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Variation in cricket acoustic mate attraction signalling explained by body morphology and metabolic differences

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Males often signal to attract mates and can show extensive variation in how much time they spend signalling. In crickets, some males signal extensively, spending over 50% of their adult lives attempting to attract a mate. At the other end of the continuum, some males are rarely observed to signal. Given that signalling efforts are usually correlated with mating success, all individuals should be selected to signal with high effort. Why then, do males show such variability? Signalling effort variation may stem from differences in physiological capacity that result from disparities in energy stores, metabolic capacities of the muscles used for sexual signalling, the comparative size of the signalling organs, or overall differences in body size. To address the proximate causes underlying variation in signalling effort, we quantified the morphological, physiological and biochemical variation among male European house crickets, *Acheta domesticus*, and assessed whether it correlated with signalling effort variation. Surprisingly, we found no correlation between signalling effort and activity of the β-oxidation enzyme HOAD, suggesting that the capacity for lipid metabolism is not associated with signalling effort. Instead, signalling effort variation was associated with differences in overall body size and differences in the activity of the glycolytic enzyme pyruvate kinase. Together our findings suggest that the ability to locate and assimilate high-quality diets both during development (to grow large) and into adulthood (capacity for carbohydrate catabolism) may explain some of the variation in signalling effort in this species.

Individuals with the largest, brightest and/or loudest mate attraction displays typically obtain the most mates (Ryan & Keddy-Hector 1992). Therefore, over evolutionary time, sexual selection should result in both the trait's enhancement and a reduction in the trait's genetic variation. However, most sexual signals exhibit high levels of genetic variation (Pomiankowski & Møller 1995). The mystery of how genetic variation is maintained has largely been resolved with the introduction of the genic capture hypothesis (Rowe & Houle 1996). The genic capture hypothesis posits that (1) because mate attraction signals are costly to produce they are dependent upon overall condition, and (2) because condition is dependent upon genes at many loci, genetic variation should persist in sexually selected traits. The genic capture hypothesis' fundamental assumption is that variation in sexually selected traits is constrained by an organism's condition (Rowe & Houle 1996). But what is condition? Genic capture defines it as the amount of resources available for allocation to traits that enhance fitness (Rowe & Houle 1996). Tomkins et al. (2004, page 326) extended this definition by suggesting that 'consideration must be given to whether it is appropriate to either measure acquisition efficiency or the magnitude of the resource pool'. Together these publications draw on the importance of understanding the physiological and biochemical mechanisms that fuel variation in sexually selected traits.

Variation in physiological capacity could stem from variation in energy stores, variation in the metabolic flux capacity (the flow of metabolites through metabolic pathways) of the muscles used for sexual signalling, variation in the comparative size of the signalling organs, or variation in body size. For example, Zimmitti (1999) found a strong positive correlation between spring peeper, *Pseudacris crucifer*, signalling rate and activity of citrate synthase, a reference enzyme for aerobic metabolism. Signalling rate was also positively correlated with β-hydroxyacyl-CoA dehydrogenase activity, a reference enzyme for lipid metabolism (Zimmitti 1999). Additionally, there were cardiovascular correlates: spring peepers with high signal rates had larger heart ventricles and higher concentrations of blood haemoglobin than those with low signal

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rates. Zimmitti's (1999) findings suggest that variation in a suite of functionally interrelated anatomical, physiological and biochemical traits could underlie variation in condition and may, therefore, be responsible for the maintenance of variation in the spring peeper's sexual signalling activity. Conversely, these correlates could be a consequence of signalling effort variation, as exercise training is known to have anatomical, physiological and biochemical effects in frogs and other vertebrates (Cummings 1979).

Here we examine the physiological and biochemical differences that correlate with interindividual variation in an insect's sexual signals. Male crickets show extensive variation in how much they signal to attract a mate through the course of their adult lives. Males produce acoustic mate attraction signals to attract females and repel males by rubbing their raised forewings together. Each closing stroke produces a pulse of sound, and pulses are concatenated into chirps. Males vary extensively in several aspects of their acoustic mate-signalling behaviour, including the duration of their pulses, the duration of their chirp rates, their carrier frequencies, the amplitudes at which that they signal and the amount of time they spend signalling through the day and night (Cade 1981; Wagner et al. 1995; Gray 1997; Bertram & Johnson 1998; Gray & Cade 1999a, b; Bertram 2000; Bertram et al. 2004; Bertram & Bowen 2006; Jacot et al. 2007; Judge et al. 2008; Verburgt & Ferguson 2010; Judge 2011; Verburgt et al. 2011; Whattam & Bertram 2011). For example, some male crickets spend over 50% of their adult lives signalling to attract a mate, while others spend less than 5% of their adult lives signalling, a 10-fold difference (Cade 1980; Bertram 2000; Hunt et al. 2004; Bertram et al. 2009; Rodríguez-Muñoz et al. 2010).

Here we quantified the physiological and biochemical correlates of variation in cricket mate-signalling effort to see whether we could explain this 10-fold difference in signalling effort. We recorded every acoustic mate attraction signal produced by each male over a week-long period. We then quantified variation in the males' aerobic potential, capacity for lipid and carbohydrate metabolism, signalling muscle size and body size. We focused on physiological variation because the metabolic power associated with acoustic stridulation comes from the work caused by the thoracic muscles closing the forewings against the file and plectrum (Prestwich & Walker 1981; Prestwich & Breuer 1987; Pfau & Koch 1994; Prestwich 1994; Prestwich & O'Sullivan 2005). Males use their five dorsoventral muscles to power the closing (sound-producing) wing stroke, and they use their basalar and subalar muscles to power the opening (silent) wing stroke (Pfau & Koch 1994).

Signalling is thought to be fuelled by aerobic metabolism (Prestwich 1994), thus variation in signalling effort could be dependent on variation in aerobic metabolic capacity, or in the breakdown of either lipids or carbohydrates. There is evidence supporting both fuel pathways. First, cricket flight is fuelled by the breakdown of lipids and is coupled with higher mitochondrial loads in the flight muscles (Zhao & Zera 2002; Zera & Zhao 2003; Harshman & Zera 2006). Given that flying insects use lipids to fuel their flights, many cricket species have the machinery in place to also utilize lipid breakdown to fuel the energetic demands of their acoustic signalling (Roff & Fairbairn 1994; Zera & Zhao 2003). Second, Maklakov et al. (2008) revealed that the availability of carbohydrates in the diet strongly influences male sexual signalling effort. Male crickets provided with diets containing a high ratio of carbohydrate to protein signalled with significantly higher efforts than males provided with high protein to carbohydrate diets. Maklakov et al.'s (2008) results suggest that the breakdown of carbohydrates may be involved in fueling acoustic signalling displays. Alternatively, crickets might be converting excess carbohydrates into lipids to fuel their mate signalling. It is thus important explore whether one or multiple fuel sources are important for mate signalling.

In an attempt to explain the extensive variation in effort that crickets put into attracting a mate, we explored multiple levels of organization. At the whole-organism level, we quantified how body size and residuals of mass correlated with signalling effort. At the organ level, we explored the relationship between the comparative size of the signalling organs and cricket-signalling effort. At the biochemical level, we explored the variation in metabolic flux capacity of the muscles used for sexual signalling. Given the present state of knowledge in this field, our multilevel approach provides the initial step towards understanding the factors influencing signalling effort variation in crickets.

METHODS

Acheta domesticus were obtained from a commercial supplier (Port Credit Pet Centre, Mississauga, ON, Canada) in March 2008 and then bred in our insect-rearing facility at Carleton University, Ottawa, ON. Our study was conducted in accordance with the guidelines of the Canadian Counsel on Animal Care. Crickets were reared on a 12:12 h light:dark cycle with lights on at 0700 hours, at a temperature of 26 ± 4 °C (mean and range). Communal rearing containers consisted of six 68-litre rectangular ($64 \times 40 \times 41.9$ cm) plastic containers where crickets were provided with ad libitum water and food (Harlan Teklad Rodent diet 8604, Madison, WI, U.S.A.) and ample shelter in the form of staggered egg cartons. Communal containers were checked daily for any males that had undergone final (imaginal) moult and reached adulthood. Adult males were transferred to individual 500 ml plastic containers ($N = 113$). Crickets were examined daily to ensure they were still alive, and to replenish their food and water.

Male house crickets typically may begin signalling for mates as early as 2–3 days post final moult and usually have begun signalling by 10–11 days post final moult. To capture variation in early mate-signalling behaviour, we monitored male cricket's mate-signalling behaviour from 7 to 14 days post final moult by placing the males into a real time electronic acoustic recording system (EARs II). The EARs II recording system consisted of 96 individually recording microphones. Each microphone was coupled with a single LED light that provided the males with the same LD 12:12 h cycle as the rearing facility. Each microphone was housed in an acoustically isolated enclosure (a cooler box made of 5 cm thick Styrofoam and internally lined with 2.5 cm thick acoustic foam) to eliminate sound contamination from neighbouring males. The microphones were continuously monitored by CricketSong software (Cambridge Electronic Design Ltd, Cambridge, U.K.). CricketSong automatically analysed the sound wave recorded by the microphone and calculated, in real time, each cricket's pulse duration, interpulse duration (time between pulses), number of pulses per chirp, chirp duration, interchirp duration (time between chirps), amplitude, carrier frequency and time spent signalling each hour. Pulse onset was determined when the amplitude passed a species-specific threshold (40.0 dB for *A. domesticus*). However, this threshold was also adjusted dynamically by CricketSong to account for individuals that signalled at higher-than-average amplitudes. For these individuals, the threshold was raised to a level proportional to the amplitude of the pulse and decayed back to the original value within 1–8000 ms (the exact rate of decay was proportional to the size of the pulse, allowing the system to be self-scaling). For very quiet individuals, the minimum threshold was manually reset to values below the species-specific value. Raw data from CricketSong were processed and summarized using Spike2 audio software (Cambridge Electronic Design Ltd). We calculated the means for all signal parameters from days 7 to 14 of adulthood ($N = 113$). The signal components were measured for every single pulse of sound (or chirp, in the case of chirp-related components, such as chirp

duration) recorded. Means were then calculated based on the total number of pulses (or chirps). Thus, measurements taken when a male was signalling continuously were weighed more heavily than when he was signalling sporadically. For this reason, we believe that averaging these measurements over each 24 h period accurately reflects values of these signal components.

Morphometric Analyses

Males were weighed to the nearest 0.1 mg at 2 weeks post imaginal moult ($N = 113$) using a Denver Instruments Precision Analytical Balance (model P-114) immediately following their removal from the acoustic recording system. They were then photographed using a calibrated Zeiss Discovery V12 microscope to obtain size measures. All size measurements (maximum head capsule width along with pronotum width, length and area) were strongly positively correlated. We thus used the first principal component of a principal component analysis as an overall size measure (PC1 size explained 90% of the variation in size measures; eigenvalue = 3.598).

To minimize stress and pain, males were euthanized immediately prior to dissection. We euthanized crickets by quickly separating the head from the body with a scalpel. The wings, legs, abdomen and gut were then quickly removed and these tissues were stored for later measurements. The crickets' dorsoventral, basalar and subalar signalling muscles were immediately removed from the thorax and weighed. Following weighing, the signalling muscles and cricket thoraxes were stored in separate labelled Eppendorf tubes and immediately frozen to −80 °C (dorsoventral, basalar and subalar muscles were stored together in one Eppendorf tube; all remaining thorax material was stored in a separate tube). Cricket muscles and thoraxes were then transported in liquid nitrogen to the University of Ottawa and stored at −80 °C until biochemical analyses were conducted.

Following removal and storage of the crickets' signalling muscles and thoraxes, the crickets' file length and harp area on the right forewing were measured using the Zeiss Discovery V12 microscope noted above. The harp area was measured by tracing the outline of the wing veins surrounding the harp, while the file length was measured to include all visible teeth.

Biochemical Analyses

To assess metabolic capacity of muscle used during signalling, we measured the maximal activity (V_{max}) of the beta-oxidation enzyme β-hydroxyacyl-CoA dehydrogenase (HOAD), the glycolytic enzyme pyruvate kinase (PK) and glycogen phosphorylase (GP). In animals that use various fuels to power aerobic metabolism, HOAD is used as an indicator enzyme for lipid catabolism as it is involved in a recurring and essential step to the mitochondrial beta-oxidation cycle (Table 1). The enzyme PK is used as an indicator of glycolytic flux capacity as it is necessary for the production of pyruvate from glucose. The enzyme GP is a regulatory enzyme that indicates the ability for glycogen breakdown. We also quantified citrate synthase activity (CS; Table 1), as the activity of this mitochondrial matrix enzyme correlates with variation in mitochondrial content. Lastly, to further characterize the metabolic phenotype of these muscle tissues, we calculated the ratio of PK:CS and HOAD:CS to quantify the relative flux of the glycolytic, mitochondrial and beta-oxidation pathways. To assess whether overall capacity of individuals is correlated with signalling effort, we measured the activity of enzymes on the whole thorax composed mainly of the remaining muscles (signalling muscles removed). While we wanted to measure the activity of all the enzymes on the signalling muscles themselves (dorsoventral, basalar and subalar muscles used to power the sound-producing and silent wing strokes; Pfau & Koch 1994), they were only large enough to obtain an accurate measure of CS activity. We assumed that the activity of these enzymes, which are found in all muscle tissues, would show similar interindividual variation in activity that characterizes individual metabolic capacity, and therefore, also be correlated with signalling effort.

Table 1
Enzyme activity (V_{max}) used as indicators of metabolic pathways flux capacity in European house crickets, *Acheta domesticus*

Enzyme	Abbreviation	Biochemical pathway	Function	Prediction
Glycogen phosphorylase	GP	Glycogen breakdown	Glycogen catabolism	Positive correlation
Pyruvate kinase	PK	Glycolysis	Carbohydrate catabolism	Positive correlation
β-hydroxyacyl-CoA dehydrogenase	HOAD	β-oxidation	Lipid catabolism	Positive correlation
Citrate synthase	CS	Mitochondrial citric acid cycle	Oxidative phosphorylation	Positive correlation

Cricket thoraxes and muscles were homogenized in separate vials in 9 volumes of chilled buffer relative to the mass of the tissue analysed (with an assumed tissue density of approximately 1 mg/μl). The homogenization buffer consisted of 50 mmol Tris–HCl (pH 7.5 at 4 °C) and 2 mmol ethylenediaminetetraacetic acid (EDTA), to which 0.5% (vol./vol.) triton X100 and 5 mmol DTT were added on each individual day that assays were preformed. Samples were homogenized in this buffer three times for 10 s at 30 s intervals, using a Omni-Prep homogenizer with a 7 mm Rotor Stator tip (Omni International, Marietta, GA, U.S.A.). Homogenates were centrifuged for 2 min at 2000 g at 4 °C (Sorvall Legend micro21R, Germany). The resulting supernatant (at a 1/10 dilution) was used for the GP assay and was diluted to 1/40 for the HOAD assay and 1/80 for the CS and PK assays. Enzyme activities are reported in U/g of tissue, where 1 U = 1 μmol substrate/min.

All assays were performed in triplicate at 37 °C 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 reaction was 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 were subtracted from rates obtained with all substrates present.

Assay conditions and substrate concentrations required to elicit V_{max} were as follows: CS: 42 mmol of Tris–HCl, pH 8.0 at ambient temperature, 0.5 mmol of oxaloacetate (omitted from control), 0.3 mmol of acetylCoA and 0.1 mmol of DTNB; PK: 40 mmol of Imidazol–HCl, pH 7.0 at ambient temperature, 10 mmol of $MgCl_2$, 100 mmol of KCl, 0.16 mmol of NADH, 5 mmol of ADP, 5 mmol of phosphoenolpyruvate (omitted from control) and 5 U of Lactate dehydrogenase; HOAD: 40 mmol of Imidazol–HCl, pH 7.0 at ambient temperature, 10 mmol of $MgCl_2$, 100 mmol of KCl, 0.16 mmol of NADH, 1 mmol of EDTA, 5 mmol of dithiothreitol (DTT) and 0.1 mmol of acetoacetylCoA (omitted from control); GP: 72 mmol of potassium phosphate, pH 7.4 at ambient temperature, 10 mmol of $MgCl_2$, 4 μmol of glucose 1,6-bisphosphate, 0.75 mmol of NADP, 2 mmol of AMP, 8 mg/ml of glycogen (omitted from control), 2.5 U of phosphoglucomutase and 2.5 U of glucose 1,6-diphosphatedehydrogenase.

Statistical Analyses

All data were analysed using JMP 8.0.2 statistical software (SAS Institute Inc., Cary, NC, U.S.A.). We used a goodness-of-fit test to test

for assumptions of normality. Non-normal data were normalized using Box–Cox transformations (pulse duration, signalling time, thorax PK, thorax CS, signalling muscle CS, PC1 size). We tested for repeatability of signalling effort following the equation $r = s_A^2/(s^2 + s_A^2)$, according to Lessells & Boag (1987). We tested for relationships between signalling parameters and body size, weight, condition, harp size (relative to body size), file length (relative to body size) and the combined mass of the signalling muscles (relative to body size) using a standard least squares regression model with all the morphological measures included as independent variables. We tested for relationships between signalling parameters, body size and enzyme activities (thorax HOAD, thorax PK, thorax CS, signalling muscle CS) using standard least squares regressions. We examined correlations among signalling parameters using pairwise correlation analyses.

RESULTS

Signalling Effort

Crickets varied considerably in their overall signalling effort (how much time they spent signalling on average over each 24 h period). Some males rarely signalled, producing acoustic mate attraction signals for only a few seconds during the entire week-long monitoring period. Others signalled extensively, signalling up to 10 h per day. Signalling effort was fairly repeatable (repeatability: $r = 0.61$, $P < 0.0001$). Thirty-six per cent of the variation in male signalling effort could be explained by variation in male body size and relative stridulatory file length (Table 2). Males that had larger bodies and longer files relative to their body size signalled with higher effort.

Given that signalling performance could be dependent on the breakdown of either carbohydrates or lipids, we examined whether variation in signalling effort was influenced by variation in enzyme V_{max} values (maximum rate of substrate conversion to product) for GP, PK, HOAD, CS, PK:CS and HOAD:CS. Enzyme V_{max} values varied across individuals (PK thorax: range 0.31–10.49, mean ± SE = 3.55 ± 0.16, CV = 46.1; HOAD thorax: range 8.48–23.13, mean ± SE = 14.64 ± 0.25, CV = 17.6; CS thorax: range 2.79–97.80, mean ± SE = 17.18 ± 1.26, CV = 76.0; CS signalling muscles: range 3.42–50.97, mean ± SE = 13.28 ± 0.81, CV = 63.2; PK:CS: range 0.02–3.28, mean ± SE = 0.37 ± 0.05, CV = 127.3; HOAD:CS: range 0.23–5.50, mean ± SE = 1.34 ± 0.10, CV = 76.9). Signalling effort variation was significantly influenced by male enzyme V_{max} values for PK. Males that signalled with higher effort had significantly higher PK activity levels compared to males that signalled with lower effort (Table 3, Fig. 1). Variation in male signalling effort was not explained by variation in HOAD or CS activities. Furthermore, rates obtained for GP activity in both cricket-signalling muscles and cricket thoraxes were not different from controls, which represents undetectable activities in these tissues. Finally, the average ratios of the thorax enzymes did not explain variation in male signalling effort.

Song Structure

Crickets also showed extensive variation in the sound of their acoustic signals. There was extensive variation among males in the duration of their pulses, interpulse durations, pulses per chirp, chirp durations, amplitudes and carrier frequencies. Seventeen per cent of the variation in pulse duration, 13% of the variation in interpulse duration, and 31% of the variation in amplitude was explained by male body size (Table 2). Males with larger bodies signalled louder and produced longer pulses with longer interpulse intervals. Fourteen per cent of the variation in carrier frequency was explained by variation in male body size and relative file length (Table 2). Males with larger bodies and longer files produced lower-frequency signals. None of the variation in the number of pulses per chirp or chirp duration was explained by variation in body size, condition, harp size, file length or signalling muscle mass (Table 2).

We also examined whether variation in song structure was influenced by variation in enzyme V_{max} values. There was a relationship between cricket PK activity and interpulse durations. Crickets that signalled with low interpulse durations had higher PK activity levels in their thorax than crickets that signalled with high interpulse durations. Enzyme V_{max} values did not appear to explain any of the variation in pulse duration, chirp duration, amplitude or carrier frequency (Table 3).

Several of the signalling parameters were strongly correlated with each other (Table 4). For example, high-effort signallers produced louder chirps, with more pulses per chirp, shorter interpulse durations and longer pulse durations than low-effort signallers. Furthermore, crickets that signalled at high amplitudes produced chirps of long duration with shorter interpulse durations; crickets that chirped at a lower carrier frequency produced chirps of long duration, with more pulses per chirp and longer pulse

Table 2
Relationships between acoustic signals and morphology/physiology of European house crickets, *Acheta domesticus*, assessed using standard least squares regressions

Signalling trait	Trait	*F*	*P*	R^2_{adj}	*df*
Signalling effort	Whole model	12.32	<0.0001	0.3590	5,96
	PC1 size	51.26	<0.0001		
	Residual mass	3.21	0.0762		
	Relative harp size	0.90	0.3446		
	Relative file length	3.79	0.0545		
	Relative muscle mass	0.56	0.4567		
Pulse duration	Whole model	4.79	0.0006	0.1709	5,87
	PC1 size	20.18	<0.0001		
	Residual mass	2.12	0.1486		
	Relative harp size	0.03	0.8726		
	Relative file length	2.06	0.1548		
	Relative muscle mass	0.34	0.5625		
Interpulse duration	Whole model	2.62	0.0293	0.1311	5,87
	PC1 size	12.20	0.0008		
	Residual mass	0.19	0.6632		
	Relative harp size	0.01	0.9406		
	Relative file length	0.00	0.9853		
	Relative muscle mass	0.70	0.4046		
Pulses/chirp	Whole model	1.1543	0.3382	0.0083	5,87
	PC1 size	2.47	0.1198		
	Residual mass	0.15	0.6974		
	Relative harp size	0.09	0.7649		
	Relative file length	2.54	0.1144		
	Relative muscle mass	0.31	0.5764		
Chirp duration	Whole model	1.6984	0.1435	0.0366	5,87
	PC1 size	5.36	0.0230		
	Residual mass	0.94	0.3351		
	Relative harp size	0.16	0.6931		
	Relative file length	2.10	0.1511		
	Relative muscle mass	0.85	0.3579		
Amplitude	Whole model	9.18	<0.0001	0.3077	5,87
	PC1 size	44.19	<0.0001		
	Residual mass	1.70	0.1958		
	Relative harp size	0.03	0.8662		
	Relative file length	0.29	0.5946		
	Relative muscle mass	0.07	0.7855		
Carrier frequency	Whole model	3.88	0.0032	0.1354	5,87
	PC1 size	6.28	0.0141		
	Residual mass	0.01	0.9041		
	Relative harp size	0.04	0.8457		
	Relative file length	13.92	0.0003		
	Relative muscle mass	0.35	0.5562		

Table 3
Relationships between acoustic signals and biochemistry of European house crickets, *Acheta domesticus*, assessed using standard least squares regressions

Signalling trait	Trait	t	P	R^2_{adj}	df
Signalling effort	HOAD thorax	0.10	0.9204	0.0000	1,106
	PK thorax	2.28	0.0246	0.0385	1,104
	CS thorax	4.23	0.5851	0.0000	1,106
	CS muscle	1.03	0.3033	0.0007	1,104
	PK:CS thorax	1.55	0.1237	0.0132	1,104
	HOAD:CS thorax	0.62	0.5345	0.0058	1,106
Pulse duration	HOAD thorax	1.46	0.1479	0.0114	1,97
	PK thorax	−0.44	0.6593	0.0000	1,95
	CS thorax	1.57	0.1199	0.0147	1,97
	CS muscle	0.61	0.5419	0.0000	1,94
	PK:CS thorax	−1.56	0.1226	0.0146	1,95
	HOAD:CS thorax	−1.33	0.1880	0.0077	1,99
Interpulse duration	HOAD thorax	1.36	0.1768	0.0086	1,97
	PK thorax	−2.15	0.0339	0.0365	1,95
	CS thorax	0.36	0.7204	0.0000	1,97
	CS muscle	−1.07	0.2852	0.0016	1,94
	PK:CS thorax	−1.22	0.2238	0.0000	1,95
	HOAD:CS thorax	−0.08	0.9382	0.0000	1,97
Pulses/chirp	HOAD thorax	0.12	0.9053	0.0000	1,97
	PK thorax	1.45	0.1508	0.0113	1,95
	CS thorax	−0.60	0.5532	0.0000	1,97
	CS muscle	0.14	0.8871	0.0000	1,94
	PK:CS thorax	1.27	0.2071	0.0000	1,95
	HOAD:CS thorax	0.66	0.5082	0.0000	1,97
Chirp duration	HOAD thorax	1.38	0.1716	0.0091	1,97
	PK thorax	−0.52	0.6013	0.0000	1,95
	CS thorax	0.65	0.5163	0.0000	1,97
	CS muscle	−0.14	0.8857	0.0000	1,94
	PK:CS thorax	−0.66	0.5107	0.0000	1,95
	HOAD:CS thorax	−0.43	0.6692	0.0000	1,97
Amplitude	HOAD thorax	0.48	0.6355	0.0000	1,97
	PK thorax	0.32	0.7489	0.0000	1,95
	CS thorax	0.46	0.6454	0.0000	1,97
	CS muscle	1.57	0.1192	0.0153	1,94
	PK:CS thorax	−0.42	0.6785	0.0000	1,95
	HOAD:CS thorax	−0.39	0.6966	0.0000	1,97
Carrier frequency	HOAD thorax	−0.40	0.6896	0.0000	1,97
	PK thorax	0.40	0.6876	0.0000	1,95
	CS thorax	−0.88	0.3818	0.0000	1,97
	CS muscle	0.33	0.7424	0.0000	1,94
	PK:CS thorax	0.99	0.3240	0.0002	1,95
	HOAD:CS thorax	0.80	0.4257	0.0037	1,97

durations; and crickets that signalled with more pulses per chirp produced chirps with reduced interpulse durations (Table 4).

DISCUSSION

Our study examined how multiple levels of organization (whole body, organs and enzymes) correlated with acoustic mate attraction signalling performance among individual male crickets. Our whole-body level results revealed that males that signalled with the highest efforts were larger than males that signalled with lower efforts (two-fold difference in body size). However, our organ-level results revealed that high-effort signallers did not differ from low-effort signallers in the relative weight of their signalling muscles, their relative harp sizes, or their relative file lengths. Our enzyme-level results revealed that high-effort signallers had double the pyruvate kinase (PK) activity compared to low-effort signallers. High-effort signallers did not differ from low-effort signallers in their β-hydroxyacyl-CoA dehydrogenase (HOAD) or their citrate synthase (CS) activity. The crickets' glycogen phosphorylase (GP) activity levels were too low to detect, which indicates that in situ glycogen is unlikely to be a main fuel source. Therefore, our

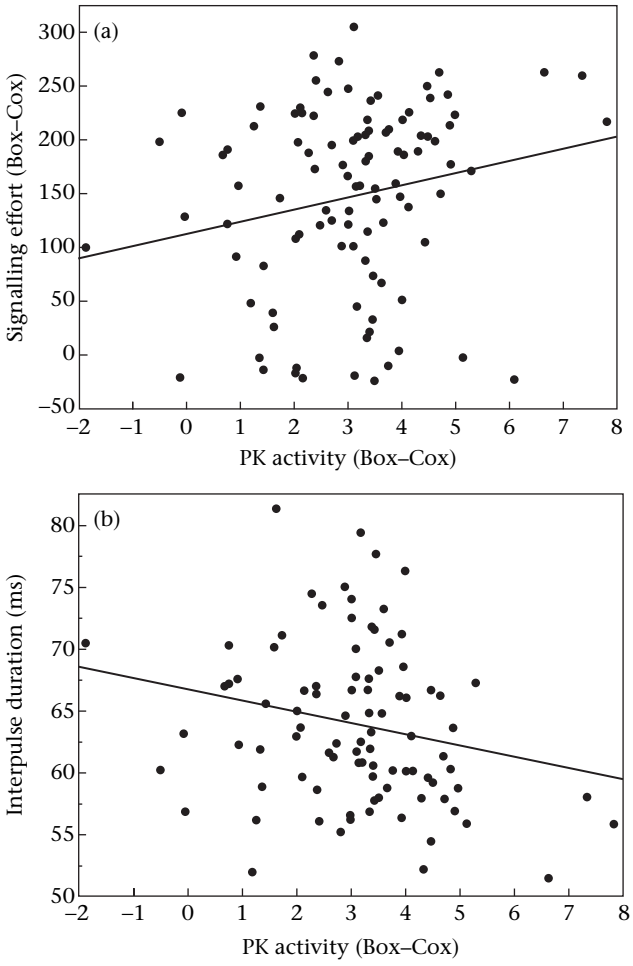

Figure 1. Influence of pyruvate kinase (PK) activity in the thorax on (a) signalling effort (average time spent signalling per night in min) and (b) average interpulse duration (in ms) in European house crickets, *Acheta domesticus*.

biochemical results suggest that circulatory carbohydrate oxidation may act as the source of energy that supplies acoustic mate attraction signalling in *A. domesticus*.

Together these enzymatic and whole-body differences between high- and low-effort signallers suggest that the ability to assimilate more nutrients during all life stages may drive some of the variation among males in their mate-signalling efforts. When all of these variables are considered together, high-effort signalling seems to be associated with the ability to grow large during juvenile stages and to acquire more carbohydrates, assimilate more carbohydrates, mobilize more carbohydrates and/or metabolize carbohydrates faster during adulthood. Future research should include experimental treatments to test these hypotheses.

Signalling Effort Morphology and Physiology

We quantified how morphological variation affected signalling variation because several studies had shown a relationship between signal structure and body morphology. Moradian & Walker (2008), for example, found that larger male *A. domesticus* tended to have larger harps (Moradian & Walker 2008). This was an important finding because Bennet-Clark (2003) revealed that the size of the file, the harp and the anal portion of the wing influences the mass of the harp, which, in turn, influences the carrier frequency of the sound (Bennet-Clark 2003). Carrier frequency is also negatively correlated with body size and harp area (Simmons &

Table 4
Relationships among acoustic signalling parameters of European house crickets, *Acheta domesticus*, assessed using correlation analyses

	IPD	PD	PPC	CD	CF	AMP	EFFORT
IPD		0.2228	<0.0001	0.0003	0.3442	<0.0001	<0.0001
PD	−0.1230		0.5221	<0.0001	<0.0001	<0.0001	<0.0001
PPC	−0.5198	0.0648		0.3029	0.0481	0.0607	0.0127
CD	0.3567	0.6448	0.1040		<0.0001	0.0044	0.3869
CF	0.0956	−0.4393	−0.1982	−0.3836		0.0703	0.1237
AMP	−0.4073	0.6476	0.1882	0.2824	−0.1818		<0.0001
EFFORT	−0.5368	0.3889	0.2485	0.0875	−0.1550	0.6379	

IPD: interpulse duration; PD: pulse duration; PPC: pulses per chirp; CD: chirp duration; CF: carrier frequency; AMP: amplitude; EFFORT: signalling effort.

Zuk 1994; Simmons 1995; Simmons & Ritchie 1996; Bennet-Clark 1998; Prestwich et al. 2000). These findings suggest that body morphology variation influences variation in signal structure.

We found that larger crickets signalled more often (had higher efforts), and also produced signals with longer pulse durations, shorter interpulse durations, longer chirp durations, at lower carrier frequencies and at higher amplitudes than smaller crickets. Given that body size is fixed at adulthood, our findings suggest that the ability to consume quality food during development may drive variation in body size, which in turn may drive variation in signalling effort and signal quality. Hunt et al.'s (2004) study on the effects of protein availability during development suggests that males that consume protein-enriched diets are larger at adulthood. Our study suggests that following adulthood, carbohydrate availability may be important for enhancing signalling efforts. Thus, to signal at high efforts and with attractive sexual signals, a male may need to consume protein rich diets to grow large during the juvenile stages, and then switch to a carbohydrate-rich diet to fuel high signalling efforts during adulthood. These ideas are speculative and need to be formally tested.

We found no significant relationship between carrier frequency and relative harp area. At first glance, this finding appears to conflict the findings of Moradian & Walker (2008), who revealed that carrier frequency is negatively correlated with harp size in European house crickets. Note, however, that our model utilized relative harp area, not actual harp area. We used relative harp area because we did not want it confounded with body size, which was a separate independent variable in our model. Carrier frequency was negative correlated with actual harp area ($F_{1,98} = 10.54$, $P = 0.0016$, $R^2_{adj} = 0.09$).

Signalling Effort Biochemistry

The relationship between signalling effort and thoracic PK activity, combined with an undetectable activity of GP, suggest that the capacity to metabolize circulatory carbohydrates may explain some of the variation in signalling effort. When comparing the ratio of activities between the glycolytic enzyme PK over the mitochondrial CS, *A. domesticus* shows an average ratio of 0.37, approaching values obtained for species powering flight muscle metabolism solely on carbohydrates, such as adult bumblebees, *Bombus impatiens* (PK/CS ratio: 0.70; Skandalis et al. 2011), or adult honeybees, *Apis mellifera* (PK/CS ratio: approximately 0.40–0.60; Schippers et al. 2010). Nevertheless, the average ratio of activities of HOAD/CS was 1.35, which also agrees with the ratio obtained for *Locusta migratoria* (range 1.22–1.31 in adults of various ages; Van den Hondel-Franken et al. 1980), a species known to power long-duration flight using fatty acids (Beenakkers 1969). Thus, it appears that thoracic muscle tissue of *A. domesticus* may be able to power aerobic metabolism using both fatty acids and circulatory carbohydrates, a metabolic phenotype observed in many insect groups (Beenakkers 1969). The significant correlation between signalling effort and PK activity alone suggests that circulatory carbohydrate is recruited during signalling, and variation in glycolytic pathway flux capacity is associated with variation in signalling effort. A more detailed description of thoracic muscle metabolic phenotype will clarify this apparent link, and manipulative studies would be necessary to assess causation.

Carbohydrates appear to be important to adult male crickets. Our findings closely align with those of Maklakov et al. (2008), who found that adult crickets fed high carbohydrate diets had higher signalling efforts. Maklakov et al. (2008) fed *Teleogryllus commodus* diets that differed in the ratio of carbohydrate to protein. Males fed the high carbohydrate to protein (5:1) diet had higher lifetime signalling efforts than males fed diets with equal (1:1) or low (1:5) carbohydrate. Together, the results from Maklakov et al. (2008) and our study seem to contradict those of Hunt et al. (2004), who found that crickets reared on low carbohydrate to protein diets had higher signalling efforts than crickets reared on high carbohydrate to protein diets (Maklakov et al. 2008). However, in Hunt et al.'s (2004) study, crickets were fed their diets from the day after hatching through to death, whereas in our study and in that of Maklakov et al. (2008), crickets were only fed their diets from adulthood onward. One possible explanation for these seemingly contradictory findings is that the availability of protein during development may be important for crickets to grow large, thus enabling them to signal with high effort in adulthood. Crickets may, however, make a switch, at adulthood, to consuming more carbohydrate-based foods to fuel their signalling efforts. These ideas are speculative and have yet to be formally tested.

Although the current study contributes to the line of evidence linking carbohydrate metabolism and signalling effort, the functional nature of the observed correlation remains to be studied. Among the possibilities, interindividual variation in metabolic flux capacity for carbohydrate metabolism could arise from genetic determinants, which in turn could limit signalling efforts. Genetic studies (Monthooth et al. 2003) showed quantitative trait loci associated with variation in flight performance and metabolic enzyme activity in *Drosophila melanogaster*, supporting the idea that genetic determinants link variation in performance and metabolic phenotype. Muscle metabolic phenotypes could also be affected by dietary fuels (e.g. Nagahuedi et al. 2009), where the level of circulatory fuels influence the expression of muscle metabolic enzymes. However, to our knowledge, studies have yet to document the effect of circulatory carbohydrates on metabolic enzyme expression in insects. This possibility can therefore not be ruled out and could in part explain the correlation observed in our study.

Finally, it should be noted that *Acheta domesticus* were obtained commercially. As such, these captive-bred crickets experienced a history of laboratory-based artificial selection, so that they thrive on artificial (unnatural) diets. Captive-bred crickets are likely to have also experienced bottlenecks and inbreeding and could, as a result, be relatively homogeneous genetically. Furthermore, laboratory-based artificial selection, inbreeding and bottlenecks

could have affected some of *A. domesticus*' physiological and biochemical traits. *Acheta domesticus* may not, therefore, be representative of other cricket species, although captive-bred crickets display similar male aggressiveness, calling, courtship and female mate choice behaviours as their wild counterparts.

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References

Beenakkers, A. M. T. 1969. Carbohydrates and fat as a fuel for insect flight: a comparative study. *Journal of Insect Physiology*, **15**, 353–361.

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

Bennet-Clark, H. C. 2003. Wing resonances in the Australian field cricket *Teleogryllus oceanicus*. *Journal of Experimental Biology*, **206**, 1479–1496.

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

Bertram, S. M. & Bowen, M. 2006. Field cricket species differences in the temporal patterns of long-distance mate attraction signals. *Ethology*, **112**, 850–857.

Bertram, S. M. & Johnson, L. 1998. An electronic technique for monitoring the temporal aspects of acoustic signals of captive organisms. *BioAcoustics*, **9**, 107–118.

Bertram, S. M., Johnson, L. A., Clark, J. & Chief, C. 2004. An electronic acoustic recorder for quantifying when, how much, and how loud individuals signal acoustically. *Technical Acoustics*, **20**, 1–15.

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.

Cade, W. H. 1980. Alternative male reproductive behaviors. *Florida Entomologist*, **63**, 30–45.

Cade, W. H. 1981. Alternative male strategies: genetic differences in crickets. *Science*, **212**, 563–565.

Cummings, J. W. 1979. Physiological and biochemical adaptations to training in *Rana pipiens*. *Journal of Comparative Physiology*, **134**, 345–350.

Gray, D. A. 1997. Female house crickets, *Acheta domesticus*, prefer the chirps of large males. *Animal Behaviour*, **54**, 1553–1564.

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

Gray, D. A. & Cade, W. H. 1999b. Quantitative genetics of sexual selection in the field cricket, *Gryllus integer*. *Evolution*, **53**, 848–854.

Harshman, L. G. & Zera, A. J. 2006. The cost of reproduction: the devil in the details. *Trends in Ecology & Evolution*, **22**, 80–86.

Hunt, J., Brooks, R., Jennions, M. D., Smith, M. J., Bentsen, C. L. & Bussiere, L. F. 2004. High-quality male field crickets invest heavily in sexual display but die young. *Nature*, **432**, 1024–1027.

Jacot, A., Scheuber, H. & Brinkhof, M. W. G. 2007. The effect of age on a sexually selected acoustic display. *Ethology*, **113**, 615–620.

Judge, K. A. 2011. Do male field crickets, *Gryllus pennsylvanicus*, signal their age? *Animal Behaviour*, **81**, 185–194.

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**, 868–878.

Lessells, C. M. & Boag, P. T. 1987. Unrepeatable repeatabilities: a common mistake. *Auk*, **104**, 116–121.

Maklakov, A. A., Simpson, S. S., Zajitschek, F., Hall, M. D., Dessmann, J., Clissolg, F., Raubenheimer, D., Bonduriansky, R. & Brooks, R. 2008. Sex-specific fitness effects on nutrient intake on reproduction and lifespan. *Current Biology*, **18**, 1–5.

Monthooth, K. L., Marden, J. H. & Clark, A. G. 2003. Mapping determinants of variation in energy metabolism, respiration and flight in *Drosophila*. *Genetics*, **165**, 623–635.

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.

Nagahuedi, S., Popesku, J. T., Trudeau, V. L. & Weber, J. M. 2009. Mimicking the natural doping of migrant sandpipers in sedentary quails: effects of dietary n-3 fatty acids on muscle membranes and PPAR expression. *Journal of Experimental Biology*, **212**, 1106–1114.

Pfau, K. & Koch, U. 1994. The functional morphology of singing in the cricket. *Journal of Experimental Biology*, **195**, 147–167.

Pomiankowski, A. & Møller, A. P. 1995. A resolution of the lek paradox. *Proceedings of the Royal Society B*, **260**, 21–29.

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

Prestwich, K. N. & Breuer, C. K. 1987. The design of advertisement calls when energy is a limiting factor. *American Zoologist*, **27**, 43A.

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). *Journal of Experimental Biology*, **208**, 1495–1512.

Prestwich, K. N. & Walker, T. J. 1981. Energetics of singing in crickets: effects of temperature in three trilling species (Orthoptera: Gryllidae). *Journal of Comparative Physiology*, **143**, 199–212.

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. *Journal of Experimental Biology*, **203**, 585–596.

Rodríguez-Muñoz, R., Bretman, A., Slate, J., Walling, C. A. & Tregenza, T. 2010. Natural and sexual selection in a wild insect population. *Science*, **328**, 1269–1272.

Roff, D. A. & Fairbairn, D. J. 1994. The evolution of alternative morphologies: fitness and wing morphology in male sand crickets. *Evolution*, **47**, 1572–1584.

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

Ryan, M. J. & Keddy-Hector, A. C. 1992. Directional patterns of female mate choice and the role of sensory biases. *American Naturalist, Supplement*, **139**, S4–S35.

Schippers, M.-P., Dukas, R. & McClelland, G. B. 2010. Lifetime- and caste-specific changes for flight metabolic rate and muscle biochemistry of honeybees, *Apis mellifera*. *Journal of Comparative Physiology*, **180**, 45–55.

Simmons, L. W. 1995. Correlates of male quality in the field cricket, *Gryllus campestris* L: age, size, and symmetry determine pairing success in field populations. *Behavioral Ecology*, **6**, 376–381.

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

Simmons, L. W. & Zuk, M. 1994. Age structure of parasitized and unparasitized populations of the field cricket *Teleogryllus oceanicus*. *Ethology*, **98**, 333–340.

Skandalis, D. A., Chinmay, R. & Darveau, C. A. 2011. Behavioural, morphological, and metabolic maturation of newly emerged adult workers of the bumblebee, *Bombus impatiens*. *Journal of Insect Physiology*, **57**, 704–711.

Tomkins, J. L., Radwan, J., Kotiaho, J. S. & Tregenza, T. 2004. Genic capture and resolving the lek paradox. *Trends in Ecology & Evolution*, **19**, 323–328.

Van den Hondel-Franken, M. A. M., Van den Broek, A. T. M. & Beenakkers, A. M. T. 1980. Flight muscle development in *Locusta migratoria*: effects of implantation of corpora allata on the attainment of metabolic enzyme activities. *General and Comparative Endocrinology*, **41**, 477–486.

Verburgt, L. & Ferguson, J. W. 2010. Mate choice in field crickets: can females acoustically detect male body size? *Journal of Ethology*, **28**, 141–151.

Verburgt, L., Ferreira, M. & Ferguson, J. W. H. 2011. Male field cricket song reflects age, allowing females to prefer young males. *Animal Behaviour*, **81**, 19–29.

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

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.

Zhao, Z. & Zera, A. J. 2002. Differential lipid biosynthesis underlies a tradeoff between reproduction and flight capability in a wing-polymorphic cricket. *Proceedings of the National Academy of Sciences, U.S.A.*, **99**, 16829–16834.

Zera, A. J. & Zhao, Z. 2003. Life-history evolution and the microevolution of intermediary metabolism: activities of lipid-metabolizing enzymes in life-history morphs of a wing-dimorphic cricket. *Evolution*, **57**, 583–596.

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**, 666–676.