Variation in cricket acoustic signaling explained by body morphology, energy stores, and muscle enzymes

by

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Abstract

In multiple cricket species some males acoustically signal for much of their life to attract mates, while others males rarely signal. Given signaling efforts are usually correlated with mating success, males with high-effort signaling should be selected. To address the proximate causes underlying signaling variation, I quantified the bodily energy stores and the activities of key enzymes in signalling muscle metabolism in two species of field crickets and assessed whether they correlated with signaling. *Gryllus assimilis* males with high signaling quality were smaller, had increased enzyme activities of glycogen phosphorylase, and thoracic glycogen contents. *Gryllus texensis* males with high signaling quality were larger, had increased trehalase activities, and thoracic carbohydrate and abdominal lipid contents. In order to account for variation in signalling, one must measure the ability to metabolise carbohydrates in *G. assimilis* and the ability to metabolise both carbohydrates and lipids in *G. texensis*.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

Amp	Amplitude
ATP	Adenosine triphosphate
CD	Chirp Duration
$ChCl_3$	Chloroform
CS	Citrate Synthase
Dom Freq	Dominant Frequency
DTNB	5,5-Dithiobis-2-Nitrobenzoic Acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic
GLM	Generalized Linear Model
GP	Glycogen Phosphorylase
HK	Hexokinase
HOAD	β-hydroxyacyl-CoA Dehydrogenase
ICD	Inter-chirp Duration
IPD	Inter-pulse Duration
Na_2SO_4	Sodium Sulphate
NADH	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
PCA	Principal Component Analysis
PD	Pulse Duration
PK	Pyruvate Kinase
PPC	Pulses per Chirp
TRE	Trehalase
TSC	Time Spent Calling
V_{Max}	Maximal Enzyme Activity

Introduction

It is common for females to choose males even in species where there are no obvious direct benefits to the female (Kirkpatrick & Ryan 1991; Møller & Alatalo 1999). One explanation for why females choose males in these systems is that females receive indirect benefits in the form of "good genes" from the males they mate with, resulting in genetic benefits to her offspring (Fisher 1930). Males are thought to be able to honestly display their quality or condition through costly sexual ornaments (Zahavi 1975; Maynard-Smith 1976; Zahavi 1977; Møller 1991; Strohbach et al. 1998; Møller et al. 1999). Empirical evidence showing that males advertise their quality to potential mates has been found in numerous taxa including: plumage in birds (von Schantz et al. 1989; Hill 1991; Møller 1991), pheromones in cockroaches (Moore 1989; Moore et al. 1999), male size in moths (Phelan & Baker 1986) and acoustic signalling in field crickets (Simmons 1987; Hunt et al. 2004; Maklakov et al. 2008). In addition to the many studies that have documented condition-dependent phenotypic variation in signalling behaviour, various studies have also documented genetic variation in sexually selected traits (Hedrick 1988, Hedrick et al 1998, David et al. 2000; Hunt et al. 2007).

Female choice for a male trait should impose directional or stabilizing selection on the trait. This should rapidly result in the reduction of genetic variation until the genes for that trait in a population go to fixation (Rowe & Houle 1996; Tomkins et al. 2004). If a female's choice should inherently reduce genetic variation, and by doing so females eliminate the variation in male traits that makes their choice necessary, why then, does variation persist? This paradox is known as the lek paradox (Rowe & Houle 1996; Tomkins et al. 2004; Kotiaho et al. 2008), and is one of the major questions facing evolutionary biologists: why, if female choice imposes

directional or stabilizing selection, do we observe high genetic and phenotypic variation in most sexually selected traits?

Despite selective pressure from female choice, we observe great variation in the mate attraction behaviour of many animals (Houle 1992). Three possible explanations for the maintenance of this genetic variation are fluctuating selection (Hedrick et al. 1976; Hedrick 1986), mutation-selection balance (Lande 1976; Kondrashov 1982), and condition dependent genic capture (Tomkins et al. 2004). The fluctuating selection hypothesis suggests that the optimal phenotype chosen by females is not constant but instead fluctuates over time and space or across females (Jia et al. 2000). The mutation-selection hypothesis suggests that mutations that create genetic variation do so at a rate that equals the rate of genetic reduction by selection, and therefore variation persists (Charlesworth 1990). My MSc thesis explores the third hypothesis: genic capture. Genic capture posits that 1) there is high genetic variation in condition and 2) an individual's ability to display sexually selected traits is dependent on condition (Rowe & Houle 1996). This hypothesis argues that fitness conferring traits are highly genetically variable since they depend on underlying physiological, morphological, and biochemical traits that affect condition (Rowe & Houle 1996). Since there are many underlying traits that affect condition at a multitude of different genetic loci, the large amount of genetic variation across these loci provides the means for mutation-selection balance to occur (Rowe & Houle 1996; Tomkins et al. 2004).

The genic capture hypothesis rests on the assumption that variation in condition drives variation in sexually selected traits. But, what is condition and how do we measure it? The genic capture hypothesis suggests that condition is an individual's ability to acquire, store, and expend resources (Tomkins et al. 2004). A more traditional measure of condition is Fulton's K (Fulton

1902; Nash et al. 2006), often referred to as a *ratio index* (Jakob et al. 1996), which gives a simple estimation of the ratio of mass over body length. The accuracy of this method has been found to be dependent on the population being studied, and is therefore unsuitable for most species and populations (Bolger & Connolly 1989; Jakob et al. 1996). More recently, it is common to use the residuals from an ordinary least squares linear regression of body mass against a measure of size (Gould 1975). This results in a measure of mass corrected for body size. My thesis explores condition at a deeper level as it measures metabolic efficiency (the ability to expend resources) and an individual's energy and fuel stores (the ability to acquire and store resources).

The genic capture hypothesis posits that since sexually selected traits (such as signalling) are costly to produce and maintain, a male's ability to overcome these costs depends on his condition. Additionally, there will be variation in the genes that code for behaviours that affect condition (Tomkins et al. 2004), which would presumable include behaviours such as the ability to locate and store carbohydrates or lipids. Therefore, genetic differences would be responsible for differences in condition, which in turn would be responsible for differences in the physiology of signalling, such as the metabolic capacities of signalling muscles. My MSc thesis explores the next link in this series, the link between the physiology/biochemistry of signalling and the expression of this sexually selected trait. By exploring some of the potential proximate mechanisms underlying signalling in field crickets, I gained more understanding of the proximate factors maintaining variation in a sexually selected trait.

Field crickets (Orthoptera: Gryllidae) provide a convenient model organism for the study of acoustic mate attraction signalling since males produce loud calls to attract females and repel rival males (Alexander 1957; Walker 1957; Cade & Cade 1992). Males produce their calls by

raising their forewings and rubbing their scraper (or plectrum) on the top of one wing against the file on the bottom on the other (Walker 1957). The sound pulses produced by this stridulation are amplified by an area of the wing called the harp (Walker 1957; Walker 1962; Miyoshi et al. 2007). Each closing stroke of the wings produces one pulse of sound, and crickets will concatenate many pulses of sound into chirps (Figures 1) or trills (Figure 2). Crickets trill when numerous closing strokes occur with no pauses (e.g., 20 pulses or more); they chirps when a series of closing strokes are followed by a pause (Walker 1962).

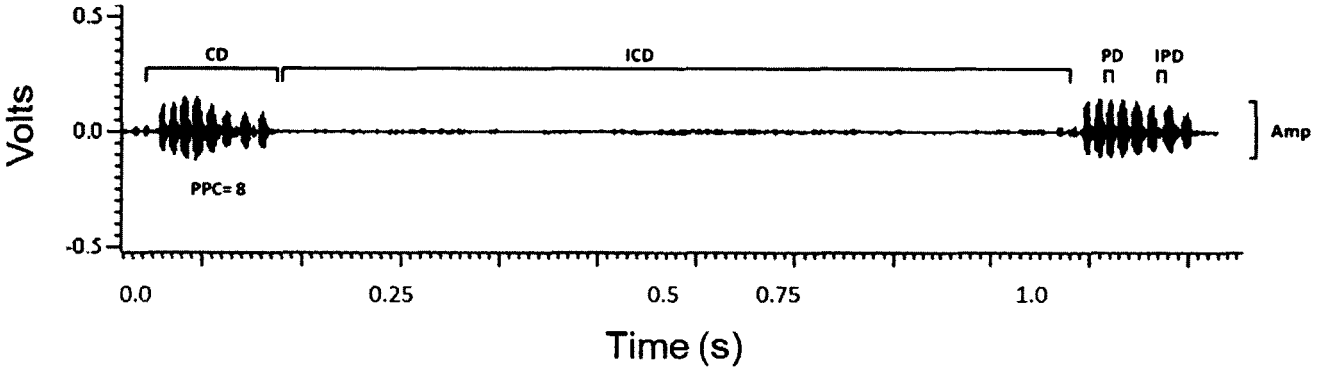

Figure 1: One second of signalling by *G. assimilis* showing a chirp with chirp duration (CD), inter-chirp duration (ICD), pulse duration (PD), inter-pulse duration (IPD), amplitude (Amp) and pulses per chirp (PPC) identified.

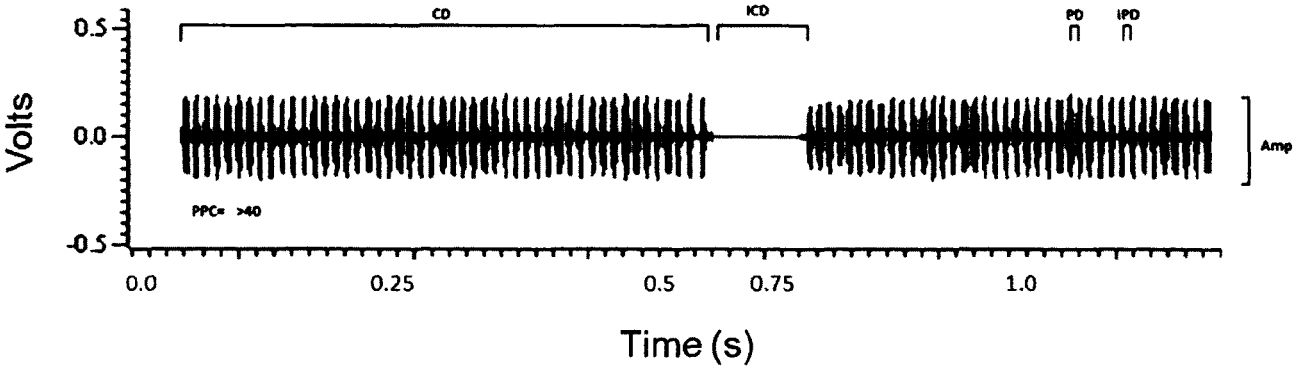

Figure 2: One second of signalling by *G. texensis* showing a trill with chirp duration (CD), interchirp duration (ICD), pulse duration (PD), inter-pulse duration (IPD), amplitude (Amp), and pulses per chirp (PPC) identified.

Acoustic mate attraction signalling can be a particularly energetically demanding behaviour (Prestwich 1994). Prestwich and Walker (1981) found, using two trilling cricket species, that acoustic signalling accounts for 26%-52% of the total daily respiratory budget. Moreover, signalling in crickets consumes energy at a rate many times basal metabolic rate (Bennet-Clark 1987; Bailey et al. 1993; Prestwich 1994; White et al. 2008). Acoustic signalling can also approach the maximal metabolic rate in some species (Prestwich 1994). Preliminary observations suggest that the Jamaican field cricket, *Gryllus assimilis* (Fabricius 1775), and the Texas field cricket, *Gryllus texensis* (formerly *G. integer*, Cade & Otte 2000), are two cricket species that use different signalling strategies. Male *G. texensis* produce a trilling call (Figure 2) which appears to be highly energetic since it has many pulses per unit time (Prestwich & O'Sullivan 2005), but seems to be performed less frequently in a given night compared to the less energetic (fewer pulses per unit time) chirping call (Figure 1) produced by *G. assimilis* males (personal observation).

Crickets are an ideal study organism for examining factors influencing variation in sexual displays because males exhibit much variation in their signalling effort. Signalling effort is typically measured as the amount of time spent signalling per night and varies greatly within a species (Cade 1991). Further, even within a species, some males may signal for most of the night, while others have been found to signal rarely, if at all (Bertram 2000). Given that females tend to prefer males with high signalling effort (Cade 1979; Cade & Cade 1992; Crnokrak & Roff 1995; Holzer 2003; Hunt et al. 2004; Judge et al. 2008), males should be strongly selected to exhibit minimal variation in signalling effort, and yet substantial variation exists within populations. Can this variation in signalling effort be explained by variation in body morphology or energy stores? Males also exhibit extensive variation in several of their fine-scale signalling

parameters (Bertram & Warren 2005; Bertram et al. 2011a). Given females tend to prefer males that signal with high chirp rates (Simmons 1988), long chirp durations (Wagner 1996) and more pulses per chirp/trill (Wagner et al. 1995), what is maintaining the variation in fine-scale signalling parameters? Some signalling parameters have been shown to be dependent on cricket body morphology, likely due to mechanical reasons. For example, wing harp mass (Bennet-Clark 1998) and area (Prestwich et al. 2000; Scheuber et al. 2003) are correlated with carrier frequency, where heavier and larger harps produce lower frequency sounds. This relationship is so predictable that in some species dominant frequency is a reliable indicator of male size (Simmons & Zuk 1992). However, can any of this variation in fine-scale signalling effort also be explained by variation in energy stores?

Signalling Metabolism and Biochemistry

Signaling is thought to be fuelled by aerobic metabolism (Prestwich 1994), suggesting that signaling is fueled by mainly lipid or carbohydrate metabolism. The vast majority of insect fuel is stored as lipid, and most of the lipids in an insect body are stored in an organ called the fat body. The fat body is responsible for the synthesis of the two main energy reserves, glycogen and fatty acids, used for fueling muscles and several other processes in the insect body (Arrese & Soulages 2010). The production of ATP in aerobic respiration is performed by the breakdown of lipids by mitochondria or by the breakdown of carbohydrates by the glycolytic pathway (Klowden 2007). There is evidence that supports both of these options.

Much of the earlier work done on insect muscle metabolisms was done by measuring respiratory quotients of insects performing high energy activities, typically flight (Drummond & Black 1960). When insects are measured to have a respiratory quotient of 1, they are consuming

just as much oxygen as they are producing carbon dioxide. A respiratory quotient of 1 therefore indicates that an insect is using purely carbohydrates as a fuel source (Chadwick 1947), whereas a quotient close to 0.75 would indicate that lipids are being consumed as a fuel source (Drummond & Black 1960). Zera et al. (1997) studied flight-muscle polymorphism of flightless and flight capable crickets during rest, and found that short-winged, flightless crickets had lower basal respiratory quotients than did long-winged, flight-capable crickets. This indicates that flight-capable crickets are using carbohydrates to fuel basal respiration while flight-incapable crickets are using lipids to fuel their basal respiration. Zera et al. (1997) proposed that flight-capable crickets use carbohydrates as a basal respiration fuel so that they can conserve lipids for use as their primary flight fuel. These studies suggest that crickets might be capable of using both lipids and carbohydrates to fuel their signalling behaviour.

Studying the maximal activities of various enzymes has also provided valuable information about insect metabolic processes (Beenakkers 1969; Darveau et al. 2005; Suarez et al. 2005a; Suarez et al. 2005b). For example, citrate synthase can be used as a reference enzyme for aerobic oxidative capacity (Suarez et al. 2005b) and has been shown to be consistently related to oxygen consumption (Beenakkers 1969). Further, enzymes such as glycogen phosphorylase can be used as reference enzymes for carbohydrate metabolism while β-hydroxyacyl-CoA dehydrogenase can be used to find activities of lipid metabolism (Zimmitti 1999; Bertram et al. 2011b).

Studies by Maklakov et al. (2008) and those of Bertram et al. (2011b) both indicate that acoustic signaling may be fueled by carbohydrates. Black field crickets, *Teleogryllus commodus*, fed high carbohydrate to protein diets, signal with higher effort than those fed with high protein to carbohydrate diets (Maklakov et al. 2008). Further, *Acheta domesticus* males who call more

often also have higher activities of pyruvate kinase, a reference enzyme for carbohydrate catabolism (Bertram et al. 2011b). Besides glycogen, there are other forms of carbohydrates used to fuel insect muscles. Trehalose (Figure 3), a disaccharide formed by two glucose subunits (Arrese & Soulages 2010), is present in circulating haemolymph in high concentrations, and is typically used during the first few seconds of flight in insects such as locusts and mosquitos (Klowden 2007). Trehalose must be synthesized in the fat body from glucose obtained by the breakdown of lipids (Arrese & Soulages 2010). Levels of trehalose are therefore dependent on both the enzymatic ability for it to be metabolised and the lipid stores available (Arrese & Soulages 2010).

Generally, insects that perform extended, high-intensity activities such as flight use lipids for fuel, whereas those that perform high-intensity but short duration activities, such as flying for short distances or jumping, use carbohydrates (Klowden 2007). Zera and Zhao (2006) showed that the flight-capable female morph of *Gryllus firmus* converts more fuel into lipid (mainly triglyceride) to fuel flight, whereas the flight-incapable morph converts more fuel into amino acid for the production of ovarian protein. This finding indicates that crickets may have the potential to utilize lipids for energetically costly behavior, such as flight or acoustic signaling. It also indicates that there are life-history trade-offs between fuel types depending on the needs of the cricket. Additionally, Bertram *et al.* (2009) showed that crickets fed diets rich in phosphorus had higher signaling effort than those with low phosphorus diets. This might imply that it is differences in mitochondrial density and the mitochondria's ability to breakdown lipids that are responsible for differences in signaling effort.

These studies highlight the necessity of measuring not only cricket size as a proxy for condition, but also specific fuel stores and enzyme capacities. Bertram et al. (2011b) also studied

the effect of multiple muscular enzymes on calling variation in *A. domesticus*, but failed to detect any noticeable activities of certain enzymes, such as GP and HOAD. This may be because crickets are not using glycogen stores to fuel signalling, but it is also possible that the small size of signalling muscles in *A. domesticus* makes it impossible to detect activities of these enzymes.

By studying two large cricket species, *G. assimilis* and *G. texensis*, my MSc thesis explores more robust differences in fuel levels and enzyme activities. In my MSc research, I successfully detected GP and HOAD, and I also improved on the methods of Bertram et al. (2011b) by measuring fuel stores in the abdomens and thoraxes of male crickets, and by using two additional enzymes that serve as markers for carbohydrate catabolism: trehalase and hexokinase (Table 1). The six enzymes used were pyruvate kinase, trehalase, and hexokinase (PK, TRE, and HK: reference enzymes for carbohydrate catabolism), citrate synthase (CS: marker for oxidative phosporylation), glycogen phosphorylase (GP: a marker for glycogen metabolism into sugars), β-hydroxyacyl-CoA dehydrogenase (HOAD: a reference enzyme for lipid metabolism). I measured the maximal capacity (V_{max}) for each enzyme. An individual with a high V_{max} value for a given enzyme should have more of this particular enzyme present in their muscles, and would therefore be better able to use the fuel the enzyme is involved in catabolising. The pathways that these enzymes are involved in are shown in Figures 3.

Table 1: Key enzymes used to measure muscle enzyme activities.

Enzyme	Abbreviation	Function
Pyruvate Kinase	PK	Carbohydrate catabolism
Glycogen Phosphorylase	GP	Glycogen catabolism
β-hydroxyacyl-CoA Dehydrogenase	HOAD	Lipid catabolism
Citrate Synthase	CS	Oxidative phosporylation
Trehalase	TRE	Carbohydrate catabolism
Hexokinase	HK	Carbohydrate catabolism

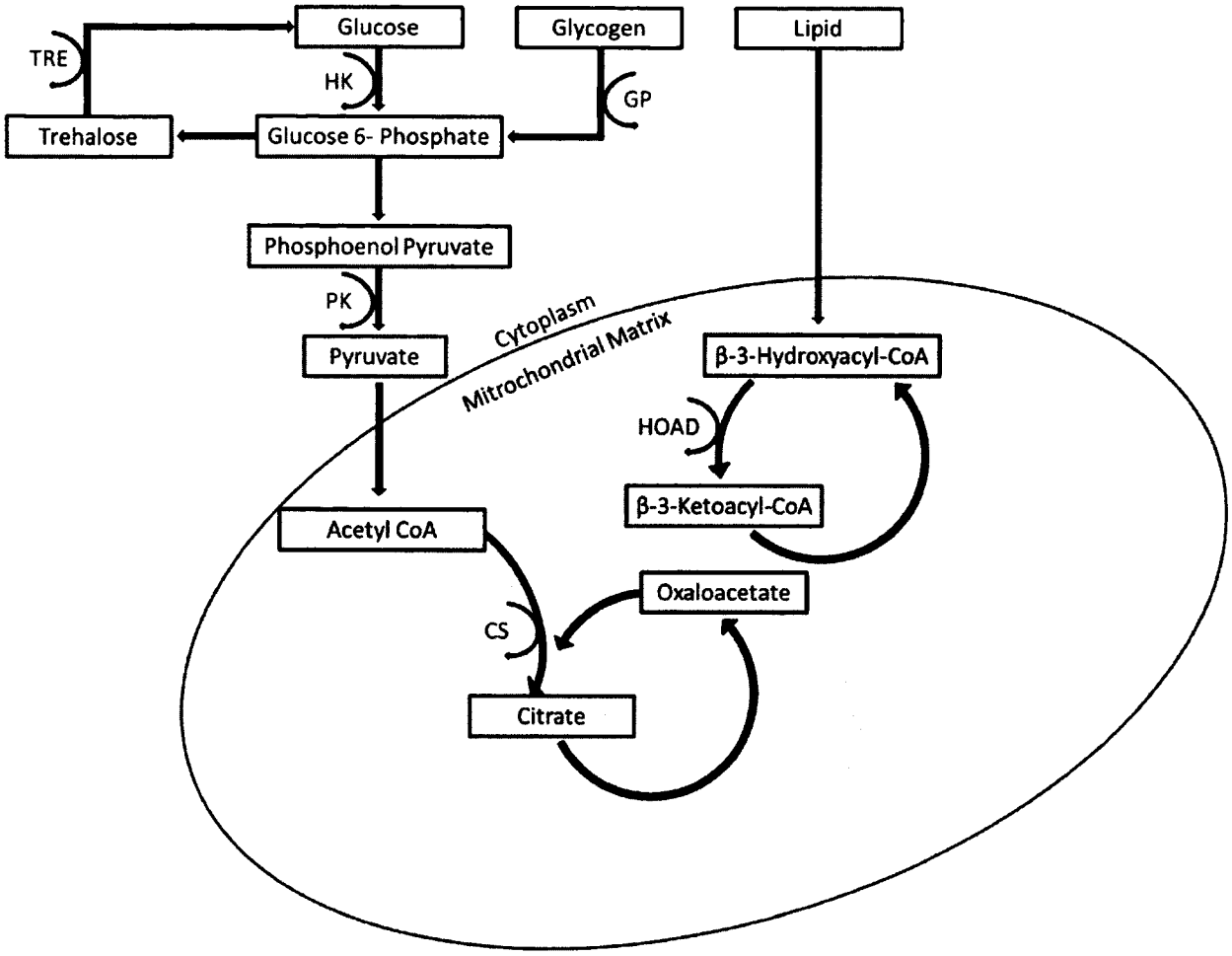

Figure 3: Metabolic pathways of six enzymes used to study muscle metabolism.

Research Objectives and Predictions

My thesis research sought to determine if physiology and energetics can explain the observed variation in cricket signalling by quantifying: a) the existing variation in signalling behaviour, enzyme activities, and body composition, and b) the univariate and multivariate relationships between signalling behaviour, enzyme activities, and body composition. The underlying assumption of my research is that male crickets exhibit substantial variation both in

their long distance signalling efforts and in their body morphology, enzyme activities, and body composition that may account for some of the variation in signalling effort.

I hypothesize that 1) sexual signalling will be correlated to measures of condition, and that 2) high-effort signallers will have a higher capacity to accumulate and store carbohydrates or lipids. To address these two hypotheses, I quantified cricket condition (size and weight), signalling morphological features (signalling muscle weight, wing file length, and wing harp area), and specific biochemistry (fuels present in the abdomen and thorax and muscular enzymes used), and I was able to determine what fuels, lipid or carbohydrate, males use to signal. I predicted, based on previous evidence using *A. domesticus*, that signalling effort in *G. assimilis* and *G. texensis* would be correlated with carbohydrate levels in the body such that high-effort signallers should have higher amounts of carbohydrate in their bodies and higher enzyme capacities to metabolise carbohydrates in their muscles. Alternatively, there may be differences between *G. assimilis* and *G. texensis* due to their markedly different signalling strategies. I therefore predicted that high-effort *G. texensis* signallers would have higher amounts of carbohydrates and lipids and higher enzyme capacities to metabolise both fuels, while high-effort *G. assimilis* signallers would only have high amounts of carbohydrate and high enzyme capacities to metabolise carbohydrates.

Methods

Cricket Rearing

Gryllus assimilis and *G. texensis* were collected in Bastrop County, Texas, United States, from 15 to 24-September, 2008 and brought back to the laboratories at Carleton University, Ottawa, Canada. The crickets were reared in communal plastic containers (64cm x 40cm x 41.9cm) with a light cycle of 12:12h at 26°C and fed *ad libitum* water and food (Harland's Tekland Rodent diet 8604; 24.3% protein, 40.2% carbohydrate, 4.7% lipid, 16.4% fiber, 7.4% ash) for 5^{th}-20^{th} generation. Crickets were reared from egg to final juvenile instar in the communal plastic containers and were checked daily for any crickets that had undergone final moult and were thus ready to be housed individually. Newly moulted male *G. assimilis* (N=169) and *G. texensis* (N=87) were removed from the colony, of which N=92 and N=63, respectively, were recorded in the EARS II, measured for morphological data, dissected, and used for enzyme assays. Individually housed adult males were housed in 500mL clear plastic containers with unbleached paper towel for shelter and *ad libitum* food and water throughout the experiment with light cycles and temperatures identical to those of the communal rearing bins. The lids for the containers had a section of about 4cm x 4cm removed and replaced by mesh as to allow air and sound to pass through. Since all males were raised in the same environment, variation among males in signalling behaviour and physiology should be largely caused by genetic differences (Wagner et al. 2012).

Acoustic Recording

On day-7 post imaginal moult, male crickets' individual containers were transferred into the EARS II (Electronic Acoustic Recording System II) which records and monitors all signalling

behaviour for each individual cricket (for details, refer to Whattam et al. 2011). The EARS II system houses up to 96 individuals in sound-proof Styrofoam containers that avoid sound contamination by neighbouring crickets. Each cricket container is equipped with a microphone that is monitored by a program called CricketSong (Cambridge Electronic Design Ltd., Unit 4, Science Park, Cambridge, UK). The program sets a specific amplitude threshold for not only different cricket species, but it also automatically adjusts its thresholds for quiet or loud individuals to ensure it records all pulses of sound that are produced. Each cricket's light cycle was set to a 12:12h light: dark cycle using individual LED lights (one per container). Crickets were checked daily and water and food were replenished as needed.

The EARS II system was used to quantify eight important aspects of signalling behaviour for each individual cricket: time spent signalling (hourly, daily, or weekly), call amplitude (Volts), dominant frequency of the call (Hz), chirp duration (ms), interchirp duration (ms), number of pulses per chirp, pulse duration (ms), interpulse duration (ms; Figure 1). The average call amplitude for each cricket (in Volts) was converted to dB for all further analyses. For *G. assimilis*, any silent durations less than 0.1s was considered to be an interpulse, and any silent durations longer than 0.1s was considered to be an interchirp. For *G. texensis*, this time threshold was set to 0.06s since pulses are produced at a high rate. See Figure 1 (page 4) for a visual representation of the fine scale signalling parameters.

Each male cricket was monitored and their long distance acoustic mate attraction calls were quantified 24 h a day over a 7 day study period (from 7-14 days post final moult).

Morphometric Analysis and Dissection

At day 14, morphological characteristics were measured and the crickets were dissected. Immediately preceding morphometric measurements and dissection, crickets were weighed to the nearest mg using a Denver Instrument PI-114 scale. For each cricket, the pronotum width, length, and trace outline, as well as the head capsule distance, were then measured in μm using a Zeiss Discovery V12 microscope and accompanying Axiovision software version 4.8.2.0 (Carl Zeiss MicroImaging, Jena, Germany). Each cricket was then dissected on ice and its signalling muscles were removed. Crickets were humanely euthanized by removing their heads with sharp scissors. Once all legs and wings were removed, the abdomen was separated and stored in an Eppendorf tube in liquid nitrogen. Cricket thoraxes were then dissected ventrally and the signalling muscles (dorsoventral, basalar and subalar muscles) were removed, weighed, and frozen in Eppendorf tubes in liquid nitrogen. The thorax was placed in a third Eppendorf tube and also frozen in liquid nitrogen. All tubes were stored in freezers at a temperature of -80°C and were then transported in dry ice to the University of Ottawa for biochemical analyses.

The wings of each cricket were also used to measure file length and harp area using the same microscope and software as described above. The harp area was measured by tracing the veins that surround the harp (Miyoshi et al. 2007) and the stridulatory file length was measured to include all visible teeth.

Biochemical Analysis

Biochemical methodology is based on that of Bertram et al. (2011b). Signalling muscles were analysed to determine which key enzymes and specific energy sources are used to fuel signalling behaviour. To determine how crickets are using lipids and carbohydrates to fuel

signalling, the maximum activity (V_{max}) of six different reference enzymes was measured. Cricket signalling muscles were homogenized in separate vials in 19 volumes of buffer relative to the mass (fresh weight) of the muscle tissue. The homogenization buffer consisted of 50 mM Tris-HCl (pH 7.5 at 21°C) and 2 mM ethylenediaminetetraacetic acid (EDTA), to which 0.5% (vol./vol.) triton X100 and 5 mM DTT were added on each individual day that assays were performed. Samples were homogenized in this buffer three times for 10 s at 30 s intervals, using an Omni-Prep homogenizer with a 7 mm Rotor Stator tip (Omni International, Marietta, GA, U.S.A.). Homogenates were centrifuged for 2 min at 10000 rpm at 21°C (Sorvall Legend micro21R, Germany). The resulting supernatant (at a 1/20 dilution) was diluted to 1/40 for the GP assay, 1/80 for the PK, CS, TRE and HK assays, and 1/600 for the HOAD assay. Enzyme activities are reported in U/g of tissue, where 1 U = 1 μmol substrate/min. All assays were performed in triplicate at room temperature using a Biotek Synergy 2 plate spectrophotometer (Biotek, Winooski, VT, U.S.A.). The CS reaction was monitored using 5,5-dithiobis-2-nitrobenzoic acid (DTNB) at 412 nm. The PK and HOAD reactions were monitored using nicotinamide adenine dinucleotide (NADH) at 340 nm. The GP, TRE, and HK reactions were monitored using nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm. For the enzyme activity calculation, the millimolar extinction coefficient used was 6.22 for NADH and NADPH, and 13.6 for DTNB, correcting for pathway length. Control rates were measured without one specific substrate (as indicated below), and experimental assays were only run once the control assay activities stabilized. Assay conditions and substrate concentrations required to elicit V_{max} were as follows: PK: 40 mM of Imidazole HCl, pH 7.0 at ambient temperature, 10 mM of $MgCl^2$, 100 mM of KCl, 0.16 mM of NADH, 5 mM of ADP, 5 mMof phosphoenolpyruvate (omitted from control) and 5 U of Lactate dehydrogenase; CS: 42 mM of

Tris HCl, pH 8.0 at ambient temperature, 0.5 mM of oxaloacetate (omitted from control), 0.3 mM of acetylCoA and 0.1 mM of DTNB; GP: 72 mM of potassium phosphate, pH 7.4 at ambient temperature, 10 mM of MgCl2, 1.25 U of glucose 6-phosphate dehydrogenase, 0.75 mM of NADP, 2 mM of AMP, 8 mg/ml of glycogen (omitted from control), 2.5 U of phosphoglucomutase and 2.5 U of glucose 1,6-diphosphatedehydrogenase; HOAD: 40 mM of ImidazoleHCl, pH 7.0 at ambient temperature, 10 mM of $MgCl^2$, 100 mM of KCl, 0.16 mM of NADH, 1 mM of EDTA, 5 mM of dithiothreitol (DTT) and 0.1 mM of acetoacetylCoA (omitted from control); TRE: 100 mM KPO^4, 1.1 mM of $MgCl^2$, 0.75 mM of NADP, 1.1 mM ATP, 10 mM trehalose (omitted from control), 1.25 U of glucose 6-phosphate dehydrogenase. 1.25 U of hexokinase; HK: 100 mM ImidazoleHCl, pH 8.1 at ambient temperature, 10 mM of $MgCl^2$, 100 mM KCl, 1 mM NADO, 5 mM ATP, 5 mM D-glucose (omitted from control) and 1.25 U of glucose 6-phosphate dehydrogenase.

Extraction of Energy Stores from Thoraxes and Abdomens

Due to time constraints, about 30 crickets of each species were chosen for body content analysis (Table 2). These 30 crickets were comprised of equal numbers of high effort, medium effort, and low effort callers, and were chosen from among the crickets with complete enzyme assay data. The methodology for the extraction of lipids, proteins, glycogen, and free carbohydrate from cricket samples was based on that of Lorenz (2003). Cricket thoraxes or abdomens were weighed (fresh weight) and placed separately into Eppendorf tubes before 20mg Na_2SO_4 and 200µl MeOH (75% in water) were added to each sample. Each sample was homogenized by first mincing with a pair of fine scissors, and then using an Omni-Prep homogenizer with a 7 mm Rotor Stator tip (Omni International, Marietta, Georgia). The

homogenizer tip was then rinsed with 600µl $CHCl_3$ (1:1) directly into the original tube. Tubes were then vortexed and centrifuged for 10 min at 21000 g at 4°C (Sorvall Legend, Germany). The resulting supernatant was removed for the separation of lipid and free carbohydrate, and the pellet was removed for separation of protein and glycogen.

Protein and glycogen

The pellet from the initial centrifugation was vortexed in 300µl of $CHCl_3$ (1:1), and sonicated (Sonics Vibra-cell, six heads) for 5 minutes with sonication occurring at 40 kHz for 10 s intervals with 30 s breaks between intervals. The pellet was then vortexed again and centrifuged. The resulting supernatant was added to the lipid and free carbohydrate tube, and the pellet was dried in a thermoblock at 70°C. Once dry, 300µl Na_2SO_4 saturated 66% EtOH was added and the solution was vortexed, put in the ultrasonic bath, vortexed again, and centrifuged. The supernatant was discarded quantitatively, and the pellet dried in a thermoblock at 70°C. Once dry, 200µl of KOH (10%) was added, the solution was vortexed, put in the sonicator, vortexed, then macerated for 30min at 100°C (while vortexing every 10mins). Then, 50µl of this sample was mixed with 150 µl of water and was used later for the determination of protein content. The remained had 150 µl of EtOH added to it, was vortexed, left standing for 10min, and centrifuged. The supernatant was discarded qualitatively and the pellet was dried in a thermoblock at 70°C. The final dried pellet was re-dissolved in 400 µl of water at 100°C and kept for the determination of glycogen.

Lipid and free carbohydrate

The following steps were carried out on the combined supernatants from the initial centrifugation, and from the first centrifugation on the protein and glycogen extraction. 500µl $CHCl_3$ and 300µl NaCl (1M) were added, and the sample was vortexed and centrifuged. Both the

organic and the aqueous epiphases were removed quantitatively and dried separately in a thermoblock at 80°C. Once dry, 500 µl of H_2O was added to the aqueous epiphase residue and it was kept for the determination of free carbohydrate. The organic hypophase was re-dissolved in 1000 µl of hexane and 500 µl of NaCl (1M) was added. This tube was vortexed, centrifuged, and kept for the determination of lipid content.

Colourimetric Determination of Body Energy Stores

All colourimetric assays were performed in plastic 96 well plates (Corning Costar 21, location) in triplicate at 37°C using a Biotek Synergy 2 plate spectrophotometer (Biotek, Winooski, VT, USA). Individual standard curves were made for each plate that was read.

Total protein was measured using the bicinchoninic acid assay (BCA1 and B9642, Sigma-Aldrich, St. Louis, MO) with bovine serum albumin as the standard, according to the manufacturer's specifications.

Total lipid was measured using the phospho-vanillin method (Kaufmann 2011), with the exception that soy bean oil was dissolved in hexane instead of chloroform so that plastic 96-well plates could be used. The phosphor-vanillin reagent was made by dissolving vanillin (at a concentration of 6 mg/mL) in de-ionized hot water and then adding 85% phosphoric acid to achieve a final ratio of 20% water to 80% acid. Solutions for the standard curve were prepared by dissolving soy bean oil in hexane to obtain final concentrations of 2.5, 5, 10, 40, 80 and 160 µg/µl per well. The pure lipid extract from either cricket thorax or abdomen was diluted 20x. In triplicate, the correct amount of standard or 10 µl of the sample was added to the wells and the plate and placed in a thermoblock at 90°C to evaporate any solvent. Then, 10 µl of sulfuric acid was added to each well, and the plate was heated for an additional 10 minutes in a thermoblock

at 90°C. Then, 240 µl of phosphor-vanillin reagent was added, the plate was allowed to cool, and a reddish colour was allowed to develop for about 5 minutes before the plate was read in the spectrophotometer at 625 nm.

Total glycogen and free carbohydrate were measured using the anthrone method (Kaufmann & Brown 2008). Anthrone reagent was made by adding 95-98% sulfuric acid to de-ionized water to achieve a final ratio of 28% water to 72% acid. Solutions for the standard curve were prepared by dissolving anhydrous glucose in dH_2O at final concentrations of 0, 1, 5, 25, and 50 µg/µl per well. In triplicate, 5 µl of the glycogen standards or the free carbohydrate extracts (diluted 2X) were added to each well. Then, 245 µl of anthrone reagent was added to each well, and the plate was heated in a thermoblock for 17 minutes at 90°C. The plate was then allowed to cool, and was read in the spectrophotometer at 625 nm.

Data Analysis

All data were analyzed using JMP 10.0.0 statistical software (SAS Institute Inc., 100 SAS Campus Drive, Cary, North Carolina, USA). Due to inconsistencies in enzyme activities between days, all the PK and CS samples run on 11-Dec were excluded (N=11). Final sample sizes are stated in Table 2. To further minimize any effects of the date of assay on V_{max}, the residuals of the enzyme activities versus date of enzyme assay were taken and used for all further analyses.

All values obtained from the spectrophotometer were compared to the standard curves and total protein, carbohydrate, glycogen, and lipid amounts were calculated for both the thorax and abdomen of each cricket. These values were then converted to a percentage to control for differences in body size.

Male body size parameters and distance signalling effort were incorporated into a principal component analysis. We included all principal components with Eigenvalues above 1.00 in subsequent analyses of variation. Loading components with Eigenvectors above 0.35 were considered to be significant. Since signalling parameters used in calculating the Effort PC1 (daily mean time spent calling, total pulses, total interpulses, total chirps, and total interchirps) were skewed to the left and not normally distributed, these parameters were all BoxCox transformed before being incorporated into the PCA.

I tested for relationships between enzymes activities, body contents, signalling parameters, and body morphology using bivariate correlations (see Tables 13 and 14). I also tested whether enzymes activities, body composition, and size had an effect on signalling effort and quality by conducting generalized linear models (GLM). Since only a subset of crickets underwent body content analyses, separate GLMs had to be conducted for the effects of enzyme activities and size on signalling, and body content and size on signalling.

Table 2: Sample sizes for each enzyme and body content analysis combination.

	G. assimilis (*N*=92)		G. texensis (*N*=63)	
	Signalling Data	Body Content	Signalling Data	Body Content
PK	89	30	54	31
GP	92	30	61	31
CS	89	30	54	31
HOAD	92	30	63	31
TRE	92	30	63	31
HK	92	30	63	31

Results

Characterizing the Variation in Traits

The signalling, morphometric, and biochemical traits were highly variable across individuals in both cricket species. Both species had many individuals that did not signal acoustically at all during the 7 day recording period, and some individuals that signalled for an average of over 6 hours (*G. assimilis*) or 12 hours (*G. texensis*) a night (Tables 3 and 4; Figure 4a,d). There was dramatic variation in average chirp duration for *G. texensis* (Figure 4b), but not for *G. assimilis* (Figure 4e). There was little variation in dominant frequency within each species, but the two species differed in their mean dominant frequencies (Tables 3 and 4; Figure 4c,f). Between species, the average number of pulses produced in a given night was markedly different, with the most energetic *G. texensis* producing more than 10X the pulses that the most energetic *G. assimilis* did (Tables 3 and 4).

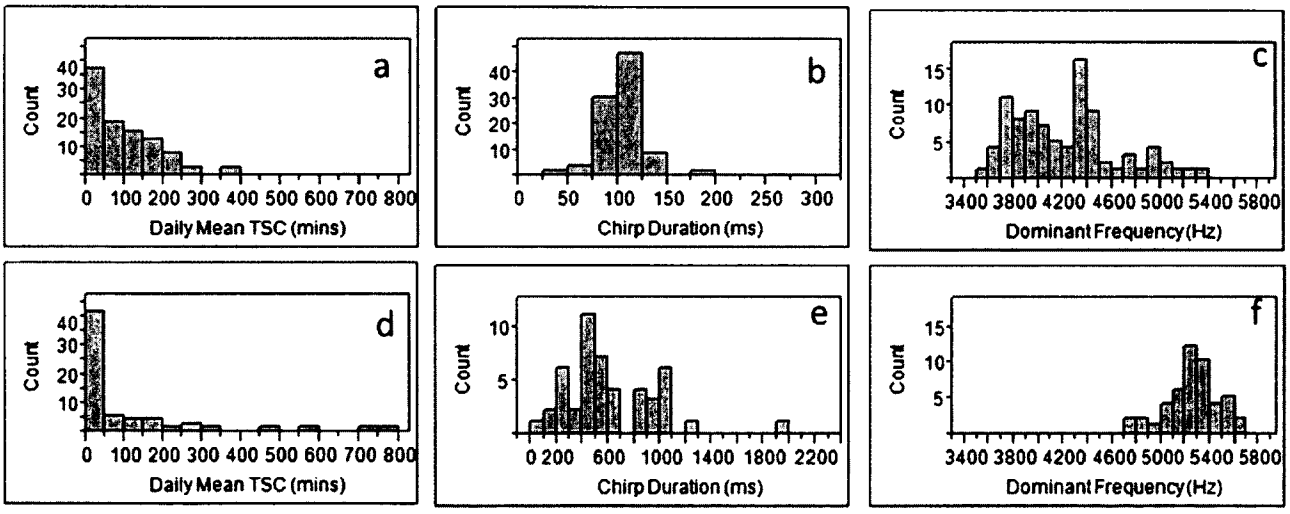

Figure 4: Distributions of signalling behaviours: daily mean time spent signalling (a,d), average chirp duration (b,e) and dominant frequency (c,f) for *G. assimilis* (a,b,c) and *G. texensis* (d,e,f). Chirp duration (b,e) are on different scales due to large differences between species.

Fine scale signalling parameters (pulse, interpulse, chirp, and interchirp durations, pulses per chirp, dominant frequency, and amplitude) had much lower coefficients of variation than the parameters indicative of signalling effort (time spent calling and total number of pulses, interpulses, chirps, and interchirps; Table 3 & 4). Additionally, for both species the coefficients of variation for morphological traits were much lower than those for signalling behaviour (comparing Table 3 with 5 and Table 4 with 6).

Table 3: Distribution of signalling behaviours for *G. assimilis*.

Trait	Mean	Min	Max	St Error	CV	Median	Difference	
TSC	94.00	0.00	364.06	8.51	86.81	73.55		
#Pulses	35052.07	0.00	200207.00	3946.61	108.00	24644.94		
#Ipulses	30714.21	0.00	184169.50	3508.98	109.58	21631.38		
#Chirps	4337.84	0.00	18231.50	452.57	100.07	3159.94		
#Ichirps	4314.07	0.00	18162.88	451.60	100.41	3137.94		
Pulse Dur	8.83	4.97	11.51	0.12	13.05	9.04	2.3	X
IP Dur	16.17	12.87	28.42	0.29	16.87	15.58	2.2	X
PPChirp	7.44	3.11	12.88	0.14	17.97	7.68	4.1	X
Chirp Dur	104.79	49.67	180.74	2.04	18.40	105.38	3.6	X
Ichirp Dur	1574.80	575.86	2634.49	45.84	27.46	1573.04	4.6	X
Dom Freq	4221.24	3599.24	5324.89	43.91	9.81	4215.95	1.5	X
Amp	41.12	11.32	63.99	1.27	29.04	43.33	5.7	X

* The *Difference* column represents the fold difference between the minimum and the maximum values. Abbreviations are as follows (units of measurement at the end of parentheses): TSC (daily average time spent calling, mins), #Pulses (daily average number of pulses), #IPulses (daily average number of interpulses), #Chirps (daily average number of chirps), #IChirps (daily average number of interchirps), Pulse Dur (average pulse duration, ms), IP Dur (average interpulse duration, ms), PPChirp (average number of pulses per chirp), Chirp Dur (average chirp duration, ms), IChirp Dur (average interchirp duration, ms), Dom Freq (average dominant frequency of pulses, Hz), Amp (average amplitude of pulses, dB).

Table 4: Distribution of signalling behaviours for *G. texensis*.

	Mean	Min	Max	St Error	CV	Median	Difference	
TSC	87.64	0.00	756.93	20.68	187.25	15.79		
#Pulses	264562.22	0.00	2362084.00	60502.59	181.52	58225.25		
#Ipulses	258905.49	0.00	2316240.00	59276.97	181.73	56611.00		
#Chirps	5656.60	0.00	53477.00	1432.74	201.04	1150.00		
#Ichirps	5641.49	0.00	53383.00	1429.38	201.10	1148.00		
Pulse Dur	7.70	5.06	11.14	0.17	15.11	7.85	2.2	X
IP Dur	13.79	7.41	17.10	0.21	10.65	13.92	2.3	X
PPChirp	50.58	8.61	220.20	5.12	70.81	39.65	25.6	X
Chirp Dur	613.15	78.78	1992.68	50.90	58.11	529.44	25.3	X
Ichirp Dur	414.56	83.23	1453.24	43.20	72.94	303.38	17.5	X
Dom Freq	5242.11	4141.26	5658.09	38.45	5.13	5265.70	1.4	X
Amp	59.75	36.46	79.46	1.55	18.17	61.12	2.2	X

* Refer to Table 3 for descriptions of abbreviations

There were very few notable differences in any of the morphological traits observed both within a species and between species (Tables 5 and 6). The exception was the average mass of the dissected signalling muscles. *Gryllus assimilis* males were also slightly larger on average than *G. texensis* males.

Table 5: Distribution of size traits for *G. assimilis* (*N*=92).

Trait	Mean	Min	Max	St Error	CV	Median	Difference	
Body Mass	593.92	170.00	796.60	10.06	16.24	612.45	4.7	X
Muscle Mass	7.51	1.00	14.00	0.24	29.49	7.70	14.0	X
Pro Area	23.59	16.52	30.46	0.31	12.61	23.40	1.8	X
Pro L	3.98	3.31	4.74	0.03	8.34	3.98	1.4	X
Pro W	6.22	5.33	7.03	0.04	6.50	6.25	1.3	X
Head W	5.17	4.52	5.98	0.03	5.59	5.16	1.3	X
Harp Area	13.89	11.12	16.70	0.11	7.84	13.81	1.5	X
File L	4.16	3.39	4.72	32.07	7.35	4.22	1.4	X

* The *Difference* column represents the fold difference between the minimum and the maximum values. Abbreviations are as follows (units of measurement at the end of parentheses): Body Mass (mg), Muscle Mass (mg), Pro Area (pronotum area, mm^2), Pro L (pronotum length, mm), Pro W (pronotum width, mm), Head W (head capsule width, mm), Harp Area (area of the wing harp, mm^2), File L (wing file length, mm).

Table 6: Distribution of size traits for *G. texensis* (*N*=63).

Trait	Mean	Min	Max	St Error	CV	Median	Difference	
Body Mass	540.89	361.80	823.30	13.76	20.19	530.30	2.3	X
Muscle Mass	6.18	1.30	11.80	0.20	25.17	5.85	9.1	X
Pro Area	22.86	14.17	33.20	0.47	16.28	22.80	2.3	X
Pro L	3.80	2.77	4.56	0.04	9.26	3.85	1.6	X
Pro W	6.06	4.97	7.31	0.06	8.12	6.06	1.5	X
Head W	5.19	4.31	6.98	0.06	9.47	5.13	1.6	X
Harp Area	10.64	8.32	13.79	0.15	10.56	10.60	1.7	X
File L	3.57	3.06	4.38	42.36	9.20	3.55	1.4	X

* Refer to Table 4 for descriptions of abbreviations

All enzyme activities varied within species. Notably, HOAD in *G. assimilis* displayed a 16.4X difference (Tables 7 and 8, Figure 5b), and GP in *G. texensis*, which displayed a 20.0X difference (Figure 5d). Values between species were not notably different (comparison of Table 7 with Table 8).

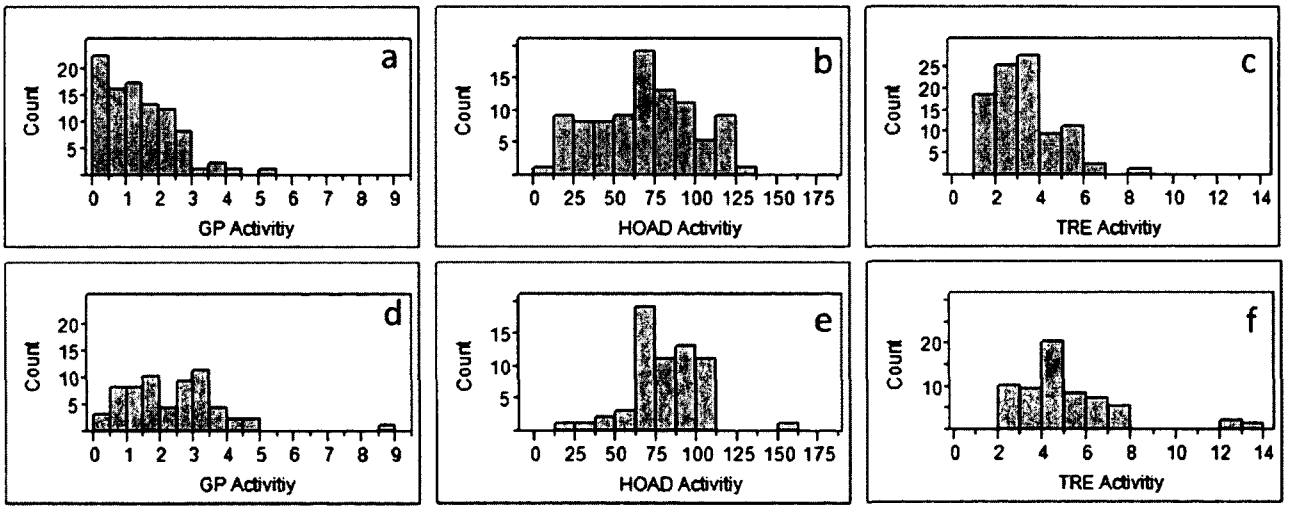

Figure 5: Distributions of enzyme activities: GP (a,d), HOAD (b,e) and TRE (c,f) activity (in U/g of muscle tissue, where 1 U = 1 μmol substrate/min) for *G. assimilis* (a,b,c) and *G. texensis* (d,e,f).

Table 7: Distribution of enzyme activities behaviours for *G. assimilis*.

Enzyme	Mean	Min	Max	Std Err	CV	Median	Difference	
PK	108.49	52.45	163.28	1.90	16.53	110.07	3.1	X
GP	1.42	0.23	5.02	0.10	70.41	1.178	22.0	X
CS	36.68	14.67	51.59	0.68	53.36	34.63	3.5	X
HOAD	68.25	7.98	131.13	3.18	44.74	71.26	16.4	X
TRE	3.30	1.06	8.36	0.15	42.17	3.04	7.9	X
HK	4.27	1.82	8.51	0.17	38.11	4.11	4.7	X

* The *Difference* column represents the fold difference between the minimum and the maximum values. All activities are in U/g of muscle tissue, where 1 U = 1 μmol substrate/min.

Table 8: Distribution of enzyme activities behaviours for *G. texensis*.

Enzyme	Mean	Min	Max	Std Err	CV	Median	Difference	
PK	104.73	47.13	131.35	2.26	15.87	107.49	2.8	X
GP	2.17	0.23	4.56	0.15	53.75	2.13	20.0	X
CS	38.59	5.56	51.38	1.04	19.76	41.16	9.2	X
HOAD	81.23	20.52	156.21	2.67	26.05	83.24	7.6	X
TRE	5.01	2.05	13.38	0.28	45.01	4.56	6.5	X
HK	8.64	3.80	20.52	0.41	37.46	8.21	5.4	X

* The *Difference* column represents the fold difference between the minimum and the maximum values. All activities are in U/g of muscle tissue, where 1 U = 1 μmol substrate/min.

In *G. assimilis*, body composition in the thorax and abdomen varied from a two-fold to a six-fold difference, depending on the particular fuel store. However, the percent mass of the abdomen that was composed of protein varied by a 13.3X difference (Table 9). In *G. texensis*, body composition in each compartment varied from a 2X to a 5X difference, with the exception of thoracic and abdominal glycogen content, which varied by a 13.5X and 12.7X difference, respectively (Table 10). The most notable difference in body composition between species was thoracic glycogen content, where thoracic glycogen levels in *G. texensis* were higher and more variable (Figure 6).

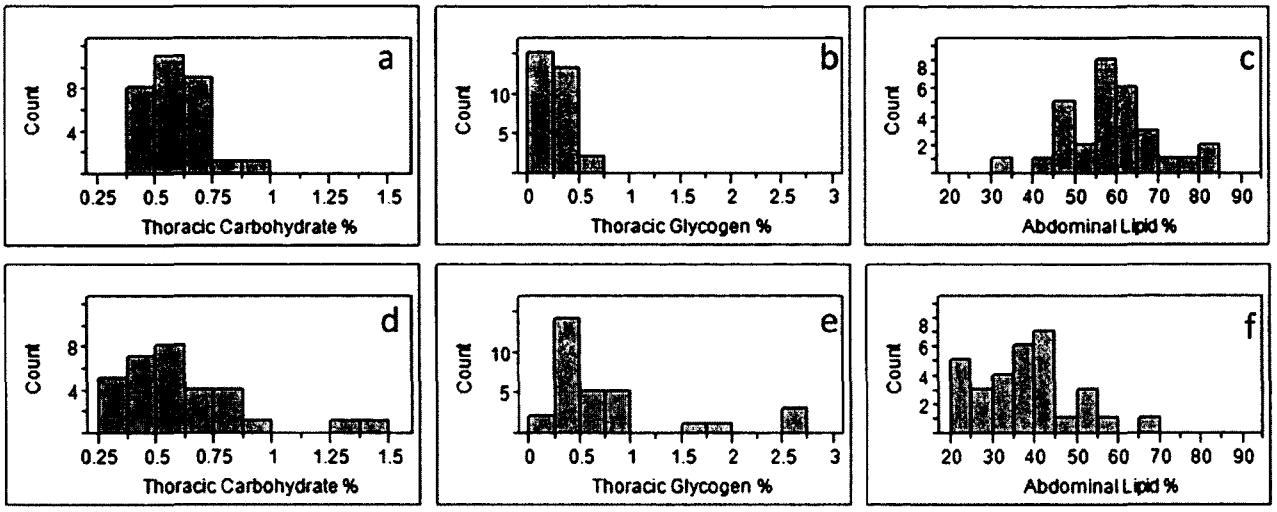

Figure 6: Distributions of body compositions; thoracic carbohydrate (a,d) and glycogen (b,e) and abdominal lipid content (c,f) for *G. assimilis* (a,b,c) and *G. texensis* (d,e,f).

Table 9: Distribution of thoracic and abdominal percentages of protein, carbohydrate, glycogen, and lipid for *G. assimilis*.

Body Content	Mean	Min	Max	St Error	CV	Median	Difference	
Thor Pro %	2.17	0.99	3.65	0.12	29.30	2.11	3.7	X
Thor Carb %	0.59	0.43	0.94	0.02	20.21	0.57	2.2	X
Thor Gly %	0.29	0.11	0.74	0.02	45.32	0.25	6.5	X
Thor Lipid %	20.38	8.36	39.87	1.31	35.31	19.26	4.8	X
Ab Pro %	1.67	0.23	3.02	0.13	42.58	1.72	13.3	X
Ab Carb %	1.29	0.49	2.62	0.10	43.67	1.17	5.3	X
Ab Gly %	1.43	0.54	3.54	0.15	56.71	1.23	6.6	X
Ab Lipid %	59.05	32.81	82.83	1.98	18.39	58.77	2.5	X

* The *Difference* column represents the fold difference between the minimum and the maximum values.

Table 10: Distribution of thoracic and abdominal percentages of protein, carbohydrate, glycogen, and lipid for *G. texensis*.

Body Content	Mean	Min	Max	St Error	CV	Median	Difference	
Thor Pro %	2.72	1.64	4.10	0.11	22.90	2.64	2.5	X
Thor Carb %	0.60	0.29	1.39	0.05	42.29	0.52	4.7	X
Thor Gly %	0.78	0.20	2.70	0.13	91.23	0.46	13.5	X
Thor Lipid %	24.16	9.36	43.45	1.80	41.50	23.59	4.6	X
Ab Pro %	1.82	0.81	2.95	0.08	25.35	1.81	3.6	X
Ab Carb %	1.21	0.43	2.16	0.08	38.75	1.06	5.0	X
Ab Gly %	2.12	0.65	8.22	0.30	77.64	1.54	12.7	X
Ab Lipid %	38.20	21.49	68.33	1.98	28.83	36.09	3.2	X

* The *Difference* column represents the fold difference between the minimum and the maximum values.

Principal Component Analyses

I used principal component analyses to reduce the large number of traits associated with size, signalling effort, and signalling quality (Tables 11 and 12). The first principal component for body size (Size PC1) accounted for 76.4% of the variation in *G. assimilis* (eigenvalue = 2.29), and 72.7% of the variation in *G. texensis* (eigenvalue = 2.18). Males with higher Size PC1 scores had larger pronotums (length, width, and area trace), larger head capsule widths, larger wing harp areas, longer wing files, and were heavier than individuals with lower Size PC1 scores.

The first principal component for signalling effort (Effort PC1) accounted for 97.3% of the signalling effort variation in *G. assimilis* (eigenvalue = 4.87) and 98.8% of the signalling effort variation in *G. texensis* (eigenvalue = 4.94). Males with higher Effort PC1 scores had greater total numbers of pulses, interpulses, chirps, and interchirps and signalled for longer through a given night than individuals with lower Effort PC1 scores.

The first principal component of fine scale temporal signalling parameters of distance calls (Quality PC1) accounted for 50.3% of the variation in distance signalling for *G. assimilis*

(eigenvalue = 3.52) and males with higher Quality PC1 scores signalled with more pulses per chirp, longer pulse and chirp durations, higher dominant frequencies, and louder amplitude than individuals with lower Quality PC1 scores. The second principal component accounted for 22.0% of the variation in signalling for *G. assimilis* (eigenvalue = 1.54) and males with higher Quality PC2 scores had longer interpulse and interchirp durations, more pulses per chirp and longer chirp durations than males with lower Quality PC2 scores. These two principal components were used in further analyses as together they explained 72.5% of the variation in signalling quality.

The first principal component of fine scale temporal signalling parameters of distance calls (Quality PC1) accounted for 40.7% of the variation in distance signalling for *G. texensis* (eigenvalue = 2.85) and males with higher Quality PC1 scores signalled with more pulses per chirp, longer chirp durations, and louder amplitudes than individuals with lower Quality PC1 scores. The second principal component accounted for 19.9% of the variation in signalling for *G. texensis* (eigenvalue = 1.39) and males with higher Quality PC2 scores had shorter pulse durations and higher dominant frequencies than males with lower Quality PC2 scores. These two principal components were used in further analyses as together they explained 60.7% of the variation in signalling quality.

Table 11: PCA loading scores for size, signalling effort, and signalling quality for *G. assimilis*.

PCA		Components	PC1	PC2
Overall Size	**% of Variation**		76.4	
	Eigenvalue		2.291	
	Eigenvector	Body Mass	**0.570**	
		Size PC1	**0.611**	
		Wing Size PC1	**0.548**	
Effort	**% of Variation**		97.3	
	Eigenvalue		4.866	
	Eigenvector	Lifetime TSC (min)	**0.441**	
		Total Pulses	**0.449**	
		Total IPs	**0.444**	
		Total Chirps	**0.450**	
		Total IChirps	**0.450**	
Quality	**% of Variation**		50.3	22.0
	Eigenvalue		3.517	1.537
	Eigenvector	Pulse duration (ms)	**0.428**	-0.105
		IP Duration (ms)	-0.277	**0.483**
		PPChirp	**0.416**	0.329
		Chirp Duration (ms)	**0.373**	**0.485**
		IC Duration (ms)	-0.054	**-0.580**
		Dom Frequency (Hz)	**-0.461**	0.236
		Amplitude (dB)	**0.459**	-0.133

Table 12: PCA loading scores for size, signalling effort, and signalling quality for *G. assimilis*.

PCA		Components	PC1	PC2
Overall Size	**% of Variation**		72.7	
	Eigenvalue		2.181	
	Eigenvector	Body Mass	**0.638**	
		Size PC1	**0.624**	
		Wing Size PC1	**0.452**	
Effort BoxCox	**% of Variation**		98.8	
	Eigenvalue		4.941	
	Eigenvector	Lifetime TSC (min)	**0.448**	
		Total Pulses	**0.447**	
		Total IPs	**0.447**	
		Total Chirps	**0.447**	
		Total IChirps	**0.447**	
Quality	**% of Variation**		40.7	19.9
	Eigenvalue		2.851	1.395
	Eigenvector	Pulse duration (ms)	0.180	**-0.726**
		IP Duration (ms)	0.068	-0.057
		PPChirp	**0.547**	0.077
		Chirp Duration (ms)	**0.555**	0.007
		IC Duration (ms)	-0.256	0.202
		Dom Frequency (Hz)	0.256	**0.648**
		Amplitude (dB)	**0.474**	-0.053

Univariate Relationships Between Traits

Muscle enzymes vs. muscle enzymes (energetic phenotypes)

In *G. assimilis*, crickets that exhibited high activities of HK also had high activities of TRE, GP, and PK (Table 13, Figure 7). Furthermore, crickets with high activities of PK also had high activities of GP. While there are correlations within glycolytic enzymes, there were no correlations within the mitochondrial enzymes or between glycolytic and mitochondrial enzymes in *G. assimilis* (Table 13).

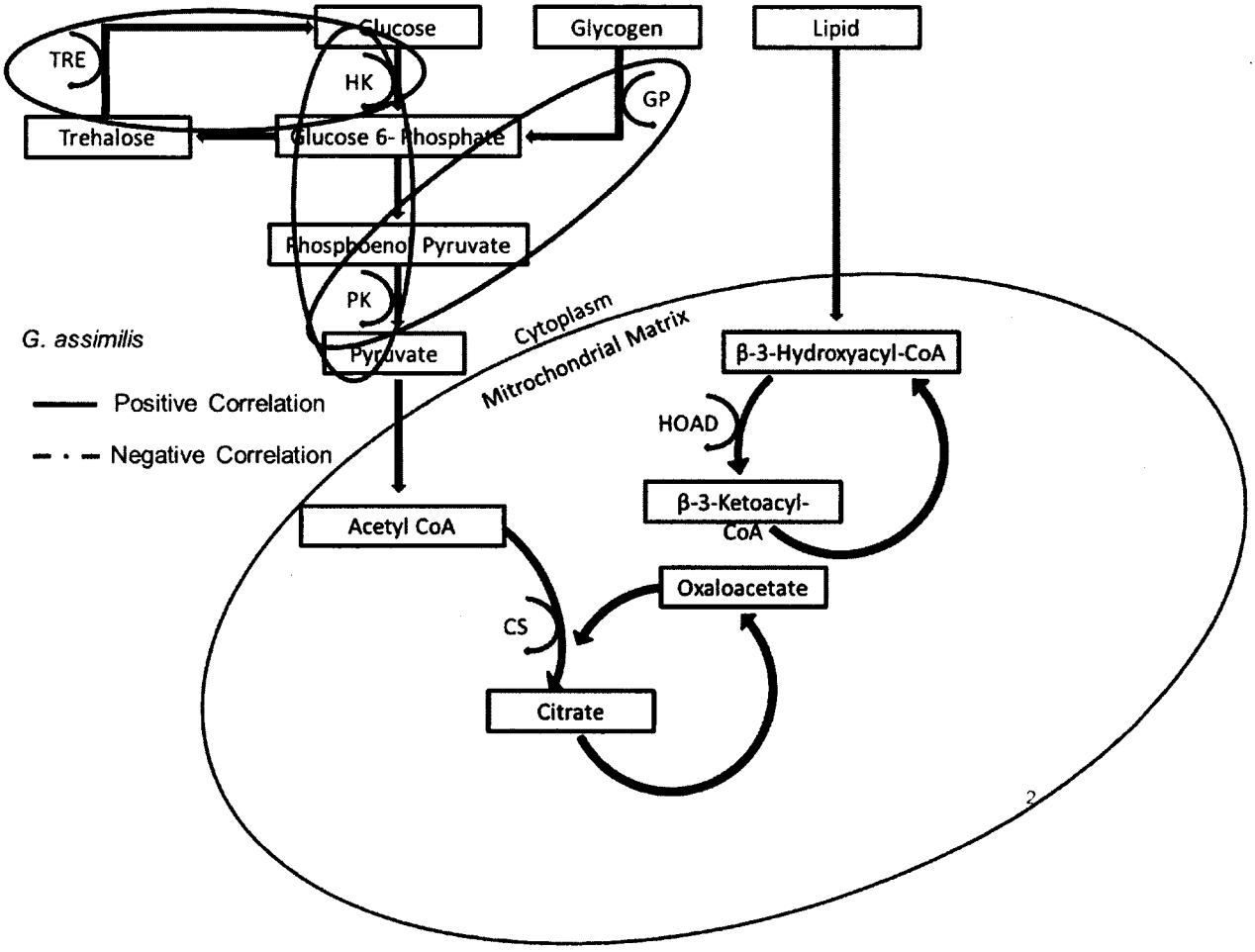

Figure 7: Correlations among enzyme capacities (V_{max}) in *G. assimilis*. Ovals joining two enzymes represent positive or negative correlations between those two enzymes.

In *G. texensis*, crickets that exhibited high activities of HK also had high activities of TRE and GP (Figure 8). However, there was a negative correlation between HK and PK, which is the opposite of the result obtained in *G. assimilis* (Figure 7 and 8). Also, crickets with high activities of TRE had high activities of GP. Additionally, there was a negative correlation between activities of TRE and HOAD, a mitochondrial enzyme (Table 14).

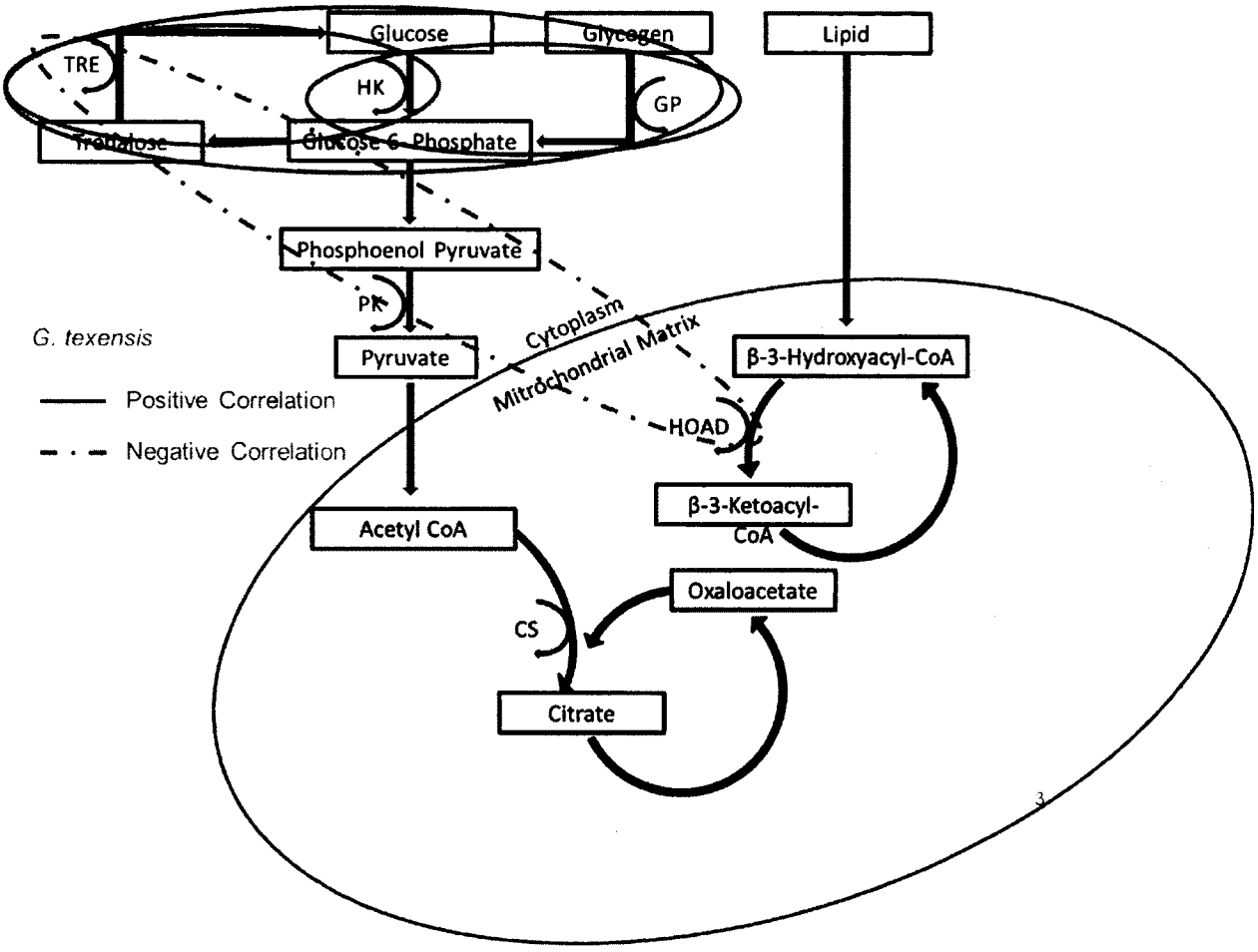

Figure 8: Correlations among enzyme capacities (V_{max}) in *G. texensis*. Ovals joining two enzymes represent positive or negative correlations between those two enzymes.

Muscle enzymes vs. body content

In *G. assimilis*, there were very few significant correlations between enzyme activities and body content, but these correlations suggest that carbohydrate may play an important role in cricket metabolism. There were positive correlations between abdominal glycogen content and the activities of PK and GP (Table 13), as well as a correlation between abdominal carbohydrate content and GP (Table 13).

These relationships were not present in *G. texensis*. However, thoracic carbohydrate content was correlated with TRE activity (Table 14), which further supports the importance of carbohydrate in cricket metabolism.

Muscle enzymes and body content vs. signalling behaviour

My univariate analysis revealed no significant relationships between signalling effort (Effort PC1) and any morphometric measures, enzyme activity measures, or body composition measures in either species with the exception that smaller *G. assimilis* males had lower signalling efforts (Tables 13 and 14, Figure 9).

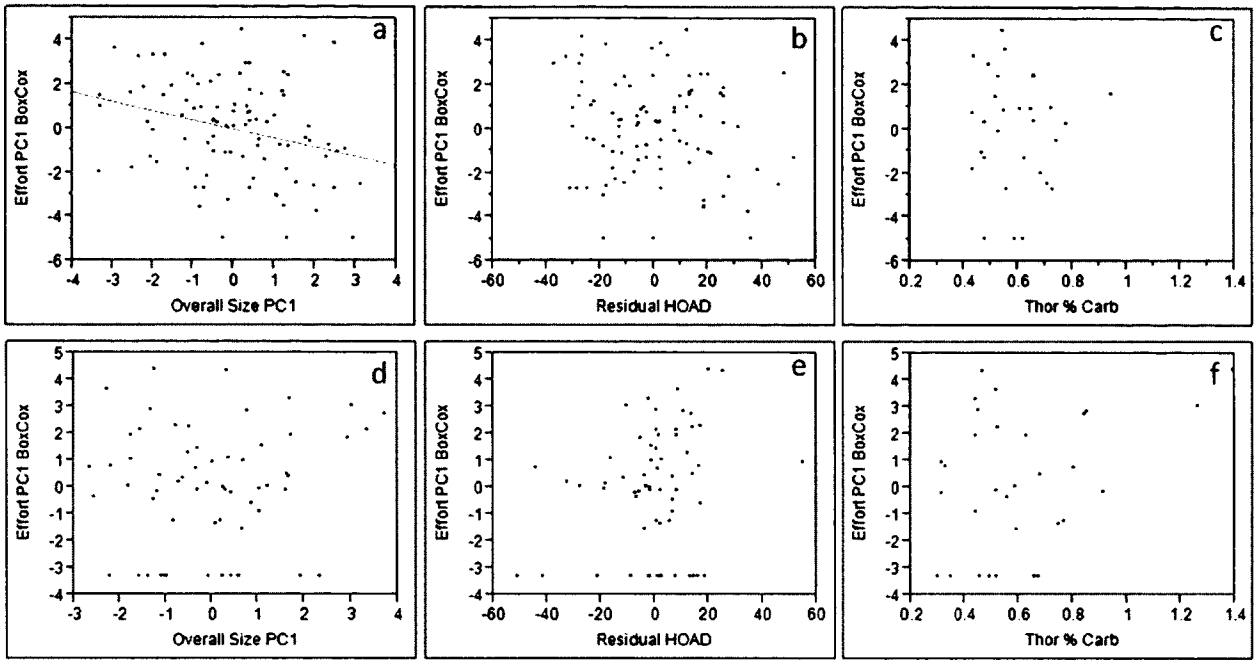

Figure 9: Univariate regressions of Size PC1 (a,d), HOAD activity (b,e), and thoracic carbohydrate % (c,f) on signalling Effort PC1 BoxCox for *G. assimilis* (a,b,c) and *G. texensis* (d,e,f). Significant relationships include a line of best fit. P-values are included in Tables 11 and 12.

My univariate analysis revealed that signalling quality (Quality PC1) was significantly correlated to GP activity in *G. assimilis* and TRE activity in *G. texensis*. I found no other significant relationships with Quality PC1 (Tables 13 and 14, Figure 10). Signalling quality (Quality PC2) was, however, significantly negatively correlated TRE activity in *G. texensis*. I found no other significant relationships with Quality PC2 (Tables 13 and 14, Figure 11).

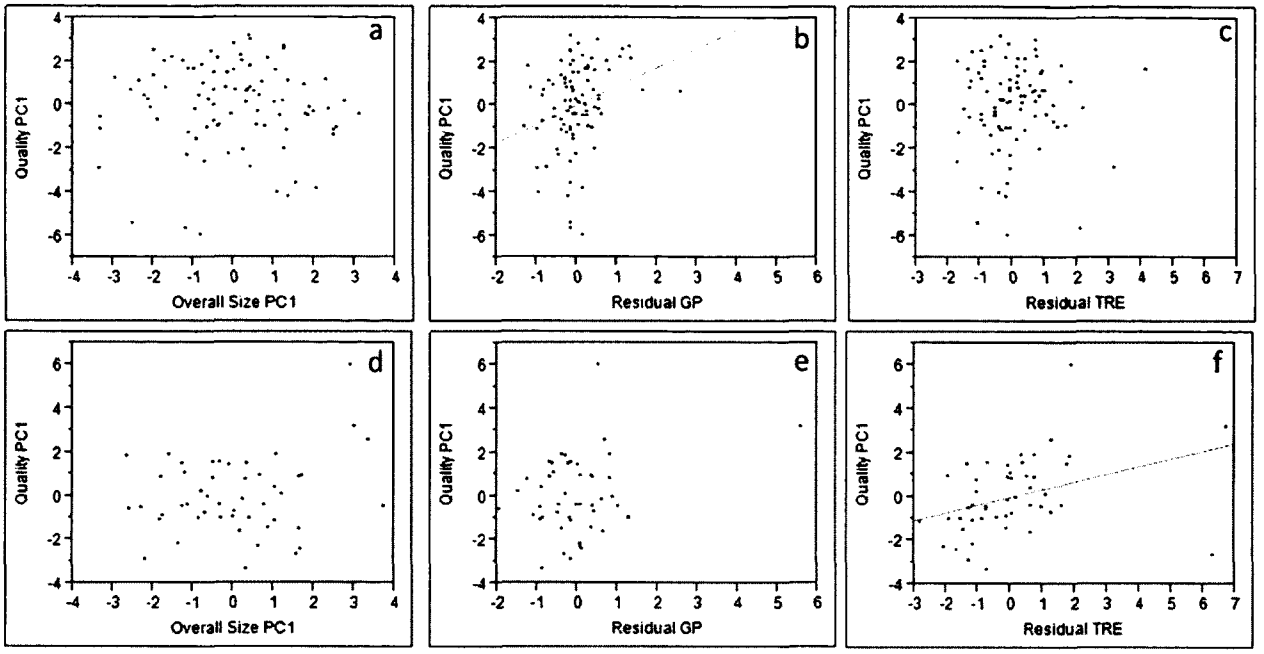

Figure 10: Univariate regressions of Size PC1 (a,d), GP activity (b,e) and TRE activity (c,f) on signalling Quality PC1 for *G. assimilis* (a,b,c) and *G. texensis* (d,e,f). Significant relationships include a line of best fit. P-values are included in Tables 11 and 12.

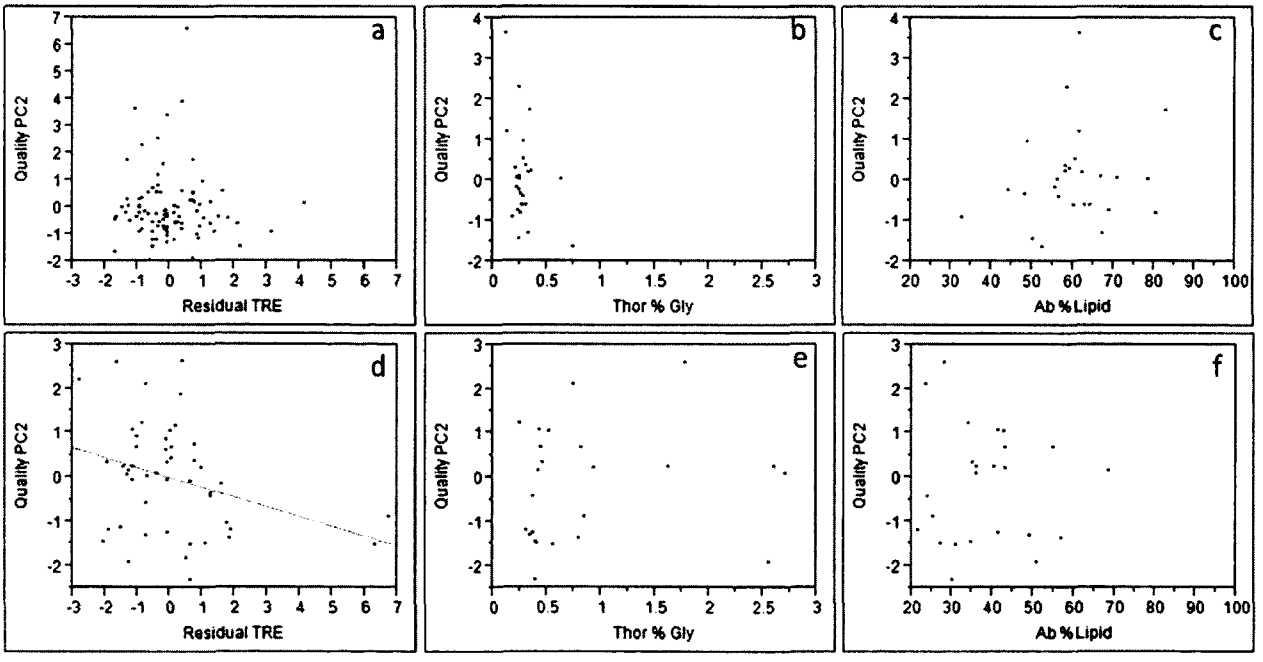

Figure 11: Univariate regressions of TRE activity (a,d), thoracic glycogen % (b,e), and abdominal lipid % (c,f) on signalling Quality PC2 for *G. assimilis* (a,b,c) and *G. texensis* (d,e,f). Significant relationships include a line of best fit. P-values are included in Tables 11 and 12.

Table 13: Matrix of Pearson correlations between enzyme activities, body content, signalling behaviour, and morphology for *G. assimilis*. Each X-Y pair represents a single regression test. P-values are displayed above the diagonal line, and r^2 adjusted values are displayed below the line. Significant p-values are highlighted, and negative relationships are displayed in **bold**.

	Signalling Variables			Enzyme Activities						Body Composition								Size
	Effort PC1	Attr. PC1	Attr. PC2	PK Act	GP Act	CS Act	HOAD Act	TRE Act	HK Act	Thor Pro	Thor Carb	Thor Gly	Thor Lip	Ab Pro	Ab Carb	Ab Gly	Ab Lip	Size PC1
Signalling Variables																		
Effort PC1		**<0.001**	**<0.001**	0.337	0.566	0.508	0.126	0.697	0.809	0.262	0.861	0.186	0.849	0.933	0.668	0.812	0.119	**0.006**
Attr. PC1	**0.606**		1.000	0.271	**0.009**	0.479	0.631	0.981	0.961	0.274	0.528	0.716	0.910	0.450	0.178	0.421	0.960	0.810
Attr. PC2	**0.512**	0.000		0.283	0.157	0.417	0.109	0.682	0.848	0.784	0.784	0.076	0.751	0.772	0.867	0.804	0.371	0.570
Enzyme Activities																		
PK Act	-0.101	0.117	-0.114		**0.001**	0.929	0.915	0.792	**<0.001**	0.254	0.601	0.714	0.661	0.715	0.152	0.045	**<0.001**	0.186
GP Act	-0.060	**0.273**	-0.150	**0.349**		0.203	0.932	0.122	0.033	0.066	0.460	0.148	0.308	0.597	0.032	**0.001**	0.613	0.014
CS Act	-0.070	0.076	0.087	0.009	0.133		0.398	0.246	0.728	0.755	0.953	0.501	0.733	0.287	0.563	0.757	0.278	0.289
HOAD Act	-0.160	-0.051	-0.170	-0.011	-0.009	0.089		0.798	0.660	0.561	0.814	0.953	0.232	0.075	0.509	0.641	0.725	0.702
TRE Act	0.041	0.002	-0.044	0.028	0.162	0.121	-0.027		**<0.001**	0.130	0.478	0.431	0.503	0.351	0.262	0.171	0.035	0.390
HK Act	0.025	-0.005	-0.021	**0.383**	0.221	0.037	0.046	**0.543**		0.415	0.386	0.831	0.731	0.522	0.502	0.275	**0.004**	0.333
Body Composition																		
Thor % Pro	0.212	0.218	-0.055	-0.215	-0.340	0.059	0.110	0.283	0.155		0.092	0.164	0.524	0.216	0.835	0.356	0.253	0.499
Thor % Carb	-0.033	-0.127	-0.055	0.100	-0.140	0.011	0.045	0.135	0.164	-0.313		0.551	0.518	0.373	0.369	0.527	0.637	0.857
Thor % Gly	-0.248	0.073	-0.347	0.070	0.271	0.128	0.011	-0.149	0.041	-0.261	0.113		0.100	0.256	0.405	0.435	0.634	0.517
Thor % Lip	-0.036	0.023	-0.064	-0.084	-0.193	0.065	-0.225	-0.127	-0.065	0.121	0.123	0.306		0.714	0.127	0.064	0.306	0.571
Ab % Pro	0.016	0.152	0.059	0.069	-0.101	-0.201	-0.330	-0.176	-0.122	-0.233	0.169	0.214	0.070		0.937	0.885	0.737	0.263
Ab % Carb	0.082	0.267	-0.034	0.268	0.393	0.110	0.126	0.212	0.128	0.040	0.170	0.158	-0.285	-0.015		**<0.001**	0.244	0.658
Ab % Gly	0.045	0.162	-0.050	0.369	**0.596**	0.059	0.089	0.257	0.206	-0.175	0.120	0.148	-0.342	-0.028	**0.790**		0.880	0.837
Ab % Lip	0.291	-0.010	0.179	**-0.618**	-0.096	-0.205	0.067	-0.387	**-0.511**	-0.215	-0.090	-0.090	-0.193	0.064	-0.219	-0.029		0.099
Size																		
Size PC1	**-0.285**	-0.026	-0.061	0.138	0.254	0.111	0.040	0.090	0.102	-0.128	-0.034	0.123	-0.108	-0.211	-0.084	-0.039	-0.307	

Table 14: Matrix of Pearson correlations between enzyme activities, body content, signalling behaviour, and morphology for *G. texensis*. Table is split over two pages. P-values are displayed above the diagonal line, and r^2 adjusted values are displayed below the line. Significant p-values are highlighted, and negative relationships are displayed in **bold**.

	Signalling Variables			Enzyme Activities						Body Composition								Size
	Effort PC1	Attr. PC1	Attr. PC2	PK Act	GP Act	CS Act	HOAD Act	TRE Act	HK Act	Thor Pro	Thor Carb	Thor Gly	Thor Lip	Ab Pro	Ab Carb	Ab Gly	Ab Lip	Size PC1
Signalling Variables																		
Effort PC1		0.050	**0.008**	0.877	0.784	0.859	0.071	0.674	0.361	0.821	0.062	0.110	0.169	0.748	0.271	0.396	0.347	0.436
Attr. PC1	0.284		0.999	0.210	0.064	0.706	0.275	0.012	0.032	0.917	0.033	0.037	0.662	0.807	0.089	0.020	0.717	0.127
Attr. PC2	**0.379**	0.000		0.360	0.827	0.184	0.330	0.025	0.276	0.957	0.538	0.564	0.491	0.314	0.814	0.744	0.836	0.625
Enzyme Activities																		
PK Act	-0.020	-0.184	0.135		0.009	0.271	0.847	0.134	**0.002**	0.683	0.916	0.742	0.957	0.231	0.716	0.650	0.162	0.338
GP Act	0.036	0.269	-0.032	-0.329		0.051	0.917	**<0.001**	**<0.001**	0.271	0.021	0.510	0.830	0.251	0.146	0.522	0.136	**0.006**
CS Act	0.023	0.056	0.195	0.142	-0.249		0.373	**0.001**	0.392	0.145	0.915	0.613	0.592	0.340	0.882	0.954	0.130	0.172
HOAD Act	0.231	-0.161	0.144	0.025	-0.013	0.115		**0.003**	0.920	0.173	0.490	0.085	0.073	0.476	0.394	0.013	0.766	0.503
TRE Act	0.055	0.360	-0.323	-0.193	**0.479**	**-0.412**	**-0.368**		**0.001**	0.899	**0.002**	0.870	0.762	0.791	0.199	0.563	0.686	0.052
HK Act	0.118	0.310	0.161	**-0.387**	**0.573**	-0.111	0.013	**0.414**		0.925	0.342	0.643	0.986	0.352	0.868	0.840	0.972	0.035
Body Composition																		
Thor % Pro	0.042	0.023	0.012	-0.076	-0.204	0.268	-0.251	0.024	0.018		0.821	0.222	0.887	0.071	0.077	0.605	0.038	0.233
Thor % Carb	0.339	0.437	0.132	-0.020	0.413	-0.020	-0.129	**0.541**	0.177	0.042		0.510	0.400	0.083	0.362	0.068	0.741	0.315
Thor % Gly	0.293	-0.428	0.124	0.062	0.123	-0.094	0.315	-0.031	0.087	0.226	-0.123		0.395	0.820	**0.003**	**<0.001**	0.766	0.624
Thor % Lip	0.253	-0.094	-0.148	0.010	-0.040	0.100	0.326	-0.057	0.003	-0.027	-0.157	0.158		0.602	0.210	0.358	0.110	0.367
Ab % Pro	0.060	0.053	-0.215	-0.221	-0.213	0.177	-0.133	-0.050	0.173	0.329	-0.316	-0.043	0.097		0.288	0.590	**0.004**	0.499
Ab % Carb	-0.204	0.355	0.051	0.068	0.267	0.028	-0.159	0.237	0.031	-0.323	0.169	**-0.518**	-0.231	-0.197		0.126	0.013	**0.003**
Ab % Gly	0.158	-0.473	0.070	0.085	0.120	-0.011	0.442	-0.108	0.038	-0.097	-0.332	**0.805**	0.171	-0.101	-0.281		0.867	0.892
Ab % Lip	0.175	0.078	-0.045	0.257	-0.274	0.278	-0.056	-0.076	-0.007	0.374	-0.062	0.056	0.293	**0.503**	-0.440	0.031		**<0.001**
Size																		
Size PC1	0.101	0.224	0.072	-0.124	**0.343**	-0.176	0.087	0.248	0.269	-0.221	0.187	0.092	-0.168	-0.126	**0.512**	0.025	**-0.649**	

Multivariate Relationships with Signalling Effort and Quality

Since I found significant correlations across several traits, I built multivariate models to explore the effects of enzyme activities, body composition, and size on signalling effort and quality.

Factors affecting signalling variation: G. assimilis

My multivariate model exploring the different factors influencing signalling in *G. assimilis* revealed that signalling effort is significantly influenced by body size (Size PC1). Males with larger pronotums (length, width, and area trace), larger head capsule widths, and larger harp areas and file lengths produced more pulses, chirps, and had greater daily average time spent signalling (Effort PC1, Table 15). Signalling effort was not influenced by enzyme activity or body content in *G. assimilis.* However, some of the variation in signal quality variation could be explained by glycogen phyosphorylase (GP) activity. Males with high activities of GP had longer pulse and chirp durations, more pulses per chirp, lower dominant frequencies, and louder amplitudes (Quality PC1, Table 15). There was also a trend for males with higher glycogen concentrations in their thoraxes to have longer chirp durations and shorter interchrip durations, but also longer interpulse durations (Quality PC2, Table 15).

Factors affecting signalling variation: G. texensis

My multivariate model exploring the factors influencing signalling in *G. texensis* revealed that signalling effort is significantly influenced by body size (Size PC1) and thoracic carbohydrate levels. Larger and heavier males with high percentages of carbohydrates in their thoraxes produced a greater number of pulses, interpulses, chirps, interchirps, and had greater daily average time spent signalling (Effort PC1, Table16). There was also a trend for male *G. texensis* with high HOAD activities to have higher signalling Effort PC1. Some of the variation in signal quality was also explained by TRE activity. Males with low TRE activity signalled with

shorter pulse durations and higher dominant frequencies (Quality PC2). There were non-significant trends for large and heavy males with high HK activities and low abdominal protein and lipid contents to signal with shorter pulse durations and higher dominant frequencies (Quality PC2, Table 16). There was also a non-significant trend for males with high activities of TRE to have more pulses per chirp, longer chirp durations, and signals with higher amplitudes (Quality PC1, Table 16).

Table 15: Multivariate regression model for factors influencing male signalling effort and quality in *G. assimilis*. Significant values (*) and strong trends are displayed in **bold**. Negative significant relationships are marked with a (-).

Behaviour	Model Parameter	Wald χ^2	df	p-value
Effort PC1	PK Activity	0.883	1	0.347
vs.	GP Activity	0.071	1	0.791
Size and Enzymes	CS Activity	0.112	1	0.738
***N*=93**	HOAD Activity	2.336	1	0.126
	TRE Activity	0.019	1	0.890
	HK Activity	0.454	1	0.500
	Size PC1	**7.493**	**1**	**0.006***
Effort PC1	Thor Protein	0.061	1	0.805
vs.	Thor Carb	0.510	1	0.475
Size and Body Content	Thor Glycogen	1.487	1	0.223
***N*=30**	Thor Lipid	0.520	1	0.471
	Abdo Protein	0.277	1	0.598
	Abdo Carb	0.952	1	0.329
	Abdo Glycogen	0.043	1	0.836
	Abdo Lipid	1.453	1	0.228
	Size PC1	0.021	1	0.886
Quality PC1	PK Activity	0.281	1	0.596
vs.	**GP Activity**	**6.490**	**1**	**0.011***
Size and Enzymes	CS Activity	0.321	1	0.571
***N*=93**	HOAD Activity	0.157	1	0.692
	TRE Activity	0.009	1	0.926
	HK Activity	0.251	1	0.616
	Size PC1	0.797	1	0.372
Quality PC1	Thor Protein	1.341	1	0.247
vs.	Thor Carb	0.862	1	0.353
Size and Body Content	Thor Glycogen	0.000	1	0.984
***N*=30**	Thor Lipid	0.724	1	0.395
	Abdo Protein	2.509	1	0.113
	Abdo Carb	1.711	1	0.191
	Abdo Glycogen	0.000	1	0.998
	Abdo Lipid	0.948	1	0.330
	Size PC1	1.438	1	0.230
Quality PC2	PK Activity	0.784	1	0.376
vs.	GP Activity	1.465	1	0.226
Size and Enzymes	CS Activity	1.489	1	0.222
***N*=93**	HOAD Activity	3.352	1	0.067
	TRE Activity	0.483	1	0.487
	HK Activity	0.497	1	0.481
	Size PC1	0.037	1	0.847
Quality PC2	Thor Protein	0.810	1	0.368
vs.	Thor Carb	0.105	1	0.746
Size and Body Content	**Thor Glycogen**	**3.932**	**1**	**0.047**
***N*=30**	Thor Lipid	0.266	1	0.606
	Abdo Protein	0.068	1	0.795
	Abdo Carb	0.405	1	0.524
	Abdo Glycogen	0.316	1	0.574
	Abdo Lipid	0.384	1	0.536
	Size PC1	0.775	1	0.379

Table 16: Multivariate regression model for factors influencing male signalling effort and quality in *G. texensis*. Significant values (*) and strong trends are displayed in **bold**. Negative significant relationships are marked with a (-).

Behaviour	Model Parameter	Wald χ^2	df	p-value
Effort PC1	PK Activity	0.004	1	0.949
vs.	GP Activity	0.393	1	0.531
Size and Enzymes	CS Activity	0.185	1	0.667
***N*=93**	**HOAD Activity**	**4.396**	**1**	**0.036**
	TRE Activity	1.191	1	0.275
	HK Activity	0.342	1	0.558
	Size PC1	0.157	1	0.692
Effort PC1	Thor Protein	0.060	1	0.806
vs.	**Thor Carb**	**5.602**	**1**	**0.018***
Size and Body Content	Thor Glycogen	0.253	1	0.615
***N*=30**	Thor Lipid	1.896	1	0.169
	Abdo Protein	0.000	1	0.987
	Abdo Carb	3.256	1	0.071
	Abdo Glycogen	0.665	1	0.415
	Abdo Lipid	2.703	1	0.100
	Size PC1	**6.911**	**1**	**0.009***
Quality PC1	PK Activity	0.418	1	0.518
vs.	GP Activity	0.006	1	0.941
Size and Enzymes	CS Activity	3.164	1	0.075
***N*=93**	HOAD Activity	1.116	1	0.291
	TRE Activity	**4.233**	**1**	**0.040**
	HK Activity	1.227	1	0.268
	Size PC1	1.295	1	0.255
Quality PC1	Thor Protein	0.121	1	0.728
vs.	Thor Carb	1.863	1	0.172
Size and Body Content	Thor Glycogen	0.353	1	0.552
***N*=30**	Thor Lipid	0.009	1	0.923
	Abdo Protein	0.046	1	0.830
	Abdo Carb	0.074	1	0.785
	Abdo Glycogen	0.011	1	0.917
	Abdo Lipid	1.138	1	0.286
	Size PC1	0.775	1	0.379
Quality PC2	PK Activity	2.367	1	0.124
vs.	GP Activity	0.022	1	0.883
Size and Enzymes	CS Activity	0.388	1	0.533
***N*=93**	HOAD Activity	0.009	1	0.922
	TRE Activity (-)	**6.579**	**1**	**0.010***
	HK Activity	**4.142**	**1**	**0.042**
	Size PC1	0.719	1	0.396
Quality PC2	Thor Protein	0.729	1	0.393
vs.	Thor Carb	0.100	1	0.751
Size and Body Content	Thor Glycogen	0.809	1	0.368
***N*=30**	Thor Lipid	0.906	1	0.341
	Abdo Protein (-)	**4.307**	**1**	**0.038**
	Abdo Carb	0.990	1	0.320
	Abdo Glycogen	0.491	1	0.483
	Abdo Lipid (-)	**6.399**	**1**	**0.011***
	Size PC1	**10.122**	**1**	**0.001***

Discussion

Many studies have attempted to explain variation in male cricket acoustic signalling. Studies explore the effect of male condition (Bertram 2000), environmental effects (temperature, time, and date: Bertram & Bellani 2002; Bertram 2002), phenotypic plasticity (Souroukis et al. 1992; Bertram 2002), hind-wing morphology (Souroukis et al. 1992) and muscle and thoracic enzymes (Bertram et al. 2011b). In my thesis I built on these studies by producing a comprehensive exploration of the physiological and biochemical traits correlated with signalling on multiple levels of organization (muscular enzymes and compartmentalized fuel stores). I examined and quantified the variation in signalling behaviours, morphology, and physiology/biochemistry in two species of field crickets: *G. assimilis* and *G. texensis*. I then explored the relationships between signalling behaviour (effort and quality) and multiple measures of condition: muscle enzyme activities, whole body energy stores, and morphology.

Variation in Traits

I observed a large amount of phenotypic variation in both species with respect to signalling effort and quality, morphology, enzyme activities, and body composition. The signalling behaviour of *G. texensis* was much more variable than the signalling behaviour of *G. assimilis*. For example, some *G. texensis* individuals did not call at all during a given 24 hour period, whereas others produced upwards of two million pulses in the same period (which can be compared to two hundred thousand pulses per day for *G. assimilis*). In *G. texensis*, since pulses per chirp and interchirp duration are both linked to chirp duration, it was not surprising that these three signalling components were all highly variable (Table 4). My findings support many previous studies that report that there is variation in signalling behaviours, in particular the

amount of time spent signalling throughout the night (Cade 1981; Kolluru 1999; Bertram et al. 2005) and the number of pulses per chirp (Wagner et al. 1995; Gray & Cade 1999).

I found that many of the signalling parameters associated with signalling effort were skewed toward the left, revealing that most crickets signalled with lower effort. This frequency distribution pattern matches that found in most other cricket species (Cade 1991; Kolluru 1999; Bertram 2000; Bertram 2002; Bertram & Warren 2005; Bertram et al. 2007). Since females select males that signal with high effort, these skewed distributions imply that there is some other opposing mechanism maintaining low signalling efforts. Predators or parasitoids (such as the acoustically locating parasitoid fly, *Ormia ochracea*) may be selecting for low signalling effort (Gray & Cade 1999). In fact, Gray and Cade (1999) suggest that the balance between selection from females and predators may maintain genetic variation in male calling song. However, this hypothesis would only explain these signalling patterns in natural hosts of *O. ochracea*, such as *G. texensis* (Cade 1975; Cade & Otte 2000), and not in cricket species that have no natural risk of parasitization, such as *G. assimilis* and *A. domesticus* (Thomson et al. 2012). It remains possible, however, that other predators could be imposing selective pressures against calling.

Another possible explanation for the skewed distributions is the high energetic costs of signalling. Many males may simply be incapable of producing such highly energetic signals due insufficient fuel stores and/or insufficient enzyme capacities.

Signalling behaviours have been found to be influenced by temperature (Alexander & Walker 1962; Souroukis et al. 1992), time of night (Souroukis et al 1992), and age (Cade & Wyatt 1984; Zuk 1987; Bertram 2000; Fitzsimmons & Bertram 2011), but I controlled for all of these effects in my thesis. Some of these signalling traits have not only been shown to be phenotypically variable, but genetically variable as well (Hedrick 1988, David et al. 2000; Hunt

et al. 2007). Hedrick (1988) found there to be large amounts of variation in *G. integer* calling bout length, which was subsequently found to be a heritable trait (Hedrick et al. 1998).

I also observed variation in all muscle enzyme activities. Bertram et al. (2011b) found activities of GP to be undetectable in *A. domesticus*, a significantly smaller cricket species than both *G. assimilis* and *G. texensis*. I was able to detect GP activity in both *G. assimilis* and *G. texensis*, although activities were relatively low compared to other enzymes, in particular PK and HOAD. Low V_{max} values indicate that there are fewer enzymes or lower amounts of the required substrate. Low GP activities likely contribute to the relatively high coefficients of variation for this enzyme (Tables 7 and 8) compared to the other enzymes studied.

Variation in body composition was consistently high for all four components in both the thoraxes and abdomens of both species. In particular, glycogen percentages in the thorax and abdomen were the most variable. This is likely due to the nature of glycogen storage in muscles of the fat body where glycogen is a molecule that is in constant flux since it is readily used as glycolytic fuel (Steele 1982). Interestingly, it seems that both the capacity to use glycogen (GP activity) and the amount of glycogen present in the body (thoracic and abdominal glycogen %) are the most variable aspects of the biochemistry of these two species. Estimates of body composition in both *G. assimilis* and *G. texensis* were very similar to previous measurements by Lorenz and Anand (2004) on 16 day old *Gryllus bimaculatus* where the following body contents (in % wet weight of the fat body) were found: 5.5% protein, 0.2% carbohydrate, 0.8% glycogen, and 25.0% lipid.

Relationship Between Signalling Behaviours and Morphology

Body morphology can play a large role in the biomechanics of signalling, and therefore in signalling quality as a whole (Simmons & Zuk 1992; Simmons & Ritchie 1996; Bennet-Clark

1998; Moradian & Walker 2008). Morphometrics correlate with signalling behaviour variation in *G. texensis*, but there are conflicting results in *G. assimilis*. For *G. assimilis*, smaller males signalled with greater effort (Effort PC1), which is the opposite result from that of Bertram and Rook (2012) who found that larger *G. assimilis* called more often. For *G. texensis*, larger males signalled with greater effort (Effort PC1). With respect to the relationship between body size and signalling behaviours, many studies present differing results. Souroukis (1992) did not find any relationship between cricket weight and song structure in *G. integer* (now *G. texensis*), and Zuk (1987) found no correlation between body and pronotum length and signalling frequency or attractiveness to females in *G. veletis* and *G. pennsylvanicus.* However, Simmons (1988) found that body size was correlated with increased pulse rates and signal amplitudes in *G. bimaculatus.* Bertram et al. (2011a) found that signalling traits were dependent on body size in *A. domesticus* and *G. veletis*, but not in *G. assimilis* or *G. texensis*. All of these studies have large sample sizes, and even though they measure signalling behaviours differently, they all measure signalling efforts in ways that give some indication of the costs of signalling. Some of these differences could instead be attributed to differences in morphological measures, where researchers measured either weight, size of various body parts, principle components of size, or principle components of size and weight (such as in my methods). However, all these morphological measures are typically correlated, so it is not likely that this would change the direction of the relationships. Further investigation is needed to determine the cause of these conflicting results.

I also found a significant negative relationship between harp area and signal dominant frequency (carrier frequency) for *G. texensis* ($F_{1,63} = 6.525$, P = 0.0140, $R^2_{adj} = 0.105$) although there was no significant relationship observed for *G. assimilis*. This relationship was unsurprising since mechanically, larger harps should produce lower frequency sounds, as has

already been found in tree crickets (Prestwich et al. 2000) and field crickets (Scheuber et al. 2003).

I did not find that any traditional measure of male condition (residuals of mass and size) was correlated with signalling effort. These findings are consistent with other studies using *G. assimilis* (Bertram 2000; Bertram et al. 2011b; Bertram & Rook 2011). This may indicate that this traditional measure of condition is not adequate to account for variation in signalling behaviours (Green 2001) since size difference between individuals may not actually be attributed to energy stores, but instead could be attributed to individual differences in body shape or even hydration levels (Tomkins et al. 2004).

Relationship between Signalling Behaviours and Physiology/Biochemistry

The ability to mobilize and/or metabolize carbohydrates in *G. assimilis*, and carbohydrates and lipids in *G. texensis*, may drive some of the variation in signaling effort. In *G. assimilis*, carbohydrates are important in explaining variation in signalling effort and quality (Table 15). Males that produced calls with higher effort (Effort PC1) and more attractive fine scale components (Quality PC1 and PC2) had increased GP activity and had increased thoracic glycogen contents. High-effort and high-quality *G. assimilis* signallers did not significantly differ from low-effort and low-quality signallers with respect to PK, CS, HOAD, TRE, and HK activities or thoracic or abdominal protein, carbohydrate, or lipid contents. Therefore, when measuring condition to account for variation in signalling effort and quality, I recommend future studies take into account the ability of *G. assimilis* males to accumulate thoracic glycogen stores and their capacity to break down glycogen in their muscles.

In *G. texensis*, the availability of both carbohydrates and lipids may be important in explaining variation in signalling (Table 16). Males that signal with higher effort (Effort PC1)

had significantly increased thoracic carbohydrate contents and a non-significant trend for increased HOAD activities. There was also a non-significant trend for males with attractive signals (Quality PC1) to have increased TRE activities. Additionally, males with unattractive signals (Quality PC2) had significantly lower activities of TRE and lower levels of abdominal lipids. High-effort and high-quality *G. texensis* signallers did not significantly differ from low-effort and low-quality signallers with respect to PK, GP, CS, or HK activities, thoracic protein, glycogen, or lipid contents, or abdominal carbohydrate or glycogen contents. Therefore, when measuring condition to account for variation in signalling effort and quality, I recommend future studies take into account the ability of *G. texensis* males to accumulate abdominal lipid stores, accumulate circulating thoracic carbohydrates, and their capacity to break down both lipids and carbohydrates in their muscles.

My finding that carbohydrates may be an important fuel source for signalling in field crickets is supported by previous work on *Teleogryllus commodus* (Maklakov et al. 2008) and on *A. domesticus* (Bertram et al. 2011b). Maklakov et al. (2008) manipulated diet in adult crickets and found that males fed carbohydrate rich diets (with a 5:1 carbohydrate to protein ratio) signalled with significantly more effort than crickets fed diets with moderate (1:1) or low (1:5) carbohydrate levels. Bertram et al. (2011b) found that crickets with high activities of PK in their thoraxes also had high signalling efforts (daily average time spent calling). These results, along with my own, suggest that carbohydrate availability and carbohydrate metabolic capacity during adulthood may be proximately linked to high-effort signalling in multiple cricket species. One conflicting study, however, indicates that crickets fed on high protein to carbohydrate diets signal with the greatest effort (Hunt et al. 2004). However, Hunt et al. (2004) raised *T. commodus* on these diets as juveniles, and it is possible that protein availability during growth and development is necessary to grow to a large size (body size is fixed at adulthood). Large size

has been shown to be linked with high calling efforts in some species (Simmons 1988; Bertram & Rook 2011; Bertram et al. 2011a). While the proximate relationship between carbohydrates and signalling effort is slowly unfolding, there have not been any studies, that I am aware of, that link lipids to signalling effort. Lipid are, however, known to fuel other highly energetic behaviours in insects, namely flight.

Insect muscles can use carbohydrates, lipids, or both carbohydrates and lipids to fuel behaviours (Zebe & McShan 1957, Beenakers 1969). Highly energetic behaviours, such as long duration flying in locusts, are initially fueled by circulating carbohydrates (Beenakers 1963, Beenakers 1969). For example, in *L. migratoria*, trehalose is the major fuel for flight in the first 20-30 minutes of flight and decreases to a steady state of around 35% of resting levels thereafter (Beenakkers et al. 1984). From then on, trehalose supplies about 25% of the total energy, with the remainder being supplied primarily by lipids (van Antwerpen et al. 1988). My result that signalling in *G. assimilis* (a moderately energetic behaviour) is fueled primarily by carbohydrates and signalling in *G. texensis* (a more highly energetic behaviour my comparison) is fueled by both carbohydrates and lipids is similar to fuel use patterns in flight-capably insects. The trade-off observed in *G. texensis* (Figure 8) between enzyme activities of TRE (a glycolytic enzyme) and HOAD (a mitrochondrial enzyme) could indicate that there may be two different energetic phenotypes in this species: low-effort would favour using carbohydrates and high-effort signallers would favour using lipids. When comparing high-effort to non-signallers with respect to enzyme activities for *G. texensis*, the top 25% of crickets with respect to daily time spent calling (high-effort signallers) had significantly higher activities of HOAD than the bottom 25% (non-signallers) (ANOVA: $F_{1,38}=4.598$, $R^2_{adj}=0.089$, $p=0.0388$) and there was no difference with respect to TRE activities (ANOVA: $F_{1,38}=0.085$, $R^2_{adj}=-0.025$, $p=0.772$). This supports my argument that high-effort signallers use a combination of lipids and carbohydrates, while non-

signallers use only carbohydrates. The relationship is more unclear with the remaining 50% of crickets that have intermediate signalling effort. It is also possible that since trehalose is synthesized in the fat body from existing lipid stores (Arrese & Soulages 2010) that high-effort *G. texensis* signallers are fuelling their muscles using only carbohydrates and the glycolytic pathway, and are instead using their high lipid stores to metabolise more trehalose. This, however, would not explain the high activity of HOAD in these individuals.

The amount and quality of food available to crickets can also influence calling effort. Judge et al. (2008) manipulated diet and found that *G. pennsylvanicus* fed on high or medium quality diets invested more in signalling effort than crickets fed on low quality diets. Holzer et al. (2003) manipulated diets in *G. campestris* in the wild and found that males receiving more food called more and attracted more females. Crnokrak & Roff (1998) food limited both long-winged and short-winged morphs of *G. firmus* and found that food limited crickets of both morphs signalled less. Wagner & Hoback (1999) also found that *G. lineaticeps* fed low-nutrition diets signalled less, but there was no significant decrease in weight compared to crickets on high-nutrition diets. This has interesting implications with regards to using the residuals of mass vs. size as a measure of condition. Low-nutrition diets would result in crickets of lower condition and quality, but measurements of condition would not find significant differences when comparing crickets fed on low- and high-nutrition diets since their weights would be the same.

Since all my crickets were raised on the same *ad libitum*, high-quality diet, all crickets should have been in relatively high condition. This would result in there being little variation in condition, which could mask some of the effects of physiology and biochemistry on signalling effort or quality. This could explain why there were no significant correlations between male condition (residuals of mass vs. size) and any signalling parameter. In the future, one could

manipulate diet amounts or compositions to yield study crickets with a wide range of energy stores (lipid or carbohydrate).

Other external factors, such as the hormonal triggers underlying the production of behaviours, also need to be formally quantified in this system. Octopamine is used by some skeletal muscle systems to modulate the effects of many neurotransmitters. Octopamine is released during activity, and increases power output and leads to the release of adipokinetic hormone (Candy 1978). Adipokenetic hormone mobilizes stored lipids from insect fat bodies to be used as fuel muscles (Klowden 2007). In locusts, there are both decapeptide and octapeptide adipokenetic hormones produced. Both of these hormones stimulate the release of diacylglycerols (lipids) from the fat body and are also responsible for the re-organization of already circulating lipids in the haemolymph resulting in the provision of fuel to power behaviours (Goldsworthy & Mordue 1989). Since hormones such as octopamine can have such a dramatic effect on the fuel stores and fuel use of insects, future work should investigate hormones as a measure of condition.

The physiology of many species, including orthopterans, can be influenced by their environment. Grasshoppers (*Melanoplus femurrubrum*) that are stressed from being exposed to spider predators have body compositions with increased amounts of carbohydrate relative to nitrogen (Hawlena et al. 2012). This implies that stress can affect the physiology of an individual, and ultimately their behaviour.

Perhaps variation in signalling effort and quality is maintained due to phenotypic plasticity, where males make different decisions regarding resource allocation. The social environment in which juvenile crickets were raised could have created social hierarchies and differences in boldness which could have impacted our findings. For example, bolder crickets might position themselves higher in their shelters and therefore closer to the microphones, and

would therefore be recorded with higher amplitudes than less bold crickets. Behavioural syndromes that include activity, boldness, exploration, mating decisions, and aggression (Kortet & Hedrick 2007; Wilson et al. 2010) could have emerged during development and could ultimately affect individual cricket's motivation to signal. In an attempt to control for such confounding effects of the social environment during cricket development, I raised all males in identical, high density environments as juveniles and in isolation as adults to ensure that all experimental males were virgins. I also kept temperature and light cycles, and crickets were not exposed to predation threats.

Implications and Future Directions

There were no significant relationships between any of the signalling parameters and residual mass as a measure of condition supporting my initial prediction that it is necessary to investigate condition physiologically, by quantifying an individual's ability to acquire, store, and expend resources (Tomkins et al. 2004). Further, since different body energy stores and different enzyme capacities were important in each of species studied, condition measures may be species dependent.

Two other possible mechanisms that may explain the variation in calling behaviour are the ploidy levels of signalling muscles and the basal and signalling aerobic metabolic rates of male crickets. I explored each of these mechanisms while acting as a mentor to two undergraduate students (Chris Séguin and Sami Majdalany) completing their Honour's research projects. Insect skeletal muscles consist of many elongated muscle fibers, each of which is a single multinucleate cell (Klowden 2007). This increase in nuclear DNA content in skeletal muscle has been shown to increase muscular function through increased protein turnover, maintenance of myonuclear domains, and reduction in nuclear transport distances (Bruusgaard et

al. 2003; Sandri 2008; Hardy et al. 2009; Jimenez et al. 2010). It is therefore possible that high-effort signallers would have greater numbers of nuclei in their cells than low-effort signallers. Since flow-through cytometry had not been attempted on cricket muscles, Chris Séguin and I developed a technique for extracting muscle nuclei. While performing flow-through cytometry on cricket signalling muscles, we discovered that high-effort *G. assimilis* had lower proportions of polyploidic nuclei in their muscles ($F_{1,10}$=16.519, R^2_{adj}=0.660, p=0.0048), but there was no correlation in *G. texensis*. There was also no significant correlation between density of nuclei in the signalling muscles and signalling effort. However, it is possible that the very small sample sizes for this project masked any significant effects. In the future, this project could be repeated with greater sample sizes and improved methods.

It is also possible that differences in metabolic rate could be correlated with high-effort signalling (Ryan 1988; Prestwich et al. 1989; Gillooly & Ophir 2010). While performing measurements of the respirometry of male crickets, Sami Majdalany and I discovered that attaining measurements of metabolic rate during signalling was not logistically possible because the crickets would not signal in our chambers. Instead we quantified basal metabolic rate to test the hypothesis that crickets that signal with higher effort have lower basal metabolic rates. There were no significant relationships between signalling effort or quality and basal metabolic rate in either *G. assimilis* or *G. texensis*. Future researchers should construct respirometry chambers that more closely resemble field cricket natural habitats or burrows (for example, see Prestwich et al. 2005) which may compel male crickets to call while their CO_2 production is being recorded.

Some of the variation in signalling effort and quality may be driven by muscular enzyme activities and thoracic and abdominal fuel stores. Although I have discovered links between signalling behaviours and carbohydrate and lipid metabolism, these relationships remain purely correlational, and the causational relationships between signalling and fuel need to be explored.

Combining studies that manipulate resource acquisition (through diet studies) with studies that manipulate resource allocation (through imposing artificial selection on survival, signalling effort, etc.) would provide insight into the life-history based allocation decisions of crickets.

References

Alexander, R. 1957. The taxonomy of the field crickets of the Eastern United States (Orthoptera: Gryllidae: *Acheta*). *Annals of the Entomological Society of America*, **50**, 585–602.

Alexander, R., & Walker, T. J. 1962. Two introduced field crickets new to Eastern United States (Orthoptera: Gryllidae). *Annals of the Entomological Society of America*, **55**, 90–94.

van Antwerpen, R., Linnemans, W. a, Van der Horst, D. J., & Beenakkers, A. M. 1988. Immunocytochemical localization of lipophorins in the flight muscles of the migratory locust (*Locusta migratoria*) at rest and during flight. *Cell and tissue research*, **252**, 661–8.

Arrese, E. L., & Soulages, J. L. 2010. Insect fat body: energy, metabolism, and regulation. *Annual Review of Entomology*, **55**, 207–25.

Bailey, W. J., Withers, P. C., Endersby, M., & Gaull, K. 1993. The energetic costs of calling in the bushcricket *Requena verticalis* (Orthoptera: Tettigonidae: *Listroscelidinae*). *Journal of Evolutionary Biology*, **178**, 21–37.

Beenakkers, A. M. 1969. Carbohydrate and fat as a fuel for insect flight. A comparative study. *Journal of Insect Physiology*, **15**, 353–61.

Beenakkers, A. M., Van der Horst, D. J. J., & Van Marrewijk, W. 1984. Insect flight muscle metabolism. *Insect Biochemistry*, **14**, 243–260.

Bennet-Clark, H. C. 1987. The tuned singing burrow of mole crickets. *Journal of Experimental Biology*, **128**, 383–409.

Bennet-Clark, H. C. 1998. Size and scale effects as constraints in insect sound communication. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **353**, 407–419.

Bertram, S. M. 2000. The influence of age and size on temporal mate signalling behaviour. *Animal Behaviour*, **60**, 333–339.

Bertram, S. M. 2002. The influence of rearing and monitoring environment on temporal mate signaling patterns in the field cricket, *Gryllus texensis*. *Journal of Insect Behavior*, **15**, 127–137.

Bertram, S. M., & Bellani, R. 2002. Influence of photoperiod on temporal mate signaling patterns in the Texas field cricket , *Gryllus texensis*. *Journal of Insect Behavior*, **15**, 607–615.

Bertram, S. M., Fitzsimmons, L. P., McAuley, E. M., Rundle, H. D., & Gorelick, R. 2011a. Phenotypic covariance structure and its divergence for acoustic mate attraction signals among four cricket species. *Ecology and Evolution*, **1**, 1–15.

Bertram, S. M., Kemp, D. J., Johnson, J. S., Orozco, S. X., & Gorelick, R. 2007. Heritability of acoustic signalling time in the Texas field cricket, *Gryllus texensis*. *Evolutionary Ecology Research*, **9**, 975–986.

Bertram, S. M., & Rook, V. L. M. 2011. Relationship between condition, aggression, signaling, courtship, and egg yaying in the field cricket, *Gryllus assimilis*. *Ethology*, **118**, 360–372.

Bertram, S. M., Thomson, I. R., Auguste, B., Dawson, J. A., & Darveau, C.-A. 2011b. Variation in cricket acoustic mate attraction signaling explained by body morphology and metabolic differences. *Animal Behaviour*, 1255–1261.

Bertram, S. M., & Warren, P. 2005. Trade-offs in signalling components differ with signalling effort. *Animal Behaviour*, **70**, 477–484.

Bertram, S. M., Whattam, E. M., Visanuvimol, L., Bennett, R., & Lauzon, C. 2009. Phosphorus availability influences cricket mate attraction displays. *Animal Behaviour*, **77**, 525–530.

Bolger, T., & Connolly, P. L. 1989. The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology*, **34**, 171–182.

Bruusgaard, J. C., Liestøl, K., Ekmark, M., Kollstad, K., & Gundersen, K. 2003. Number and spatial distribution of nuclei in the muscle fibres of normal mice studied in vivo. *The Journal of physiology*, **551**, 467–78.

Cade, W. H. 1975. Acoustically orienting parasitoids: fly phonotaxis to cricket song. *Science*, **190**, 1312–1313.

Cade, W. H. 1979. The evolution of alternative male reproductive strategies in field crickets. In: *Sexual selection and reproductive competition in insects*, (Ed. by M. Blum), Academic Press, New York.

Cade, W. H. 1981. Alternative male strategies: genetic differences in crickets. *Science*, **212**, 563–564.

Cade, W. H. 1991. Inter- and intraspecific variation in nightly calling duration in field cricekts, *Gryllus integer* and *G. rubens* (Orthoptera: Grillidae). *Journal of Insect Behavior*, **4**, 185–194.

Cade, W. H., & Cade, E. 1992. Male mating success, calling and searching behaviour at high and low densities in the field cricket, *Gryllus integer*. *Animal Behaviour*, **43**, 49–56.

Cade, W. H., & Otte, D. 2000. *Gryllus texensis* n. sp.: a widely studied field cricket (Orthoptera; Gryllidae) from the southern United States. *Transactions of the American Entomological Society*, **126**, 117–123.

Cade, W. H., & Wyatt, D. R. 1984. Factors affecting calling behaviour in field crickets, *Teleogryllus* and *Gryllus* (age, weight, density, and parasites). *Behaviour*, **88**, 61–75.

Candy, D. 1978. The regulation of locust flight muscle metabolism by octopamine and other compounds. *Insect Biochemistry*, **8**, 177–181.

Chadwick, L. E. 1947. The Respiratory Quotient of Drosophila in Flight. *Biological Bulletin*, **93**, 229–239.

Charlesworth, B. 1990. Mutation-selection balance and the evolutionary advantage of sex and recombination. *Genetical Research*, **55**, 199–221.

Crnokrak, P., & Roff, D. A. 1995. Fitness differences associated with calling behaviour in the two wing morphs of male sand crickets, *Gryllus firmus*. *Animal Behaviour*, **50**, 1475–1481.

Crnokrak, P., & Roff, D. A. 1998. The contingency of fitness: an analysis of food restriction on the macroptery-reproduction trade-off in crickets. *Animal Behaviour*, **56**, 433–441.

Darveau, C.-A., Hochachka, P. W., Roubik, D. W., & Suarez, R. K. 2005. Allometric scaling of flight energetics in orchid bees: evolution of flux capacities and flux rates. *The Journal of Experimental Biology*, **208**, 3593–602.

David, P., Bjorksten, T., Fowler, K., & Pomiankowski, A. 2000. Condition-dependent signalling of genetic variation in stalk-eyed flies. *Nature*, **406**, 186–8.

Drummond, G. I., & Black, E. C. 1960. Comparative physiology: fuel of muscle metabolism. *Annual Review of Physiology*, **22**, 169–90.

Fisher, R. A. 1930. *The genetical theory of natural selection.* Oxford: Clarendon Press.

Fitzsimmons, L. P., & Bertram, S. M. 2011. The calling songs of male spring field crickets (*Gryllus veletis*) change as males age. *Behaviour*, **148**, 1045–1065.

Fulton, T. W. 1902. The rate of growth of fishes. *20th Anual Report of the Fishery Board of Scotland*, **3**, 326–446.

Gillooly, J., & Ophir, A. 2010. The energetic basis of acoustic communication. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 1325–31.

Goldsworthy, G., & Mordue, W. 1989. Adipokinetic Hormones: Functions and Structures. *Biological Bulletin*, **177**, 218.

Gould, S. J. 1975. Allometry in primates, with emphasis on scaling and thte evolution of the brain. *Contributions to Primatology*, **5**, 244–292.

Gray, D. A., & Cade, W. H. 1999. Sex, death, and genetic variation: natural and sexual selection on cricket song. *Proceedings of the Royal Society B: Biological Sciences*, **266**, 707–709.

Green, A. J. 2001. Mass/Length Residuals: Measures of Body Condition or Generators of Spurious Results? *Ecology*, **82**, 1473–1483.

Hardy, K. M., Dillaman, R. M., Locke, B. R., & Kinsey, S. T. 2009. A skeletal muscle model of extreme hypertrophic growth reveals the influence of diffusion on cellular design. *American journal of physiology. Regulatory, integrative and comparative physiology*, **296**, R1855–67.

Hawlena, D., Strickland, M. S., Bradford, M. a., & Schmitz, O. J. 2012. Fear of predation slows plant-litter decomposition. *Science*, **336**, 1434–1438.

Hedrick, P. W. P. 1986. Genetic polymorphism in heterogeneous environments: a decade later. *Annual Review of Ecology and Systematics*, **17**, 535–566.

Hedrick, A. V. 1988. Female choice and the heritability of attractive male traits: an empirical study. *The American Naturalist*, **132**, 267–276.

Hedrick, P. W. P., Ginevan, M. E., & Ewing, E. P. 1976. Genetic polymorphism in heterogeneous environments. *Annual Review of Ecology and Systematics*, **7**, 1–32.

Hedrick, A., & Weber, T. 1998. Variance in female responses to the fine structure of male song in the field cricket, *Gryllus integer*. *Behavioral Ecology*, **9**, 582–591.

Hill, G. E. 1991. Plumage coloration is a sexually selected indicator of male quality. *Nature*, **350**, 337–339.

Holzer, B. 2003. Condition-dependent signaling affects male sexual attractiveness in field crickets, *Gryllus campestris*. *Behavioral Ecology*, **14**, 353–359.

Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics*, **130**, 195–204.

Hunt, J., Blows, M. M. W., Zajitschek, F., Jennions, M. D., & Brooks, R. C. 2007. Reconciling strong stabilizing selection with the maintenance of genetic variation in a natural population of black field crickets (*Teleogryllus commodus*). *Genetics*, **177**, 875–80.

Hunt, J., Brooks, R. C., Jennions, M. D., Smith, M. J., Bentsen, C. L., & Bussière, L. F. 2004. High-quality male field crickets invest heavily in sexual display but die young. *Nature*, **432**, 1–4.

Jakob, E. E. M., Marshall, S. D., & Uetz, G. W. 1996. Estimating fitness: a comparison of body condition indices. *Oikos*, **77**, 61–67.

Jia, F. Y., Greenfield, M. D., & Collins, R. D. 2000. Genetic variance of sexually selected traits in waxmoths: maintenance by genotype x environment interaction. *Evolution*, **54**, 953–67.

Jimenez, A., Kinsey, S. T., Dillaman, R. M., & Kapraun, F. 2010. Nuclear DNA content variation associated with muscle fiber hypertrophic growth in decapod crustaceans. *Genome*, **53**, 161–171.

Judge, K. A., Ting, J. J., & Gwynne, D. T. 2008. Condition dependence of male life span and calling effort in a field cricket. *Evolution*, **62**, 868–78.

Kaufmann, C., & Brown, M. 2008. Determination of lipid, glycogen and sugars in mosquitoes. *Journal of Insect Physiology*, **54**, 367–377.

Kirkpatrick, M., & Ryan, M. J. 1991. The evolution of mating preferences and the paradox of the lek. *Nature*, **350**, 33–38.

Klowden, M. J. 2007. *Physiological Systems in Insects.* 2nd edn. Academic Press.

Kolluru, G. R. 1999. Variation and repeatability of calling behavior in crickets subject to phonotactic parasitoid fly. *Journal of Insect Behavior*, **12**, 611–626.

Kondrashov, A. 1982. Selection against harmful mutations in large sexual and asexaul populations. *Genetical Research*, **40**, 325–332.

Kortet, R., & Hedrick, A. V. 2007. A behavioural syndrome in the field cricket *Gryllus integer*: intrasexual aggression is correlated with activity in a novel environment. *Biological Journal of the Linnean Society*, **91**, 475–482.

Kotiaho, J. S., LeBas, N. R., Puurtinen, M., & Tomkins, J. L. 2008. On the resolution of the lek paradox. *Trends in Ecology & Evolution*, **23**, 1–3.

Lande, R. 1976. The maintenance of genetic variability by mutation in a polygenic character with linked loci. *Genetical Research*, **26**, 221–235.

Lorenz, M. W. 2003. Adipokinetic hormone inhibits the formation of energy stores and egg production in the cricket *Gryllus bimaculatus*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, **136**, 197–206.

Lorenz, M. W., & Anand, A. N. 2004. Changes in the biochemical composition of fat body stores during adult development of female crickets, *Gryllus bimaculatus*. *Archives of Insect Biochemistry and Physiology*, **56**, 110–9.

Maklakov, A. A., Simpson, S. J., Zajitschek, F., Hall, M. D., Dessmann, J., Clissold, F. J., Raubenheimer, D., Bonduriansky, R., & Brooks, R. C. 2008. Sex-specific fitness effects of nutrient intake on reproduction and lifespan. *Current Biology*, **18**, 1062–6.

Miyoshi, A. R., Zefa, E., Martins, L. D. P., Dias, P. G. B. S., Drehmer, C. J., & Dornelles, J. E. F. 2007. Stridulatory file and calling song of two populations of the tropical bush cricket *Eneoptera surinamensis* (Orthoptera , Gryllidae , Eneopterinae). *Série Zoologia*, **97**, 461–465.

Moore, A. J. 1989. Sexual selection in Nauphoeta cinerea: inherited mating preference? *Behavioral Genetics*, **19**, 717–724.

Moore, A. J., & Moore, P. J. 1999. Balancing sexual selection through opposing mate choice and male competition. *Proceedings of the Royal Society B: Biological Sciences*, **266**, 711–716.

Moradian, N. R., & Walker, S. E. 2008. Relationships between body size and sound-producing structures in crickets: do large males have large harps? *Invertebrate Biology*, **127**, 444–451.

Møller, A. P. 1991. Viability is positively related to degree of ornamentation in male swallows. *Proceedings of the Royal Society B: Biological Sciences*, **243**, 145–148.

Møller, A. P., & Alatalo, R. V. 1999. Good-genes effects in sexual selection. *Proceedings of the Royal Society B: Biological Sciences*, **266**, 85–91.

Nash, R. D. M., Valencia, A. H., Geffen, A. J., & Meek, A. 2006. The Origin of Fulton's Condition Factor — Setting the Record Straight. *Fisheries*, **31**, 236–238.

Phelan, P. ., & Baker, C. 1986. Male-size-related courtship success and intersexual selection in the tobacco moth, *Ephestia elutellal. Experientia*, **42**, 1291–1293.

Prestwich, K. N. 1994. The energetics of acoustic signaling in Anurans and Insects. *American Zoologist*, **34**, 625–643.

Prestwich, K. N., Brugger, K., & Topping, M. 1989. Energy and communication in three species of hylid frogs: power input, power output and efficiency. *Journal of Experimental Biology*, **144**, 53–80.

Prestwich, K. N., Lenihan, K. M., & Martin, D. M. 2000. The control of carrier frequency in cricket calls: a refutation of the subalar-tegminal resonance/auditory feedback model. *The Journal of Experimental Biology*, **203**, 585–96.

Prestwich, K. N., & O'Sullivan, K. 2005. Simultaneous measurement of metabolic and acoustic power and the efficiency of sound production in two mole cricket species (Orthoptera: Gryllotalpidae). *The Journal of Experimental Biology*, **208**, 1495–512.

Prestwich, K. N., & Walker, T. J. 1981. Energetics of Singing in Crickets□: Effect of Temperature in Three Trilling Species (Orthoptera: Gryllidae). *Journal Of Comparative Physiology*, **143**, 199–212.

Rowe, L., & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society B: Biological Sciences*, **263**, 1415–1421.

Ryan, M. 1988. Energy, calling, and selection. *American Zoologist*, **28**, 885–898.

Sandri, M. 2008. Signaling in muscle atrophy and hypertrophy. *Physiology (Bethesda, Md.)*, **23**, 160–70.

von Schantz, T., Goransson, G., Andersson, G., Froberg, I., Grahn, M., Helgée, A., & Wittzell, H. 1989. Female choice selects for a viability-based male trait in pheasants. *Nature*, **337**, 166–169.

Scheuber, H., Jacot, A., & Brinkhof, M. W. G. 2003. The effect of past condition on a multicomponent sexual signal. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 1779–84.

Simmons, L. W. 1987. Heritability of a male character chosen by females of the field cricket, *Gryllus bimaculatus*. *Behavioral Ecology and Sociobiology*, **21**, 129–133.

Simmons, L. W. 1988. The calling song of the field cricket, *Gryllus bimaculatus* (De Geer): constraints on transmission and its role in intermale competition and female choice. *Animal Behaviour*, **36**, 380–394.

Simmons, L. W., & Ritchie, M. G. 1996. Symmetry in the songs of crickets. *Proceedings of the Royal Society B: Biological Sciences*, **263**, 1305–1311.

Simmons, L. W., & Zuk, M. 1992. Variability in call structure and pairing success of male field crickets, *Gryllus bimaculatus*: the effects of age, size and parasite load. *Animal Behaviour*, **44**, 1145–1152.

Smith, J. M. J. 1976. Sexual selection and the handicap principle. *Journal of Theoretical Biology*, **57**, 239–242.

Souroukis, K., Cade, W. H., & Rowell, G. 1992. Factors that possibly influence variation in the calling song of field crickets□: temperature, time, and male size, age, and wing morphology. *Canadian Journal of Zoology*, **70**, 950–955.

Steele, J. E. 1982. Glycogen phosphylase in insects. *Insect Biochemistry*, **12**, 131–147.

Strohbach, S., Curio, E., Bathen, A., Epplen, J. T., & Lubjuhn, T. 1998. Extrapair paternity in the great tit (*Parus major*): a test of the "good genes" hypothesis. *Behavioral Ecology*, **9**, 388–396.

Suarez, R. K., Darveau, C.-A., & Hochachka, P. W. 2005a. Roles of hierarchical and metabolic regulation in the allometric scaling of metabolism in Panamanian orchid bees. *The Journal of Experimental Biology*, **208**, 3603–7.

Suarez, R. K., Darveau, C.-A., Welch, K. C., O'Brien, D. M., Roubik, D. W., & Hochachka, P. W. 2005b. Energy metabolism in orchid bee flight muscles: carbohydrate fuels all. *The Journal of Experimental Biology*, **208**, 3573–9.

Thomson, I. R., Vincent, C. M., & Bertram, S. M. 2012. Success of the parasitoid fly Ormia ochracea (Diptera□: Tachinidae) on natural and unnatural cricket hosts. *Florida Entomologist*, **95**, 43–48.

Tomkins, J. L., Radwan, J., Kotiaho, J. S., & Tregenza, T. 2004. Genic capture and resolving the lek paradox. *Trends in Ecology & Evolution*, **19**, 323–8.

Wagner, W. E. 1996. Convergent song preferences between female field crickets and acoustically orienting parasitoid flies. *Behavioral Ecology*, **7**, 279–285.

Wagner, W. E., Beckers, O. M., Tolle, A. E., & Basolo, A. L. 2012. Tradeoffs limit the evolution of male traits that are attractive to females. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 2899–2906.

Wagner, W. E., & Hoback, W. 1999. Nutritional effects on male calling behaviour in the variable field cricket. *Animal Behaviour*, **57**, 89–95.

Wagner, W. E., Murray, A. M., Cade, W. H., & Wagner Jr, W. E. 1995. Phenotypic variation in the mating preferences of female field crickets, *Gryllus integer*. *Animal Behaviour*, **49**, 1269–1281.

Walker, T. T. J. 1957. Specificity in the response of female tree crickets (Orthoptera, Gryllidae, Oecanthinae) to calling songs of the males. *Annals of the Entomological Society of America*, **50**, 626–636.

Walker, T. 1962. Factors responsible for intraspecific variation in the calling songs of crickets. *Evolution*, **16**, 407–428.

Whattam, E. M., & Bertram, S. M. 2011. Effects of juvenile and adult condition on long-distance call components in the Jamaican field cricket, *Gryllus assimilis*. *Animal Behaviour*, **81**, 135–144.

White, C. R., Matthews, P. G. D., & Seymour, R. S. 2008. In situ measurement of calling metabolic rate in an Australian mole cricket, *Gryllotalpa monanka*. *Comparative Biochemistry and Physiology. Part A, Molecular & integrative physiology*, **150**, 217–21.

Wilson, A. D. M., Whattam, E. M., Bennett, R., Visanuvimol, L., Lauzon, C., & Bertram, S. M. 2010. Behavioral correlations across activity, mating, exploration, aggression, and

antipredator contexts in the European house cricket, *Acheta domesticus*. *Behavioral Ecology and Sociobiology*, **64**, 703–715.

Zahavi, A. 1975. Mate selection-a selection for a handicap. *Journal of Theoretical Biology*, **53**, 205–14.

Zahavi, A. 1977. The cost of honesty (further remarks on the handicap principle). *Journal of Theoretical Biology*, **67**, 603–5.

Zera, A. J., Sall, J., & Grudzinski, K. 1997. Flight-muscle polymorphism in the cricket Gryllus firmus: muscle characteristics and their influence on the evolution of flightlessness characteristics and their influence on the evolution of flightlessness. *Physiological Zoology*, **70**, 519–529.

Zera, A. J., & Zhao, Z. 2006. Intermediary metabolism and life-history trade-offs: differential metabolism of amino acids underlies the dispersal-reproduction trade-off in a wing-polymorphic cricket. *The American Naturalist*, **167**, 889–900.

Zimmitti, S. J. 1999. Individual variation in morphological, physiological, and biochemical features associated with calling in spring peepers (*Pseudacris crucifer*). *Physiological and Biochemical Zoology*, **72**, 666–76.

Zuk, M. 1987. Variability in attractiveness of male field crickets (Orthoptera□: Gryllidae) to females. *Animal Behaviour*, **35**, 1240–1248.