-chained hydrocarbons were not significantly affected by diet. Therefore, although the environment may affect the relative strength of the CHC signal in females, it does not influence its content, as overall the signal remains relatively intact over both environments and genotypes.

The design of our study manipulated the nutritional environment of females over their entire developmental lifetime and found only a marginal effect of diet on overall quantity of CHCs, suggesting, perhaps, some phenotypic plasticity for these cues. However, individual recognition cues need not remain stable over the entire lifetime of an individual, as long as any phenotypic changes happen gradually enough to allow for updates to the template of recognition (Liebert & Starks, 2004). We know from previous work that *G. sigillatus* females show behavioural discrimination against previous mates for at least 24–28 h after initial copulation (Ivy *et al.*, 2005), but it is unclear how long the CHC cues last after they are transferred to males. Females have been observed to move over greater distances than males of this species, travelling an average distance of 2 m over consecutive nights (Sakaluk, 1987). If females are likely to move to a new area over the course of several days, reducing the likelihood of encountering previous mates during that time, then a brief efficacy may be all that is required to maintain recognition of self and facilitate polyandry.

In contrast to females, we did find significant GEIs for males, indicating that phenotypic expression of CHCs for different male genotypes is dependent on the

nutritional rearing environment. This suggests that CHCs of male *G. sigillatus* are condition dependent. Moreover, for components of variation in CHCs that did show significant GEIs, we found that the genotypic rank orders of reaction norms were highly correlated across environments, indicating that for a particular genotype, phenotypic expression of male CHC traits in the high-quality nutritional environment is a good predictor of trait expression for that genotype in the low-quality environment (Greenfield & Rodriguez, 2004; Hunt *et al.*, 2004b). Additionally, genetic analyses yielded high additive genetic variance in CHCs of males in both nutritional environments, indicating a high degree of genetic determination relative to environmental determination in male CHCs. High, positive genetic covariance in CHCs across nutritional environments indicates that male CHCs are likely to share a common genetic basis, and likely coevolve in the same direction under both direct and indirect selection (Via & Lande, 1985, 1987; Lynch & Walsh, 1998).

Consistent with our predictions, our results indicate that male CHCs show several of the characteristics of evolutionarily reliable cues of quality: male CHCs are condition dependent and have a higher degree of environmental determination relative to females (Dale *et al.*, 2001). Differential expression of male CHCs among genotypes due to environmental variation indicates that some genotypes are unable to acquire sufficient resources under certain environmental conditions to allocate towards the production and/or maintenance of optimal levels of CHC expression. This suggests that CHC expression may be costly for males, so that levels of expression are dependent on the phenotypic condition of the male and may be reliable indicators of male quality. In many species of crickets, nutritional environment has been shown to have a direct effect on the acoustical signals that males use to attract females for mating (Wagner & Hoback, 1999; Holzer *et al.*, 2003; Scheuber *et al.*, 2003a,b; Hunt *et al.*, 2004a; Tolle & Wagner, 2010), and in some cases, to affect the attractiveness of song components to females (Holzer *et al.*, 2003; Hunt *et al.*, 2004a). Here, we have demonstrated that nutritional rearing environment can generate genotype specific trade-offs in the CHCs of *G. sigillatus* males in terms of absolute quantity of CHCs (PC1) and between short-chained and long-chained hydrocarbons (PC2). This is consistent with our recent finding that females of this species favour males that produce a greater overall quantity, and an intermediate mixture of short- and long-chained CHCs (J. Hunt, K. Jensen, C. Mitchell, S. Steiger, S.N. Gershman & S.K. Sakaluk, unpublished data). Trade-offs between short-chained and long-chained hydrocarbons are often associated with desiccation tolerance in insects, with a greater relative increase in long-chained CHCs providing greater desiccation resistance (Gibbs *et al.*, 1997; Gibbs, 1998; Foley & Telonis-Scott, 2011). The differential responses of male genotypes in terms of these trade-offs may reflect a relative physiological cost to the maintenance of long-chained hydrocarbons under low-quality nutritional conditions, such that these cues provide reliable cues of individual male quality.

GEIs for male sexual traits have been documented in several mating systems, and can have profound effects on the maintenance of variation in these traits (Bussière *et al.*, 2008; Ingleby *et al.*, 2010). According to the 'lek paradox', directional selection imposed by female preference for signals of quality is expected to erode genetic variance in these traits, which is an essential prerequisite for indirect genetic benefits to be maintained (Kirkpatrick & Ryan, 1991). However, evolutionary models have shown additive variance in sexually selected traits can be maintained if condition-dependant sexual traits combine with GEIs (Hunt *et al.*, 2004a,b). Because phenotypic condition (i.e. traits associated with acquiring and allocating resources) is likely to be coded by many genes, additive genetic variance in condition should be more robust to erosion by direct selection than traits coded by fewer loci (Rowe & Houle, 1996; Tomkins *et al.*, 2004). Additionally, strong GEIs can lead to ecological crossover, where no single genotype is superior across all environments and can thus prevent fixation of allelic frequencies for sexually selected traits (Greenfield & Rodriguez, 2004).

GEIs can also affect the reliability of sexually selected traits comprising signals of genetic quality (Higginson & Reader, 2009; Ingleby *et al.*, 2010). Even weak GEIs without ecological crossover can, in theory, reverse positive correlations between trait expression and male genetic quality (Higginson & Reader, 2009). However, under these conditions, signal reliability (and associated female trait preferences) can still be preserved if trait heritability is high relative to environmental effects (Higginson & Reader, 2009; Ingleby *et al.*, 2010), particularly if male trait expression and genetic quality respond in the same way to the environment. Because we found significant GEI with no ecological crossover, but high additive genetic variance in male CHCs in both environments, this suggests that if male *G. sigillatus* CHCs have evolved as cues of mate quality, then that signal reliability might be maintained by the high amount of genetic variation relative to environmental variation in the phenotypic variance of these cues. It is important to note, however, that the high genetic variation and lack of ecological crossover that we observe for male CHCs does not necessarily mean that signal reliability will be maintained across environments (Higginson & Reader, 2009). For example, Higginson & Reader (2009) showed in their stochastic simulation model that even weak GEIs without any obvious ecological crossover was sufficient to disrupt the signal content of a male sexual trait when a heterogeneous environment is encountered during development.

It is also possible that CHCs could be important cues of quality for social interactions between conspecific males, providing 'badge-of-status' cues about a male's social or dominance rank (Moore *et al.*, 1997; Tibbetts & Curtis, 2007). Because cues such as these convey information about individual quality, they are also expected to be condition dependent in their expression because the reliability (or 'honesty') of their information content is reinforced by social costs (Smith *et al.*, 1988; Tibbetts & Dale, 2004). Indeed, the CHC profiles of male *T. oceanicus* have been shown to change based on the outcome of social dominance interactions between males, with individual male CHC profiles changing with a change in dominance status from dominant to subordinate (Thomas & Simmons, 2009b, 2011). Future experiments designed to determine whether male CHCs are correlated with fitness could provide further evidence for male CHCs as reliable cues of quality. Although we have no behavioural evidence at this time to indicate that *G. sigillatus* males use CHC as cues of quality for either mate choice or dominance interactions, this would be an exciting new area of research.

The differential response of male and female CHC profiles to variation in nutritional environment suggests that these chemical cues may be under sex-specific selection pressures for signal reliability. Female CHCs show the evolutionary characteristics of reliable signals of identity: high genetic variability, low condition dependence and a high degree of genetic determination (Dale *et al.*, 2001; Thom & Hurst, 2004; Tibbetts & Dale, 2007). This indicates that female CHCs are under selection for recognition of individual identity (i.e. self) via chemosensory self-referencing to maximize the benefits of polyandry. However, the CHC profiles of males showed higher condition dependence with significant GEI, suggesting that for males of this species, these lipid cues may have secondarily evolved as signals of quality, either for mate attraction or in social dominance interactions (Dale *et al.*, 2001; Tibbetts & Curtis, 2007).

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