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RESEARCH ARTICLE

Respiratory dynamics of discontinuous gas exchange in the tracheal system of the desert locust, *Schistocerca gregaria*

Berlizé Groenewald[1], Stefan K. Hetz[2], Steven L. Chown[3] and John S. Terblanche[1,*]

[1]Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland 7602, Stellenbosch, South Africa, [2]Department of Animal Physiology, Humboldt-Universität zu Berlin, Philippstr. 13, 10115 Berlin, Germany and [3]Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, Stellenbosch, South Africa

*Author for correspondence (jst@sun.ac.za)

SUMMARY

Gas exchange dynamics in insects is of fundamental importance to understanding evolved variation in breathing patterns, such as discontinuous gas exchange cycles (DGCs). Most insects do not rely solely on diffusion for the exchange of respiratory gases but may also make use of respiratory movements (active ventilation) to supplement gas exchange at rest. However, their temporal dynamics have not been widely investigated. Here, intratracheal pressure, $\dot{V}_{CO_2}$ and body movements of the desert locust *Schistocerca gregaria* were measured simultaneously during the DGC and revealed several important aspects of gas exchange dynamics. First, *S. gregaria* employs two different ventilatory strategies, one involving dorso-ventral contractions and the other longitudinal telescoping movements. Second, although a true spiracular closed (C)-phase of the DGC could be identified by means of subatmospheric intratracheal pressure recordings, some CO_2 continued to be released. Third, strong pumping actions do not necessarily lead to CO_2 release and could be used to ensure mixing of gases in the closed tracheal system, or enhance water vapour reabsorption into the haemolymph from fluid-filled tracheole tips by increasing the hydrostatic pressure or forcing fluid into the haemocoel. Finally, this work showed that the C-phase of the DGC can occur at any pressure. These results provide further insights into the mechanistic basis of insect gas exchange.

Key words: ventilation, water loss, passive suction ventilation, diffusion, insect, metabolic rate.

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INTRODUCTION

Insects living in terrestrial environments face significant metabolic challenges. For example, they have to regulate internal O_2 levels necessary for aerobic metabolism and excrete metabolic by-products (e.g. CO_2), while minimizing water loss (e.g. Chown, 2002; Woods and Smith, 2010; Matthews and White, 2011a). Insects possess numerous morphological and physiological adaptations for coping with these simultaneous pressures. Among the most controversial of the proposed adaptations is the use of discontinuous gas exchange cycles (DGCs) associated with the possession of occludible spiracles (Lighton, 1994; Chown et al., 2006). Indeed, the underlying mechanistic and evolutionary reasons for variation in patterns of insect gas exchange are receiving renewed attention (White et al., 2007; Terblanche et al., 2008; Chown, 2011; Contreras and Bradley, 2011; Matthews and White, 2011b). These studies have all argued that a mechanistic understanding of insect respiratory dynamics and gas exchange patterns is central to understanding evolved variation in insect gas exchange patterns.

The respiratory organs of terrestrial insects consist of semi-rigid tracheal tubes that penetrate the body and tissues. These tracheae are air filled and transport gases between the atmosphere, spiracles and internal tissues or organs. The tracheae divide into tubes of decreasing diameter and eventually branch into microscopic tubes, or tracheoles. The tracheoles are the main sites of gas exchange with tissues (e.g. Wigglesworth, 1929) (for reviews, see Wigglesworth, 1965; Wigglesworth, 1983); they closely surround cells and might even penetrate them to lie close to mitochondria in some cases (Quinlan and Gibbs, 2006). In most insects the terminal part of the tracheole is filled with fluid (Chapman, 1998; Woods et al., 2009), and the final stages of gas transport between the tracheoles and haemolymph or mitochondria take place *via* diffusion through a liquid (Miller, 1964). Thus, the spiracles regulate the flow of gases and water vapour into and out of the tracheae (Hadley, 1994; Chapman, 1998). By closing the spiracles, tracheal P_{O_2} and pressure can be regulated, perhaps minimizing oxidative damage (Hetz and Bradley, 2005), while the loss of water from the internal environment of the insect to the external atmosphere can be limited (e.g. Loveridge, 1968).

In insects, efficient gas exchange is dependent on the morphological structure of the tracheal system, as well as on adequate ventilation. Passive diffusion or active muscular movements (i.e. convective gas exchange), and the selective opening of the spiracles can be used to modulate whole-animal ventilation patterns (Miller, 1964; Lighton, 1996; Chown et al., 2006). In small insects and in the inactive stages of some larger species, an adequate rate of O_2 uptake can be obtained predominantly by diffusion, and there is thus little need for respiratory movements (Kestler, 1985). However, in larger or more active insects diffusion has to be supplemented with convection to maintain an adequate O_2 supply (Chown and Nicolson, 2004). These larger insects probably rely on diffusion only for O_2 transport along the finer terminations of the tracheae, while mechanically ventilating the larger tracheal trunks

Table 1. Summary of literature measuring insect gas exchange, intratracheal pressure and body movements

Family	Species	Reference	Method	Cannulated spiracle	Measurements: Gas exchange	Measurements: Pressure	Measurements: Body movements	Findings
Acrididae	*Dissosteira carolina*	McCutcheon, 1940	Manometer	Metathoracic	No	Yes	Yes	Mean pressure 266.6 Pa
Acrididae	*Melanoplus differentialis; Schistocerca americana*	Krolikowski and Harrison, 1996	Differential pressure transducer	Metathoracic	No	Yes	Yes	Pressure range 300–3500 Pa No subatmospheric pressures
Formicidae	*Cataglyphis bicolor*	Lighton et al., 1993	Differential pressure transducer	Mesothoracic	Yes	Yes	No	O-phase pressure ~0 Pa C-phase pressure falls to –50 Pa Pressure pulses of up to 40 Pa during C and F phases
Saturniidae	*Attacus atlas*	Hetz et al., 1993	Differential pressure transducer	2nd or 3rd abdominal spiracle	Yes	Yes	No	O-phase pressure ~0 Pa C-phase pressure –250 to –750 Pa
Saturniidae	*Attacus atlas*	Hetz and Bradley, 2005	Differential pressure transducer	?	Yes	Yes	Yes	O-phase pressure ~0 Pa C-phase pressure below atmospheric
Saturniidae	*Attacus atlas*	Wobschall and Hetz, 2004	Differential pressure transducer	2nd or 3rd thoracic spiracle	Yes	Yes	Yes	O-phase pressure ~0 Pa C-phase pressure falls
Saturniidae	*Hyalophora cecropia*	Levy and Schneiderman, 1966	Manometer	3rd abdominal spiracle	Yes	Yes	No	O-phase pressure within 0.7 Pa of atmospheric C-phase pressure –466.6 Pa**
Saturniidae	*Hyalophora cecropia*	Schneiderman and Schechter, 1966	Electronic pressure transducer	?	Yes	Yes	Yes	Min. pressure –546.6 Pa C-phase pressure falls O-phase pressure ~0 Pa*
Saturniidae	*Hyalophora cecropia*	Brockway and Schneiderman, 1967	Strain-gauge transducer	3rd or 4th abdominal spiracle	No	Yes	Yes	O-phase pressure ~0 Pa C-phase pressure min. –667 Pa During about 95% of the DGC, pressure is below atmospheric*
Saturniidae	*Hyalophora cecropia*	Burkett and Schneiderman, 1974)	Strain-gauge transducer	?	No	Yes	No	C-phase mean pressure –313.3 Pa*
Saturniidae	*Samia cynthia*	Terblanche et al., 2008	Differential pressure transducer	2nd or 3rd abdominal spiracle	Yes	Yes	No	O-phase pressure ~0 Pa C-phase pressure falls Some positive peaks observed during C- and O-phases (max. peaks ±50 Pa and ±100 Pa, respectively)
Saturniidae; Sphingidae	*Actias selene; Sphinx Ligustri; Hyalophora cecropia; Manduca sexta*	Slama, 1988	Anemometric transducer	?	Yes	Yes	No	*Hyalophora cecropia*: C-phase pressure below 0 Pa
Scarabaeidae	*Circellium bacchus*	Duncan et al., 2010	Differential pressure transducer	2nd abdominal spiracle	Yes	Yes	Yes	Positive and subatmospheric pressure pulses

C-phase, closed phase; O-phase, open phase; DGC, discontinuous gas exchange cycle; max., maximum; min., minimum.
*Because of the large dead air space (tracheal system, cannula and transducer), the recorded pressure changes were smaller than the actual pressure changes that occur in the tracheal system.
**The first direct and repeated measurements of the barometric pressure within an insect's tracheal system.

(Wigglesworth, 1972). Although the significance of ventilation is widely acknowledged, the temporal dynamics of gas exchange and variation in pressure associated with such ventilatory movements have only been explored in a limited range of species (mostly Lepidoptera, some Orthoptera and one species of Hymenoptera; see Table 1). Most work to date, drawn primarily from the classic gas exchange model of diapausing moth pupae, suggests that the closed (C)-phase of the DGC is typically associated with negative intratracheal pressures, while during the open (O)-phase intratracheal pressure remains at or near atmospheric levels (Table 1). Moreover, only a handful of studies combine gas exchange, intratracheal pressure and body movement measurements simultaneously

(Table 1). Given the diversity of gas exchange patterns among tracheated arthropods, and the fact that the DGC has evolved independently on at least five occasions within the Insecta (Marais et al., 2005), further investigation of patterns and mechanisms underlying gas exchange is required, perhaps encompassing a wider range of species.

Here, we therefore simultaneously measured intratracheal pressure, $\dot{V}_{CO_2}$ and body movements (i.e. pumping body movements) of the desert locust *Schistocerca gregaria*. This study aimed to assess the role of ventilatory movements over the entire gas exchange cycle and the relative importance of convection *vs* diffusion in this species. Intratracheal pressure was determined for the different DGC phases, as well as for the start of each of the DGC phases. Specifically, we aimed to test whether the DGC is accompanied by a specific intratracheal pressure pattern. We hypothesized that the C-phase of the DGC is accompanied by a gradual decrease in intratracheal pressure, followed by rising pressure during the flutter (F)-phase, until the pressure reaches and stabilizes at or near atmospheric levels during the O-phase.

MATERIALS AND METHODS

Animals

Adult *S. gregaria* (Forskål 1775) (Orthoptera: Acrididae) were supplied by Grillenzucht Hildner (Gerhardshofen, Germany). Individuals were kept separated from each other in plastic containers. Animals were fed fresh lettuce every second day and were kept at ambient temperature (18–22°C), relative humidity and air conditions. Both male and female adult grasshoppers were used. Animals were fasted for at least 8 h before respirometry commenced.

Respirometry

Twelve individuals were recorded for 8 h each at 15°C. Rate of CO_2 release ($\dot{V}_{CO_2}$), body movements (i.e. activity) and intratracheal pressure were recorded with 8 samples s^{-1}. Carbon dioxide release was measured in a custom-made flow-through respirometer, with a length of 60 mm and a volume of ~10 ml. The temperature of the cuvette was controlled by a custom-made Peltier-cooling unit with a computer-controlled feedback (accuracy ±0.1°C). Ambient air (from outside the lab) was scrubbed of CO_2 and water vapour by passing it through two columns containing a 5 mol l^{-1} NaOH solution. The scrubbed air was then driven through two 200 ml columns filled with distilled water, which were kept at a constant temperature of 8°C, to keep the water vapour pressure constant. The flow rate of the air was regulated at 250 ml min^{-1} by a mass flow controller (MKS 1179, MKS Instruments, Wilmington, MA, USA). The air was then fed through the reference cell of a pressure-compensated differential infrared gas analyser (URAS 14, range 200 p.p.m., ABB Analytical, Frankfurt, Germany), whereafter it flowed through the respirometry cuvette, and then through the analysing cell of the gas analyser, to measure the CO_2 output of the locust. During a trial, the body movements of each individual were recorded with three different infrared sensors, located dorsally above the animal. These consisted of reflective interrupters (Osram SFH 9201, Osram, Germany) that sent infrared light (wavelength 850–950 nm) to the animal and measured the reflected changes in radiation due to body movements. Data were recorded to a computer hard disk using a customized recording program in TurboLab 4.03 (Bressner Technology, Gröbenzell, Germany).

Intratracheal pressure

Each individual was weighed before and after spiracle intubation, as well as after each trial. Body movements, $\dot{V}_{CO_2}$ and intratracheal pressure were recorded following methods outlined elsewhere (Moerbitz and Hetz, 2010; Wobschall and Hetz, 2004). To measure intratracheal pressure, the animal's mesothoracic spiracle was cannulated. Before intubation of the spiracle commenced, animals were restrained by binding the mesothoracic and metathoracic legs to the abdomen with adhesive tape (Fig. 1). The restraints did not confine the abdomen or physically impede abdominal ventilatory movements. These measures were taken to ensure that the tubing could not be removed from the cannulated spiracle by the animal's leg movements.

Fig. 1. *Schistocerca gregaria* individual with a steel cannula inserted into a mesothoracic spiracle; the area around the spiracle was sealed with dental wax. Mesothoracic and metathoracic legs were restrained with adhesive tape. The short piece of polyethylene tubing, with the thin steel cannula (0.25 mm i.d.) at the end, was placed underneath the adhesive tape, as well as being secured with wax to the tape, in order to stabilize the cannulated tubing.

For the cannulation, a short piece of polyethylene tubing (PE10) was modified to include a thin steel tube (0.25 mm i.d.) at the end, which was inserted into the animal's spiracle. The surrounding of the spiracle was then sealed with dental wax. The tubing was connected to a precision micro-pressure transducer (SenSym SDXL010, SensorTechnics, Pucheim, Germany). The other port of the transducer opened into the respirometry chamber to measure pressure differences between the chamber and tracheal system. The pressure sensor electrically consisted of a Wheatstone bridge that was driven with a precision voltage reference of 6.000 V (REF02, Burr Brown, Texas Instruments, Dallas, TX, USA) buffered with a precision operational amplifier (OPA177, Burr Brown). Differential voltage output of the pressure sensor bridge was amplified with a differential amplifier (INA131, Burr Brown).

After intubation, animals were allowed 20–30 min to recover from the handling stress of the intubation procedure, before recording of their CO_2 release commenced. After this period animals no longer struggled and appeared to ventilate normally.

Data extraction and analysis

The data for each individual were imported into EXPEDATA Version 1.1.25 (Sable Systems, Las Vegas, NV, USA) data acquisition and analysis software. The data from the first hour after an individual was placed into the respirometer were discarded. For each individual the first three consecutive DGCs were selected for data analysis. An individual's breathing pattern was defined as a DGC when, according to the CO_2 trace, a burst (O-phase) and interburst [closed/flutter (CF)-phase] period were clearly discernible. The C-phase and F-phase were combined to form the interburst period because the F-phase could not be separated from the C-phase by using the rate of CO_2 release. In addition, there was no obvious

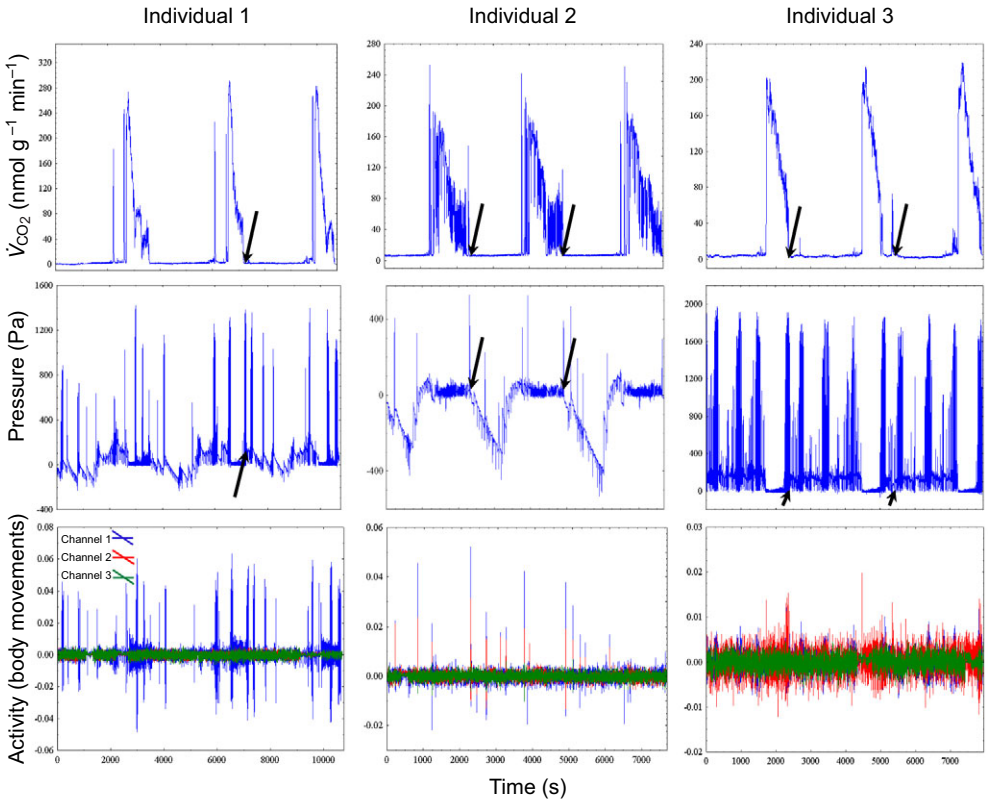

Fig. 2. Recordings of CO_2 release rate ($\dot{V}_{CO_2}$, nmol g^{-1} min^{-1}), intratracheal pressure (Pa) and body movements for three different *Schistocerca gregaria* individuals (mass 2.2869 g, 1.4529 g and 2.2971 g, respectively). All individuals were recorded at 15°C, 250 ml min^{-1} flow rate, and sampling frequency of 1 s for $\dot{V}_{CO_2}$ and 0.125 s for body movements and pressure. Note that axes scaling varies for the different parameters between individuals for clarity. Arrows indicate the start of the interburst period where the intratracheal pressure is not sub-atmospheric.

pattern in the intratracheal pressure trace that could be used to distinguish these two phases. The beginning of the interburst period was used as the onset of a DGC. To examine the relationships between the different variables (phase duration, pressure, rate of CO_2 release, volume of CO_2 released) and to assess whether any pattern of intratracheal pressure exists that accompanies the DGC, correlation analysis was performed using STATISTICA v. 10 (StatSoft, Tulsa, OK, USA). Data collected from the three different infrared sensors were converted to *z*-scores (standard scores) before being used in correlation analysis, as the baselines of the channels from each individual differed. The data were converted using the following formula:

$$z = \frac{x - u}{\text{s.d.}}, \quad (1)$$

where z is the standardised body movement score, x is the observed body movement score (raw signal from the activity detector), u is the mean body movements, and s.d. is the standard deviation of the body movement channel.

RESULTS

All 12 individuals exhibited DGCs when recorded at 15°C (mean ± s.d. body mass 1.761±0.325 g). There was no significant relationship between body mass and any DGC phase duration, $\dot{V}_{CO_2}$ during the O-phase and interburst period, or volume of CO_2 released during the O-phase (P>0.39 in all cases).

The mean duration of the interburst period increased with mean DGC duration (r=0.922, N=12, P<0.0001). Mean interburst $\dot{V}_{CO_2}$ was positively correlated with mean interburst duration (r=0.608, N=12, P=0.0358). Mean O-phase volume was positively related to mean interburst duration (r=0.734, N=12, P=0.0066). Moreover, the interburst period of a DGC commenced at any intratracheal pressure and was not restricted to sub-atmospheric pressures (Fig. 2, Table 2).

Mean O-phase $\dot{V}_{CO_2}$ was negatively, but not significantly, related to mean O-phase duration (r=–0.524, N=12, P=0.0806). Mean O-phase volume was positively correlated with mean O-phase duration (r=0.726, N=12, P=0.0075).

To examine whether any relationship exists between the duration of the different phases of a DGC and the intratracheal pressure pattern of an individual, correlations between these variables were assessed. No significant relationship existed between mean O-phase duration and mean pressure during an O-phase within those same DGCs (r=–0.509, N=12, P=0.0914), or between mean interburst duration and mean pressure during that same interburst period (r=–0.098, N=12, P=0.7615).

There were no characteristic patterns for intratracheal pressure changes that were common among all individuals, but rather intratracheal pressure traces showed high among-individual variability. In *S. gregaria* the O-phase could include periods of negative intratracheal pressure, while the interburst period sometimes showed high positive pressure peaks (Table 3).

The mean intratracheal pressure of individual *S. gregaria* during the interburst period ranged from –112.1 to 224.6 Pa, with four individuals having a negative mean intratracheal pressure during the interburst period and eight individuals having a positive mean intratracheal pressure. Mean $\dot{V}_{CO_2}$ during the interburst period ranged from 1.49 to 14.67 nmol g^{-1} min^{-1}. In eight individuals, $\dot{V}_{CO_2}$ during the interburst period was statistically greater than 0, regardless of whether the mean intratracheal pressure was negative or positive. Four of the 12 individuals recorded had a negative mean intratracheal pressure during the interburst period, and in three out of these four individuals $\dot{V}_{CO_2}$ during the interburst period was statistically greater than the infrared gas analyser baseline (see e.g. Fig. 2, individual 2).

Table 2. Intratracheal pressure data at the start of interburst periods and O-phases for three consecutive DGCs per individual, for each of the 12 recorded *S. gregaria* individuals

Individual	Cycle	Pressure at start of interburst period (Pa)	Pressure at start of O-phase (Pa)
1	1	49.9	18.7
	2	18.9	–4.4
	3	70.9	41.3
2	1	404.2	170.6
	2	124.6	213.2
	3	73.5	160.5
3	1	–1.3	–2.6
	2	–4.9	0.9
	3	3.1	–2.5
4	1	159.3	170.6
	2	34.8	209.3
	3	344.5	404.9
5	1	–11.9	11.2
	2	11.4	74.4
	3	4.8	29.7
6	1	–26.3	56.3
	2	–16.7	72.0
	3	–32.3	85.7
7	1	–19.9	18.5
	2	44.3	32.1
	3	51.5	40.8
8	1	149.5	105.5
	2	118.7	196.1
	3	161.3	174.9
9	1	48.7	80.4
	2	113.2	234.1
	3	93.5	121.2
10	1	178.2	115.1
	2	98.1	90.4
	3	172.8	179.6
11	1	371.9	38.7
	2	40.0	68.9
	3	179.7	182.3
12	1	–40.2	256.8
	2	38.3	246.5
	3	124.9	97.1

O-phase, open phase; DGC, discontinuous gas exchange cycle.

Table 3. Summary statistics for different components of the DGC in *S. gregaria* for all individuals

	Mean ± s.d.
Mass (g)	1.761±0.325
DGC duration (s)	3189±953
Interburst duration (s)	1968±772
O-phase duration (s)	1233±382
DGC $\dot{V}_{CO_2}$ (nmol g^{-1} min^{-1})	36.10±4.52
Interburst $\dot{V}_{CO_2}$ (nmol g^{-1} min^{-1})	6.25±4.16
O-phase $\dot{V}_{CO_2}$ (nmol g^{-1} min^{-1})	84.83±19.18
O-phase area (nmol g^{-1} CO_2)	27.82±7.35
Mean DGC pressure (Pa)	56.8±63.3
Mean DGC pressure min. (Pa)	–166.7±132.5
Mean DGC pressure max. (Pa)	1166.5±399.2
Mean Interburst pressure (Pa)	46.2±82.8
Mean Interburst pressure min. (Pa)	–159.9±137.2
Mean Interburst pressure max. (Pa)	1068.5±443.7
Mean O-phase pressure (Pa)	71.1±43.0
Mean O-phase pressure min. (Pa)	–66.3±65.1
Mean O-phase pressure max. (Pa)	1128.4±404.9

O-phase, open phase; DGC, discontinuous gas exchange cycle; max., maximum; min., minimum; $\dot{V}_{CO_2}$, rate of CO_2 release.
N=12 in all cases.

Examples of DGCs, together with intratracheal pressure and body movements, are presented in Fig. 2. These traces clearly show that *S. gregaria* employs two of the four different ventilatory movements described by Miller for resting locusts (Miller, 1960). Dorso-ventral muscles are contracted at a high rate, while longitudinal muscles are used rarely, but coincide with higher positive pressure pulses (Fig. 2, individual 1 and individual 2). Also, strong pumping actions do not necessarily lead to CO_2 release, and large outputs of CO_2 are not always associated with abdominal pulsation body movements (Fig. 2, individual 3).

DISCUSSION

Several authors have demonstrated that a comprehensive understanding of gas exchange dynamics in insects should include measurements of intratracheal pressure, as these measurements can provide novel information about both the kinetics of gas exchange and insect respiration in general (e.g. Buck, 1958; Levy and Schneiderman, 1966; Terblanche et al., 2008). This was certainly the case here, where we found for the first time that for *S. gregaria* the O-phase can include periods of negative intratracheal pressure, as well as pressures well above atmospheric levels (Table 3), and the interburst period can start at any pressure amplitude (Table 2) and can include periods of high intratracheal pressure (Table 3; Fig. 2). Rather than the patterns found here being unusual forms of the DGC, several aspects of the DGCs recorded conformed well with established patterns for Orthoptera and other arthropod taxa. For example, the positive relationship observed between O-phase duration and DGC duration is similar to that reported previously for a variety of dung beetles (Davis et al., 1999) and for *Garypus californicus* (Lighton and Joos, 2002) (for review, see Chown and Nicolson, 2004).

The pumping movements of an insect's abdomen lead to volume changes of the tracheal system, which in turn lead to pressure fluctuations within the internal body (detected as intratracheal or haemolymph pressure changes) of the insect (Buck, 1962). In locusts, the pumping movement of the abdomen is generally thought to ventilate the tracheae and larger tracheal trunks (Wigglesworth, 1972), and both inspiration and expiration are active processes (Miller, 1964). In an actively ventilating orthopteran, the first four pairs of spiracles open during the inspiratory phase of abdominal ventilation, while the remaining pairs open during the expiratory phase (Weis-Fogh, 1967; Harrison, 1997), leading to an anterior to posterior (unidirectional) flow of air through the insect (Henderson et al., 1998). However, the path of this unidirectional flow of air is subject to variation, and occasionally any of the spiracles might serve for both inspiration and expiration (Steen, 1971). If there is a unidirectional flow of air through the insect, it could be possible for the insect to have a negative pressure in one part of its tracheal system, while a positive pressure is present in another part, depending on coordinated spiracle and ventilation movements. This could explain the periods of negative intratracheal pressure during the O-phase (Table 3).

The pressure patterns we recorded in *S. gregaria* are similar to those reported for other grasshoppers [e.g. *Melanoplus differentialis* (Krolikowski and Harrison, 1996)] in which tracheal pressures varied substantially among individuals, although this is unlike the situation for lepidopteran pupae (e.g. Levy and Schneiderman, 1966; Wobschall and Hetz, 2004; Terblanche et al., 2008). Moreover, the negative intratracheal pressure during the O-phase is unexpected as the spiracles typically remain open, indicating that the intratracheal pressure should be close to atmospheric levels. This result also

differs from findings in lepidopteran pupae in which intratracheal pressure during the O-phase was maintained at levels similar to atmospheric pressure, and intratracheal pressure never rose much above atmospheric levels (e.g. Wobschall and Hetz, 2004; Terblanche et al., 2008). By contrast, in *M. differentialis* grasshoppers, intratracheal pressure measured in the thoracic trachea never reached atmospheric levels when these individuals were at rest (Krolikowski and Harrison, 1996). In lepidopteran pupae, intratracheal pressure generally falls during the C-phase, rises during the F-phase and remains constant near atmospheric levels during the O-phase (Schneiderman and Schechter, 1966; Hetz et al., 1993). However, for *S. gregaria*, the C-phase can start at any intratracheal pressure (above or below atmospheric pressure) (Table 2) and can include periods of high intratracheal pressure (Table 3; Fig. 2). Therefore, in *S. gregaria* the respiratory cycle is not accompanied by a clearly distinguishable sub-atmospheric intratracheal pressure cycle, as is the case with lepidopteran pupae.

The body movements accompanying gas exchange at rest are similar to those reported previously for this species. Indeed, it was established more than 50 years ago through visual observation and mechanical recordings that dorso-ventral contractions and longitudinal telescoping movements of the abdomen occur in grasshoppers at rest (e.g. McCutcheon, 1940; Weis-Fogh, 1967; Lewis et al., 1973). More recent work that included gas exchange recordings showed that gas exchange may also take place when no abdominal pumping occurs, suggesting that gas exchange is primarily diffusive (although some micro-ventilation pumping actions can be employed, are barely visible to the naked eye and may aid convection) (e.g. Harrison, 1997; Greenlee and Harrison, 1998). However, our study is unique because we recorded body movement patterns, gas exchange and intratracheal pressure simultaneously, which has never previously been done for any Orthoptera (Table 1).

Hamilton (Hamilton, 1964) found that in adult *Schistocerca* bursts of CO_2 coincided with ventilation, and postulated this as being a means to conserve respiratory water. Kestler also explained these coordinated ventilatory movements (involving both diffusive and convective exchanges) as being a means to reduce respiratory water loss (Kestler, 1978). By compressing the abdomen and closing the spiracles, pressure inside the abdomen can be increased, thereby also compressing the tracheal system and gases within the tracheae. But what could the advantage of such high pressures achieved during the interburst period be? One potential explanation is that this might slow down evaporation of water. The pressure cycle theory suggests that changes in intratracheal pressure could help with the regulation of the influx and efflux of water vapour, by making it possible for the animal to regulate the hydrostatic pressure within the trachea (Corbet, 1988). Within the tracheal system, respiratory gases occur at a vapour–liquid interface between the air-filled trachea and insect body fluids. Water can move from the body fluids to the trachea and *vice versa*, and by regulating intratracheal pressure (and thereby also hydrostatic pressure) it should thus be possible to reduce the rate or reverse the direction of water movement. Water evaporates into subsaturated air, but if the vapour pressure within the air exceeds the supersaturation ratio, water will condense. An increase in intratracheal pressure will lead to increased hydrostatic pressure within the tracheal system, and an increase above the supersaturation ratio will cause condensation. The trachea are lined with cuticle that is impermeable to water, but the tracheoles are liquid filled and have permeable walls, making it possible for water and respiratory gases to move through these walls (Krogh, 1941; Wigglesworth, 1965; Kerkut and Gilbert, 1985; Mill, 1985). Therefore, increasing the hydrostatic pressure in the tracheal system above the supersaturation ratio will lead to the condensation of water and the movement of water from the trachea into the tracheoles. The remarkably high intratracheal pressure pulses observed during the interburst period in *S. gregaria* could be high enough to meet, or perhaps even exceed, the supersaturation ratio for water vapour, thus reversing the direction of water movement. According to Henry's law, at a given temperature the partial pressures of gases, like water vapour, within a gas mixture are elevated when the total pressure is elevated. In contrast, evaporation from a surface (like the tracheoles) occurs faster if the pressure is lower. The osmolality of the fluid within the tracheoles should set the equilibrium water partial pressure within the tracheal system.

If the osmolality of the tracheole fluid is assumed to be 300 mosmol l^{-1} – which is equivalent to a water chemical activity (a_w) of 0.995, or 99.5% relative humidity, and approximates values for insect haemolymph (see Edney, 1977) – at this high relative humidity tracheal pressure can be directly interpreted as water vapour pressure, assuming the system is in equilibrium. Therefore, the highest pressure pulses within an individual of around 1971 Pa are equivalent to water vapour pressure of 14.78 mmHg. At 15°C the saturation partial pressure of distilled water is 12.76 mmHg. This partial pressure exceeds the saturation pressure of water vapour and air in the tracheal system is therefore likely to be oversaturated during the interburst phase of the DGC. Another possibility is that these high pressure pulses could be used to ensure effective mixing of gases in the closed tracheal system of the insect, which could perhaps allow the insect to increase the duration of the interburst period as a result of more efficient distribution of O_2.

Terrestrial insects display at least three different patterns of gas exchange when at rest (for reviews, see Chown et al., 2006; Quinlan and Gibbs, 2006; Bradley, 2007). A DGC consists of three phases, which are typically described in terms of spiracular behaviour; namely a C, F and O spiracle phase (Schneiderman, 1960). In some of the recorded individuals, although a true C-phase of the DGC could be identified by means of subatmospheric intratracheal pressure recordings, some CO_2 continued to be released (e.g. Fig. 2, individual 2). This particularly novel finding has important implications for the identification of patterns solely on the basis of $\dot{V}_{CO_2}$ traces, although to date discussion has mostly focused on the use of appropriate flow rates or temperatures for pattern identification (e.g. Gray and Bradley, 2006; Terblanche and Chown, 2010; Contreras and Bradley, 2011).

In conclusion, this study highlights the importance of using multiple parameters when examining the mechanisms of gas exchange and suggests that much is still to be learned about the fundamentals of insect respiration.

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