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RESEARCH ARTICLE

Ontogeny can provide insight into the roles of natural and sexual selection in cricket cuticular hydrocarbon evolution

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ABSTRACT

The often complex cocktails of hydrocarbon compounds found on the cuticles of insects can serve both naturally and sexually selected functions, contributing to an individual's ability to withstand water loss and attract mating partners. However, whether natural and sexual selection act synergistically or antagonistically on a species' cuticular hydrocarbon (CHC) profile remains unclear. Here, we examined the ontogeny of the CHC profile in a species of cricket, *Teleogryllus oceanicus*, while manipulating humidity during development. We predicted that juvenile crickets should produce only those compounds that contribute to desiccation resistance, while those compounds contributing specifically to male attractiveness should be produced only at sexual maturity. Further, if attractive CHCs come at a cost to desiccation resistance as predicted by some models of sexual selection, then males reared under low humidity should be constrained to invest less in attractive CHCs. Crickets reared under low humidity produced more long-chain methyl-branched alkanes, alkenes and alkadienes than did crickets reared under high humidity. The abundance of *n*-alkanes was unaffected by humidity treatment. Sexual dimorphism in the CHC profile was not apparent until adult emergence and became exaggerated 10 days after emergence, when crickets were sexually mature. Males produced more of the same compounds that were increased in both sexes under low humidity, but the humidity treatment did not interact with sex in determining CHC abundance. The data suggest that CHC profiles which protect crickets from desiccation might have synergistic effects on male attractiveness, as there was no evidence to suggest males trade-off a CHC profile produced in response to low humidity for one associated with sexual signalling.

KEY WORDS: Sexual signalling, Waterproofing, Trade-off, Insect, Mate choice, Desiccation resistance, Chemical signal, Magic trait

INTRODUCTION

Sexual selection acts on traits that enhance an individual's ability to gain access to members of the opposite sex and persuade them to mate, with significant evolutionary consequences for sexual dimorphism, population divergence and speciation (Andersson, 1994; Darwin, 1871). The costs of sexual trait expression have long been central to the development of sexual selection theory (Kotiaho, 2001; Kuijper et al., 2012). Nevertheless, while a large body of empirical work has focused on the reproductive benefits of sexual trait expression, far less attention has been given to the costs and constraints that are predicted to impose antagonistic natural selection against sexual trait expression (Podos, 2022). Moreover, some theoretical models have suggested that if a trait subject to natural selection is also beneficial in a mating context, then speciation can proceed more rapidly because of the synergistic effects of natural and sexual selection (Servedio et al., 2011). The concept of so-called 'magic traits' has long been controversial and empirical support for the idea is limited (Jiggins et al., 2001; Seehausen et al., 2008; Villa et al., 2019). The insect cuticular hydrocarbon (CHC) profile is a multivariate chemical trait that contributes to both sexual attractiveness and desiccation resistance. The dual function of insect CHC profiles makes them good models with which to explore how natural and sexual selection might interact in the evolution of trait expression.

Insect CHCs are long (C_{20} or longer) straight or methyl-branched carbon chains, with (unsaturated) or without (saturated) double-bonds. They are synthesised in the oenocytes and transported via the epidermal cells to the external cuticle of the insect (Blomquist and Bagneres, 2010; Wigglesworth, 1933). Generally, the CHC coating of insects comprises a cocktail of hydrocarbons, with as many as 100 or more different compounds constituting the CHC profile (Blomquist and Bagneres, 2010). At ambient temperatures, the CHC coating exists as a biphasic solid–liquid mixture with a viscosity approaching that of motor oil (Menzel et al., 2019). The CHC coating is thought to protect insects from desiccation (Gibbs, 1998) and the compounds within it to serve as semiochemicals involved in the transfer of information between conspecifics (Blomquist and Bagneres, 2010). A significant body of recent research has focused on sexual selection and the signalling properties of insect CHCs (Steiger and Stökl, 2014). Their naturally selected functions, however, have been relatively less well documented in the evolutionary ecology literature, and the precise function(s) of individual compounds within the CHC profile remains poorly understood (Blomquist and Bagneres, 2010; Brückner et al., 2017).

The waterproofing properties of insect CHCs are thought to depend on their ability to form a tightly packed hydrophobic layer across the insect cuticle (Geiselhardt et al., 2010). There is evidence from *Drosophila* that longer-chain CHC compounds might provide greater desiccation resistance than shorter-chain compounds (Foley and Telonis-Scott, 2011; Ingleby et al., 2014; Toolson, 1982). A comparative analysis of *Drosophila* sp. found associations between the average length of CHC chains and climatic variables; CHC chain length tends to be longer in species from regions of low precipitation (Jezovit et al., 2017). Moreover, female flies respond to desiccating environments by rapidly increasing the proportion of long-chain saturated hydrocarbons in their CHC profiles (Stinziano et al., 2015). Likewise, for both *Temnothorax* (Sprenger et al., 2018) and *Myrmica* ants (Menzel et al., 2018), acclimation to dry and warm conditions is associated with an increase in the abundance of longer *n*-alkanes.

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The presence of double bonds and methyl groups changes the shape of the molecule, which can effect lipid packing. It has been suggested, therefore, that the protective properties of a CHC blend might depend on its composition, with the presence of alkenes and methyl alkanes potentially compromising the ability of an insect to resist water loss (Blomquist and Bagneres, 2010; Gibbs and Pomonis, 1995). In contrast, methyl-branched alkanes and alkenes appear critical for the communicative function of insect CHCs (Blomquist and Bagneres, 2010). A recent comparative study of 85 ant taxa found that the CHC profiles of species from dry climates had fewer alkenes than those from wet habitats, and that species living in mutualistic association with other ant species had CHC profiles consisting of greater quantities of methyl-branched alkenes and alkadienes (hydrocarbons with two double bonds) (Menzel et al., 2017). The functional composition of an insect's CHC blend may thereby reflect the relative strengths of different selection pressures acting on it. In *Drosophila serrata*, alkenes, alkadienes, methyl-branched alkanes and methyl-branched alkenes all contribute to a male's attractiveness to females (Hine et al., 2011). However, the presence of these compounds in the CHC profile is predicted to make males more susceptible to water loss, generating antagonistic natural selection that impedes their evolutionary exaggeration. Indeed, populations of flies selected for increased amounts of these compounds returned to pre-selection levels following the relaxation of selection, consistent with antagonistic natural selection against them (Hine et al., 2011; see also Sharma et al., 2012). In contrast to these findings, methyl-branched alkanes were found to both increase male attractiveness and improve male survival under desiccation stress (Chung et al., 2014), leading to the suggestion that insect CHCs in general may be magic traits capable of promoting ecological divergence (Chung and Carroll, 2015). Indeed, when Krupp et al. (2020) applied synthetic hydrocarbons to flies lacking oenocytes, and thus unable to manufacture their own CHCs, they found that the application of blends of either *n*-alkanes or methyl-branched alkanes increased desiccation resistance, with either class of compound seemingly equivalent in its rescuing efficacy. These apparently conflicting findings for *Drosophila* call for further studies of insect CHC profiles and the interacting roles of natural sexual selection in their evolution.

Changes in CHC profiles during ontogeny can provide insight into both naturally and sexually selected functions. The CHC profiles of adult insects are known to exhibit considerable plasticity, with quantitative and qualitative changes in their composition associated with temperature, humidity, social experience and age (Otte et al., 2018). For example, changes in female CHC profiles during the first few days following adult emergence have been linked to ovary development and are responsible for age-related changes in the attractiveness of females to males (Otte et al., 2018). In *D. melanogaster*, female-specific CHCs replace longer-chain CHCs that are common to both sexes after the moult to adulthood (Arienti et al., 2010). However, little is known of the developmental ontogeny of CHC blends in male insects, or of the ontogeny of CHC profiles across different life-history stages. Here, we analysed the ontogeny of the CHC profile in male and female field crickets, *Teleogryllus oceanicus*, in order to gain insight into the potential roles of natural and sexual selection in their evolution.

In *T. oceanicus*, CHC profiles are sexually dimorphic and used by both sexes in mate choice (Simmons et al., 2013; Thomas and Simmons, 2009, 2010). For males, there appears to be a trade-off between the production of CHC profiles that are attractive to females and those that protect a male from desiccation (Berson et al., 2019), suggesting that natural selection may oppose sexual selection acting on the composition of CHC profiles. We analysed the ontogeny of CHC profiles in this species, from the 8th nymphal instar when sex can first be determined through to adulthood and sexual maturity. We expected to see little sexual dimorphism in CHC profiles when crickets are in their nymphal life-history stages and are required only to avoid desiccation, but to see increased sexual dimorphism at sexual maturity when sexual signalling becomes important. Moreover, we reared crickets in either high or low humidity environments, predicting that compounds which contribute to desiccation resistance should be upregulated in low humidity environments where crickets are more susceptible to evaporative water loss. If CHC attractiveness incurs a desiccation cost to males, the production of those compounds contributing to sexual signalling should be compromised in low humidity environments. In contrast, if CHCs are magic traits with synergistic effects on male attractiveness and desiccation resistance, the compounds that are elevated in response to low humidity should be the same compounds that are elevated in males at sexual maturity.

MATERIALS AND METHODS

Male and female crickets, *Teleogryllus oceanicus* (Le Guillou 1841), at the 8th nymphal instar were collected from a large outbred laboratory stock that is seeded annually with >50 freshly captured individuals sourced from tropical fruit plantations in Carnarvon, Western Australia, and held at the University of Western Australia. The crickets were housed in individual boxes (7×7×5 cm) with wire fly screen lids and provided with cat chow and a small piece of green bean, which was changed every 2 days.

Crickets were assigned haphazardly to one of two humidity-controlled environments that were maintained at constant temperature (26°C) and on a 12 h:12 h light:dark cycle. To manipulate humidity, crickets were placed within their individual boxes inside 149 l storage boxes, on a wire rack that rested above a tray containing a humidifying agent. Once crickets had been stacked inside these boxes, the boxes were sealed with a layer of plastic food wrap before securing the lid. High humidity was maintained using a saturated solution of potassium chloride. Low humidity was maintained using silicon dioxide (silica gel), which was dried in an oven at 60°C and changed every 2 days. Humidity within the storage boxes was recorded daily throughout the course of the experiment, a total of 41 days: the high humidity treatment had a mean (±s.e.m.) relative humidity of 77.5±0.38% (range 72–82%) while the low humidity treatment had a mean humidity of 25.4±0.16% (range 25–29%).

Crickets (20 males and 20 females) were sampled from each humidity treatment at four different developmental stages: as 8th nymphal instars 2–4 days after being introduced into the humidity treatments, 5 days after moulting to the 9th nymphal instar, 1 day after adult eclosion and 10 days after adult eclosion, when crickets are sexually mature. Crickets were removed from the treatments at the designated sampling stage and frozen at −20°C. They were then weighed and placed individually into clean glass vials with 5 ml of hexane (Merck) for exactly 5 min. The hexane contained two internal standards, nonacosane (Sigma-Aldrich) and tetratriacontane (Sigma-Aldrich) at a concentration of 0.02 g l^{-1}. When removed from the hexane, crickets were placed in an oven overnight at 60°C. Dry mass was measured the following day. Vials were left in a fume cupboard overnight to allow the hexane to evaporate fully, after which the sample was resuspended in 0.5 ml of hexane with standards as above. The extract was transferred to a GCMS vial and stored at −20°C until analysis.

We injected 1 µl of this sample via an Agilent 7693 Autosampler into a gas chromatograph and mass spectrometer (Agilent GCMS

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7890B/5977E) operating in the split mode (ratio 2:1) and fitted with an Agilent VF-WAXms column of 30 m×0.25 mm internal diameter with 0.5 µm coating (PN: CP9222, Agilent). Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}. We optimized separation of the extract using a column temperature profile in which the analysis began at a temperature of 150°C for 1 min and rose to 250°C by 25°C min^{-1} for 16 min. The final temperature was set at 255°C for 8 min. The MS transfer line, the ion source and the quadpole temperatures were 280°C, 230°C and 150°C, respectively. The certified saturated alkanes standard (C7–C40, 49452-U, Merck) was used for concentration calibration and retention index calculation.

Agilent Mass Hunter Software was used to acquire and analyse extracts. These were randomised and analysed in two independent iterations. The mass spectra of unknown compounds were deconvoluted and identified by AMDIS_32 with the NIST MS database 2020. The retention index was also used to help identify the compounds. Quantitation of metabolite features, peak detection, deconvolution, filtering, scaling and integration were all processed by Mass Hunter Quantitative Analysis for GCMS (v.7.045.7). All statistical analyses were conducted using JMP 15. Data reduction was achieved using a principal components analysis, and ANOVA was used to test the significance of treatment, sex and stage of development effects. We included log-transformed dry mass as a covariate in these analyses to control for any variation in CHC abundance that might be due to variation in body size. We also assessed the effects of treatment, developmental stage and sex on water content by analysing log cricket wet mass in an ANOVA with log dry mass as a covariate. For all analyses, interaction effects that were not statistically significant were removed from the final models. *Post hoc* contrasts were conducted using Tukey HSD. All data required to replicate the analyses reported in this article are available from Dryad (https://doi.org/10.5061/dryad.5x69p8d5z).

RESULTS

We identified 34 compounds from the extracts of 320 crickets, with carbon chains ranging in length from C_{28} to C_{35}, and mean abundance ranging from 0.1 to 241 ppm (Table 1). Principal components analysis returned two major axes of variation that each explained ≥10% of the variation in the CHC profile (Table 1). PC1 contrasted the abundance of alkanes of relatively shorter chain length (C_{28}–C_{31}) with the abundance of methyl alkanes, alkenes and alkadienes of relatively longer chain length (C_{31}–C_{33}), with the latter classes of compounds contributing most strongly to the principal axis. PC2 was loaded most strongly by the shorter-chain alkanes (Table 1).

We found significant differences in CHC profiles between crickets raised in high and low humidity environments, as well as significant differences among developmental stages and between the sexes (Table 2). For PC1, there were significant two-way interaction effects between developmental stage and humidity and between developmental stage and sex, requiring interpretation of the main effects at these levels. PC1 was greater in nymphal crickets (stages A and B) raised under low humidity but this difference was initially lost on adult emergence (stage C) and then restored at greater magnitude once crickets were 10 days of adult age (stage D) (Fig. 1A). Thus, low humidity was associated with a greater abundance of longer-chain methyl-branched alkanes, alkenes and alkadienes. Males also tended to have a greater abundance of these compounds, and thus higher scores on PC1, than did females at adult eclosion (stage C) and this sexual dimorphism had increased by 10 days after their emergence (stage D) (Fig. 1B). For PC2, there was a three-way interaction between treatment, sex and

Table 1. Cuticular hydrocarbons (CHCs) identified in whole-body hexane extracts of 320 *Teleogryllus oceanicus* that differed in sex, developmental stage and rearing humidity

Peak	Hydrocarbon	Abundance (ppm)	PC1 (29%)	PC2 (18%)
1	C_{28}	3.07±7.56	–0.167	**0.272**
2	Unresolved	0.32±0.30	–0.129	**0.292**
3	C_{30}	0.91±2.14	–0.087	0.136
4	C_{30}	0.34±1.02	–0.131	**0.255**
5	C_{30}	0.32±0.35	–0.118	**0.302**
6	C_{30}	0.68±0.90	–0.108	**0.305**
7	C_{30}	0.18±0.37	–0.141	**0.291**
8	C_{30}	0.13±0.22	–0.125	0.198
9	C_{31}	0.09±0.22	–0.103	0.106
10	C_{31}	4.73±4.19	0.029	0.009
11	C_{31}	0.99±0.74	0.012	0.098
12	Unresolved	2.55±1.62	0.143	**0.271**
13	Unresolved	3.49±5.31	–0.009	**0.299**
14	x-meC_{31}	64.72±31.97	**0.240**	0.147
15	Unresolved	8.13±3.32	0.123	0.090
16	$C_{31:1}$	3.41±1.95	**0.237**	0.081
17	$C_{31:1}$	5.09±2.89	0.076	–0.057
18	$C_{31:1}$	56.09±50.49	**0.252**	0.166
19	$C_{31:2}$	5.44±3.61	0.140	0.041
20	$C_{31:2}$	35.40±18.40	**0.262**	0.109
21	$C_{31:2}$	161.51±107.00	**0.261**	0.152
22	$C_{31:2}$	19.76±21.42	**0.224**	0.178
23	x-meC_{33}	19.21±7.75	0.181	0.118
24	$C_{32:2}$	1.80±2.59	0.152	0.163
25	$C_{33:1}$	1.86±0.75	**0.232**	–0.022
26	$C_{33:1}$	39.29±18.80	**0.278**	0.077
27	$C_{33:1}$	2.55±1.25	0.127	–0.123
28	$C_{33:1}$	6.38±3.93	**0.205**	0.058
29	$C_{33:2}$	33.63±16.38	0.116	–0.147
30	$C_{33:2}$	151.87±63.01	0.139	–0.182
31	$C_{33:2}$	241.44±124.93	**0.260**	0.027
32	$C_{33:2}$	11.22±6.70	**0.246**	0.041
33	$C_{35:2}$	2.78±1.93	0.085	–0.036
34	$C_{35:2}$	8.54±5.49	0.139	–0.078

The mean (±s.d.) abundance and contribution to the first two principal axes of variation (% variance explained) in the hydrocarbon profile are shown (eigenvectors in bold are interpreted as contributing significantly to the axis of variation, being ≥70% of the largest eigenvector; Mardia et al., 1979).

Table 2. Effect of humidity treatment, developmental stage and sex on CHC profiles of *T. oceanicus*

Source	d.f.	SS	*F*	*P*
PC1				
Treatment	1	170.13	37.44	<0.0001
Sex	1	457.08	100.59	<0.0001
Stage	3	406.37	29.81	<0.0001
Treatment×Stage	3	63.53	4.66	0.0034
Sex×Stage	3	419.36	30.76	<0.0001
log Dry mass	1	25.81	5.68	0.0178
Error	307	1394.93		
PC2				
Treatment	1	43.28	13.91	0.0002
Sex	1	15.18	4.88	0.0280
Stage	3	306.26	32.81	<0.0001
Treatment×Sex	1	3.13	1.01	0.3168
Treatment×Stage	3	12.76	1.37	0.2529
Sex×Stage	3	17.81	1.91	0.1283
Treatment×Sex×Stage	3	25.09	2.69	0.0466
log Dry mass	1	0.49	0.16	0.6921
Error	303	942.75		

Analysis of variance in the first two major axes of variation in the CHC profiles of *T. oceanicus* developing under high or low humidity treatments and sampled at the eighth and ninth nymphal stages, on adult emergence and 10 days after adult emergence when sexually mature.

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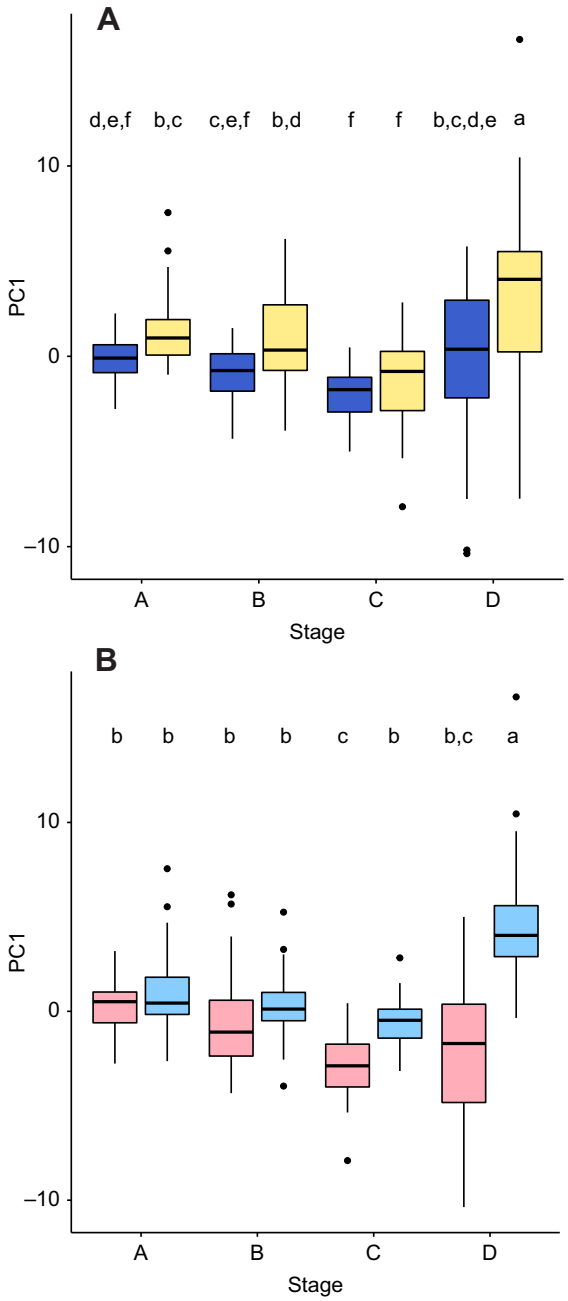

Fig. 1. Differences in cuticular hydrocarbon (CHC) profiles of *Teleogryllus oceanicus* raised in high and low humidity environments and between sexes. Boxplots (median, upper and lower quartiles and 1.5× interquartile range; circles are outliers) of the scores on the first major axis of variation (PC1) in the CHC profiles of (A) crickets developing under high (blue) or low (yellow) humidity and (B) males (blue) and females (pink), at the eighth (stage A) and ninth nymphal stages (stage B), 1 day after adult emergence (stage C) and 10 days after adult emergence when sexually mature (stage D). The breakdown in A illustrates the two-way interaction between stage and treatment, and in B the two-way interaction between stage and sex. Thus, within each plot, categories without a lowercase letter in common differ significantly at $P<0.05$ using Tukey HSD.

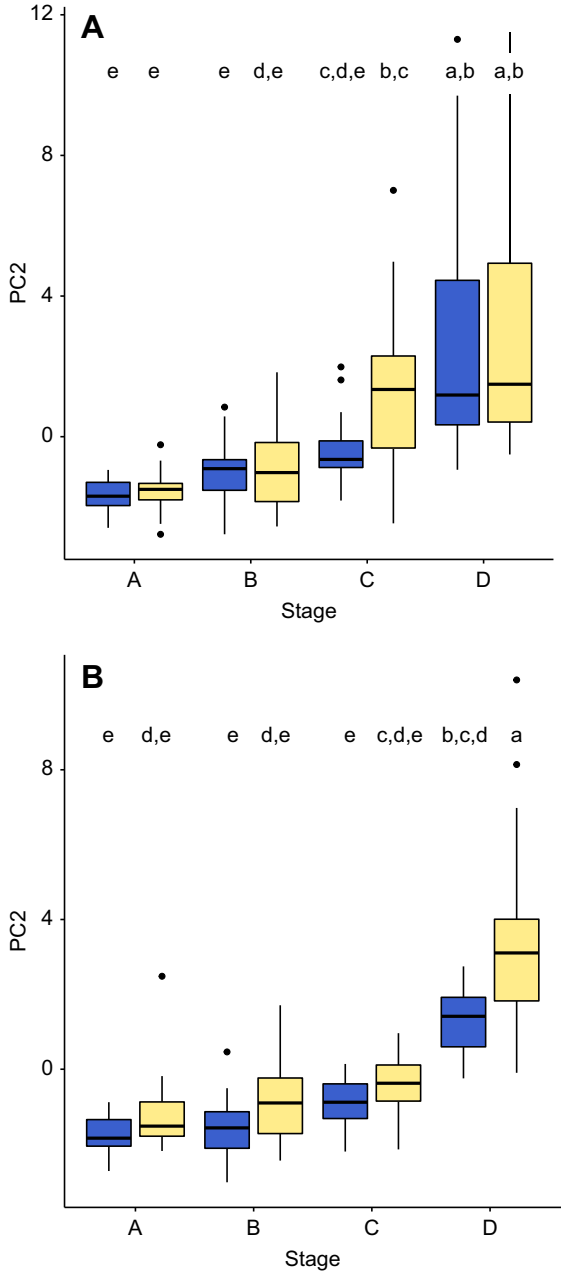

Fig. 2. Differences in CHC profiles of male and female crickets raised in high and low humidity. Boxplots of the scores on the second major axis of variation (PC2) in the CHC profiles of (A) female and (B) male *T. oceanicus* reared under high (blue) and low (yellow) humidity, at the eighth (stage A) and ninth nymphal stages (stage B), 1 day after adult emergence (stage C) and 10 days after adult emergence when sexually mature (stage D). The breakdown of the data illustrates the three-way interaction between treatment, stage and sex. Thus, within and between plots, categories without a lowercase letter in common differ significantly at $P<0.05$ using Tukey HSD.

developmental stage (Table 2). Scores on PC2 increased throughout development for both males and females (Fig. 2). For females, PC scores did not differ significantly between humidity treatments at any developmental stage (Fig. 2A). In contrast, for males, PC2 scores were higher in the low humidity treatment 10 days after adult emergence (Fig. 2B). Thus, crickets increased the relative abundance of shorter-chain alkanes as they developed and there was no evidence of sexual dimorphism at any stage. Among males, those housed in a low humidity environment produced a greater abundance of short-chain alkanes as sexually mature adults compared with those housed in a high humidity environment.

There was a significant effect of treatment and a significant two-way interaction effect between developmental stage and sex on the water content of crickets (Table 3). Crickets reared in the low

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Table 3. Effect of humidity treatment, developmental stage and sex on the water content of *T. oceanicus*

Source	d.f.	SS	*F*	*P*
Treatment	1	0.057	45.90	<0.0001
Sex	1	0.036	29.32	<0.0001
Stage	3	0.176	47.25	<0.0001
Sex×Stage	3	0.028	7.58	<0.0001
log Dry mass	1	0.930	749.53	<0.0001
Error	310	0.385		

Analysis of variance in the water content of *T. oceanicus* developing under high or low humidity treatments and sampled at the eighth and ninth nymphal stages, on adult emergence and 10 days after adult emergence when sexually mature.

humidity environment generally had a lower water content (log wet mass corrected for log dry mass: low humidity, 2.668±0.003; high humidity, 2.697±0.003). Males tended to have the greater water content until adult emergence, after which there was no longer a difference in water content between the sexes (Fig. 3).

DISCUSSION

Here, we manipulated the potential for water loss in crickets, *T. oceanicus*, by manipulating humidity during their development from juvenile to adult. Crickets reared under low humidity had a lower body water content than crickets reared under high humidity, confirming that our experimental manipulation of humidity had a significant effect on water balance. We found that crickets exposed to the low humidity environment increased the abundance of methyl-branched alkanes and longer-chain alkenes and alkadienes in their CHC profiles. Sexual dimorphism in CHC profiles appeared only after the adult moult and increased as adults aged, with males having a greater abundance of methyl-branched alkanes and longer-chain alkenes and alkadienes in their CHC profiles. There was no evidence to suggest that sexual dimorphism in the CHC profile was moderated by environmental humidity. These patterns of CHC development shed light on the potential role of natural and sexual selection in the evolution of the cricket CHC profile.

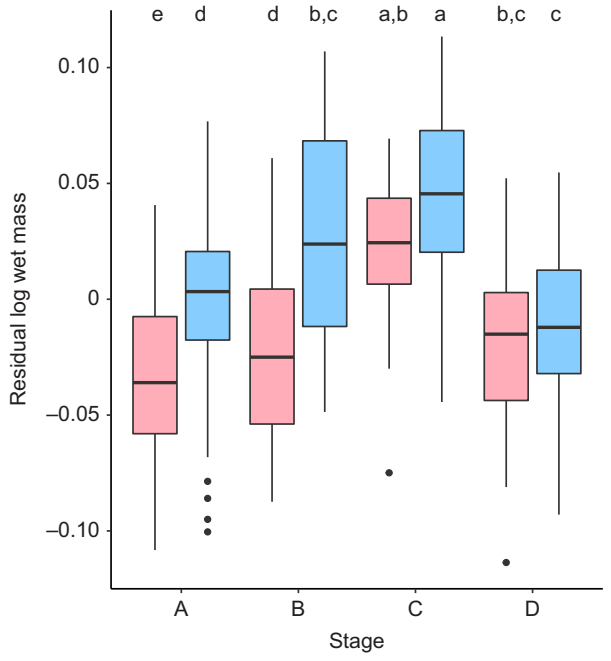

Fig. 3. Differences in the water content of crickets between sexes. Boxplots of the water content of male (blue) and female (pink) *T. oceanicus* at the eighth (stage A) and ninth nymphal stages (stage B), 1 day after adult emergence (stage C) and 10 days after emergence when sexually mature (stage D). Water content is shown as the residual wet mass (mg) after controlling for dry mass. Categories without a lowercase letter in common differ significantly at $P<0.05$ using Tukey HSD.

Longer-chain CHC compounds are predicted to afford greater resistance to desiccation than relatively shorter-chain compounds, because of their higher melting temperatures (Blomquist and Bagneres, 2010). Consistent with this prediction, we found that crickets raised under low humidity increased their scores on the first major axis of CHC variation compared with crickets raised under high humidity. This axis of variation was loaded positively by the abundance of compounds with chain lengths ranging between C_{31} and C_{35} but loaded negatively by compounds in the CHC blend with relatively shorter chain lengths (C_{28}–C_{30}). Similar increases in the production of longer-chain CHCs have been found in studies that have acclimated flies (Stinziano et al., 2015) and ants (Menzel et al., 2018; Sprenger et al., 2018) to xeric conditions, suggesting that in general these longer-chain hydrocarbons are key players in desiccation resistance across insect taxa.

The phenotypic plasticity in longer-chain CHC production observed in our study supports the hypothesis that these hydrocarbons play an important role in desiccation resistance in *T. oceanicus*. In support of this conclusion, a recent quantitative genetic study of *T. oceanicus* examined genetic correlations between individual hydrocarbon compounds within the CHC profile and the ability of males to survive desiccation (Berson et al., 2019). The authors found significant positive genetic correlations between desiccation resistance and the abundance of two of the longer-chain compounds, one alkene ($C_{33:1}$) and one alkadiene ($C_{33:2}$). These compounds contributed positively to PC1 in our study, and showed increased abundance in response to low humidity, as would be expected from their contribution to desiccation resistance. In theory, straight-chain *n*-alkanes should afford the greatest desiccation resistance as they can pack more tightly to form a protective layer (Geiselhardt et al., 2010). However, in *T. oceanicus*, straight-chain *n*-alkanes appear to be present in very low abundance and those that are produced are of relatively short chain lengths compared with the majority of compounds found in the profile. Moreover, the second major axis of variation, which was loaded predominantly by these shorter *n*-alkanes, remained very low throughout juvenile development and was generally unaffected by differences in humidity. This suggests that *n*-alkanes within the CHC profile of *T. oceanicus* are unlikely to be important for reducing water loss in drying environments.

We found sexual dimorphism in the CHC profile of crickets only after they had moulted to adulthood, both 1 day after the adult moult and to a significantly greater extent 10 days after the moult when crickets were sexually mature. Males scored significantly higher than females on PC1, producing a greater amount of longer-chain methyl-branched alkanes, alkenes and alkadienes than did females. This effect of sex was not moderated by rearing humidity. Thus, the same suite of compounds that were upregulated in response to low humidity were found in greater abundance in the sexually mature adult male CHC profile. In their study of sexual selection acting on the male CHC profile of male *T. oceanicus*, Thomas and Simmons (2009) found that males with a greater abundance of alkenes and alkadienes of chain length 31 had the greater mating success, suggesting that sexual selection may act to increase the abundance of these compounds in males. These C_{31} compounds were among those found to be elevated in males at sexual maturity, raising the

possibility that these CHC compounds may be under synergistic natural and sexual selection. Such a possibility holds important implications for ecological divergence and speciation.

If a trait subject to divergent ecological selection also generates non-random mating then speciation can proceed in the presence of gene flow because of the synergistic effects of natural and sexual selection (Servedio et al., 2011). Work on *D. serrata* has reported how longer-chain methyl-branched compounds within the CHC profile both are attractive to females and increase desiccation resistance (Chung et al., 2014). Our finding that the axis of variation in the CHC profile of *T. oceanicus* which responded to variation in humidity was the same axis of variation that was exaggerated in males at sexual maturity might be interpreted as consistent with the CHC profile of *T. oceanicus* acting as a magic trait. However, quantitative genetic analyses have reported significant negative genetic correlations between male attractiveness and three longer-chain compounds in the CHC profile, the methyl-branched alkane (meC_{33}), an alkadiene ($C_{33:2}$) and an alkene ($C_{33:1}$), the last of which also had positive effects on desiccation resistance (Berson et al., 2019). These patterns of genetic covariance suggest that the compounds which protect a male from desiccation resistance may have negative effects on his attractiveness to females. Thus, Berson et al.'s (2019) findings are more consistent with antagonistic natural and sexual selection acting on the CHC profile. These different findings for *T. oceanicus* could perhaps be reconciled if different compounds within the CHC profile contributed differently to mating success and desiccation resistance. For example, if C_{31} compounds promote male mating success but C_{33} compounds promote desiccation resistance at the expense of male mating success, the negative effects of C_{33} compounds could be offset by a relatively greater production of C_{31} compounds. In the parasitic wasp *Lariophagus distinguendus*, there are in excess of 48 compounds in the CHC profile ranging in chain length from C_{25} to C_{48}, yet just one compound, 3-meC_{27}, appears to be necessary and sufficient for courtship and mating (Kühbandner et al., 2012; Steiner et al., 2007). This example shows that different compounds in a CHC profile can serve different functions. While it is clear that male *T. oceanicus* increase their production of longer-chain CHCs at sexual maturity, and that these compounds are elevated in response to low humidity environments, the extent to which their joint or independent elaboration under sexual selection might be opposed by natural selection for desiccation resistance requires further functional analysis.

Although we are unable to draw firm conclusions regarding the relative directions of natural and sexual selection, there is good evidence that selection on the CHC profile of *T. oceanicus* has resulted in significant divergence across the species range. Studies of three genetically isolated populations introduced onto different islands in the Hawaiian archipelago have found that the abundance of longer-chain compounds (C_{33}) relative to shorter-chain compounds (C_{31}) appears to be associated with annual rainfall on different islands, with males from drier populations having a greater abundance of longer-chain compounds (Simmons et al., 2014). There is also evidence that the evolutionary loss of male song on the islands of Oahu and Kauai is associated with an increased abundance of C_{31} hydrocarbons, which might be expected if silent males in these populations rely more heavily on their CHC profiles to persuade females to mate (Simmons et al., 2014). More significantly, a comprehensive study of *T. oceanicus* across its indigenous range in Australia found that CHC divergence was strongly associated with an ecological gradient from the wet tropics in the north to the drier southern reaches of its distribution (Moran et al., 2020). Interestingly, this divergence was significant only for males, which might be the case if the major axis of variation in the CHC profile of males were a magic trait promoting ecological speciation.

In conclusion, by manipulating environmental humidity during development and examining the ontogeny of CHC profiles in male and female *T. oceanicus*, we have identified a single axis of variation in the blend of compounds that exhibits both phenotypic plasticity in response to environmental humidity and sexual dimorphism. Crickets produced more methyl-branched alkanes, alkenes and alkadienes when developing in a low humidity environment. Sexual dimorphism in the CHC profile did not develop until adult emergence, after which males produced a greater abundance of the same compounds that increased in abundance under low humidity. Contrary to theoretical expectation (Geiselhardt et al., 2010), straight-chain *n*-alkanes occurred in generally low abundance and were not upregulated in response to low humidity, suggesting that these compounds play little role in protecting these insects from desiccation. While our findings suggest that some compounds found previously to contribute to male mating success may constitute magic traits capable of promoting ecological divergence, other compounds in the CHC profile may be under antagonistic natural and sexual selection (Berson et al., 2019). Future research will be needed to explore the interactions between different compounds within the *T. oceanicus* profile, their relative contributions to desiccation resistance and attractiveness, and the extent to which they can evolve independently in response to natural and sexual selection.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: L.W.S.; Methodology: L.W.S., X.D., M.T.; Formal analysis: L.W.S.; Investigation: L.W.S., M.L., X.D.; Resources: Y.R.; Data curation: L.W.S., M.L., X.D., M.T.; Writing - original draft: L.W.S.; Writing - review & editing: L.W.S., X.D., Y.R., M.T.; Supervision: Y.R., M.T.; Project administration: L.W.S.; Funding acquisition: L.W.S.

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Data availability
Data are available from Dryad (Simmons et al., 2022): https://doi.org/10.5061/dryad.5x69p8d5z

References

Andersson, M. (1994). *Sexual Selection*. Princeton: Princeton University Press.
Arienti, M., Antony, C., Wicker-Thomas, C., Delbecque, J.-P. and Jallon, J.-M. (2010). Ontogeny of *Drosophila melanogaster* female sex-appeal and cuticular hydrocarbons. *Integr. Zool.* **5**, 272-282. doi:10.1111/j.1749-4877.2010.00213.x
Berson, J. D., Zuk, M. and Simmons, L. W. (2019). Natural and sexual selection on cuticular hydrocarbons: a quantitative genetic analysis. *Proc. R. Soc. B Biol. Sci.* **286**, 20190677. doi:10.1098/rspb.2019.0677
Blomquist, G. J. and Bagneres, A.-G. (2010). *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*. Cambridge: Cambridge University Press.
Brückner, A., Heethoff, M. and Blüthgen, N. (2017). The relationship between epicuticular long-chained hydrocarbons and surface area - volume ratios in insects (Diptera, Hymenoptera, Lepidoptera). *PLoS One* **12**, e0175001. doi:10.1371/journal.pone.0175001
Chung, H. and Carroll, S. B. (2015). Wax, sex and the origin of species: dual roles of insect cuticular hydrocarbons in adaptation and mating. *BioEssays* **37**, 822-830. doi:10.1002/bies.201500014
Chung, H., Loehlin, D. W., Dufour, H. D., Vaccarro, K., Millar, J. G. and Caroll, S. B. (2014). A single gene affects both ecological divergence and mate choice in *Drosophila*. *Science* **343**, 1148-1151. doi:10.1126/science.1249998
Darwin, C. (1871). *The Descent of Man and Selection in Relation to Sex*. London: John Murray.

Foley, B. R. and Telonis-Scott, M. (2011). Quantitative genetic analysis suggests causal association between cuticular hydrocarbon composition and desiccation survival in *Drosophila melanogaster*. *Heredity* **106**, 68-77. doi:10.1038/hdy.2010.40

Geiselhardt, S. F., Lamm, S., Gack, C. and Peschke, K. (2010). Interaction of liquid epicuticular hydrocarbons and tarsal adhesive secretion in *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). *J. Comp. Physiol. A* **196**, 369-378. doi:10.1007/s00359-010-0522-8

Gibbs, A. G. (1998). Water-proofing properties of cuticular lipids. *Am. Zool.* **38**, 471-482. doi:10.1093/icb/38.3.471

Gibbs, A. and Pomonis, J. G. (1995). Physical properties of insect cuticular hydrocarbons: the effects of chain length, methyl-branching and unsaturation. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **112**, 243-249. doi:10.1016/0305-0491(95)00081-X

Hine, E., McGuigan, K. and Blows, M. W. (2011). Natural selection stops the evolution of male attractiveness. *Proc. Natl. Acad. Sci. USA* **108**, 3659-3664. doi:10.1073/pnas.1011876108

Ingleby, F. C., Hosken, D. J. and Hunt, J. (2014). Sexual selection and genotype-by-environment interactions in Drosophila cuticular hydrocarbons. In *Genotype-by-Environment Interactions and Sexual Selection* (ed. J. Hunt and D. J. Hosken), pp. 265-281. Chichister: John Wiley.

Jezovit, J. A., Levine, J. D. and Schneider, J. (2017). Phylogeny, environment and sexual communication across the *Drosophila* genus. *J. Exp. Biol.* **220**, 42-52. doi:10.1242/jeb.143008

Jiggins, C. D., Naisbit, R. E., Coe, R. L. and Mallet, J. (2001). Reproductive isolation caused by colour pattern mimicry. *Nature* **411**, 302-305. doi:10.1038/35077075

Kotiaho, J. S. (2001). Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. *Biol. Rev.* **76**, 365-376. doi:10.1017/S1464793101005711

Krupp, J. J., Nayal, K., Wong, A., Millar, J. G. and Levine, J. D. (2020). Desiccation resistance is an adaptive life-history trait dependent upon cuticular hydrocarbons, and influenced by mating status and temperature in *D. melanogaster*. *J. Insect Physiol.* **121**, 103990. doi:10.1016/j.jinsphys.2019.103990

Kühbandner, S., Sperling, S., Mori, K. and Ruther, J. (2012). Deciphering the signature of cuticular lipids with contact sex pheromone function in a parasitic wasp. *J. Exp. Biol.* **215**, 2471-2478. doi:10.1242/jeb.071217

Kuijper, B., Pen, I. and Weissing, F. J. (2012). A guide to sexual selection theory. *Annu. Rev. Ecol. Evol. Syst.* **43**, 287-311. doi:10.1146/annurev-ecolsys-110411-160245

Mardia, K. V., Kent, J. T. and Bibby, J. M. (1979). *Multivariate Analysis*. London: Academic Press.

Menzel, F., Blaimer, B. B. and Schmitt, T. (2017). How do cuticular hydrocarbons evolve? Physiological constraints and climatic and biotic selection pressures act on a complex functional trait. *Proc. R. Soc. B Biol. Sci.* **284**, 20161727. doi:10.1098/rspb.2016.1727

Menzel, F., Zumbusch, M. and Feldmeyer, B. (2018). How ants acclimate: impact of climatic conditions on the cuticular hydrocarbon profile. *Funct. Ecol.* **32**, 657-666. doi:10.1111/1365-2435.13008

Menzel, F., Morsbach, S., Martens, J. H., Räder, P., Hadjaje, S., Poizat, M. and Abou, B. (2019). Communication versus waterproofing: the physics of insect cuticular hydrocarbons. *J. Exp. Biol.* **222**, jeb210807. doi:10.1242/jeb.210807

Moran, P. A., Hunt, J., Mitchell, C., Ritchie, M. G. and Bailey, N. W. (2020). Sexual selection and population divergence III: interspecific and intraspecific variation in mating signals. *J. Evol. Biol.* **33**, 990-1005. doi:10.1111/jeb.13631

Otte, T., Hilker, M. and Geiselhardt, S. (2018). Phenotypic plasticity of cuticular hydrocarbon profiles in insects. *J. Chem. Ecol.* **44**, 235-247. doi:10.1007/s10886-018-0934-4

Podos, J. (2022). Costs, constraints and sexual trait elaboration. *Anim. Behav.* **184**, 209-214. doi:10.1016/j.anbehav.2021.05.021

Seehausen, O., Terai, Y., Magalhaes, I. S., Carleton, K. L., Mrosso, H. D. J., Miyagi, R., van der Sluijs, I., Schneider, M. V., Maan, M. E., Tachida, H. et al. (2008). Speciation through sensory drive in cichlid fish. *Nature* **455**, 620-626. doi:10.1038/nature07285

Servedio, M. R., Doorn, G. S. V., Kopp, M., Frame, A. M., Nosil, P. (2011). Magic traits in speciation: 'magic' but not rare? *Trends Ecol. Evol.* **26**, 389-397. doi:10.1016/j.tree.2011.04.005

Sharma, M. D., Hunt, J. and Hosken, D. J. (2012). Antagonistic responses to natural and sexual selection and the sex-specific evolution of cuticular hydrocarbons in *Drosophila simulans*. *Evolution* **66**, 665-677. doi:10.1111/j.1558-5646.2011.01468.x

Simmons, L. W., Thomas, M. L., Simmons, F. W. and Zuk, M. (2013). Female preferences for acoustic and olfactory signals during courtship: male crickets send multiple messages. *Behav. Ecol.* **24**, 1099-1107. doi:10.1093/beheco/art036

Simmons, L. W., Thomas, M. L., Gray, B. and Zuk, M. (2014). Replicated evolutionary divergence in the cuticular hydrocarbon profile of male crickets associated with the loss of song in the Hawaiian archipelago. *J. Evol. Biol.* **27**, 2249-2257. doi:10.1111/jeb.12478

Simmons, L. W., Lovegrove, M., Du, B., Ren, Y. and Thomas, M. L. (2022). Data from: Ontogeny can provide insight into the roles of natural and sexual selection in cricket cuticular hydrocarbon evolution. Dryad, Dataset. doi:10.5061/dryad.5x69p8d5z

Sprenger, P. P., Burkert, L. H., Abou, B., Federle, W. and Menzel, F. (2018). Coping with the climate: cuticular hydrocarbon acclimation of ants under constant and fluctuating conditions. *J. Exp. Biol.* 221, jeb171488. doi:10.1242/jeb.171488

Steiger, S. and Stökl, J. (2014). The role of sexual selection in the evolution of chemical signals in insects. *Insects* **5**, 423-438. doi:10.3390/insects5020423

Steiner, S., Mumm, R. and Ruther, J. (2007). Courtship pheromones in parasitic wasps: comparison of bioactive and inactive hydrocarbon profiles by multivariate statistical methods. *J. Chem. Ecol.* **33**, 825-838. doi:10.1007/s10886-007-9265-6

Stinziano, J. R., Sové, R. J., Rundle, H. D. and Sinclair, B. J. (2015). Rapid desiccation hardening changes the cuticular hydrocarbon profile of *Drosophila melanogaster*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **180**, 38-42. doi:10.1016/j.cbpa.2014.11.004

Thomas, M. L. and Simmons, L. W. (2009). Sexual selection on cuticular hydrocarbons in the Australian field cricket, *Teleogryllus oceanicus*. *BMC Evol. Biol.* **9**, 162. doi:10.1186/1471-2148-9-162

Thomas, M. L. and Simmons, L. W. (2010). Cuticular hydrocarbons influence female attractiveness to males in the Australian field cricket, *Teleogryllus oceanicus*. *J. Evol. Biol.* **23**, 707-714. doi:10.1111/j.1420-9101.2010.01943.x

Toolson, E. C. (1982). Effects of rearing temperature on cuticle permeability and epicuticular lipid composition in *Drosophila pseudoobscura*. *J. Exp. Zool.* **222**, 249-253. doi:10.1002/jez.1402220307

Villa, S. M., Altuna, J. C., Ruff, J. S., Beach, A. B., Mulvey, L. I., Poole, E. J., Campbell, H. E., Johnson, K. P., Shapiro, M. D., Bush, S. E. et al. (2019). Rapid experimental evolution of reproductive isolation from a single natural population. *Proc. Natl. Acad. Sci. USA* **116**, 13440-13445. doi:10.1073/pnas.1901247116

Wigglesworth, V. B. (1933). The physiology of the cuticle ond of ecdysis in *Rhodnius prolixus* (Triatomidae, Hemiptera); with special reference to the function of the oenocytes and the dermal glands. *Q. J. Microsc. Sci.* **76**, 269-318. doi:10.1242/jcs.s2-76.302.269