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Resting metabolism and critical thermal maxima of vespine wasps (*Vespula* sp.)

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ABSTRACT

Vespine wasps are known for their high endothermic capacity. Endothermic activity is directly linked to respiration. However, knowledge on wasp respiration is sparse and almost nothing is known about their resting metabolism.

We investigated the yellowjackets' CO_2 production in a flow-through respirometer chamber overnight. Endothermic and behavioral activity was observed by real-time infrared thermography. Most resting wasps were ectothermic or only slightly endothermic (thoracic temperature excess against abdomen < 0.6 °C). In the investigated temperature range (T_a = 2.9–42.4 °C) mean CO_2 production rate of resting wasps increased steeply according to an exponential function, from 5.658 μl g^{-1} min^{-1} at 8.3 °C to 8.504 μl g^{-1} min^{-1} at 20.2 °C, 58.686 μl g^{-1} min^{-1} at 35.3 °C and 102.84 μl g^{-1} min^{-1} at 40 °C. The wasps' respiratory critical thermal maximum (CT_{max}), marking the upper edge of their viable temperature range, was 45.3 °C. The respiratory CT_{max} did not differ significantly from the activity CT_{max} of 44.9 °C. CT_{max} values were considerably below that of honeybees (48.9 and 49.0 °C for respiration and activity, respectively). This allows honeybees to kill wasps by heat-balling.

Comparison with other arthropods showed that vespine wasps are among the insects with the highest mass-specific resting metabolic rate and the steepest increase of metabolism with ambient temperature.

1. Introduction

Vespine wasps of the genus *Vespula* are capable of a very impressive thermoregulatory performance (Coelho and Ross, 1996; Heinrich, 1989; Kovac and Stabentheiner, 1999; Kovac et al., 2009). Endothermy improves muscular function (Coelho, 1991), which improves agility and enables them to carry heavy loads during foraging (Kovac and Stabentheiner, 1999; Kovac et al., 2009). Endothermy is also used to regulate the nest temperature (Himmer, 1927; Schmolz et al., 1993; Steiner, 1930). A high nest temperature in honeybees speeds up larval development (Petz et al., 2004). However, in the nest of honeybees, which have a comparable social thermoregulatory capacity, most bees are ectothermic (Stabentheiner et al., 2003, 2010). The same has to be assumed for the nest of vespine wasps. Basal metabolism of the ectothermic insects provides a considerable amount of heat for social thermoregulation (Kovac et al., 2007; Petz et al., 2004; Schmolz et al., 1993; Stabentheiner et al., 2010). As in the wasps' nests temperature varies more than in honeybee nests (e.g. Büdel, 1955; Himmer, 1962; Klingner et al., 2005, 2006; Simpson, 1961; Steiner, 1930) the temperature dependence of their resting metabolism is of special interest. The resting metabolism as a measure of the basal metabolism, however, has not yet been well investigated in vespine wasps. Wasp nests may cool considerably during cold nights (Himmer, 1962; Klingner et al., 2005, 2006; Steiner, 1930), and the individuals' resting metabolism is important also outside their thermal optimum. To gain a comprehensive overview of an insect's physiological reaction to environmental changes, analysis over the animal's entire viable temperature range is a necessity. Therefore we measured the CO_2 production of resting *Vespula vulgaris* and *Vespula germanica* foragers in the entire range of temperatures they are likely exposed to in a breeding season (2.9–42.4 °C) in Central Europe.

Vespula often builds its nests under the roofing tiles of old farmhouses. These nests are sometimes abandoned at an early stage. On the one hand this may be caused by an accident or illness of the nest-founding queen. On the other hand, however, this may be caused by the increasingly higher temperatures in the course of the early breeding season. Temperatures at these locations may become as high as 45.8 °C when the sun shines on the tiles on warm days (our own unpublished observations). This is in the range of the wasps' suggested upper thermal limit (Käfer et al., 2011). Although wasps are known to cool their nests with water spread on the combs (Klingner et al., 2005; Kovac et al., 2009; Steiner, 1930), these nest temperatures may be higher than single insects or small colonies can survive. In this context the wasps' critical thermal maximum (CT_{max}) is of special interest. Some vespine wasps are known to be more susceptible to high temperatures

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than honeybees (Ono et al., 1987, 1995). This allows honeybees to kill wasps by heat-balling (Ono et al., 1987; Papachristoforou et al., 2007; Stabentheiner, 1996; Tan et al., 2005). Stabentheiner (1996) and Stabentheiner et al. (2007) investigated this aggressive interaction between *Apis mellifera carnica* and *Vespula* sp. However, while the upper lethal temperature has been determined in *Vespa mandarinia japonica* (44–46 °C, Ono et al., 1995), *Vespa velutina* (45.7 °C, Tan et al., 2005), and *Vespa orientalis* (50.6 °C, Papachristoforou et al., 2011) the upper thermal limit of *Vespula* has not yet been investigated. Because it is thought to be more relevant to natural conditions we choose the temperature ramping procedure (Terblanche et al., 2011). We applied behavioral observations (Klok et al., 2004) and thermolimit respirometry (Lighton and Turner, 2004) to determine the wasps' upper critical thermal maximum (activity and respiratory CT_{max}).

2. Material and methods

2.1. Animals

Experiments took place in late summer and autumn 2008 (September, October, November) and 2009 (October), and in summer 2010 (August). Foraging yellowjackets (*V. vulgaris* (Linnaeus 1758) and *V. germanica* (Fabricius 1793) – subsequently referred to as *Vespula* sp.) were caught at an artificial feeding station provided with sucrose solution. Animals were collected for immediate analysis. In some cases (8 of 35 wasps) they were stored in cages overnight in a dark and cool area (12–15 °C, food provided) for use on the following day. Individuals were weighed before and after the experiments.

2.2. CO_2 measurement

Individuals were put into a flow-through respirometer measurement chamber made of brass and immersed into an electronically controlled water bath (Julabo F33 HT) regulated within ±0.1 °C of the set temperature. The chamber volume was 18 ml (3 × 3 × 2 cm). This allowed unrestricted movement of the wasps at a high measurement sensitivity. Because of the wasps' long stay in the chamber (typically overnight, >6 h) they were also provided with a food source (1.5 M sucrose solution ad libitum). Experimental ambient temperature (T_a) for the wasps was set via the water bath from 2.5 to 45 °C in steps of 5 °C. Most individuals (23 of 35) were tested at only one T_a. Six individuals were tested at two T_as, five individuals at three T_as, two individuals at four T_as, and one individual at five T_as. Experiments lasted at least 3.5 h at each set temperature.

Individuals were transferred into the respirometer chamber directly from the outside or from storage and had time to accustom to the adjusted T_a for at least 15 min. Because the chamber was not completely submersed and the chamber's top lid window was covered with a thin plastic film, the inside temperature deviated somewhat from the temperature of the water bath. Therefore, actual ambient air temperature was measured with a thermocouple inside the chamber near the insect (~1 cm), sensing the actual experimental temperature.

The air for the flow-through respirometry was taken from an inlet outside the laboratory. Before entering the measurement system it had to pass a 10 l canister and a 5 l bottle to smooth any variations in outside CO_2 concentration. Relative humidity was kept at 50% down to 15 °C, 60% at 12.5 °C, 70% at 10 °C, 80% at 7.5 °C, 90% at 5 °C and 100% at 2.5 °C. To control relative humidity, the measuring gas was passed through two humidifying bottles filled with distilled water prior to the measurement chamber, saturating the air with water vapor. The bottles were submersed in a second Julabo F33 HT water bath adjusted to the according dew point temperature required for the desired relative humidity in the measurement chamber (Stabentheiner et al., 2012).

CO_2 production was measured with a differential infrared gas analyzer (DIRGA) sensitized to carbon dioxide in serial mode (Advance Optima URAS14, ABB; compare Kovac et al., 2007; Stabentheiner et al., 2012; Petz et al., 2004). Air flow was set to 150 ml min^{-1} and regulated by a Brooks 5850S mass flow controller (0–1000 ml/min; Brooks Instrument, Hatfield, USA). As a result of the tube length between the measuring chamber and the URAS a delay of 35.0 s was measured. The wasps' CO_2 production was recorded at intervals of 1 s. The amount of CO_2 production (μl g^{-1} min^{-1}) reported in this paper refer to standard (STPS) conditions (0 °C, 101.32 kPa = 760 Torr). Considering the duration of each experiment, the URAS gas analyzers were set to automatic zero and end point calibration every 3 h using the internal calibration cuvettes. During evaluation, the data were corrected for any remaining offset and drift.

2.3. Activity and body temperature

The top lid of the measurement chamber was covered with a plastic film transparent to infrared (IR) radiation in the range of 3–13 μm. It enabled us to record both the wasps' body surface temperature and activity with an infrared thermography camera (ThermaCam SC2000 NTS; FLIR Systems Inc.). An IR emissivity of 0.97 of the wasp cuticle was used to calculate surface temperatures (for details see Kovac et al., 2007; Schmaranzer and Stabentheiner, 1988; Stabentheiner et al., 2012; Stabentheiner and Schmaranzer, 1987). The measurement accuracy of 0.7 °C was achieved by using a self-constructed Peltier driven reference source of known temperature and emissivity. Infrared data were recorded digitally on hard disk at 3, 5 or 10 frames s^{-1}. Evaluation of the surface temperatures of head (T_{hd}), thorax (T_{th}) and abdomen (T_{ab}) was done with AGEMA Research software (FLIR Systems Inc.) controlled by a proprietary Excel (Microsoft Corporation) VBA macro. The thermographic video sequences also allowed judgment of active and resting periods without behavioral impairment. Endothermy was assessed by the difference between T_{th} and T_{ab}. As these temperatures were both surface temperatures measured via IR, we minimized measurement errors which possibly might occur when calculating T_{th} from IR and T_a from thermocouple data. Our definition of rest (classification according to Crailsheim et al., 1999; Stabentheiner and Crailsheim, 1999; Stabentheiner et al., 2003) was: (1) The individual was ectothermic (no visibly heated thorax) and (2) there were no or marginal signs of bodily activity (i.e. movements of antennae, single movement of legs allowed) for a duration of at least 10 min (reduced to 5 min at temperatures >27.6 °C if no 10 min intervals were available). However, we were forced to take into account that individuals, although being obviously at rest (sitting still for an hour or more), could be slightly endothermic. Therefore we had to define "rest" in terms of "scarce movement" and "only weak endothermy" with $T_{th} - T_{ab} < 2$ °C during a few periods of the experiment.

2.4. CO_2 production calculation

Before we determined the amount of carbon dioxide produced in a certain experimental trial, the IR video sequences were analyzed concerning the wasps' activity. Sections assessed as "resting periods" (defined in Section 2.3) were divided up into 10 min intervals. At high T_a (27.6 °C and above) phases of inactivity in some individuals decreased in duration as well as in number to such an extent that we had to reduce the minimal interval for our definition of "rest" to 5 min. URAS 14 CO_2 data from these time intervals were used for further calculations. Integrating the gas

exchange cycles over the 10 min intervals, the mean production rate of CO_2 (M_{CO_2} and V_{CO_2}) was calculated. All data analysis and statistics were carried out using custom-made peak and valley finding formulas and macros in Excel (Microsoft Corporation), OriginPro 8.5 (OriginLab Corporation) and Stathgraphics Centurion XVI (StatPoint Technology Inc.). In the figures mean values are given with their standard deviations (SD).

2.5. Critical thermal maximum (CT_{max})

As the combination of respirometry data and activity detection had shown the most accurate results in previous studies concerning the upper thermal maximum (Klok et al., 2004; Lighton and Turner, 2004; Stevens et al., 2010), respiration and activity as well as body surface temperatures were assessed simultaneously via flow-through respirometry and IR thermography as described in Section 2.3. The wasps' critical thermal maximum (CT_{max}) was assessed following a standardized method of driving a temperature ramp from 25 ° to 53 °C at a dT = 0.25 °C min^{-1} (e.g. Chown et al., 2009; Stevens et al., 2010; Terblanche and Chown, 2010). The CT_{max} was defined via observation of activity (activity CT_{max}, cease of controlled motoric activity, e.g. start of muscle spasms, for further information see Hazell et al., 2008; Klok and Chown, 1997; Lighton and Turner, 2004; Lutterschmidt and Hutchison, 1997), and via thermolimit respirometry (respiratory CT_{max}, cease of cyclic gas exchange, Lighton and Turner, 2004). The absolute difference sum of CO_2 production (*r*ADS) is a measure of cumulative dynamic variability (Lighton and Turner, 2004). To determine the respiratory CT_{max} more accurately, the inflection point of the *r*ADS residual values from 10 min before to 10 min after the suggested activity CT_{max} was determined. This inflection point helps to determine the minute point of the respiratory CT_{max}. For detailed information on the procedure and detailed comparison among different methods see Stevens et al. (2010).

3. Results

As the yellowjackets were collected during foraging at a feeding station and were provided with food in the measurement chamber they had sufficient energy reserves to survive the experimental periods. Before starting the experiments their mean body weight was 0.1019 g. On average the individuals were slightly lighter after the experiments (−7.9 mg, see Table 1). Some wasps left the measurement chamber even heavier than they entered it.

3.1. Activity and body temperature

After being inserted into the measurement chamber the wasps were generally agitated and very active. At this point the CO_2 production was high (Fig. 1A) and the individuals were highly endothermic (Fig. 2A). After some time the wasps calmed down and were "at rest" with a strongly decreased metabolic rate. This is represented in the gas exchange pattern (Fig. 1B) as well as in body temperature (Fig. 2B). Individuals were not resting over the entire period of the experiment. Except for the lowest temperature

Table 1
Mean body mass of tested yellowjackets at the start and the end of the experiment and their mean weight loss.

	Weight in (g)	Weight out (g)	Weight difference (g)
Mean	0.1019	0.0939	−0.0079
SD	0.0198	0.0230	0.0221
Min	0.0692	0.0523	−0.0609
Max	0.1450	0.1520	0.0357

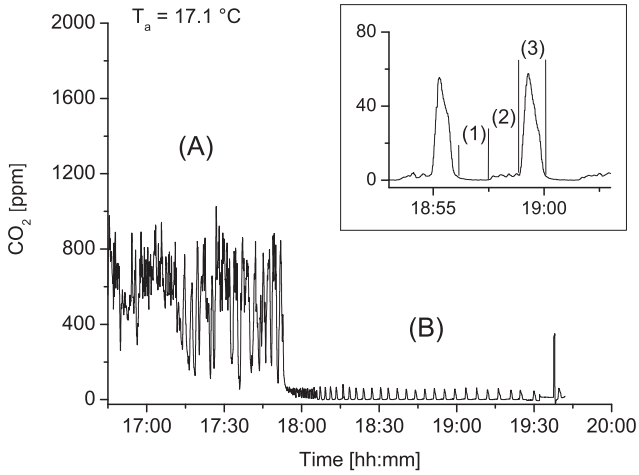

Fig. 1. Gas exchange pattern of a wasp (*Vespula* sp.) during an experiment. (A) agitated, active, continuous respiration; (B) at rest, discontinuous gas exchange (DCG); the exceptional high peak at ~19:37 h shows automatic system end point calibration. Insert: Discontinuous gas exchange DCG: (1) closed phase, (2) flutter phase, (3) open phase. Experimental ambient temperature (T_a) = 17.1 °C.

(T_a = 2.9 °C) almost all wasps sometimes showed some kind of activity, be it self-grooming, feeding or just relocating inside the chamber. At high experimental temperatures ($T_a \geqslant 27.6$ °C) some individuals were not inactive for 10 min between active periods. In these cases we had to reduce the minimal interval for "rest" to 5 min.

Although being obviously resting, the wasps were not always ectothermic (Fig. 3). Between 15 °C and 30 °C some individuals showed a slightly elevated T_{th} over the T_{ab} (thoracic temperature excess up to 0.6 °C), nevertheless sitting motionless over long periods and matching our definition of being "at rest" (Fig. 2C). Below 15 °C most individuals were ectothermic, again with some individuals deviating from the main fraction, especially at temperatures of 10 °C and below. Some maintained a mean thoracic temperature excess of up to 1.9 °C, with a high standard deviation. Even at the highest experimental temperature of 42.4 °C the wasps showed "rest" according to our definition at least for some minutes (Fig. 2D, data point (D) in Fig. 3). Some wasps like the individual in Fig. 2E (T_a = 38.5 °C) showed an unusually cool spot at the head which was caused by wetting of the mouthparts with regurgitated liquid droplets. This behavior cools the head and to some extent also the thorax at high temperatures. However, those wasps were usually active, cooling individuals at rest were an exception. Negative values of the thoracic temperature excess (i.e. the thorax was cooler than the abdomen) may have been caused by the aforementioned evaporative cooling of head and thorax in some individuals, but may also have occurred due to slight vertical temperature gradients inside the measurement chamber and the orientation of the wasp body in this gradient (Fig. 3, e.g. individual at T_a = 12 °C).

3.2. CO_2 production

Respiration data from clearly identified *V. vulgaris* and *V. germanica* (Bellmann, 1995; Clapperton et al., 1989) did not differ significantly (ANOVA: $P = 0.4857$, $F = 0.49$), so results of all individuals were pooled (*V. vulgaris*: $n = 26$, *V. germanica*: $n = 12$). With increasing experimental ambient temperature (T_a), CO_2 production rate increased exponentially, from 5.658 µl g^{-1} min^{-1} at 8.3 °C to 18.504 µl g^{-1} min^{-1} at 20.2 °C, 58.686 µl g^{-1} min^{-1} at 35.3 °C, and approaching 102.84 µl g^{-1} min^{-1} at 40 °C (Fig. 4). The following exponential function fitted the data best:

$$V_{CO_2} = A1 * \exp^{Ta/t1} + A2 * \exp^{Ta/t2} + A3 * \exp^{Ta/t3} + y0$$

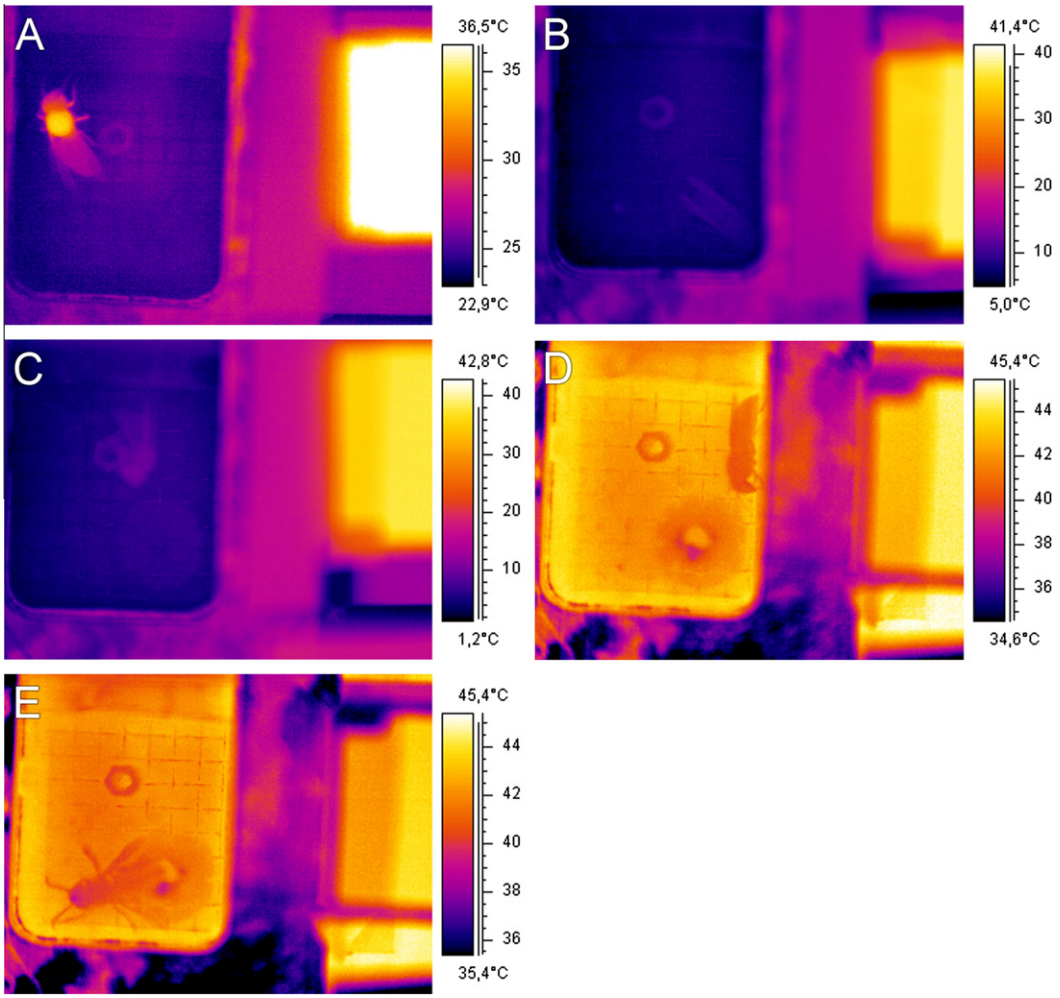

Fig. 2. Thermograms of wasps (*Vespula* sp.) inside the flow-through respirometer chamber at different temperatures (T_a) and different thermic (metabolic) conditions. (A) active individual, endothermic, T_a = 15 °C; (B)–(E) resting individuals: (B) ectothermic wasp with all body parts and the chamber at the same temperature, T_a = 7.7 °C; (C) slightly endothermic wasp with elevated T_{th}, but no bodily activity, close to the thermocouple for measurement of T_a = 5.8 °C; (D) ectothermic wasp at high T_a = 42.5 °C; (E) ectothermic wasp with wetted mouth parts (evaporative cooling) at T_a = 35.8 °C. Rectangle in right part of thermograms: Peltier driven reference radiator for IR camera calibration.

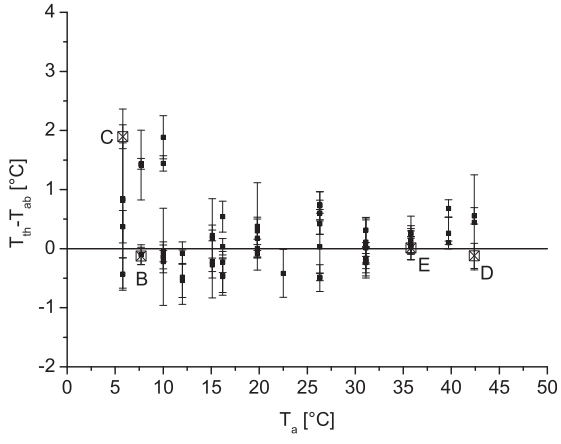

Fig. 3. Thoracic temperature excess over the abdomen (T_{th}–T_{ab}) of resting yellow-jackets (*Vespula* sp.) in the investigated temperature range. Marked data (crossed squares) match thermograms (B)–(E) in Fig. 2.

where V_{CO_2} is carbon dioxide production rate [µl g^{-1} min^{-1}] and T_a is the ambient temperature [°C] in the measurement chamber (R^2 = 0.96275, n = 846, 38 individuals; the range of validity is 7.7–42.4 °C). Parameters: A1 = 9.7023∗10^{-5}, $t1$ = 3.11195, A2 = 4.63097, $t2$ = 14.6382, A3 = 56769.01521, $t3$ = 3.81259∗10^{84}, y0 = −56770.80269. The mean Q_{10} was 2.27 (SD = 0.30, n = 23). However, with this function the Q_{10} was not constant. It decreased from 2.98 at a mean T_a of 13 °C (±5 °C) to 1.97 at a T_a of 23 °C and increased to 2.84 at a T_a of 35 °C. This function fitted the data better than a conventional exponential equation ($V_{CO_2} = a * b^{Ta}$; R^2 = 0.9404; a = 1.37152, b = 1.11652) particularly in the range of T_a = 20 to 35 °C. At high T_a above 35 °C (Fig. 4, dashed line) CO_2 production increased steeply until the wasp's upper respiratory critical thermal maximum (resp CT_{max}).

3.3. Survival rate

Individual wasps differed in their thermal tolerance. Our experiments were not conducted to determine the lethal temperature, nevertheless some wasps died due to continuous exposure to high experimental temperatures. Below 35 °C all wasps survived at least for 6 h (which was the minimal duration of an experiment). At higher temperatures some wasps died already at a T_a below the mean CT_{max}. At T_a = 35.8 °C one individual of six (17%) did not survive past 9 h, at T_a = 39.7 °C three of four wasps (75%) died within 9–12.5 h. At T_a = 42.4 °C all four individuals (100%) died within 1.7

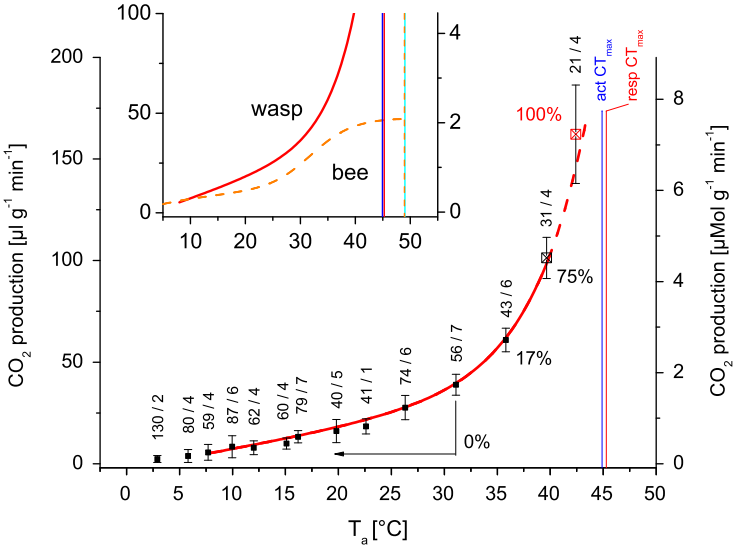

Fig. 4. Metabolic rate of resting wasps (*Vespula* sp.) in dependence on ambient temperature (T_a). Valid range of fit curve is 7.7–42.4 °C. Number of measurements/individuals above data points. Crossed box at T_a = 39.7 °C represents one individual of four measured in heat stupor, shortly after cyclic respiration stopped. Red crossed box at T_a = 42.4 °C: all individuals measured in heat stupor. Percentage quotes show the mortality at the respective T_a. The insert compares wasps' CO_2 production (continuous line) with that of honeybees (dotted line, from Kovac et al., 2007) with their CT_{max} values (wasps: voluntary activity CT_{max} = 44.9 °C, blue; respiratory CT_{max} = 45.3 °C, red; bees: activity CT_{max} = 49.0 °C, turquoise; respiratory CT_{max} = 48.9 °C, orange dotted, this study).

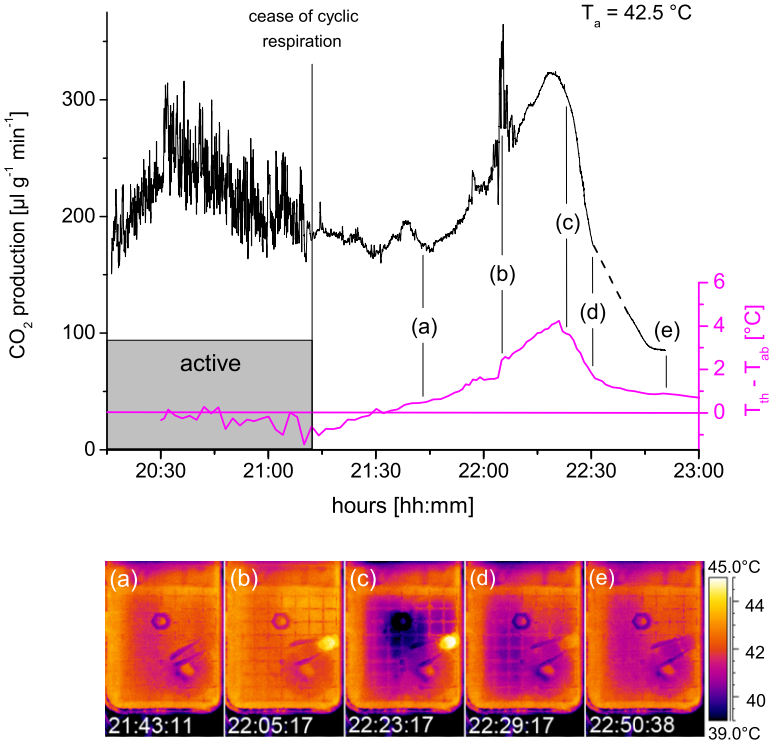

Fig. 5. CO_2 production curve and thorax temperature excess of an individual wasp (*Vespula* sp.) with experimental ambient temperature (T_a) kept constantly at 42.5 °C. Although the temperature was kept below that of the yellowjackets' CT_{max} the individual did not survive the experiment. Visible in the CO_2 recording are the cease of cyclic respiration and the postmortal plateau as well as the spike before it (see Fig. 6A). The dotted section of the curve represents estimated CO_2 values during an internal calibration. Thoracic temperature excess over the abdomen (T_{th}–T_{ab}) follows a pattern similar to that during a thermolimit experiment (Fig. 6B) with an ectothermic period followed by thoracic heating. Representative thermograms show the individual at certain stages during the experiment: (a) ectothermy, (b) after start of thoracic heating, (c) after maximum endothermy, (d) end of endothermy, (e) ectothermy. Markings in the CO_2 recording correspond with the respective thermograms. Weight of the wasp was 0.0958 g.

to 2.5 h. In Fig. 4 the percentage of mortality at the tested T_a is indicated. Fig. 5 displays the CO_2 production and the thoracic temperature excess ($T_{th} - T_{ab}$) of a wasp that did not survive the experiment. After cease of cyclic respiration the individual showed

a characteristic pattern of CO_2 release. This was accompanied by a distinct endothermic phase. The thermograms show that it was induced by thoracic heating activity.

3.4. Critical thermal maxima (CT_{max})

In these experiments solely *V. vulgaris* foragers were investigated. Fig. 6 shows a representative thermolimit experiment. With increasing temperature the wasps were more agitated, they ran around looking for an exit from the measurement chamber, gnawed into the chamber's fittings and showed self-grooming as well as cooling behavior. Coordinated bodily activity ceased with mortal fall (Fig. 6, stage 4). The averaged values of mortal fall provided the knockdown temperature (Klok et al., 2004; Stevens et al., 2010) or activity CT_{max} of 44.9 °C (Table 1). However, spasms as well as occasional abdominal movements (which might evade automated activity detection because of diminutive appearance) could be observed in the IR recordings of some individuals until the postmortal peak.

CO_2 production followed the stages of response to rising ambient temperature first described by Lighton and Turner (2004) (Fig. 6). The respiratory CT_{max} was determined via the inflection point of the *r*ADS residual values 10 min before and after the mortal fall. Averaged values were considered as the respiratory CT_{max} amounting to 45.3 °C. Activity CT_{max} and respiratory CT_{max} did not differ significantly ($P = 0.357507$, *t*-test, Table 1). For comparison, we determined both the activity and also the respiratory CT_{max} in honeybee foragers (*A. mellifera carnica*). Their activity CT_{max} of 49.0 °C was nearly identical with their respiratory CT_{max} of 48.9 °C ($P = 0.899966$, *t*-test, Table 1). The honeybees' activity CT_{max} was 4.1 °C and their respiratory CT_{max} was 3.6 °C higher than that of the wasps. Values differed significantly between both species ($P < 0.001$, *t*-test, see Table 1).

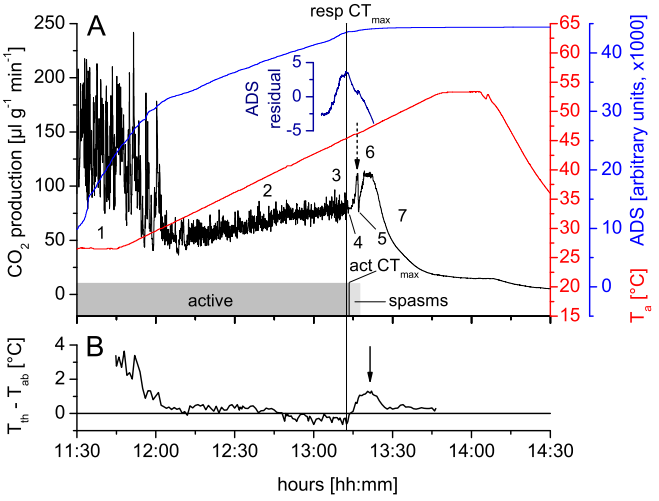

Fig. 6. (A) Representative thermolimit respirometry of a *V. vulgaris* individual. Visible in the CO_2 recording are the seven typical stages of response to increasing ambient temperature: 1 equilibration, 2 increase during ramping, 3 premortal plateau, 4 mortal fall (i.e. activity critical thermal maximum = act CT_{max}), 5 postmortal valley, 6 postmortal peak, 7 exponential decay (Lighton and Turner, 2004). Absolute difference sum (ADS) of CO_2 production is a measure of cumulative dynamic variability (Lighton and Turner, 2004). The reversal point of respiratory ADS residual (arbitrary units, ×100) helps to determine the minute point of respiratory critical thermal maximum (resp CT_{max}). Although bodily activity (dark grey) ceased with mortal fall, spasms (light grey) could be observed until the postmortal peak in some individuals. Characteristic CO_2 release pattern of *Vespula* before the postmortal valley is marked with a dotted arrow. Weight of the wasp was 0.142 g. (B) Thoracic temperature excess over the abdomen (T_{th}–T_{ab}) of the same wasp. While the individual was ectothermic from equilibrium to the premortal plateau, heating of the thorax occurred shortly after the mortal fall (arrow).

Vespula showed a characteristic CO_2 release pattern before the postmortal valley (Fig. 6A, dotted arrow) which could not be found in other hymenopteran CO_2 curves evaluated from thermolimit respirometry (e.g. *A. mellifera*, Käfer et al., 2011; *Pogonomyrmex rugosus*, Lighton and Turner, 2004). Fig. 6B also shows the typical thermal reaction (T_{th}–T_{ab}) of the same wasp, following failure of respiration at the respiratory CT_{max}. The postmortal peak of CO_2 release was accompanied by a heating bout in the thorax (compare also Fig. 5). The mean increase of the thoracic temperature excess over the abdomen at the peak of this bout was as high as 2.5 °C (SD = 0.7 °C, $n = 8$, maximum = 3.6 °C). The honeybees tested for their CT_{max} showed a similar heating bout with a mean increase of 1.9 °C (SD = 0.6 °C, $n = 8$, maximum = 2.5 °C).

4. Discussion

4.1. Resting metabolism and body temperature

Right after insertion into the measurement chamber the yellowjackets were active and highly endothermic. After some time they calmed down. Discontinuous gas exchange with periods of zero gas exchange and a distinct spiracle flutter phase (Fig. 1, insert; Hetz and Bradley, 2005; Lighton and Lovegrove, 1990) as well as a strongly decreased metabolic rate was an unmistakable sign of rest. Furthermore, IR-thermography video sequences gave us confirmation that the individual showed scarce or no movement and no active thermoregulation. Active thermoregulation, manifested in the thoracic temperature excess over the abdomen, was always accompanied by increased metabolic activity. Resting wasps were ectothermic on average (Fig. 3, thoracic temperature excess <0.6 °C). However, great individual variations could be observed at comparable experimental temperatures (Fig. 3, see means and standard deviations). Deviating values could have been based on several factors: There was a slight vertical temperature gradient inside the measurement chamber from the bottom (immersed into the water bath) to the lid (plastic cover outside the water for IR recording) if the water bath temperature deviated from ambient room temperature. If the individual positioned itself in this gradient, the abdomen was cooler or warmer than the thorax, causing slightly positive or negative values of the thorax temperature excess (Fig. 3). At higher temperatures ($T_a > 30$ °C), cooling behavior resulted in a slightly decreased head and thorax temperature. Cooling by regurgitation of fluid droplets is a common behavior at high temperature observed during similar experiments with honeybees (Kovac et al., 2007), or during experiments on *Vespula* thermoregulation (Coelho and Ross, 1996).

At low temperatures some individuals showed signs of weak endothermy (Fig. 3C). Some individuals alternated between ectothermy and weak endothermy. As the wasps were provided with sufficient fuel, they obviously went against cooling with this heating behavior at low T_a (10 °C to 5 °C). At present the importance of this behavior is unclear. A slightly activated flight musculature might keep them in a more activated state for possible reaction to their environment (e.g. escape).

In honeybee nests, the resting metabolism plays a significant role in generating heat for social thermoregulation (Kovac et al., 2007; Petz et al., 2004; Schmolz et al., 1995; Stabentheiner et al., 2010). During cold nights in wasp nests the temperature may drop significantly (Himmer, 1962; Klingner et al., 2006; Steiner, 1930), probably due to a lack of fuel (carbohydrate reserves) for continuous social thermoregulation. As temperatures in wasp nests are somewhat lower than in honeybee nests and vary in a broader range, one should surmise that *Vespula* needs to economize its resources. Foraging yellowjackets usually regulate thoracic temperatures somewhat lower (~0.5 to 3 °C) than honeybees if

measured at the same food source and under the same ambient conditions (Kovac and Stabentheiner, 1999, 2011; Kovac et al., 2009, 2010; Schmaranzer and Stabentheiner, 1988). According to the life-style hypothesis (Reinhold, 1999) we had expected that this would result also in a lower resting metabolism. However, it was a surprising result that *Vespula* stands out not only with a considerably higher resting metabolism compared to *A. mellifera* (Fig. 4, insert, wasp CO_2 production at 15 °C 41%, at 25 °C 63%, at 35 °C 57% higher than in bees, respectively) but also with a much steeper increase (higher mean Q_{10} value) with rising ambient temperature. The wasps' CO_2 production (Fig. 4) follows basically an exponential course. Slight deviations of single data points have been well documented in similar investigations on resting insects (Kovac et al., 2007; Lighton and Bartholomew, 1988; Lighton, 1989; Stabentheiner et al., 2003) and could be regarded as slight plateaus in an otherwise exponential increase. While the CO_2 curve of honeybee resting metabolism follows a sigmoidal progression with the inflection point at around 37 °C (Kovac et al., 2007), the wasps' curve is described best by an adapted exponential function (see Fig. 4) with an assumed sudden drop-off at the wasps' upper critical thermal maximum.

Honeybee foragers feed on a diet consisting predominantly of carbohydrates, which results in a respiratory quotient (RQ) of 1 (Rothe and Nachtigall, 1989). As the wasps were caught on an artificial feeding station provided with sucrose solution and were also supplied with carbohydrates during the experiment (1.5 M sucrose solution ad libitum), also a RQ = 1 could be assumed. So, as the wasp and bee RQ should show minimal – if any – differences under these experimental conditions, a direct comparison of their resting metabolism seems to be possible from the CO_2 recordings. A comparison of the resting metabolism of *Vespula* with that of honeybees (Kovac et al., 2007) and *Polistes* (Weiner et al., 2009, 2010) shows that the metabolism of *Vespula* is not optimized to save energy in the resting state. Their unexpected high basal metabolic rate and the steep incline with ambient temperature surely have consequences for their social thermoregulation. Similar as was reported in honeybees (Stabentheiner et al., 2010), nest temperature regulation in Vespine wasps (Himmer, 1962; Klingner et al., 2005, 2006; Steiner, 1930) can be assumed to be the result of behavioral measures, active (endothermic) heat production "on demand" and "passive effects". An important passive effect is the reinforcement of passive heat production (in the ectothermic state) of resting individuals due to social nest temperature homeostasis (Stabentheiner et al., 2010). Thermally inactive wasps are shifted to considerably higher (resting) energy turnover rates along their steep resting metabolic curve (Fig. 4). This passive effect in nest thermoregulation is considerably higher in wasps than in honeybees (see insert of Fig. 4; compare also Kovac et al., 2007). A wasp RQ below 1 would shift the curve of wasp metabolism in terms of O_2 consumption to even higher values, and this way increase the difference in energy turnover between bees and wasps. In phases of regulated nest temperature, therefore, a certain number of ectothermic wasps produce a higher amount of heat than the same number of ectothermic individuals in honeybee colonies at a certain ambient temperature. This has also the consequence that fewer wasps are needed for active (endothermic) heat production. Relatively few thermally active wasps may take away much burden from other individuals which can stay passive.

4.1.1. Metabolism at high temperatures (T_a > 35 °C)

At the upper range of experimental temperatures (from ~35 °C upwards) the wasps showed rest only sparsely. Both, number and duration of resting periods decreased with rising T_a and agitated movement predominated. Furthermore, many individuals showed cooling behavior, an indication that the individuals were not comfortable under these circumstances, and mainly wanted to escape the hostile environment. From 39.7 °C onwards only 37.5% (3 of 8 individuals) of the wasps could be measured in a true resting state (Fig. 4, crossed boxes), all other individuals were measured during "rest" in their "deleterious range" (Klok et al., 2004) or heat stupor (Fleurat-Lessard and Dupuis, 2010), right after cyclic respiration had ceased (see Fig. 6, after stage 4). Other individuals tested did not show rest at all at these high temperatures and therefore were not included in this study. As a consequence, one could reason that *Vespula* generally does not show resting behavior at ambient temperatures above $T_a \approx 40$ °C (Fig. 4, dashed line). In any case occasional rest (observed only for one or two minutes) at these temperatures is at a very high energetic level. With rising ambient temperatures, an increasing number of individuals did not survive the experiments (see Fig. 4, mortality in %) in spite of T_a being way under their CT_{max} (see Table 1). The time of exposure obviously plays a considerable role in the wasps' thermal tolerance when T_a reaches the upper edge of viability (compare e.g. Terblanche et al., 2011; Willmer et al., 2004). Activity CT_{max} ("knockdown temperature" as defined by Klok et al., 2004) and respiratory CT_{max} ("mortal fall", (Lighton and Turner, 2004)) of *V. vulgaris* were proved to be within narrow thermal margins (average 0.4 °C, Table 1). This has to be expected under normobaric conditions (Stevens et al., 2010). The use of the residual of the absolute difference sum of CO_2 production (*r*ADS residual, see Fig. 6) proved eligible in determining the end point of cyclic respiration and respiratory CT_{max}. Visual determination of the activity CT_{max} via IR recordings revealed additional information in comparison to other investigations, where automated activity detection was used (Lighton et al., 2004). In our observations coordinated motor activity ceased with activity CT_{max}, but spastic leg movements and slight bending and relaxing of the abdomen (which resembled slow motion respiration movements, but clearly were not) could be observed in almost all individuals until the post mortal valley and the post mortal peak, respectively (Fig. 6). These small-scale spasms might escape automated activity measurement, but were distinctly visible in our IR video sequences. We conclude from these observations that for determination of the CT_{max} video analyses are of great benefit if it is to judge activity in fine detail (Hazell et al., 2008; Hazell and Bale, 2011).

Our thermographic temperature measurements revealed that the final bouts of CO_2 release after the loss of respiratory control are caused by heating bouts (Figs. 5 and 6). The respiratory peaks, therefore, are the result of activation of the flight muscles (Fig. 5, thermograms). They are not caused by a general derailment of cellular metabolism, nor are they exclusively the consequence of a final diffusive loss of CO_2 due to spiracle opening. As heat produced by the thoracic muscles still reaches the head (Fig. 5, thermograms (b) and (c)), blood circulation (via heart and aorta) seems to be still active. Such final metabolic postmortal peaks (Lighton and Turner, 2004) were also observed in beetles (*Gonocephalum simplex*, Klok et al., 2004; *Tenebrio molitor*, Stevens et al., 2010) and even in ants (*Pogonomyrmex rugosus*, Lighton and Turner, 2004). In *Polistes dominulus* we also observed such thoracic heating bouts (our own unpublished results) though this species is known to be only weakly endothermic (Kovac et al., 2009; Weiner et al., 2009). It would be interesting whether the postmortal metabolic peaks in other species are also caused by (flight) muscle activation. The increase in CO_2 production as well as thoracic heating shortly after the wasps' CT_{max} (see arrows in Figs. 6 A and B) might result from a loss of nervous control of the flight musculature. To answer this question, however, electrophysiological recordings of the motoneurons and neuronal centers controlling flight would be needed.

Heat-induced mortality in hornets and bees has been determined so far strictly in the context of defensive behavior (heatballing of predating wasps by bees) in LD_{50} tests (Ono et al., 1995; Sugahara and Sakamoto, 2009; Tan et al., 2005). In Central

European wasps, which are also combated *via* heat by bees (Stabentheiner, 1996; Stabentheiner et al., 2007), such information was missing. The difference in wasp and honeybee respiratory and activity CT_{max} of 3.6 °C and 4.2 °C, respectively, might be large enough to enable honeybees to kill predating yellowjackets by heat-balling. Papachristoforou et al. (2007), however, reported that the increased CO_2 concentration inside such clusters decreases the CT_{max} of *V. orientalis* considerably. Sugahara and Sakamoto (2009) reported a similar effect in *V. mandarinia* attacked by *Apis cerana japonica*. Therefore we suggest that an increased CO_2 concentration inside heat clusters probably also makes *Vespula* more susceptible for high temperatures. As the terminal wasp body temperature inside a honeybee heat cluster can be below the wasps' CT_{max} (Stabentheiner et al., 2007) but nevertheless suffices to kill them, we suggest that the high CO_2 level inside such clusters lowers the CT_{max} also in *Vespula*, this way reducing the necessary exposure time (Stevens et al., 2010; Sugahara and Sakamoto, 2009).

Our findings suggest that ambient temperatures above the wasps' upper thermal limit may be critical for the survival and progress of foundress nests at an early time of colony development. Extended periods of high solar radiation may increase temperatures under roof tiles to 45.8 °C (our own unpublished observations). This is above the CT_{max} of adult wasps (44.9–45.3 °C). The CT_{max} of the brood, however, remains to be investigated to further support this suggestion. The cooling capacity of the queen alone or of small colonies by fanning and spreading of water (Kovac et al., 2009) may be too low to provide viable temperatures for wasps and brood over longer time spans. So we suggest foundress nests sometimes may be abandoned because of increased heat stress.

4.1.2. Metabolism at low temperatures (T_a < 15 °C)

At low temperatures (T_a < 15 °C) the wasps' CO_2 production rate approximates that of honeybees (Fig. 4, insert; Kovac et al., 2007). Bees show occasional thoracic heating during rest at low ambient temperatures down to T_a = 13 °C (Kovac et al., 2007). The same behavior could be observed in wasps. Some individuals showed a thorax temperature excess of up to 1.9 °C. In contrast to honeybees this occurred in the wasps mainly at $T_a \leqslant 10$ °C. The variation in these measurements leads to the conclusion that weak endothermy (as a measure to counteract cooling) alternates with ectothermy. However, while in honeybees controlled movement and regulated ventilation cease at body temperatures <10 °C as a consequence of chill coma (Esch, 1960, 1964; Free and Spencer-Booth, 1960; Kovac et al., 2007; Lighton and Lovegrove, 1990), the wasps' respiration functioned well down to 2.9 °C over longer periods (in one case tested for 24 h). Therefore, the wasps' respiratory critical thermal minimum (CT_{min}) can be assumed to be below T_a = 2.9 °C. As all wasps regained full motility after these experiments their lower lethal temperature must be below this value. The wasps' activity CT_{min} is not easily defined according to the assessment of Hazell and Bale (2011) or Stevens et al. (2010). As individuals sat motionless over long periods of time (several hours at 5.8 °C) one could guess that activity CT_{min} was already reached. However, we found the animals capable of coordinated movement down to 5.8 °C if the need arose, e.g. in case of loss of foothold at the measurement chamber's wall or lid. During such events every single movement of legs was accompanied by exceptional spikes in the CO_2 production curve at these low temperatures, which could be clearly distinguished from the common resting gas exchange pattern (our own unpublished results). Thus we assume the wasp forced activity CT_{min} to be below 5.8 °C (our lowest experimental temperature with IR video observation). In any case our investigations demonstrate an increased cold hardiness of *Vespula* sp. foragers in comparison to *A. mellifera*. MacMillan and Sinclair (2011) proposed that in insects chill coma and CT_{min} are not caused by failure of cell respiration or the circulatory system but by disruption of signal transmission leading to failure in the neuromuscular system. Hazell et al. (2008) and Hazell and Bale (2011) opine that voluntary and forced activity show an insects' CT_{min}. Respiration data seem not to be of so much significance for them regarding the lower thermal limit. Insect respiration, however, depends on active spiracle control and abdominal respiratory movements to achieve sufficient exchange of respiration gases via the tracheae. So respiration and muscular and neural activity are closely related. Like in CT_{max} determination, the combination of respiratory and behavioral data seems to provide the most accurate results in defining CT_{min}.

4.2. Resting metabolism in arthropods – a comparison over taxa

Our investigation showed that even closely related groups like honeybees and wasps may show significant differences of resting metabolism (Fig. 7, compare No. 7 *A. mellifera* (Kovac et al., 2007), No. 8 *Vespula* sp. (this study) and No. 9 *P. dominulus* (Weiner et al., 2009)). In a comparison over several taxa *Vespula* sp. stands out with a high resting metabolic rate over the entire temperature range (Fig. 7). At T_a = 20 °C the CO_2 production of *Vespula* sp. is 60% higher than that of *A. mellifera*, an insect with similar body shape, weight and active thermoregulation: 18.054 $\mu l\ mg^{-1}\ min^{-1}$ vs. 11.16 $\mu l\ mg^{-1}\ min^{-1}$. This might be based on differences in the thermal activity range as well as diverse overwintering strategies (single *Polistes*- and *Vespula*-queens vs. whole *Apis* colony). *Nowickia* sp. has a comparable body mass, but an even lower resting metabolism of only 2.304 $\mu l\ mg^{-1}\ min^{-1}$ (Chappell and Morgan, 1987). This is only 13% of *Vespula*'s turnover. Measurements at only one temperature (Fig. 8) or in the species' preferred temperature range do not always show differences between species clearly. Only respiratory data gathered over the animals' entire active temperature range allows profound comparison.

The metabolic theory of ecology links the metabolic rate to mass and ambient temperature. It predicts a general decrease of mass-specific metabolism with body mass for all organisms (see e.g. Clarke, 2006). In a comparison of several cockroach species Coelho and Moore (1989) reported that an increase in body mass comes along with a decrease in mass-specific resting metabolism. Sharing the same basic body shape, their weight ranged from 0.055 to 5.2 g (Table 3). Basal energy turnover diminished with increasing body mass also in locusts (Harrison et al., 2010) and in

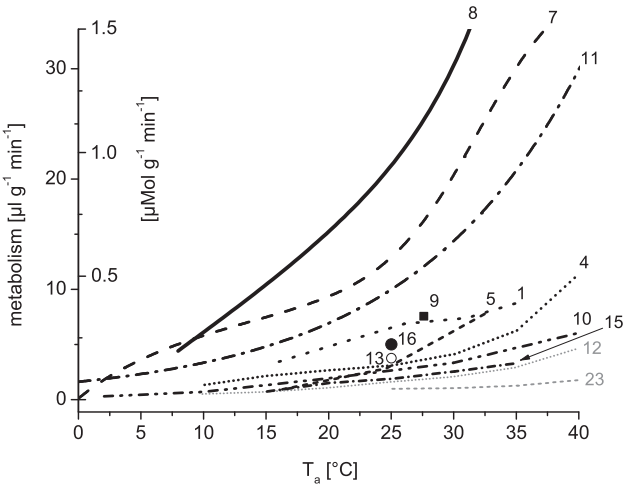

Fig. 7. Resting metabolism (CO_2 production or O_2 consumption) of several arthropods in dependence on ambient temperature (T_a). Numbers at the curves correspond with species in Table 3.

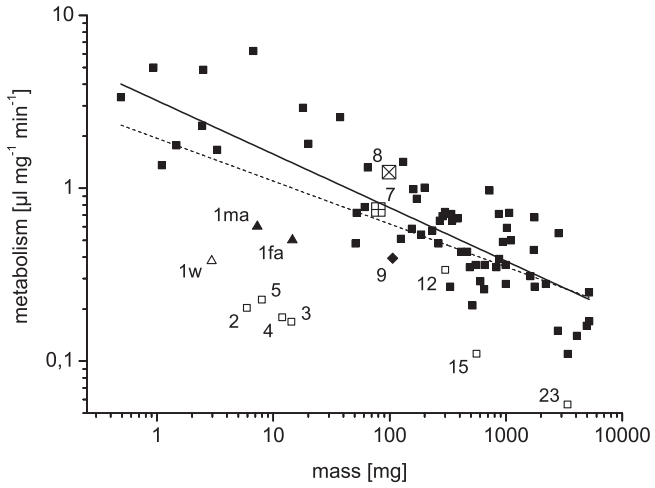

Fig. 8. Double-log plot of the allometric relationship between mass-specific resting metabolism and body mass in various arthropod species. Solid squares represent flying (Niven and Scharlemann, 2005), unfilled squares non-flying arthropods (Table 3, Fig. 7). *Vespula* sp. (8) and *A. mellifera* (7) data points are marked particularly. 1w = worker, 1ma = male alate, 1fa = female alate of *Solenopsis* ants. Ambient temperature was normalized to T_a = 22 °C (Q_{10} = 2); O_2 consumption or CO_2 production were normalized assuming a RQ of 1 (see Niven and Scharlemann, 2005). The value of honeybees was corrected according to Kovac et al. (2007). Resting metabolism scaled negatively with body mass in flying arthropods (solid line, least-squares regression: $\log_{10}$ metabolism [µl mg^{-1} min^{-1}] = 0.50701–0.30953∗$\log_{10}$ mass [mg]; R^2 = 0.66195) and to a lesser degree in flying and non-flying arthropods (dotted line, least-squares regression, $\log_{10}$ metabolism [µl mg^{-1} min^{-1}] = 0.28883–0.24769∗$\log_{10}$ mass [mg]; R^2 = 0.38742).

honeybee larvae (Petz et al., 2004). Niven and Scharlemann (2005) came to similar findings comparing resting metabolism of many flying insects. If also non-flying arthropod species are included, the decrease of mass specific resting metabolism with body mass is smaller (Fig. 8). Nonetheless there is an enormous variation in (resting) metabolism measurements of even closely related taxa of arthropods (compare Figs. 7 and 8). There are several hypotheses concerning this variation.

The evolutionary trade-off hypothesis tries to explain the relationship between resting metabolic rate and ambient temperature, and the cause of variation on all taxonomic levels (order, family, inter- as well as intra-species; e.g. Clarke, 2006; Riveros and Enquist, 2011).

The aerobic capacity hypothesis (developed for mammals by Hayes and Garland, 1995) states that the higher the maximal metabolic rates that can be achieved by animals the higher the resting metabolism. Transferring this hypothesis to insects with a similar energetic capacity than mammals, species with a highly energetic life-style (see Riveros and Enquist, 2011) like yellowjackets and honeybees should have a higher mass-specific resting metabolism than insects with a more settled way of life like *Eupsilia* sp. (Heinrich, 1987) and *P. dominulus* (Kovac et al., 2009; Weiner et al., 2009). Our findings support this hypothesis (see Figs. 7 and 8).

Another explanation for differences in resting metabolism is provided by the life-style hypothesis (Reinhold, 1999; Riveros and Enquist, 2011). If one compares the tachinid fly *Nowickia* sp. (Chappell and Morgan, 1987) and the winter flying cuculinid moth *Eupsilia* sp. (Heinrich, 1987; Heinrich and Mommsen, 1985) which weigh 0.130 g and 0.155 g, respectively, they differ highly in resting metabolism – and also in way of life (Table 2; Figs. 7 and 8, No. 10 *Nowickia* sp. and No. 11 *Eupsilia* sp.). The fly with the higher metabolism lives "on the wing" whereas the moth is rather inactive and sits still most of the day. However, Terblanche and Anderson (2010) showed that the resting metabolic rate in the hawkmoth *Macroglossum trochilus* and the long-proboscid fly

Table 2
Mean critical thermal maximum (CT_{max}) of *V. vulgaris* and *A. mellifera* with SD and number of individuals (*n*). While there was no significant difference in respiratory CT_{max} vs. activity CT_{max} (in both wasps and bees), CT_{max} between the species differed significantly.

	n	Respiratory CT_{max} (°C)	SD (°C)	*t*-test ← →	Activity CT_{max} (°C)	SD (°C)
V. vulgaris	10	45.3	0.5	t = 0.944327 p = 0.357507	44.9	1.3
A. mellifera	11	48.9	2.8	t = 0.127311 p = 0.899966	49.0	2.6
t-test		t = 4.05661 p = 0.00067			t = 4.5485 p = 0.00022	

Table 3
Body weight (mass) and resting metabolic rate (metabolism) in various taxa of arthropod species from literature and this study. Experimental temperatures were normalized to 22 °C assuming Q_{10} = 2.

No.	Species	Mass (mg)	Metabolism		Method	References
			(µl mg^{-1} h^{-1})	(µMol mg^{-1} min^{-1})		
1	*Solenopsis invicta*	2.96	0.38	0.017	O_2	Vogt and Appel (1999)
1	*S. invicta* male alates	14.6	0.5	0.0223		
1	*S. invicta* female alates	7.3	0.6	0.0268		
2	*Pogonomyrmex californicus*[*]	5.92	0.2	0.0089	CO_2	Quinlan and Lighton (1999)
3	*Pogonomyrmex occidentalis*[*]	14.3	0.17	0.0089		
4	*Camponotus fulvopilosus*	11.9	0.18	0.0076	O_2	Lighton (1989)
5	*Pogonomyrmex rugosus*[*]	7.96	0.23	0.0103	CO_2	Quinlan and Lighton (1999)
7	*Apis mellifera*	80.0	0.75	0.0335	CO_2	Kovac et al. (2007)
8	*Vespula* sp.	89.7	1.24	0.0554	CO_2	This study
9	*Polistes dominulus*	106	0.39	0.0174	CO_2	Weiner et al. (2009)
10	*Nowickia nitida/rostrata*	130.4	1.42	0.0634	O_2	Niven and Scharlemann (2005)
11	*Eupsilia* sp.	155	0.58	0.0259	O_2	Heinrich (1987)
12	*Centruroides sculpturatus*	300	0.34	0.0152	O_2	Hadley and Hill (1969)
13	*Blatta orientalis*	331	0.27	0.0121	O_2	Niven and Scharlemann (2005)
15	*Acheta domesticus*	558	0.11	0.0049	CO_2	Lachenicht et al. (2010)
16	*Periplaneta americana*	990.5	0.36	0.0161	O_2	Niven and Scharlemann (2005)
23	*Hadrurus arizonensis*	3380.23	0.06	0.0027	O_2	Hadley (1970)

[*] Animals decapitated.

Moegistorhynchus longirostrus differs despite a similar size and life-style (in this case foraging behavior).

As in fact resting metabolic rate within the arthropods seems to reflect the association between the variation of gas exchange physiology and anatomy (Riveros and Enquist, 2011), as well as the organisms' life-style in terms of energetic performance (flying – nonflying, Riveros and Enquist, 2011; active – inactive; acoustically advertising – mute) or feeding ecology (chaser – lurker, Reinhold, 1999; foraging rate, Terblanche and Anderson, 2010), energetic costs of activity may explain part of inter-taxon variation in mass-specific resting metabolism.

In conclusion it can be said that each of the above hypotheses may explain part of the variation between species. However, a quantitative prediction for a species based on measurement of another one cannot be made due to the complexity of physiology and ecology. Only empirical data are appropriate to gain insight in the metabolism of a particular arthropod species.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jinsphys.2012.01.015.

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