RESEARCH ARTICLE

The metabolic costs of sexual signalling in the chirping katydid *Plangia graminea* (Serville) (Orthoptera: Tettigoniidae) are context dependent: cumulative costs add up fast

Marcé Doubell[1], Paul B. C. Grant[1,2], Nanike Esterhuizen[1,3], Corinna S. Bazelet[1], Pia Addison[1] and John S. Terblanche[4],*

ABSTRACT

Katydids produce acoustic signals via stridulation, which they use to attract conspecific females for mating. However, direct estimates of the metabolic costs of calling to date have produced diverse cost estimates and are limited to only a handful of insect species. Therefore, in this study, we investigated the metabolic cost of calling in an unstudied sub-Saharan katydid, *Plangia graminea*. Using wild-caught animals, we measured katydid metabolic rate using standard flow-through respirometry while simultaneously recording the number of calls produced. Overall, the metabolic rate during calling in *P. graminea* males was 60% higher than the resting metabolic rate (0.443 ± 0.056 versus 0.279 ± 0.028 ml CO_2 h^{-1} g^{-1}), although this was highly variable among individuals. Although individual call costs were relatively inexpensive (ranging from 0.02 to 5.4% increase in metabolic rate per call), the individuals with cheaper calls called more often and for longer than those with expensive calls, resulting in the former group having significantly greater cumulative costs over a standard amount of time (9.5 h). However, the metabolic costs of calling are context dependent because the amount of time spent calling greatly influenced these costs in our trials. A power law function described this relationship between cumulative cost (y) and percentage increase per call (x) ($y=130.21x^{-1.068}$, $R^2=0.858$). The choice of metric employed for estimating energy costs (i.e. how costs are expressed) also affects the outcome and any interpretation of costs of sexual signalling. For example, the absolute, relative and cumulative metabolic costs of calling yielded strongly divergent estimates, and any fitness implications depend on the organism's energy budget and the potential trade-offs in allocation of resources that are made as a direct consequence of increased calling effort.

KEY WORDS: Communication, Evolution, Sound production, Energy, Phylogenetic signal

INTRODUCTION

Calling is a conspicuous way of attracting a potential mate and is thus thought to be associated with elevated energy usage and evolutionary fitness costs (Symes et al., 2015). These signals have associated metabolic costs that are influenced by sexual selection and constrained by abiotic and biotic factors (Greenfield, 1997). Surprisingly little information is available, however, on the energetic costs of calling, and there are few firm theoretical expectations (White et al., 2008). The wing muscles used by tettigoniids during stridulation are of the very fast synchronous type and in some species the wing stroke (WS) frequency during stridulation may even exceed that of flight (Stevens and Josephson, 1977). Based on the high frequency of wing muscle contraction during stridulation, one would expect calling in these insects to incur a pronounced metabolic cost (Heath and Josephson, 1970). This expectation has been confirmed for three trilling katydid species – *Neoconocephalus robustus*, *Euconocephalus nasutus* (Conocephalinae) (Stevens and Josephson, 1977) and *Mecopoda* sp. (Erregger et al., 2017) – through increased oxygen uptake rates (an indirect measure of metabolic rate, MR) and a rise in thoracic temperature during stridulation (Heath and Josephson, 1970; Nespolo et al., 2003; Erregger et al., 2017). Empirical evidence from other Orthoptera indicate diverse estimates of energy expenditure, typically measured in terms of indirect calorimetry (either as CO_2 production or O_2 consumption rates, or converted to MR) during acoustic signalling (e.g. Prestwich and Walker, 1981; Kavanagh, 1987; Prestwich and O'Sullivan, 2005; White et al., 2008; Erregger et al., 2017). However, the cost of work performed depends on the elastic contribution to mechanical efficiency. Because insect muscle efficiency depends on its resilin content and the role of elastic tension in the cuticle (Dickinson and Lighton, 1995), any predictions of direct metabolic costs might be influenced by the relative amount of elasticity in a system and this may, in turn, vary in a species-specific manner (e.g. Burrows et al., 2008; reviewed in Qin et al., 2012). It is therefore unclear how metabolically expensive calling activity is, and particularly what the fitness consequences of elevated MR might be if raised a few percent above baseline resting levels. Regardless, this is particularly significant for understanding the evolution of calling and sexual selection from theoretical and empirical perspectives (e.g. White et al., 2008; Erregger et al., 2017; reviewed in e.g. Gerhardt and Huber, 2002).

Signalling to attract conspecifics is predominantly a male feature in the majority of Orthoptera species, owing to the increased risk of predation and higher reproductive investment for females (Kavanagh, 1987; Bailey, 1991; Riede, 1998; Korsunovskaya, 2009). In katydids, sound is produced by tegminal stridulation, by specialised forewings (tegmina) that are rapidly opened and closed. During the closing stroke, a file of minute teeth (pars stridens) along the underside of the left forewing moves over a hardened scraper (plectrum) on the upper surface of the right forewing. Each individual tooth strike across the plectrum causes an associated

[1]Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland 7602, Stellenbosch, South Africa. [2]Grant Scientific Services Ltd, 4901 Cherry Tree Bend, Victoria BC V8Y1S1, Canada. [3]Department of Botany and Zoology, Faculty of Science, Stellenbosch University, Private Bag X1, Matieland 7602, Stellenbosch, South Africa. [4]Centre for Invasion Biology, Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland 7602, Stellenbosch, South Africa.

*Author for correspondence (jst@sun.ac.za)

J.S.T., 0000-0001-9665-9405

List of symbols and abbreviations

BLED	band limited energy detector
CMR	calling metabolic rate
FMR	flying metabolic rate
ICC	intraclass correlation coefficient
MR	metabolic rate
MR_{ms}	mass-specific metabolic rate
RMR	resting metabolic rate
RMR_c	resting metabolic rate at the lowest MR period (even if an individual did call during this period)
RMR_n	resting metabolic rate at no calls
RQ	respiratory quotient
$\dot{V}_{CO_2}$	carbon dioxide production rate
$\dot{V}_{O_2}$	oxygen consumption rate
WS	wing stroke
WSR	wing stroke rate

membrane (mirror) surrounded by a sclerotised U-shaped frame to resonate and amplify sound (Greenfield, 1997). Thus, a complete cycle of the wing opening and closing (one WS) can generate a single chirp, comprising multiple pulses of sound, which are the smallest amplitude modulations within signals (Bailey et al., 1993). Wing stroke rates (WSRs) may vary from a few to several hundred per second, and are characteristic in each species for a given temperature (Prestwich and Walker, 1981). If a few WSs (i.e. chirps) are followed by a pause and then more WSs, the calling pattern is referred to as chirping. However, if many WSs occur continuously, the calling song is termed a trill (Gerhardt and Huber, 2002). Katydids have a very wide range of calling strategies in terms of the temporal structure of the call (Bailey et al., 1993). Calls may be nearly continuous, such as in many coneheads (Tettigoniidae: Conocephalinae; Josephson, 1973; Counter, 1977), or extremely brief sounds, as in the chirps of some phaneropterines (Tettigoniidae: Phaneropterinae; Heller, 1990).

For many invertebrate species, the energy expenditure during calling may approach or even surpass the level reached during other activities (Prestwich, 1994). Calling can therefore expend a large proportion of the insect's total daily energy budget, especially if maintained over a long period (Prestwich and Walker, 1981). Moreover, the cost of calling is additional to other metabolic or fitness costs that can be involved during mating (Calow, 1979). For example, the production and exchange of a nuptial gift in the form of a spermatophylax is an important reproductive strategy in katydids (Lehmann, 2012). As reproductive investment by the male entails more than just the donation of its sperm, any metabolic cost of calling could energetically constrain its mating behaviour (Arak, 1983). Furthermore, because reproductive success of singing insects is closely related to their calling success, they are under selective pressure to optimise their calling efficiency (Bennet-Clark, 1998). If females prefer males that invest more energy in their calls, their genetic material will spread through the population and, consequently, result in greater fitness (Bailey et al., 1993). If this is indeed the case, it becomes important to determine a robust estimate of the metabolic costs or energetic consequences of calling and understand the contexts in which trade-offs might be made (Symes et al., 2015).

Previous studies examining the metabolic costs of calling in Orthoptera have mainly focused on a handful of species of crickets and mole crickets, most of which call relatively continuously (Prestwich and Walker, 1981; Kavanagh, 1987; Lee and Loher, 1993; Prestwich and O'Sullivan, 2005; White et al., 2008). To our knowledge, the metabolic cost of calling has only been investigated in four katydid species, of which three produce trilling calls (Stevens and Josephson, 1977; Erregger et al., 2017). In the fourth species, Bailey et al. (1993) focused on the energetic costs of calling in the chirping katydid, *Requena verticalis* (Listroscelidinae). The present study aims to add to Bailey et al. (1993)'s findings, and the global database across taxa, by investigating the metabolic cost of calling in an unstudied sub-Saharan katydid, *Plangia graminea* (Serville 1838) (Phaneropterinae) (Hemp et al., 2015), which also produce chirping calls. We predict that, as in the case of the other orthopterans, there is a cost associated with calling; but, as found with *R. verticalis*, the cost is likely to be relatively low compared with the costs observed in trilling katydids. However, we also aim to assess diverse metrics of calling costs and how these might influence understanding of the costs. We therefore specifically compare the estimates of calling costs in absolute, relative and cumulative terms, including consideration of the power (dB) of sound produced. To better understand the intrinsic variability of our estimates, we aimed to perform a comprehensive repeatability assessment of metabolic rates and calling cost estimates across our trials for controlled and more variable conditions, using temperature in the latter case. Our final study objective was to compare the relative amounts of these costs between different activity states by interspecific comparison of literature estimates to date (following e.g. White et al., 2008) in both ordinary and phylogenetically informed statistical approaches.

MATERIALS AND METHODS
Animals
The metabolic cost of sound production was measured in adult male *P. graminea* katydids (N=11). All individuals were collected in vineyards surrounding Stellenbosch in the Western Cape of South Africa and were kept in vivaria with *ad libitum* access to lettuce, grapevines and water. The vivaria were kept at room temperature (25±5°C) in an air-conditioned laboratory at Stellenbosch University.

Experimental design
Experimental trials were conducted during late austral summer. Experiments were typically run within 2 weeks of collection but at randomised start dates to minimise any laboratory acclimation effects (e.g. Terblanche et al., 2004). Combined respirometry and calling trials were started just before dusk and continued throughout the night to cover the period when katydids usually sing in the field (Stevens and Josephson, 1977), except for two individuals on which trials were conducted during the day and night. Only one experimental trial consisting of a single male katydid was conducted per night, and each male was only tested once. Individuals were randomly selected from the vivarium and weighed to the nearest 0.1 mg using a digital microbalance (Model MS104S, Mettler Toledo, Greifensee, Switzerland) before and after their metabolic rates were measured. After each trial, the male was placed in a designated vivarium for used males to prevent it from being selected more than once.

Each experimental respirometry and calling trial consisted of three phases: (1) initial baseline period, (2) respirometry and calling period with a katydid and (3) second baseline period. During the baseline periods, respirometry measurements were taken without a katydid for ~10 min to measure potential instrument drift and to allow for baseline corrections, which were typically negligible. During the respirometry and calling period, a katydid was placed inside the 50 ml respirometry cuvette coupled to an open flow-through system. Respirometry consisted of simultaneous

measurements of CO_2 and H_2O production using a standard flow-through, push-system respirometry set-up. Compressed air, generated by an aquarium pump, was passed through sodalime and Drierite (W. A. Hammond Drierite Co., Xenia, OH, USA) scrubber columns to remove CO_2 and H_2O. Scrubbed air was fed through a flow control valve (Model 840, Side-Trak, Sierra Instruments, Monterey, CA, USA) and regulated at a fixed rate of 200 ml min^{-1} using a mass flow control unit (Sable Systems International, MFC-2, Las Vegas, NV, USA). Thereafter, air flowed through the zero channel of an infra-red gas analyser (Li-7000, Li-Cor, Lincoln, NE, USA) and through the cuvette containing the katydid. Air leaving the cuvette then entered the gas analyser through another channel, resulting in differential recordings of insect CO_2 and H_2O production logged at 1 Hz. The output of the analyser ($\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$) was recorded via Li-7000 software on a standard desktop computer. Data were exported as text files into a respirometry software program (Expedata Data Acquisition & Analysis Program, Sable Systems International) for further analysis.

Air temperature inside the respirometry cuvette was recorded at 1 Hz using a 36-standard wire gauge Type T thermocouple connected to a PicoLog TC-08 digital recording logger, with data captured by the standard PicoLog software (PicoLog for Windows 5.20.3, Pico Technology, UK). Temperature recordings were temporally synchronised with respirometry and audio recordings. Animal activity was monitored using an infrared activity detector (AD-2 Activity Detector, Sable Systems International). The cuvette containing the insect was wrapped with aluminium foil to improve activity detector readings, and placed inside an insulated cooler box container with a sound recording device. Temperature was allowed to vary (i.e. not strictly controlled) during trials, as a constant temperature might not have encouraged natural calling to be induced.

Calls produced during experimental trials were acoustically monitored and recorded in real-time using a Song Meter wildlife recorder (Model SM2+, Wildlife Acoustics, Inc., Concord, MA, USA) fitted with an omnidirectional weatherproof acoustic microphone for SM2 [sensitivity: −36±4 dB (0 dB=1 V/pa@1 kHz), frequency response: 20–20,000 Hz; Model SMX-II, Wildlife Acoustics] directly onto the left channel of the recorder. Sound recordings were made at a sample rate of 96 kHz (16 bit resolution). The recorder was pre-amplified to 48 dB gain and digitally configured to an additional 12 dB gain. Sound recordings were made continuously in 10-min intervals, with no gaps between consecutive recordings. For all experimental trials, the start and end times of sound recordings were synchronised with the start and end times of MR measurements. The total duration of the baseline, respirometry and calling periods were recorded for each experimental trial.

Respirometry and temperature data processing and analysis
In Expedata, CO_2 and H_2O data were transformed from ppm to ml CO_2 h^{-1} and ppt to mg H_2O h^{-1}, respectively. Using the marker tool in Expedata, the data were divided into the two baseline periods and the intermediate 10-min time intervals that correspond with the acoustic recording intervals per individual. H_2O data were discarded as the lag times were too large to analyse meaningfully. After correcting for baseline drift, the mean CO_2 production values for each 10-min interval were extracted for each individual. CO_2 production rate ($\dot{V}_{CO_2}$; ml CO_2 h^{-1}) was then converted to oxygen consumption rate ($\dot{V}_{O_2}$; ml O_2 h^{-1}) and to microwatts (μW) assuming a respiratory quotient (RQ) of 0.84 (Lighton, 2008) and an oxyjoule equivalent of 20.3 J ml^{-1} (Lighton et al., 1987). Resting metabolic

rates (RMRs) were considered to be the lowest MR 10-min interval recorded per individual, which was confirmed as intervals without activity by visually inspecting the activity detector recordings. Katydids were quiescent for the majority of time during the respirometry trials, and when small activity bouts were observed these never coincided with calling periods. Calling metabolic rates (CMRs) were considered to be the MR recorded during the 10-min interval with the highest calling rate for every individual. Temperature data of each individual were exported from the PicoLog software and the mean temperature was calculated for all 10-min intervals that correspond to the audio and respirometry recordings.

Acoustic data processing and metrics of calling costs
The Song Meter recorder stored each 10-min audio recording as individual uncompressed .WAV files logged to a 32 GB memory card by the Song Meter device. All audio files were analysed in Raven Pro (v. 1.5; Cornell Lab of Ornithology, Bioacoustics Research Program, NY, USA) and extraneous low-frequency sounds were filtered out using the band filtering feature [specifically, Raven uses the Window Method for FIR filter design; see Oppenheim et al. (1998) and Charif et al. (2010) for a complete description of this method]. The number of chirps (or WSs) were counted in every audio file over each 10-min interval for all individuals using the band limited energy detector (BLED). Target signal parameters for the BLED were acquired by making multiple selections for each parameter on the spectrogram and then by extracting the most appropriate values from the selection table provided by Raven for each parameter. Selections were made according to the following measures: minimum and maximum frequencies (kHz) determined the frequency range of the pulses in which the detector searched; minimum and maximum duration (ms) specified the length of signal that could be considered a single detection (or one chirp); and minimum separation corresponded to the time interval between adjacent chirps (i.e. chirp intervals) (for a complete description of this method, see Mills, 2000). After the BLED was run, a visual scan through the spectrogram was performed to ensure all detections were accurate and that no calls were missed. The number of chirps counted by the BLED represented the number of calls made during a specific interval and were correlated with the corresponding respirometry interval to obtain an estimate of metabolic rate relative to a specific calling effort. From 18 respirometry trials, 11 individuals called sufficiently throughout the trial to be included in analyses. The other seven individuals either did not call or only chirped briefly once or twice, thus limiting us from comparing calling and resting periods; therefore, they were excluded. Additionally, the peak power (dB) of the calls (detected by the BLED) was extracted from the selection table provided by Raven for a subset of six individuals (individuals 6–11). We could only perform these analyses in a subset of individuals as the hard drive storing the calling data of individuals 1–5 was corrupted. The dB values of the calls were averaged over their respective 10-min intervals and represented the call power for corresponding time intervals.

The cost of calling was estimated using a set of different metrics for each individual. The metrics employed were: (1) MR (ml CO_2 h^{-1}); (2) mass-specific MR (MR$_{ms}$; ml CO_2 h^{-1} g^{-1}); (3) MR per call (ml CO_2 h^{-1} call^{-1}); MR$_{ms}$ per call (ml CO_2 h^{-1} g^{-1} call^{-1}); (4) Δcost of calls (calculated as the difference between CMR$_{ms}$ and RMR$_{ms}$ and expressed per call); (5) percentage cost of calls (metabolic cost of calling expressed as the % change over resting rates divided by the number of calls); and finally (6) the cumulative energy cost of calling

inferred from the total number of calls and each call's relative cost summed over time. The cumulative cost was calculated over the longest single contiguous period (17:30 to 03:00 h, i.e. 9.5 h) for each individual trial. However, individuals 6 and 9 had only 9 h overlap during this period, and individual 4 only had 3.5 h overlap. As a result, the cumulative cost calculated for individuals 4, 6 and 9 over their respective time periods was extrapolated to a 9.5 h period.

Repeatability assessment

Repeated measurements of mean MR and number of calls recorded over the 10-min intervals during respirometry trials of the 11 calling males were used to estimate the repeatability of MR and various metrics of calling costs. The intraclass correlation coefficient (ICC) was estimated, following methods described by Wolak et al. (2012), in the icc package (v. 2.3.0) run in R version 3.3.1 (R Core Team, 2013). Measurements tested for repeatability were MR (ml CO_2 h^{-1}); MR_{ms} (ml CO_2 h^{-1} g^{-1}); number of calls; MR per call (ml CO_2 h^{-1} $call^{-1}$); MR_{ms} per call (ml CO_2 h^{-1} g^{-1} $call^{-1}$); Δcost of calls; and percentage cost of calls. These repeatability estimates were obtained from the full data set (N=11, allowing temperature variation) as well as only using a subset of the data (N=10, 10-min sections representing controlled temperature conditions between 22 and 24°C), to test whether repeatability is affected by extrinsic factors – temperature in this case. One individual was excluded from the subset of data because the mean temperature during its trial was >27°C. To assess whether temporal autocorrelation might be influencing the repeatability results, we examined this per individual in STATISTICA (StatSoft, Tulsa, OK, USA) using time-series forecasting tools on the 10-min extracted data and found little to no significant autocorrelation.

Interspecific data comparison

To better understand the range of variation we observed and place it into context of other activities and species, RMR and CMR of *P. graminea* measured in this study were compared with MRs of other resting Orthoptera, calling Orthoptera and flying insects. Values for MRs and body mass were compiled from the literature (Table S1). Mass-specific MRs were converted back to MR by multiplying with fresh (wet) body mass. Data were available in a variety of units, and these were converted to microwatts (μW) assuming an RQ value of 0.84 and an oxyjoule equivalent of 20.3 J ml^{-1}. When necessary, MR data were adjusted to 25°C assuming a Q_{10} of 2.0, with MR roughly doubling with a 10°C increase in temperature (Nespolo et al., 2003; Terblanche et al., 2007; Irlich et al., 2009; reviewed in Dell et al., 2011), which was also the case in *P. graminea* here. In the case where several studies had measured the same species' MR, we calculated the mean across these studies. All data were normalised by logarithmic ($\log_{10}$) transformation.

RMR of 393 insect species from 16 orders and 87 families were obtained from published data (Chown et al., 2007). For this study, however, we only focused on a sub-set of those data and included the 32 species of resting Orthoptera for the purpose of clarity on the graph and also because of the level of variation that exists in RMR amongst insects for various physiological, ecological and evolutionary reasons (see discussions in e.g. Nespolo et al., 2003; White, 2011; White and Kearney, 2014). Additionally, we obtained CMR values of 14 orthopteran species from previously published data (Table S1), and flying MR (FMR) values for 56 insect species from six orders from Niven and Scharlemann (2005). FMR values were included as an upper boundary on metabolic rates that might be expected across the Insecta (following e.g. White et al., 2008). The effect of phylogenetic signal on the relationship of metabolic rate to

body mass was investigated by means of a phylogenetic generalised least-squares (PGLS) analysis (details in Appendix).

Statistical analysis

Statistical tests were performed using STATISTICA v.13 or R v.3.2.4 using the 'lme4' library for the linear mixed-effects model. Data were checked for normality using the Shapiro–Wilk test. RMR and CMR data were compared using appropriate pairwise tests for dependent samples based on the normality of the distribution of the data. When comparing mean RMR with mean CMR, the RMR was expected to be the lowest MR period for each individual when it was not calling, and CMR was considered to be the MR during the period with the highest calling rate for each individual. However, we discovered that the period with the lowest MR for each individual was not necessarily a period without calls for every individual. This was true for three individuals. Therefore, we decided to include both scenarios in the analysis: (1) comparing the RMR at no calls (RMR_n) with CMR, and (2) comparing the RMR at the lowest MR period (even if an individual did call during this period, RMR_c) with CMR. We also compared RMR_n and RMR_c with each other. Non-parametric sign tests were used to compare RMR_n, RMR_c and CMR with each other because the data were not normally distributed.

To test whether temperatures at RMR_n, RMR_c and CMR were similar, dependent samples t-tests were performed to compare the three pairs. Temperature data at RMR and CMR were compared using dependent samples t-tests, after verifying that the dependent variable was approximately normally distributed and that there were no significant outliers.

For the repeatability assessment, significantly different ICC values between the complete dataset and the subset of data were determined through inspection of the 95% confidence intervals of the respective parameters. If the 95% confidence intervals of the parameters did not overlap between the complete dataset and the subset, the ICC values were considered significantly different from each other (P<0.05).

RMR and CMR values for *P. graminea* measured in this study were compared with RMR and CMR measurements of other orthopteran species and FMRs of flying insects based on literature on scaling of energy use. To investigate how RMR and CMR of *P. graminea* compared with RMRs, CMRs and FMRs of other species, P-values were determined using prediction levels in relation to the respective regression lines, following Cooper and Withers (2006). In all tests, we assumed P=0.05 as the critical value for rejecting a null hypothesis.

RESULTS

The insects were typically quiescent during daylight hours in the laboratory, as they are in the field (Stevens and Josephson, 1977). At dusk they became active and started calling. However, placing them in a dark chamber during the day was sufficient to disrupt quiescence. Two trials were initiated during the day and in both cases the insects started calling within an hour of placement in the darkened respirometry chamber. The other nine trials were started just before dusk and continued until the next morning. Activity detector readings showed that the animals were mostly inactive for the entire time, and traces showed very little activity even when calling. Calling never coincided with other activities.

Overall, calling activity was significantly positively correlated with $\dot{V}_{CO_2}$ (R=0.73, P<0.0001), where $\dot{V}_{CO_2}$ increased with an increase in number of calls, although this was variable among individuals (Fig. 1A). However, when comparing a generalised linear model (GLM) versus a general linear mixed-effects (GLME) model
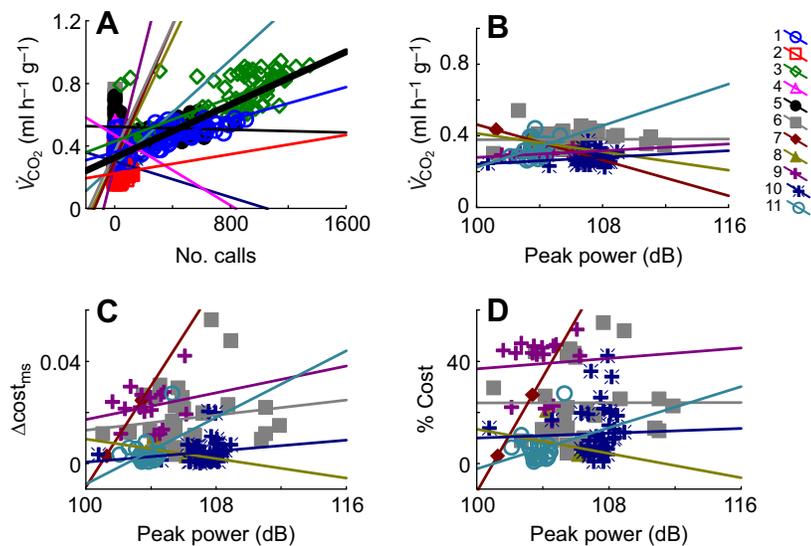
Fig. 1. Scatterplots showing the relationship between various calling (sound) estimates and metabolic rate of call cost estimates in katydids. (A) $\dot{V}_{CO_2}$ was significantly positively correlated with number of calls ($y=0.0004x+0.327$; $R=0.73$, $P<0.0001$). (B) $\dot{V}_{CO_2}$ versus peak power of calls recorded (overall trend: $P=0.189$). (C) Metabolic cost of calling expressed as the difference between the mass-specific metabolic rate during calling minus mass-specific resting metabolic rate when not calling divided by the number of calls plotted against peak calling power recorded (overall trend: $P=0.76$). (D) Metabolic cost of calling expressed as the percentage change over resting rates divided by the number of calls versus peak calling power (overall trend: $P=0.521$). Each individual is shown as a unique line colour; the overall trendline is shown in black [bold indicates statistical significance (only in the case of A)]. Note that power graphs were only for a subset of six individuals for which these data could be estimated.

(accounting for individuals as random effects), we found that the GLME model is a significantly better model (ΔAIC: 626.8) and, therefore, accounting for individuals is important. There was no relationship between the power of calls and $\dot{V}_{CO_2}$ ($P=0.189$) (Fig. 1B), and call power was not related to Δcost ($P=0.76$) or percentage cost ($P=0.521$) as estimates of the metabolic cost of calling (Fig. 1C,D).

The mean total number of calls per trial was 347.6±137.8 (range: 9–1350). The mean percentage increase in MR from RMR to CMR was 60.1±12.7% in calling males (maximum 154%). However, the mean (±s.e.m.) percentage increase per call (or per WS) was ca. 1± 0.5% (Table 1). Expressing the cost of calling in different metrics yielded different results. An individual with a relatively high calling rate experienced a small percentage increase in MR (individual 5, call rate=826; absolute increase=13.6%), whereas an individual with a low calling rate (individual 9, with a maximum calling rate of 9) experienced an absolute increase in MR of ca. 50% (Table 1). However, the cost of calling for a 'cheap' caller with a high calling rate accumulated rapidly over time and, therefore, individual 5 experienced a high cumulative cost (3735.2 ml CO_2 h^{-1} g^{-1}) compared with a more expensive caller (individual 9, 19.8 ml CO_2 h^{-1} g^{-1}) with a low call rate (Table 1). A power law

function described this relationship between cumulative cost and percentage increase per call ($y=130.21x^{-1.068}$, $R^2=0.858$), where y is cumulative cost and x is percentage increase per call.

Sign tests indicated that both RMR$_n$ and RMR$_c$ were significantly different from CMR ($Z=3.015$, $P=0.003$), but they were not significantly different from each other ($Z=1.155$; $P=0.248$; Fig. 2A). There was no significant difference in air temperature within the respirometry cuvette between RMR$_n$ (23.6±3.1°C) and RMR$_c$ (22.9 ±2.2°C; $t=-1.384$, $P=0.196$, $N=11$) as well as RMR$_n$ and CMR (23.5 ±2.0°C) ($t=0.229$, $P=0.824$, $N=11$), but there was a small yet significant difference between RMR$_c$ and CMR ($t=-2.329$, $P=0.042$, $N=11$; Fig. 2B). From here onwards we refer to RMR$_n$ as RMR and exclude RMR$_c$ from further analysis, as there was no significant difference in mean RMR$_n$ and RMR$_c$, and no significant difference in temperature at RMR$_n$ and CMR. Across all individuals for the 'no calling' periods only, the RMR$_n$ was positively related to temperature [$y=0.0320±0.002x-0.410±0.040$ (mean±s.e.m.); $R^2=0.39$; $F_{1,529}=329.18$, $P<0.0001$] and had a typical Q_{10} effect ($Q_{10}=2.09$).

Mean CO_2 production rate for calling males was 0.443± 0.056 ml CO_2 h^{-1} g^{-1}. This was an increase of approximately 1.6 times the mean resting rate of 0.279±0.028 ml CO_2 h^{-1} g^{-1} (Table 1).

Table 1. Summary statistics from *Plangia graminea* respirometry and acoustic recordings

Individual	Body mass (g)	Mean temperature (°C)	RMR (not calling) (ml CO_2 h^{-1} g^{-1})	CMR (max. call effort) (ml CO_2 h^{-1} g^{-1})	Number of calls	Increase in MR (%)	Increase per call (%)	Cumulative cost (ml CO_2 h^{-1} g^{-1})
1	0.64	24.6	0.287	0.568	905	98.0	0.1	2468.7
2	0.83	21.5	0.170	0.226	118	33.0	0.3	191.0
3	0.77	27.1	0.359	0.915	1350	154.5	0.1	4614.6
4	0.88	26.0	0.385	0.407	159	5.7	0.04	579.2
5	0.70	27.4	0.476	0.540	826	13.6	0.02	3735.2
6	0.79	22.8	0.258	0.456	49	76.8	1.6	122.0
7	0.58	22.0	0.233	0.433	62	86.2	1.4	139.0
8	0.65	21.9	0.244	0.357	38	46.5	1.2	89.1
9	0.60	22.7	0.219	0.326	9	48.6	5.4	19.8
10	0.75	22.0	0.223	0.319	150	43.1	0.3	318.1
11	0.68	21.8	0.213	0.330	158	54.9	0.3	320.7
Mean±s.e.m.	0.71±0.03	23.6±0.7	0.279±0.028	0.443±0.056	347.64±137.80	60.1±12.7	1.0±0.5	1145.2±500.3

Percentage increase in metabolic rate (MR; CO_2 ml h^{-1} g^{-1}) from resting metabolic rate (RMR; CO_2 ml h^{-1} g^{-1}) to calling metabolic rate (CMR; CO_2 ml h^{-1} g^{-1}), and expressed per call (at maximum calling effort) for 11 *Plangia graminea* individuals.
Cumulative costs of calling estimated as the mean cost of calling multiplied by the number of calls per individual over 9.5 h. Note that estimates are for 10 min interval summaries and are conditional on the number of calls being at maximum (full details in Materials and methods).
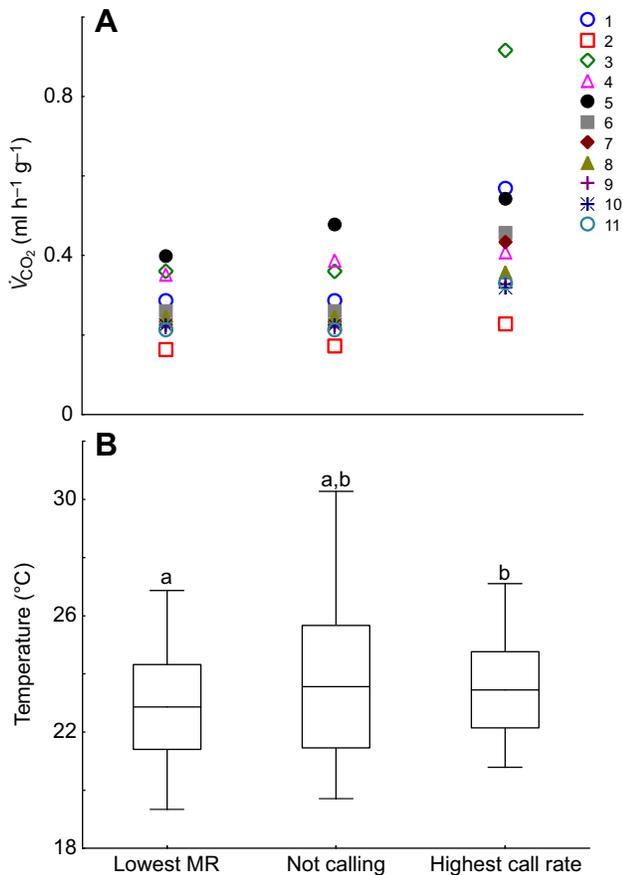
Fig. 2. Comparison of mean CO_2 production rates and temperature in different metabolic and activity states. (A) Mean CO_2 production ($\dot{V}_{CO_2}$; ml CO_2 h^{-1} g^{-1}) in respirometry trials from 11 individuals. (B) Air temperature (°C) measured inside the respirometry cuvette during each of the 11 experimental trials in A. The data are presented for the mean of the lowest MR interval, the period when katydids were not calling and the interval with the highest calling rates recorded. Box plot boundaries show the 95% confidence intervals and the solid horizontal line is the mean. Error bars above and below the box indicate minimum and maximum temperatures. Different letters indicate statistically significant homogeneous groups.

The energy required for production of the calling song was the total energy used during calling minus the resting metabolic rate (Kavanagh, 1987). For *P. graminea* this was 2280.34−1430.25=850.09 µW.

In the complete dataset (with temperature varying between ca. 20 and 30°C), repeatability was high for MR, MR_{ms} and the number of calls produced, but low for the various measurements of cost of calling (MR per call, Δcost and %cost). However, when considering only the subset of data representing a controlled temperature range (22–24°C), repeatability increased for all parameters, and significantly so for MR per call, MR_{ms} per call and Δcost (Table 2).

In the interspecific comparison, resting, calling and maximum recorded calling MR of *P. graminea* all fall within the 95% prediction level of resting Orthoptera (P=0.822, 0.65 and 0.203, respectively) as well as the 95% prediction level of calling Orthoptera (P=0.062, 0.132 and 0.29, respectively), but are all significantly lower than MR from flying insects (P<0.001; Fig. 3). There was significant phylogenetic signal for FMR–mass scaling, but not in the case of RMR or calling Orthoptera mass scaling (Table S2, Fig. S4).

DISCUSSION
While it is widely postulated that the energetic costs of sexual signalling are likely to be high (e.g. Heller and von Helversen,
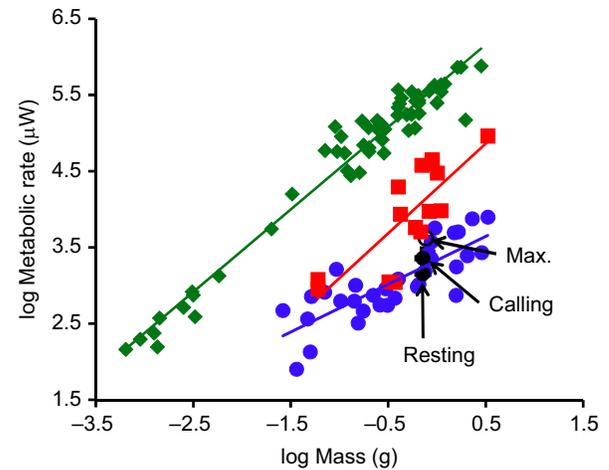

Fig. 3. Scatterplot showing the linear relationships between metabolic rate (MR, µW) and body mass (*M*, g) for resting, calling and flying insects. Resting and calling MR of *Plangia graminea* is shown in black filled circles (mean±s.d.). The maximum metabolic rate recorded in this study for a calling *P. graminea* individual is shown as a black open circle. Metabolic rates of flying insects [green filled diamonds; ordinary least-squares (OLS) regression (y=mx+c): y=1.081x+5.617; phylogenetic generalised least squares (PGLS) regression: y=0.906x+5.245], resting Orthoptera (blue filled circles; OLS: y=0.626x+3.33; PGLS: y=0.561x+3.351) and other calling Orthoptera (red filled squares; OLS: y=1.176x+4.274; PGLS: y=1.27x+4.32). Metabolic rates are normalised to 25°C assuming a Q_{10} of 2.0 (our estimate for *P. graminea* Q_{10} was 2.09). PGLS fits are not shown here because they were virtually indistinguishable from the OLS lines in each case (Fig. S4). Names of species included in this graph with their respective values are provided in Table S1.

1993), and the efficiency of calling is often argued to be significant in the evolution of sexual signalling (e.g. Gerhardt and Huber, 2002) and in trade-offs or the evolution of calling (Symes et al., 2015), the direct estimates of costs typically do not support these views. Indeed, the metabolic costs of communication are often small (or insignificant) relative to other activities based on direct estimates (e.g. Bailey et al., 1993; White et al., 2008). Therefore, we provided a detailed comprehensive assessment of diverse cost metrics expressed in different ways and across more controlled versus more variable conditions. One major novel outcome of the approach we undertook is that it provides strong support for the view that an estimate of metabolic cost of calling or communicative sound production depends heavily on the context. More specifically, the cumulative costs are pronounced for those individuals with low calling metabolic cost per call and are described by a power law function. If cumulative energy costs were extrapolated to multiple nights spent calling and/or calls were sustained for a long period owing to extrinsic factors (e.g. windy or noisy environments), for example, then such energy-related expenses could well become a significant proportion of an insect's lifetime energy budget and result in significant trade-offs. Our results show a small but significant metabolic cost associated with calling effort in *P. graminea*, although the approach we used likely maximised these costs, an inadvertent consequence of our efforts to obtain robust metabolic rate estimates during calling bouts. The CMR of *P. graminea* males was significantly higher than their RMR based on these estimates. The RMR for *P. graminea* (0.332 ml O_2 h^{-1} g^{-1} at ~22–27°C) is similar to that of most other insects (0.30–0.48 ml O_2 h^{-1} g^{-1}; Bailey et al., 1993) and to that predicted from the scaling relationship in Chown et al. (2007) (predicted MR of a 0.71 g individual=3.138 µW versus our estimate of MR=3.155 µW; t_{391}=0.653, P=0.743). The MR of calling in *P. graminea*

Table 2. Repeatability estimated from the intraclass correlation coefficient (ICC) with 95% confidence intervals, sample size (n), mean number of observations per *Plangia graminea* individual (k), for various metabolic or calling cost measurements using the complete dataset (with temperature variation ~20–30°C) and a subset of the data (measurements only between 22 and 24°C)

| | ICC (lower 95% CI – upper 95% CI) | | | | | |
	Complete dataset	n	k	Subset (22–24°C)	n	k
MR (ml CO_2 h^{-1})	0.7757 (0.6231–0.9149)	11	41.2	0.8462 (0.7083–0.9496)	10	12.3
MR_{ms} (ml CO_2 h^{-1} g^{-1})	0.7616 (0.6041–0.9086)	11	41.2	0.8154 (0.6600–0.9382)	10	12.3
Number of calls	0.5741 (0.3890–0.8086)	11	41.2	0.8631 (0.7362–0.9557)	10	12.3
MR per call (ml CO_2 h^{-1} $call^{-1}$)	**0.0892 (0.0334–0.2614)**	**11**	**41.2**	**0.5040 (0.2919–0.7846)**	**10**	**12.3**
MR_{ms} per call (ml CO_2 h^{-1} g^{-1} $call^{-1}$)	**0.1005 (0.0396–0.2840)**	**11**	**41.2**	**0.5604 (0.3458–0.8189)**	**10**	**12.3**
ΔCost	0.0938 (0.0359–0.2708)	11	41.2	0.4712 (0.2625–0.7629)	10	12.3
$ΔCost_{ms}$	**0.1041 (0.0416–0.2911)**	**11**	**41.2**	**0.5130 (0.3002–0.7903)**	**10**	**12.3**
%Cost	0.2008 (0.0978–0.4531)	11	41.2	0.5467 (0.3322–0.8108)	10	12.3

$$\Delta \text{Cost} = \frac{\text{MR} - \text{RMR}^*}{\text{Number of calls}}$$

$$\Delta \text{Cost}_{ms} = \frac{\text{MR}_{ms} - \text{RMR}^*_{ms}}{\text{Number of calls}}$$

$$\% \text{Cost} = \left[\frac{\text{MR}}{\text{RMR}} \times 100 \right] / \text{Number of calls}$$

*RMR=lowest MR period recorded per individual; MR_{ms}=mass-specific MR.
Values in bold indicate significantly different ICC values between the complete dataset and the subset (22–24°C) for specific parameters.

(0.443 ml CO_2 h^{-1} g^{-1}) is elevated to approximately 1.6 times over resting levels. This translates to an approximate 60% increase in RMR in the 11 calling males analysed in this study, and the energy required for the production of the calling song (±850 µW).

Although we observed a significant increase in MR owing to calling in this study, this increase is perhaps relatively low when compared with other calling Orthoptera (Fig. 3). This can be explained by the nature of the calling song. The calls of chirping species consist of short bursts of sound followed by a pause (Lee and Loher, 1993; Gerhardt and Huber, 2002), with WSRs an order of magnitude slower than species that produce trilled calls (Weissman et al., 1980), where a number of sound chirps are produced in rapid succession without extended pauses (Prestwich and O'Sullivan, 2005). The order of magnitude greater WSRs of trilling species mean that more mechanical work is performed, assuming constant costs, which, in turn, translates to the higher rates of metabolism reported in these species (Prestwich and Walker, 1981; Kavanagh, 1987; Lee and Loher, 1993; Hack, 1998; Prestwich and O'Sullivan, 2005). However, it is important to note that muscle work may not necessarily correlate with calling effort owing to elasticity, which can result in muscle contractions being far more efficient than might be expected (e.g. Dickinson and Lighton, 1995; Qin et al., 2012). Therefore, actual muscle work depends on the elastic contribution of resilin and the cuticle, which, in turn, could mask the detection of metabolic costs of calling and influence estimates of mechanical efficiency. In jumping insects [e.g. the froghoppers (Hemiptera, Cercopoidea)], energy needed for jumping is stored by means of a composite structure of chitinous cuticle and resilin (Burrows et al., 2008). In the same way, energy needed for wing movement in katydids to produce their calling songs can perhaps be stored, similar to the sound-producing tymbals of cicadas (e.g. *Cyclochila australasiae*, Bennet-Clark, 1997; *Tympanistalna gastrica*, Fonseca and Bennet-Clark, 1998), and therefore, work performed may be low while sounds appear costly. Like *P. graminea*, there are many other orthopterans that produce chirping songs. The elevation in MRs reported for three chirping crickets – *Acheta domesticus* (1.5× resting; Hack, 1998), *Teleogryllus comoddus* and *Teleogryllus oceanicus* [both ca. 2× resting; Lee and Loher (1993); however, Kavanagh (1987) reported a fourfold increase]

– is similar to the values reported for two chirping katydids *R. verticalis* (Bailey et al., 1993) and *P. graminea* (present study), both of which experienced an increase of approximately 1.6× their resting rates. By contrast, elevated MRs reported for trilling species ranged from five to 13 times that of their respective resting MRs (Stevens and Josephson, 1977; Prestwich and Walker, 1981; Kavanagh, 1987; Bailey et al., 1993; Lee and Loher, 1993; Hack, 1998; Prestwich and O'Sullivan, 2005; White et al., 2008; Erregger et al., 2017). The conehead katydids *E. nasutus* and *N. robustus* produce trilling calls and experience a more than sixfold increase in MR during stridulation (Stevens and Josephson, 1977). This is fourfold the increase reported for *P. graminea*, which is a similarly sized katydid. Interestingly, the trilling tree cricket *Oecanthus quadripunctatus* reaches a calling MR comparable to that of *P. graminea* during stridulation, even though it has a significantly smaller body size (Prestwich and Walker, 1981). In other words, the relative cost of calling likely varies across taxa and is partly dependent on the nature of the call (see also Erregger et al., 2017) and the number of file teeth struck per WS (Prestwich and Walker, 1981).

Resting and calling metabolic rates are significantly lower compared with the MR–mass scaling relationships of flying insects (Fig. 3), a result that is largely in keeping with previous studies (e.g. Prestwich and Walker, 1981; White et al., 2008). For example, White et al. (2008) reported that the CMR of the mole cricket, *Gryllotalpa monanka*, is only 10% that of the MR predicted for a 0.89 g insect based on the scaling relationship that they derived for flying insects using data acquired from the available literature (MR=$59.7M^{0.82\pm0.09}$ [95% CI]). Using the same trendline to predict the FMR for an insect with the average mass of *P. graminea* (0.71 g), the CMR for *P. graminea* would be less than 1% that of the predicted value (0.38 ml O_2 h^{-1}). In contrast, a flying female of the same mass consumes a similar amount in only 30 s spent searching for a male, assuming similar muscle mechanical efficiencies between different activities (e.g. flying and calling). It is interesting to note that the same set of muscles used to move the wings during flight are used by katydids and crickets during stridulation (Stevens and Josephson, 1977; Lee and Loher, 1993). However, Stevens and Josephson (1977) reported that the katydids *E. nasutus* and *N. robustus* had WSRs an order of magnitude higher during stridulation than a similar-sized desert locust during flight. Even so, the MR of the wing

muscles for the two katydids was less than in a flying locust. Why, then, is flight so much more expensive than stridulation? Weis-Fogh (1964) concluded that the aerodynamic work during flight is three to five times greater than the inertial work, i.e. the work required to accelerate the oscillating wings of a flying locust. This suggests that there are other aspects of flight that add to the overall metabolic cost of flight and likely contribute to this variation.

It is widely accepted that ambient or body temperature affects many aspects of the functional performance of insects, including biochemical and physiological processes (Nespolo et al., 2003; Terblanche et al., 2007; Irlich et al., 2009; Dell et al., 2011; Halsey et al., 2015). Therefore, an increased MR may be a result of increased temperature and is not necessarily due to activities such as calling. Moreover, it has been shown that calling in itself may also increase the insect's body temperature (Heath and Josephson, 1970; Stevens and Josephson, 1977; Erregger et al., 2017). This is especially true for species that produce trilling calls, where WSRs are high and sound is produced almost continuously (Bailey et al., 1993). The thoracic temperature of the katydid N. robustus, for example, is 5–15°C higher than that of the environment when producing its trilling calls (Heath and Josephson, 1970). Stevens and Josephson (1977) also reported an average increase of 16.6°C in body temperature of calling N. robustus specimens. These katydids seemingly depend on this heat production in order to achieve greater acoustic power outputs (Heath and Josephson, 1970; Stevens and Josephson, 1977). Where chirping species are concerned, however, heat production as a result of calling is low and thoracic temperatures typically remain similar to ambient levels (Bailey et al., 1993). In this study, although there was a typical Q_{10} effect overall, the variation in temperature was relatively small within individuals over each trial and therefore the mean levels remained quite similar between MR and calling cost estimates within individuals.

Although the overall increase in MR with calling was significant for the 11 individuals analysed in this study, there was considerable variation noted among individuals. The total number of calls produced per individual ranged from nine to 1350, and the percentage increase in RMR ranged from 5 to almost 155% (Table 1). In addition to temperature, body mass is another immediate determinant of MR in insects (e.g. Nespolo et al., 2003; Chown et al., 2007; Riveros and Enquist, 2011), which may have attributed to the variation among individuals in this study. However, the differences in mass in P. graminea were not to such a degree that could explain the level of variation found in MR recorded here. The more plausible explanation for the variation in MR experienced by the males in this study is that some males merely called less actively than others.

Male crickets can facultatively adjust their calling strategy to fit local conditions (Hack, 1998). According to Hack (1998), the relative prevalence of calling and non-calling strategies among conspecific male crickets appears to be mediated by population density. For example, the daily calling durations of individuals in Gryllus campestris field populations vary widely, and males within the same population vary independently of each other (Rost and Honegger, 1987). Presumably, changes in the social environment or small-scale interactions among individuals, rather than larger-scale changes in the ecological or physical environment [e.g., temperature (Walker, 1983)], which would affect individuals in the same population similarly, give rise to this variation (Hack, 1998).

Previous studies on field crickets have indeed shown that increases in local density cause males to abandon calling for a non-calling mate-searching strategy, and to abandon site defence

(Alexander, 1961; French and Cade, 1989; Hissmann, 1990; Cade and Cade, 1992). Although both strategies can occur at high densities, the majority of males in high density environments pursue a non-calling rather than calling strategy (e.g. Hissmann, 1990). Although katydids are distantly related to crickets and no studies have established whether katydid mating behaviour is similarly affected by population density, it is possible that the density of males kept in vivaria in this study may have caused some of the males to switch to a non-calling strategy and presumably caused some of the variation in calling frequency recorded during the respirometry experiments. This behaviour, however, should be more prevalent in trilling species because a trilling male expends energy at roughly the same rate whether calling or walking and, therefore, would be more prone to switch between the two strategies (Hack, 1998). In contrast, the energy cost differential between calling and walking in chirping species is much greater, making it less beneficial for chirpers to abandon a calling strategy (Hack, 1998).

The ICC values reported for MR of P. graminea in this study are within range of what is expected from measurements of other insects over shorter time scales (<24 h) (e.g. Marais and Chown, 2003; Nespolo and Franco, 2007; reviewed in Wolak et al., 2012). Under conditions with temperature variation, repeatability of MR and number of calls of P. graminea was high but for some estimates of the cost of calling, repeatability was low (Table 2). However, under conditions where costs were estimated only for a controlled temperature range (22–24°C), repeatability increased significantly. This indicates that repeatability, and costs associated with activities such as calling, are context dependent. Most importantly, estimates of metabolic costs depend on the context in which they are measured and how such costs are expressed. Partly this is an issue of choice of units of measurement (e.g. percentage increase versus absolute increase will naturally yield divergent estimates of cost) but also because a 'snapshot' view of energy costs may be wholly inadequate. Estimates of the cumulative energy cost of calling inferred from the total number of calls and each call's relative costs in our study yielded an entirely different view. From this analysis, individuals with a 'cheap' call spend far more time calling and thus incur a high cumulative cost; individuals with expensive calls spend very little time calling and have low cumulative cost. This is a novel and important demonstration of the value of an energy budget approach to considering the problem of communication and its energetic consequences.

APPENDIX
Testing for phylogenetic signal
Because we expected more closely related species to behave more similarly (i.e. presence of phylogenetic signal in data; Blomberg et al., 2003), we conducted three analyses to analyse the presence and extent of phylogenetic signal in the data, and to take this signal into account when analysing the relationship of mass to metabolic rate for three insect behaviours: flying, resting and calling.

Three phylogenetic trees were constructed: for flying insects, for resting Orthoptera and for calling Orthoptera (Figs S1–S3). For flying insects, a tree was constructed using the R package rotl (Michonneau et al., 2016), which searches the Open Tree of Life for taxa and constructs a tree with no branch lengths. Both Orthoptera phylogenies were constructed manually in Newick format according to phylogenetic relationships among subfamilies as published in Song et al. (2015) and Chintauan-Marquier et al. (2016) and drawn in figtree (http://tree.bio.ed.ac.uk/software/figtree/). In order for the trees to be usable in PGLS analyses, node labels were added and

polytomies were resolved randomly to dichotomies using the ape package in R (Paradis et al., 2004). Owing to the absence of available information for most species, branch lengths were uniformly set to 1.00 manually for all trees.

In order to test for strength of phylogenetic signal, two metrics were calculated. Blomberg's K was estimated for log metabolic rate of each group of species using the function Kcalc in the picante package in R (Kembel et al., 2010). K close to 0 indicates no phylogenetic signal, K approaching 1 indicates a trait signal as would be expected under Brownian motion, and $K>1$ indicates a strong phylogenetic signal in the trait (Blomberg et al., 2003; Erregger et al., 2017). Pagel's λ was estimated in the R package caper (www.R-project.org/package=caper). The λ value ranges from 0 to 1, with the closer the value to 1, the stronger the phylogenetic signal (Pagel, 1999).

PGLS analysis was run in the package caper, using the function pgls. The relationship of log metabolic rate (response variable) to log mass (explanatory) was modelled for each of the three insect behaviours and species groups. The K-value was read in as the calculated value from Kcalc. For each of the three behaviours, an OLS which did not take phylogenetic signal into account was compared with PGLS using Akaike's information criterion (AIC). The lower the AIC value, the better the performance of the tested model (Burnham and Anderson, 2002).

Results

The relationship of metabolic rate to body mass had a relatively strong phylogenetic signal for flying insects ($\lambda=0.953$) which approached Brownian motion ($K=0.635$). For these species, PGLS performed better than OLS, indicating it is important to take phylogenetic signal into account (Table S2, Fig. S4).

For resting and calling Orthoptera, there was no phylogenetic signal in the relationship of metabolic rate to mass ($\lambda=0.000$, $K_{resting}=0.301$, $K_{calling}=0.500$). For both groups of insects, OLS performed better than PGLS, indicating no need to take phylogenetic signal into account (Table S2, Fig. S4).

Data availability
Raw data are available from the authors upon request.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.160036.supplemental

References

Alexander, R. D. (1961). Aggressiveness, territoriality, and sexual behaviour in field crickets (Orthoptera: Gryllidae). *Behaviour* **17**, 130-223.

Arak, A. (1983). Male–male competition and mate choice in anuran amphibians. In *Mate Choice* (ed. P. Bateson), pp. 181-210. Cambridge: Cambridge University Press.

Bailey, W. (1991). *Acoustic Behaviour of Insects. An Evolutionary Perspective.* London: Chapman and Hall.

Bailey, W., Withers, P., Endersby, M. and Gaull, K. (1993). The energetic cost of calling in the bushcricket *Requena verticalis* (Orthoptera: Tettigoniidae: Listroscelidinae). *J. Exp. Biol.* **178**, 21-37.

Bennet-Clark, H. C. (1997). Tymbal mechanics and the control of song frequency in the cicada *Cyclochila australasiae*. *J. Exp. Biol.* **200**, 1681-1694.

Bennet-Clark, H. C. (1998). Size and scale effects as constraints in insect sound communication. *Philos. Trans. R. Soc. Lon., B Biol. Sci.* **353**, 407-419.

Blomberg, S. P., Garland, T., Jr and Ives, A. R. (2003). Testing for phyolgenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**, 717-745.

Burnham, K. P. and Anderson, D. R. (2002). *Model Selection and Multimodel Inference.* New York: Springer.

Burrows, M., Shaw, S. R. and Sutton, G. P. (2008). Resilin and chitinous cuticle form a composite structure for energy storage in jumping by froghopper insects. *BMC Biol.* **6**, 41.

Cade, W. H. and Cade, E. S. (1992). Male mating success, calling and searching behaviour at high and low densities in the field cricket, *Gryllus integer*. *Anim. Behav.* **43**, 49-56.

Calow, P. (1979). The cost of reproduction – a physiological approach. *Biol. Rev.* **54**, 23-40.

Charif, R., Strickman, L. and Waack, A. (2010). *Raven Pro 1.4 User's Manual.* Ithaca, NY: The Cornell Lab of Ornithology.

Chintauan-Marquier, I. C., Lengendre, F., Hugel, S., Robillard, T., Grandcolas, P., Nel, A., Zuccon, D. and Desutter-Grandcolas, L. (2016). Laying the foundations of evolutionary and systematic studies in crickets (Insecta, Orthoptera): a multilocus phylogenetic analysis. *Cladistics* **32**, 54-81.

Chown, S. L., Marais, E., Terblanche, J. S., Klok, C. J., Lighton, J. R. B. and Blackburn, T. M. (2007). Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. *Funct. Ecol.* **21**, 282-290.

Cooper, C. E. and Withers, P. C. (2006). Numbats and aardwolves – how low is low? A re-affirmation of the need for statistical rigour in evaluating regression predictions. *J. Comp. Physiol. B.* **176**, 623-629.

Counter, S. A. (1977). Bioacoustics and neurobiology of communication in the tettigoniid *Neoconocephalus robustus*. *J. Insect Physiol.* **23**, 993-1008.

Dickinson, M. H. and Lighton, J. R. B. (1995). Muscle efficiency and elastic storage in the flight motor of *Drosophila*. *Science* **268**, 87-90.

Dell, A. I., Pawar, S. and Savage, V. M. (2011). Systematic variation in the temperature dependence of physiological and ecological traits. *Proc. Natl. Acad. Sci. USA* **108**, 10519-10596.

Erregger, B., Kovac, H., Stabentheiner, A., Hartbauer, M., Römer, H. and Schmidt, A. K. D. (2017). Cranking up the heat: relationships between energetically costly song features and the increase in thorax temperature in male crickets and katydids. *J. Exp. Biol.* **220**, 2635-2644.

Fonseca, P. J. and Bennet-Clark, H. C. (1998). Asymmetry of tymbal action and structure in a cicada: a possible role in the production of complex songs. *J. Exp. Biol.* **201**, 717-730.

French, B. W. and Cade, W. H. (1989). Sexual selection at varying population densities in male field crickets, *Gryllus veletis* and *G. pennsylvanicus*. *J. Insect Behav.* **2**, 105-121.

Gerhardt, C. and Huber, F. (2002). *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions.* Chicago, IL: The University of Chicago Press.

Greenfield, M. (1997). Acoustic communication in Orthoptera. In *The Bionomics of Grasshoppers, Katydids and their Kin* (ed. S. Gangwere, M. Muralirangan and M. Muralirangan), pp. 197-230. Oxford: CAB International.

Hack, M. A. (1998). The energetics of male mating strategies in field crickets (Orthoptera: Gryllinae: Gryllidae). *J. Insect Behav.* **11**, 853-867.

Halsey, L. G., Matthews, P. G. D., Rezende, E. L., Chauvaud, L. and Robson, A. A. (2015). The interactions between temperature and activity levels in driving metabolic rate: theory, with empirical validation from contrasting ectotherms. *Oecologia* **177**, 1117-1129.

Heath, J. and Josephson, R. (1970). Body temperature and singing in the katydid, *Neoconocephalus robustus* (Orthoptera, Tettigoniidae). *Biol. Bull.* **138**, 272-285.

Heller, K. (1990). Evolution of song pattern in east Mediterranean Phaneropterinae: Constraints by the communication system. In *The Tettigoniidae: Biology, Systematics and Evolution* (ed. W. Bailey and D. Rentz), pp. 130-152. Berlin: Springer Verlag.

Heller, K.-G. and von Helversen, D. (1993). Calling behaviour in bushcrickets of the genus *Poecilimon* with differing communication systems (Orthoptera: Tettigonioidea, Phaneropteridae). *J. Ins. Behav.* **6**, 361-377.

Hemp, C., Heller, K.-G., Warchałowska-Śliwa, E., Grzywacz, B. and Hemp, A. (2015). Review of the *Plangia graminea* (Serville) complex and the description

of new *Plangia* species from East Africa (Orthoptera: Phaneropteridae, Phaneropterinae) with data on habitat, bioacoustics, and chromosomes. *Org. Divers. Evol.* **15**, 471-488.

Hissmann, K. (1990). Strategies of mate finding in the European field cricket (*Gryllus campestris*) at different population densities: a field study. *Ecol. Entomol.* **15**, 281-291.

Irlich, U. M., Terblanche, J. S., Blackburn, T. M. and Chown, S. L. (2009). Insect rate-temperature relationships: environmental variation and the metabolic theory of ecology. *Am. Nat.* **174**, 819-835.

Josephson, R. (1973). Contraction kinetics of the fast muscles used in singing by a katydid. *J. Exp. Biol.* **59**, 781-801.

Kavanagh, M. (1987). The efficiency of sound production in two cricket species, *Gryllotalpa Australis* and *Teleogryllus commodus* (Orthoptera: Grylloidea). *J. Exp. Biol.* **130**, 107-119.

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P. and Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**, 1463-1464.

Korsunovskaya, O. S. (2009). Acoustic signals in katydids (Orthoptera, Tettigoniidae). Communication 2. *Entomol. Rev.* **89**, 16-20.

Lee, H.-J. and Loher, W. (1993). The mating strategy of the male short-tailed cricket *Anurogryllus muticus* de Geer. *Ethology* **95**, 327-344.

Lehmann, G. (2012). Weighing costs and benefits of mating in bushcrickets (Insecta: Orthoptera: Tettigoniidae), with an emphasis on nuptial gifts, protandry and mate density. *Front. Zool.* **9**, 1-12.

Lighton, J. R. B. (2008). *Measuring Metabolic Rates: A Manual for Scientists.* Oxford: Oxford University Press.

Lighton, J. R. B., Bartholomew, G. A. and Feener, D. H. (1987). Energetics of locomotion and load carriage and a model of the energy cost of foraging in the leaf-cutting ant *Atta colombica* Guer. *Physiol. Zool.* **60**, 524-537.

Marais, E. and Chown, S. L. (2003). Repeatability of standard metabolic rate and gas exchange characteristics in a highly variable cockroach, *Perisphaeria* sp. *J. Exp. Biol.* **206**, 4565-4574.

Michonneau, F., Brown, J. W. and Winter, D. J. (2016). rotl: an R package to interact with the Open Tree of Life data. *Methods Ecol. Evol.* **7**, 1476-1481.

Mills, H. (2000). Geographically distributed acoustical monitoring of migrating birds. *J. Acoust. Soc. Am.* **108**, 2582.

Nespolo, R. F. and Franco, M. (2007). Whole-animal metabolic rate is a repeatable trait: a meta-analysis. *J. Exp. Biol.* **210**, 2000-2005.

Nespolo, R. F., Lardies, M. A. and Bozinovic, F. (2003). Intrapopulational variation in the standard metabolic rate of insects: repeatability, thermal dependence and sensitivity (Q_{10}) of oxygen consumption in a cricket. *J. Exp. Biol.* **206**, 4309-4315.

Niven, J. E. and Scharlemann, J. P. (2005). Do insect metabolic rates at rest and during flight scale with body mass? *Biol. Lett.* **1**, 346-349.

Oppenheim, A., Schafer, R. and Buck, J. (1998). Filter design techniques. In *Discrete-Time Signal Processing* (ed. A. Oppenheim, R. Schafer and J. Buck), pp. 474-476. Upper Saddle River, NJ: Prentice Hall.

Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature* **401**, 877-884.

Paradis, E., Claude, J. and Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289-290.

Prestwich, K. N. (1994). The energetics of acoustic signalling in anurans and insects. *Am. Zool.* **34**, 625-643.

Prestwich, K. N. and O'Sullivan, K. (2005). Simultaneous measurement of metabolic and acoustic power and the efficiency of sound production in two mole cricket species (Orthoptera: Gryllotalpidae). *J. Exp. Biol.* **208**, 1495-1512.

Prestwich, K. and Walker, T. (1981). Energetics of singing in crickets: effect of temperature in three trilling species (Orthoptera: Gryllidae). *J. Comp. Physiol.* **143**, 199-212.

Qin, G., Hu, X., Cebe, P. and Kaplan, D. L. (2012). Mechanisms of resilin elasticity. *Nat. Commun* **3**, 1003.

R Core Team (2013). *R: A Language and Environment for Statistical Computing.* Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/.

Riede, K. (1998). Acoustic monitoring of Orthoptera and its potential for conservation. *J. Insect Conserv.* **2**, 217-223.

Riveros, A. J. and Enquist, B. J. (2011). Metabolic scaling in insects supports the predictions of the WBE model. *J. Insect Physiol.* **57**, 688-693.

Rost, R. and Honegger, H. W. (1987). The timing of premating and mating behaviour in a field population of the cricket *Gryllus campestris* L. *Behav. Ecol. Sociobiol.* **21**, 279-289.

Song, H., Amédégnato, C., Cigliano, M. M., Desutter-Grandcolas, L., Heads, S. W., Huang, Y., Otte, D. and Whiting, M. F. (2015). 300 million years of diversification: elucidating the patterns of orthopteran evolution based on comprehensive taxon and gene sampling. *Cladistics* **31**, 621-625.

Stevens, E. D. and Josephson, R. K. (1977). Metabolic rate and body temperature in singing katydids. *Physiol. Zool.* **50**, 31-42.

Symes, L. B., Ayres, M. P., Cowdery, C. P. and Costello, R. A. (2015). Signal diversification in *Oecanthus* tree crickets is shaped by energetic, morphometric, and acoustic trade-offs. *Evolution* **69**, 1518-1527.

Terblanche, J. S., Klok, C. J., Marais, E. and Chown, S. L. (2004). Metabolic rate in the whip-spider, *Damon annulatipes* (Arachnida: Amblypygi). *J. Insect Physiol.* **50**, 637-645.

Terblanche, J. S., Janion, C. and Chown, S. L. (2007). Variation in scorpion metabolic rate and rate-temperature relationships: implications for the fundamental equation of the metabolic theory of ecology. *J. Evol. Biol.* **20**, 1602-1612.

Walker, T. (1983). Diel patterns of calling in nocturnal Orthoptera. In *Orthopteran mating systems: Sexual Competition in a Diverse Group of Insects* (ed. D. Gwynne and G. Morris), pp. 45-72. Boulder, CO: Westview Press.

Weis-Fogh, T. (1964). Biology and physics of locust flight. VII. Lift and metabolic rate of flying locusts. *J. Exp. Biol.* **41**, 257-271.

Weissman, D., Rentz, D., Alexander, R. and Loher, W. (1980). Field crickets (*Gryllus* and *Acheta*) of California and Baja California. *Trans. Am. Entomol. Soc.* **106**, 327-356.

White, C. R. (2011). Allometric estimation of metabolic rates in animals. *Comp. Biochem. Physiol. Part A.* **158**, 346-357.

White, C. R. and Kearney, M. R. (2014). Metabolic scaling in animals: methods, empirical results, and theoretical explanations. *Compr. Physiol.* **4**, 231-256.

White, C. R., Matthews, P. G. D. and Seymour, R. S. (2008). *In situ* measurement of calling metabolic rate in an Australian mole cricket, *Gryllotalpa monanka*. *Comp. Biochem. Phys. A* **150**, 217-221.

Wolak, M. E., Fairbairn, D. J. and Paulsen, Y. R. (2012). Guidelines for estimating repeatability. *Methods Ecol. Evol.* **3**, 129-137.