THE EVOLUTIONARY RESPONSES OF CUTICULAR HYDROCARBON PRODUCTION AND COMPENSATORY FEEDING BEHAVIOURS WHEN CRICKETS HAVE EVOLVED IN DIFFERENT NUTRITIONAL ENVIRONMENTS

Submitted by

Alexandria Williams

BNatSc (AnimSc)

A thesis submitted in requirement for the degree of

Master of Research

Hawkesbury Institute for the Environment

Western Sydney University

22 February 2020

Supervisory panel:

Professor John Hunt, Dean's Unit School of Science, Western Sydney University (Principal Supervisor)

Dr Clarissa House, School of Science, Western Sydney University

ACKNOWLEDGEMENTS

The work undertaken and challenges faced to complete the Master of Research would not have been possible without the help of many people. I would not have been able to accomplish what I have without the support and guidance of my supervisors Professor John Hunt and Dr Clarissa House. Thank you for your constant support, expertise and advice over the course of my project and completion of this thesis. I greatly appreciate the constructive critique, late night contributions, assistance with cricket maintenance, and for ensuring I understood the scientific concepts I was working with.

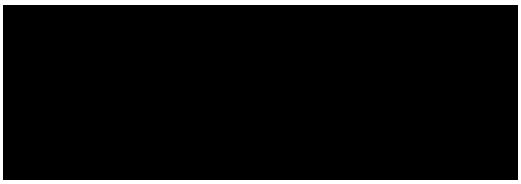
I thank the Hawkesbury Institute for the Environment (HIE) for supporting the involved expenses and for providing me with the opportunity to present the progression of my thesis and offering constructive criticism throughout the course of my research.

I would not have been able to put as much time and effort into my research without the immense support and collaborative work of my fellow students Alejandro Rios Villamil and Corinne Letendre, whose teamwork ensured the cricket populations used in this study were maintained throughout the experiment. I also appreciate their encouragement and constructive comments, which allowed me to achieve the best results from my laboratory work. Special mention must also be made to laboratory technician Carly Brown, whose assistance in the laboratory allowed for the intensive work to be carried out as smoothly as possible. Also, Dominika Wojtalewicz from HIE was instrumental in ensuring my work with the Gas Chromatography-Mass Spectrometry machine and extractions went as smoothly as possible in spite of numerous delays which were out of our control, for which I am most grateful.

Thank you to my family for their support, especially during the last few months of writing this thesis, providing me with a roof over my head and financial assistance so I could focus solely on my research.

STATEMENT OF AUTHENTICATION

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

. 

22 February 2020

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CHC	Cuticular hydrocarbon
HC	High nutritional value, carbohydrate-biased
HCN	High nutritional value, carbohydrate-biased – normal diet
HCS	High nutritional value, carbohydrate-biased – switched to standard cricket diet
HP	High nutritional value, protein-biased
HPN	High nutritional value, protein-biased – normal diet
HPS	High nutritional value, protein-biased – switched to standard cricket diet
LC	Low nutritional value, carbohydrate-biased
LCN	Low nutritional value, carbohydrate-biased – normal diet
LCS	Low nutritional value, carbohydrate-biased – switched to standard cricket diet
LP	Low nutritional value, protein-biased
LPN	Low nutritional value, protein-biased – normal diet
LPS	Low nutritional value, protein-biased – switched to standard cricket diet
PC	Principal component
P:C	Protein : carbohydrate ratio
SCD	Standard cricket diet

ABSTRACT

Condition dependent traits are reliant upon the acquisition and allocation of resources, with studies theorising that those individuals able to acquire more resources are then able to allocate more resources to these sensitive traits. These traits are often related to sexual reproduction, one of the most important aspect of an organism's life cycle. One such trait is chemical signalling, where the production of these chemicals is known to be linked to adequate, over- or under-consumption. However, relatively little is known on compensatory feeding behaviours and dietary adaptation to offset the nutritional value of resources. This includes either consuming more or less depending on the overall quality of the food sourced. Furthermore, little research has been conducted into how populations respond to diet over multiple generations, analysing whether feeding behaviours has the potential to evolve in response to different dietary environments. Thus, little is also known about whether potentially evolved dietary responses have an effect on condition-dependent traits. Here, nutritional geometry was used to determine the effect of protein and carbohydrate ratios and overall nutrition on compensatory feeding behaviours and cuticular hydrocarbon (CHC) production in the decorated cricket (*Gryllodes sigillatus*), studying the effect of different ratios on multiple generations. A common garden experiment was employed for to determine whether responses were genetic rather than phenotypic in nature. Populations of crickets were raised on either low or high nutritional diets, which were also either protein or carbohydrate biased. A population was also kept on a standard balanced diet (SCD) for comparison. For study into possible genetic dietary responses, some populations raised on the altered diets were switched back to the standard diet (SCD).

Individuals maximised consumption when faced with low nutritional value diets, while those raised on high nutritional value diets consumed less. Individuals switched back to SCD consumed an amount similar to their ancestral diet, however still maintained a noticeable difference in consumption, suggesting an evolution of genetics behind compensatory feeding behaviour as well as the typical phenotypic response. This adaptation to differing diets has a direct influence on CHC production, impacting the overall amount and blend of short and long chain CHCs across and within all diet treatments. CHC composition was sex-specific, however, diet altered the overall production. Switched diet individuals showed evolved feeding behaviour, consequently affecting CHC production in line with altered macronutrient and caloric intake. CHC compositions indicated that while individuals changed from a protein or carbohydrate-biased diet to a more balanced diet, there is the possibility of a genetic tendency to produce CHC blends similar to their ancestors. Theoretically, this has a consequential effect on male fitness, as females are attracted to specific male CHC blends.

INTRODUCTION AND STUDY AIMS

Introduction

The condition of an organism is dependent upon the acquisition of resources and the allocation of these resources to traits (Delcourt & Rundle 2011). The theory of condition-dependence predicts that organisms with higher attainment of resources (which is influenced by genes and the environment), are able to allocate more resources to traits that are sensitive to condition. Thus, they have higher fitness due to superior, competitive ability and improved physiology (Cotton, Fowler & Pomiankowski 2004; Franzke & Reinhold 2012; Wang et al 2019). Many fitness traits have been shown to be condition-dependent, such as growth (Barton et al 2017), reproductive effort (Delcourt & Rundle 2011) and sexual traits (Cotton, Fowler & Pomiankowski 2004; Rapkin et al 2017). Furthermore, support for condition-dependence theory has been shown in a wide range of taxa, such as fish (Aday, Wahl & Phillip 2003; Nordeide et al 2008; Barton et al 2017), amphibians (Wang et al 2019), birds (Birkhead, Fletcher & Pellatt 1999), arachnids (Uetz, Papke & Kilinc 2002), and insects (House et al 2016; Rapkin, Jensen, House et al 2018).

Due to the importance of resource acquisition for trait expression, dietary manipulation is one of the most common methods of testing condition dependence (Ricklefs & Wikelski 2002; Urrejola, Nespolo & Lardies 2011). Most studies subjected their experimental animals to a "good" vs. "bad" diet, focusing on diets that were either nutritionally rich or poor in calories, however, this method only tests a small range of diets (Wehi, Raubenheimer & Morgan-Richards 2013) that don't specify the caloric value or nutrient composition (Rapkin et al 2017), making it hard to determine which nutrients are having the impact. Recently studies have begun using a geometric nutritional framework to test the impact of caloric content and specific macronutrient ratios i.e. protein and carbohydrate ratios (P:C) for trait expression (Clark, Zera & Behmer 2015; House et al 2016; Simpson et al 2017; Rapkin, Jensen, Archer et al 2018). Studies focusing on P:C ratios found these macronutrients play an essential role in growth and development (Zajitschek et al 2012; Kurpad 2018), with animals relying on specific macronutrient ratios for optimal trait expression (Hochuli 2001; Joern, Provin & Behmer 2012). The amino acids in protein, and the sugars, starch and fibre from carbohydrates are used in biological reactions, providing required energy and essential compounds for phenotypic traits at each stage of an organism's life (Wehi, Raubenheimer & Morgan-Richards 2013). Thus, restriction of these two macronutrients can have adverse impacts on the phenotype (Flatten & Heyland 2011).

Condition-dependence studies have, until recently, overlooked other aspects of resource acquisition; including compensatory feeding and dietary adaptation. Ideally, in studies of condition-dependent trait expression the measurement of animal feeding behaviour and of food consumption is measured (Rapkin et al 2017). Animals on low caloric diets and/or nutritionally imbalanced diets may regulate their feeding behaviour and therefore consume an equal amount of nutrients as those provided with high calorie and/or nutritionally balanced diets (Raubenheimer & Simpson 1997; Berner, Blanckenhorn & Körner 2005). This raises the possibility that condition-dependent trait expression is not detected, even though it does indeed occur. Furthermore, the majority of studies focus on one to two generations, testing the immediate impact of dietary manipulations on trait expression. Studying the impact of different nutritional compositions on immediate feeding behaviours is important to determine an animal's ability to adjust to diet stress. However, this approach does not reveal whether the response to diet has the potential to evolve. Expanding studies to include multiple generations would show whether dietary feeding behaviours improve nutrient intake,

particularly in systems where compensatory feeding occurs and has a genetic basis and the potential to evolve due to directional selection (Johnston et al 2013). Combining this generational approach with a transplant or common garden experiment would establish a method to devise whether there is a genetic effect behind feeding behaviours, transplanting those from biased diets to an environment with balanced resources. Previously, this method has been employed in analysing plasticity among several phenotypes (e.g. Ingleby, Hunt & Hosken 2013; Pitchers et al 2013).

Typically, tests on the impact of nutrition for phenotypes often involve the measurement of condition dependent, sexually selected traits such as acoustic signals (e.g. Franzke & Reinhold 2012; Duffield et al 2018), courtship displays (Kotiaho 2002) and morphological traits (e.g. Clark, Zera & Behmer 2015; House et al 2016; Christie et al 2018). In contrast, influence of diet for the expression of condition dependent, chemical signals and cues (e.g. Shelly, Edu & Pahio 2007; Steiger et al 2013; Clark, Zera & Behmer 2015) are often overlooked. Such chemical signals include pheromones (d'Ettorre & Moore 2008; Fischman, Woodard & Robinson 2011). In particular, the contact pheromones known as cuticular hydrocarbons (CHCs). These are found in terrestrial arthropods, which consist of particular blends of hydrocarbons, acidic resins and lipids, which are excreted on the exoskeleton of insects (Golebiowski et al 2012; Renou 2014). Specific CHCs are important to prevent water loss and water logging (Baker et al 1960), however CHCs also influence male–male competition (Lane et al 2016) and female-male interactions (Ingleby 2015; Steiger et al 2015; Lane et al 2016) at different stages during reproduction (Weddle et al 2013). Despite the importance of CHCs for fitness, surprisingly few studies have investigated the influence of nutrition for the expression of condition dependent CHCs (South et al 2011; Rapkin et al 2017) and whether insects compensatory feed to optimize CHC expression when faced with dietary imbalance (Rapkin et al. 2017). Furthermore, to our knowledge, no studies have investigated whether insects evolve feeding behaviour and ultimately CHC expression when populations have been, for generations, supplied a novel food source.

The decorated cricket, *Gryllodes sigillatus*, regulates the acquisition of protein and carbohydrates to maximise reproductive effort (Rapkin, Jensen, Archer et al 2018). Like in other cricket species, cuticular hydrocarbons are important chemical cues that are likely to be important during male dominance interactions and mate choice (Weddle et al. 2012; Ingleby 2015). Females have also been found to use CHCs as a means of detecting viable males, choosing those which have particular CHC phenotypes that they find favourable (Steiger et al 2015). They also imbue males they have mated with their own cuticular hydrocarbons so as to avoid them and find new males (Ivy et al 2005; Gershman & Sakaluk 2010). Weddle et al (2012) found that nutrition affects CHC production in *G. sigillatus.* However, this study, like others, overlooked the possibility of compensatory feeding and did not measure consumption. Subsequently, it has been found that there is an immediate increase or decrease in feeding behaviour when faced with holidic diets, with cuticular hydrocarbon production linked to protein and carbohydrate ratios and caloric content (Rapkin, Jensen, House 2018).

Study Aims and Expected Outcomes

Here, a common garden experiment was employed to determine whether there is a genetic basis to compensatory feeding behaviours to regulate protein and carbohydrate intake and CHC production in *G. sigillatus*. Crickets were derived from replicate populations that evolved on altered calorie value, macronutrient-biased diets, resulting in either high protein, low protein, high carbohydrate, low carbohydrate, or on a standard diet. Populations raised on

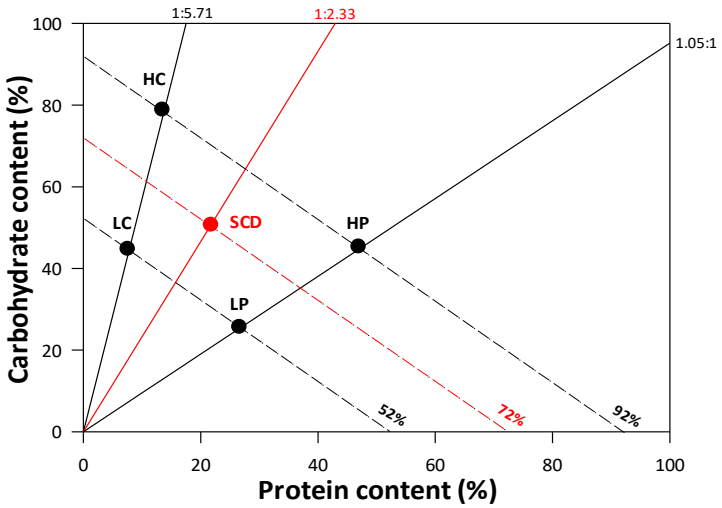
the biased diets were either maintained on their original, ancestral diet or switched to a 'standard' balanced diet, splitting the populations in two. It was predicted that there will be a difference in the feeding behaviour of crickets derived from protein versus carbohydrate and high versus low caloric diets. Populations raised on high nutritional value, protein or carbohydrate-biased diets were expected to exhibit significantly lowered intake as the combination of high nutrition value and the macronutrient bias will constrain the individuals to consuming less as they reach one nutrient target before the other (Simpson et al 2017). Alternatively, the populations on nutritionally low diets will consume more food, however, they will differ due to the constraints placed by the exceeded level of either protein or carbohydrate (Simpson, Le Couteur & Raubenheimer 2015). In populations placed in the common garden set-up, and therefore changed from their original, ancestral diet to the 'standard' balanced diet, we tested the hypothesis that feeding behaviour is similar to their ancestral diet. Selection should favour dietary adaptation to high protein, low protein, high carbohydrate, low carbohydrate or a standard diet and therefore, those switched to the standard diet are expected to have similar feeding behaviours to their normal diet counterparts. Next, we tested the hypothesis that the quantity of cuticular hydrocarbons produced is different across each diet. This is predicted to occur as protein is required for CHC production (Zajitschek, Lailvaux, Dessmann & Brooks 2012), and therefore populations on protein-biased diets are expected to produce a larger amount of these chemical compounds, compared to those on carbohydrate-biased diets. However, high nutrition has the potential to place a constraint on CHC expression as individuals consuming high nutritional value resources can theoretically reach their caloric intake target before their macronutrient target (Simpson, Le Couteur & Raubenheimer 2015; Simpson et al 2017). It is expected that the low nutrition, protein-biased diet populations will excrete the most CHCs as the diet may allow for macronutrient targets to be the limiting factors. Thus, it is predicted that the nutritionally high, carbohydrate-biased diet will produce the least amount of CHCs out of all diets tested as either the carbohydrate bias or caloric value will be the limiting factor. Larger CHCs (longer chain) require more protein compounds to create, therefore the populations consuming a higher amount of protein will produce on average more long chain CHCs over short chains, and vice versa.

METHODOLOGY

Rearing of Populations and Diet Manipulation

For this study, the decorated cricket *Gryllodes sigillatus* was used. Crickets were sourced from lines of this species already established by Professor John Hunt at the university. These cricket populations originated from a line in New Mexico (Weddle et al 2012; Rapkin, Jensen, Archer et al 2018), and were used to establish a large laboratory population, raised on a standard cricket diet (SCD). For the means of enabling studies on diet and nutrition effects, lines were raised on new diets, either protein or carbohydrate biased (P or C), and simultaneously either of high or low caloric value (H or L). These resulted in diets which were labelled Low Protein, Low Carbohydrate, High Protein, and High Carbohydrate (Zajitschek, Lailvaux, Dessmann & Brooks 2012). Protein and carbohydrate biases were created by changing the ratio of P:C. Protein-biased diets had a ratio of 1.05:1, while carbohydrate-biased diets contained a ratio of 1:5.71 (Fig. 1), compared to the standard cricket diet (SCD) maintaining a ratio of 1:2.33. The caloric value of the diets differed from the SCD by 20%, with low diets having a 52% nutritional value, and high value diets sitting at 92%. These diets were made in the laboratory, using weighed out portions of ground commercial cat biscuits (Friskies Adult), and rat food pellets for the SCD, and altered weights of the cat and rat food with the addition of powdered protein and carbohydrate sources. All of the altered diets contained varied amounts casein, albumen and peptone (proteins), and sucrose and dextrin (carbohydrates) (Wehl et al 2013; Rapkin, Jensen & Archer et al 2018; Rapkin, Jensen & House et al 2018). They also contained cellulose, Wesson salts, cholesterol, and vitamin mix (Rapkin, Jensen, Archer et al 2012; Harrison et al 2014). The cellulose was added in varying amounts, acting as a filler to ensure nutrient intake varied among the diets due to cellulose's low digestibility in insects (Harrison et al 2014; Bunning et al 2016).

Figure 1. Comparison of nutrition level of diets from the Standard Cricket Diet (SCD). HC = high carbohydrate, LC = low carbohydrate, HP = high protein, LP = low protein. Includes P:C ratios within the diets and total nutrition as shown by the percentage above the x-axis

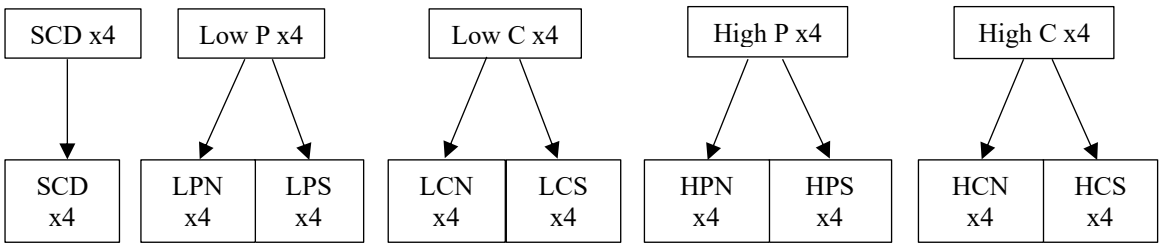


Each of the populations were on at least their 35th generation, having been raised on one of the specified diets since the first generation. Each line was raised in the same environmental conditions (aside from diet), with a 12:12 light:dark cycle (Pascoal 2015), constant access to water, shelter in the form of egg carton stacks, and ambient temperatures of 30°C (± 2°C)

(Rapkin, Jensen Archer et al 2018). This resulted in lines that were well established on these "new" diets. There were four replicates for each diet, resulting in, for example Low Protein, replicate 1, 2, 3 and 4.

The crickets used in this experiment were randomly selected from these diet lines as newly hatched nymphs retaining the same diet and replicate number. Each replicate consisted of around 200 individuals. To allow for analysis of possible genetic alterations nymphs from each diet line were allocated to populations that were switched to the SCD (Fig. 2). As a result, each replicate was split and either kept on the original diet (normal = N) or switched (S), creating a common garden experiment (Pitchers et al 2013). For example, this created four replicates of LP(N) and LP(S). Nymphs from the line on SCD were used to create populations to generate a baseline upon which the other populations were compared to as well as to each other. Each population was fed *ad libitum*, provided with the same environmental conditions as their predecessors – the 12:12 light:dark cycle, access to water (50ml test tubes filled with water and plugged with cotton wool), egg cartons for shelter, and ambient temperature of 30°C (±2°C).

Figure 2. Nymphs from diets Low Protein, Low Carbohydrate, High Protein and High Carbohydrate are randomly split between remaining on their normal diet (N) and being switched to SCD (S) to analyse any genetic differences. Populations on SCD were continued to be used as a baseline for comparison. Each diet box pictured consisted of four replicates of 200 individuals, totalling 800 individuals per diet. Replicate populations were split into normal (N) or switched (S) to build 4 replicates of 200 individuals for the resultant diet test groups.



When the majority of a population reached maturity, they were given egg pads (petri dish bases filled with a mound of wet cotton) to lay eggs for the new generation. The egg pads were removed after one week and placed into a small container to allow for the nymphs to hatch, creating the new generation. This continued for two generations allow for parental effects to be ruled out, with the third generation used for feeding behaviour experiments and cuticular hydrocarbon analysis. The nymphs creating the third generation were kept together in a small container in densities of 100 individuals with their specified diet fed *ad libitum*, two 10ml test tubes plugged with cotton wool for water bottles, and egg cartons for shelter (Rapkin, Jensen, Archer et al 2018). Once they reached 2 weeks of age, the nymphs were isolated into small individual containers to ensure each individual was a virgin and that there was no transfer of CHCs between individuals (Steiger et al 2015). For this study each replicate had 15 females and 15 males randomly chosen for feeding measurements. This resulted in a total of 30 individuals per replicate, and therefore 120 individuals per diet, with a total of 1080 individuals for the experiment. To ensure there were enough adults available,

200 individuals were isolated per diet. Within these containers, each nymph was given food, a small water bottle, and an egg carton section for shelter (Steiger et al 2015).

Feeding

The isolated nymphs were fed *ad libitum*, with food and water replaced once a week until they eclosed and became adults. The day an individual became an adult, they were recorded and numbered until each replicate had the 15 females and 15 males required. In preparation for the feeding experiment, feeding dishes were constructed by gluing a small test tube lid onto the inverse side of a small petri dish lid. This ensured any fall-out of the food from the test tube lid was caught by the dish. Batches of each diet were made and placed in containers in an oven set to 40°C to remove any water so as to measure a true dry weight (Harrison et al 2014; Rapkin, Jensen, Archer et al 2018).

The feeding dishes were placed on a scale and their weights recorded to the fourth decimal place. The scale was zeroed while the feeding dish was still on it, and food was spooned into the test tube lid section and patted down into the compartment until it was filled. The weight of the food was then recorded, ensuring the dish was assigned to an individual (e.g. SCD, Repetition 1, Female 1). These dishes were given to their allocated individuals. Each adult cricket had their food dish for four days, with a new dish replacing the first one on the fourth day. This occurred for an eight-day period, with two feedings per adult. When the feeding dish was removed, it was dried in the 40°C oven for 24 hours, with faecal and other matter removed, and then reweighed to get the combined total of dish and remaining food. This weight was deducted from the total of the initial feeding dish weight and starting amount of food, giving the amount of food consumed. All measurements of the feeding dish, starting food and end weight was measured precisely to the fourth decimal place to ensure the smallest of variances in feeding behaviour were accounted for (Archer et al 2009).

On the eighth day of feeding (and eight days from eclosion) each cricket was freeze-killed in a -20°C freezer (Tregenza & Wedell 1997). As the most humane and quickest method, freeze-killing lessened the possibility of stress and any impact on cuticular hydrocarbon production. The eight-day period was deemed sufficient time for sexual maturation to occur and production of targeted cuticular hydrocarbons to be produced (Wagner Jr & Hoback 1999). After 24 hours in the freezer, the crickets were placed into labelled Eppendorfs and placed back in a -20°C freezer until required for CHC extraction.

Extraction of Cuticular Hydrocarbons

Extraction of the cuticular hydrocarbons involved the use of hexane (Tregenza & Weddell 1997; Thomas & Simmons 2008). Each individual was submerged in a 4ml extraction vial containing 3ml of a hexane solution containing 10ppm of dodecane. They were submerged for five minutes, ensuring the CHCs dissolved within the non-polar solvent (Thomas & Simmons 2008; Kilani-Morakchi et al 2009; Touhara 2013; Sharma et al 2015).

After extraction, the crickets were removed and replaced back into their respective Eppendorfs so as to measure pronotums. Of the cuticular hydrocarbon/hexane solution, 1ml was pipetted into a 2ml autosampler vial, preparing all 1080 samples for analysis through a Gas Chromatography Mass Spectrometer (GCMS) machine (Tregenza & Wedell 1997).

Analysis of Quantity and Composition

Each sample was loaded onto an Agilent Technologies 7890A GC which was attached to an Agilent Technologies 5975B MS and run through a pre-prepared method. The machine

extracted 1µl of the solution (Tregenza & Wedell 1997; Harari, Zahavi & Thiéry 2011) and injected it into the gas chromatograph, where the extract was carried by the inert gas hydrogen. The programmed method used was adapted from a previous analysis method used by Rapkin et al (2017) and is as follows: both inlet lines were set at 325°C, with injection set to pulsed splitless. Temperature began at 50°C for a total of 30 seconds, and then increased to 320°C at a pace of 20°C per minute. It then increased to 350°C at a pace of 7.5°C per minute and was held at 350°C for 5 minutes. There was a flow rate of 10mL of hydrogen (Steiger et al 2015) per minute, resulting in a total run time of 23 minutes per sample.

The retention times detected by an ionised detector in the GCMS and were graphed using the CHEMSTATION program (Agilent Technologies), resulting in peaks for each sample (Thomas & Simmons 2008). These peaks were manually integrated, obtaining the area of each targeted compound – a total of 18 CHC peaks. As a means of calculating the relative peak area of the cuticular hydrocarbons, the dodecane retention time and peak was also recorded.

Pronotum Measurements

After cuticular hydrocarbon extraction, the crickets were measured for the width of their pronotums. These measurements were taken to remove the variable of size from affecting any differences in food consumption and feeding behaviour. This involved the use of a microscope with graticules etched into the glass. A ruler was initially placed under the lens, with the magnification set to 1.6, so as to allow for conversion of graticules to millimetres. An individual was placed on the base and the width of the pronotum was taken and recorded in both graticules and millimetres. After measurement, the crickets were returned to their Eppendorfs and replaced in the -20°C in case of further need.

Statistical Analysis

Overall mean consumption and protein and carbohydrate intake were graphed to visually analyse any variances within diets (e.g. LPN to LPS) and across diets. The mean for the crickets raised on SCD was mapped as a baseline, to allow for comparisons.

Statistical analysis of the feeding behaviour involved the use of ANCOVA for the mean dietary consumption, and a MANCOVA analysing the mean intake of protein and carbohydrate. For analysis of feeding behaviour, the P:C ratio was established as the first fixed effect, and total nutrition as the second fixed effect as they were the two non-random variables. Interactions between these two factors were analysed as a fixed effect. Sex was also included in analysis as fixed effects. The response variables were noted as the amount of food consumed, the intake of protein and carbohydrates, and the quantity of cuticular hydrocarbons expressed as well as the principal components.

Principal component (PC) analysis of cuticular hydrocarbon expression was used to calculate eigenvalues of the PC scores. Eigenvalues that exceeded 1 were deemed biologically important (Steiger et al 2015), and further analysis of the PC loadings had absolute 0.30 as also deemed biologically important. PC1 was positively loaded to all of the CHC components, The PC2 scores were positively loaded to shorter chain CHCs, while the PC3 scores were positively loaded to four CHCs specific to males. The PC3 score was further analysed as in Steiger et al (2015), to theorise the impact of diets and feeding behaviours on male cuticular hydrocarbon production and attractiveness. The mean PC scores and theoretical male attractiveness were graphed for visual analysis, with the SCD crickets mapped as a baseline, to allow for comparisons, as done for feeding behaviour.

RESULTS

Compensatory Feeding Behaviour

The main effects of normal diet, switched diet and sex were significant for the mean consumption of food across all diets (Table A1) for both females and males (Table A1), with each diet differing from each other and the SCD baseline (solid red line is mean consumption ± SE in animals evolved on SCD; Figure A1; a & b). The two-way interactions between normal diet and switched diet and diet and sex had significant effects on dietary consumption (Table A1). However, the three-way interaction and body size were non-significant (Table A1). Food consumption was significantly higher in females than males, and consumption was higher in individuals feeding on the normal diets (i.e. HP, LC and LP) compared to those that were switched to the S diet except for HC, the trend was reversed. The difference was not significant when the normal diet was HP compared to those switched to S diet and consumption was lower than the SCD baseline (Figure A1; a & b – ▲indicates significant difference in consumption when switched). In both sexes, food consumption was highest on the LP diet, both in the normal and switched set-ups, followed by the LC diets (N+S), with both normal diet and switch sitting above the baseline. In contrast, the sexes on the normal HC and HP diets and switched diets (S) consumed significantly less than the SCD baseline. The difference in consumption in females and males on normal HP or switched HP was not significant.

Protein and Carbohydrate Intake

There was a multivariate effect of an individual's original diet, diet switching, sex and an interaction between all three factors that influenced the intake of protein and carbohydrate (Table A2). Diet (N or S) and sex had different effects on the nutrient intake. Once again, body size was not a significant contributor to nutrient intake (Table A2).

There was significant difference in protein intake from the baseline within (Figure A1; c & d – ▲) and across all four diets for both females and males (Figure A1; c & d - *). Intake of proteins was highest in the protein-biased diets, with HPN, LPN and LPS sitting above the baseline. However, HPS populations consumed less protein than those raised on the SCD diet. The populations exposed to carbohydrate-biased diets (HC and LC) consumed less protein, with HCN, HCS and LCN falling below the baseline. LCS populations had an average protein intake that was higher than the SCD baseline populations (Figure A1; c & d - *). Mean protein intake was higher in females than males, although both sexes show a similar intake pattern. In females, protein intake differed significantly in populations exposed to HC, LC, HPN and LPN. Populations switched to HPS did not differ from HC in protein intake and populations switched to LPS did not differ from HPN (Figure A; 1c & d). In males, protein intake differed significantly in populations exposed to HC, LC, HPN and LPN. Populations switched to HPS did not differ from HC in protein intake and populations switched to LPS did not differ from HPN.

There were significant differences in carbohydrate intake from the baseline for all diets but the difference between the normal and switched diets (i.e. N & S) were only significant for HC and LP (Figure A1; e & f), in females and males. HCN, LC and LPS diets showed carbohydrate intakes above the SCD baseline in both sexes, while HCS, HP and LPN diets sat below the baseline. Mean carbohydrate intake was higher in females than males, although both sexes show a similar intake pattern. In females, carbohydrate intake differed significantly in populations exposed to HC, LC, HPN and LP. Populations switched to HPS did not differ from HC in carbohydrate intake (Figure A1; e). In males, carbohydrate intake

differed significantly in populations exposed to HC, LC, HPN and LPN. Populations switched to HPS did not differ from HC in carbohydrate intake and populations switched to LPS did not differ from HPN.

Cuticular Hydrocarbon Production

PC analysis of 18 CHCs yielded three PCs with eigenvalues >1, which account for 74.35% of the total variation (Table A3). PC1 accounts for 39.94% of the variance in CHC expression and is positively loaded to each CHC compound, representing the total amount of CHCs. PC2 accounts for a further 25.99% of the total variation and is positively loaded to short and long chained CHCs below C_{37}, and negatively loaded to long chained CHCs equal to or greater than C_{37}. PC3 explains another 8.42% of total variation and is positively loaded to two short chain and negatively to two long chain CHCs.

There was a significant multivariate effect of an individual's normal diet, diet switching, sex and their two-way interactions that influenced CHC expression, but the three-way interaction and body size were non-significant (Table A4). For PC1 there are main effects of the normal diet, diet switch, sex and the interaction between normal diet and diet switch (Table A4). Females and males exhibited significant differences in total cuticular hydrocarbon production (PC1) across the four diets compared to the SCD baseline, as well as significant differences between diets and within diets (N compared to S) (Figure A2; a & b). In females, PC1 scores were negative for HPN and LPN diets whereas PC1 scores were positive for HCS, LCS, HP and LP. In males, PC1 scores were negative for HC and LC and positive for HP and LP (Figure A2; a & b).

For PC2 and PC3, the main effect of normal diet and diet switch were non-significant but differences in the expression of CHC blends are driven by the significant, higher order interactions between sex and the normal diet and switch diet (Table A4). In terms of the blend of longer CHCs (i.e. PC2) in the normal diet, females had longer CHC chains (i.e. negative scores) in their exoskeleton compared to males that had shorter, long CHCs (i.e. positive scores). In the switch diet, females had a reduction in longer CHCs and males had a reduction in shorter, long CHCs (Figure A2; c & d). In terms of the blend of short and long CHCs (i.e. PC3) in the normal diet, females had shorter CHCs (i.e. positive scores) compared to males that have long CHCs (i.e. negative scores) (Figure A2: e & f). Whereas, in the diet switch, females had a reduction in short CHCs and males had a reduction in long CHCs. This pattern was consistent for PC2 and PC3, across all four normal and switched diets (Figure A2; c, d, e & f).

Predicted Attractiveness of Males

There was a significant effect of an individual's normal diet and the interaction between normal diet and switch diet for male predicted attractiveness which is based on average male CHC profile (Table A5). Male body size was non-significant (Table A5). All diets varied significantly from the baseline and there were significant differences between the normal and switched diet populations for each diet with the exception of HP and LPS (Table 5; Figure A3). Males in the HP and LP populations had a greater abundance of longer chain CHCs on average compared to the HC and LC populations and the SCD baseline (Table 3; Figure A2; d & f; Figure A3). Theoretically, as LP males had more of the longer CHCs they would be most attractive to females, while LC males, on average, are least attractive.

DISCUSSION

Implications of Nutrition on Feeding Behaviour and CHC production

Condition-dependent traits are influenced by the acquisition of resources, which in turn is affected by environment and behaviour (Cotton, Fowler & Pomiankowski 2004; Franzke & Reinhold 2012; Wang et al 2019), with resource acquisition affecting the quality of an organism (e.g. Clark, Zera & Behmer 2015). It was predicted that feeding behaviour would change when animals are faced with imbalanced diets, and in the long term, adapt to a novel diet. The findings of this study are consistent with this prediction. *G. sigillatus* altered their feeding behaviour in response to diet composition and this consumption pattern was similar when individuals switched from their normal diet, (where the lines had evolved for 35 years) to a standard diet, suggesting that there is a genetic predisposition to consume a certain amount of macronutrient, even when this results in under or over consumption of macronutrients. Similar feeding behaviour was exhibited even though the diet composition was changed, supporting the prediction that feeding behaviour disposition evolves to some extent (Ledón-Rettig, Pfennig & Crespi 2010), impacting the effectiveness of macronutrient acquisition to either the individuals' benefit or detriment. Nonetheless, there were differences between the HC, LC and LP normal and switched diets, as expected if there is still plasticity in feeding behaviour in response to the ratio of protein to carbohydrate and total caloric content of the diet.

The caloric content of each diet was an important determinant of the amount of diet that was consumed. Both female and male crickets raised and kept on low calorie diets (i.e. LP and LC) showed higher intakes compared to those kept on high calorie diets (i.e. HC and HP; Figure A1; a & b). This has been found in other cricket species (Hunt, Brooks & Jennions 2005; Harrison et al 2014), and species of rodents (Speakman, Mitchell & Mazidi 2016) and horses (Laut et al 1985), and was expected as individuals on lower calorie diets are required to consume more to reach their daily caloric target. I also show that it is not simply the total intake of calories that is important for the amount of diet that is consumed. Consumption was highest on the LP diets where protein was diluted with indigestible cellulose and carbohydrate, consistent with studies in other insects such as beetles (Shareefi & Cotter 2019), as well as mice (Sørensen et al 2008; Solon-Biet et al 2014) and mink (Mayntz et al 2009), that show that animals are highly responsive to the amount of protein in the diet. They compensate for an imbalance in protein by ingesting more, in an attempt to gain limited protein (i.e. the protein leverage hypothesis) (Simpson & Raubenheimer 2004; Gosby et al. 2013; Simpson et al. 2017) to reach their 'intake target' (Simpson & Raubenheimer 2004; Simpson et al. 2017). However, in situations where feeding behaviour is limited by an unbalanced diet it may be impossible to reach the target intake for P and C and instead, ingestion is stopped when the optimal, total energy intake is reached but there is an energy deficit in one macronutrient and energy surplus for another (Simpson & Raubenheimer 2004). Simpson et al (2017) suggest that this occurs when the maximal target for one macronutrient is reached before another. Furthermore, Clark, Zera and Behmer (2015) theorise that at the optimal total energy intake, there are costs to overeating that limit how much macronutrient can be consumed and processed at one time. The results of this study support the idea that the overall nutritional value and the ratio of P and C constrain the intake of essential macronutrients and whether individual *G. sigillatus* reach their target intake for P and C. On the normal diet, individuals of both sexes, overconsumed protein on the protein biased diets and overconsumed carbohydrate on the carbohydrate-biased diets, relative to the standard cricket diet. We infer that this occurs as females and males must compromise between eating more carbohydrate in the carbohydrate-biased diet in order to approach the

target amount of P – although on the HC normal and switched diet females and males fail to reach this target whereas LC switched male and females do. The pattern is similar in the normal protein diets, with HP & LP males overconsuming protein to reach the same target intake of carbohydrate as in the baseline diet. In contrast, females overconsume protein in the protein-biased normal diets but never reach the target amount of carbohydrate as compared to the SCD. In HP, switched diet females and males, the consumption of protein is lower than the baseline and females fail to reach the baseline consumption of C whereas males do. In contrast, LP, switched diet females and males overconsume P (compared to the baseline) and overconsume C also.

Both sexes exhibited similar patterns of feeding behaviour and protein and carbohydrate intake, suggesting females and males regulate their P and C intake in the same way, which is consistent with earlier studies in other cricket species (Maklakov et al 2008) and a cockroach (Bunning et al 2016). However, total consumption was higher in females compared to males, likely due to the different costs of sex-specific reproductive efforts (Harrison et al 2014; Rapkin, Jensen, House et al 2018). The total expression of cuticular hydrocarbons also mirrors the sex-specific feeding behaviours and patterns of P and C intake. Females produce entirely more CHCs compared to males, after standardising size within sex. Individuals feeding on protein-biased diets produce more CHCs, while animals on carbohydrate-biased diets produce less (Figure A2; a & b). Higher protein intake has been suggested to support the production of more CHCs, as protein determines CHC expression (Zajitschek, Lailvaux, Dessmann & Brooks 2012; Shelly, Edu & Pahio 2007; Rapkin et al 2017) and therefore, it was unsurprising that individuals from LC treatments consumed more diet than individuals from any other treatments and had the lowest expression of CHCs in males and females (Figure A2; c & d). This is consistent with other studies of condition-dependent traits such as CHCs (Rapkin et al 2017) immune function (Kelly, Neyer & Gress 2014) and reproductive encapsulation (Rapkin, Jensen & Archer et al 2018) that have shown that high nutrition and protein are required for optimal trait expression. In contrast, despite of the diet composition and overall nutritional value of the SCD, populations switched to the SCD showed similar patterns in total CHC expression to their normal diet counterparts. However, the significant difference between the normal diets to their respective switched populations (i.e. LPN to LPS) suggests the feeding behaviours of the previous generations have had an effect on the ability of the current generation to acquire resources for CHC production (Figure A1; a & b; Figure A2; a & b). This indicates there may be a history of feeding behaviour selection (i.e. those eating more or less to reach their intake targets for successful reproduction), which in turn is impacting this condition-dependent trait (Johnston et al 2013).

Sex-specific differences in female and male cuticular hydrocarbon production have been found in a number of insects (Singer 1998; Sharma, Hunt & Hosken 2012; Weddle et al 2012; Steiger et al 2015; Lane et al. 2016; Rapkin et al 2017; Berson & Simmons 2018). Consistent with these studies, I found that the blend of CHCs differed in the sexes. Females produced more short (i.e. 35 to 36) and longer chain CHCs whereas males produced more shorter (i.e. 37 chain), or very long chain CHCs (i.e. 39 chain). However, the amount of the specific blends of CHCs was dependent on individuals' diet, supporting the prediction that individuals that consume more protein acquire more resources and can allocate more resources to costly long chain CHCs. Females typically have CHC blends that are independent of condition (Weddle et al 2012), producing more shorter chain CHCs. However, the amount of nutrients available allows for more or less total CHCs to be produced, as shown here. In contrast, males rely heavily on condition for CHC expression (Weddle et al. 2012; Rapkin et al 2017), producing less or more CHCs depending on the

nutritional environment, and trading off between short and long chain hydrocarbons when under stress. Within sexes, populations kept on, and descended from LP diet, produced more shorter (i.e. 37 chain), or very long chain CHCs (i.e. 39 chain) compared to those from LC treatments that acquired the lowest amount of protein and total nutrition and had the least concentrated blend of CHCs among the diet treatments for both females and males (Figure A2; e & f). This suggests that access to a combination of more macronutrients and energy influences the ability to produce a broader range of CHCs which, in this cricket species, influences male attractiveness and mate attraction (Steiger et al 2015).

Theoretical Impact on Male Attractiveness and Mating Success

Typically, female decorated crickets have been expected to prefer males with CHC profiles that significantly differ from their own. As CHCs provide insight into an individuals' underlying genetic quality (Thomas & Simmons 2011; Ingleby 2015), selecting those mates with significantly different genotypes, and therefore CHCs, from their own theoretically provides the most viable mate and offspring (Ingleby 2015; Bertram et al 2017) as an individual avoids inbreeding and increases the viability of their offspring. However, recent studies suggest that female mate choice in decorated crickets is partly based on the CHC profiles of the males (Steiger et al 2015). More specifically, those males with a CHC blend containing an increase in two alkadienes (9,31-C_{38}diene and 9,31-C_{39}diene) were found most attractive by a majority of the tested female population. In this study, males exposed to the protein-biased diets (i.e. normal diet LP and HP) had larger amounts of long chain CHCs within their profile blends, and therefore more of these two alkadienes (i.e. PC3). Furthermore, when switched to SCD, males ancestrally from the high nutrition, protein-biased (HP) diet produced more of these specific long chain CHCs compared to LC treatment males, even though they consumed on average less protein than those on LC treatments (Figure A1: c & d; Figure A3). This suggests that these lines have evolved to maximize the amount of P that they extract from the diet and/or process the protein more efficiently during digestion and are able to lay down more of the longer chained CHCs in their cuticle, despite of the lower amount of protein in the SCD. Similarly, it seems likely that males from the switched LC lines lack the capacity to efficiently extract P as they have evolved, primarily, to feed on and digest carbohydrate and therefore produce smaller amounts of CHCs with prominently short chain CHCs (Figure A1; c & d). The genetically derived differences in the CHC profile of males from the switched diets are predicted to influence male reproductive fitness as females are more attracted to males with CHC profiles with longer chain CHCs. Males from the switched LP diets are theoretically the most attractive followed by HP, HC, and finally males from the former LC diet are the least attractive.

In conclusion, this study shows feeding behaviour changes when faced with diets of different nutritional value and macronutrient bias. It also suggests that feeding behaviour has evolved, creating a disposition to consume more or less resources even as an individual adjusts to a novel diet. Nonetheless, I show that there is an immediate behavioural adjustment to meet nutrient and caloric requirements (Wehi, Raubenheimer & Morgan-Richards 2013), although these behaviours are influenced by underlying genetic changes that may have evolved via directional selection (Johnston et al 2013). While there are differences in consumption, it is not clear if nutritional value or the P:C ratio is the ultimate limiting factor (Simpson et al 2017), it may be the result of which target is reached first (Simpson, Le Couteur & Raubenheimer 2015; Simpson et al 2017). Ultimately, this adaption to differing diets has a direct influence on the expression of CHCs with differences in the overall amount and blend

of short and long chain CHCs across and within all diets. Weddle et al (2012) and Rapkin et al (2017) previously determined female total hydrocarbon production but not composition was impacted by resource acquisition, while male CHC total expression and blend was condition dependent. This suggests while the CHC compositions are sex-specific, diet alters the overall production of CHCs and blend of CHCs. Switching diets showed that feeding behaviour had evolved and consequently affected CHC production in line with altered macronutrient and caloric intake. Consequently, although individuals changed from carbohydrate-biased (and thus protein constrained) or protein-biased diets to more balanced nutritional environments, there is a possible genetic tendency to produce similar CHC blends as their ancestors which has consequences for male fitness.

REFERENCES

Aday, DD, Wahl, DH & Phillip, DP 2003, 'Assessing population-specific and environmental influences on bluegill life histories: a common garden approach', *Ecology*, vol. 84, no. 12, pp. 3370-3375, ESA Journals, DOI 10.1890/02-3139.

Archer, CR, Royle, N, South, S, Selman, C & Hunt, J 2009, 'Nutritional geometry provides food for thought', *The Journals of Gerontology: Series A*, vol. 64A, no. 9, pp. 956-959, Oxford Academic, DOI 10.1093/gerona/glp069.

Baker, G, Pepperz, JH, Johnson, LH & Hastings, E 1960, 'Estimation of the composition of the cuticular wax of the Mormon cricket, Anabrus simplex Hald.', *Journal of Insect Physiology*, vol. 5, no. 1, pp. 47-60, Science Direct, DOI 10.1016/0022-1910(60)90022-6.

Barton, MB, Moran, JR, Vollenweider, JJ, Heintz, RA & Boswell, KM 2017, 'Latitudinal dependence of body condition, growth rate, and stable isotopes of juvenile capelin (Mallotus villosus) in the Bering and Chukchi seas', *Polar Biology*, vol. 40, no. 7, pp. 1451-1463, Springer Link, DOI 10.1007/s00300-016-2041-8.

Berner, D, Blanckennhorn, WU & Körner, C 2005, 'Grasshoppers cope with low host plant quality by compensatory feeding and food selection: N limitation challenged', *Oikos*, vol. 111, no. 3, pp. 525-533, Wiley Online Library, DOI 10.1111/j.1600-0706.2005.14144.x.

Berson, JD & Simmons, LW 2018, 'A costly chemical trait: phenotypic condition dependence of cuticular hydrocarbons in a dung beetle', *Journal of Evolutionary Biology*, vol. 31, no. 12, pp. 1772-1781, Wiley Online Library, DOI 10.111/jeb.13371.

Bertram, SM, Harrison, SJ, Ferguson, GL, Thomson, IR, Loranger, MJ, Reifer, ML, Corlett, DH & Adair Gowaty, P 2017, 'What is driving male mate preference evolution in Jamaican field crickets?', *Ethology: International Journal of Behavioural Biology*, vol. 123, no. 11, pp. 793-799, Wiley Online Library, DOI 10.1111/eth.12649.

Birkhead, TR, Fletcher, F & Pellatt, EJ 1999, 'Nestling diet, secondary sexual traits and fitness in the zebra finch', *Proceedings of the Royal Society B: Biological Sciences*, vol. 266, no. 1417, pp. 385-390, PubMed Central, DOI 10.1098/rspb.1999.0649.

Bunning, H, Bassett, L, Clowser, C, Rapkin, J, Jensen, K, House, CM, Archer, CR & Hunt, J 2016, 'Dietary choice for a balanced nutrient intake increases the mean and reduces the variance in the reproductive performance of male and female cockroaches', *Ecology and Evolution*, vol. 6, no. 14, pp. 4711-4730, Wiley Online Library, DOI 10.1002/ece3.2243.

Christie, MR, McNickle, GG, French, RA & Blouin, MS 2018, 'Life history variation is maintained by fitness trade-offs and negative frequency-dependent selection', *PNAS: Proceedings of the National Academy of Sciences of the United States of America*, vol. 115, no. 17, pp. 4441-4446, PNAS, DOI 10.1073/pnas.1801779115.

Clark, RM, Zera, AJ & Behmer, ST 2015, 'Nutritional physiology of life-history trade-offs: how food protein-carbohydrate content influences life-history traits in the wing-polymorphic

cricket Gryllus firmus', *The Journal of Experimental Biology*, vol. 218, pp. 298-308, The Company of Biologists: JEB database, DOI 10.1242/jeb.112888.

Cotton, S, Fowler, K & Pomiankowski, A 2004, 'Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis?', *Proceedings of the Royal Society B: Biological Sciences*, vol. 271, no. 1541, pp. 771-783, The Royal Society Publishing, DOI 10.1098/rspb.2004.2688.

Delcourt, M & Rundle, HD 2011, 'Condition dependence of a multicomponent sexual display trait in Drosophila serrata', *The American Naturalist*, vol. 177, no. 6, pp. 812-823, JSTOR, DOI 10/1086/659949.

Duffield, KR, Hampton, KJ, Houslay, TM, Hunt, J, Rapkin, J, Sakaluk, SK & Sadd, BM 2018, 'Age-dependent variation in the terminal investment threshold in male crickets' *Evolution*, vol. 72, no. 3, pp. 578-589, The Society for the Study of Evolution, DOI 10.1111/evo.13443.

d'Ettorre, P & Moore, AJ 2008, 'Chemical communication and the coordination of social interactions in insects', in *Sociobiology of communication: an interdisciplinary perspective*, Oxford University Press, Oxford, pp. 81-96.

Fischman, BJ, Woodard, SH & Robinson, GE 2011, 'Molecular evolutionary analyses of insect societies', *PNAS Proceedings of the National Academy of Sciences of the United States of America*, vol. 108. no. 2, pp. 10847-10854, PNAS, DOI 10.1073/pnas.1100301108.

Flatten, T & Heyland, A 2011, *Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs*, Oxford University Press USA – OSO, ProQuest Ebook Central.

Franzke, A & Reinhold, K 2012, 'Limited condition dependence of male acoustic signals in the grasshopper Chorthippus biguttulus', *Ecology and Evolution*, vol. 2, no. 8, pp. 1914-1921, Wiley Online Library, DOI 10.1002/ece3.309.

Gershman, SN & Sakaluk, SK 2010, 'Mate quality and novelty influence post-copulatory female choice in decorated crickets, Gryllodes sigillatus', *Ethology: International Journal of Behavioural Biology*, vol. 116, no. 11, pp. 1113-1117, Wiley Online Library, DOI 10.1111/j.1439-0310.2010.01826.x.

Golebiowski, M, Bogus, MI, Paskiewicz, M, Wieloch, W, Wlóka & Stepnowski, P 2012, 'The composition of the cuticular and internal free fatty acids and alcohols from Lucilla sericata males and females', *Lipids*, vol. 47, no. 6, pp. 613-622, NCBI Springer, DOI 10.1007/s11745-012-3662-5.

Harrison, SJ, Raubenheimer, D, Simpson, SJ, Godin, J-GJ & Bertram, SM 2014, 'Towards a synthesis of frameworks in nutritional ecology: interacting effects of protein, carbohydrate and phosphorus on field cricket fitness', *Proceedings of the Royal Society B: Biological Sciences*, vol. 281, no. 1792, 9 pages, The Royal Society Publishing, DOI 10.1098/rspb.2014.0539.

Hochuli, DF 2001, 'Insect herbivory and ontogeny: how do growth and development influence feeding behaviour, morphology and host use?', *Austral Ecology: A Journal of Ecology in the Southern Hemisphere*, vol. 26, no. 5, pp. 563-570, Wiley Online Library, DOI 10.1046/j.1442-9993-2001.01135.x.

House, CM, Jensen, K, Rapkin, J, Lane, S, Okada, K, Hosken, DJ & Hunt, J 2016, 'Macronutrient balance mediates the growth of sexually selected weapons but not genetalia in male broad-horned beetles', *Functional Ecology*, vol. 30, no. 5, pp. 769-779, Wiley Online Library, DOI 10.1111/1365-2435.12567.

Hunt, J, Brooks, R & Jennions, MD 2005, 'Female mate choice as a condition-dependent life-history trait', *American Naturalist*, vol. 166, no. 1, pp. 79-92, EBSCOhost, DOI 10.1086/430672.

Ingleby, FC 2015, 'Insect cuticular hydrocarbons as dynamic traits in sexual communication', *Insects*, vol. 6, no. 3, pp. 732-742, Agricultural & Environmental Science database, ProQuest, DOI 10.3390/insects6030732.

Ingleby, FC, Hunt, J & Hosken, DJ 2013, 'Genotype-by-environment interactions for female mate choice of male cuticular hydrocarbons in Drosophila simulans', *PLoS ONE*, vol. 8, no. 6, pp. 1-12, EBSCOhost, DOI 10.1371/journal.pone.0067623.

Ivy, TM, Weddle, CB & Sakaluk, SK 2005, 'Females use self-referent cues to avoid mating with previous mates', *Proceedings of the Royal Society B: Biological Sciences*, vol. 272, no. 1580, pp. 2475-2478, The Royal Society Publishing, DOI 10.1098/rspb.2005.3222.

Joern, A, Provin, T & Behmer, S.T. 2012, 'Not the usual suspects: insect herbivore populations and communities are associated with multiple plant nutrients', *Ecology: Ecological Society of America*, vol. 93, no. 5, pp. 1002-1015, Wiley Online Library, DOI 10.1890/11.1142.1.

Johnston, SE, Gratten, J, Berenos, C, Pilkington, JG, Clutton-Brock, TH, Pemberton, JM & Slate, J 2013, 'Life history trade-offs at a single locus maintain sexually selected genetic variation', *Nature*, vol. 502, pp. 93-96, Macmillan Publishers Ltd., DOI 10.1038/nature12489.

Kelly, CD, Neyer, AA & Gress, BE 2014, 'Sex-specific life history responses to nymphal diet quality and immune status in a field cricket', *Journal of Evolutionary Biology*, vol. 27, no. 2, pp. 381-390, Wiley Online Library, DOI 10.1111/jeb.12304.

Kilani-Morakchi, S, Aribi, N, Farine, JP, Smagghe, G & Soltani, N 2009, 'Halofenozide affects sexual behaviour, cuticular hydrocarbons and reproduction in the female German cockroach Blatella germanica (Dictyoptera, Blattellidae)', *Belgian Journal of Zoology*, vol. 139, no. 2, pp. 147-155, Royal Belgian Zoological Society Archives.

Kotiaho, JS 2002, 'Sexual selection and condition dependence of courtship displays in three species of horned dung beetles', *Behavioural Ecology*, vol. 13, no. 6, pp. 791-799, Oxford Academic, DOI 10.1093/beheco/13.6.791.

Kurpad, AV 2018, '90th anniversary commentary: amino acid imbalances: still in the balance', *The Journal of Nutrition*, vol. 148, no. 10, pp. 1647-1649, Oxford Academic, DOI 10.1093/jn/nxy195.

Lane, SM, Dickinson, AW, Tregenza, T & House, CM 2016, 'Sexual selection on male cuticular hydrocarbons via male-male competition and female choice', *Journal of Evolutionary Biology*, vol. 29, no. 7, pp. 1346-1355, Wiley Online Library, DOI 10.1111/jeb.12875.

Laut, JE, Houpt, KA, Hintz, HF & Houpt, TR 1985, 'The effects of caloric dilution on meal patterns and food intake of ponies', *Physiology & Behaviour*, vol. 35, no. 4, pp. 549-554, Science Direct, DOI 10.1016/0031-9384(85)90139-8.

Ledón-Rettig, CC, Pfennig, DW & Crespi, EJ 2010, 'Diet and hormonal manipulation reveal cryptic genetic variation: implications for the evolution of novel feeding strategies, *Proceedings B: Biological Sciences*, vol. 277, no. 1700, pp. 3569-3578, Royal Society Publishing, DOI 10.1098/rspb.2010.0877

Maklakov, AA, Simpson, SJ, Zajitschek, F, Hall, MD, Dessmann, J, Clissold, F, Raubenheimer, D, Bonduriansky, R & Brooks, RC 2008, 'Sex-specific fitness effects of nutrient intake on reproduction and lifespan', *Current Biology*, vol. 18, no. 14, pp. 1062-1066, Science Direct, DOI 10.1016/j.cub.2008.06.059.

Pascoal, S, Mendrok, M, Mitchell, C, Wilson, AJ, Hunt, J & Bailey, NW 2015, 'Sexual selection and population divergence I: The influence of socially flexible cuticular hydrocarbon expression in male field crickets (Teleogryllus oceanicus)', *Evolution: International Journal of Organic Evolution*, vol. 70, no. 1, pp. 82-97, Wiley Online Library, DOI 10.1111/evo.12839.

Pitchers, WR, Brooks, R, Jennions, MD, Tregenza, T, Dworkin, I & Hunt, J 2013, 'Limited plasticity in the phenotypic variance-covariance matrix for male advertisement calls in the black field cricket, Teleogryllus commodus', *Journal of Evolutionary Biology,* vol. 26, no. 5, pp. 1060-1078, Wiley Online Library, DOI 10.1111/jeb.12120.

Rapkin, J, Jensen, K, House, CM, Sakaluk, SK, Sakaluk, JK & Hunt, J 2017, 'The complex interplay between macronutrient intake, cuticular hydrocarbon expression and mating success in male decorated crickets', *Journal of Evolutionary Biology*, vol. 39, no. 4, pp. 711-727, Wiley Online Library, DOI 10.1111/jeb.13036.

Rapkin, J, Jensen, K, Archer, CR, House, CM, Sakaluk, SK, del Castillo, E & Hunt, J 2018, 'The geometry of nutrient space-based life-history trade-offs: sex specific effects of macronutrient intake on the trade-off between encapsulation ability and reproductive effort in decorated crickets.', *American Naturalist*, vol. 191, no. 4, pp. 452-474, EBSCOhost, DOI 10.1086/696147.

Rapkin, J, Jensen, K, House, CM, Wilson, AJ & Hunt, J 2018, 'Genotype-by-sex-by-diet interactions for nutritional preference, dietary consumption, and lipid deposition in a field cricket', *Hereditary*, vol. 121, pp. 361-373, Nature, DOI 10.1038/s41437-018-0130-x.

Raubenheimer, D & Simpson, SJ 1997, 'Integrative models of nutrient balancing: application to insects and vertebrates', *Nutrition Research Reviews*, vol. 10, no. 1, pp. 151-179, Cambridge Core, Cambridge University Press, DOI 10.1079/NRR19970009.

Renou, M 2014, 'Pheromones and general odor perception in insects', in *Neurobiology of Chemical Communication*, Mucignat-Caretta, CRC Press, Taylor & Francis, chapter 2.

Ricklefs, RE & Wikelski, M 2002, 'The physiology/life-history nexus', *Trend in Ecology & Evolution*, vol. 17, no. 10, pp. 462-468, Science Direct, DOI 10.1016/S0169-5347(02)02578-8.

Shareefi, EA & Cotter, SC 2019, 'The nutritional ecology of maturation in a carnivorous insect', *Behavioural Ecology*, vol. 30, no. 1, pp. 256-266, Oxford Academic, DOI 10.1093/beheco/ary142.

Sharma, KR, Enzmann, BL, Schmidt, Y, Moore, D, Jones, GR, Parker, J, Berger, SL, Reinberg, D, Zwiebel, LJ, Breit, B, Liebig, J & Ray, A 2015, 'Cuticular hydrocarbon pheromones for social behaviour and their coding in the ant antenna', *Cell Reports*, vol. 12, no. 8, pp. 1261-1271, Science Direct, DOI 10. 1016/j.celrep.2015.07.031.

Sharma, MD, Hunt, J & Hosken, DJ 2012, 'Antagonistic responses to natural and sexual selection and the sex-specific evolution of cuticular hydrocarbons in Drosophila simulans', *Evolution: International Journal of Organic Evolution*, vol. 66, no. 3, pp. 665-677, Wiley Online Library, DOI 10.1111/j.1558-5646.2011.01468.x.

Shelly, TE, Edu, J & Pahio, E 2007, 'Condition-dependent mating success in male fruit flies: ingestion of a pheromone precursor compensates for a low-quality diet', *Journal of Insect Behaviour*, vol. 20, no. 3, pp. 347-365, Nature database, Springer Link, DOI 10.1007/s10905-007-9082-3.

Simpson, SJ, Le Couteur, DG, James, DE, George, J, Gunton, JE, Solon-Biet, SM & Raubenheimer, D 2017, 'The geometric framework for nutrition as a tool in precision medicine', *Nutrition and Healthy Aging*, vol. 4, no. 3, pp. 217-226, National Center for Biotechnology Information, U.S. National Library of Medicine Online, Royal Society Publishing database, DOI 10.3233/NHA-170027.

Simpson, SJ, Le Couteur, DG & Raubenheimer, D 2015, 'Putting the balance back in diet', *Cell*, vol. 161, no. 1, pp. 18-23, ScienceDirect, DOI 10.1016/j.cell.2015.02.033.

Speakman, JR, Mitchell, SE & Mazidi, M 2016, 'Calories or protein? The effect of dietary restriction on lifespan in rodents is explained by calories alone', *Experimental Gerontology*, vol. 86, pp. 28-38, Science Direct, DOI 10.1016/j.exger.2016.03.011.

Solon-Biet, SM, McMahon, AC, Ballard, JWO, Ruohonen, K, Wu, LE, Cogger, VC, Warren, A, Huang, X, Pichuad, N, Melvin, RG, Gokarn, R, Khalil, M, Turner, N, Cooney, GJ, Sinclair, DA, Raubenheimer, D, Le Couteur, DG & Simpson, SJ 2014, 'The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad-libitum mice', *Cell Metabolism,* vol. 19, no. 3, pp. 418-430, Science Direct, DOI 10.1016/j.cmet.2014.02.009.

Sørensen, A, Mayntz, D, Raubenheimer, D & Simpson, SJ 2008, 'Protein-leverage in mice: the geometry of macronutrient balancing and consequences for fat deposition', *Obesity: A Research Journal*, vol. 16, no. 3, pp. 566-571, Wiley Online Library, DOI 10.1038/oby.2007.58.

South, SH, House, CM, Moore, AJ, Simpson, SJ & Hunt, J 2011, 'Male cockroaches prefer and high carbohydrate diet that makes them more attractive to females: implications for the study of condition-dependence', *Evolution: International Journal of Organic Evolution*, vol. 65, no. 6, pp. 1594-1606, Wiley Online Library, DOI 10.1111/j.1558-5646.2011.01233.x.

Steiger, S, Ower, GD, Stökl, J, Mitchell, C, Hunt, J & Sakaluk, SK 2013, 'Sexual selection on cuticular hydrocarbons of male sagebush crickets in the wild', *Proceedings of the Royal Society B: Biological Sciences*, vol. 280, no. 1773, 10 pages, DOI 10.1098/rspb.2013.2353.

Steiger, S, Capondeanu-Nägler, A, Gershman, SN, Weddle, CB, Rapkin, J, Sakaluk, SK & Hunt, J 2015, 'Female choice for male cuticular hydrocarbon profile in decorated crickets is not based on similarity to their own profile', *Journal of Evolutionary Biology*, vol. 28, pp. 2175-2186, European Society for Evolutionary Biology, DOI 10.1111/jeb.12740.

Thomas, ML & Simmons, LW 2008, 'Cuticular hydrocarbons are heritable in the cricket Teleogryllus oceanicus', *Journal of Evolutionary Biology*, vol. 21, no. 3, pp. 801-806, Wiley Online Library, DOI 0.1111/j.1420-9101.2008.01514.x.

Thomas, ML & Simmons LW 2011, 'Crickets detect the genetic similarity of mating partners via cuticular hydrocarbons', *Journal of Evolutionary Biology,* vol. 24, no. 8, pp. 1793-1800, Wiley Online Library, DOI 10.1111/j.1420-9101.2011.02319.x.

Touhara, K 2013, *Pheromone signalling: methods and protocols*, Springer Protocols, Humana Press, Springer New York Heidelberg Dordrecht London, Springer Link, DOI 10.1007/978-1-62703-619-1.

Tregenza, T & Wedell, N 1997, 'Definitive evidence for cuticular pheromones in a cricket', *Animal Behaviour*, vol. 54, no. 4, pp. 979-984, Science Direct, DOI 10.1006/anbe.1997.0500.

Uetz, GW, Papke, R & Kilinc, B 2002, 'Influence of feeding regime on body size, body condition and a male secondary sexual character in schizocosa ocreata wolf spiders (Aranae, Lycosidae): condition-dependence in a visual signaling trait', *The Journal of Arachnology*, vol. 30, no. 3, pp. 461-469, BioOne, DOI 10.1636/0161-8202(2002)030[0461:IOFROB]2.0.CO:2.

Urrejola, S, Nespolo, R & Lardies, MA 2011, 'Diet-induced developmental plasticity in life histories and energy metabolism in a beetle', *Revista Chilena de Historia Natural*, vol. 84, no. 4, pp. 523-533, SciELO, DOI 10.4067/S0716-078X2011000400005.

Wagner Jr, WE & Hoback, WW 1999, 'Nutritional effects on male calling behaviour in the variable field cricket', *Animal Behaviour*, vol. 57, no. 1, pp. 89-95, Science Direct, DOI 10.1006/anbe.1998.0964.

Wang, X, Zhao, Z, Cao, Y, Cui, J, Tang, Y & Chen, J 2019, 'Condition dependence of advertisement calls in male African clawed frogs', *Journal of Ethology*, vol. 37, no. 1, pp. 75-81, Springer Link, DOI 10.1007/s10164-018-0570-z.

Weddle, CB, Mitchell, C, Bay, SK, Sakaluk, SK & Hunt, J 2012, 'Sex-specific genotype-by-environment interactions for cuticular hydrocarbon expression in decorated crickets, Gryllodes sigallatus: implications for the evolution of signal reliability', *Journal of Evolutionary Biology*, vol. 25, no. 10, pp. 2112-2125, Wiley Online Library, DOI 10.1111/j.1420-9101.2012.02593.x.

Weddle, CB, Steiger, S, Hamaker, CG, Ower, GD, Mitchell, C, Sakaluk, SK & Hunt J 2013, 'Cuticular hydrocarbons as a basis for chemosensory self-referencing in crickets; a potentially universal mechanism facilitating polyandry in insects', *Ecology Letters*, vol. 16, no. 3, pp. 346-353, Wiley Online Library, DOI 10.1111/ele.12046.

Wehi, PM, Raubenheimer, D & Morgan-Richards, M 2013, 'Tolerance for nutrient imbalance in an intermittently feeding herbivorous cricket, the Wellington tree weta', *PLoS ONE*, vol. 8, no. 12, pp.1-11, EBSCOhost, DOI 10.1371/journal.pone.0084641.

Zajitschek, F, Lailvaux, SP, Dessmann, J & Brooks, J 2012, 'Diet, sex and death in field crickets', *Ecology and Evolution'*, vol. 2, no. 7, pp. 1627-1636, Wiley Online Library, DOI 10.1002/ece3.288.

APPENDICES

Appendix 1: Figure A1. Mean (±SE) consumption of diet, protein intake and carbohydrate intake across female and male *Gryllodes sigillatus*

Appendix 2: Table A1. ANCOVA for mean dietary consumption

Appendix 3: Table A2. MANCOVA for protein (P) and carbohydrate (C) intake

Appendix 4: Table A3. Principal component analysis (PC) of CHCs in *Gryllodes sigillatus*

Appendix 5: Figure A2. Mean (±SE) PC1 score, PC2 score, and PC3 score across female and male *Gryllodes sigillatus*

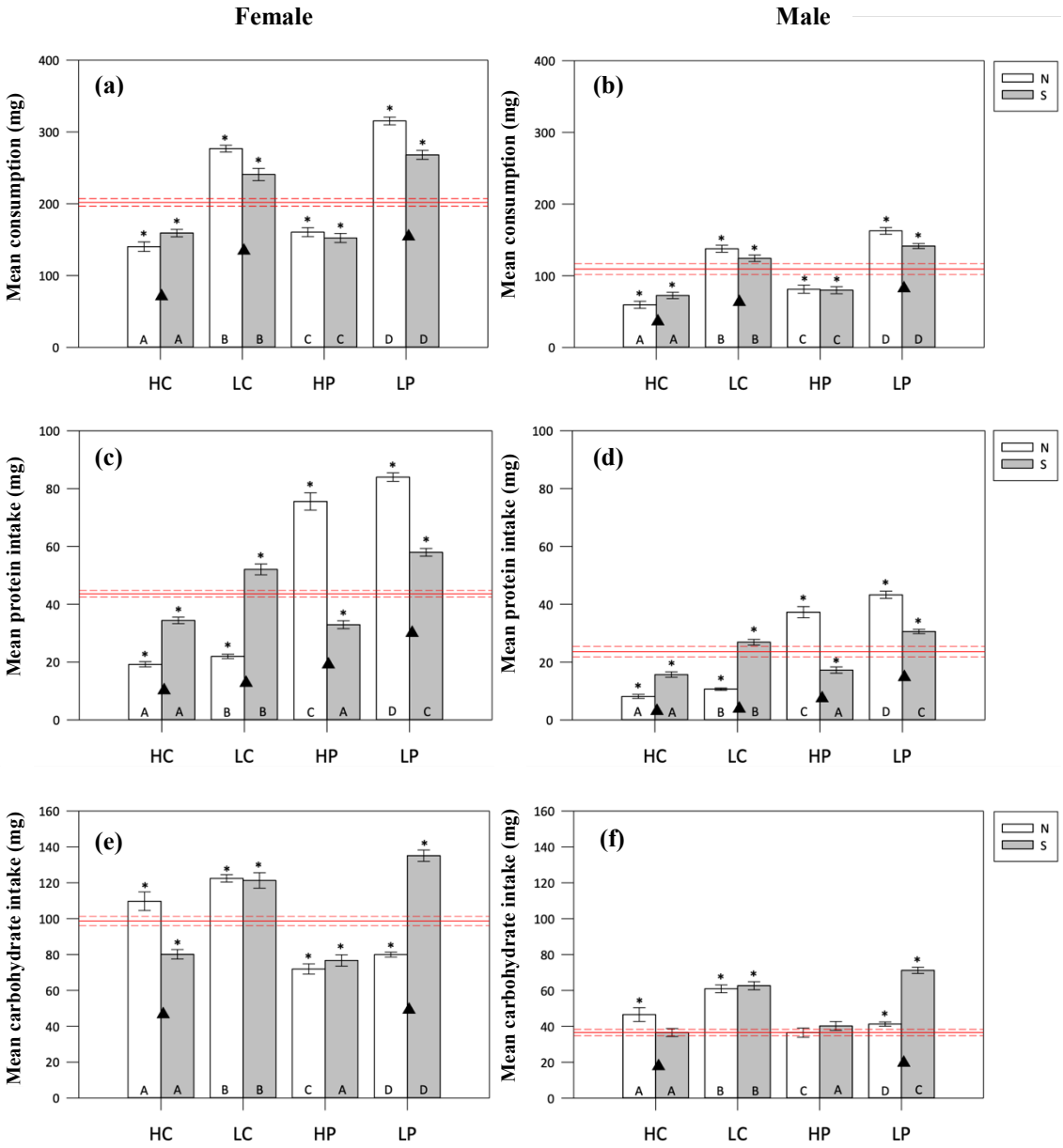
Appendix 6: Table A4. MANCOVA for cuticular hydrocarbon expression (PC1, PC2 and PC3)

Appendix 7: Table A5. ANCOVA for male attractiveness based on cuticular hydrocarbon expression (taken from Steiger et al 2015)

Appendix 8: Figure A3. Mean (±SE) predicted attractiveness of males across diets based on the average cuticular hydrocarbon profile (based on Steiger et al 2015)

Appendix 1: Figure A1. Mean (±SE) consumption of diet, protein intake and carbohydrate intake across female and male *Gryllodes sigillatus*

Figure A1(a) – (f). Mean (±SE) (a & b) consumption of diet (mg), (c & d) protein intake (mg) and (e & f) carbohydrate intake (mg) across female and male *Gryllodes sigillatus*. SCD baseline represented by the solid red line, with ±SE indicated by the red broken lines. Triangle (▲) indicates significant difference between normal (N) and switched (S) diets within diet. A, B, C, D indicates a significant difference from the diet treatment to all other diets. Significant differences from baseline indicated by (*).



Appendix 2: Table A1. ANCOVA for mean dietary consumption

Table A1. ANCOVA for mean dietary consumption (mg). Significance tests: $P < 0.001$ and $P < 0.01$ were deemed significant.

Model term	*F*	*df*	*P*
Initial diet (A)	292.41	3,47	0.0001
Switch (B)	7.12	1,47	0.01
Sex (C)	437.84	1,47	0.0001
A x B	15.09	3,47	0.0001
A x C	32.14	3,47	0.0001
B x C	5.07	1,47	0.029
A x B x C	1.79	3,47	0.16
PW	0.30	1,47	0.58

Appendix 3: Table A2. MANCOVA for protein (P) and carbohydrate (C) intake

Table A2. MANCOVA for protein (P) and carbohydrate (C) intake (mg). Significance tests: $P < 0.001$, $P < 0.01$. Interactions between initial diet, switch and sex analysed by MANCOVA and ANCOVAs to determine any significant two or three-way interactions.

	MANCOVA			
Model term	Pillai's Trace	*F*	*df*	*P*
Initial diet (A)	1.77	119.09	6,94	0.0001
Switch (B)	0.81	95.26	2,46	0.0001
Sex (C)	0.89	176.87	2,46	0.0001
A x B	1.67	79.85	6,94	0.0001
A x C	1.19	23.21	6,94	0.0001
B x C	0.30	9.66	2,46	0.0001
A x B x C	1.12	20.00	6,94	0.0001
PW	0.03	0.81	2,46	0.45
	Univariate ANCOVAs			
	Traits	*F*	*df*	*P*
Initial diet (A)	P	112.79	3,47	0.0001
	C	114.72	3,47	0.0001
Switch (B)	P	17.92	1,47	0.0001
	C	13.63	1,47	0.001
Sex (C)	P	288.51	1,47	0.0001
	C	357.07	1,47	0.0001
A x B	P	175.30	3,47	0.0001
	C	57.75	3,47	0.0001
A x C	P	33.34	3,47	0.0001
	C	11.47	3,47	0.0001
B x C	P	4.77	1,47	0.03
	C	0.08	1,47	0.79
A x B x C	P	288.25	3,47	0.0001
	C	328.81	3,47	0.0001
PW	P	0.07	1,47	0.80
	C	0.22	1,47	0.64

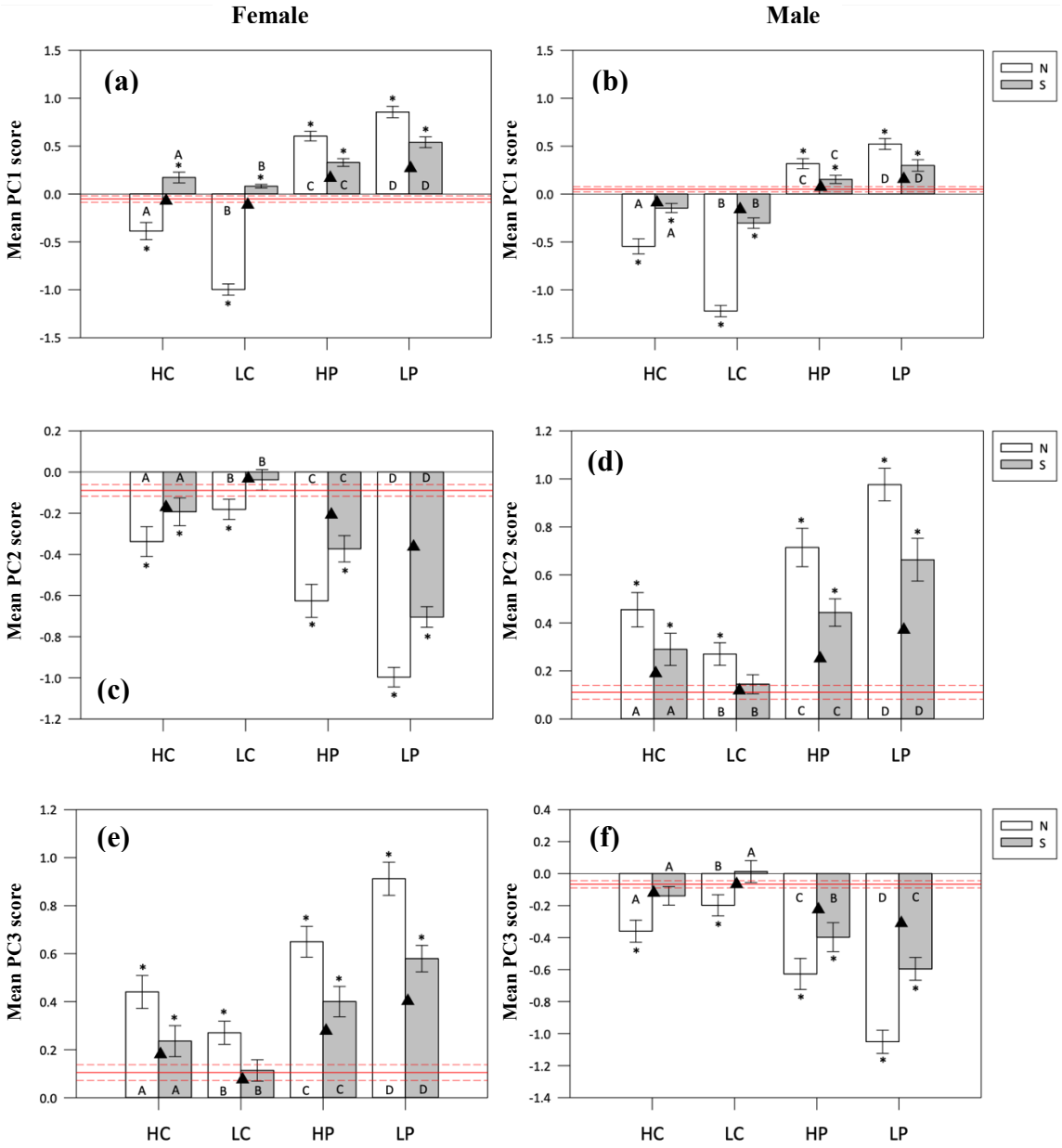
Appendix 4: Table A3. Principal component analysis (PC) of CHCs in *Gryllodes sigillatus*

Table A3. Principal component analysis (PC) of CHCs in *Gryllodes sigillatus*. PCs which exceeded an eigenvalue of 1 were considered biologically important and kept for further multivariate analysis. An absolute of 0.30 (in bold) was used as biologically important for the loadings (Steiger et al 2015). CHCs are named where they are known, while unnamed CHCs are described by their structure. They are listed in order of increasing length in carbon chains (Rapkin et al 2017).

	PC1	PC2	PC3
Eigenvalue	7.82	4.68	1.52
% variance	39.94	25.99	8.42
Loading			
7-MeC_{33}	**0.94**	0.09	0.05
5-MeC_{33}	**0.91**	0.12	0.14
3-MeC_{33}	**0.88**	0.09	0.20
3,7-diMeC_{33}	**0.89**	0.11	0.04
7-C_{35}ene	**0.52**	0.21	**0.47**
3,13-diMeC_{36}	**0.56**	0.08	0.26
5,9-diMeC_{36}	**0.47**	0.16	**0.65**
5,9-C_{37}diene	0.07	**0.85**	0.04
3,9-C_{37}diene	0.09	**0.84**	0.03
9,31-C_{37}diene	0.11	**0.90**	-0.09
7,31-C_{37}diene	0.10	**0.90**	0.01
9,31-C_{38}diene	**0.78**	0.10	-0.17
Alkatriene ($C_{39}H_{74}$)	**0.57**	0.25	**-0.55**
Alkatriene ($C_{39}H_{74}$)	**0.64**	-0.09	**-0.55**
9,31-C_{39}diene	**0.88**	-0.01	-0.25
7,31-C_{39}diene	**0.72**	**-0.42**	-0.04
Alkatriene ($C_{41}H_{78}$)	**0.50**	**-0.73**	-0.15
9,31-C_{41}diene	**0.42**	**-0.74**	0.09

Appendix 5: Figure A2. Mean (±SE) PC1 score, PC2 score, and PC3 score across female and male *Gryllodes sigillatus*

Figure A2 (a) – (f). Mean (±SE) (a & b) PC1 score, (c & d) PC2 score, and (e & f) PC3 score across female and male *Gryllodes sigillatus*. SCD baseline represented by the solid red line, with ±SE indicated by the red broken lines. Triangle (▲) indicates significant difference between normal (N) and switched (S) diets within diet. A, B, C, D indicates a significant difference from the diet treatment to all other diets. Significant differences from baseline indicated by (*).



Appendix 6: MANCOVA for cuticular hydrocarbon expression (PC1, PC2 and PC3)

Table A4. MANCOVA for cuticular hydrocarbon expression (PC1, PC2 and PC3). Significance test: $P < 0.001$, $P < 0.01$. Interactions between initial diet, switch and sex analysed by MANCOVA and ANCOVAs to determine any significant two or three-way interactions.

	MANCOVA			
Model term	Pillai's Trace	*F*	*df*	*P*
Initial diet (A)	0.95	7.23	9,141	0.0001
Switch (B)	0.39	9.44	3,45	0.0001
Sex (C)	0.89	126.66	3,45	0.0001
A x B	0.82	5.90	9,141	0.0001
A x C	0.93	6.99	9,141	0.0001
B x C	0.67	30.15	3,45	0.0001
A x B x C	0.26	1.46	9,141	0.17
PW	0.09	1.40	3,45	0.26
	Univariate ANCOVAs			
	Traits	*F*	*df*	*P*
Initial diet (A)	PC1	148.54	3,47	0.0001
	PC2	0.08	3,47	0.97
	PC3	1.30	3,47	0.29
Switch (B)	PC1	29.06	1,47	0.0001
	PC2	0.45	1,47	0.51
	PC3	0.08	1,47	0.77
Sex (C)	PC1	15.71	1,47	0.0001
	PC2	212.25	1,47	0.0001
	PC3	163.56	1,47	0.0001
A x B	PC1	62.05	3,47	0.0001
	PC2	0.27	3,47	0.85
	PC3	0.43	3,47	0.74
A x C	PC1	0.55	3,47	0.65
	PC2	75.34	3,47	0.0001
	PC3	60.38	3,47	0.0001
B x C	PC1	0.38	1,47	0.54
	PC2	43.22	1,47	0.0001
	PC3	55.76	1,47	0.0001
A x B x C	PC1	1.69	3,47	0.18
	PC2	1.44	3,47	0.24
	PC3	1.55	3,47	0.22
PW	PC1	1.52	1,47	0.22
	PC2	1.28	1,47	0.26
	PC3	1.15	1,47	0.29

Appendix 7: Table A5. ANCOVA for male attractiveness based on cuticular hydrocarbon expression (based on Steiger et al 2015)

Table A5. ANCOVA for male attractiveness based on cuticular hydrocarbon expression (based on Steiger et al 2015). Significance test: $P < 0.0001$, $P < 0.001$.

Model term	***F***	***df***	***P***
Initial diet (A)	61.19	3,23	0.0001
Switch (B)	0.52	1,23	0.48
A x B	9.28	3,23	0.0001
PW	2.99	1,23	0.10

Appendix 8: Figure A3. Mean (±SE) predicted attractiveness of males across diets based on the average cuticular hydrocarbon profile (based on Steiger et al 2015)

Figure A3. Mean (±SE) predicted attractiveness of males across diets based on the average cuticular hydrocarbon profile (based on Steiger et al 2015).