



Endothermy in the temperate scarab *Cyclocephala signaticollis*

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ABSTRACT

The increase in body temperature over that of the environment has been frequently reported in insects, in particular in relation with flight activity. Scarab beetles of the genus *Cyclocephala* living in tropical areas are known to exploit the heat produced by thermogenic plants, also producing heat by endothermy. Here, we report the first case of endothermy in a species of this genus living in a temperate region, *Cyclocephala signaticollis*. We characterised the phenomenon in this beetle using infrared thermography and exposing them to different thermal conditions. We evaluated the frequency of endothermic bouts, the nature of their periodic occurrence and their association with the activity cycles of the beetles. We found that endothermy occurs in both males and females in a cyclic fashion, at the beginning of the night, around 21:00 local time. The mean temperature increase was of 9 °C, and the mean duration of the bouts was 7 min. During endothermic bouts, the temperature of the thorax was on average 3.6 °C higher than that of the head and 4.8 °C above that of the abdomen. We found no differences between females and males in the maximum temperature attained and in the duration of the endothermy bouts. The activity period of the beetles extends throughout the whole night, with maximum activity between 22:00 and 23:00. By subjecting the beetles to different light regimes we were able to determine that the rhythm of endothermy is not controlled by the circadian system. Finally, we experimentally tested if by performing endothermy the scarabs try to reach a particular body temperature or if they invest a given amount of energy in heating up, instead. Our results indicate that at lower ambient temperature beetles show higher increase in body temperature, and that endothermy bouts last longer than at relatively higher ambient temperatures. We discuss our findings in relation to the ecology and behaviour of this beetle pest.

1. Introduction

Endothermy has been documented in several species across the Hexapoda. Its occurrence has been linked to distinct aspects of the life of insects, being mainly associated with flight, as in moths, bumblebees and tsetse flies (Heinrich, 1993; Lahondère and Lazzari, 2015). Although beetles move slowly and rarely fly, which makes endothermy unlikely for the majority of the species (Heinrich, 1993), it has been shown that endothermy can play an important role in various activities in diverse taxa within this group. In relation to flight or flight preparation, endothermy has been observed in several families, there being exceptional examples in which body temperature during flight can reach values 30 °C over ambient temperature (Chown and Scholtz, 1993; Heinrich and McClain, 1986; Merrick and Smith, 2004; Oertli, 1989; Verdu et al., 2004, 2006). Endothermy among beetles is only present in flying species, as it is generally only possible due to flight muscles activity (Heinrich, 1993), but its occurrence has also been

linked to terrestrial activities, i.e., decoupled from flight. These include examples of endothermy during stationary or walking periods (Bartholomew and Casey, 1977a,b; Chappell, 1984; Morgan, 1987; Morgan and Bartholomew, 1982), in relation to foraging (Chappell, 1984) and mate guarding (Saeki et al., 2005), and, in dung beetles, to ball making and rolling, as well as to the competition for dung balls (Bartholomew and Heinrich, 1978; Heinrich and Bartholomew, 1979; Ybarrondo and Heinrich, 1996).

Endothermy represents a high energetic investment for the animal which performs it. For instance, oxygen consumption rates in endothermic rain beetles can match those of flying birds, and can be up to 13 times higher in beetles when performing endothermy than in a resting, non-endothermic situation (Morgan, 1987). Particularly for small insects, which possess high surface to volume ratios and therefore high rates of heat loss, alternative behavioural mechanisms have been described which allow attaining high body temperatures at relatively low energy expenses. These behaviours aim at seeking warmer

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environments (i.e., where heat loss is lower), either by temporarily basking in the sun for diurnal species or by staying within heated areas for nocturnal species (e.g., within thermogenic flower chambers) (Heinrich, 1993; Seymour et al., 2009). However, other nocturnal species from temperate regions must deal with heat-producing trade-offs without relying on an association with external thermal sources (Kingsolver, 1985).

The Argentine scarab, *Cyclocephala signaticollis* Burmeister (Scarabaeidae: Dynastinae), is a species native to South America that inhabits grasslands, and it is mainly distributed in temperate regions of Argentina and Uruguay (Remedi de Gavotto, 1964). In natural habitats larvae feed mainly on tubercles and grass roots, but also on roots of agricultural plants such as potato, sunflower and some cereals. Due to its larvae feeding habits, *C. signaticollis* is considered an agricultural pest for many cultivated plants in Argentina and other areas of the world (Alvarado et al., 1981; Carne, 1957; Potter, 1998). Studies on the life cycle of *C. signaticollis* have mainly described the morphological characteristics and the duration and the survival rates of each stage (Alvarado, 1980; Carne, 1957; López et al., 1994), typically under laboratory conditions. However, in spite of its economic importance, its ecology and physiology have been scarcely investigated (Frew et al., 2016; Souza et al., 2014), particularly for the adult stage. Adults, similar to other *Cyclocephala*, are nocturnal and crepuscular, remaining buried in the soil during the day (Ratcliffe, 2008; Albuquerque et al., 2016). Their habits have not been well documented, but estimates from sampling rates in the field suggest that their activity peak occurs between 19:00 and 01:00 local time (Alvarado, 1980). The Cyclocephalini tribe is known to include highly specialised pollinators, which are often associated with thermogenic plant species from which they obtain their food (Bernhardt, 2000; Gottsberger, 1986, 1990, 1999; Gibernau et al., 1999; Moore and Jameson, 2013; Seymour et al., 2003). Contrary to other species of the genus, no feeding activity has been reported for *C. signaticollis* adults, nor any association with a particular plant species. It is frequently assumed that these beetles do not feed at all and that the main activities they perform during the night are related to dispersal and reproduction above the ground (Carne, 1957; López et al., 1994). However, association of this species with some plant species cannot be discarded for three reasons: 1) no studies of adult behaviour have been conducted in the field; 2) adults possess fully-developed mouthparts and digestive tracts and 3) other nocturnal *Cyclocephala* species such as *C. putrida*, sympatric to *C. signaticollis*, have been reported to be pollinators and feed on floral resources (Moore and Jameson, 2013; Wiersema, 1987).

Endothermy in southern, temperate scarabs which do not seem to exploit external heat sources, nor to feed, has not been studied before. The biology of *C. signaticollis*, as well as its geographic distribution, renders it a good model for studying the capacity for endogenous heat production under these conditions. Using infrared thermography we studied first the occurrence of endothermy. Once confirmed, we characterised the phenomenon, investigating its relationship to the activity cycle of the insects and its dependence on environmental temperature. These approach allowed us to inquire into the adaptive value of endothermy in this temperate species.

2. Material and methods

2.1. Insects sampling and maintenance

Third instar *C. signaticollis* larvae were collected in a field in Tortuguitas, Buenos Aires Province, Argentina (−34.476778 S, −58.785232 W). Larvae were kept individually in 30 ml opaque plastic jars with commercial soil at 25 ± 1 °C and were weekly fed with thin carrot slices upon reaching the pupal stage. Adults were identified and sexed as they emerged and were individually kept in 50 ml Falcon tubes externally covered up to half of their length with opaque black plastic foil and containing 20 ml of commercial soil (*Tierra Fértil*, Carrefour).

Tubes were kept in a rearing room at 25 ± 1 °C, $60 \pm 10\%$ RH, and under a 12 h:12 h light-dark regime, and insects were only used in experiments after at least 3 days of exposure to such conditions. In the experiments, adults ranging from 3 to 15 days after emergence were used (mean maximal lifespan: 30 days). The body length of adult males and females of this species is 1.3–1.4 cm and 1.3–1.6 cm, respectively (Remedi de Gavotto, 1964). The mass was not individually recorded on the insects used in this study. However, the mass of 57 adult insects was recorded in the field. The mean mass is of 0.22 ± 0.06 g. Females are heavier than males, weighing 0.24 ± 0.06 g (N = 28) and 0.20 ± 0.04 g (N = 29), respectively (t-test, $p = 0.03$).

2.2. Endogenous heat production, thermal cycle and activity cycle

In order to determine whether or not the beetles are capable of endogenous heat production, as well as the timing and frequency of its occurrence, 38 females and 26 males were filmed with an infrared thermographic camera (PYROVIEW 380L compact, DIAS infrared GmbH, Germany; spectral band: 8–14 μm, uncooled detector 2D, 384×288 pixels). Beetles were placed individually in 30 ml black plastic jars, which had the bottom covered with humidified filter paper (0.1 ml distilled water) to both avoid desiccation and provide a substrate on which individuals could walk without sliding. Also, jars were sealed using laboratory sealing film DuraSeal™ to avoid desiccation and escape of the insects. This film is a transparent polyethylene material, also clear for infrared thermal radiation. However, given that any film could attenuate IR transmission, we calibrated the camera recordings following the recommendations by Stabentheiner et al. (2012). Briefly, we first constructed a portable cavity black body radiator, by immersing a cylinder with the inner surface coated with black lacquer into a water bath. Then, we measured the temperature registered by the camera at different known temperatures (from 25 to 50 °C, every 5 °C) with and without interposing the sealing film, and derived an equation relating known to recorded temperature, which we used to correct the temperature values after the experiments.

The size of the beetles in the thermographic recordings averaged 10×6 pixels, which allowed accurate measurement of their surface temperature. Thermographic recordings were made during 4 consecutive days and nights with a sample frequency of 1.6 Hz. Data storing was done in an external hard drive capturing images from the recordings every 5 s. Emissivity value was fixed at 0.97, accepted to be the emissivity of insects' cuticle (Stabentheiner and Schmaranzer, 1987). During the experiment, the temperature of the room was kept at 21 ± 1 °C and the light regime was of 12 h:12 h L/D. The insects were then returned to the maintenance chamber (25 °C, 12:12 h L/D).

Surface temperature of each individual was tracked using Pyrosoft Software (DIAS Infrared Systems, Germany). Individuals moved within the containers, and hence, no particular pixel or set of pixels could be identified in order to perform temperature tracking. Instead, assuming that maximum temperature increase would only be evident on the insect and not on the background (which was corroborated by visually examining each recording), each container was identified as a region of interest (ROI) and its maximum temperature value was extracted as value of interest (VOI) for each ROI during the whole recording. Given that it was observed that the thoracic temperature of the insects during the endothermy events was always higher than that of the rest of the body (see below and Section 3), the maximum temperature of each ROI was considered to be the thoracic temperature of the individuals (T_{th}). As even under constant ambient temperature and when beetles are not performing endothermy their temperature might vary slightly, we decided to use the following criterion to determine the occurrence of endothermy: an endothermy event had occurred in a given insect when its thoracic temperature was at least 3 °C higher than its thoracic mean temperature throughout the experiment.

For those insects that showed endothermy events, thoracic temperature was extracted during each endothermy peak and four variables

were measured: the maximum temperature attained (in any of the endothermy bouts performed by an individual), the maximum temperature difference recorded for each individual with respect to its mean temperature throughout the experiment, the mean maximum temperature per individual and the duration of the bouts of endothermy. Comparisons between females and males with respect to these four variables were made by means of t-tests. Also, for these same insects we generated a daily thermal profile in which we could identify the time intervals in which endothermy events occurred along the day. In order to analyse temperature differences among body parts during those intervals, surface temperatures of the head (T_{hd}), and the abdomen (T_{abd}) were also measured. For this, we identified the head and abdomen regions and manually computed the maximum temperature of the pixels corresponding to each region at the peak of each endothermy bout (head and abdomen temperature when thoracic temperature was maximum). Temperatures of the different parts of the body were compared by means of a Kruskal-Wallis test, followed by a *posteriori* Dunn test (Dinno, 2016).

In the thermal recordings, individuals are clearly distinguishable from the background even when they do not generate endogenous heat, because of the different emissivity of the cuticle and the background. This allowed us to also determine their activity pattern. For that purpose, we graphically divided each container into two semi-circular zones and we counted the number of times each individual crossed from one zone to the other in every 15 min period throughout the whole recording. The number of crossings over time was considered to be an estimator of the activity of the insects. We measured activity patterns only in those insects that showed endothermy. We only focused on the activity of thermogenic animals with the aim of linking these two processes. However, we know from other experiments in the laboratory and from field observations that the activity cycle of thermogenic and non-thermogenic individuals is similar. With these data, we built activity profiles over time, which we compared against the heat production profile obtained as above.

2.3. Nature of the thermogenesis rhythm

In the previous experiments, we found that beetles show endogenous heat production in a cyclic manner (see Section 3). In order to determine whether this cycle is endogenously time-driven (i.e., constitutes a true circadian rhythm), a second experiment was performed in which individuals were kept in complete darkness during the assays. Individuals that had been previously maintained under 12 h:12 h L:D conditions were placed in containers as described before and were filmed with the thermal camera during 4 consecutive days in constant darkness (D:D) ($N = 52$, 21 males, 31 females). In these experiments, we utilised both naïve and some of the beetles that had showed endothermy in the previous experiment in order to ensure that at least some individuals were capable of heat production under normal conditions ($N = 31$, 14 males, 17 females). In these cases, beetles were kept in the maintenance room during at least 3 days between experiments to allow their recovery from previous experimental experience.

Data analysis was performed as before, tracking maximum thoracic temperature, determining whether endothermy events had occurred and profiling temperature differences throughout the experience.

2.4. Endothermy at different ambient temperatures

Having characterised endothermy under constant conditions, we aimed to determine whether insects invest a fixed amount of energy for heat production or if, conversely, they are able to utilise variable amounts of energy in order to reach a fixed minimal temperature. In order to test this, we measured body temperature of individuals subjected to different ambient temperatures: 19 °C, 25 °C and 30 °C. These temperatures comprise the range of summer temperatures in the Pampas region. In a room at 19 ± 1 °C, we constructed a thermal

gradient using an aluminium plate, where temperature was regulated using a heating resistance (Thermo, 15W) pad connected to a thermostat (Votcraft). We covered the surface of the gradient with humidified filter paper and we placed an extruded polystyrene foam plate (2 cm height) on top of it, in which we carved 3 cm diameter wells in positions that corresponded to 30 °C, 25 °C and 19 °C. Each of these temperatures was measured and set using a thermocouple. Inside the wells, we placed plastic cylinders (open on both ends, 3×2 cm) to contain the insects, and we covered the system with an IR material window (Edmund Optics, #88-610), which allows infrared radiation to pass through and to be recorded by the camera, while preventing the insects from escaping. In this way, insects would be in direct contact with the surface at a given temperature but insulated from the rest of the environment, and their body temperature could be recorded using the thermographic camera, as before. Given that the IR material window could attenuate IR radiation, we performed a calibration process as before, measuring actual and recorded temperatures of an object in a temperature range from 25 to 50 °C and derived an equation to correct the temperature values. Beetles were individually introduced into the tubes and their temperature was measured during three consecutive days in 12 h:12 h L:D.

Twenty-two beetles (9 females and 13 males) were used in this experiment, some of which were tested in the three different treatments. For those, the order in which beetles were tested at each temperature was randomly assigned and no beetle was used in two consecutive experiments. Instead, individuals were kept under rearing conditions for five days between one assay and the next one. In this way, we could consider each measurement as an independent value, hence giving $N = 4$ (all males) at 30 °C, $N = 14$ (6 females and 8 males) at 25 °C, and $N = 12$ (6 females and 6 males) at 19 °C. As before, we considered an insect had shown endothermy when its thoracic temperature was at least 3 °C higher than the mean maximum temperature recorded throughout the experiment. For those insects that showed endothermy bouts, we measured the mean difference between the maximum thoracic temperature and the ambient temperature, and the mean duration of the endothermy bouts, when exposed to each temperature. Due to the low number of individuals used in the experiment (they cannot be reared in the laboratory), and given that we found no differences in the endothermy parameters between males and females (i.e., maximum temperature attained, endothermy bouts duration; see Section 3), both sexes were analysed together. We used Mann-Whitney tests to compare between temperature treatments pairwise for each variable.

3. Results

3.1. Endogenous heat production, thermal cycle and activity rhythm

We recorded endothermy events (i.e., increase in body temperature due to endogenous heat production) in 37 of the 54 beetles tested (20 females and 17 males, within all the age range), at ambient temperature of 21 ± 1 °C. In these beetles, body temperature was always maximal on the thorax (T_{th}) during the endothermy events (see Fig. 1A for an example). The mean maximum temperature attained by an individual during endothermy events was 34.0 ± 2.3 °C (max = 39.0 °C, min = 30.1 °C), which represents a mean increase of 9.0 ± 2.9 °C over mean thoracic temperature throughout the experiment (max increase recorded = 12.8 °C). During endothermy events, the temperature of different parts of the body was significantly different ($H = 59.337$, $p < 0.001$, an example shown in Fig. 1). T_{th} was always higher than T_{hd} ($Z = -5.034$, $p < 0.001$), and higher than T_{abd} ($Z = -7.567$, $p < 0.001$). Also, T_{hd} was higher than T_{abd} ($Z = -2.533$, $p = 0.006$). At the peaks of maximum T_{th} , the mean difference between T_{th} and T_{hd} was 3.6 ± 1.7 °C, and the mean difference between T_{th} and T_{abd} was 4.8 ± 2.1 °C. Although we did not measure it quantitatively, we observed that in all individuals that showed endothermy the temperature

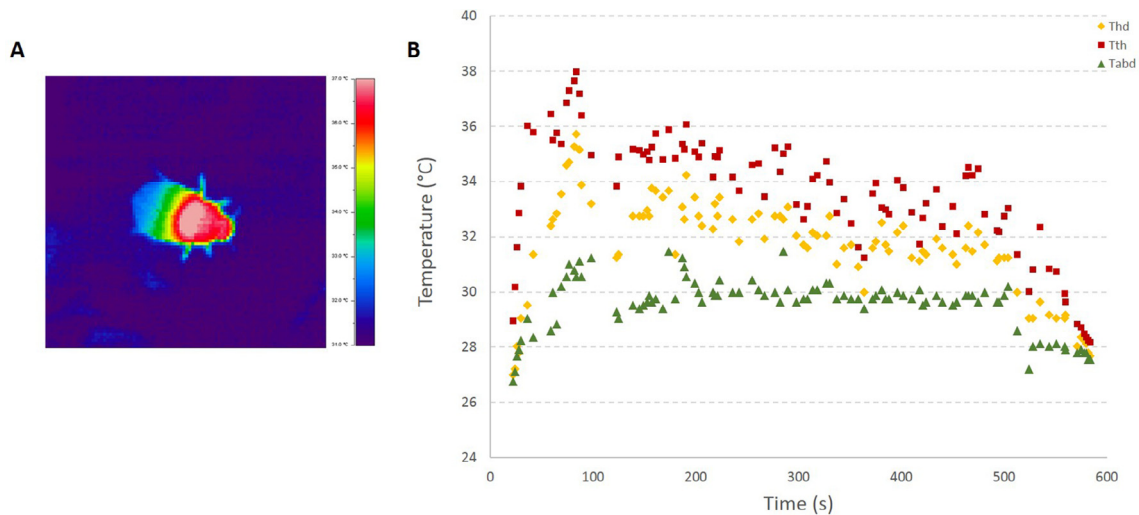


Fig. 1. Example of an endothermy event in a male of *Cyclocephala signaticollis*. A: Thermal image showing the temperature of distinct body regions during an endothermy event. B: Temperature profile of the distinct body regions during an endothermy event. T_h , T_{th} and T_{abd} are maximum temperatures of the head, the thorax and the abdomen, respectively.

of the legs was low and usually similar to that of the abdomen.

Of the 37 insects that performed endothermy, in 11 (2 females and 9 males) these events were repeated in a cyclic fashion (i.e., around the same time in consecutive days). An example of such cyclic endothermy is shown in Fig. 2 for a male beetle. The endothermy events occurred at the beginning of the night, presenting a maximum peak around 21:00; the earliest time at which an individual showed an endothermy bouts was 20:01 and the latest time was 3:53.

The maximum number of endothermy events per night (separated from each other by at least 1 min) was 7, observed in a single male, and the mean number of events was 1.92 ± 1.36 , with 20 individuals (54%) performing a single endothermy bout per night. The mean duration of the endothermy events was 6.80 ± 4.40 min (max = 40.27 min, min = 0.08 min). The endothermy peaks coincide with the beginning of nocturnal activity, but they do not persist for the whole active period. Instead, activity extends from 20:00 up until lights-on (Fig. 3). No endothermy event was recorded during the day, even though we observed a few active insects during that period.

Comparison between males and females showed no difference between sexes in the maximum temperature attained, the maximum

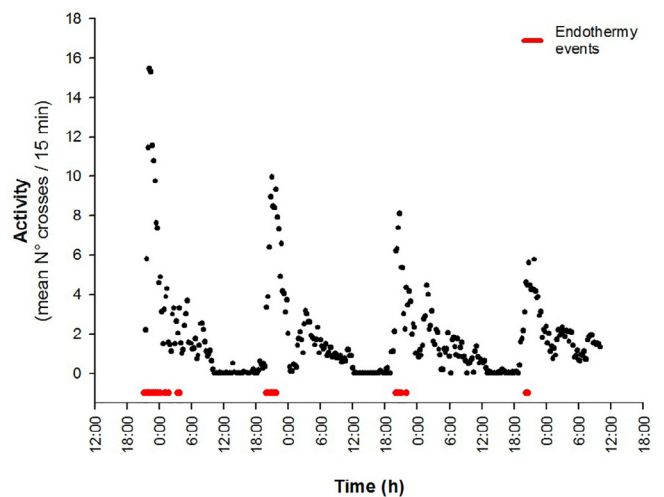


Fig. 3. Activity pattern and endothermy events over time in adults of *Cyclocephala signaticollis*. Activity was estimated by the number of crossings from one zone to the other of the container where individuals were kept. $N = 37$.

Table 1

Comparison between females and males which performed endothermy events. Variables measured: maximum temperature attained (in any of the endothermy bouts performed by an individual, max T_{th} attained); maximum temperature difference recorded for each individual with respect to its mean temperature throughout the experiment (max ΔT , max T_{th} -mean T_{th}); mean maximum temperature per individual (mean max T_{th}); and mean duration of the bouts of endothermy. Ambient temperature during the experiment was $21 \pm 1^\circ\text{C}$.

Variable	Mean value \pm s.d.		t-value	p-value
	Male	Female		
max T_{th} attained ($^\circ\text{C}$)	36.2 ± 2.3	34.7 ± 3.1	1.695	0.099
max ΔT ($^\circ\text{C}$)	9.8 ± 2.3	8.4 ± 3.2	1.580	0.123
mean max T_{th} ($^\circ\text{C}$)	34.1 ± 1.6	34.0 ± 2.8	0.131	0.897
mean duration (min)	5.87 ± 2.65	7.58 ± 5.38	-1.187	0.243

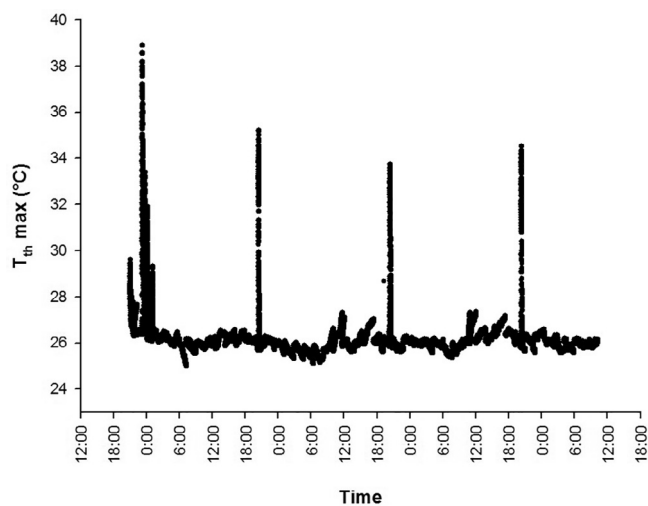


Fig. 2. Example of the endothermy cycle in a male *Cyclocephala signaticollis*. Endothermy peaks (increase in thoracic maximum temperature, T_{th} max) repeat during four consecutive nights, between 20:00 and 21:00.

temperature difference with respect to the insect's mean temperature throughout the experiment, the mean maximum temperature or the duration of the bouts of endothermy (Table 1).

Concerning the activity cycles, we confirmed that individuals of both sexes are predominantly active during the night, from 20:00 to 8.00, and that only occasionally they are active during the day (Fig. 3). The maximum activity (estimated as the maximum mean number of crosses) was recorded between 22:00 and 23:00 (over 10 crosses every 15 min) during the first day of experimentation. In successive days, maximum activity was recorded around the same hour, but with decreasing intensity. In all cases, endothermy events were associated with the beginning of the activity cycle, but activity was recorded during much longer periods.

3.2. Nature of the thermogenesis rhythm

When exposed to a regime of complete darkness during the experiment, we recorded endothermy events in 29 of the 52 beetles tested, 13 males and 16 females (of which 11 and 9 had been used in previous experiments where they had shown endothermy, respectively). Endothermy events were only recorded during the first night for all but 2 males, which showed endothermy events during the first two consecutive nights. All such events occurred around the same time range in which the thermal cycle was described in the previous experiment (earliest time: 18:43, latest time: 21:25). We did not register any flight attempt during the experiments. All parameters measured were of similar magnitude to those previously determined: mean maximum number of endothermy events per night = 1.83 ± 0.93 ; mean duration of endothermy events = 6.32 ± 7.07 min (max = 23.52 min, min = 0.08 min); mean maximum temperature = 33.0 ± 2.2 °C (max = 38.3 °C, min = 30.0 °C), representing a mean increase of 8.5 ± 2.4 °C over the mean thoracic temperature throughout the experiment (max increase recorded = 13.0 °C). No thermal activity was recorded during the day. The insects, however, remained active in a cyclic fashion during the whole recording period, showing activity only during the nights, as described above.

3.3. Endothermy at different ambient temperatures

Beetles exposed to different temperatures showed differences in their thermal behaviour. For instance, none of the insects subjected to 30 °C showed endothermy (N = 4), while 10 out of 14 (5 females and 5 males) showed endothermy when exposed to 25 °C, and 10 out of 12 (4 females and 6 males) did so when exposed to 19 °C. The mean maximum temperature difference with respect to ambient temperature was significantly higher in insects at 19 °C than in insects at 25 °C (12.2 ± 3.3 °C and 7.1 ± 1.4 °C, respectively; U = 29, p = 0.001, Table 2). Similarly, the mean duration of endothermy bouts was significantly longer for beetles at 19 °C than for beetles at 25 °C (5.67 ± 2.77 min and 1.70 ± 1.97 min, respectively; U = 29, p = 0.002, Table 2).

4. Discussion

In this study, we first investigated whether adult *C. signaticollis* are capable of generating endogenous heat. Our results show that in this

temperate species both males and females are capable of increasing their body temperature to up to 39 °C over an ambient temperature of 21 °C. This value is intermediate between values reported for Neotropical *Cyclocephala* species: stationary *C. colasi* can keep body temperature 4–17 °C above ambient temperature (mean thoracic temperature = 33 °C, at ambient temperatures from 16 to 29 °C, Seymour et al., 2009), while *C. caelestis* beetles can reach a body temperature of 27 °C at ambient temperatures between 20° and 25 °C (Dieringer et al., 1998). As in other *Cyclocephala*, and as shown in other relatively large insects, *C. signaticollis* presents endogenous thermo-regulatory mechanisms that allow body temperature increase. These results, together with the determination of the cyclic nature of endothermy in *C. signaticollis*, further discussed below, represent the first characterization of endothermy in a temperate *Cyclocephala* species.

In concordance with all other previous findings in Coleoptera, thoracic flight muscles seem to be responsible for heat production in *C. signaticollis* during endothermy bouts, as evidenced by thoracic temperature always being higher than the temperature of the head and the abdomen during these periods. Furthermore, we observed that walking activity does not correlate with body temperature increase and that leg temperature always remains low, hence excluding non-flight locomotion muscles as actors in the endothermy process. Indeed, it has been argued that beetles that lack the ability to fly (i.e., have atrophied flight muscles) are incapable of performing endothermy at all (Heinrich, 1993).

Bouts of endothermy in *C. signaticollis* were of variable duration and relatively short when compared to their activity period, which extended for several hours. These bouts could be related with preparation for flight, which takes place early at night, and which is in turn related to dispersal in males (Potter, 1998) and to finding suitable oviposition sites in females (Carne, 1957; López et al., 1994). As the endothermy bouts are quite short, it is possible that, as seen in tropical flower scarabs *Pachnoda sinuata* and *P. cincta* (Heinrich and McClain, 1986), beetles could be minimizing the amount of energy spent to increase their body temperature by performing endothermy only when it is strictly necessary, i.e., in preparation for flight.

Endothermy constitutes a very costly process and has been proposed to only represent a selective advantage in large beetles, heavier than 1 g, as for smaller insects the surface to volume ratio would incur high heat losses that would render endothermy too costly of a process (Heinrich, 1993). However, endothermy has been reported in fairly small, though not minute, active-flyer scarabs (Seymour et al., 2009; Dieringer et al., 1998), and shown for *C. signaticollis* (0.22 g) in the present study. We currently lack metabolic data to estimate energetic costs of endothermy for this species. While other scarabs from tropical and subtropical regions are closely associated with heat-generating flowers, and gain thermal energy through such association (Seymour et al., 2003, 2009; Seymour and Schultze-Motel, 1997), it remains unknown whether *C. signaticollis*, a temperate species, is able to exploit any such advantage. In any case, limiting heat production to just when it is needed, may be advantageous as a mechanism for energy saving. We identify a decrease in the occurrence of thermogenic bouts with time, as well as in the general activity of the insects. This decrease could be due to some extent to the depletion of energetic reserves, but we

Table 2

Endothermy events at different ambient temperatures. N_{total}: number of individuals tested; N_{endot}: number of individuals that performed endothermy; MaxTempDifference: maximum temperature difference between mean thoracic temperature and maximum thoracic temperature attained (mean calculated from all individuals that performed endothermy). Given that none of the insects at 30 °C performed endothermy, comparisons presented are between insects at 25 °C and at 19 °C.

	30 °C	25 °C	19 °C	U value	p value
N _{total}	4	14	12	–	–
N _{endot}	0	10	10	–	–
MaxTempDifference (°C)	–	7.1 ± 1.4	12.2 ± 3.3	29	0.001
mean duration (min)	–	1.70 ± 1.97	5.67 ± 2.77	29	0.002

cannot exclude an influence of the reduction in the oxygen level inside the recipient as a consequence of the metabolism of the beetle. Further work, keeping constant the amount of oxygen in the container, should allow evaluating the relative influence of these factors.

Temperature can modify both how and if an insect performs a particular behaviour (Kovac et al., 2010; Abram et al., 2016). Certain activities require that the temperature of the body, or of the part of it involved in the activity, reaches particular values. For instance, pre-flight shivering is common among insects belonging to diverse taxa (i.e., from Odonata to Hymenoptera), as a mechanism to reach temperature thresholds that allow flight initiation (Heinrich, 1996; Lahondère and Lazzari, 2015). These temperature thresholds correspond to values that maximise muscle power output under given temperature conditions (Krogh and Zeuthen, 1941; Stevenson and Josephson, 1990; Lehmann, 1999). When ambient conditions are variable (i.e., temperature lowers), insects must adjust in order to overcome such variations and continue to develop their activities. In those situations, increasing body temperature could follow two possible pathways: a) expending as much energy as needed to ensure reaching particular temperature thresholds, or b) expending only pre-set amounts of energy and reaching whatever temperature is possible given that investment. Insects that actively feed and can count on continuous food sources are expected to utilise the first strategy, as their energy reserves can be constantly replenished. Honeybees and *Deilephila nerii* moths constitute good examples of insects using this strategy (Dorsett, 1962; Heinrich, 1996), although in honeybees the energy investment strategy may vary according to conditions such as solar radiation (Stabentheiner and Kovac, 2014). Conversely, insects that live on low calories food or that do not feed at all during their adult stage, are expected to either avoid performing the activity or to do it using the second strategy. Energy supply for insects that do not feed is limited to the reserves they have accumulated in previous stages, and the use of such reserves is inversely related to the survival of the insect (Chown and Nicolson, 2004). Therefore, when confronted with lower temperatures, which call for higher energy expenditures, non-feeding insects undergo a trade-off between reaching necessary temperature to carry on a specific activity and reducing their overall life expectancy. In our study, we show that *C. signaticollis* seems to invest more energy in heat production when subjected to lower temperatures, even though has been proposed that adults do not to feed and only depend on the reserves acquired in their larval stage for survival (Carne, 1957; López et al., 1994). Given this fact, we hypothesise that endothermy in this beetle could constitute an essential process, and one which might be associated with an activity that could impact the fitness of the individual. For instance, if mating chances significantly increase with dispersal, and dispersal flights are dependent on body temperature rising, dedicating large amounts of energy to raising body temperature could render this behaviour adaptive even if shortening the lifespan of the beetle. Previous studies have not found evidence that this species feeds as adults. In order to test these observations, we conducted preliminary experiments in the laboratory (Zermoglio et al., unpublished), offering to adult beetles, starved from emergence, different commonly used laboratory diets (e.g., pollen, sugar and honey solutions), but beetles never fed. However, these results are not conclusive and to fully discard feeding in this species, further research is needed in which the content of the digestive tract is analysed in wild animals.

Once we determined that *C. signaticollis* is capable of generating endogenous heat and that it does so in a cyclic fashion, we aimed at determining if the endothermy cycle in this species is governed by external or internal factors. In chronobiology, a circadian rhythm is a daily-period oscillation in a biological process or behaviour, characterised by its persistence over time in the absence of environmental signals (Saunders, 2002). Our results show that most *C. signaticollis* beetles, although they present cyclic endothermy events, cease to produce heat if they are kept in complete darkness. Such interruption presents no delay with respect to the suppression of the stimulus to

which the cycle is synchronised (i.e., environmental light cycle). This suggests that, in spite of light triggering endothermy in a cyclic fashion, this does not constitute a circadian rhythm *sensu stricto*, as it seemingly lacks endogeneity. Finding evidence of a circadian pattern might be influenced by the time beetles are able to perform endothermy (i.e., if beetles are only able to produce heat during the first couple of days after emergence). However, given that we tested beetles of different ages ranging up to half of their maximum lifespan, we believe the results we found are consistent with a lack of endogeneity. The physiological mechanisms and molecular pathways underlying the heat production cycle switching-on remain to be investigated.

Endothermy within the genus *Cyclocephala* has only been shown for tropical species which are associated with thermogenic plants (Cramer et al., 1975; Gottsberger, 1988; Seymour and Matthews, 2006). In this work we present evidence showing that endothermy is also possible in temperate species. While there are aroid species in Argentina that could potentially be attractive to *Cyclocephala* species (Crisci 1968, 1971), in the Pampas, where *C. signaticollis* are found, thermogenic plants are rare and are only associated with intrusions of others habitat types in marginal areas (Cabrera, 1971). However, with the available evidence, we cannot fully discard that the species could have an association with thermogenic plants, as seen in tropical species. Further research is needed to address the ecological interactions of the beetles with the plants of the region, to date unknown, and to understand the role of endothermy of this temperate species from an evolutionary point of view.

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Competing interests

No competing interests declared.

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