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Endothermy of dynastine scarab beetles (*Cyclocephala colasi*) associated with pollination biology of a thermogenic arum lily (*Philodendron solimoesense*)

Roger S. Seymour[1,*], Craig R. White[2] and Marc Gibernau[3]

[1]Ecology and Evolutionary Biology, School of Earth and Environmental Sciences, Darling Building, University of Adelaide, Adelaide, SA 5005, Australia, [2]School of Biological Sciences, University of Queensland, St Lucia, QLD 4072, Australia and [3]Laboratoire d'Evolution et Diversité Biologique UMR 5174, Université Paul Sabatier, 31062 Toulouse, Cedex 9, France

*Author for correspondence (roger.seymour@adelaide.edu.au)

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SUMMARY

Cyclocephala colasi **beetles are facultative endotherms that spend most of their adult lives inside the inflorescences of** ***Philodendron solimoesense*****, where ambient temperature (T_a) averages about 28°C due to floral thermogenesis. Measurements of respiration within a range of T_a showed that active beetles became spontaneously endothermic at T_a below 28°C but were rarely endothermic above it. There was no evidence of endothermy within the inflorescences, indicating that activities in the floral chamber can occur without the high energy expense of endothermy. Bouts of endothermy occurred at lower T_a in respirometer chambers mainly in the evening, when the insects normally fly from one inflorescence to another, and during the night, when they normally eat and mate within the inflorescence. Patterns of endothermy in individual episodes were studied in non-flying beetles with respirometry and infrared thermal imaging. Heat was generated in the thorax by oscillatory waves of respiration that were coupled with thoracic temperature (T_{th}) increases. Stationary beetles could regulate T_{th} at about 33°C independently of T_a between 16 and 29°C. At T_a=20°C, this represents a 116-fold increase in metabolic rate over resting, ectothermic values. Endothermy was clearly a requirement for flight, and beetles departing inflorescences warmed to about 30°C before take-off. During flight, T_{th} was dependent on T_a, decreasing from 37 to 28°C at T_a of 37 to 20°C, respectively. The lowest T_a at which flight could occur was about 20°C. Thermal conductance of stationary, endothermic beetles increased at higher metabolic rates, probably because of increased ventilatory heat loss.**

Key words: beetle, endothermy, pollination biology, *Cyclocephala*, *Philodendron*.

INTRODUCTION

Patterns of activity and energy expenditure differ greatly between animals whose body temperatures roughly equal their environment (ectotherms) and those that are able to raise body temperature by producing significant metabolic heat (endotherms). Lower environmental temperatures cause ectotherms to become more sluggish but may not affect the activity of endotherms. The price endotherms pay for their independence from environmental temperature, however, is the energy they expend to remain warm. The cost is higher with greater differences between body and ambient temperature or with relatively higher surface areas in smaller animals. Many insects become endothermic when they are active, a response termed 'facultative endothermy', and elevated thoracic temperature is often a prerequisite for locomotion (Heinrich, 1993). The increase in metabolic rate can be enormous, for example, about 40-fold between rest and pre-flight warm-up in moths (Bartholomew et al., 1981).

Despite the extreme diversity of beetles, relatively little research has been done on their energetics and thermal biology, specifically associated with endothermy. However, it is clear that there is a wide variety of endothermic responses in beetles, from complete ectothermy (Chown et al., 1995) to long periods of facultative endothermy (Morgan, 1987). Because of the physics of heat exchange, large beetles are more likely to show higher temperature elevations (Bartholomew, 1981) but even beetles weighing about 100 mg can physiologically thermoregulate several degrees above the environment (Oertli, 1989).

Most previous studies involved measuring metathoracic temperatures (T_{th}) in relation to ambient temperature (T_a) and activity. Typically, temperatures are recorded at rest and in beetles encouraged to warm up to flight temperature, or caught in the air or just after landing. Such measurements can be very informative, especially if placed in the context of the animal's natural history (Chown and Nicolson, 2004). For example, intense endothermy that results in high T_{th} is associated with flying in cold weather. Male scarab rain beetles, *Pleocoma australis*, are endothermic when flying (mean T_{th}=37.6°C) at T_a from 3 to 15°C during the winter, when they are searching for females (Morgan, 1987). An African scarab beetle, *Sparrmannia flava*, which flies late at night, presumably to avoid predators, can maintain T_{th} just above 38°C at T_a down to at least 13°C (Chown and Scholtz, 1993). Scarab fig beetles, *Cotinus texana*, show T_{th} in the region of 30–40°C during flight at T_a down to 20°C (Chappell, 1984). There are other examples of high body temperatures of flying beetles (Bartholomew and Casey, 1977a; Heinrich and McClain, 1986; Merrick and Smith, 2004; Oertli, 1989; Verdu et al., 2004; Verdu et al., 2006).

The association between endothermy and flight is clear – strong flight demands warm thoracic muscles and the high rates of muscular work can usually produce more than enough heat to keep them warm (Heinrich, 1993). However, the role of endothermy is less clear in beetles that are not flying. It may be associated with terrestrial locomotion, escape from predators, competition for resources, feeding, assimilation of nutrients or other factors. Sustained endothermy without flight has been reported in many species of

beetles, chiefly scarabs. For example, male rain beetles naturally maintain a mean T_{th} near 31.5°C while walking on the ground at T_a below 15°C (Morgan, 1987). Inactive fig beetles show T_{th} up to 12°C higher than T_a, independently of flight intentions (Chappell, 1984). Elephant beetles, *Megasoma elephas*, spontaneously become endothermic while completely stationary if T_a is decreased below 20°C (Morgan and Bartholomew, 1982). Large cerambycid beetles, *Stenodontes molarium*, and scarab beetles, *Strategus aloeus*, raise T_{th} several degrees above T_a while walking voluntarily or stationary, without preparing to fly (Bartholomew and Casey, 1977b). Other tropical beetles show various degrees of endothermy when artificially stimulated to be active in a revolving respirometry chamber (Bartholomew and Casey, 1977a). Male Japanese beetles, *Popillia japonica*, have higher thoracic temperatures when guarding mates than when solitary (Saeki et al., 2005). In dung beetles, *Scarabaeus*, *Kheper*, *Gymnopleurus* and *Heliocopris* species, endothermy is associated with not only flight to dung heaps but also ball making, ball rolling and competition for dung and finished balls (Bartholomew and Heinrich, 1978; Heinrich and Bartholomew, 1979; Ybarrondo and Heinrich, 1996). The studies on dung beetles give an elegant picture of how elevated body temperatures confer a selective advantage during these terrestrial activities.

Endothermy in beetles is energetically expensive. For example, 1.1 g rain beetles can reach rates of oxygen consumption of 0.75 μmol s^{-1} (Morgan, 1987), which, when analysed allometrically, lies on a line derived from hovering moths and flying birds (Bartholomew, 1981). Of course the cost of endothermy can be reduced if the environment is warmer, because the rate of heat loss diminishes in proportion to the temperature difference between the animal and the environment. To attain higher body temperatures without the energy expense, insects are well known to choose warmer environments, for example, by basking in the sun (Heinrich, 1993).

We proposed that such energy savings might be afforded to insects that visit heat-producing flowers (Seymour and Schultze-Motel, 1997). The present study was therefore initiated to test the hypothesis by evaluating the energy expenditures of dynastine scarab beetles that are the main pollinators of hundreds of species of neotropical thermogenic flowers (Schatz, 1990). Specifically, in 2003, we examined the effect of temperature on the energy expenditure of *Cyclocephala colasi*, which is the major pollination vector in thermogenic *Philodendron solimoesense* (Barabé et al., 2002; Gibernau and Barabé, 2000; Gibernau et al., 1999). This insect–plant association is very tight, with adult beetles spending most of their lives in the large inflorescences. Typically the beetles fly to fresh inflorescences as they open just after sunset and they remain there until the following evening. This ensures that pollen is brought to the protogynous inflorescence during the female phase and carried away during the male phase (Gottsberger and Amaral, 1984). The beetles benefit from the relationship, because the inflorescence is a focus of mating behaviour, and the sterile male florets provide food.

The results from the 2003 expedition represented the first demonstration of an energy reward to pollinators directly in the form of heat rather than indirectly as nectar or pollen food. These results contrast with suggestions that floral heating is not a benefit to pollinators (Dieringer et al., 1998; Dieringer et al., 1999; Fægri and van der Pijl, 1979). The report of these findings was necessarily short (Seymour et al., 2003). The present paper is a more complete presentation of our research on endothermy in *C. colasi* from 2003 and also from further work in 2007 that involved respirometry associated with infrared (IR) imaging thermometry. The main aim of these studies was to understand the energy role of endothermy in the daily lives of adult beetles. Thus, we examined patterns of endothermy in beetles in relation to environmental temperature and time of day. We linked respiration rate to T_{th} elevations during bouts of spontaneous endothermy. We measured T_{th} and observed the behaviour of beetles within natural inflorescences, to determine whether endothermy occurred during activities not associated with flight. Finally, we experimentally determined the relationship between flight temperature and T_a to assess temperature regulation during flight. Aside from the implications for the natural history of the species in particular, these studies provide information about beetle thermoregulation in general.

MATERIALS AND METHODS

Species and study site

Cyclocephala colasi Endrödi, which are the main pollinators of *Philodendron solimoesense* A.C. Smith, were studied at the Laboratoire Environnement de Petit Saut research facility in French Guiana in July and August 2003 and June 2007. Inflorescences of the plants were located at km 97 on the national highway between Kourou and Sinnamary, which was one of the sites studied by Gibernau and Berabé (Gibernau and Berabé, 2000). Many trees were felled during the construction of the national road 25 years ago, and several specimens of this normally arboreal epiphyte were growing at ground level. Beetles were usually captured from inside inflorescences or at the bright lights of an insect-attracting station at Petit Saut. They were fed sterile and fertile male florets and were used within a day of capture.

Beetle respirometry

The respirometry system in 2003 consisted of a train of instruments. Air was pumped from outside the building through a condensation trap, a pump (Gilair model 3, Sensidyne, Clearwater, FA, USA), a 21 surge tank and a Y-junction. One side of the Y was a needle valve vent, and the other side passed through a sapphire orifice [0.2 mm internal diameter (i.d.)] that created a constant resistance to flow. By varying the pump speed and vent, it was possible to set a flow within 5% of desired. The air stream passed through a column of Drierite®, Ascarite® and Drierite® to create dry, CO_2-free air. This passed at about 100 ml min^{-1} through a mass flowmeter (Mass Trak model 822 Sierra Instruments, Monterey, CA, USA) and then through the glass animal chamber (10 or 25 ml) or through a bypass. The outflow from the chamber passed through a 5 ml tube containing regenerated Drierite® to minimise washout effects (White et al., 2006) and into a combination O_2 and CO_2 analyser (model 280, David Bishop Instruments, Leamington Spa, Warks., UK) that was buffered against temperature change with an insulation layer and an air-conditioned room. An analog–digital converter (TX3 digital voltmeter and WaveStar 2.2 software, Tektronix, Beaverton, OR, USA) recorded the CO_2 output in a computer.

Calibration of the CO_2 analyser was carried according to principles set out by Withers (Withers, 2001). First, the O_2 and CO_2 analysers were read at barometric pressure with dry, CO_2-free air. The outflow vent of the analyser was attached to a tube that could be submerged in a container of water to create a 2.45 kPa backpressure in the system. This provided two calibration points for the O_2 analyser and eliminated the need to zero the instrument. To calibrate the CO_2 analyser, a Gilair 3 pump sucked air through a can containing an ethanol lamp into a Douglas bag. The gas was then pumped through Drierite® into the instrument, and the fractions of O_2 and CO_2 (F_{O_2}, F_{CO_2}) recorded. Combustion of ethanol produces a respiratory quotient (RQ) of 0.667 but the ratio of ΔF_{CO_2}:ΔF_{O_2} is 0.717 in the analysers, because of dilution effects. This permitted the calculation of ΔF_{CO_2} from measured ΔF_{O_2}.

Apparent rates of CO_2 production ($\dot{M}_{CO_2}$) were calculated according to Withers (Withers, 2001). The accuracy of the CO_2 analyser was limited by that of the O_2 analyser, which was ±0.2%. The flow meter was calibrated with a 3.5 l calibrator (model 1057A Vol-U-Meter Calibrator; Brooks Instruments, Hatfield, PA, USA) with an accuracy better than 5% of the reading. The precision was much better, however, with resolution of 0.000001 kPa. The air flow rate was sufficient to make corrections for instantaneous respiration unnecessary.

Temperature of the animal chamber was controlled with a small constant temperature (CT) cabinet (internal dimensions: 55×80×120 mm, with 40 mm polystyrene foam walls) illustrated earlier (Seymour, 2004). Cooling was performed by two 40×40 mm Peltier elements, and heating was performed with a 27 Ω resistance heater. Air was circulated around aluminium pin heat exchangers with a 12 VDC, 0.06 A, 40×40 mm computer chip fan. The Peltier elements were cooled with a 12 V pump, reservoir and 13×13 cm copper radiator with cooling fan. The cabinet temperature was controlled with a Peltier temperature control circuit (Oatley Electronics, Oatley, NSW, Australia), powered from a 12 VDC, 6 A source. The animal chamber was viewed from above with an Olympus model R080-024-045-50 'borescope', under weak red light from a single LED of a headlamp covered with a red cellophane filter. The temperatures inside the chamber and CT cabinet were measured with T-type thermocouples and a thermometer (model 52, Fluke Australia, Castle Hill, NSW, Australia). The thermocouples were calibrated in water with a precision mercury thermometer.

T_a was set near one of four levels (20, 25, 30 and 35°C), because this range encompasses the ambient and floral temperatures in July (Gibernau and Barabé, 2000). Flow rate was set at 20 ml min^{-1} for single beetles and 50 ml min^{-1} when 'trios' of three beetles (two males and one female) were in the chamber. Readings were taken at each temperature for approximately 1 h, and the temperature was changed according to a randomly derived sequence. Beetles were observed briefly from time-to-time during this period. Their activity was noted as 'resting' if no movements were visible or 'active' if they were crawling. Data were accepted for the last 20–40 min of each constant temperature treatment. After the last treatment, the beetle was removed and its T_{th} taken by holding it in a cheese-cloth bag to prevent conduction from fingers and immediately puncturing the thorax with a 25 gauge needle containing a T-type thermocouple, read with a calibrated thermometer (DuaLogR^{TM} model 600-1050, Barnant Company, Barrington, IL, USA), taking care to do it quickly to avoid temperature change (Stone and Willmer, 1989). Then the beetle was weighed to 2 mg with a balance (model 1210-100, Tanita Corporation, Tokyo, Japan).

Respirometry and IR thermometry

Respirometry was coupled with IR thermometry in 2007. The flow-through respirometry system was similar to that described above except that the CO_2 analyser was improved (model LI-820, LI-COR, Lincoln, NE, USA) and a flow rate of about 160 ml min^{-1} occurred through a 25 ml chamber. The analyser was calibrated with CO_2-free air and a precision 0.49% CO_2 in N_2 mixture and the output was recorded with LI-COR software. The respirometry chamber was temperature controlled with a 0.6 l, Peltier mini CT cabinet similar to that described above.

Surface temperatures of beetles were measured with an IR camera (Avio TVS-500, Nippon Avionics, Tokyo, Japan). This was placed on a tripod under a table with a hole in it, with the upward-facing lens directly under the respirometry chamber. A thin polyethylene floor permitted transmission of IR radiation from the beetle's surface. The system was calibrated by warming a stack of coins with a thermocouple taped to the bottom and photographing it as it cooled in the chamber. The actual temperature was linearly related to the apparent temperature according to the equation: actual= 1.21×apparent–6.81. IR images were obtained every 15 s and analysed with ThermoMovieEditor module of the Advanced Package, Version 1.1 from Nippon Avionics. Chamber temperature was measured on the coolest pixel away from animal, and maximum surface temperature was found on the beetle. The dorsal and ventral sides could be distinguished, because the insect often turned over. The bases of the legs could usually be seen over the surface of the thorax on the ventral side but not on the dorsal side. Temperatures could often be read from beetles crawling head-up on the side of the chamber but were slightly lower than those measured perpendicularly. On rare occasions, when the beetles were lying on their sides, it was possible to measure both ventral and dorsal temperatures. Frames were omitted when the angle of the beetle prevented measurement or when excessive movement blurred the picture.

Beetle temperatures in inflorescences and a flight room

T_{th} was measured in beetles removed from two thermogenic inflorescences on the first evening of blooming. Freshly captured beetles either flew spontaneously or were encouraged to fly by tossing them into the air inside of a walk-in drying room equipped with a heater and a refrigerated air conditioner to control air temperature between 19 and 36°C. After at least 1 min of flight, which was sometimes interrupted by brief landings, they were captured in the cloth bag and flight temperatures were measured immediately as described above.

Beetle behaviour in inflorescences

Beetles (mainly *C. colasi* and a few *Cyclocephala emarginata*) were observed in five inflorescences of *P. solimoesense* in the field. Observations were made on each inflorescence at about 1 h intervals between 18:45 h (prior to sunset) and 06:30 h (after sunrise) during the first night of blooming on 4–5 August 2003. Observations were made through the borescope using illumination from a head torch. Because the beetles are highly photosensitive, it was necessary to count the number of individuals quickly as they rapidly moved away from the light to the non-illuminated side of the spadix. Beetles were scored either as 'active' if they were obviously moving as the light was inserted, 'resting' if they were not moving or 'mating' if they were attached. The number of rows of sterile male florets partly consumed by the beetles was estimated through the borescope.

The IR camera was also used in the field to record the temperatures of beetles in an inflorescence of *P. solimoesense*. The output of the camera was recorded in cine form with a digital media recorder (model 504, Archos, Igny, France). This format was incompatible with the Nippon Avionics software, so individual pixel temperatures could not be evaluated but they could be estimated visually against the recorded temperature scale.

Statistics

Data are summarised with means and 95% confidence intervals (CI), and a Student's *t*-test was used to compare means. To compare least-square regression lines for slope and elevation, analysis of covariance (ANCOVA) was carried out according to Zar (Zar, 1998), with beetle ID included as a random factor to account for the non-independence of repeated measures of individuals. Where slopes were significantly different, the regions of overlap with significantly different data were identified with the Johnson–Neyman (J–N) test (White, 2003).

RESULTS

Respiration rates in relation to T_a and circadian cycle

The main aim of the present study was to assess the energy expenditure of adult *C. colasi* during the period that they were resident in the inflorescences of *P. solimoesense*. Therefore, it was important to measure respiration during the entire 24-h cycle and average it every hour. Furthermore, it was necessary to spread experimentally imposed T_a evenly throughout the day. Therefore, assignment of the test temperatures was determined by a random number generator. Single beetles were measured during 1-h periods and data were averaged from the last 20–40 min of each period. Data for single beetles represent 129 h of respirometry from 51 males and 26 females. Twenty-one measurements were made at each of 20, 25, 30 and 35°C nominally but actual temperature was recorded. An example of a record from a single beetle is shown in Fig. 1. In addition, a series of measurements was made involving trios of two males and one female beetle in the respirometer together, and seven measurements were made at each temperature. There was no sexual body size dimorphism, and mean body mass of all beetles was 272 mg±12 CI.

Both male and female beetles were recorded as resting or active in the chamber, so the data were combined. Rate of resting CO_2 production ($\dot{M}_{CO_2}$, nmol s^{-1} g^{-1}) of single beetles increased with T_a according to the exponential equation $\dot{M}_{CO_2}=0.960e^{0.0616T_a}$ (R^2=0.42), with a Q_{10} of 1.9 (Fig. 2). Mean rates from beetles that were observed to be active in the respirometer during the measurement period were higher and described by a polynomial equation, $\dot{M}_{CO_2}=0.794T_a^2-51.7T_a+850$ (R^2=0.40), although there was considerable variation at the lower T_a. Respiration from trios was calculated assuming that only one of the trio was active. Thus, the respiration by two inactive beetles was estimated from the equation and subtracted from the total. There was no difference between the data from single active beetles and from trios under this assumption but if all beetles in a trio were assumed to be active then the result was unreasonably low.

A circadian rhythm in metabolic rate was evident in beetles measured throughout the 24-h cycle (Fig. 3). Single insects were generally quiescent in the respirometry vial during the day but often became active after sunset, and mean metabolic rate increased, especially in the evening. The pattern was similar in trios but these were not measured during the day.

T_{th} of 107 beetles, measured by needle thermocouples inserted into the metathorax upon removal from the respirometry chamber were close to ambient chamber temperature, although the regression line has a slope (0.71±0.03 CI) significantly less than 1.0 (Fig. 4). Resting beetles had T_{th} slightly higher than T_a when the temperatures were low, and *vice versa*. Their temperatures could have been affected by endothermy just before the final observation but because they were not observed continuously the effect is not known.

T_{th} of 50 beetles encouraged to fly in the temperature-controlled room were significantly higher than T_a (Fig. 4). By contrast, data from 42 resting beetles in the flight room were only slightly higher than T_a. Compared with resting beetles in the room, flying beetles had a significantly lower regression slope (0.58±0.06 CI), which was also significantly lower than 1.0 (F=86.3; P<0.001). Data above a T_a of 36.2°C were not significantly different, according to a J–N test. Values from 106 resting beetles upon removal from the respirometer showed a significantly lower slope than resting beetles in the flight room (F=63.8; P<0.001) and the regression lines crossed. However, the J–N test showed that the data above a T_a of 25.0°C were significantly different. Fourteen beetles (6 male; 8 female) removed from the floral chambers of two *P. solimoesense* inflorescences when chamber temperature was 27.5±0.2°C had a mean T_{th} of 27.9±0.3°C, which was slightly but significantly higher (2-tailed t=–2.49; P=0.019).

Respirometry and IR thermometry

Because the analysis of 24-h records obtained in 2003 was limited to infrequent spot observations of beetle activity, yet respiration was averaged over 20–40 min periods, the data for 'active' beetles in Fig. 2 included periods in which the beetles were inactive. In 2007, therefore, we looked more closely at individual episodes of endothermy with respirometry combined with IR thermometry.

Respirometry was undertaken between about 18:00 h and 01:00 h on beetles collected in second-day inflorescences that were brought to the laboratory. Bouts of endothermy were indicated by spikes in $\dot{M}_{CO_2}$ and IR images. If endothermy was not evident within about 30 min of introduction, then the beetle was removed and replaced with another. Of 25 beetles tested on the evening of capture, 14 showed endothermy and 11 did not. There were 12 endothermic males and two females. Sex was not determined in all members of the

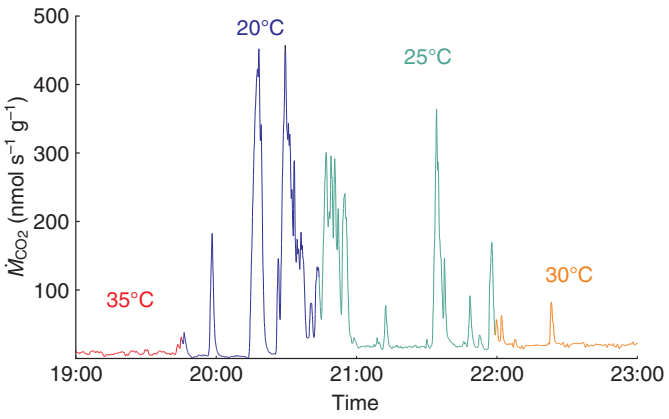

Fig. 1. Example of respiration in a *Cyclocephala colasi* beetle subjected to four experimental ambient temperatures of at least 40 min duration. Bouts of endothermy are marked by spikes in the record, which are more numerous and extreme at lower temperatures.

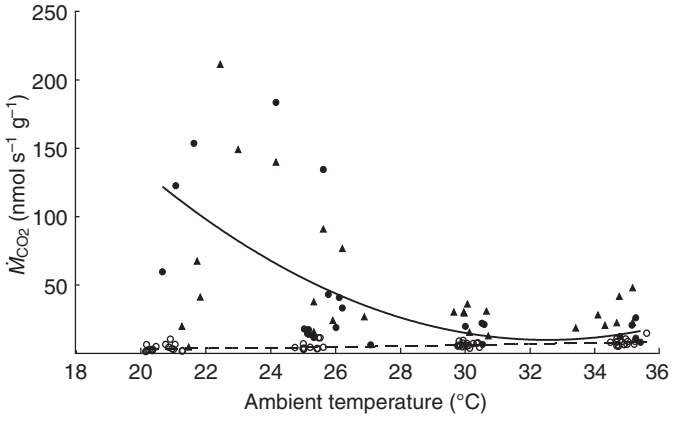

Fig. 2. Respiration rate of *Cyclocephala colasi* beetles in relation to ambient temperature. They represent single beetles described as either resting (open circles) or active (closed circles) during the period of measurement. Data for active trios (triangles) assume that only one of the three beetles was active. Lines are regressions for resting (broken) and active (solid) beetles.

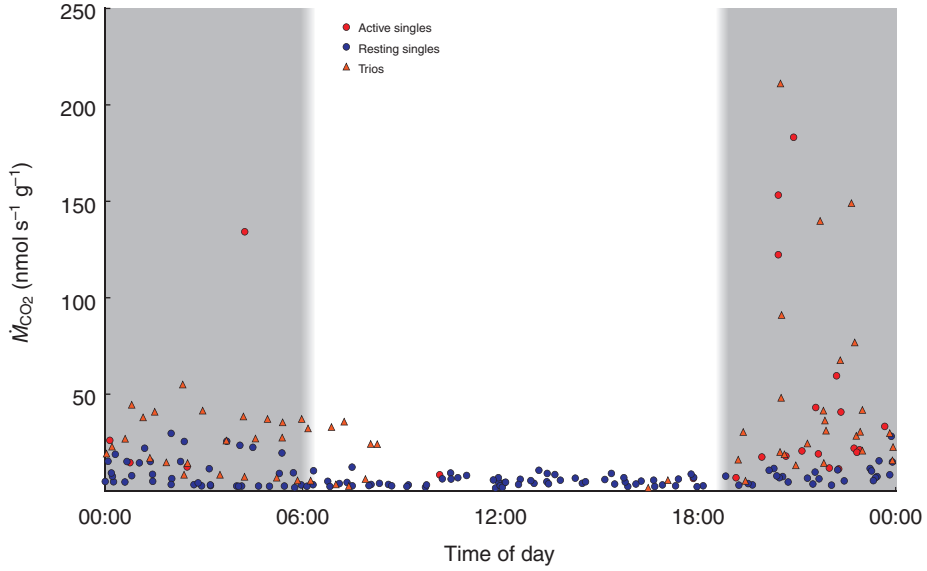

Fig. 3. Pattern of metabolic rate in *Cyclocephala colasi* throughout the circadian cycle. Civil dawn and dusk are indicated. Single beetles were described as resting or active from spot observations during the measurement periods that were spread evenly throughout the 24-h period. Data from trios of two males and one female beetle were calculated assuming only one of the three was active.

unresponsive group but it did include males. Six beetles were tested after being in captivity for a day and none became endothermic.

IR images clearly showed that heat was generated in the meta- and mesothorax, associated with the flight muscles but not in the prothorax, associated with the forelegs (Fig. 5). Heat was conducted to the surface more easily on the ventral side than on the dorsal side, which was on average about 1°C cooler (Fig. 5C). Often the temperature on the right side of the dorsal thorax was slightly warmer than the left. This difference was not quantified but was noticed on many beetles. It is also noteworthy that the temperature of the abdomen was always cool but the head was warmer than the prothorax.

Values of T_{th} and T_a were recorded from thermal images every 15 s. $\dot{M}_{CO_2}$ was measured every 15 s and four points were averaged over 1 min rolling intervals. These data were superimposable (Fig. 6). Endothermy occurred in conspicuous cycles within each bout, and changes in $\dot{M}_{CO_2}$ were tracked by changes in the difference: T_{th}–T_a.

Mean ventral T_{th} of 14 endothermic beetles was 33.3°C (±1.2 CI) within a T_a range of 15.7–29.3°C. Data from two beetles tested throughout the range demonstrate that as T_a decreased, $\dot{M}_{CO_2}$ significantly increased (slope=–28.7; CI=2.6; P<0.001) (Fig. 7) and T_{th} slightly, but significantly, increased (slope=–0.12; CI=0.05; P<0.001) (Fig. 8). $\dot{M}_{CO_2}$ was higher in these experiments than in long-term records that included periods of inactivity (Fig. 2). $\dot{M}_{CO_2}$ oscillated widely during endothermic episodes (Fig. 6). Although oscillations in $\dot{M}_{CO_2}$ were matched to T_{th} changes quite well, there was a significant lag, particularly during the initial warm-up and final cool-down phases. This can be seen as a hysteresis in the relationship between temperature excess and $\dot{M}_{CO_2}$ when changes in respiration rate precede changes in temperature (Fig. 9).

When data from all 12 beetles were analysed without the initial and final non-equilibrium data, there was a significant relationship between respiration rate and temperature elevation (Fig. 10). However, it was not linear but was better represented by a power curve.

Beetle behaviour in inflorescences

Counts of beetles in five field inflorescences indicated that arrival was just after sunset, and the number of residents was stable throughout the night (Fig. 11). They were usually inactive when first observed but about a third were active at any time, either moving about or mating. This activity was consistent throughout the night, ending only after sunrise. During this time, the beetles continued to eat the tops of sterile male florets, as evidenced by a consistent rise in the number of rows of florets eaten (Fig. 11). The florets were observed with the borescope illumination overnight but with natural illumination after dawn, which resulted in less contrast and slight underestimation of the final point.

Body temperatures of beetles within inflorescences were very near the temperatures of the floral chamber (Fig. 4). The IR camera was used in the field to photograph beetles in the inflorescence of *P. solimoesense* on one occasion. This was in the evening of a second-day inflorescence, when the spathe was closing and beetles were leaving. At the time, T_a was 23°C and spadix male florets were 29°C. Thoracic surface temperatures of beetles ranged from 25°C on the spathe to 28°C on the spadix. However, we recorded pre-flight warm-up to 30°C in one individual before it climbed to the top of the spathe and took off.

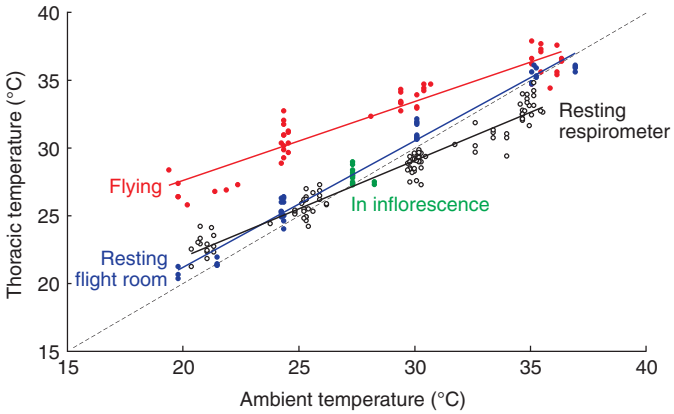

Fig. 4. Thoracic temperatures of *Cyclocephala colasi* in relation to ambient temperature and situation. Data for beetles flying in a temperature-controlled room (red) were taken after at least 1 min of flight and within a few seconds of landing. The equation for the line is T_{th}=0.58T_a+16.0 (R^2=0.89). Resting beetles in the flight room (blue) were measured while stationary on wood. The equation for the line is T_{th}=0.93T_a+2.5 (R^2=0.98). Resting beetles in the respirometer (white) were measured upon removal from chamber. The regression is T_{th}=0.71T_a+7.7 (R^2=0.95). Beetles were also measured upon removal from two thermogenic inflorescences of *Philodendron solimoesense* (green). The broken line is isothermal.

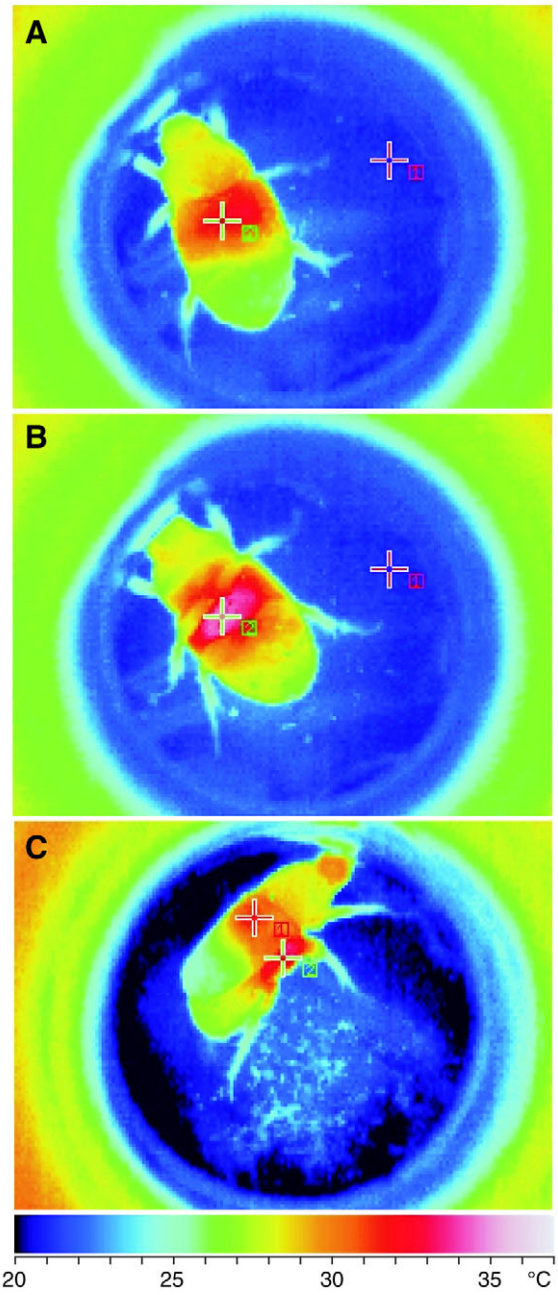

Fig. 5. Thermal images of *Cyclocephala colasi* beetles in the respirometry chamber, photographed from below, showing the dorsal (A), ventral (B) and side (C) surfaces. A and B are from the same beetle, photographed 15 s apart. C is another beetle briefly on its side. The temperatures of the pixels marked by 1 and 2 are, respectively, A: 20.9, 33.0°C. B: 21.1, 34.5°C. C: 31.1, 32.1°C.

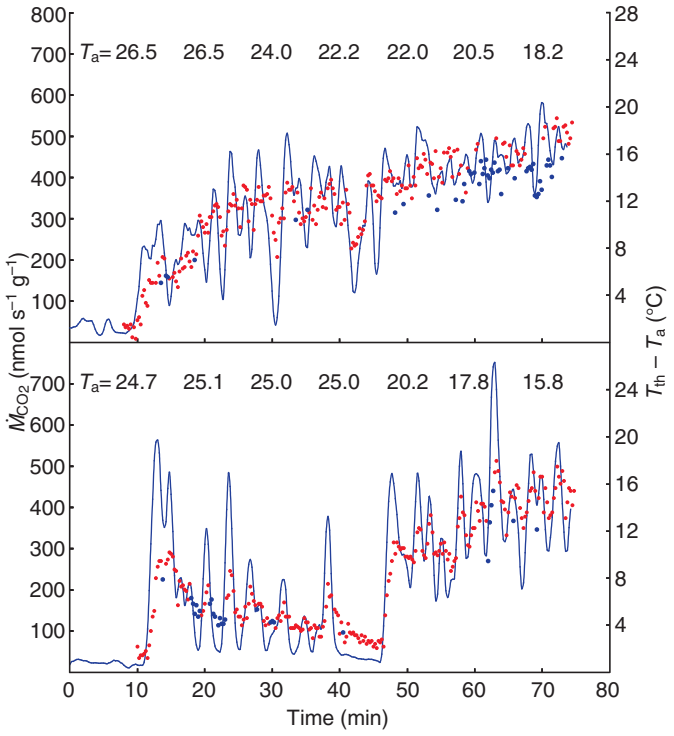

Fig. 6. Rates of CO_2 production ($\dot{M}_{CO_2}$) of a 340 mg male (top) and a 324 mg female (bottom) *Cyclocephala colasi* during episodes of endothermy (blue line). The differences between thoracic and ambient temperature (T_{th}–T_a) are superimposed, with symbol colour designating ventral (red) and dorsal (blue) surfaces of the insect. Rising metabolic rate was in response to decreasing T_a, as indicated every 10 min.

DISCUSSION

Role of endothermy in nature

This study was designed primarily to evaluate the effect of environmental temperature on energy expenditure of facultative endothermic *C. colasi* beetles that inhabit thermogenic inflorescences of *P. solimoesense*. It demonstrated that the beetles exhibit spontaneous bouts of endothermy that were more frequent and more intense at lower T_a (Fig. 1). When measured at a range of T_a spread evenly over the 24-h period, the mean metabolic rate of active beetles increased at T_a below about 28°C (Fig. 2). When placed in the context of temperatures inside and outside of the floral chamber, the results suggested that the daily energy expenditure of active beetles inside of the inflorescence would be about a half to a quarter of that outside of it, although the chamber averaged only 4°C warmer than the surroundings (Seymour et al., 2003).

There is no evidence of endothermy within field inflorescences, as T_{th} values of beetles removed from inflorescences were all similar to floral chamber temperature, *ca*. 28°C (Fig. 4), which was confirmed by IR images of beetles on the spadix in the field. Intense endothermy

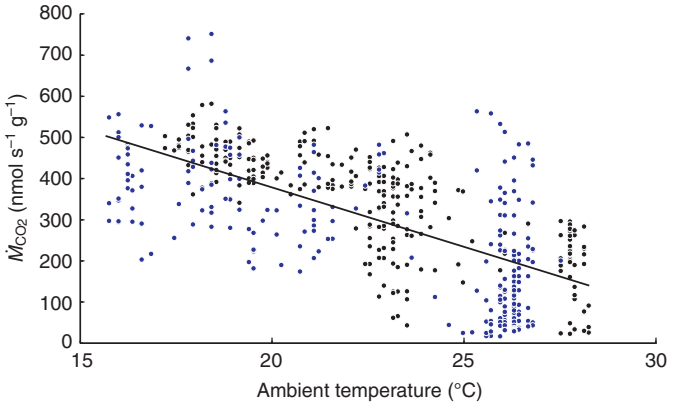

Fig. 7. Relationship between rate of CO_2 production ($\dot{M}_{CO_2}$) and ambient temperature in the two endothermic *Cyclocephala colasi* beetles illustrated in Fig. 6. The equation for the linear regression is $\dot{M}_{CO_2}$=–28.7T_a+950 (SE_b=1.34; CI=±2.6). The line extrapolates to 33°C when $\dot{M}_{CO_2}$ is zero.

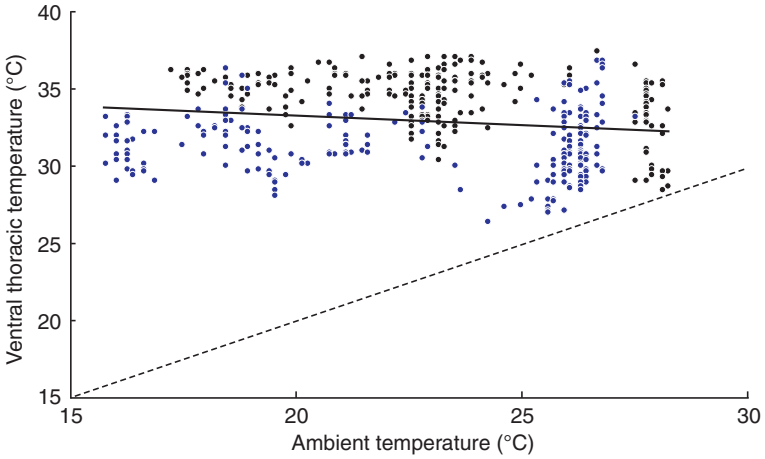

Fig. 8. Relationship between ventral metathoracic temperature and ambient temperature in two endothermic *Cyclocephala colasi* beetles illustrated in Fig. 6. The equation for the linear regression is T_{th}=–0.12T_a+35.7 (SE_b=0.03; CI=±0.05). The broken line is isothermal.

is also rare in respirometry chambers above 28°C (Fig. 2). The present data are similar to mean T_{th} (26.8°C) of active *Cyclocephala caelestis* in thermogenic *Magnolia tamaulipana* flowers in Mexico (Dieringer et al., 1998). Mean T_{th} of many active *Cyclocephala hardyi* was 33.2°C within first-night Amazon water lilies, *Victoria amazonica*, which was not significantly higher than floral chamber temperature at the time (Seymour and Matthews, 2006). We conclude that endothermy with appreciable body temperature rise does not usually occur when beetles are inside thermogenic flowers.

At present, we do not fully understand the biological significance of bouts of endothermy recorded in respirometers. It could be argued that endothermy at cooler temperatures was related to attempts to escape. There is no question that endothermy is associated with preparation for flight in beetles (Bartholomew and Casey, 1977a; Bartholomew and Casey, 1977b; Bartholomew and Heinrich, 1978; Chown and Scholtz, 1993; Merrick and Smith, 2004; Oertli, 1989). When averaged data from 24-h experiments are associated with time of day, there was a clear circadian rhythm in endothermy, despite all measurements being made in a darkened respirometer (Fig. 3). Bouts of endothermy were largely absent during the day but were most prevalent in the early evening when the insects normally fly from one inflorescence to another. Bouts continuing throughout the night, especially at the cooler respirometer temperatures, could be interpreted as preparations for escape or associated with some other activities. Field observations of beetles within inflorescences revealed activity, including eating and mating, throughout the night (Fig. 11).

Endothermy in beetles is not exclusively associated with flight or pre-flight warm-up. Spontaneous endothermy over long periods of time can occur in scarab and cerambycid beetles, either walking or completely motionless, without any indications of imminent flight (Bartholomew and Casey, 1977a; Bartholomew and Casey, 1977b). This occurs particularly at lower T_a and appears to be attempts at homeothermy, presumably to facilitate terrestrial activity. In the case of *C. colasi*, the activity could be eating, digesting and mating, all of which occur in the floral chamber. Vigorous fighting between male *C. hardyi* for access to females occurs in the floral chamber of *V. amazonica* (Seymour and Matthews, 2006), and warm body temperatures may be as important in this case as they are in rain beetles searching for females (Morgan, 1987) or in dung beetles fighting for dung balls (Heinrich and Bartholomew, 1979). We attempted to measure respiration during competition for mates in *C. colasi* by introducing trios into the respirometry chamber (Fig. 3) but were unable to associate any values with fighting or mating.

Endothermy is clearly required for flight in *C. colasi*. T_{th} of flying beetles were above 25°C (Fig. 4), and IR records showed take-off temperatures of about 30°C. In lowland French Guiana, ambient air temperature did not drop below 20°C during the study. We judge that 20°C might be close to the lower limit for flight, because it was difficult to get beetles to fly near this temperature, and those that did were generally slower and seemed to be struggling to remain aloft. Their T_{th} values were below the regression line for all flying individuals (Fig. 4). This contrasts with other scarabs that are able to

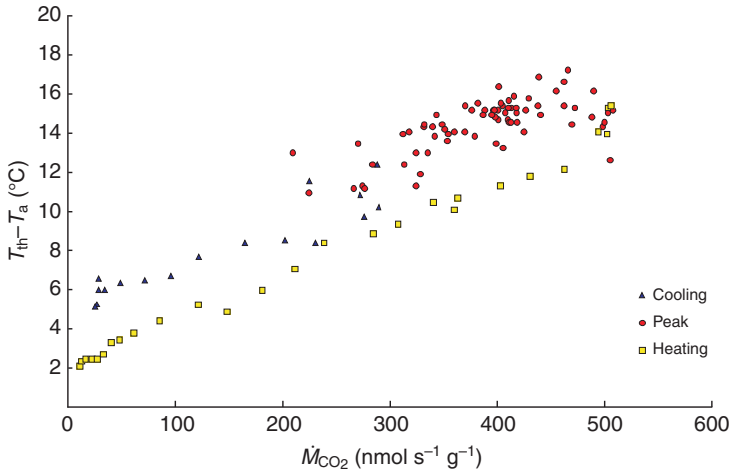

Fig. 9. Example of hysteresis of temperature elevation in relation to rate of CO_2 production in a *Cyclocephala colasi* beetle. The heating data begin at the left and proceed up the line of yellow squares. Peak temperatures in red represent a thermoregulating beetle. Cooling data in blue proceed from right to left. The difference between thoracic and ambient temperatures is less during initial heating than during final cooling.

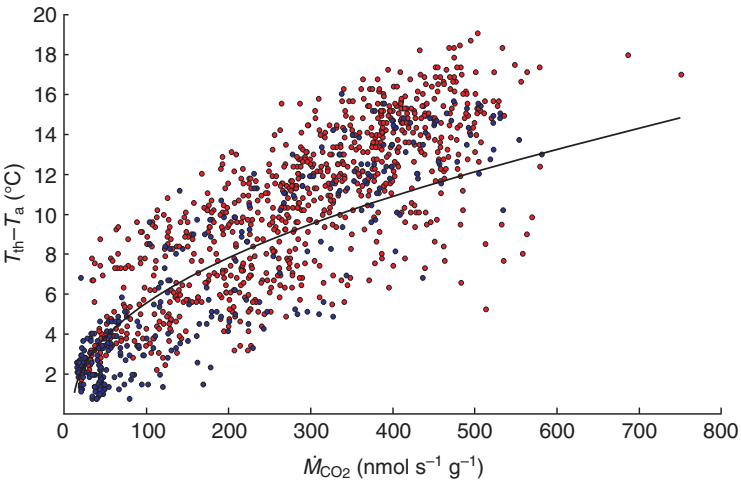

Fig. 10. Relationship between temperature elevation ($T_{th}-T_a$) and CO_2 production ($\dot{M}_{CO_2}$) in *Cyclocephala colasi*. Data derived from 12 beetles of mean mass 280 mg, without data during initial warm-up and final cool-down. Ventral (red) and dorsal (blue) surfaces are indicated; however, the power regression ($T_{th}-T_a=0.577\ \dot{M}_{CO_2}^{0.49}$; $SE_b=0.01$; CI=±0.02) is for ventral data only.

fly at T_a close to freezing (Morgan, 1987), and suggests that the flight capabilities of *C. colasi* are matched to their warm environment.

Temperature regulation and thermal conductance

Beetles in this study exhibited regulation of T_{th} during bouts of endothermy. The standard metric for the precision of temperature regulation is the slope of the regression line relating T_{th} with T_a (e.g. Chown and Scholtz, 1993; Merrick and Smith, 2004; Morgan, 1987; Oertli, 1989; Stone and Willmer, 1989; Verdu et al., 2004; Verdu et al., 2006). While a simplistic regression approach such as this ignores much of the detail regarding an insect's thermoregulatory ability (Chown and Nicholson, 2004), it nevertheless provides a coarse index of thermoregulatory precision under the conditions of measurement. A slope of zero indicates perfect independence of T_{th} and T_a, implying precise thermoregulation whereas a slope of 1 shows no regulation. By this measure, *C. colasi* beetles in the respirometer demonstrated perfect temperature regulation, maintaining a mean T_{th} of 33.3°C independently of T_a down to 15.7°C by increasing $\dot{M}_{CO_2}$ as T_a decreased (Figs 7 and 8). However, data from *C. colasi* from the flight room provided a slope of 0.54, which shows only moderate independence (Fig. 4). This supports the majority of reports that show that thermoregulatory precision is generally lower in flying insects than stationary ones. For example, dung beetles, *S. sacer*, have a significant slope during flight but almost no slope at take-off when T_{th} is about 37°C at T_a ranging between 16 and 30°C (Verdu et al., 2004). Without high convective losses associated with flight movement and raised elytra and wings, heat retention is better and thermoregulation more 'precise'. The slopes are quite variable in other beetles that are measured as they fly into light traps or in experimental enclosures. They range from essentially zero to 0.64 in several species (Chown and Scholtz, 1993; Merrick and Smith, 2004; Morgan, 1987; Oertli, 1989; Verdu et al., 2004; Verdu et al., 2006). This variation shows that the slope of the temperature relationship provides limited information about the factors influencing thermoregulatory precision, because the slope is affected by the balance between heat production and heat loss, both of which are independently variable. The high slopes observed in some species during flight are likely to be influenced by a relatively faster rate of heat loss (affected by small body size, lack of insulation, high convective losses during flight, etc.) or a lower rate of heat production from the flight muscles (affected by flight speed, wing loading, etc.). Unfortunately the relationships between these factors have not been elucidated and they cannot be deduced from the temperature relationship alone.

The high variability in respiratory and thermal data from individual beetles during bouts of endothermy (Figs 7 and 8) is partly due to oscillations in respiration (Fig. 6). Profound oscillations are common in endothermic beetles and the frequency increases with greater temperature elevations (Bartholomew and Casey, 1977b; Morgan, 1987). It is tempting to speculate that the level of heat production is determined simply by frequency changes in an on/off heat-generating mechanism. As in other advanced insects, the heat is apparently

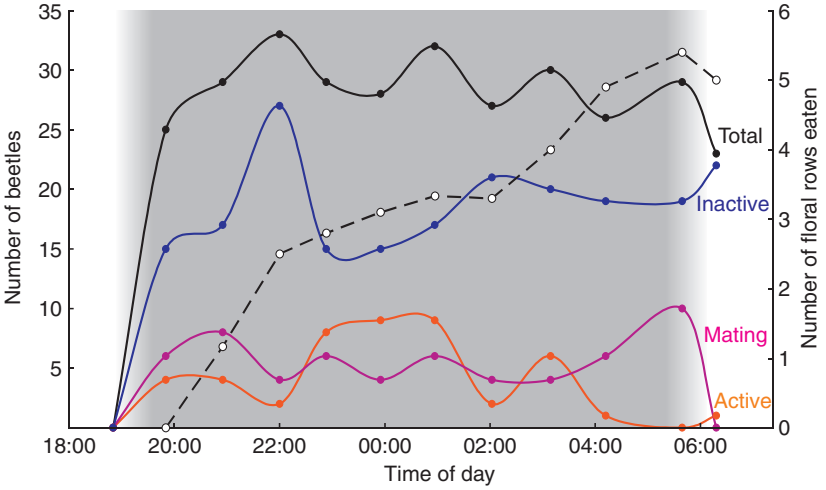

Fig. 11. Numbers of *Cyclocephala colasi* beetles in the floral chambers of five inflorescences of *Philodendron solimoesense* during the first night of blooming. Data are totals for designated activities observed at each point. Mean number of rows of sterile male florets partly consumed by the beetles is indicated by open circles. Shading indicates night and times of dusk and dawn are shown.

derived from simultaneous myogenic contractions of opposing sets of meta- and mesothoracic flight muscles (Heinrich, 1995).

Data for respiration and body temperature make it possible to estimate thermal conductance of the beetles without having to heat a dead insect artificially and calculate from a cooling curve in still air (Casey and Joos, 1983; Chown and Scholtz, 1993; Merrick and Smith, 2004; Morgan, 1987). The slope of the curve in Fig. 7 is $-28.7\,\mathrm{nmol\,s^{-1}\,g^{-1}\,°C^{-1}}$, which corresponds to $13.6\,\mathrm{mW\,°C^{-1}\,g^{-1}}$ in the average 330 mg beetle. This compares favourably with $10.9\,\mathrm{mW\,°C^{-1}\,g^{-1}}$ in a 796 mg scarab *S. flava* (Chown and Scholtz, 1993). The difference can be ascribed to a combination of larger body size and thicker insulation on the ventral surface of *S. flava*. However, the present study shows clearly that thermal conductance is not simply a function of temperature difference. The curved relationship between temperature elevation with respiration rates indicates an increase in thermal conductance at higher temperatures (Fig. 10). Assuming $0.47\,\mathrm{J\,\mu mol^{-1}}$ CO_2 (RQ=1), the thermal conductance increases from 7.6 to $24.0\,\mathrm{mW\,°C^{-1}\,g^{-1}}$ when the temperature elevation increases from 5 to 15°C. This is to be expected, because heat loss should increase as ventilation increases. Not only does ventilation of the tracheal system increase with greater respiration, causing greater convective heat loss but also water vapour density increases exponentially with temperature, increasing the gradient with the atmosphere and enhancing evaporative cooling. Evaporative cooling is also evident by lower T_{th} of resting beetles in the dry air of the respirometers compared with those in the more humid flight room (Fig. 4).

Conclusions

Endothermy is important in the energy balance of scarab beetles visiting thermogenic flowers. The warm floral chamber saves the insects energy by allowing them to be active while reducing the incidence and intensity of energetically expensive bouts of endothermy. Endothermy is clearly necessary for flight outside of the inflorescences but whether it is associated with competition for mates within the floral chamber remains unknown. This study focused on the phenomenon in the lowlands of French Guiana, where the rather warm environment (T_a>20°C) minimised the energy-saving value of floral thermogenesis in *P. solimoesense*. It will be interesting to compare the energetics and patterns of endothermy of scarab beetles (*Erioscelis emarginata*) that pollinate *Philodendron selloum* at T_a as low as 6°C in the highlands of Brazil (Gottsberger and Silberbauer-Gottsberger, 1991).

LIST OF ABBREVIATIONS

CI	95% confidence interval
J–N	Johnson–Neyman test
$\dot{M}_{CO_2}$	rate of CO_2 production
RQ	respiratory quotient
SE_b	standard error of the slope
T_a	ambient temperature
T_{th}	thoracic temperature

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REFERENCES

Barabé, D., Gibernau, M. and Forest, F. (2002). Zonal thermogenetic dynamics of two species of *Philodendron* from two different subgenera (Araceae). *Bot. J. Linn. Soc.* **139**, 79-86.

Bartholomew, G. A. (1981). A matter of size: an examination of endothermy in insects and terrestrial vertebrates. In *Insect Thermoregulation* (ed. B. Heinrich), pp. 46-78. New York: Wiley.

Bartholomew, G. A. and Casey, T. M. (1977a). Body temperature and oxygen consumption during rest and activity in relation to body size in some tropical beetles. *J. Therm. Biol.* **2**, 173-176.

Bartholomew, G. A. and Casey, T. M. (1977b). Endothermy during terrestrial activity in large beetles. *Science* **195**, 882-883.

Bartholomew, G. A. and Heinrich, B. (1978). Endothermy in African dung beetles during flight, ball making, and ball rolling. *J. Exp. Biol.* **73**, 65-83.

Bartholomew, G. A., Vleck, D. and Vleck, C. M. (1981). Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *J. Exp. Biol.* **90**, 17-23.

Casey, T. M. and Joos, B. A. (1983). Morphometrics, conductance, thoracic temperature, and flight energetics of noctuid and geometrid moths. *Physiol. Zool.* **56**, 160-173.

Chappell, M. A. (1984). Thermoregulation and energetics of the green fig beetle (*Cotinus texana*) during flight and foraging behavior. *Physiol. Zool.* **57**, 581-589.

Chown, S. L. and Nicolson, S. W. (2004). *Insect Physiological Ecology: Mechanisms and Patterns*. New York: Oxford University Press.

Chown, S. L. and Scholtz, C. H. (1993). Temperature regulation in the nocturnal melolonthine *Sparrmannia flava*. *J. Therm. Biol.* **18**, 25-33.

Chown, S. L., Scholtz, C. H., Klok, C. J., Joubert, F. J. and Coles, K. S. (1995). Ecophysiology, range contraction and survival of a geographically restricted African dung beetle (Coleoptera, Scarabaeidae). *Funct. Ecol.* **9**, 30-39.

Dieringer, G., Reyes-Castillo, P., Lara, M., Cabrera, R. L. and Loya, L. (1998). Endothermy and floral utilization of *Cyclocephala caelestis* (Coleoptera: Scarabaeoidea; Melolonthidae): a cloud forest endemic beetle. *Acta Zool. Mexicana, Nueva Serie* **73**, 145-153.

Dieringer, G., Cabrera, R. L., Lara, M., Loya, L. and Reyes-Castillo, P. (1999). Beetle pollination and floral thermogenicity in *Magnolia tamaulipana* (Magnoliaceae). *Int. J. Plant Sci.* **160**, 64-71.

Fægri, K. and van der Pijl, L. (1979). *The Principles of Pollination Ecology*. Oxford: Pergamon Press.

Gibernau, M. and Barabé, D. (2000). Thermogenesis in three *Philodendron* species (Araceae) of French Guiana. *Can. J. Bot.* **78**, 685-689.

Gibernau, M., Barabé, D., Cerdan, P. and Dejean, A. (1999). Beetle pollination of *Philodendron solimoesense* (Araceae) in French Guiana. *Int. J. Plant Sci.* **160**, 1135-1143.

Gottsberger, G. and Amaral, Jr, A. (1984). Pollination strategies in Brazilian *Philodendron* species. *Ber. Dtsch. Bot. Ges.* **97**, 391-410.

Gottsberger, G. and Silberbauer-Gottsberger, I. (1991). Olfactory and visual attraction of *Erioscelis emarginata* (Cyclocephalini, Dynastinae) to the inflorescences of *Philodendron selloum* (Araceae). *Biotropica* **23**, 23-28.

Heinrich, B. (1993). *The Hot-blooded Insects: Strategies and Mechanisms of Thermoregulation*. Cambridge, MA: Harvard University Press.

Heinrich, B. (1995). Insect thermoregulation. *Endeavour* **19**, 28-33.

Heinrich, B. and Bartholomew, G. A. (1979). Roles of endothermy and size in inter- and intraspecific competition for elephant dung in an African dung beetle, *Scarabaeus laevistriatus*. *Physiol. Zool.* **52**, 484-496.

Heinrich, B. and McClain, E. (1986). 'Laziness' and hypothermia as a foraging strategy in flower scarabs (Coleoptera: Scarabaeidae). *Physiol. Zool.* **59**, 273-282.

Merrick, M. J. and Smith, R. J. (2004). Temperature regulation in burying beetles (*Nicrophorus* spp.: Coleoptera: Silphidae): effects of body size, morphology and environmental temperature. *J. Exp. Biol.* **207**, 723-733.

Morgan, K. R. (1987). Temperature regulation, energy metabolism and mate-searching in rain beetles (*Pleocoma* spp.), winter-active, endothermic scarabs (Coleoptera). *J. Exp. Biol.* **128**, 107-122.

Morgan, K. R. and Bartholomew, G. A. (1982). Homeothermic response to reduced ambient temperature in a scarab beetle. *Science* **216**, 1409-1410.

Oertli, J. J. (1989). Relationship of wing beat frequency and temperature during take-off flight in temperate-zone beetles. *J. Exp. Biol.* **145**, 321-338.

Saeki, Y., Kruse, K. C. and Switzer, P. V. (2005). Physiological costs of mate guarding in the Japanese beetle (*Popillia japonica* Newman). *Ethology* **111**, 863-877.

Schatz, G. E. (1990). Some aspects of pollination biology in Central American forests. In *Reproductive Ecology of Tropical Forest Plants*, vol. 7 (ed. K. S. Bawa and M. Hadley), pp. 69-84. Paris: UNESCO.

Seymour, R. S. (2004). Dynamics and precision of thermoregulatory responses of eastern skunk cabbage *Symplocarpus foetidus*. *Plant Cell Environ.* **27**, 1014-1022.

Seymour, R. S. and Matthews, P. G. D. (2006). The role of thermogenesis in the pollination biology of the Amazon waterlily *Victoria amazonica*. *Ann. Bot.* **98**, 1129-1135.

Seymour, R. S. and Schultze-Motel, P. (1997). Heat-producing flowers. *Endeavour* **21**, 125-129.

Seymour, R. S., White, C. R. and Gibernau, M. (2003). Heat reward for insect pollinators. *Nature* **426**, 243-244.

Stone, G. N. and Willmer, P. G. (1989). Endothermy and temperature regulation in bees: A critique of 'grab and stab' measurement of body temperature. *J. Exp. Biol.* **143**, 211-223.

Verdu, J. R., Diaz, A. and Galante, E. (2004). Thermoregulatory strategies in two closely related sympatric Scarabaeus species (Coleoptera: Scarabaeinae). *Physiol. Entomol.* **29**, 32-38.

Verdu, J. R., Arellano, L. and Numa, C. (2006). Thermoregulation in endothermic dung beetles (Coleoptera: Scarabaeldae): effect of body size and ecophysiological constraints in flight. *J. Insect Physiol.* **52**, 854-860.

White, C. R. (2003). Allometric analysis beyond heterogeneous regression slopes: use of the Johnson-Neyman technique in comparative biology. *Physiol. Biochem. Zool.* **76**, 135-140.

White, C. R., Portugal, S. J., Martin, G. R. and Butler, P. J. (2006). Respirometry: anhydrous Drierite equilibrates with carbon dioxide and increases washout times. *Physiol. Biochem. Zool.* **79**, 977-980.

Withers, P. C. (2001). Design, calibration and calculation for flow-through respirometry systems. *Aust. J. Zool.* **49**, 445-461.

Ybarrondo, B. A. and Heinrich, B. (1996). Thermoregulation and response to competition in the African dung beetle *Kheper nigroaeneus* (Coleoptera: Scarabaeidae). *Physiol. Zool.* **69**, 35-48.

Zar, J. H. (1998). *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice Hall.