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RESEARCH ARTICLE

Cranking up the heat: relationships between energetically costly song features and the increase in thorax temperature in male crickets and katydids

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ABSTRACT

Sexual displays of acoustically signalling insects are used in the context of mate attraction and mate choice. While energetic investment in sound production can increase the reproductive success of the sender, this entails metabolic costs. Resource allocation to sexually selected, reproductive traits can trade off against allocation to naturally selected traits (e.g. growth, immunity) when individuals' energy budgets are limited. Estimating the magnitude of the costs invested in acoustic signalling is necessary to understand this trade-off and its influence on fitness and life history. To compare the costs associated with acoustic signalling for two ensiferan species, we simultaneously took respiratory measurements to record the rate of CO_2 production and used infrared thermography to measure the increase in thorax temperature. Furthermore, to identify what combinations of acoustic parameters were energetically costly for the sender, we recorded the calling songs of 22 different cricket and katydid species for a comparative analysis and measured their thorax temperature while they sang. Acoustic signalling was energetically costly for *Mecopoda* sp. and *Anurogryllus muticus*, requiring a 12- and 16-fold increase over resting levels in the CO_2 production rate. Moreover, calling increased thorax temperature, on average by 7.6 and 5.8°C, respectively. We found that the song intensity and effective calling rate, not simply the chirp/trill duty cycle or the pulse rate alone, were good predictors for the thorax temperature increase in males.

KEY WORDS: Respiratory metabolic costs, Calling energetics, Insects, Infrared thermography, Thorax temperature, Acoustic signalling

INTRODUCTION

In many anuran and insect species, acoustic signalling is the primary form of communication used to attract or choose mates (Gerhardt and Huber, 2002; Bradbury and Vehrencamp, 2011). To produce sound signals, however, muscular energy must be expended, so the sender inevitably incurs metabolic costs (Reinhold, 1999). These costs vary considerably across species (Prestwich, 1994). During calling, aerobic metabolic rates increase between 2 and 21 times in insects, and 6 and 24 times in frogs compared with the resting rate (Prestwich, 1994; Wells, 2010; Bradbury and Vehrencamp, 2011), and up to 56% of the daily respiratory budget is devoted to calling in *Oecanthus celerinictus*, for example (Prestwich and Walker, 1981). Resource allocation to sexually selected life-history traits such as acoustic signalling is subjected to a variety of selective forces and constraints. For example, the acoustic performance of an adult male is limited by its ability to acquire and assimilate resources, the availability of these resources during juvenile development, or the predation and parasitisation costs associated with calling (Ryan, 1988; Cade, 1975; Belwood and Morris, 1987; Höglund and Sheldon, 1998; Hunt et al., 2004; Zuk et al., 2006; Houslay et al., 2016). While the energetic investment in sound production increases the male's reproductive success, the inherent metabolic and predation costs as well as the female's preference for high energy calls can decrease the overall survival rate of males (Ryan, 1988; Hunt et al., 2004). Indeed, females display clear preferences for males that call at higher rates, spend more time calling, or produce calls at greater intensities and, therefore, invest more energy in their acoustic display (Ryan, 1988; Wagner and Hoback, 1999; Wagner and Harper, 2003; Bussière et al., 2005; Bradbury and Vehrencamp, 2011). In a study of two chirping species of field crickets with slow pulse rates, multivariate female preference functions even revealed cryptic preferences for long trills (Gray et al., 2016).

Ensiferans (crickets and katydids) produce calling songs by stridulation, whereby one wing with a file-like structure and a number of sclerotised teeth is rubbed against a hardened vein (scraper) (Bennet-Clark, 1989; Otte, 1992; Gerhardt and Huber, 2002). A single wing stroke creates a sound pulse which is the basic element of the calling song, and the number of pulses (i.e. the pulse rate) corresponds to the number of wing strokes produced per unit of time. The pulse rate and, correspondingly, the wing stroke rate can vary considerably among species, ranging from <10 Hz (e.g. *Cycloptilum albocircum*; Love and Walker, 1979) up to 280 Hz (*Neoconocephalus punctipes*; Walker and Greenfield, 1983; Bush and Schul, 2010). These high wing stroke rates are made possible by specific physiological characteristics of the mesothoracic synchronous muscles, which are used during singing and flight, and had been thought to be limited to 100 Hz (Josephson and Halverson, 1971; Elder, 1971; Young and Josephson, 1983; Bennet-Clark, 1989; Syme and Josephson, 2002). Moreover, the timing of pulses results in the emergence of species-specific song patterns composed either of continuously repeated pulses (trills) or pulses that are grouped into units (chirps) (Otte, 1992; Gerhardt and Huber, 2002). Crickets and katydids are known to produce an impressive variety of species-specific acoustic advertisement signals, and it is not surprising that some of these are energetically more demanding than others (Prestwich, 1994; Gerhardt and Huber, 2002). The energetically costly workload of sound production in these insects can be directly correlated with the

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pulse rate and the chirp/trill duty cycle. Song intensity is also dependent on muscular power output and is regulated by the force with which the wings are moved against each other. Therefore, the aerobic metabolic rates are generally considered to be higher for trilling species than for chirping species, and calling songs that are produced at high amplitudes are more energetically demanding than those produced at low amplitudes (Lee and Loher, 1993; Prestwich, 1994; Gerhardt and Huber, 2002).

In insects, thoracic heating is another striking physiological feature associated with calling songs produced by wing frequency rates higher than 200 Hz (Heath and Josephson, 1970; Stevens and Josephson, 1977; Heller, 1986). The extensive use of wing muscles at such high rates generates heat that significantly increases thorax temperature. However, the exact relationship between thoracic heating and metabolic costs during calling has remained elusive because of technical difficulties faced while measuring the two physiological parameters simultaneously (Heath and Josephson, 1970).

In this study, we examined whether a predictable relationship could be drawn between different calling song parameters in crickets and katydids, the thorax temperature increase during singing, and the resulting metabolic costs. We took respiratory measurements and used infrared thermography to quantify the relationship between the CO_2 production rate and increase in thoracic temperature for two trilling ensiferan species that produce calling songs with a high pulse rate (*Anurogryllus muticus*; 140 Hz) and an extremely high intensity [*Mecopoda* sp.; 97 dB sound pressure level (SPL) at 30 cm distance]. While researchers agree that isolated call features such as call duration, loudness and pulse rate increase metabolic rates during singing, no qualitative analysis has been conducted so far that has examined a combination of different acoustic parameters and their associated energetic costs. Therefore, we additionally performed a comparative analysis of 22 ensiferan species from tropical and temperate habitats to identify combinations of song parameters that predicted the increase in thoracic temperature during singing.

MATERIALS AND METHODS

Animals

Male individuals of two paleotropical katydid species of the genus *Mecopoda* (*M. elongata* and *Mecopoda* sp.) and the neotropical cricket species *Anurogryllus muticus* were taken from laboratory colonies maintained at the Department of Zoology in Graz. The ambient temperature in the breeding room was about 27°C with a relative humidity of about 70%. Insects were reared under a 12 h:12 h light:dark cycle; they were fed on fish food, oat flakes, water gel, lettuce and apple pieces *ad libitum*.

To evaluate the effects of different song parameters on body temperature, we captured three species native to Central Europe (*Tettigonia cantans*, *Ruspolia nitidula* and *Metrioptera roeselii*; Table 1) from late spring to autumn in the countryside around Graz, Austria, in 2015 and 2016 and 16 tropical species on Barro Colorado Island (9°9′N, 79°51′W) in Panama during the dry seasons in 2015 and 2016. Animals were kept in plastic boxes and fed on cat food, oat flakes, lettuce and water gel *ad libitum*.

Respiratory measurements

CO_2 production rates of individual males were continuously measured using a differential CO_2 gas analyser (DIRGA; URAS 14 Advance Optima, ABB, Zürich, Switzerland; Stabentheiner et al., 2012; Stabentheiner and Kovac, 2016). Measurements were taken for *Mecopoda* sp. males in a small acrylic box (11×8×3.5 cm; volume: 308 ml) placed in an incubator (Kendro B12 Function

Table 1. Summary of species-specific acoustic parameters, thoracic temperature increases and body size (pronotum width)

	Pulse rate (Hz) (*N*)	Chirp duration (ms)	Chirp pause (ms)	Chirp/Trill DC	Eff. calling rate (Hz)	Song intensity (dB SPL)	Pronotum width (mm) (*N*)	Thorax temp. increase (°C) (*N*)
Crickets								
Anurogryllus muticus	141.1±11 (10)	–	–	1	141.1	89	4.81±0.58 (63)	5.2±1.2 (36)
Paroecanthus podagrosus	206.2±17 (26)	23.7±3.3	13.2±2.1	0.64	132.2	73	2.82±0.18 (5)	2.7 (1)
Orocaris sp.	90.6±3.4 (18)	1050.3±144.7	3197.1±366.8	0.25	22.4	88	4.21±0.29 (5)	0.45 (1)
Podoscirtinae	46.5±5.1 (14)	44.4±5.7	269.4±69.5	0.14	6.6	84	3.01±0.08 (5)	0.3 (1)
Anaxipha sp. 1	54.9±9.8 (32)	137±20.1	1045.8±390.3	0.12	6.4	74	1.77±0.10 (12)	0.3 (1)
Anaxipha sp. 2	70.1±5.7 (31)	151.4±42.2	883.6±305.1	0.15	10.3	68	1.25±0.07 (15)	0 (1)
Amblyrhethus sp.	16.7±1.5 (20)	–	–	1	16.7	75	4.22±0.11 (4)	0 (1)
Diatrypa sp.	134.9±4.3 (14)	137.3±13.4	318.5±69.7	0.3	40.6	64	2.64±0.20 (6)	0 (1)
Gryllodes sigillatus	53.7 (1)	55.9	80	0.41	22.1	65	3.48±0.24 (4)	0 (2)
Gryllus sp.	87.5±2.4 (3)	98.7±6.8	418.5±167.8	0.15	13.3	82	6.62±1.87 (3)	0 (1)
Lerneca sp.	40.2±3.2 (5)	586±94	990.8±434.8	0.39	15.8	74	2.48±0.11 (3)	0 (1)
Makroanaxipha sp.	253±5.2 (3)	49.5±20.7	113.4±94.7	0.35	89.1	60	2.00±0.02 (2)	0 (1)
Miogryllus sp.	74.8±5.3 (4)	590.4±39.9	1832.5±312.2	0.14	10.7	66	4.68±0.43 (3)	0 (1)
Katydids								
Neoconocephalus maxillosus	263.3±9.8 (2)	–	–	1	263.3	92	6.07 (1)	8.1±1.2 (5)
Mecopoda sp.	67.7±1.4 (4)	–	–	1	67.7	97	8.3±0.3 (11)‡	7.6±0.4 (17)
Ruspolia nitidula	107.5 (3)	–	–	1	107.5	92	4.38±0.12 (8)	7.1±1.5 (8)
Neoconocephalus affinis	29.6±1 (2)	–	–	1	29.6	90	6.75 (1)	1.7±0.6 (8)
Metrioptera roeselii	77.6±3.1 (3)	–	–	1	77.6	65		1.6±0.1 (3)
Tettigonia cantans	42.2 (1)	6433.5	3650.1	0.64	27.3	81	7.5 (1)	1.3 (1)
Mecopoda elongata	56.5*	273	1729.0	0.14	7.7	75	6.8±0.3 (14)‡	0.9±0.4 (5)
Thamnobates sp.	48.9±2.3 (3)	35.93±3.48	554.4±199	0.07	3.3	60	5.34 (3)	0 (3)
Bucrates sp.	36.28 (1)	55.13	701	0.17	6.0	49		0 (1)

Data are means±s.d. DC, duty cycle. *Hartbauer et al., 2012; ‡I. Krobath and M.H., unpublished. Cases where there was no increase in thorax temperature were treated as 0.001 values for further analyses (see Materials and methods, ‘Statistical analyses’).

Line, 230 V, 50/60 Hz; Newton, CT, USA) kept at a constant ambient temperature of 27.3±0.8°C (mean±s.d.). Males of *A. muticus* were placed in a smaller box (inner dimensions 3×3×2 cm; volume: 18 ml) placed in a water bath to maintain a constant ambient temperature of 26.07±1.23°C (mean±s.d.). One side of the measurement chambers was covered with an infrared-transparent film that allowed thermographic measurements to be taken simultaneously (see below). The ambient temperature and humidity in the laboratory, incubator, measurement boxes and water bath were recorded using a multichannel data logger (ALMEMO® 2690-8 and 2890-9, Ahlborn, Holzkirchen, Germany) equipped with thermocouples for the boxes and water bath, as well as a humidity sensor (FHA646-R, Ahlborn) for the incubator and room temperature. To determine singing activity, we either used a G.R.A.S microphone (type 40AC; Sound and Vibration A/S, Holte, Denmark) placed next to the animals (~5 cm distance) and digitised at a sampling rate of 20 kHz (PowerLab 4/26, ADInstruments, Sydney, Australia) or we recorded sounds with a lavalier microphone (LMLM-09, frequency range: 50–16,000 Hz; Hama, Monheim, Germany) connected to a standard laptop using the software Cool Edit Pro (Syntrillium Inc., Phoenix, AZ, USA).

Because these animals are nocturnal, we recorded the metabolic rate and sound production of each male during the dark phase of a 12 h:12 h light:dark cycle. While respiration was being measured, males were denied access to food but were provided with water gel. The software Centrol 5 (Harnisch, Austria) was used to record the CO_2 production rate of the males (Stabentheiner et al., 2012; Hartbauer et al., 2012). The CO_2 production rates of *Mecopoda* sp. and *A. muticus* males were calculated by multiplying the gas flow (~165 and ~145 ml min^{-1}, respectively) by the drift-corrected CO_2 concentration (in parts per million, ppm) of the measurement channel, divided by 1000. To determine the resting metabolic rate, only those sections of data were evaluated in which insects had remained motionless and the thermograms had indicated an absence of endothermic activity (thorax temperature not elevated more than 0.2°C above abdomen surface temperature, i.e. ectothermy; for details, see Kovac et al., 2007). The average metabolic rate associated with song production was determined by integrating the amount of CO_2 generated during a song bout, including the decline after the song terminated, until respiration regained a steady value close to the resting level. We subtracted the amount of CO_2 that corresponded to the resting metabolism from this integral value and divided the result by the duration of the sound signal.

Metabolic power (*P*, in W) was calculated by multiplying the O_2 consumption rate by the caloric equivalent (oxyjoule equivalent). The O_2 consumption rate was estimated on the basis of the CO_2 production rate using a respiratory quotient (RQ=$\dot{V}_{CO_2}/\dot{V}_{O_2}$) of 0.847. The RQ was determined by assessing the relative amount of carbohydrates (RQ=1.0), lipids (RQ=0.7) and proteins (RQ=0.8) consumed by nine *Mecopoda* sp. individuals. Based on the RQ of 0.847, the calculated caloric equivalent was 20.51 kJ l^{-1} O_2.

Infrared thermography during singing

In this study, we used the non-invasive method of infrared thermography to measure surface temperature as a proxy for energetic costs incurred during sound production. However, the actual thoracic core temperature is expected to be higher than the surface temperature, as in endothermic honeybees where temperature below the thoracic cuticle was found to be at least ~1°C higher than surface temperature (Stabentheiner and Schmaranzer, 1987). To study alterations in the body surface temperature associated with CO_2 production rate, we recorded the surface temperature of males simultaneously inside a respiratory chamber using an infrared-sensitive camera (ThermaCam SC2000-NTS, FLIR, Stockholm, Sweden for *Mecopoda* sp.; T420, FLIR, for *A. muticus*). These cameras detect energy within the infrared range of the electromagnetic spectrum (λ=7.5–13 µm) and produce a thermal image. Measurements were taken from either the lateral (*Mecopoda*) or the dorsal view (*A. muticus*) of males. Infrared images of the two species were recorded at intervals of 0.54 and 0.13 s, respectively. The captured sequences were evaluated using ThermaCam Researcher 2002 software (FLIR), with an insect cuticular infrared emissivity of 0.97 (Stabentheiner and Schmaranzer, 1987; Kovac and Stabentheiner, 1999). For calibration purposes, we used a Peltier-driven reference radiator that was partly covered with the same infrared-transparent film that was used to cover the measurement chamber. The surface temperature difference of the reference radiator with and without the film was then used for data correction of temperature measurements in the respiratory chamber (Stabentheiner et al., 2012). This method of correction for attenuation of infrared radiation by the film covering the measurement chambers has the benefit that it compensates for camera drift and off-set and for variations in reflections of ambient radiation by the films automatically. The measured surface temperature of the thorax represented an average of 16–18 of the hottest pixels within the measurement spot fitted to the males' thoracic area, whereby 16 pixels represented 6.9 mm^2. A proprietary Microsoft Excel (Microsoft Corporation) VBA-macro, controlling the ThermaCam Researcher 2002 software, was used to evaluate the body surface temperatures of insects in intervals of 3 s while they sang. This macro was also used to extract data for ambient temperature from the logger files. Thorax temperature of resting individuals was evaluated within the same time period as the resting metabolic rate (for more details, see Stabentheiner et al., 2012).

Correlation between song parameters and body temperature

We used infrared thermography to determine whether the body temperature of male individuals from 22 tropical and temperate katydid and cricket species was elevated during song production and identify the combinations of song parameters that correlated with elevated thoracic temperatures. Surface temperature for these males was recorded using an infrared-sensitive camera (T420, FLIR) and songs were recorded using a microphone for sound analysis (sampling rate up to 100 kHz) in the laboratory at an ambient temperature of approximately 24°C.

The following acoustic parameters were evaluated manually in CoolEdit Pro (Syntrillium Inc.): pulse duration and pulse pause, the number of pulses per chirp, chirp duration and chirp pause (see Fig. 1). The song amplitudes were measured 30 cm above the singing insects using a ½ inch microphone (type 2540, Larson Davis, Depew, NY, USA) that was connected to a sound level meter (CEL 414, Casella, Bedford, UK; combined with the filter set CEL 296) with a flat frequency response operating in fast reading mode (time constant: 125 ms).

To account for body size in our analyses we measured the animals' pronotum width using a calliper or based on digital photo images using ImageJ 1.5.

Statistical analyses

To test the effect of the acoustic signal parameters pulse rate, duty cycle, song intensity and effective calling rate (independent

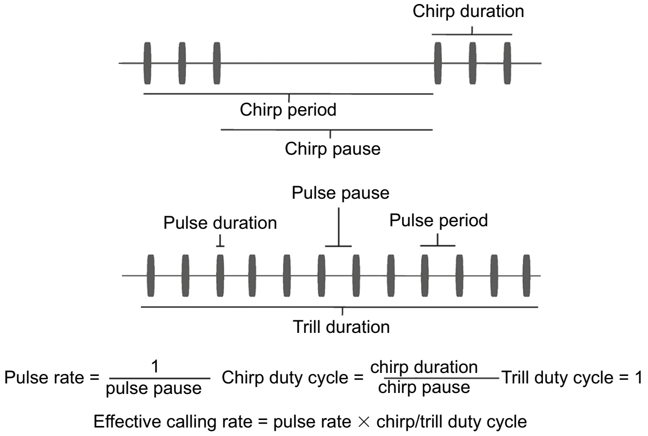

Fig. 1. Illustration of calling song types (chirp and trill) and the terminology of temporal features.

variables) on male thorax temperature (dependent variable), we ran a multiple regression analysis (SigmaPlot 13.0, Systat Software Inc., San Jose, CA, USA). We used log-transferred species-specific average values of song parameters and thorax temperature (Table 1) to ensure normality of data. Effective calling rate is the product of pulse rate and duty cycle and is therefore co-linear and highly correlated with these two variables. Consequently, we performed two separate analyses using pulse rate, duty cycle and song intensity, and, in subsequent analyses, song intensity and effective calling rate to predict thorax temperature. Further statistical tests were performed according to standard parametric and non-parametric procedures (*t*-test, Mann–Whitney rank sum test, ANOVA, Pearson product moment correlation). If not stated otherwise, we measured means±s.d.

Testing for phylogenetic signal

Because of the current lack of knowledge about the phylogenetic relationship of Panamanian cricket and katydid species, we built a phylogenetic tree based on recent phylogenetic studies of Orthopterans (Song et al., 2015; Chintauan-Marquier et al., 2016) using Mesquite software 3.2 (http://mesquiteproject.org). The taxonomic positions of relevant subfamilies in these studies were taken as a template for the 22 species used in this study, where polytomies were randomly resolved into a series of dichotomies (see tree in Fig. S1). To incorporate phylogenetic signals (i.e. related species have similar traits) for all acoustic signal parameters and species' body size, we calculated Pagel's λ (Pagel, 1999) and Blomberg's *K* (Blomberg et al., 2003) using R 3.3.2 (http://www.R-project.org/). Pagel's λ ranges between 0 (no phylogenetic signal) and 1 (strong phylogenetic signal) and was determined using the *pgls* function in the *caper* package (https://CRAN.R-project.org/package=caper). The *pgls* function was also used to correct for phylogeny in bivariate correlations (see below; Fig. S2). Blomberg's *K* was calculated with the *phylosignal* function in the *picante* package (Kembel et al., 2010); *K* close to 0 indicates no phylogenetic signal, *K* close to 1 suggests trait distribution as expected under Brownian motion, and *K*>1 indicates a strong phylogenetic signal. The statistical significance of Blomberg's *K* was determined by comparing observed phylogenetic independent contrasts of traits with a null model of randomly shuffled traits across phylogeny (number of iterations=1000).

RESULTS

Respiratory metabolic rate

Trilling *Mecopoda* sp. and *A. muticus* males exhibited an average resting CO_2 production rate ($\dot{V}_{CO_2}$) of 26.6±4.79 and 3.82±1.29 µl min^{-1}, respectively (Mann–Whitney rank sum test: P<0.001, N=9). $\dot{V}_{CO_2}$ during singing was on average 308.0±48.93 µl min^{-1} for *Mecopoda* sp. and 57.64±11.78 µl min^{-1} for *A. muticus* (Mann–Whitney rank sum test: P<0.001, N=9; see Figs 2 and 3). The factorial scope (the ratio of the metabolic rate during singing to that while resting) was on average 12.0±3.18 for *Mecopoda* sp. and 16.36±5.61 for *A. muticus*. We also calculated the average mass-specific CO_2 production rate, which differed significantly between singing *Mecopoda* sp. and *A. muticus* males (94.21±19.0 versus 146.89±22.71 µl min^{-1} g^{-1}; *t*-test: t=5.337, P<0.001, N=9; Fig. 3B). The resting metabolic rate was similar in the two species: *Mecopoda* sp. individuals displayed a rate of 8.28±2.33 µl min^{-1} g^{-1} and *A. muticus* individuals had a rate of 9.53±2.03 µl min^{-1} g^{-1} (*t*-test: t=1.217, P=0.241, N=9). The mean mass of the *Mecopoda* sp. males was 3.33±0.52 g and that of *A. muticus* males was 0.40±0.09 g.

Infrared thermography during singing

By simultaneously measuring the body surface temperature of males in the respiratory chamber using infrared thermography, we discovered a significant, positive correlation between the increase in thoracic temperature and the respiration rate while singing (Fig. 2). The average increase in the thoracic temperature was 6.9±0.8°C for *Mecopoda* sp. males (resting temperature: 29.2±0.7°C; mean peak temperature: 36.2±1.2°C; N=10) and 4.5±1.0°C for *A. muticus* males (resting temperature: 27.3±1.0°C; mean peak temperature: 31.8±1.1°C, N=9).

We identified a strong positive correlation between mean $\dot{V}_{CO_2}$ and the thoracic temperature of individual males in these two species during calling (*Mecopoda* sp.: R^2=0.913–0.989; *A. muticus*: R^2=0.78–0.95; Fig. 4, Fig. S3). We also analysed the relationship between song duration and the increase in temperature, which revealed that the thoracic temperature increased strongly within approximately 250 s of song onset before a plateau was reached (Fig. 5). The average duration of the *Mecopoda* sp. males' songs during respiratory measurements was 361.78±101.35 s, while songs of *A. muticus* males lasted an average of 419.34±216.42 s.

In addition to surface temperature measurements using infrared thermography in the respiratory chamber, we measured the thoracic temperature of males that were free to move in larger cages. When males sang, thoracic temperature increased an average of 7.6±0.4°C (N=14) in *Mecopoda* sp. and 5.8±1.1°C (N=23) in *A. muticus* (Fig. 6).

To investigate the relationship between energy expenditure and song parameters, we also measured thoracic heating in a closely related *Mecopoda* species (*M. elongata*), which produces chirps of about 300 ms duration at regular intervals of 2 s. When singing, temperature increased in the chirping species by 0.9±0.4°C (N=5). We compared the overall metabolic costs of singing among all three species by calculating the metabolic power expended (Fig. 6). The average metabolic power calculated was 9.93±1.94 mW g^{-1} for chirping *M. elongata* (Hartbauer et al., 2012), 38.03±7.67 mW g^{-1} for trilling *Mecopoda* sp. males and 59.3±9.17 mW g^{-1} for *A. muticus*. Statistical analyses revealed highly significant differences between these species in both metabolic power (ANOVA: F=124.035, P<0.001) and measured increases in thoracic temperature (ANOVA: F=107.411, P<0.001). Multiple species comparisons (Holm– Šidák method) revealed P<0.001 for each test.

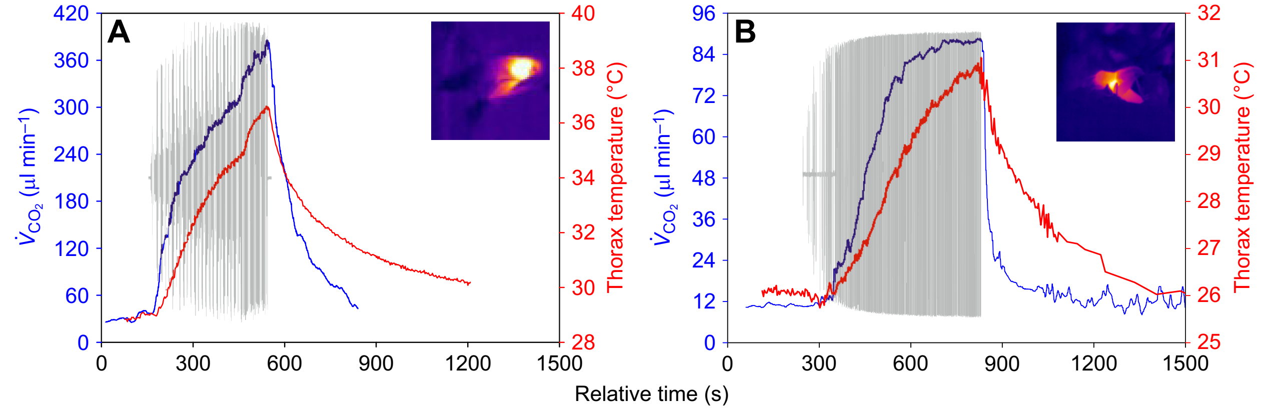

Fig. 2. CO$_2$ production rate ($\dot{V}_{CO_2}$) and thorax temperature during calling in two ensiferan species. Data are for *Mecopoda* sp. (A) and *Anurogryllus muticus* (B) males. Note that the calling song of *Mecopoda* consists of two different motifs, an amplitude-modulated motif (AM-motif) that is usually followed by a high-amplitude trill, which leads to an additional increase in CO$_2$ production rate and temperature. The insets show thermograms of singing males recorded outside the respiratory chamber.

Furthermore, based on our results and data reported in the literature, we found a significant linear correlation between species-specific metabolic power and thoracic heating during calling (Pearson product moment correlation: R^2=0.86, P=0.023, N=5; Fig. 7).

Correlations between song parameters and body temperature

In eight of the 22 insect species, the thoracic temperature increased during calling by 1.3–8.1°C on average (see Table 1). A multiple regression analysis of the acoustic parameters song intensity, chirp/trill duty cycle and pulse rate revealed that these had significant effects on the increase in thoracic temperature (R^2=0.625, F=10.001, P<0.001). Examining the contribution of each of the parameters revealed that song intensity affected the increase in temperature most strongly (t=3.319, P=0.004), while the parameters chirp/trill duty cycle (t=1.775, P=0.09) and pulse rate (t=1.315, P=0.205) alone were not significant predictors. To more effectively interlink temporal features of chirping and trilling species, we calculated the effective calling rate (i.e. the product of pulse rate and chirp/trill duty cycle; see Fig. 1). The multiple regression analysis revealed a significant overall effect (R^2=0.621, F=15.598, P<0.001): both effective calling rate (t=2.358, P=0.029) and song intensity (t=3.596, P=0.002) predicted the thoracic temperature increase, as also illustrated by bivariate correlations shown in Fig. 8A,B. However, in these single regression models, the resulting R^2 values were 0.45 and 0.46 and had therefore less explanatory power than when combined in the multiple regression analysis (R^2=0.621). We explored the multidimensional relationships of both signal parameters with thorax temperature increase in more detail and found that the production of songs with either high sound intensity and low effective calling rate (<25 Hz) or high effective calling rate and low sound intensity (<65 dB SPL) is not a sufficient predictor for increased thoracic temperature (Fig. 8C). However, songs that combine sound intensities of at least 65 dB SPL and effective calling rates of more than 50 Hz do increase the thoracic temperature. Greater increases in thoracic temperature are associated primarily with higher song intensities, and saturate for an effective calling rate above 100 Hz.

To account for body size in these analyses, we conducted several bivariate correlations (Fig. S2). There was a moderate, yet significant influence of body size on thorax temperature (R^2=0.26, t=2.48, P=0.02) and song intensity (R^2=0.28, t=2.62, P=0.02), but not on effective calling rate (R^2=0.02, t=0.62, P=0.55), pulse rate (R^2=0.03, t=−0.70, P=0.50) or duty cycle (R^2=0.10, t=1.43, P=0.17).

Testing for phylogenetic signal

Using Pagel's λ and Blomberg's K, we found no significant phylogenetic signal in thorax temperature and acoustic signal

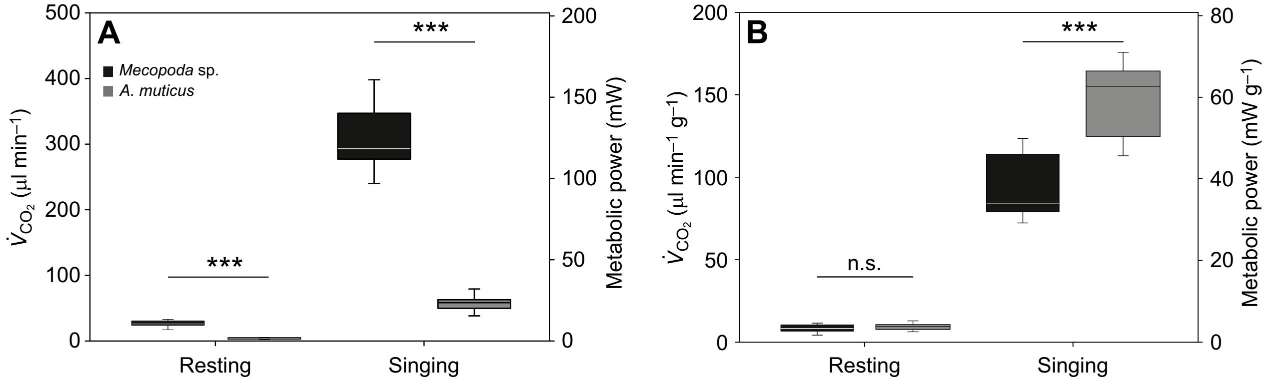

Fig. 3. Comparison of $\dot{V}_{CO_2}$ during resting and singing. Absolute values (A) and mass-specific values (B) of CO$_2$ production are shown. The box dimensions represent the 25th to 75th percentiles; solid horizontal lines indicate the median value. ***P<0.001; n.s. not significant. N=9 for *Mecopoda* sp. and *A. muticus*.

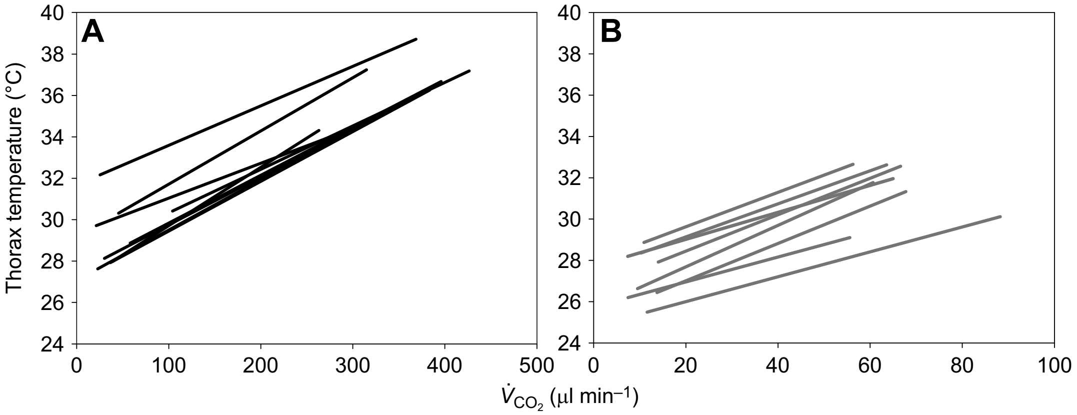

Fig. 4. Linear relationship between thoracic temperature and $\dot{V}_{\mathrm{CO_2}}$. Data were obtained from *N*=9 *Mecopoda* sp. males (A) with R^2 values ranging between 0.91 and 0.99, and *N*=8 *A. muticus* males (B) with R^2 values ranging between 0.78 and 0.95. Regression lines were derived from 20–38 and 14–30 data points for *Mecopoda* sp. and *A. muticus*, respectively (see Fig. S3).

parameters (Table S1). In contrast, a strong phylogenetic signal was detected in body size, which generally might be explained by size differences between crickets (3.38±1.49 mm) and katydids (6.45±1.32 mm). However, in bivariate correlations comprising call parameters, thorax temperature and body size, we found no significant differences between the outputs of phylogenetic generalised least square models compared with linear models (Fig. S2 and Table S2).

DISCUSSION

Production of sexual signals by male insects, or their repetition over prolonged periods of time, is known to be energetically expensive. In this study, we analysed the energetic costs of calling inherent to sound production and identified what combinations of song parameters required the highest energetic expenditure by the sender. In this comparative analysis of 22 cricket and katydid species, we assumed that increased surface temperature during singing was indicative of the energetic expenditure of singing, as an increase in thoracic temperature linearly correlated with $\dot{V}_{\mathrm{CO_2}}$ and metabolic power expended during calling (Figs 2, 4 and 7). Indeed, thoracic heating was used as an estimator for energy expenditure of signalling behaviour in a previous study (Heath and Josephson, 1970).

The results of the analyses of 22 calling songs (Table 1) allowed us to draw some general conclusions about the predictability of acoustic parameters with respect to surface temperature increases in ensiferan species. The parameters of duty cycle and pulse rate alone did not account for the ability to predict an increase in temperature. This is unsurprising: some species have high duty cycles but rather low pulse rates (e.g. *Amblyrhethus* sp.); for others, the opposite is the case (e.g. *Gryllus* sp.; Table 1). However, by combining the two temporal parameters to form a new variable, the effective calling rate (duty cycle×pulse rate), we could considerably improve our ability to predict surface temperature increases (Fig. 8B). We identified song parameter combinations of song intensity and effective calling rate, resulting in a significant thorax temperature increase (Fig. 8C).

While comparing two species used in the comparative analysis in more detail, we identified rather obvious differences of calling features that can be regarded as significant predictors of the energetic expenditure of calling. *Anurogryllus muticus* males use loud calling songs (89 dB SPL measured at 30 cm distance) that are characterised by a rather high effective calling rate of 140 Hz,

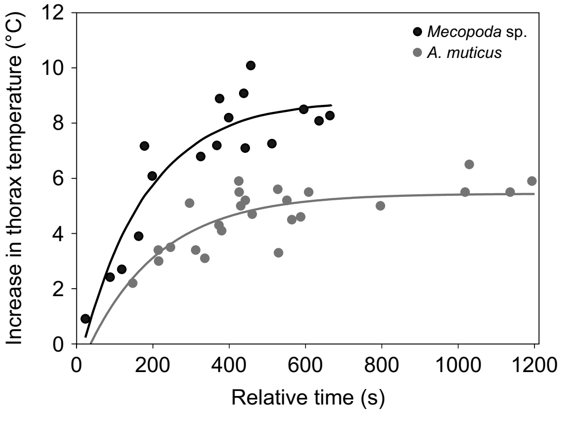

Fig. 5. Non-linear dynamics showing thoracic temperature increase during singing. Non-linear regression analyses: exponential rise to maximum, single, 3-parameter equation: $f(x)=y_0+a[1-\exp(-bx)]$; *Mecopoda* sp.: $y_0=-1.5157$, $a=10.3451$, $b=0.0061$, $R^2=0.89$, $P<0.001$, $N=17$; *A. muticus*: $y_0=-0.4328$, $a=6.1583$, $b=0.0041$, $R^2=0.62$, $P<0.001$, $N=25$. The three lowest values of *Mecopoda* males were obtained by interrupting the song production.

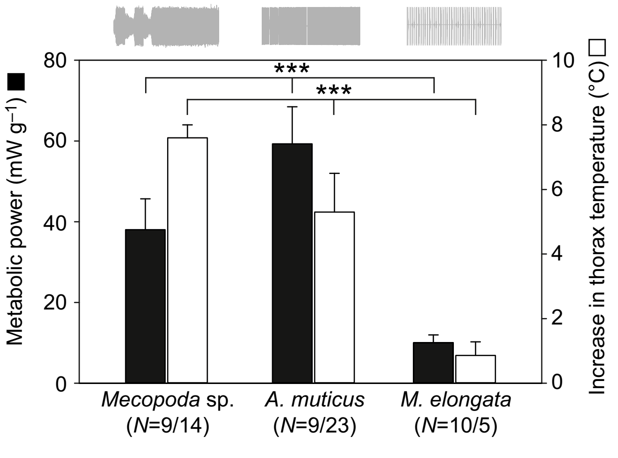

Fig. 6. Metabolic power of calling and increase in thoracic temperature of trilling and chirping insect species. The chirping *Mecopoda elongata* with a low effective calling rate of 7.7 Hz showed only a marginal thoracic temperature increase and rather low metabolic power compared with the two continuously trilling species *Mecopoda* sp. and *A. muticus*. Data for *M. elongata* were obtained from Hartbauer et al. (2012). ***$P<0.001$.

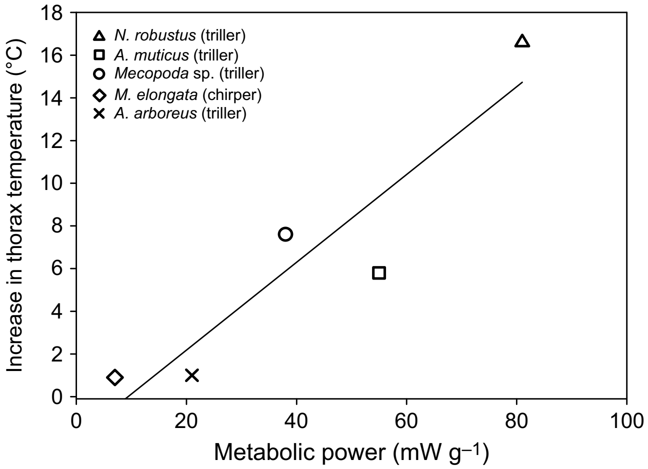

Fig. 7. Relationship between metabolic power and thoracic temperature increase in different cricket and katydid species. A polynomial linear equation following $f(x)=y_0+ax$ was used for regression analyses ($y_0=-1.9297$, $a=2.057$, $R^2=0.86$, $P=0.023$, $N=5$). Data presented for *Neoconocephalus robustus*, *Anurogryllus arboreus* and *M. elongata* were taken from Stevens and Josephson (1977), Prestwich and Walker (1981) and Hartbauer et al. (2012), respectively.

whereas calling rate is much lower in trilling *Mecopoda* males (68 Hz), which, in contrast, produce extremely intense calling songs (97 dB SPL). This high song amplitude is mirrored in the morphological characteristics of this species: the pronotum that houses the flight and singing muscles is significantly wider in males than in females, which are larger overall. Thus, male pronotum width clearly deviates from the expected allometry (I. Krobath and M.H., unpublished). Furthermore, the pronotum width of males is positively correlated with the SPL of the calling song, indicating that a direct relationship exists between muscular power and song amplitude. However, in our cross-species analysis we found that pronotum width was a less sufficient explanatory variable of song intensity ($R^2=0.28$; Fig. S2) and thorax temperature increase ($R^2=0.26$) compared with a correlation of song intensity with thorax temperature increase ($R^2=0.46$; Fig. 8A; Table S2). This suggests that song intensity is not simply the result of absolute sound-producing muscle mass but might also be the result of physiological differences, such as muscle contraction kinetics (Josephson and Young, 1987) and the effect of temperature on it, or differences in the effectiveness of sound radiation (Montealegre-Z et al., 2011).

Acoustic signalling in the trilling *Mecopoda* and *Anurogryllus* species investigated was associated with high energetic demands, which is reflected in the factorial scopes of 12 and 16, respectively. These values fall within the range of the highest net metabolic costs of calling yet measured for ensiferan species such as *Anurogryllus arboreus*, *Gryllotalpa monanka* and *Gryllotalpa australis*, which display factorial scopes of 13 (Prestwich, 1994; White et al., 2008). Lee and Loher (1993) analysed the metabolic costs of singing in *A. muticus*. However, we are quite confident that we studied a different subspecies of *A. muticus* in the present study. Not only were the species-specific carrier frequencies very different from those reported by Lee and Loher (1993: 3.5–4 kHz, versus 7 kHz in this study) but also the factorial scope calculated in our study was 10-fold higher than that reported by Lee and Loher (1993).

Considering the absolute energetic costs of calling, the respiratory measurements revealed an average net metabolic power of 38 mW g^{-1} for *Mecopoda* sp. and 59 mW g^{-1} for *A. muticus*. These values are considerably higher than those reported for a closely related *Mecopoda* species, the chirping *M. elongata* katydid (9.9 mW g^{-1}; factorial scope: 3.9; Hartbauer et al., 2012; Fig. 6) and the trilling cricket *A. arboreus* (21 mW g^{-1}; Prestwich and Walker, 1981) but are lower than the net metabolic power reported for other trilling katydids such as *Euconocephalus nasutus* (∼93 mW g^{-1}) and *Neoconocephalus robustus* (∼81 mW g^{-1}) (Stevens and Josephson, 1977; Prestwich, 1994). The overall higher factorial scope and metabolic power during calling of *A. muticus* compared with *Mecopoda* sp. could be due to the higher proportion of sound-producing muscles relative to body mass in the former. Relative sound-producing muscle mass is known to vary considerably within ensiferan species (between 0.8% and 8.9%; Ophir et al., 2010).

Energetically demanding calling songs produced by *Mecopoda* and *A. muticus* males were also accompanied by a relatively linear increase in thoracic temperature (1.2 and 0.6°C min^{-1}, respectively; Fig. 4). This difference in the increase in relative temperature per minute might be explained by the differences in body mass (3.3 g for *Mecopoda* sp. and 0.4 g for *A. muticus*). *Mecopoda* sp. is predicted to have a higher proportion of thoracic muscle mass and, hence, greater muscular power (Bennet-Clark, 1989) than *A. muticus*, whereas the heat loss is expected to be more rapid in the smaller species (*A. muticus*) because of its higher surface-to-volume ratio.

In some katydids with extremely fast stridulatory movements, warm-up phases prior to singing have been reported, which increase thoracic temperature (Stevens and Josephson, 1977; Heller, 1986). Intuitively, one may assume that the temperature of the singing muscles must be elevated to maintain stridulatory movements at such high rates. Thus, a more general question in this context is whether the increase in temperature can be regarded as a cause or a result of the species' (high) pulse rate. If the thorax temperature increase is required to produce high pulse rates, we would expect to observe a warm-up phase prior to singing and higher pulse rates over the course of calling. However, this was not the case: no individuals of the 22 species studied in this analysis displayed a warm-up phase prior to calling (observed using infrared thermography), and we observed only a marginal shift in pulse rate within a range of 4% for *Mecopoda* sp. and *A. muticus*, several minutes after the onset of singing, at a time when the highest thoracic temperature was recorded for the males. Such a minor shift is not expected to result from a temperature change but, instead, falls within the range of within-individual variability for this parameter (usually ∼4%; Gerhardt and Huber, 2002). In insects, the possible power output of the flight muscles depends strongly on the temperature of these muscles (Heinrich, 1993). The fact that the calling parameters remain quite stable, therefore, suggests that regulatory neural circuits counteract the effects of alterations in the temperature of muscles on singing parameters.

Another question arising with energetically demanding calling songs is if elevated thorax temperature constitutes a physiological problem for males caused by heat stress, because maximum surface temperature during calling reached up to 38.9°C (*Ruspolia nitidula*) and even 42.9°C in the thorax core of *Neoconcephalus robustus* (Stevens and Josephson, 1977). The recently proposed heat dissipation limit theory (Speakman and Król, 2010) states that energy expenditure is not limited by the energy supply in the environment but is instead limited by the capacity to dissipate heat and therefore to avoid hyperthermia. Thus, the behavioural performance of acoustic signalling might be limited by its physiological heat tolerance (Neven, 2000) or the ability to efficiently dissipate heat from the thorax.

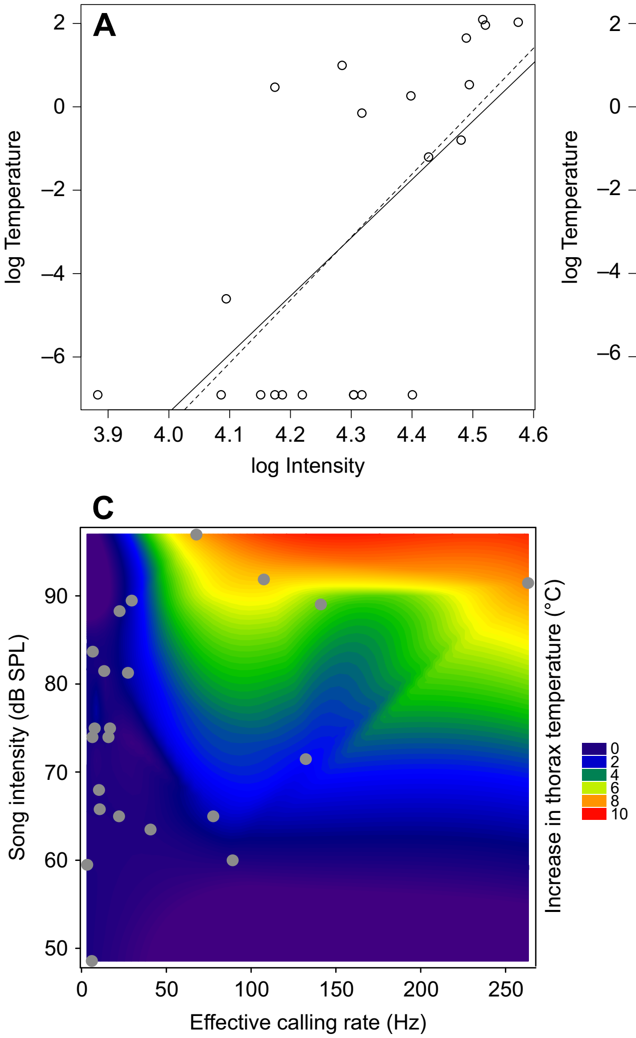

Fig. 8. Relationship of thorax temperature to song intensity and effective calling rate. (A) Thorax temperature (°C) versus song intensity (dB SPL). (B) Thorax temperature (°C) versus effective calling rate (Hz). Regression lines of the linear model (dashed line; A: R^2=0.46; B: R^2=0.44) and phylogenetic generalised least square model (solid line; A: R^2=0.51; B: R^2=0.55) are shown. (C) Contour plot showing the multidimensional relationship between thoracic temperature increase and the statistically most relevant song parameters effective calling rate and sound intensity (grey dots represent species).

A major disadvantage of using sound for intraspecific communication in insects is the inefficiency of sound production. Only a fraction of the muscular energy used to activate the sound-producing apparatus can be converted into acoustic energy for the call, and much of the energy is lost as a result of frictional forces and muscle contraction (Ryan, 1988). The efficiency of sound production in most insects (the amount of energy required for calling as compared with the amount of sound power being produced) is in the range of 1.0% or even lower, and rarely exceeds a few percent (Kavanagh, 1987; Prestwich, 1994; see Bailey et al., 1993, for an exception). According to this, up to 99% of energy invested in a signal may be ‘wasted’ during acoustic signalling. However, increased thoracic temperature in males might play a role in the context of multimodal communication and potentially influence a female’s decision to mate. Thoracic heating during sound production turns the acoustic signal into an inherently bimodal signal, whereby the energy invested in sound production is not ‘wasted’ but, instead, is reflected in the elevated thoracic temperature of a male. This thermal cue could provide important, reliable information for females in mate-choice decision scenarios, as it allows them to evaluate how much cumulative energy was invested by the sender in the signal. Additionally, thermoreception would be advantageous for females in lek-like systems to expose satellite males that do not invest in costly song production but instead try to intercept arriving females attracted by other singing males (Cade, 1975). Females could assess the thoracic temperature of males to distinguish between those that had been singing for some time, but which had fallen silent upon phonotactic arrival (but were still heated up above ambient temperature) and non-singing satellite males. This information is available only through the thermal modality and not the acoustic one, because acoustic signals vanish immediately, whereas more time is required for the thorax to cool down again (see example in Fig. 2). The thoracic temperature is still elevated above the resting level by 1–2°C for several minutes after the male has ceased singing and, therefore, the female can easily detect evidence of singing using her sensory system and the thermo-receptors associated with cuticular sensilla in the form of sensory pegs that are located on the antennae (Altner and Loftus, 1985; Tichy and Gingl, 2001). We are currently investigating the role of thermal information in mate-choice decisions.

As a result of this study, we were able to demonstrate relationships among different song parameter combinations, metabolic costs and increases in thoracic temperature. Among the acoustic parameters considered, song intensity had the strongest association with thoracic temperature and is, therefore, inferred to be more energetically demanding than other parameters. The importance of song intensity in the context of mate attraction is illustrated by the fact that an increase of 6 dB doubles the transmission distance, and thus increases the active space of the signal (Brenowitz, 1982). Consequently, a male that produces louder signals that travel over longer distances and potentially can attract more mates, can enhance its fitness, because females also prefer louder calls over songs with lower amplitudes (Walker and Forrest, 1989). However, a number of trade-offs may prevent all males from producing loud calling songs. Not only are these energetically costly to produce but they might also attract unintended receivers such as predators, parasites and rivals. Moreover, Symes et al. (2015) have shown that tree cricket species with louder calls spend less time singing as compared with species with lower call amplitudes. Acoustic signal evolution is shaped by various trade-offs between sexually and naturally selected traits, and it is necessary to analyse their costs and benefits to more completely understand this process.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: B.E., H.R.; Methodology: B.E., H.K., A.S., H.R., A.K.D.S.; Validation: H.K., A.K.S.; Formal analysis: B.E., A.K.D.S.; Investigation: B.E., H.K.; Resources: B.E., A.S.; Data curation: B.E., A.K.D.S.; Writing - original draft: B.E., H.R., A.K.D.S.; Writing - review & editing: B.E., H.K., A.S., M.H., H.R., A.K.D.S.; Supervision: H.R., A.K.D.S.; Funding acquisition: M.H., H.R.

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