



Comparative thermoregulation between different species of dung beetles (Coleoptera: Geotrupinae)

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ARTICLE INFO

Keywords:

Heat thermal stress
Infrared thermography
Thermoregulation ability
Overheating
Thermal tolerance

ABSTRACT

Insects can use thermoregulation to keep their body temperatures within a certain range in response to thermal stress situations. The mechanisms to regulate internal temperature depend on whether species are endothermic or ectothermic species, i.e., if the heat source is internal and/or external. In this study, the thermal stress response due to excess heat was examined in individuals belonging to different species of the Geotrupinae subfamily by using a standardized protocol based on infrared thermography. All the measured heat stress variables allow discrimination among the considered species to a greater or lesser extent. The body heating rate in the heat stress range, the critical thermal maximum and the stress start temperature were the most important variables in discriminating between apterous and winged individuals ($R^2 = 52.5\%$, 51.1% and 50.0% , respectively). Examining the degree of association between the physiological similarity of individuals and some species traits suggest that flying capability and daily activity are related with different thermal responses. Based on the obtained results, the Geotrupinae subfamily can be divided into three ecophysiological groups: good, medium and non-thermoregulators. Within these groups, apterism appears mainly in those species with the ability to actively decrease their body temperature. Our results indicate that this ability may be partially due to the management of water loss related with evaporative cooling.

1. Introduction

Temperature is one of the most important factors affecting many biological and metabolic processes (Angilletta, 2009). Assuming that both the thermal optimum and the thermal performance breadth of individuals match body temperatures experienced under different environmental conditions, species with distinct distributional ranges and life history traits may differ in their thermal niches (Bozinovic et al., 2011). This *a priori* close relationship between physiological and environmental temperatures would explain the distribution and abundance of species. In contrast, a mismatch between physiological and environmental temperatures would indicate that behaviour, physiological plasticity and variation in thermal tolerance could be the rule, and that distribution and abundance must be explained by other types of factors.

The thermal tolerance and heat stress of phylogenetically close related species is in many cases correlated with the thermal conditions

prevailing in the environment in which the species lives (Garbuz et al., 2003; Gallego et al., 2016; Hamblin et al., 2017; Zhu et al., 2017, but see also Paterson and Blouin-Demers, 2017). However, things seem to be much more complex. Upper thermal limits in ectotherm species seem to be relatively homogeneous (Hamilton, 1973; Addo-Bediako et al., 2000) generally approaching the maximum temperature at which metabolic and growth rates are more effective without reaching inoperative lethal levels. As a consequence, if heat stress tolerances in ectotherms differ between phylogenetically related species, these differences will tend to appear close to upper extreme temperatures, an important fact that may diminish the possibilities of adaptation under drastic climatic changes (Hamilton, 1973). Here, we examine whether the response to thermal stress differs among species of an ancient monophyletic beetle group (Coleoptera, Geotrupidae, Geotrupinae) living mainly under cold temperate conditions. The existence of different thermal responses between the species of this group would suggest that the management of heat stress may played a role in the

Abbreviations: CT_{max}, Critical thermal maximum; HCR, Heat coma range; HRT, Heat regulation temperature; HSR, Heat stress range; rHCR, Rate of increase in the body temperature per unit of time during the heat coma range (HCR); rHSR, Rate of increase in the body temperature per unit of time during the heat stress range (HSR); rSAR, Rate of increase in the body temperature per unit of time during the supra-optimal activity range (SAR); rTR, Rate of increase in the body temperature per unit of time during the thermoregulation range (TR); SAR, Supra-optimal activity range; SST, Start stress temperature; TR, Thermoregulation range; ULT, Upper lethal temperature

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<https://doi.org/10.1016/j.jtherbio.2018.03.009>

Received 19 August 2017; Received in revised form 14 February 2018; Accepted 9 March 2018

Available online 13 March 2018

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diversification of this group.

Briefly, Geotrupinae species are frequently associated with the dung of mammalian herbivores as part of the dung beetle assemblages, including both diurnal and nocturnal species, with broad and narrow distribution ranges, varied morphology and behaviour, as well as different climatic and environmental conditions in the localities in which they live. This monophyletic group, whose origins date back to Cretaceous times (Krell, 2007; Cunha et al., 2011), is mainly distributed across the Holarctic region, harbouring a diversified group of derived species that is well represented in the Iberian Peninsula and North Africa (López-Colón, 2000). Interestingly, many of these Mediterranean Geotrupinae species are apterous due to the reduction of the hind wings and the fusion of elytra. This morphological characteristic is usually considered an adaptation to arid and extreme conditions because the fusion of elytra diminishes the loss of body water, and the subelytral cavity allowed the abdomen expansion to store water (Scholtz, 1981; Draney, 1993; Prange, 1996; Verdú and Galante, 2002). In other dung beetle groups with a Gondwanian origin that are adapted to tropical and hot-temperate conditions (e.g., Scarabaeidae), winged species of medium and large body are endothermic and have different mechanisms for thermoregulation in response to the excess of heat (Verdú et al., 2012). These species must cope with both the internal heat produced by the dorsoventral and longitudinal thoracic muscles during flight and the external heat of the environment (Heinrich, 1993; Denlinger and Yocum, 1998; Chown and Nicolson, 2004; Verdú et al., 2012). In regard to ectothermic species without the ability of flight, the overheating risk should be mainly due to the different external sources of heat available in their environment. However, these apterous or flightless species may also thermoregulate, although the involved mechanisms and their frequency or capacity are relatively unknown (Huey and Stevenson, 1979; Heinrich, 1995; Willott, 1997). In the case of Geotrupinae the adaptations of apterous species toward arid and extreme climates provide an opportunity to analyse the thermal strategies of a mostly cold temperate group when it has to address heat stress.

In this study, thermal stress responses due to heat excess were examined in individuals belonging to different species of the Geotrupinae subfamily using a standardized protocol based on infrared thermography (Gallego et al., 2016). By including species with varying morphological, ecological and phylogenetic characteristics, the aims of this study are to ascertain i) the existence and diversity of the thermoregulation mechanisms used to avoid overheating within this group and ii) the degree of the association between these mechanisms and different species traits, such as flying capability, daily activity and phylogeny.

2. Materials and methods

2.1. Animals, capture and acclimation conditions

Individuals belonging to 26 species of Geotrupinae (Coleoptera, Geotrupidae) were studied to estimate heat stress responses. The number of studied specimens for each species was determined by its availability under natural field conditions (see Table 1 and Table S1 in Supporting Information). The selected species represent approximately 58–64% of the Geotrupinae genera inhabiting the Palaearctic region, depending on the phylogenetic and taxonomic criteria (Branco and Ziani, 2006; Lobo et al., 2015), and 75% of the genera and 46% of the species inhabiting Europe (López-Colón, 2003). The individuals were collected in the field, immediately placed in 10 × 10 × 10 cm plastic containers with soil from the collection site and stored at 15 °C inside a portable cooler (Thermo Engel® 18 l) during transport to the laboratory. To select individuals of approximately the same age, we estimated both the cuticular deterioration of the anterior tibia and the hardness of the pronotum and elytra (Tyndale-Biscoe, 1984).

Individuals of each species were acclimated using a refrigerated chamber (MIR-153, Sanyo Electric Co. Osaka, Japan) at 25 °C, which is

the minimum temperature at which the neuromuscular system performs properly and individuals are active without heat stress (May, 1979, 1985; Vannier, 1994; Chown and Nicolson, 2004; Gallego et al., 2016). All individuals were fasted during the five-day acclimation period to mitigate the effect of diet and gut content on the physiological measurements obtained (Verdú et al., 2010; Gallego et al., 2016). These experimental procedures conform to the Spanish legal requirements, including those related to conservation and welfare. The beetle capture was performed according to the relevant permits related to animal collection and laboratory studies.

2.2. Thermal measurements

Thermal responses to heat stress were estimated using the infrared thermography protocol proposed by Gallego et al. (2016) under controlled conditions applied similarly to all the studied individuals (a model graph of the obtained response is shown in Fig. 1). The use of a thermocamera allows us to record and display the thermal sessions to estimate body temperatures as well as behavioural activities. The assays were carried out using a hotplate (rectangular precision hotplate “Plactronic”; stability ± 0.5 °C; J.P. Selecta, Barcelona, Spain). A pin was stuck to the left side of the thorax of each beetle specimen and that pin was pricked on a strip of cork by using hot melt adhesive. Between three and five individuals, placed 3 cm apart, are fixed on the strip of cork and the entire set placed 2.5 cm above this hotplate so that the ventral side of the beetle is oriented parallel to the hotplate (see Fig. S1 in Supporting Information). Thermocamera was placed at a set height above the experimental arena composed by the hotplate and the strip of cork containing dung beetle specimens.

The temperatures of living individuals were compared to freshly killed individuals (by freezing) after they were acclimated similarly, which represent operative temperature measurements (Dzialowski, 2005) that are hereinafter referred to as “model individuals”. A ramp heat treatment with a heating rate of 1.5 °C min⁻¹ was used in order to ensure that all individuals reached a stress state, as endotherm beetle species tend to have high pre-flight and flight warming rates that easily reach 2 °C min⁻¹ (Heinrich, 1993; Denlinger and Yocum, 1998; Verdú et al., 2012). The heating rate was recorded in each experimental trial using a K/J thermometer Fluke 152 (Fluke Co. California, USA) placed in the “model individual”. Following this protocol, three different body temperatures were measured (see Fig. 1): start of stress temperature (SST), critical thermal maximum (CT_{max}) and upper lethal temperature (ULT).

These three temperatures are estimated watching the video recordings carried out with the thermocamera combining with temperature data and thermal curves behaviour. Although partially subjective these temperatures are not arbitrary and are in line with those obtained using thermolimit respirometry (not showed). SST was delimited to the moment when a clear and sudden acceleration in leg and head movements was observed. CT_{max} is the temperature at which hind limb paralysis was observed, and ULT is the temperature at which individuals were completely paralyzed and dead. These three temperatures delimit the following three thermal ranges: the supra-optimal activity range (SAR; from the acclimation temperature to the SST), the heat stress range (HSR; from the SST to the CT_{max}), and the heat coma range (HCR; from the CT_{max} to the ULT). The heating rate in these three thermal ranges ($rSAR$, $rHSR$ and $rHCR$) has also been estimated and, finally, six variables that measure the response of individuals to heat stress have been considered (see Fig. 1 and Gallego et al., 2016).

All measurements were obtained using a FLIR ThermoCam P620 thermal infrared camera (resolution of 640 × 480 pixels) with a microbolometer Focal Plane Array detector (with a spectral range of 7.5–13 µm and a thermal sensitivity of 0.06 °C at 30 °C). The thermocamera was calibrated by the Standard Calibration service provided by FLIR Systems, Inc. We measured the cuticle emissivity at different temperatures (from 40 °C to 70 °C) using fresh cuticles from all

Table 1
Number of studied individuals (N) and main characteristics of the studied species. IP = Iberian Peninsula.

Species	N	Collected location	Distribution	Phylogenetic Clade	Daily Activity	Flying Capacity
<i>Jekelius albarracinus</i>	3	Valencia (Spain)	Central and southeast of IP	I	Diurnal	No
<i>Jekelius brullei</i>	3	Kütahya (Turkey)	Eastern Europe	I	Diurnal	No
<i>Jekelius castillanus</i>	3	Toledo (Spain)	Centre of IP	I	Diurnal	No
<i>Jekelius hernandezi</i>	8	Alicante (Spain)	Southeast of IP	I	Diurnal	No
<i>Jekelius hispanus</i>	3	Huelva (Spain)	Southwest of IP	I	Diurnal	No
<i>Jekelius nitidus</i>	3	Toledo (Spain)	Northwest of IP	I	Diurnal	No
<i>Jekelius punctatolineatus</i>	8	Alicante (Spain)	Southeast of IP	I	Diurnal	No
<i>Silphotrupes escorialensis</i>	2	Ávila (Spain)	Northwest and centre of IP	I	Crepuscular	No
<i>Silphotrupes orocantabricus</i>	6	Lugo/León (Spain)	Northwest of IP	I	Crepuscular	No
<i>Silphotrupes punctatissimus</i>	4	Zamora (Spain)	North and centre of IP	I	Crepuscular	No
<i>Thorectes armifrons</i>	9	Ifrane (Morocco)	North of Africa	I	Diurnal	No
<i>Thorectes baraudi</i>	3	Toledo (Spain)	Central and southern of IP	I	Diurnal	No
<i>Thorectes laevigatus</i>	3	Tetuán (Morocco)	North of Africa	I	Diurnal	No
<i>Thorectes lusitanicus</i>	10	Almería/Málaga (Spain)	Southern of IP	I	Diurnal	No
<i>Thorectes trituberculatus</i>	4	Khénifra (Morocco)	North of Africa	I	Diurnal	No
<i>Thorectes valencianus</i>	10	Alicante (Spain)	Southeast of IP	I	Diurnal	No
<i>Trypocopris pyrenaicus</i>	11	Huesca/Lugo (Spain)	Europe	I	Diurnal	Yes
<i>Anoplotrupes stercorosus</i>	4	León (Spain)	North of IP	II	Nocturnal	Yes
<i>Geotrupes ibericus</i>	3	Huelva (Spain)	IP	II	Nocturnal	Yes
<i>Geotrupes mutator</i>	3	Ávila (Spain)	West of Europe	II	Nocturnal	Yes
<i>Geotrupes stercorarius</i>	14	Huesca/Madrid/Zamora/León (Spain)	West of Europe	II	Diurnal	Yes
<i>Sericotrupes niger</i>	3	Huelva (Spain)	Southwest of IP/ North of Africa	II	Crepuscular	Yes
<i>Ceratophyus hoffmannseggii</i>	6	Madrid/Huelva (Spain)	Southwest of IP/ North of Africa	III	Nocturnal	Yes
<i>Typhaeus momus</i>	6	Cádiz/Huelva (Spain)	Southern of IP	III	Crepuscular	No
<i>Typhaeus typhoeoides</i>	7	Khémisset (Morocco)	North of Africa	III	Nocturnal	Yes
<i>Typhaeus typhoeus</i>	2	Málaga (Spain)	Europe/ North of Africa	III	Nocturnal	Yes

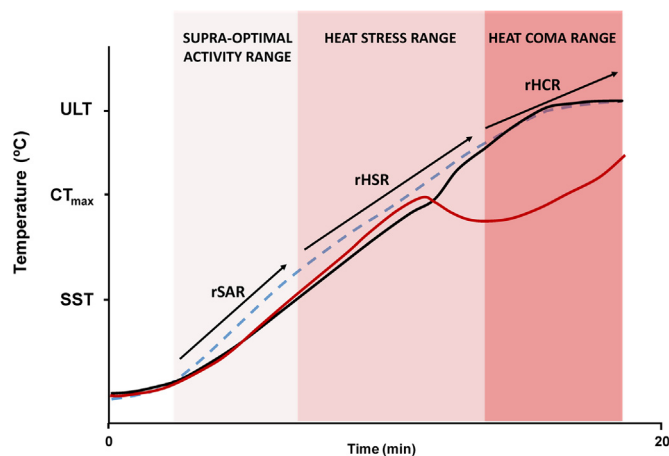


Fig. 1. Graphical representation of a heat stress response with the variables obtained by infrared thermography. SST: start stress temperature; CT_{max} : critical thermal maximum; ULT: upper lethal temperature; rSAR: heating rate on supra-optimal activity range; rHSR: heating rate on heat stress range; rHCR: heating rate on heat coma range. Red continuous line: thermoregulatory living individual; blue discontinuous line: not thermoregulatory living individual; black: model individual (death).

Geotrupinae species studied, obtaining values between 0.94 and 0.99. As a reference for the measurements, we used electrical tape (a reference method described in ISO 18434–1) and black paint (NEXTEL-Velvet-Coating 811–21). In each experimental trial, the ambient temperature in the laboratory was recorded using a Fluke 152 K/J thermometer (Fluke Co., California, USA). All individuals were weighed before and immediately after all assays using a precision balance (AG104 Analytic Balance; Mettler Toledo, Columbus, OH, US). The assays were performed with the thermocamera connected to a computer in which the video recording was stored, and the provided images were used to obtain the temperatures by selecting an area in the central part of the pronotum (ca. 20 mm²) where temperature data were obtained using the ThermoCAM Researcher Pro 2.9.

2.3. Studied traits

We examine the explanatory capacity on thermal stress responses accounted for three types of categorical predictors reflecting the main clades, the daily activity patterns and the presence or absence of wings (Table 1). Phylogenetic relationships are relatively well established based on morphology, partial mitochondrial and nuclear gene sequence data (Zunino, 1984; Martín-Piera and López-Colón, 2000; Verdú and Galante, 2000; Lobo et al., 2006; Cunha et al., 2011; Lobo et al., 2015). Such phylogenetic information was used to assign each of the species studied to one of the following three main clades derived from the phylogenetic relationships obtained by Cunha et al. (2011): *Thorectes-Jekelius-Silphotrupes-Trypocopris* (clade I), *Geotrupes-Anoplotrupes-Sericotrupes* (clade II) and *Ceratophyus-Typhaeus* (clade III). The general daily activity patterns of each species were categorized as nocturnal, diurnal or crepuscular, because this factor may influence the temperature range to which insects are exposed (Holm and Edney, 1973; Huey and Bennett, 1990; Verdú et al., 2004). Finally, we also classified the species studied as apterous (non-flying) or winged (flying) because this trait is considered an adaptation to semi-arid and arid conditions, which is related to thermoregulatory capacity through evaporative cooling due to water storage in the subelytral cavity (Draney, 1993).

2.4. Statistical analyses

Between-species differences in the thermal response to heat stress were examined using general linear models (GLMs) with an ANCOVA model design using a type III sum of squares (i.e., estimating the partial effects of each factor while controlling for the effects of the remaining predictors). Statistical analysis accounted for inter-individual variability according to Spicer and Gaston (1999). Therefore, species was not considered as a random factor. In addition to species identity (response variable), the following three continuous covariates were included in the model to statistically control for their effects on the response to heat stress variables: initial body mass, room temperature, and weight loss. The logarithm of each individual body mass was included to account for the effect of physical inertia (the resistance to a change in body temperature depending on the mass). The average air temperature

experienced in the room during each heating assay was also included to account for its possible influence on the response variables. Weight loss is the reduction in the body mass of each individual during each assay and was included here to consider the possible differences in the management of body temperatures due to evaporative cooling. Model residuals were checked for normality and homoscedasticity assumptions.

Subsequently, the relationship between the physiological similarity of the different species and the selected traits (phylogenetic clades, daily activity patterns and flight capacity categories) was assessed using a non-parametric PERMANOVA test (Anderson and Walsh, 2013). Adjusted GLM mean values of the heat stress variables for each species were used (i.e., eliminating the effects of co-variables) and were standardized to a mean of zero and one standard deviation to eliminate the effect of measurement scale differences. The non-Euclidean and non-metric Gower coefficient was used to estimate physiological similarity among all the species considered. A weight proportional to their explanatory capacity (R^2 in GLM) was given to each thermal stress variable to generate a triangular similarity matrix that is able to better reflect the physiological similarity. Finally, multidimensional scaling (MDS) was used to provide a bi-dimensional visual representation of the physiological similarity of the species according to their formerly mentioned traits.

All the statistical analyses were performed using StatSoft's STATISTICA v12.0 (StatSoft Inc, Tulsa, Oklahoma, USA) and PRIMER v6.1.14 + PERMANOVA v6.1.0.4.

3. Results

3.1. Differences in thermal response to heat stress

All the heat stress variables considered allow for discrimination, to a greater or lesser degree, among the species considered (Table 2). The variables for which the species factor showed a higher explanatory capacity are $rHSR$, SST and CT_{max} , which explained 52.5%, 51.1% and 50.0% of the observed variability, respectively. Pairwise comparisons between the species were carried out using post hoc N-HSD tests, and 5%, 14% and 27% were statistically significant for $rHSR$, SST and CT_{max} , respectively (see Fig. 2). $rSAR$ allowed for the discrimination of 19% of all pairwise comparisons, despite its relatively low explanatory capacity (Table 2). Regarding the covariates included, body mass had no effect on any variable and the ambient temperature at which the tests were carried out influenced $rSAR$, $rHSR$ and SST , explaining 17.5%, 2.2% and 2.6% of the total variability, respectively. Finally, the weight loss during the assays only had an effect on the $rHSR$ variable, explaining 6.8% of total variability (a greater weight loss occurred

Table 2

GLM results examining between-species differences in six heat stress variables after controlling for body mass of individual beetles, water loss during assays and variations in ambient temperature in the experimentation room (included as covariates in the GLM with an ANCOVA model design using species as a fixed factor). R^2 is the variance accounted for by the species factor (in %) when the effects of all covariates have been accounted for. Total R^2 is the variance accounted for the complete model. The right column shows the number of species pairs with statistically significant differences according to a post-hoc unequal N HSD test (the total number of pairwise comparisons are 325).

Heat stress variables	$F_{(25,112)}$	P	Total R^2	R^2	N° pairs
SST ($^{\circ}C$)	6.59	< 0.0001	65.3	51.1	47
CT_{max} ($^{\circ}C$)	9.86	< 0.0001	77.3	50.0	88
ULT ($^{\circ}C$)	3.89	< 0.0001	57.9	36.5	28
$rSAR$ ($^{\circ}C/min$)	5.39	< 0.0001	75.8	29.1	63
$rHSR$ ($^{\circ}C/min$)	5.35	< 0.0001	56.1	52.5	15
$rHCR$ ($^{\circ}C/min$)	3.06	< 0.0001	41.7	39.8	3

Abbreviation: SST : start stress temperature; CT_{max} : critical thermal maximum; ULT : upper lethal limit; $rSAR$: heating rate on supra-optimal activity range; $rHSR$: heating rate on heat stress range; $rHCR$: heating rate on heat coma range.

when the $rHSR$ values were low). However, this effect varies substantially depending on whether the specimen belongs to a winged or apterous species. In winged individuals $rHSR$ values are unrelated to weight loss ($R^2 = 0.93\%$; $F_{(1,51)} = 0.48$; $p = 0.41$), but they are highly related in the case of apterous individuals ($R^2 = 24.95\%$; $F_{(1,86)} = 28.59$; $p < 0.0001$). Model residuals were always normally distributed, and scatterplots of standardized residuals against fitted values did not show a systematic pattern. A visual inspection of the between-species variation in the $rHSR$, CT_{max} and SST values (Fig. 2) showed that higher differences in these variables were observed between those species of the apterous genera *Thorectes* and *Jekelius* and those winged species belonging to *Geotrupes*, *Anoplotrupes*, *Sericotrupes* or *Ceratophyus*. In general, the factor species accounts for a high proportion of the complete explained variability (from 38% to 95%), while the unexplained variability ranges from 23% to 58% (Table 2).

3.2. Correspondence with selected factors

The species belonging to phylogenetic clades II and III showed a similar thermal response to heat stress, but both differed from those belonging to clade I (Table 3). The categories of daily activity appear to form independent groups with statistically significant differences among diurnal and nocturnal species, but the responses of nocturnal and crepuscular species seem to be similar (Table 3). Finally, differences in thermal traits also appear in measures of physiological similarity between apterous and winged individuals. These results are shown in Fig. 3.

3.3. Responses to overheating

It is possible to easily group the studied species taking into account the values of the two variables with a higher interspecific discrimination capacity (the heating rate on the heat stress range, $rHSR$, and the critical thermal maximum, CT_{max}). Thus, responses to overheating in Geotrupinae could be divided into the following three categories according to the specific conditions of the laboratory experiments (Fig. 2): i) 'good thermoregulators' capable of actively decreasing their body temperatures before the appearance of irreversible injury; ii) 'non-thermoregulators' that cannot actively decrease their body temperatures; and iii) 'intermediate thermoregulators' with an intermediate response between these two groups. Good thermoregulators have low or intermediate $rHSR$ values and high or medium CT_{max} values, showing slow heating rates and relatively high heat tolerances. Non-thermoregulators would be those that warm up quickly and reach the critical state at relatively low temperatures, thereby having high or medium $rHSR$ values and low or intermediate CT_{max} values. Finally, intermediate thermoregulators would include species with varied heating rates and critical thermal maximum values that do not correspond with those of the other, more extreme categories.

4. Discussion

In this study, the thermal response to heat stress has been examined for a mainly cold temperate group. The provided results show that Geotrupinae species have two main strategies to cope with heat stress, which are associated with the apterous or winged character of the species. The existence of these strategies suggests that the Mediterranean diversification of this group and the varied response to heat stress were enabled by a trade-off with the loss of the capacity of flight.

Three main variables have higher interspecific discriminatory capacity ($rHSR$, SST and CT_{max}) and reflect the rate of heat acquired by the body during the heat stress range, the temperature at which stress starts, and the critical maximum temperature, respectively. All these variables characterize the tolerance to high temperatures of the examined individuals, and their values significantly differ between

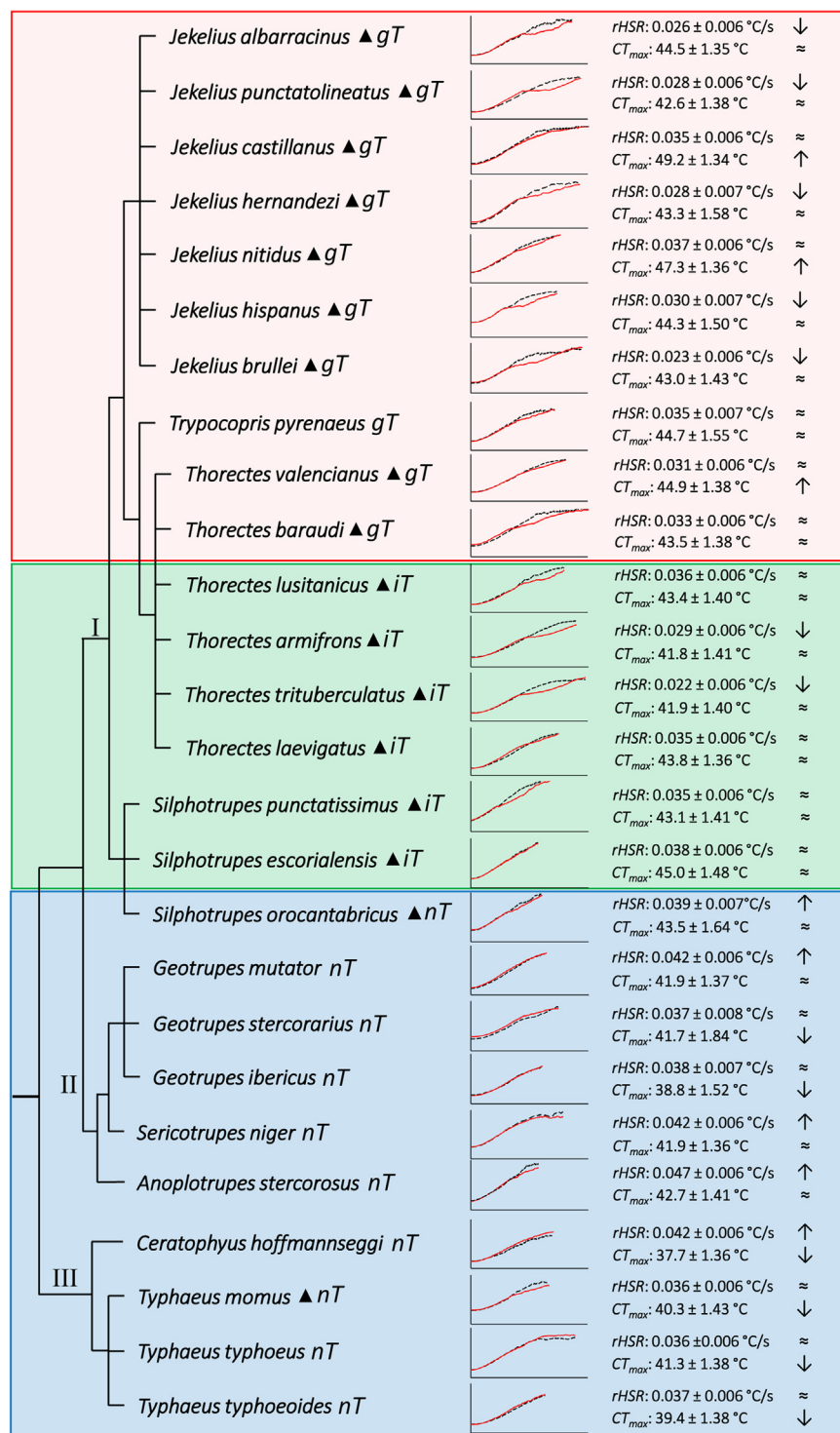


Fig. 2. Average responses to heat stress of Geotrupinae species, phylogenetic relationships and thermoregulation categories. Each graph shows in a black discontinuous line the response of the model (death) individual and in red the average response of the living studied individuals. On the left side of the graph, the phylogenetic relationships between studied species are shown and clades are indicated (based on Cunha et al., 2011 and Lobo et al., 2015). For the five species not included in these published phylogenies, the positions are assumed according to their taxonomic identity. Colours and letters indicate good thermoregulators (gT, in red), intermediate thermoregulators (iT, in green), and non-thermoregulators (nT, in blue). On the right side of the graph, the average values of heating rate on heat stress range ($rHSR \pm SD$) and critical thermal maximum temperature ($CT_{max} \pm SD$) are provided. ▲: apterous species; †: high values; ‡: low values; ≈: medium values.

apterous and winged species, as well as between diurnal (all but one are apterous; see Table 1) and crepuscular/nocturnal species. Thus, the ability to be active during diurnal and hot conditions seems to be a characteristic associated with the atrophy of the hind wings and the fusion of elytra. As a consequence, all the species belonging to the apterous genus *Jekelius*, and three species belonging also to the apterous genus *Thorectes* (*T. valencianus* (Baraud, 1966), *T. armifrons* (Reitter, 1893) and *T. trituberculatus* Reitter, 1892) can be categorized as good regulators of body excess temperature under experimental conditions ('good thermoregulators'; see Fig. 2). These species are all diurnal and generally distributed in semiarid and lowland areas; although *T.*

armifrons, distributed in North Africa, may occur at higher altitudes (Verdú et al., 2015). All species of this group belong to the phylogenetic clade I. The intermediate thermoregulator (Fig. 2) group is characterized by species distributed in mid-mountain areas, such as the apterous diurnal species *T. lusitanicus* (Jekel, 1866), *T. baraudi* López-Colón, 1981 and *T. laevigatus* Fabricius, 1798. This group also includes some apterous and crepuscular species such as *Silphotrupes punctatissimus* (Chevrolat, 1840) and *S. escorialensis* (Jekel, 1866), which are distributed in the central and northern arid mountainous areas of the Iberian Peninsula. Furthermore, this group also includes *Trypocopris pyrenaicus* (Charpentier, 1825), a flying species distributed in the

Table 3

Permutational multivariate analysis of variance (PERMANOVA) test examining the association between the physiological similarity of the studied species and three qualitative factors: daily activity, flying capability and phylogenetic group. Clade I: Species of *Thorectes*, *Silphotrupes* and *Trypocopriss* genera; clade II: Species of *Geotrupes*, *Anoplotrupes* and *Sericotrupes* genera; clade III: Species of *Ceratophyus* and *Typhaeus* genera.

Daily Activity	t	P
Nocturnal, Diurnal	6.123	0.001
Nocturnal, Crepuscular	2.601	0.557
Diurnal, Crepuscular	2.455	0.002
Flying capacity	t	P
Apterous/Winged	3.060	0.004
Phylogenetic clades	t	P
clade II/clade III	1.391	0.220
clade II /clade I	3.143	0.001
clade III/clade I	3.202	0.035

mountainous areas of Western Europe (Verdú et al., 2015) that shows a low endothermic capacity during flight compared with other Geotrupinae winged species (J. R. Verdú, unpublished data). *T. pyrenaicus* would be the only winged species with the ability to decrease their body temperature to cope the excess of heat; this represents an intermediate strategy between that of the apterous *Thorectes* species (*sensu lato*) and the winged species with a more basal position within the Geotrupinae phylogeny (Cunha et al., 2011). This second group is also formed by species of the phylogenetic clade I.

Finally, the remaining individuals belong mostly to clades II and III can be considered bad physiological thermoregulators when trapped in the hot microenvironments established by the used experimental conditions (Fig. 2). They are nocturnal or crepuscular and are distributed in cold areas with an autumn-winter phenology. The individuals of all these species can fly, except *Typhaeus* (*Chelotrupes*) *momus* (Olivier, 1789) and *Silphotrupes orocantabricus* (Verdú and Galante, 2000). *T. momus* is a vespertine-crepuscular and autumn-winter species distributed in the coastal areas of the south of the Iberian Peninsula (Gallardo de la Torre et al., 2002) and *S. orocantabricus* is the only species of the phylogenetic clade I within this group, being a vespertine-crepuscular species distributed at higher elevations in the Iberian Peninsula than the other two Iberian *Silphotrupes* species (Verdú et al., 2015). We argue that these non-thermoregulator species lack physiological adaptations to heat stress because their main physiological requirement is to maintain and generate heat rather than to eliminate it (Zunino and Palestini, 1986; Martín-Piera and López-Colón, 2000; López-Colón, 2000, 2003; Lobo and Hortal, 2006; Verdú et al., 2007). Thus, we hypothesize that the emergence of apterism would have propitiated the evolution of novel physiological mechanisms of thermoregulation in part because apterous species could no longer thermoregulate behaviourally. The consequence in Geotrupinae is that it is possible to find a high variability of responses to manage heat stress, both endothermic and ectothermic, both behavioural and physiological. Even within the studied endothermic Geotrupinae, there are both diurnal and nocturnal species, implying that a general physiological pattern to manage heat stress is not plausible (Krogh and Zeuthen, 1941; Scholtz, 2000; Verdú and Galante, 2000; Scholtz et al., 2009; Chown and Klok, 2011; Cunha et al., 2011).

Our results highlight the phylogenetic association of the detected thermal responses because apterism has a deep phylogenetic signal in Geotrupinae (Cunha et al., 2011). The association between thermoregulation and phylogenetic clade I is the consequence of the high degree of endemism of these related genera in the Western Mediterranean region, which could have acted as a diversification centre since the Lower Tertiary (Verdú and Galante, 2002; Cunha et al., 2011).

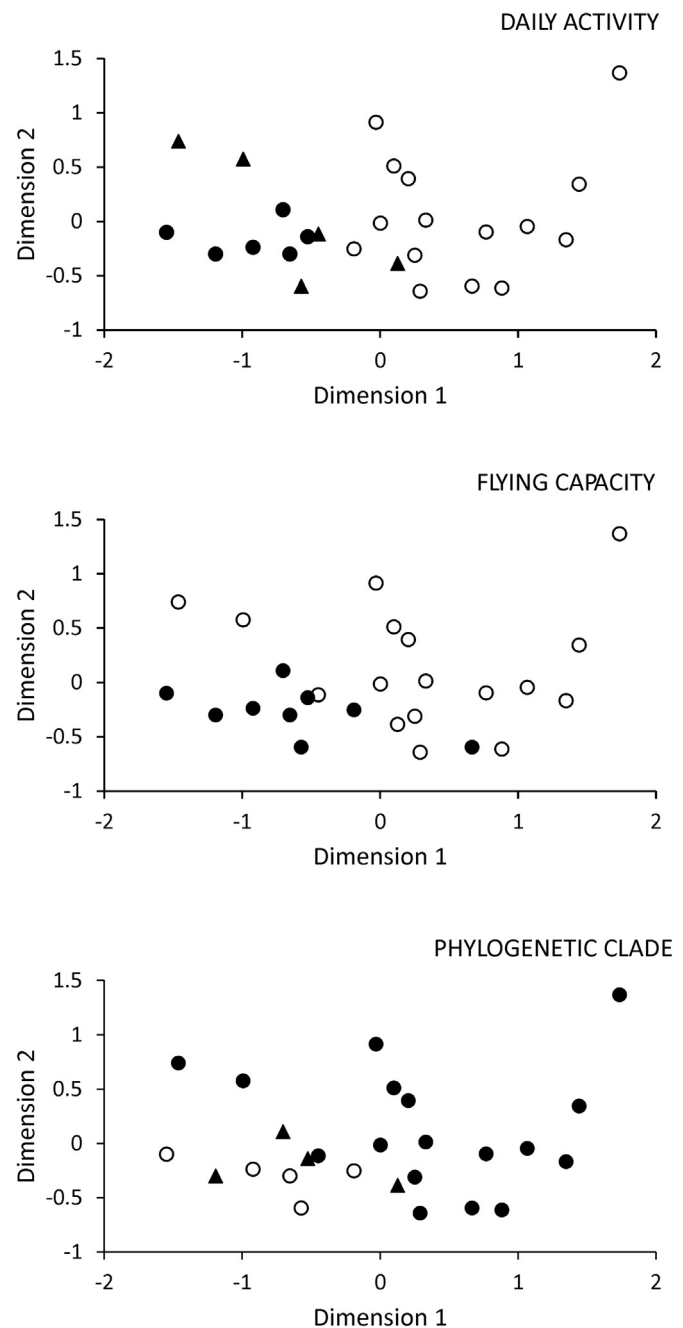


Fig. 3. Multidimensional Scaling (MDS) bi-dimensional representation of the matrix of physiological similarity according to the three studied species traits: daily activity (diurnal -white circles-, nocturnal -black circles-, crepuscular -black triangles-); flying capacity (winged -black circles-, apterous -white circles-), and phylogenetic clade (clade I -black circles-, clade II -white circles-, clade III -black triangles-).

These results highlight the importance of including phylogenetic information when estimating how different physiological traits vary within and between taxonomic levels (Chown, 2001). Further studies examining the connection of these thermal physiological variables and molecular data may help to elucidate the phylogenetic signal of these traits and the influence of evolution on the currently observed physiological patterns. Our results also show that the identity of the species explains 43% of the total physiological variability, while 38% of the variability may be due to the differences between individuals. Thus, it is necessary to continue research regarding thermal physiology that considers individual continuous traits and behavioural measures (Spicer and Gaston, 1999), which can allow measurement of thermal

responses before they reach the limits at which irreversible injuries occur. This research will enable us to obtain information on the insect strategies to handle thermal stress, which would help us better understand aspects such as the geographical distribution patterns, phenological activity or historical evolution of species or their ability to respond to climate change.

Apterism in Geotrupinae seems to have been crucial to thermoregulatory ability, and the evolution of this ability to avoid overheating might have promoted the success of this group in colonizing warmer and arid climates. Apterous species can all be considered good or intermediate thermoregulators who are able to actively decrease their body temperature when experiencing excess heat. We hypothesize that this capacity is linked with the fusion of elytra because it prevents water loss and increases the capacity to store water in the subelytral cavity (Roff, 1990; Draney, 1993). Our results indicate that water loss has a small influence on the heating rate during the heat stress range in Geotrupinae, but that weight loss may explain a quarter part of the variation in this heating rate in the case of apterous individuals (*rHSR* diminish 0.17 °C per milligram of lost weight). However, the relationship between apterism and thermoregulatory capacity cannot be only due only to the management of water and its use in the evaporative cooling process (Prange, 1996); both internal (via tracheal system) and external (by flapping or moving legs to circulate cool air) air ventilation mechanisms can be fundamental for this purpose. It is necessary to continue researching the thermal and hydric physiology of these species under heat stress conditions. The association between thermoregulation and diurnal activity has been observed in other insects (Fresquet and Lazzari, 2014) and is probably inevitable under Mediterranean conditions, where diurnal individuals need a thermoregulatory mechanism that allows them to live under these dry and hot conditions.

Geotrupinae is a lineage that likely originated in temperate climates during the Mesozoic (Cunha et al., 2011), with a relatively recent diversification related to the colonization of warm and arid environments. Thus, we can hypothesize that the ability to thermoregulate under warmer climates is a capacity acquired to manage heat stress situations associated with the loss of flying capacity. Flightless could be considered as a common strategy observed in Coleoptera inhabiting semiarid areas (Roff, 1990). In the case of dung beetles inhabiting semiarid areas, the tribe Eucraniini (except for *Emnearabds lobocephalus*) in South America, all species of the southern African genus *Pachysoma* as well as the South African *Circellium bacchus* are flightless species (Scholtz, 2000). Interestingly, apterism is the dominant condition among species of the other sister basal Geotrupidae subfamilies, such as Taurocerastinae and Lethrinae (Cunha et al., 2011). Further thermal studies on individuals of these subfamilies can shed light on the relationship between heat thermoregulation and apterism.

5. Conclusions

Within the Geotrupinae subfamily, we found three different strategies in response to heat stress, ranging from active thermoregulators to species unable to remove excess heat under the specific conditions of the laboratory experiments. These differences in the management of thermal stress are associated with the loss of flight and the fusion of elytra, as it is possible to find both winged endothermic species and apterous ectothermic species. The former would prefer to live under cold and mountainous zones and/or would have nocturnal activity, while the latter can be found in the more arid and warm areas of the Iberian Peninsula and are able to actively eliminate excess heat under thermal stress situations.

Acknowledgements

This work was supported by the Spanish research project CGL2011-25544 of the Ministerio de Economía y Competitividad, as well as by the F. P. I. fellowship BES-2012-052010 to B.G. We are grateful to two

reviewers by their valuable comments.

Conflict of interest

There are no conflicts of interest to declare

Author contributions

B.G., J.R.V. and J.M.L. conceived and designed the research, collected the biological samples, designed and performed the thermographical and behavioural analyses, performed data analyses and wrote the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jtherbio.2018.03.009>.

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Supplementary Information

Table S1. Detailed information of each one of the individuals used in this study.

Species	Province	Country	Collection	Weight	Tamb	SST (°C)	CT _{max} (°C)	ULT (°C)	rSAR	rHSR	rHCR	SEX
<i>Anoplotrupes stercorosus</i>	León	Spain	June 2014	0.393	24.5	30.00	42.90	50.20	34.97	43.14	34.43	Male
<i>Anoplotrupes stercorosus</i>	León	Spain	June 2014	0.375	24.5	30.30	42.30	50.80	38.40	44.78	36.72	Male
<i>Anoplotrupes stercorosus</i>	León	Spain	June 2014	0.286	24.5	30.10	43.50	50.60	37.91	47.02	36.32	Female
<i>Anoplotrupes stercorosus</i>	León	Spain	June 2014	0.355	24.5	30.20	42.30	50.60	38.80	45.57	34.02	Male
<i>Ceratophyus hoffmannseggi</i>	Madrid	Spain	April 2013	0.464	23.2	28.60	41.10	50.80	24.92	51.23	24.31	Female
<i>Ceratophyus hoffmannseggi</i>	Madrid	Spain	April 2013	0.718	23.2	28.30	38.30	50.30	17.73	40.98	53.93	Male
<i>Ceratophyus hoffmannseggi</i>	Madrid	Spain	April 2013	0.662	23.2	27.80	38.20	51.20	19.61	42.62	56.52	Male
<i>Ceratophyus hoffmannseggi</i>	Huelva	Spain	November 2014	0.466	23.8	27.70	36.20	45.70	26.64	36.72	19.83	Female
<i>Ceratophyus hoffmannseggi</i>	Huelva	Spain	November 2014	0.499	23.8	28.50	34.90	45.00	32.60	33.42	31.37	Male
<i>Ceratophyus hoffmannseggi</i>	Huelva	Spain	November 2014	0.52	23.8	29.80	37.80	51.00	40.75	44.69	29.27	Male
<i>Geotrupes ibericus</i>	Huelva	Spain	November 2014	0.708	21.8	31.50	39.10	48.20	33.29	40.11	27.96	Male
<i>Geotrupes ibericus</i>	Huelva	Spain	November 2014	0.890	21.8	31.30	38.50	48.70	31.23	37.99	31.34	Female
<i>Geotrupes ibericus</i>	Huelva	Spain	November 2014	1.006	21.8	30.80	38.70	49.80	32.16	41.69	34.10	Male
<i>Geotrupes mutator</i>	Ávila	Spain	May 2014	0.549	21.7	30.30	41.50	48.70	36.29	44.27	42.11	Male
<i>Geotrupes mutator</i>	Ávila	Spain	May 2014	0.300	21.7	28.90	42.20	49.00	37.36	44.48	39.65	Female
<i>Geotrupes mutator</i>	Ávila	Spain	May 2014	0.581	21.7	27.20	41.70	48.70	34.13	43.48	23.93	Female
<i>Geotrupes stercorarius</i>	Huesca	Spain	August 2013	1.010	27.0	31.10	40.90	48.30	11.15	27.68	24.63	Male

<i>Geotrupes stercorarius</i>	Huesca	Spain	August 2013	1.042	27.0	32.90	39.80	47.20	13.41	29.05	28.79	Female
<i>Geotrupes stercorarius</i>	Huesca	Spain	August 2013	0.757	27.0	30.10	42.10	47.10	0.42	24.14	29.24	Female
<i>Geotrupes stercorarius</i>	Madrid	Spain	April 2013	0.873	24.5	31.60	40.00	49.00	17.77	31.76	20.22	Female
<i>Geotrupes stercorarius</i>	Madrid	Spain	April 2013	0.779	24.5	31.80	40.40	46.00	16.71	27.13	20.29	Female
<i>Geotrupes stercorarius</i>	Madrid	Spain	April 2013	0.705	24.5	31.10	40.50	48.50	18.75	27.65	20.46	Female
<i>Geotrupes stercorarius</i>	Zamora	Spain	June 2013	0.673	24.0	29.80	41.90	48.50	17.97	38.78	33.17	Male
<i>Geotrupes stercorarius</i>	Zamora	Spain	June 2013	0.447	24.0	30.30	43.10	49.90	21.82	45.55	19.77	Female
<i>Geotrupes stercorarius</i>	Zamora	Spain	June 2013	0.787	24.0	30.30	42.90	49.80	20.47	50.00	47.42	Female
<i>Geotrupes stercorarius</i>	Zamora	Spain	June 2013	0.452	24.0	31.00	45.40	50.70	8.70	46.01	36.30	Male
<i>Geotrupes stercorarius</i>	León	Spain	June 2013	0.969	23.3	31.30	41.80	46.70	10.40	32.86	31.72	Female
<i>Geotrupes stercorarius</i>	León	Spain	June 2013	0.801	23.3	31.40	40.60	48.00	16.18	42.59	34.26	Female
<i>Geotrupes stercorarius</i>	León	Spain	June 2013	0.846	23.3	31.20	42.80	48.50	12.44	37.36	35.96	Male
<i>Geotrupes stercorarius</i>	León	Spain	June 2013	1.113	23.3	31.70	42.80	51.70	14.36	34.63	23.30	Female
<i>Jekelius albarracinus</i>	Valencia	Spain	November 2014	0.392	23.8	29.40	45.70	51.70	36.49	31.07	25.37	Male
<i>Jekelius albarracinus</i>	Valencia	Spain	November 2014	0.421	23.8	29.30	43.80	51.50	36.11	20.49	27.16	Female
<i>Jekelius albarracinus</i>	Valencia	Spain	November 2014	0.370	23.8	29.20	44.10	54.20	38.31	23.21	40.48	Male
<i>Jekelius brullei</i>	Kütahya	Turkey	November 2012	0.460	20.8	27.40	43.60	52.20	24.25	32.34	17.39	Male
<i>Jekelius brullei</i>	Kütahya	Turkey	November 2012	0.513	20.8	28.10	43.20	52.60	22.72	23.59	30.67	Male
<i>Jekelius brullei</i>	Kütahya	Turkey	November 2012	0.466	20.8	26.10	42.00	51.50	6.89	20.57	38.54	Female

<i>Jekelius castillanus</i>	Toledo	Spain	April 2013	0.429	23.0	29.20	49.10	55.40	27.02	32.20	13.18	Female
<i>Jekelius castillanus</i>	Toledo	Spain	April 2013	0.358	23.0	28.20	48.40	54.50	22.07	35.82	30.96	Male
<i>Jekelius castillanus</i>	Toledo	Spain	April 2013	0.481	23.0	31.20	50.10	53.40	24.70	38.34	20.50	Female
<i>Jekelius hernandezi</i>	Alicante	Spain	January 2013	0.470	19.9	28.70	44.00	50.80	39.23	25.48	18.04	Male
<i>Jekelius hernandezi</i>	Alicante	Spain	January 2013	0.374	19.9	29.30	43.80	54.40	37.65	32.08	18.26	Male
<i>Jekelius hernandezi</i>	Alicante	Spain	January 2013	0.490	19.9	28.90	44.20	54.10	35.38	39.38	22.73	Female
<i>Jekelius hernandezi</i>	Alicante	Spain	January 2013	0.311	19.9	31.30	45.50	55.60	43.14	35.19	17.60	Female
<i>Jekelius hernandezi</i>	Alicante	Spain	December 2013	0.367	21.1	28.30	41.00	50.40	34.82	25.76	17.60	Female
<i>Jekelius hernandezi</i>	Alicante	Spain	December 2013	0.326	21.1	29.30	43.70	49.80	36.86	34.29	27.92	Female
<i>Jekelius hernandezi</i>	Alicante	Spain	December 2013	0.385	22.5	30.90	41.90	49.60	31.80	28.72	18.76	Male
<i>Jekelius hernandezi</i>	Alicante	Spain	December 2013	0.339	22.5	30.80	41.60	49.60	32.57	24.30	21.00	Female
<i>Jekelius hispanus</i>	Huelva	Spain	October 2013	0.324	26.3	31.00	44.10	51.20	14.76	31.38	20.11	Female
<i>Jekelius hispanus</i>	Huelva	Spain	October 2013	0.247	26.3	29.90	43.40	51.80	10.52	27.66	42.42	Male
<i>Jekelius hispanus</i>	Huelva	Spain	October 2013	0.435	26.3	30.30	45.60	51.20	11.73	19.28	41.33	Female
<i>Jekelius nitidus</i>	Toledo	Spain	May 2013	0.379	22.8	28.00	47.60	55.30	29.43	35.19	29.96	Male
<i>Jekelius nitidus</i>	Toledo	Spain	May 2013	0.321	22.8	31.70	46.10	56.30	28.18	31.58	37.99	Male
<i>Jekelius nitidus</i>	Toledo	Spain	May 2013	0.464	22.8	29.40	48.20	56.00	23.94	45.14	34.51	Male
<i>Jekelius punctatolineatus</i>	Alicante	Spain	September 2012	0.538	24.3	30.30	44.50	50.70	24.68	37.42	18.73	Male
<i>Jekelius punctatolineatus</i>	Alicante	Spain	September 2012	0.451	24.3	30.00	43.50	51.80	20.26	32.93	23.51	Male
<i>Jekelius punctatolineatus</i>	Alicante	Spain	September 2012	0.455	24.3	28.40	44.90	48.70	18.83	37.89	13.69	Male

<i>Jekelius punctatolineatus</i>	Alicante	Spain	December 2013	0.402	21.2	29.49	40.04	48.57	35.99	16.03	22.42	Male
<i>Jekelius punctatolineatus</i>	Alicante	Spain	December 2013	0.340	21.2	28.65	40.28	50.05	31.29	17.04	42.76	Female
<i>Jekelius punctatolineatus</i>	Alicante	Spain	December 2013	0.388	21.2	29.48	41.25	51.02	32.49	22.42	21.17	Female
<i>Jekelius punctatolineatus</i>	Alicante	Spain	December 2013	0.354	21.2	29.11	42.78	51.16	33.84	34.30	14.60	Male
<i>Jekelius punctatolineatus</i>	Alicante	Spain	December 2013	0.461	21.2	28.73	42.89	51.03	32.90	30.95	19.13	Female
<i>Sericotrupes niger</i>	Huelva	Spain	July 2013	0.657	23.7	29.41	41.12	51.70	17.05	39.30	35.86	Male
<i>Sericotrupes niger</i>	Huelva	Spain	July 2013	0.560	23.7	29.43	42.18	50.07	18.47	42.79	35.30	Male
<i>Sericotrupes niger</i>	Huelva	Spain	July 2013	0.501	23.7	28.24	42.42	51.14	15.42	42.65	25.95	Male
<i>Silphotrupes orocantabricus</i>	Lugo	Spain	June 2014	0.238	25.5	28.90	44.00	52.10	32.90	47.71	36.00	Female
<i>Silphotrupes orocantabricus</i>	Lugo	Spain	June 2014	0.206	25.5	29.00	42.10	54.40	33.05	29.64	25.20	Female
<i>Silphotrupes orocantabricus</i>	Lugo	Spain	June 2014	0.216	25.5	31.40	43.00	51.00	33.77	38.28	22.86	Male
<i>Silphotrupes orocantabricus</i>	León	Spain	June 2013	0.239	23.4	30.40	44.50	53.40	20.30	35.61	50.42	Male
<i>Silphotrupes orocantabricus</i>	León	Spain	June 2013	0.332	23.4	29.20	44.70	52.90	18.19	39.90	27.06	Female
<i>Silphotrupes orocantabricus</i>	León	Spain	June 2013	0.298	23.4	30.10	42.60	51.80	17.99	31.53	50.00	Male
<i>Silphotrupes punctatisimus</i>	Zamora	Spain	June 2013	0.348	23.9	30.30	44.10	51.60	32.26	38.44	48.08	Male
<i>Silphotrupes punctatisimus</i>	Zamora	Spain	June 2013	0.337	23.9	30.60	44.30	54.70	34.59	38.70	57.46	Male
<i>Silphotrupes punctatisimus</i>	Zamora	Spain	June 2013	0.359	23.9	29.60	41.00	46.00	28.93	30.56	28.99	Male
<i>Silphotrupes punctatisimus</i>	Zamora	Spain	June 2013	0.271	23.9	30.00	43.00	48.20	31.13	29.08	40.31	Male

<i>Silphotrupes escorialensis</i>	Ávila	Spain	June 2014	0.206	24.3	31.00	45.20	51.30	40.94	39.55	38.49	Male
<i>Silphotrupes escorialensis</i>	Ávila	Spain	June 2014	0.245	24.3	30.90	44.90	50.90	42.26	34.78	55.81	Male
<i>Thorectes armifrons</i>	Ifrane	Morocco	January 2014	0.580	22.9	31.50	41.70	51.30	38.85	31.19	21.92	Male
<i>Thorectes armifrons</i>	Ifrane	Morocco	January 2014	0.863	22.9	27.30	42.40	51.30	33.35	33.48	27.22	Female
<i>Thorectes armifrons</i>	Ifrane	Morocco	January 2014	0.356	22.9	32.00	41.00	51.00	44.37	23.11	41.41	Female
<i>Thorectes armifrons</i>	Ifrane	Morocco	January 2014	0.479	23.4	29.70	42.90	50.30	30.10	21.75	28.24	Female
<i>Thorectes armifrons</i>	Ifrane	Morocco	January 2014	0.739	23.4	29.20	41.40	48.80	26.56	24.42	14.77	Male
<i>Thorectes armifrons</i>	Ifrane	Morocco	January 2014	0.832	23.4	28.80	42.30	48.70	24.97	29.09	16.33	Male
<i>Thorectes armifrons</i>	Ifrane	Morocco	January 2014	0.580	23.3	27.45	41.60	46.46	28.67	32.98	23.59	Female
<i>Thorectes armifrons</i>	Ifrane	Morocco	January 2014	0.511	23.3	28.04	41.10	47.96	31.38	27.12	44.69	Female
<i>Thorectes armifrons</i>	Ifrane	Morocco	January 2014	0.508	23.3	28.29	41.72	48.81	32.97	37.41	26.07	Female
<i>Thorectes baraudi</i>	Toledo	Spain	February 2013	0.405	25.1	30.00	42.00	52.90	16.33	25.78	20.30	Female
<i>Thorectes baraudi</i>	Toledo	Spain	February 2013	0.561	25.1	29.60	43.90	55.20	14.40	31.26	28.68	Female
<i>Thorectes baraudi</i>	Toledo	Spain	February 2013	0.367	25.1	29.80	44.70	53.60	15.34	35.86	35.74	Male
<i>Thorectes laevigatus</i>	Tetuán	Morocco	January 2014	0.414	22.2	25.50	43.20	51.30	31.77	38.56	24.22	Male
<i>Thorectes laevigatus</i>	Tetuán	Morocco	January 2014	0.489	22.2	26.50	44.10	50.80	39.22	40.37	20.03	Female
<i>Thorectes laevigatus</i>	Tetuán	Morocco	January 2014	0.606	22.2	27.10	44.10	48.80	31.69	30.30	19.14	Male
<i>Thorectes lusitanicus</i>	Almeria	Spain	December 2013	0.336	21.0	26.68	45.27	51.36	32.95	31.51	30.45	Male

<i>Thorectes lusitanicus</i>	Almeria	Spain	December 2013	0.361	21.0	26.60	44.17	51.82	31.14	33.25	16.42	Female
<i>Thorectes lusitanicus</i>	Almeria	Spain	December 2013	0.303	21.0	26.87	43.21	49.82	34.41	33.80	27.37	Male
<i>Thorectes lusitanicus</i>	Málaga	Spain	February 2013	0.411	24.6	32.27	45.05	53.84	21.77	45.16	24.66	Female
<i>Thorectes lusitanicus</i>	Málaga	Spain	February 2013	0.461	24.6	31.08	42.22	52.27	19.10	41.64	27.53	Female
<i>Thorectes lusitanicus</i>	Málaga	Spain	February 2013	0.416	24.6	30.11	43.06	54.07	16.62	32.83	45.12	Female
<i>Thorectes lusitanicus</i>	Málaga	Spain	November 2014	0.458	23.0	31.22	42.80	52.58	32.10	41.96	22.59	Female
<i>Thorectes lusitanicus</i>	Málaga	Spain	November 2014	0.351	23.0	31.30	43.15	52.20	32.11	42.93	21.47	Male
<i>Thorectes lusitanicus</i>	Málaga	Spain	November 2014	0.399	23.0	30.75	40.34	51.51	30.79	28.12	26.10	Male
<i>Thorectes lusitanicus</i>	Málaga	Spain	November 2014	0.307	23.0	31.61	44.31	51.38	34.38	35.52	18.75	Female
<i>Thorectes trituberculatus</i>	Khénifra	Morocco	January 2014	0.708	22.2	30.66	40.82	47.85	34.76	23.12	17.64	Male
<i>Thorectes trituberculatus</i>	Khénifra	Morocco	January 2014	0.691	22.2	30.27	43.06	48.45	30.94	21.21	20.49	Female
<i>Thorectes trituberculatus</i>	Khénifra	Morocco	January 2014	0.636	22.2	30.57	44.27	50.30	33.29	27.29	21.85	Female
<i>Thorectes trituberculatus</i>	Khénifra	Morocco	January 2014	0.448	22.2	30.79	39.45	49.94	34.50	21.12	24.51	Female
<i>Thorectes valencianus</i>	Alicante	Spain	October 2012	0.524	24.2	30.10	46.10	52.60	7.74	25.10	25.44	Female
<i>Thorectes valencianus</i>	Alicante	Spain	October 2012	0.399	24.2	29.90	46.10	54.00	9.51	23.09	46.47	Male
<i>Thorectes valencianus</i>	Alicante	Spain	October 2012	0.484	21.6	29.80	43.40	52.30	29.10	27.78	24.90	Female
<i>Thorectes valencianus</i>	Alicante	Spain	October 2012	0.430	21.6	30.20	45.10	51.90	31.72	39.21	19.24	Male
<i>Thorectes valencianus</i>	Alicante	Spain	December 2013	0.591	22.9	28.85	43.00	48.48	29.92	35.02	13.70	Male

<i>Thorectes valencianus</i>	Alicante	Spain	December 2013	0.659	22.9	30.48	43.03	52.88	34.42	35.81	23.79	Female
<i>Thorectes valencianus</i>	Alicante	Spain	November 2013	0.501	22.4	30.31	44.49	51.13	27.10	35.90	20.95	Male
<i>Thorectes valencianus</i>	Alicante	Spain	November 2013	0.475	22.4	30.85	45.99	51.56	28.97	31.35	24.32	Male
<i>Thorectes valencianus</i>	Alicante	Spain	November 2013	0.566	22.1	29.98	45.23	52.12	28.62	36.05	25.81	Male
<i>Thorectes valencianus</i>	Alicante	Spain	November 2013	0.583	22.1	30.00	46.80	51.96	30.84	27.75	19.77	Male
<i>Trypocopris pyrenaeus</i>	Huesca	Spain	August 2013	0.314	27.0	28.70	45.50	53.40	4.40	25.42	13.56	Male

<i>Trypocopris pyrenaeus</i>	Huesca	Spain	August 2013	0.298	27.0	28.60	43.40	51.50	2.60	23.99	22.63	Male
<i>Trypocopris pyrenaeus</i>	Huesca	Spain	August 2013	0.243	27.0	28.60	45.10	51.40	4.76	27.97	20.62	Male
<i>Trypocopris pyrenaeus</i>	Huesca	Spain	August 2013	0.375	27.0	28.70	45.60	52.70	2.13	28.38	16.63	Female
<i>Trypocopris pyrenaeus</i>	Lugo	Spain	June 2014	0.295	23.4	27.20	44.40	51.20	28.18	40.33	18.66	Male
<i>Trypocopris pyrenaeus</i>	Lugo	Spain	June 2014	0.254	23.4	26.80	42.90	51.90	29.71	34.51	23.41	Female
<i>Trypocopris pyrenaeus</i>	Lugo	Spain	June 2014	0.366	23.4	27.70	44.90	50.60	28.84	36.56	20.58	Male
<i>Trypocopris pyrenaeus</i>	Lugo	Spain	June 2014	0.395	23.4	26.50	43.70	51.30	27.72	33.73	31.15	Male
<i>Trypocopris pyrenaeus</i>	León	Spain	June 2013	0.415	23.7	27.79	46.02	54.41	16.63	42.54	26.30	Male
<i>Trypocopris pyrenaeus</i>	León	Spain	June 2013	0.386	23.7	27.89	44.91	48.32	19.08	44.09	14.67	Female
<i>Trypocopris pyrenaeus</i>	León	Spain	June 2013	0.606	23.7	27.82	45.39	52.59	19.00	28.41	20.25	Female
<i>Typhoeus momus</i>	Cádiz	Spain	February 2013	0.752	22.0	29.70	38.40	51.30	47.52	37.58	21.16	Female

<i>Typhoeus momus</i>	Cádiz	Spain	February 2013	0.357	22.0	28.90	39.40	51.10	33.85	39.11	27.59	Male
<i>Typhoeus momus</i>	Huelva	Spain	October 2013	0.536	26.5	28.80	41.30	50.50	7.74	32.05	24.50	Male
<i>Typhoeus momus</i>	Huelva	Spain	October 2013	0.586	26.5	28.60	40.50	50.40	4.60	30.51	26.36	Male
<i>Typhoeus momus</i>	Huelva	Spain	October 2013	0.529	26.5	28.70	41.80	50.40	7.96	33.81	17.95	Female
<i>Typhoeus momus</i>	Huelva	Spain	October 2013	0.574	26.5	28.60	40.80	48.20	5.91	30.81	15.73	Female
<i>Typhoeus typhoeoides</i>	Khémisset	Morocco	January 2014	0.313	22.4	28.20	39.50	48.40	32.40	36.51	30.58	Male
<i>Typhoeus typhoeoides</i>	Khémisset	Morocco	January 2014	0.452	22.4	26.60	39.40	49.60	27.95	39.88	34.29	Male
<i>Typhoeus typhoeoides</i>	Khémisset	Morocco	January 2014	0.654	22.4	27.00	38.40	49.80	28.91	37.01	33.68	Female
<i>Typhoeus typhoeoides</i>	Khémisset	Morocco	January 2014	0.547	22.4	31.30	41.60	49.60	30.79	42.83	27.49	Male
<i>Typhoeus typhoeoides</i>	Khémisset	Morocco	January 2014	0.502	22.0	23.50	37.80	48.00	26.38	35.01	26.32	Male
<i>Typhoeus typhoeoides</i>	Khémisset	Morocco	January 2014	0.495	22.0	23.50	39.70	50.70	41.69	37.54	33.74	Male
<i>Typhoeus typhoeoides</i>	Khémisset	Morocco	January 2014	0.506	22.0	23.70	38.90	49.30	32.13	35.51	28.26	Male
<i>Typhoeus typhoeus</i>	Málaga	Spain	February 2013	0.621	21.7	23.60	41.20	50.80	29.44	36.77	31.17	Female
<i>Typhoeus typhoeus</i>	Málaga	Spain	February 2013	0.562	21.7	23.80	41.20	50.30	38.54	39.18	34.60	Female

Weight: natural logarithm of body mass; Tamb: ambient temperature of laboratory; *SST*: start stress temperature; *CT_{max}*: critical thermal maximum; *ULT*: upper lethal temperature; *rSAR*: supra-optimal activity heating rate (value x1000); *rHSR*: heat stress range heating rate (value x1000); *rHCR*: heat coma range heating rate (value x1000).

Figure S1. Photographs showing the experimental arena used to measure the thermal response to heat stress in dung beetles.

