Respiration patterns of resting wasps (Vespula sp.)

Helmut Käfer, Helmut Kovac*, Anton Stabentheiner*

Institut für Zoologie, Karl-Franzens-Universität Graz, Universitätsplatz 2, A-8010 Graz, Austria

A R T I C L E I N F O

Article history:
Received 12 December 2012
Accepted 30 January 2013
Available online 9 February 2013

Keywords:
Wasp
Vespula
Respiration patterns
Ventilation movement
Resting metabolism
Temperature

A B S T R A C T

We investigated the respiration patterns of wasps (Vespula sp.) in their viable temperature range (2.9–42.4 °C) by measuring CO₂ production and locomotor and endothermic activity.

Wasps showed cycles of an interburst–burst type at low ambient temperatures (T_a < 5 °C) or typical discontinuous gas exchange patterns with closed, flutter and open phases. At high T_a of >31 °C, CO₂ emission became cyclic. With rising T_a, they enhanced CO₂-emission primarily by an exponential increase in respiration frequency, from 2.6 mHz at 4.7 °C to 74 mHz at 39.7 °C. In the same range of T_a, CO₂ release per cycle decreased from 38.9 to 26.4 µl g⁻¹ cycle⁻¹. A comparison of wasps with other insects showed that they are among the insects with a low respiratory frequency at a given resting metabolic rate (RMR), and a relatively flat increase of respiratory frequency with RMR.

CO₂ emission was always accompanied by abdominal respiration movements in all open phases and in 71.4% of the flutter phases, often accompanied by body movements. Results suggest that resting wasps gain their highly efficient gas exchange to a considerable extent via the length and type of respiration movements.

© 2013 Elsevier Ltd. Open access under CC BY-NC-ND license.

1. Introduction

Insects may vary stupendously in their modes of gas exchange (Gibbs and Johnson, 2004), both among (Hadley, 1994; Lighton, 1996; Sláma, 1999; Terblanche et al., 2008c) and within species (Chown et al., 2002; Ilrich et al., 2009; Kuusik et al., 2004; Marais and Chown, 2003), and even within the same individual (Chown, 2001; Kovac et al., 2007; Snelling et al., 2012). One particular respiration pattern in both flying and flightless insects is well known as discontinuous gas exchange cycle (DGC, for reviews see Chown et al. 2006b; Lighton, 1996; Sláma, 1988). Many insects show this pattern when at rest, at least at the lower to medium temperatures of their thermal range. Typical DGCs consist of a closed or constriction phase with spiracles shut and little to no external gas exchange (Bridges et al., 1980). O₂ inside the insect is metabolized, while CO₂ accumulates in the tracheae and in part is buffered in the hemolymph. This causes a drop in the total intratracheal pressure (Buck and Keister, 1955, 1958; Buck and Friedman, 1958; Hetz et al., 1994). In the following flutter phase single spiracles open and close rapidly. Gas exchange works here due to convection and diffusion. Small amounts of O₂ are inhaled to sustain a certain low level of PO₂ for a minimum O₂ delivery to the insect’s metabolizing tissues (Hetz and Bradley, 2005; Lighton, 1996). The CO₂ level keeps rising in the hemolymph during the flutter phase, as only small amounts of CO₂ are exhaled (Buck, 1958). As accumulated CO₂ reaches a trigger threshold, a massive amount exits from the tracheal system to the environment in the open-spiracle phase (Lighton, 1996; Schneiderman and Williams, 1955). CO₂ is assumed to act directly at the spiracular muscles, with little central nervous control (Hoyle, 1961); however, Bustami and Hustert (2006), Bustami et al. (2002) and Woodman et al. (2008) found contrary evidence.

Discontinuous gas exchange was hypothesized to be an adaptation aimed at minimizing water loss from the tracheae (hygric model, Chown, 2002, 2006a; Dingha et al., 2005; Duncan et al., 2002; Hadley, 1994; Kivimägi et al., 2011; Williams and Bradley, 1998; Williams et al., 1998, 2010), though findings by Contreras and Bradley (2009), Gibbs and Johnson (2004) and Sláma et al. (2007) call into question the universal validity of this model. Other explanations suggest that it developed to allow sufficient gas exchange in subterranean, CO₂ rich environments (chthonic model, Lighton and Berrigan, 1995). A combination of these two models is the hygric-chthonic hypothesis (Lighton, 1998). An alternative explanation suggests that it minimizes oxygen toxicity (Bradley, 2000; Hetz and Bradley, 2005). The variation of respiration patterns has been well investigated in different species (Basson and Terblanche, 2011; Chown et al., 2006a; Groenewald et al., 2012; Klok and Chown, 2005; Kovac et al., 2007; Nespolo et al., 2007; Terblanche et al., 2008a; Williams et al., 2010). Such an analysis is lacking in vespine wasps. This is especially interesting because Vespula sp. show an overall higher level and a steeper incline in resting metabolism with increasing ambient temperature (high Q₁₀) than many other insects (see Käfer et al., 2012). In this paper, therefore, we investigated the characteristics of the respiration pattern of resting wasps (Vespula sp.) in their viable temperature range (2.9–42.4 °C) by measuring CO₂ production and locomotor and endothermic activity.
patterns of vespine wasps, *Vespa* sp., over their entire viable temperature range. We compare the specific features of their gas exchange patterns with other flying and nonflying insects.

Respiration of adult insects is accomplished by a combination of passive diffusive gas exchange and active convective ventilation (Jógár et al., 2011; Lighton, 1996; Terblanche et al., 2008b). Ventilatory movements are usually observed via automated optical activity detection. While this technique allows for an easy, semi-quantitative assessment of general activity (Lighton, 2008) it cannot give information about the nature of an activity event. It cannot distinguish between abdominal pumping and movement of other body parts. For a general perspective on the mechanisms of respiration, therefore, we investigated the connection between gas exchange and respiratory movements in detail by infrared video observation.

2. Material and methods

2.1. Animals

A total of 37 yellow jacket foragers (24 *Vespa vulgaris* (Linnaeus 1758) and 13 *Vespa germanica* (Fabricius 1793)) were baieted with sucrose solution at an artificial feeding place and caught for immediate analysis (29 individuals) or stored in cages overnight in a dark and cool area (8 wasps, 12–15 °C, sucrose solution provided) for use at low temperatures on the next day. As we needed undisturbed, undamaged individuals for our experiments, species determination had to be accomplished after the experiments by assessment of head and thorax color markings, following the main characteristics in identification literature (temple, clypeus and pronotal markings; see Bellmann, 1995; Brohmer, 1977; Claperton et al., 1989; Witt, 1998). As color markings are highly variable (Claperton et al., 1989), this proved to be rather difficult in some cases. For example, we had 4 *V. germanica* individuals which could be easily taken for *V. vulgaris* because of their thoracic pronotal markings.

The experiments took place overnight to ensure that the wasps were at rest for long enough periods (especially at high *T*<sub>a</sub>). The animals would no longer have shown their natural resting behavior and could have been physically damaged (especially at higher *T*<sub>a</sub>s) had we extended the experimental periods even further. After insertion into the chamber, it took usually at least 90 min before the insects had calmed down enough in the measurement chamber to allow analysis of resting respiratory patterns. Individuals had time to accustom to a new experimental ambient temperature (*T*<sub>r</sub>) in the respiratory measurement chamber for a minimum of 15 min at the lowest temperatures (<10 °C). At medium to high temperatures we waited at least 30 min before an evaluation was started. Temperatures were set from 2.5 to 45 °C in steps of 2.5 or 5 °C. After every change of *T*<sub>a</sub> (ramp), however, it took time for an individual to stabilize in metabolism and behavior. So we had to optimize the measurement regime in the course of the experiments through reduction of tested *T*<sub>a</sub> s per individual. The majority of individuals (23 of 37) were tested at one *T*<sub>a</sub>, six at two *T*<sub>a</sub>s, five at three *T*<sub>a</sub>s, two at four *T*<sub>a</sub>s, and one individual at five *T*<sub>a</sub>s. Each *T*<sub>a</sub> lasted for 3.5 h minimum.

As respiration data did not differ significantly between *V. vulgaris* and *V. germanica* (*P* > 0.5, ANOVA; see Section 3.2 and Table S1; for metabolism data see (Käfer et al., 2012)), respiration data were pooled and animals were referred to as *Vespa* sp. in this paper (body mass = 0.1019 ± 0.0179 g, *N* = 37).

2.2. CO<sub>2</sub> measurement

Carbon dioxide production of the yellowjackets was measured in a flow through respirometry setup as described in Käfer et al. (2012), Kovac et al. (2007), Petz et al. (2004) and Stabentheiner et al. (2012). The test chamber dimensions (volume = 18 ml) allowed unhindered movement of the wasps during the experiment. As the wasps stayed in the chamber over long time spans (>6 h, typically overnight) they were also provided with 1.5 M sucrose solution ad libitum as a food source. Experimental temperature was set by an automatically controlled water bath (Julabo F33 HT, Julabo Labortechnik GmbH, Seelbach, Germany; temperature regulation to 0.1 °C). As the temperature inside the test chamber deviated slightly from that of the water bath we measured the actual experimental temperature (*T*<sub>e</sub>) with a thermocouple inside the chamber, close (<10 mm) to the wasp.

Outside air was led through the reference channel of a differential infrared gas analyser (Advance Optima URAS 14, ABB Analytical, Frankfurt, Germany) sensitized to carbon dioxide, the measurement chamber and subsequently through the measurement channel. Gas flow was set at 150 ml min<sup>-1</sup> by a mass flow controller (Brooks 5850S; 0–1000 ml/min; Brooks Instrument, Hatfield, USA). This flow allowed for an accurate temporal resolution as well as for a good CO<sub>2</sub> signal in terms of signal to noise peak ratio (Gray and Bradley, 2006; Stabentheiner et al., 2012). Carbon dioxide production of the tested wasps was recorded at intervals of 1 s. The measurement gas (i.e. air) was dried via Peltier element equipped cool traps prior to the reference and measurement channel. Relative humidity in the test chamber was regulated by a set of humidifying bottles filled with distilled water, immersed in another Julabo water bath adjusted to the desired dew point temperature to keep the relative humidity in the measurement chamber at the desired level (50% at 45–15 °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). Formulas for dew point calculation are given in Stabentheiner et al. (2012).

The empty test chamber was recorded for 5 min before and after each experiment to determine any initial CO<sub>2</sub> signal offset from zero as well as a possible signal drift from the start to the end of the experiment. The long duration of each experiment required regular (3 h intervals) automatic zero- and end point calibration of the URAS gas analyser, utilizing internal calibration gas cuvettes containing a defined concentration of carbon dioxide.

The tube length between measurement chamber and measurement channel of the DIRGA resulted in a signal delay that was corrected for synchronization of the CO<sub>2</sub> trace recordings with infrared video sequences.

Data analysis and statistics were conducted using custom made peak and valley finding formulas in Excel (Microsoft Corporation, Redmond, USA), OriginPro 8.5 (OriginLab Corporation, Northampton, USA) and Statgraphics Centurion XVI (StatPoint Technology Inc., Warrenton, USA). The amount (µl min<sup>-1</sup>, ppm) of CO<sub>2</sub> production refers to standard (STPS) conditions (0 °C, 101.32 kPa = 760 Torr). All gas exchange referred to as respiration in the following chapters is strictly speaking CO<sub>2</sub> emission, as O<sub>2</sub> uptake was not measured in this setup.

2.3. Behaviour and activity observation

To evaluate the wasps’ behavior and to determine the periods when the tested individuals were at rest we applied state of the art infrared thermography techniques that particularly enabled us to distinguish between rest and activity without disturbing the wasps in their natural behavior (Käfer et al., 2012; Kovac et al., 2007; Stabentheiner et al., 2012).

The top of the measurement chamber was transparent to infrared (IR) radiation (covered with plastic film permeable 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 (ThermoCam SC2000 NTS, FLIR Systems Inc., Wilsonville, USA; for details see Kovac et al., 2007; Schmaranzer and Stabentheiner, 1988; Stabentheiner and Schmaranzer, 1987; Stabentheiner et al.,
Not only visual clues (e.g., body movements), but also the thermal state of the individual (ectothermic or endothermic) could be evaluated. This thermal state was determined by the difference in thoracic and abdominal surface temperature \((T_{th} - T_{ab})\). An individual was assessed as resting when it was ectothermic \((T_{th} \approx T_{ab})\) and showed no or only scarce body movements for a minimum timespan of 10 min (see classification according to Crailsheim et al., 1999; Stabentheiner and Crailsheim, 1999; Stabentheiner et al., 2003); single flips of legs or antennae were allowed (compare Kaiser, 1988). At higher \(T_a\) (>27.6 °C) the duration was reduced to 5 min if no 10 min sections were available. In the course of evaluation we had to redefine “rest” in such a way that individuals not moving for a longer period of time were allowed to show slow endothermy \((T_{th} - T_{ab} < 2 °C, \text{ usually} < 1 °C)\) over a few periods in the experiment (see Käfer et al., 2012; Kovac et al., 2007).

IR sequences were recorded on hard disk at 3, 5 or 10 Hz. Analysis of the yellow jackets body surface temperatures was conducted with AGEMA Research software (FLIR Systems Inc., Wilsonville, USA) controlled by a proprietary Excel (Microsoft Corporation, Redmond, USA) VBA macro.

### 2.4. Respiration frequency and abdominal ventilation movements

A respiration cycle was determined from one minimum in CO₂ emission just before the open phase to the next one. For discontinuous gas exchange cycles (DGCs) this included a closed and a flutter phase. In cyclic respiration at higher temperatures the same scheme applied. From minimum emission to minimum emission, every CO₂ peak was assumed to be a respiration cycle. Abdominal ventilation movement (pumping, etc.) was assessed from IR video sequences recorded at a frequency of 10 Hz. A minimum of 10 respiration cycles were assessed in the evaluation of respiration movements, resulting in time spans of 13 min at the highest \(T_a\) (36.3 °C) and 287 min at the lowest \(T_a\) (5.9 °C) tested. The abdomen had to be well distinguishable from the background over this period of time.

Respiratory ventilation within one cycle consisted of one or several successions of single abdominal pumping movements. These successions were counted as single ventilatory events. The durations of these ventilatory events were determined, and related to the whole cycle as well as the cycle phases (open, closed or flutter).

### 3. Results

As we tested two species of vespine wasps, *Vespula vulgaris* and *V. germanica*, we had to analyze our data regarding the possibility of inter-species differences in respiration parameters. ANOVA revealed no influence of the tested wasp species on respiration cycle duration \((P = 0.5449, F\text{-quotient} = 0.39, DF = 1)\) and CO₂ release per cycle \((P = 0.9239, F\text{-quotient} = 0.01, DF = 1); \text{ see Supplementary material, Table S1; data for the two species in Table S2). Therefore, species was not considered for the further analysis.

#### 3.1. Respiration (CO₂ release) pattern

Over the entire temperature range, spiracle control was functioning well for *Vespula* sp. At the lowest experimental temperatures \((T_a = 2.9 °C)\) yellow jackets showed discontinuous gas exchange resembling an “interburst–burst” pattern similarly to that described by Marais and Chown (2003) for *Perisphaeria* sp. cockroaches. Interburst phases with a minimum of 0.6 and a maximum of 81.73 min duration (mean: 11.66 ± 12.05 min) were followed by 0.42–14.57 min long burst phases (mean: 6.19 ± 4.91 min) consisting of 1–5 initial higher peaks and several subsequent lower ones (see Fig. 1A). A flutter phase could not be observed at this \(T_a\). Sporadic single CO₂ spikes with similar peak height and duration as the initial peaks of the burst phases were counted as separate open phases. They caused the rather high SD in duration of closed as well as open phases (Fig. 3).

With increasing \(T_a\) DGC appeared in a more common fashion with a closed phase followed by a distinct flutter phase and the main peak or open phase (Fig. 2A). The open phase oscillations of the CO₂ signal merged (but remained detectable), and flutter became visible (Fig. 1B and C). At temperatures of 15–25 °C *Vespula* sp. showed typical DGC patterns (Hetz and Bradley, 2005; Lighton, 1996) with closed, flutter and open phase (Fig. 2B). Exceptional body movements, e.g. when the wasp lost and regained hold with a leg or flipped the wings (Fig. 1B–D, arrows) were clearly distinguishable from the “normal” respiration pattern. At \(T_a = 26.2 °C\) the CO₂ level inside the measurement chamber did not always reach zero between two respiration cycles. However, CO₂ emission before the open phase resembled a flutter pattern consisting of merging single peaks. In certain individuals, residues of this particular pattern could be observed in some cycles as slight increases in the CO₂ signal prior to the main respiratory peak even at \(T_a = 31.4\) and 36.4 °C (Fig. 2B, D; see large triangles in Fig. 3). At \(T_a > 3.4 °C\) all individuals showed cyclic respiration (Fig. 2C). At the highest experimental temperatures \((T_a = 39.7\) and 42.4 °C), resting periods were scarce in yellow jackets. CO₂ emission was always cyclic, sometimes on the verge of continuous respiration (Fig. 2D).

Fig. 3 shows the duration of cycles, and of open, closed and flutter phases (where present) as a function of experimental ambient temperature. The course of all components of DGC follows exponential curves. With rising ambient temperature the open phase decreased slower in duration than the flutter and the closed phases at low to medium \(T_a\). Closed phases were only detectable up to \(T_a = \leq 26.3 °C\). Fig. 4 shows the duration of the respiration cycles and cycle phases in dependence on resting metabolic rate (RMR). However, the courses of data points indicate a higher order of dependence than a simple exponential decrease. Good linear regression in a double logarithmic graph (inset) strengthens this finding.

#### 3.2. Respiration frequency and CO₂ release per cycle

With rising \(T_a\) the cycle frequency \(f\) increased (Fig. 1, Fig. 2) following an exponential curve (Fig. 5). Data fitted best with an exponential function of the type \(f = y_0 + A e^{A_{1}T_{0}}\), with \(y_0 = 0.12716, A_1 = 2.18932, r_1 = 11.2997 (R^2 = 0.51337, P = 0.0001, N = 37)\). Respiration cycle frequency was 2.55 ± 3.58 mHz at 4.7 °C, 9.33 ± 13.2 mHz at 9.8 °C, 13.0 ± 24.66 mHz at 19.8 °C, 39.92 ± 25.35 mHz at 31.1 °C and 73.97 ± 28.85 mHz at 39.7 °C. Data at 42.4 °C were not included in the fitting curve because single CO₂ “peaks” merged to “plateaus”. Comparison of variances of cycle frequency at the same \(T_a\) revealed significant differences between individuals \((P < 0.05, N = 2–10, \text{ ANOVA})\). Over the entire temperature range these tests indicated significant differences in 69.5% of comparisons.

An ANOVA with the means per animal and \(T_a\) (of both species) indicated a slight negative temperature dependence of CO₂ release per cycle \((P < 0.05; R^2 = 0.06685, N = 62, F = 5.36977, DF = 60)\). The correlation was more pronounced in an analysis with all cycles of all animals, which includes the intra-individual variation (Fig. 6). CO₂ release per cycle as estimated from the regression line changed from 39.51 µg g⁻¹ cycle⁻¹ at 2.9 °C to 25.4 µg g⁻¹ cycle⁻¹ at 42.4 °C.

Single individuals compared at the same temperature showed significant differences in the variances of mean CO₂ emission per cycle and animal \((P < 0.05, N = 2–8, \text{ ANOVA); see large circles in Fig. 6). Over the entire temperature range these within-\(T_a\) comparisons showed inter-individual differences in 56.8% of cases. This implies that the other 43.2% of cases indicated no difference.
However, measurements where data of only one individual could be evaluated indicate also considerable intra-individual variance (Fig. 6, T_a = 22.5 and 42.4°C). In direct comparison, wasps differed from honeybees significantly in slope and intercept (P < 0.0001 in both cases, ANOVA; see Fig. 6).

Cycle frequency (f) increased linearly with the mass specific RMR (Fig. 7, f (mHz) = -2.54647 + 0.65394 RMR CO₂ (μl g⁻¹ min⁻¹), R² = 0.976, P < 0.0001, N = 37, means per animal). A comparison with other (flying and non-flying) insect species revealed yellow jackets to be among the insects with the lowest respiratory frequency at a given RMR though Vespula has a rather high mass-specific RMR (Käfer et al., 2012). This comparison also showed that this relation differs largely between different insect species (Fig. 7). However, in spite of the high variation in RMR levels as well as in slopes of the single species data, a tendency is obvious in insects to increase respiration frequency with an increase in emission of CO₂.

### 3.3. Respiration movements

CO₂ emission of wasps at rest was accompanied by convective abdominal respiration movements (pumping, etc.) in all observed cases (100%) where CO₂ emission took place, during discontinuous as well as during cyclic respiration. Respiratory ventilation consisted of a succession of single abdominal pumping movements (see Supplementary material, IR video S3). Such a succession was counted as one single ventilatory event. However, typical abdominal ventilation movements were often accompanied by leg or antenna movement, flipping of the wings (see Supplementary material, IR video S4) as well as sideward jerking of the abdomen, leading to spasm-like twisting of the whole wasp body (24.2% over the tested temperature range; for details see Table 2, Fig. 8). Additional body movements, therefore, contributed to a considerable amount to respiration movements. During a DGC, some kind of respiration movement could be observed in all open phases and also in 71.4% of the flutter phases (66.7% if the distinct increase in the CO₂ signal before an open phase at T_a > 26.3°C was not counted as a flutter phase). Ventilation movements during flutter were in the majority of cases single or few abdominal movements with small amplitude often accompanied or masked by body movement. They differed visibly from the wasps’ pumping in open phases. Fig. 8A shows the percentage distribution of abdominal respiration movements (resp), abdominal respiration movements accompanied by leg and antenna movements (resp&mov), and body movements possibly masking respiration movements (mov) in closed, flutter and open phases. All types of movement occurred in all phases of respiration, though at some T_s some types were missing. Abdominal respiration movements (pumping) were in all tested individuals accompanied by other body movements in at least one phase of a respiration cycle. Whole-body movements possibly masking the abdominal ventilation movements (mov; see Table 2 and Supplementary material, IR video S5) were rather rare. They occurred in 9.7% of the cycles (over the tested temperature range), in closed as well as in flutter and open phases. Fig. 8B shows the relative amount of ventilation movements (resp, resp&mov, mov) in the closed, flutter and open phases of respiration cycles. In the open phase of the gas exchange cycle clearly definable respiration movements (resp and resp&mov) were observed at all T_s. In the flutter and closed phases, however, this did not always occur. Including those body movements that might have masked abdominal pumping (mov) did not change this result considerably.

Abdominal movements did also occur in closed phases (see also Groenewald et al., 2012; Hetz et al., 1994; Jõgar et al., 2011). The movements resembled abdominal respiration movements as observed in flutter phases (without additional leg or body

![Fig. 1. Discontinuous gas exchange (DGC) of resting wasps at ambient temperatures (T_a) < 20°C. CO₂ release changes characteristically in pattern as well as in frequency with rising T_a (A–D). Arrows mark CO₂ release exceptional in peak height (B) or pattern (C and D), caused by bodily activity (e.g. in (B) the insect lost and regained grip on the film covering the experimental chamber for two times). Dotted lines indicate mean CO₂ emission over timespan. Insets show details in the CO₂ registration.](attachment:image)
movement), but were not accompanied by CO2 emission. With increasing $T_a$ the total duration of abdominal ventilation moves decreased exponentially (Fig. 9), which coincided with the increase in cycle frequency reported in Section 3.2 (Fig. 5).

Fig. 2. Representative CO2 release patterns of resting wasps at ambient temperatures ($T_a$) >20 °C. (A) Typical DGC pattern with closed (C), flutter (F) and open (O) phases. (B) DGC on the verge of cyclic respiration. No closed phases (i.e. CO2 trace reaches zero), and flutter phases merge with open phases. (C) and (D) Cyclic respiration. Dotted lines indicate mean CO2 emission over timespan.

Fig. 3. Duration of cycles, flutter, open, and closed phases (where existent; mean values with SD). Open phase values are 100% in every cycle (i.e. no cycle without CO2 emission). Flutter data points at $T_a > 30$ °C (large triangles) represent respiration patterns resembling flutter phases without preceding closed phases (see Section 4).

Fig. 4. Duration of cycles and flutter, open, and closed phases (where existent; mean values with SD) as a function of resting metabolic rate (RMR). Large triangles represent the same respiration patterns as described in Fig. 3. Inset shows data with logarithmic scaling on both axes. Regression lines follow $\log_{10} \text{RMR} \cdot \text{VCO2} \left(\mu l \ g^{-1} \ min^{-1}\right) = a + b \cdot \log_{10} \text{Duration (s)}$. Cycle: $a = -1.221$, $b = 3.6982$, $R^2 = 0.93908$; Open phase: $a = -0.73177$, $b = 2.68333$, $R^2 = 0.95925$; Closed phase: $a = 1.1129$, $b = 3.19209$, $R^2 = 0.64334$; Flutter phase: $a = 1.09373$, $b = 3.47489$, $R^2 = 0.87907$.
**4. Discussion**

4.1. Respiration patterns

At rest, many insect species show a particular respiration pattern of discontinuous gas exchange cycles (DGC; for review see Chown et al., 2006a; Lighton, 1996; Sláma, 1988). The illustration of respiration patterns depends on flow rate, measurement chamber size (i.e. volume) and metabolic rate of the animal (Gray and Bradley, 2006; Lighton, 2008; Terblanche and Chown, 2010). A large measurement chamber dilutes the animal’s CO₂ trace, leading to a smoothed away signal at the CO₂ detector. Last but not least, the metabolic turnover of the tested animal is a crucial parameter (Gray and Bradley, 2003; Moerbitz and Hetz, 2010). In resting yellow jackets the CO₂ emission varied in a wide range, from 5.6 l lg⁻¹ min⁻¹ at 7.7 °C to 101.3 l lg⁻¹ min⁻¹ at 40 °C (Käfer et al., 2012). With a measurement chamber size of 18 ml –as small as possible, but without impairing the animal’s natural movement – and a flow rate set to 150 ml min⁻¹ the respiration patterns of Vespula sp. could be displayed throughout their entire viable temperature range.

Typical DGCs consist of a closed phase with shut spiracles and no external gas exchange (Bridges et al., 1980) followed by a flutter phase with the spiracles opened in close succession, and the open spiracle phase (Hetz and Bradley, 2005; Lighton, 1996). At the lowest experimental temperatures (Tₐ = 2.9 °C), DGC resembled an interburst–burst pattern similar to that described by Marais and Chown (2003) for Periphausia sp. cockroaches and Duncan and Dickman (2001) for Cerotaílus sp. beetles. In Vespula sp. long interburst (closed) phases alternated with long open burst phases consisting of single peaks which sometimes tended to merge at the end of the open phase (Fig. 1A), resembling to some degree “reversed” flutter phases. This seems to suffice in exchanging CO₂ and O₂ at this overall low level of metabolic rate (Fig. 1A; Hetz, 2007; Käfer et al., 2012; Moerbitz and Hetz, 2010). Nevertheless, spiracle control functioned well at this lowest experimental ambient temperature. Honeybees, in comparison, fall into chill coma at
and, losing control over their spiracles, emit CO\(_2\) continuously (Kovac et al., 2007; Lighton and Lovegrove, 1990; compare Free and Spencer-Booth, 1960).

With rising \(T_a\), wasp DGC had closed phases and distinct flutter phases as found in many other resting insects (e.g. Chown and Davis, 2003; Hadley, 1994; Hetz and Bradley, 2005; Lighton, 1996; Lighton and Lovegrove, 1990; Sláma, 1999; Vogt and Appel, 1999, 2000). Open phases consisted of consecutive merging and amplitude diminishing peaks at \(T_a\)s of about 6–16 °C (Figs. 1B and 2A). The typical DGC pattern with closed, flutter and open phase appeared more and more distinctly (Fig. 2B).

With rising \(T_a\), the DGC patterns changed in a way that the closed and flutter phases diminished in duration and then successively vanished entirely (Fig 3). This result was in accordance to the findings of Contreras and Bradley (2010) in *Rhodnius prolixus* and *Gromphadorhina portentosa*, which showed that metabolic rate affects spiracle activity, which may be an explanation for the different patterns of gas exchange in one species at different temperatures. At \(T_a = 31.1^\circ\)C no closed phases were detectable; see Fig. 3;
Table 1
Mass specific resting metabolic rate (RMR), respiration frequency (f) and cycle duration (s) data from this study and literature data. Tær = experimental ambient temperature; N ind. = number of individuals; n = number of respiration cycles (where available).

| No. | Species                  | Tær (ºC) | N ind. | n  | f  | SD | Duration (s) | SD | RMR (CO2) (µl g⁻¹ min⁻¹) | SD | References                          |
|-----|--------------------------|----------|--------|----|----|----|--------------|----|--------------------------|----|-------------------------------------|
| 1   | Vespa sp.                | 2.9      | 2      | 130| 0.99| 0.99| 1062.22      | 826.24| 2.31                | 1.71| This study and Kfäfer et al. (2012) |
| 2   | Blatella germanica       | 10.0     | 19     |    |    |    |              |    |                       |    | Dingha et al. (2005)             |
| 2a  | Blatella germanica       | 10.0     | 12     |    |    |    |              |    |                       |    | Duncan and Dickman (2001)        |
| 3   | Culiseta inornata        | 100.0    | 4      |    |    |    |              |    |                       |    | Gray and Bradley (2006)          |
| 4   | Zophobis complanata      | 25.0     | 9      | 4–90| 1.50| 0.41| 676.20       | 207.60| 2.87                | 0.82| Duncan and Byrne (2002)          |
| 5   | Zophobis punctata        | 25.0     | 10     | 4–90| 1.86| 0.77| 621.00       | 211.60| 3.25                | 1.27| Duncan and Byrne (2002)          |
| 6   | Pimelia canescens        | 25.0     | 2      | 4–90| 1.20|    * |              |    |                       |    | Duncan and Byrne (2002)          |
| 7   | Pimelia grandis          | 25.0     | 6      | 5–17| 1.24| 0.29| 14.10        | 3.23  | 1.62                | 0.15| Duncan and Byrne (2002)          |
| 8   | Onymacris multiistriata  | 23.0     | 8      | 20–55| 1.79| 0.46| 587.40       | 126.00| 4.25                | 0.96| Duncan (2003)                 |
| 9   | Pachylomerus femoralis   | 25.0     | 5      |    | 0.73| 0.30| 1384.62      |    *  | 1.64                | 0.22| Duncan and Byrne (2005)          |
| 10  | Scarabeus gariepinus     | 25.0     | 7      |    | 0.33| 0.20| 300.00       |    *  | 1.34                | 0.66| Duncan and Byrne (2005)          |
| 11  | Scarabeus striatulatus   | 25.0     | 6      |    | 0.42| 0.20| 2400.00      |    *  | 1.34                | 0.42| Duncan and Byrne (2002)          |
| 12  | Cerella lucicola         | 23.5     | 6      | 63  | 0.26| 0.05| 3829.79      | 0.86  | 0.30                |     | Duncan and Dickman (2001)        |
| 13  | Cerothria sp.            | 20.0     | 7      | 2to10| 0.63| 0.42| 2103.60      | 1084.20| 1.30                | 0.83| Duncan and Dickman (2001)        |
| 14  | Caracemus sp.            | 25.0     | 11     | 2to12| 1.54| 0.49| 712.20       | 246.00| 2.01                | 0.75| Duncan and Dickman (2001)        |
| 15  | Syniphus fasciatus       | 25.0     | 3      | 2to10| 2.11| 0.84| 537.00       | 211.20| 2.10                | 0.87| Duncan and Dickman (2001)        |
| 16  | Scarabeoidea subfasciata | 35.0     | 5      | 2to10| 2.72| 0.63| 381.60       | 207.20| 2.48                | 0.55| Duncan and Dickman (2001)        |
| 17  | Anachalos convexus       | 40.0     | 6      | 2to10| 4.90| 1.42| 228.00       | 99.60  | 3.51                | 0.68| Duncan and Dickman (2001)        |
| 18  | Scarabeoidea flavicornis | 35.0     | 3      | 2to14| 2.89| 0.62| 357.00       | 76.80  | 2.92                | 1.53| Duncan and Byrne (2000)          |
| 19  | Glossina palpalis        | 15.0     | 13     |     | 1.86| 1.40| 778.80       | 420.00| 2.62                | 0.68| Duncan and Byrne (2000)          |
| 20  | Glossina brevipalpis     | 15.0     | 21     |     | 37.45| 13.15| 26.70        |    *  | 7.40                | 13.27| Duncan and Byrne (2000)          |
| 21  | Glossina australis       | 15.0     | 21     |     | 39.09| 15.15| 25.58        |    *  | 15.44               | 18.24| Duncan and Byrne (2000)          |
| 22  | Glossina morsitans       | 14.0     | 14     |     | 84.88| 9.77 | 35.59        |    *  | 6.09                | 12.57| Duncan and Byrne (2000)          |
| 23  | Aphodius fassor           | 15.0     | 10     |     | 1.15| 0.14| 999.40       | 115.40| 2.75                | 0.12| Chown and Holter (2010)          |
| 24  | Bombyx mori              | 24.0     | 6      |     | 1.44| 0.16| 694.44       |    *  | 6.17                | 0.79| Karise et al. (2010)            |
| 25  | Apis mellifera           | 14.0     | 14     |     | 84.88| 9.77 | 35.59        |    *  | 6.09                | 12.57| Duncan and Byrne (2000)          |
| 26  | Aquarius remigis         | 15.0     | 10     |     | 1.25| 0.59| 76.23        | 74.70  | 1.08                | 0.07| Contreras and Bradley (2010)     |
| 27  | Crotanemus armatus       | 15.0     | 35     |     | 8.33| 2.00| 182.05       |    *  | 5.34                | 0.44| Nespolo et al. (2007)           |
| 28  | Rhodnius prolixus        | 15.0     | 10     |     | 4.17|    * | 238.44       | 5.11   | 2.50                | 0.03| Contreras and Bradley (2010)     |

Cells marked with * indicate missing SD due to calculation of mean duration from mean frequency literature data or vice versa, or because data was measured from figures published in literature.
Table 2
Occurrence of individual wasps’ abdominal movement during respiration cycles in per cent. Cycles were split in the three phases of discontinuous gas exchange (see Fig. 2B). All types of movement may occur in the same phase. Example: All (100%) flutter phases at \( T_a = 16.5^\circ \)C show abdominal movement (resp) as well as phases with abdominal and antennae/leg/wing movement (resp & mov) and 33.3% showed body movement (mov). \( T_a \) indicates the ambient temperature, \( n \) is the number of cycles tested. Abdominal movement in closed phases – though resembling movement in flutter phases – did not concur with CO2 release.

| \( T_a \) (°C) | Flutter (%) | Open (%) | Closed (%) | \( n \) |
|--------------|-------------|----------|------------|--------|
|              | resp        | resp & mov | mov        | resp   | resp & mov | mov        | resp   | resp & mov | mov        | resp   | resp & mov | mov        | resp   | resp & mov | mov        | resp   | resp & mov | mov        |
| 6.2          | 84.2        | 5.3       | 15.8       | 68.2    | 9.1       | 59.1       | 5.0    | –          | –          | 10.0   | 26         |
| 11.8         | –           | –         | –          | 87.5    | 7.7       | 76.9       | –      | –          | –          | 13     |
| 14.4         | –           | –         | –          | 22.2    | 33.3       | 83.3       | 9.1    | 9.1         | 27.3       | 12     |
| 16.5         | 100         | 100       | 33.3       | 81.3    | 93.8       | 43.8       | 23.1   | 30.8        | 15.4       | 16     |
| 19.8         | 87.5        | 43.8      | 81.3       | 94.7    | 26.3       | 84.2       | 46.2   | –          | 23.1       | 19     |
| 22.5         | –           | –         | 25.0       | 85.7    | –          | 14.3       | –      | –          | 9.1        | 14     |
| 31.1         | 75          | –         | –          | 82.6    | –          | 47.8       | –      | –          | –          | 28     |
| 35.8         | –           | –         | –          | 68.9    | 45.9       | 48.6       | –      | –          | –          | –      | 74         |

resp = abdominal (respiration) pumping movement.
resp & mov = abdominal (respiration) movement and concurrent movement of e.g. antennae, legs, wings.
mov = movement of the whole wasp, possibly masking abdominal (respiration) movements.

Flutter data at 31.1 °C is marked grey because flutter phase at this \( T_a \) merged with open phase (see Section 4).

Supplementary material, Table & Fig. S5). In R. prolixus, Contreras and Bradley (2010) still observed closed phases at \( T_a = 35^\circ \)C. It has to be kept in mind that they determined this relationship in a different experimental procedure, exposing insects to a temperature ramp while our insects were exposed to constant temperatures. A rough estimation of the cease temperature of closed phases can be done by determining the quotient of cycle to open phase duration (\( Q_{C/O} \)). We calculated a best fit curve of \( Q_{C/O} \) from the quotients of the original cycle and open-phase duration values. At a \( Q_{C/O} \) of 1, the open phase was as long as the respiration cycle, and the closed phase had vanished. This occurred at a temperature of 36.8 °C. This value corresponded almost exactly with the one determined from the best-fit curves for cycle and open phase duration in Fig. 3, which was 36.7 °C. Flutter phases ceased between 35.8 and 39.7 °C (see Fig. 3, Supplementary material S6). The fusion frequency of cycles should depend to a considerable degree on the relation between (basal) metabolic rate and CO2 buffer capacity of an insect. A prediction of Hetz (2007) suggests that DGCs should mainly occur in insects with large differences in metabolic rate due to changing temperatures or in insect species with huge spiracular conductance due to short-time high metabolic demands (e.g. during endothermy or flight). This applies to wasps (Käfer et al., 2012; and own unpublished measurements). Their rather high fusion frequency (despite a high RMR), therefore, suggests a high CO2 buffer capacity.

As RMR increases with \( T_a \) the curve progression of cycle duration vs. \( T_a \) (Fig. 3) seems similar to that in cycle duration vs. RMR (Fig. 4). However, while in the former case the curves are best described by the mentioned exponential functions, analysis of the latter revealed a higher order of dependence than a simple exponential growth. Good linear regression in dual logarithmic scaling (Fig. 4, inset) backs this finding. Due to high intra- and inter-individual variation in gas exchange pattern, neither switched all wasps from one pattern to another at the same experimental temperature, nor did they always show the same pattern at the same \( T_a \). Such variation was also observed in the cockroach Periplaneta sp. by Marais and Chown (2003) and in several beetle species of southern Africa by Chown (2001).

It is discussed that opening an insect’s spiracles for extended periods leads to critical tracheal water loss in dry environments (Chown et al., 2006a; Dingha et al., 2005; Duncan and Byrne, 2000; Duncan et al., 2002a,b; Hadley, 1994; Kivimägi et al., 2011; Williams et al., 1998, 2010; Williams and Bradley, 1998). Contrary findings question this hypothesis (Contreras and Bradley, 2008; Gibbs and Johnson, 2004). An alternative model suggests that possible O2 intoxication caused by high partial O2 pressure in the tracheal system is a key parameter which forced development of discontinuous gas exchange (Hetz and Bradley, 2005). In any case, the amount of accumulated CO2 is the trigger for the opening of spiracles (Lighton, 1996; Schneiderman and Williams, 1955). With rising \( T_a \) and resulting increase in RMR, yellow jackets have to balance spiracle opening, O2 ingress and CO2 emission. Short, fast openings (i.e. flutter) accompanied by single, small-scale abdominal ventilation movements could maintain a sufficient PO2 inside the wasp for longer periods (see Förster and Hetz, 2010), until it has to get rid of CO2 in a comparably short, huge burst, concurrently inhaling O2. This allows for the following closed phase with no or little O2 uptake and CO2 emission and tracheal water loss. When the CO2 level reaches a certain threshold, the cycle starts anew. However, this works only up to a certain temperature and therefore metabolic rate. As reported by Chown and Nicolson (2004) and Contreras and Bradley (2010), with increasing ambient temperature, duration of the closed phase becomes shorter and shorter first, and in succession the flutter phase vanishes. In Vespa sp., above experimental temperatures of about 30 °C, with rising temperature the CO2 trace increasingly often did not reach zero, which is said to be a criterion of a DGC (Chown et al., 2006b). However, right at the beginning of the open phase, CO2 increased in steps at a rate clearly distinguishable from that of the main peak (Fig. 2B, e.g. at 7, 12 and 18 min). Probably, these are single peaks of a flutter phase, below the temporal resolution of our measurement setup and therefore forming a graduated slope. In our opinion these graduated slopes are flutter phases merging with the consecutive open phases (Fig. 3, large triangles; Table 2, marked data). We suppose that this represents DGC on the verge of cyclic respiration. This resembles findings of Contreras and Bradley (2009) on R. prolixus. At temperatures higher than 36 °C, open phases of wasps occurred in such close succession that the peaks merged at the base and the CO2 signal never reached baseline levels. Their metabolic rate was so high that the produced and emitted CO2 could not be entirely removed from the measurement chamber before the next pulse was generated. The respiration pattern became entirely cyclic (compare Gray and Bradley, 2003).

4.2. Respiration frequency and respiration movements
The wasps’ RMR increases exponentially with rising \( T_a \) (see Käfer et al., 2012). They respond to the according demand of increased gas exchange with a likewise exponential increase in respiration frequency (Fig. 5) but not with an increasing CO2 emission per respiration cycle (Fig. 6). This was also reported for honeybees (Kovac et al., 2007) and fire ants (Vogt and Appel, 1995)
A comparison over flying and non-flying insect species reveals a positive correlation of respiration frequency and RMR (Fig. 7, Table 1). In spite of a high variation in level as well as in slope of the single species data, a trend is obvious in insects to increase CO2 emission with an increase in respiration frequency rather than in “depth of breath” or other measures.

In the lower to medium temperature range (Ta = 10–27 °C), resting yellow jackets’ respiration frequency did not differ much from that of honeybees (see Fig. 5). The increasing deviation of the curves above 27.5 °C could result from the exceptional steep increase in RMR in yellow jackets compared to honeybees (see Käfer et al., 2012). Regarding CO2 emission per respiration cycle, yellow jackets show a slight decrease with Ta similar to honeybees (Kovac et al., 2007; Fig. 6). Because of virtually identical testing arrangements in Vespuia sp. and Apis mellifera, a straight comparison of these two species is possible. At similar respiration frequencies (Fig. 5), resting yellow jackets have a much higher energetic turnover (see Käfer et al., 2012) and emit CO2 on average in much higher amounts per cycle (Figs. 6 and 7) than honeybees at similar ambient temperatures. Wasps seem to breathe more efficiently with respect to gas exchange volume per cycle than honeybees. This might base on anatomical (compare Snelling et al., 2011 on Locusta migratoria tracheae), physiological or behavioral differences between the two species. Both are known to have thoracic and abdominal air sacs serving as buffering reservoirs for CO2 laden exhalation air. These air sacs are documented in anatomical drawings by Snodgrass (1985) for honeybees, but to our knowledge there is no detailed information for yellow jackets available, and volume data of the tracheal system including the air sacs is available neither for honeybees nor for wasps. The insect hemolymph serves as a CO2 buffer (Buck and Keister, 1958; Buck and Friedman, 1958; Kaiser, 2002). However, there is also no report of differences in the buffer capacity of wasp and honeybee hemolymph available. Future investigations will have to elucidate these topics.

Another explanation might lie in differences in the respiration movements between yellow jackets and honeybees. Other than in honeybees, the wasps’ abdominal ventilation movements were not of a uniform pumping pattern, but often consisted of lateral flipping of the abdomen or single pumps accompanied by wing and leg movement (spasm-like; see Supplementary material, IR video S4). These body movements might contribute to the abdominal pumping in discharging tracheas and air sacs of CO2 laden air. We observed abdominal ventilation movements in 100% of the open phases. The wasps showed ventilation movements also in 71.4% of the flutter phases (66.7% if the distinct increase in the CO2 signal before an open phase above 26.3 °C was not counted as a flutter phase), whereas in honeybees no distinct pumping movements could be observed (Kovac et al., 2007). For a sufficiently effective gas exchange of adult insects diffusion is not enough (Hadley, 1994). The wasps seem to rely on active ventilation during the flutter phase in addition to the open phase (Table 2, Fig. 8). Some abdominal movements did also occur in closed phases (see also Groenewald et al., 2012; Hetz et al., 1994). Passive gas influx during micro openings in the closed phase leads to a gradual abdominal elongation in Attacus atlas pupae (Hetz and Bradley, 2005; Hetz, 2007) and Pieris brassicae pupae (Jögar et al., 2011). The closed phase movements observed in yellow jackets resembled the single small abdominal pumping movements observed in flutter phases but were clearly not of the passive type (see Brockway and Schneiderman, 1967).

Vespuia sp. has a high energetic turnover at rest compared to A. mellifera (Käfer et al., 2012). However, the yellow jackets’ respiration frequencies are similar to that of honeybees up to ambient temperatures of about 27.5 °C (see Fig. 5), but with overall higher CO2 emission per cycle (see Fig. 6). Despite their high resting metabolic rate (Käfer et al., 2012), wasps are among the insects with a rather low respiratory frequency at a given RMR. Variation in data between insect species is so high that no meticulous conclusions can be drawn from one species to another. However, a general trend to raise CO2 emission with an increase in respiration frequency can be seen (Fig. 7).

The amount of CO2 emission per cycle correlated positively with the duration of abdominal respiration movements (Fig. 10). Wasps reduced the duration of ventilation movements at higher temperatures (Fig. 9). Total duration of respiration movement events was up to tenfold longer than in honeybees (42.2 vs. 4.8 s at 20 °C, 27.8 vs. 2.3 s at 25 °C; mean values, honeybee data from Kovac et al., 2007). It seems that resting yellow jackets gain their efficient gas exchange to a considerable extent via the length of respiration movements per respiratory cycle. Therefore, they manage a considerably higher RMR (see Käfer et al., 2012) with a similar respiration frequency as honeybees (see Fig. 4). The high respiration volume and efficiency might be responsible for the rather high transition temperature from discontinuous to cyclic respiration.

5. Conclusion

Despite an overall high level and a steep increase of resting metabolism with increasing ambient temperature (high Q10), resting yellow jackets maintain DGC at comparably high ambient temperatures. They breathe more ‘efficiently’ than other insects, achieving more CO2 emission per respiration cycle at comparable respiration frequencies.

Abdominal ventilation movements at rest were not uniform pumping movements but also included movements of legs, antennae and wings, and lateral flipping of the abdomen. Results suggest that respiration efficiency was increased by long duration of these ventilation movements.

Acknowledgements

The research was funded by the Austrian Science Fund (FWF; P20802-B16, P25042-B16). We greatly appreciate the help with electronics by G. Stabentheiner and with data evaluation by M. Bodner, M. Brunnhoffer, M. Fink, P. Kirchberger, A. Lienhard, L. Mirwald and A. Settari. We also thank W. Schappacher for his help in clarifying some quirks with data conversion, two anonymous reviewers for helpful comments and the editor D.L. Denlinger.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2013.01.012.

References

Basson, C.H., Terblanche, J.S., 2011. Respiratory pattern transitions in three species of Glossina (Diptera, Glossinidae). Journal of Insect Physiology 57, 433–443.
Bellmann, H., 1995. Bienen, Wespen, Amiesen: Die Hautflügler Mitteleuropas. Franckh-Kosmos-Verlags-GmbH & Co, Stuttgart, p. 336.
Bradley, T.J., 2000. The discontinuous gas exchange cycle in insects may serve to reduce oxygen supply to the tissues. American Zoologist 40, 952.
Brohmer, P., 1977. Fauna Von Deutschland: Ein Bestimmungsbuch Unserer Heimischen Tierwelt. Quelle & Meyer, Heidelberg, p. 319.
Brockway, A.P., Schneiderman, H.A., 1967. Strain-gauge transducer studies on intratracheal pressure and pupal length during discontinuous respiration in diapausing silkworm pupae. Journal of Insect Physiology 13, 1413–1451.
Buck, J., 1958. Cyclic CO2 release in insects IV. A theory of mechanism. Biological Bulletin 114, 118–140.
Snelling, E.P., Matthews, P.G.D., Seymour, R.S., 2012. Allometric scaling of discontinuous gas exchange patterns in the locust Locusta migratoria throughout ontogeny. The Journal of Experimental Biology 215, 3388–3393.

Snelling, E.P., Seymour, R.S., Runciman, S., Matthews, P.G.D., White, C.R., 2011. Symmorphism and the insect respiratory system: allometric variation. The Journal of Experimental Biology 214, 3225–3237.

Snodgrass, R.E., 1985. Anatomy of the Honey Bee. Comstock Publishing Associates, p. 352.

Stabentheiner, A., Crailsheim, K., 1999. The effect of activity level and ambient temperature on thermoregulation in isolated honeybees (Hymenoptera: Apidae). Entomologia Generalis 24, 13–21.

Stabentheiner, A., Vollmann, J., Kovac, H., Crailsheim, K., 2003. Oxygen consumption and body temperature of active and resting honeybees. Journal of Insect Physiology 49, 881–889.

Stabentheiner, A., Kovac, H., Hetz, S.K., Käfer, H., Stabentheiner, G., 2012. Assessing honeybee and wasp thermoregulation and energetics–new insights by combination of flow-through respirometry with infrared thermography. Thermochimica Acta 534, 77–85.

Stabentheiner, A., Schmaranzer, S., 1987. Thermographic determination of body temperatures in honey bees and hornets: calibration and applications. Thermology 2, 563–572.

Terblanche, J.S., Chown, S.L., 2010. Effects of flow rate and temperature on cyclic gas exchange in tsetse flies (Diptera, Glossinidae). Journal of Insect Physiology 56, 513–521.

Terblanche, J.S., Clusella-Trullas, S., Deere, J.A., Chown, S.L., 2008a. Thermal tolerance in a south-east African population of the tsetse fly Glossina pallidipes (Diptera, Glossinidae): implications for forecasting climate change impacts. Journal of Insect Physiology 54, 114–127.

Terblanche, J.S., Marais, E., Hetz, S.K., Chown, S.L., 2008b. Control of discontinuous gas exchange in Samia cynthia: effects of atmospheric oxygen, carbon dioxide and moisture. Journal of Experimental Biology 211, 3272–3280.

Terblanche, J.S., White, C.R., Blackburn, T.M., Marais, E., Chown, S.L., 2008c. Scaling of gas exchange cycle frequency in insects. Biology Letters 4, 127–129.

Vogt, J.T., Appel, A.G., 1999. Standard metabolic rate of the fire ant, Solenopsis invicta Buren: effects of temperature, mass, and caste. Journal of Insect Physiology 45, 655–666.

Vogt, J.T., Appel, A.G., 2000. Discontinuous gas exchange in the fire ant, Solenopsis invicta Buren: caste differences and temperature effects. Journal of Insect Physiology 46, 403–416.

Williams, A.E., Bradley, T.J., 1998. The effect of respiratory pattern on water loss in desiccation-resistant Drosophila melanogaster. Journal of Experimental Biology 201, 2953–2959.

Williams, A.E., Rose, M.R., Bradley, T.J., 1998. Using laboratory selection for desiccation resistance to examine the relationship between respiratory pattern and water loss in insects. Journal of Experimental Biology 201, 2945–2952.

Williams, C.M., Pelini, S.L., Hellmann, J.J., Sinclair, B.J., 2010. Intra-individual variation allows an explicit test of the hygric hypothesis for discontinuous gas exchange in insects. Biology Letters 6, 274–277.

Witt, R., 1998. Wespen: Beobachten, Bestimmen. Naturbuch Verlag, Augsburg, pp. 360–360.

Woodman, J.D., Cooper, P.D., Haritos, V.S., 2008. Neural regulation of discontinuous gas exchange in Periplaneta americana. Journal of Insect Physiology 54, 472–480.