

RESEARCH ARTICLE

Gas exchange patterns and water loss rates in the Table Mountain cockroach, *Aptera fusca* (Blattodea: Blaberidae)

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SUMMARY

The importance of metabolic rate and/or spiracle modulation for saving respiratory water is contentious. One major explanation for gas exchange pattern variation in terrestrial insects is to effect a respiratory water loss (RWL) saving. To test this, we measured the rates of CO₂ and H₂O release (\dot{V}_{CO_2} and $\dot{V}_{\text{H}_2\text{O}}$, respectively) in a previously unstudied, mesic cockroach, *Aptera fusca*, and compared gas exchange and water loss parameters among the major gas exchange patterns (continuous, cyclic, discontinuous gas exchange) at a range of temperatures. Mean \dot{V}_{CO_2} , $\dot{V}_{\text{H}_2\text{O}}$ and $\dot{V}_{\text{H}_2\text{O}}$ per unit \dot{V}_{CO_2} did not differ among the gas exchange patterns at all temperatures ($P > 0.09$). There was no significant association between temperature and gas exchange pattern type ($P = 0.63$). Percentage of RWL (relative to total water loss) was typically low ($9.79 \pm 1.84\%$) and did not differ significantly among gas exchange patterns at 15°C ($P = 0.26$). The method of estimation had a large impact on the percentage of RWL, and of the three techniques investigated (traditional, regression and hyperoxic switch), the traditional method generally performed best. In many respects, *A. fusca* has typical gas exchange for what might be expected from other insects studied to date (e.g. \dot{V}_{CO_2} , $\dot{V}_{\text{H}_2\text{O}}$, RWL and cuticular water loss). However, we found for *A. fusca* that $\dot{V}_{\text{H}_2\text{O}}$ expressed as a function of metabolic rate was significantly higher than the expected consensus relationship for insects, suggesting it is under considerable pressure to save water. Despite this, we found no consistent evidence supporting the conclusion that transitions in pattern type yield reductions in RWL in this mesic cockroach.

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INTRODUCTION

Given their small body size and relatively large surface area-to-volume relationships, water balance in terrestrial environments can pose significant challenges for many insects. Most water is lost by passive diffusion to the outside air through the cuticle (i.e. cuticular water loss, CWL) or through the spiracles (respiratory water loss, RWL) while exchanging gas with their environment (reviewed in Hadley, 1994; Benoit and Denlinger, 2010; Chown et al., 2011). However, the contribution of RWL to total water loss may be influenced by gas exchange pattern (e.g. Schimpf et al., 2009; Williams et al., 2010) or metabolic rate modulation (e.g. Terblanche et al., 2010), particularly under xeric conditions (e.g. Gefen, 2011), and may therefore be linked to evolutionary fitness (e.g. Schimpf et al., 2012). Manipulation of environmental conditions (such as temperature, moisture or oxygen availability) or the insect's state (rest *versus* active, dehydrated *versus* hydrated) can have a dramatic influence on the pattern and overall flux rates exhibited (see Lighton and Turner, 2008; Schimpf et al., 2009; Terblanche et al., 2008; Terblanche et al., 2010; Williams et al., 2010; Matthews and White, 2011a; Matthews and White, 2011b).

Insects produce at least three distinct gas exchange patterns at rest: continuous gas exchange (CGE), cyclic gas exchange or burst–interburst, and discontinuous gas exchange (DGE) (e.g. Marais and Chown, 2003; Gibbs and Johnson, 2004; Marais et al., 2005; Contreras and Bradley, 2011). A DGE cycle consists of three

phases that can be identified on the basis of spiracular behaviour (Schneiderman and Williams, 1955; Schneiderman, 1960) and their associated CO₂ emission patterns: the open (O) spiracle phase, when gas exchange takes place freely through diffusion [although sometimes aided by active convection (e.g. Loveridge, 1968; Miller, 1973; Groenewald et al., 2012)]; the closed (C) spiracle phase, when there is no exchange of gas between the insect's tracheae and the outside environment; and the flutter (F) phase, when spiracles open and close rapidly and some exchange of gases occurs (reviewed in Lighton, 1996; Chown et al., 2006a; Bradley, 2007; Hetz, 2007).

There are several adaptive and non-adaptive theories that have been proposed to explain the origin and maintenance of DGE in tracheated arthropods (see Chown et al., 2006a; Matthews and White, 2011a). One of the most prominent, and perhaps well supported, is the hygric hypothesis, which states that DGE evolved to reduce RWL by extending the C phase or reducing the O phase (Kestler, 1985; Lighton, 1994; Chown, 2002; Duncan et al., 2002; Chown and Davis, 2003; Chown et al., 2006b; White et al., 2007; Schimpf et al., 2009; Terblanche et al., 2010; Williams et al., 2010; Chown, 2011). However, this notion is not without controversy (see discussions in Chown, 2011; Contreras and Bradley, 2011; Matthews and White, 2011a), at least partly owing to the mechanisms by which RWL and CWL are regulated, their relative contribution to total water loss (TWL) (Chown,

2002; Chown and Davis, 2003; Terblanche et al., 2010) and the fact that many insects abandon DGE under conditions when it is thought to be most useful for water saving [e.g. higher temperatures, dehydration (Quinlan and Hadley, 1993)]. Alternatively, DGE or cyclic patterns are present in some mesic species, such as water striders, which are expected not to be under desiccation stress (Contreras and Bradley, 2011).

Some studies have argued that RWL is a negligible component of TWL (Edney, 1977; Hadley, 1994; but see Chown and Davis, 2003). In the German cockroach, for example, ~95% of water loss occurs through cuticular transpiration, while respiratory transpiration accounts for 3.4–4.4% of TWL (Dingha et al., 2005). However, RWL can be of importance for insects that have evolved under dry conditions, as the amount saved may be the difference between life or death for a xeric species, and indeed, may therefore be the subject of natural selection for pattern variation (Chown and Davis, 2003; Duncan et al., 2002; Gibbs et al., 2003; Benoit and Denlinger, 2007; Schimpf et al., 2012). Furthermore, the regulation of metabolic rate (MR) and gas exchange patterns have considerable impacts on the way in which RWL can be adjusted (Terblanche et al., 2010; Woods and Smith, 2010; Weldon et al., 2013), which in turn may be influenced by life-style factors (e.g. aptery, diapause).

Here, we investigate gas exchange and water loss rates of the Table Mountain cockroach, *Aptera fusca* (Thunberg 1784), a poorly studied mesic insect species. We hypothesize that if DGE is a water saving strategy, *A. fusca* will favour DGE above other gas exchange patterns under desiccating conditions (e.g. high temperatures, low humidity), and that $\dot{V}_{\text{H}_2\text{O}}$ per unit \dot{V}_{CO_2} (where $\dot{V}_{\text{H}_2\text{O}}$ and \dot{V}_{CO_2} are the rates of H_2O and CO_2 release, respectively) will be lower during DGE than CGE (see Williams et al., 2010). We also expect that the percentage of RWL relative to TWL will be lower during DGE than other gas exchange patterns. Because measurement of RWL for gas exchange patterns other than DGE (i.e. CGE and cyclic gas exchange) is not straightforward, we compare three techniques for partitioning CWL and RWL, and test their repeatability (following Gray and Chown, 2008). Finally, to better comprehend how the study species fits into a broader (global) perspective, we compare our results with those for other insect species.

MATERIALS AND METHODS

Animals

Adult *Aptera fusca* (Blattodea: Blaberidae) females were collected from two localities in the Western Cape, South Africa. Ten individuals were collected from Jonaskop, Villiersdorp (33°58'00"S, 19°30'00"E), and eight individuals were collected from Landdroskop, Hottentots Holland Nature Reserve (34°0'6.48"S, 19°1'18.12"E), in late autumn/early winter. Animal field collection was undertaken under Cape Nature permit number 0056-AAA007-00006. Animals were maintained at a constant temperature of 18±4°C, a relative humidity (RH) of 50–90% and a 10h:14h light:dark photoperiod. Temperature and RH conditions were verified with iButton hygchron temperature/humidity loggers (Maxim/Dallas Semiconductors, Sunnyvale, CA, USA).

Cockroaches from the two different localities were placed in separate glass terrariums, thereby creating two separate colonies. Individuals for each experimental trial were selected at random from the different colonies, as preliminary trials suggested no body mass or resting \dot{V}_{CO_2} differences between individuals from the different colonies. The terrariums contained sterilized potting soil, empty egg cartons (to create refugia), as well as restios (Restionaceae) and wood/bark from their natural environment. Animals were fed a diet of mixed nuts (Montagu dried fruit: Mixed nuts raw; Montagu,

Western Cape, South Africa) and seeds (Montagu dried fruit: Seed & almond mix), oats, fish food (Tetra goldfish flakes; Melle, Osnabrück, Germany), fresh lettuce and apple slices *ad libitum*. Water was provided as soaked cotton wool and the containers were lightly sprayed with distilled water daily to maintain high humidity levels (~50–90%). Animals were fasted, but allowed access to water, for at least 12 h before respirometry commenced.

Respirometry

Each individual was weighed to 0.1 mg before and after each trial using an electronic microbalance (Model MS104S, Mettler Toledo, Greifensee, Switzerland). $\dot{V}_{\text{H}_2\text{O}}$ and \dot{V}_{CO_2} were recorded in a darkened 40 ml cuvette. A short plastic rod was placed in each cuvette for the animal to grasp (simulating their host plants) to reduce activity of the animal during respirometry trials. Individuals were given a period of at least 5 min to settle in the cuvette before recording commenced. When handling the animals, care was taken to minimize contact with the cuticle to avoid accidental abrasion, which could elevate CWL rates (see Johnson et al., 2011). Pilot trials with visual observation or recordings with a custom-built electronic activity detector clearly showed that activity abolished cyclic or DGE patterns, and resulted in the exhibition of CGE.

Flow-through respirometry was undertaken to record \dot{V}_{CO_2} and $\dot{V}_{\text{H}_2\text{O}}$. An infrared $\text{CO}_2/\text{H}_2\text{O}$ analyzer (Li-7000, Li-Cor, Lincoln, NE, USA) was set up as follows. For the gas switches (100% O_2 and 100% N_2), a pressurized gas cylinder (Air Products South Africa, Cape Town, South Africa) fed one of the gases into the system. Where normoxic conditions were required, an aquarium pump (AC-9610, Hailea Group, Guangdong, China) was used to feed atmospheric air into the system. The air stream was then fed through a scrubber column containing soda lime (Merck, Gauteng, RSA) and another scrubber containing silica gel/Drierite (ratio 1:1) (Merck, Gauteng, RSA/Sigma-Aldrich, St Louis, MO, USA) to remove CO_2 and water vapour from the air stream. Scrubbed air was then fed through a flow control valve (Model 840, Side-Trak, Sierra Instruments, Monterey, CA, USA) and regulated at a constant flow rate of 200 ml min^{-1} by a mass flow control unit (Sable Systems, MFC-2, Las Vegas, NV, USA). Thereafter, air flowed through the zero channel of the $\text{CO}_2/\text{H}_2\text{O}$ analyzer and through the cuvette containing the cockroach, which was placed into a programmable water bath (Huber CC-410-WL, Peter Huber Kältemaschinenbau, Offenburg, Germany) to regulate the temperature of the cockroach. Air leaving the cuvette then entered the gas analyzer through another channel, which thus recorded the difference in CO_2 and H_2O concentration of the air before and after it flowed through the respirometry cuvette, at 1 s intervals. The output of the analyzer (\dot{V}_{CO_2} and $\dot{V}_{\text{H}_2\text{O}}$) was recorded *via* Li-7000 software on a standard desktop computer. Only periods where no activity was visible, based on recordings of movement from the electronic activity detector, were used in analyses. The time constant of the cuvette was determined to be 12 s (40/200×60 s), meaning that it takes 60 s (12×5 s) for 99% of the CO_2 that is released by the animal to be removed from the cuvette and detected by the analyzer (Gray and Bradley, 2006). The shortest C-phase duration that we recorded was 564 s; therefore, we were confident that the different phases of the DGE cycles could be accurately detected.

Gas exchange pattern

Trials were conducted under normoxic conditions, at 0% RH and 15°C. Each individual ($N=13$) was run in three separate trials (allowing a rest period of 2–5 days between trials) to allow analyses of repeatability. Time of day was randomized among individuals' trials to avoid potential diurnal effects on gas

exchange. Gas exchange patterns were identified based on \dot{V}_{CO_2} . Cyclic gas exchange was identified according to methods described in Marais et al. (Marais et al., 2005), namely that if a line is drawn through the middle of the data trace, <30% of the data points should be above the line. If >30% of the data points are above the line, then the pattern would be identified as CGE. Discontinuous gas exchange was identified on the basis of a true C phase being present. The F phase of the DGE cycle could not be identified for all individuals when looking at the \dot{V}_{CO_2} trace. For this reason, and because the F phase may begin before CO₂ release is detected, C and F phases were combined to form the closed/flutter (CF) phase and analyzed as such (e.g. Wobschall and Hetz, 2004; Groenewald et al., 2012).

Hyperoxic switch

Trials were conducted at 0% RH and 15°C. Each trial began with a period of 3–4 h at normoxic conditions. Air was then switched to 100% O₂ (hyperoxic switch) for 30 min, followed by a switch to 100% N₂ (anoxic switch) for another 30 min. Thereafter, the gas was switched to normoxic air. A period of 30 min was gauged as adequate time to ensure maximum spiracle opening and closing to evaluate the highest and lowest steady-state water loss rates, respectively. Time of day was randomized among individuals' trials to avoid potential diurnal effects on gas exchange.

Temperature switches

To investigate the effects of temperature on gas exchange pattern and water loss rate (WLR), individuals ($N=10$) were subjected to increasing temperatures using a programmable water bath. The same respirometry setup was used as in the hyperoxic switch experiment. Trials were conducted under normoxic conditions at 0% RH, and individuals were exposed to temperatures in the following order: 15, 20 and 25°C for 3 h at each temperature, and 30°C for 2 h. The heating rate between each of the temperatures was 1°C min⁻¹. The temperature range of 15–30°C represents the mean annual to mean maximum annual temperatures that *A. fusca* experience in their natural environment [see table 3 in Clusella-Trullas et al. (Clusella-Trullas et al., 2009)].

Data extraction

Data were extracted using ExpeData data acquisition and analysis software (v. 1.1.25, Sable Systems) and statistical analyses were performed using STATISTICA v. 10 (StatSoft, Tulsa, OK, USA). Data were inspected for outliers, defined as individuals having a mass-specific \dot{V}_{CO_2} that is more than two standard deviations higher or lower than the mean for all animals. No overall outliers were found, although for some tests where subsets of data were used, significant outliers were excluded, and these are stated below. Individuals were assessed for different gas exchange patterns based on their \dot{V}_{CO_2} . Where individuals displayed cyclic gas exchange or DGE, data from one to five consecutive cycles per individual were extracted. A DGE cycle was measured from the onset of the C phase until the end of the O phase. Examples of the different gas exchange patterns that were detected are shown in Fig. 1A–C.

Water loss partitioning methods

Three different methods were used to partition CWL from RWL.

Traditional method

In the traditional method, RWL is calculated as the difference between TWL (O phase) and CWL (CF phase) (e.g. Lighton, 1992; Hadley and Quinlan, 1993).

Hyperoxic switch method

In the hyperoxic switch method, RWL is calculated by subtracting CWL [hyperoxic C (hypC) phase] from TWL (anoxic burst). For data extraction from the hypC phase, a period of 2 min was given after the gas switch occurred to ensure that the treatment gas had reached the individual. The hypC phase was defined as a period of at least 30 s during which mean \dot{V}_{CO_2} was less than the mean \dot{V}_{CO_2} preceding the switch (see Fig. 1D for example), as spiracle closure was not always visible. The anoxic burst was taken as the period (>120 s), after the gas switch to anoxia, during which maximum CO₂ release occurred.

Regression method

In the regression method, \dot{V}_{CO_2} is regressed against $\dot{V}_{\text{H}_2\text{O}}$ (dependent variable), yielding a positive relationship. A period (>25 min) of the data trace was chosen where the individual was at rest and where high variation in CO₂ and H₂O was visible. The y -intercept of the regression line is equivalent to CWL (Gibbs and Johnson, 2004) and RWL is calculated as the difference between TWL and CWL.

Statistical analysis

Gas exchange patterns and water loss partitioning methods

The three different gas exchange patterns (DGE, CGE and cyclic) were compared for the following variables: \dot{V}_{CO_2} , $\dot{V}_{\text{H}_2\text{O}}$, $\dot{V}_{\text{H}_2\text{O}}$ per unit \dot{V}_{CO_2} , slope of regression of \dot{V}_{CO_2} and $\dot{V}_{\text{H}_2\text{O}}$, CWL (i.e. regression method), RWL and percentage RWL (%RWL; relative to TWL) using restricted maximum likelihood (REML) ANOVA. Individual was considered a random effect, while body mass and gas exchange pattern were taken to be fixed effects. The three gas exchange patterns were coded separately except in analyses where the total number of data points was too small to create three separate categories (e.g. temperature experiments), and in analyses on subsets of the data that did not include any instances of cyclic gas exchange.

To detect bias, the three water loss partitioning methods were compared with each other using Friedman's ANOVA with CWL, RWL and %RWL as dependent variables, water loss partitioning method as the repeated effect, and individual as the categorical predictor. Values were compared only for those runs that exhibited DGE and a hypC phase ($N=6$ trials for 4 individuals). However, the hyperoxic switch method was designed specifically for insects performing CGE (Lighton et al., 2004), so CWL, RWL and %RWL estimates calculated by the hyperoxic switch method were compared among gas exchange patterns using REML ANOVA with individual as a random effect, and body mass and gas exchange pattern as fixed effects ($N=16$ trials for 8 individuals).

Among- versus within-individual variation was assessed by calculating repeatability of mean \dot{V}_{CO_2} , mean $\dot{V}_{\text{H}_2\text{O}}$, anoxic $\dot{V}_{\text{H}_2\text{O}}$ burst, CWL and RWL from each of the three methods according to the procedure described in Lessells and Boag (Lessells and Boag, 1987). Repeatability, confidence intervals (Krebs, 1999) and standard error (Becker, 1984) were calculated using values obtained from a one-way ANOVA in STATISTICA, with individual as the categorical predictor.

Effect of temperature on gas exchange

A χ^2 contingency table comparing observed with expected values was used to evaluate the association of gas exchange pattern with temperature (taken as a categorical variable). To assess the relationship of \dot{V}_{CO_2} and $\dot{V}_{\text{H}_2\text{O}}$ with a change in temperature, the mean rate and emission volume of CO₂ and H₂O, and the ratio of $\dot{V}_{\text{H}_2\text{O}}$ per unit \dot{V}_{CO_2} for the total of all phases together, were compared for CGE and DGE/cyclic gas exchange using Mann–Whitney U -

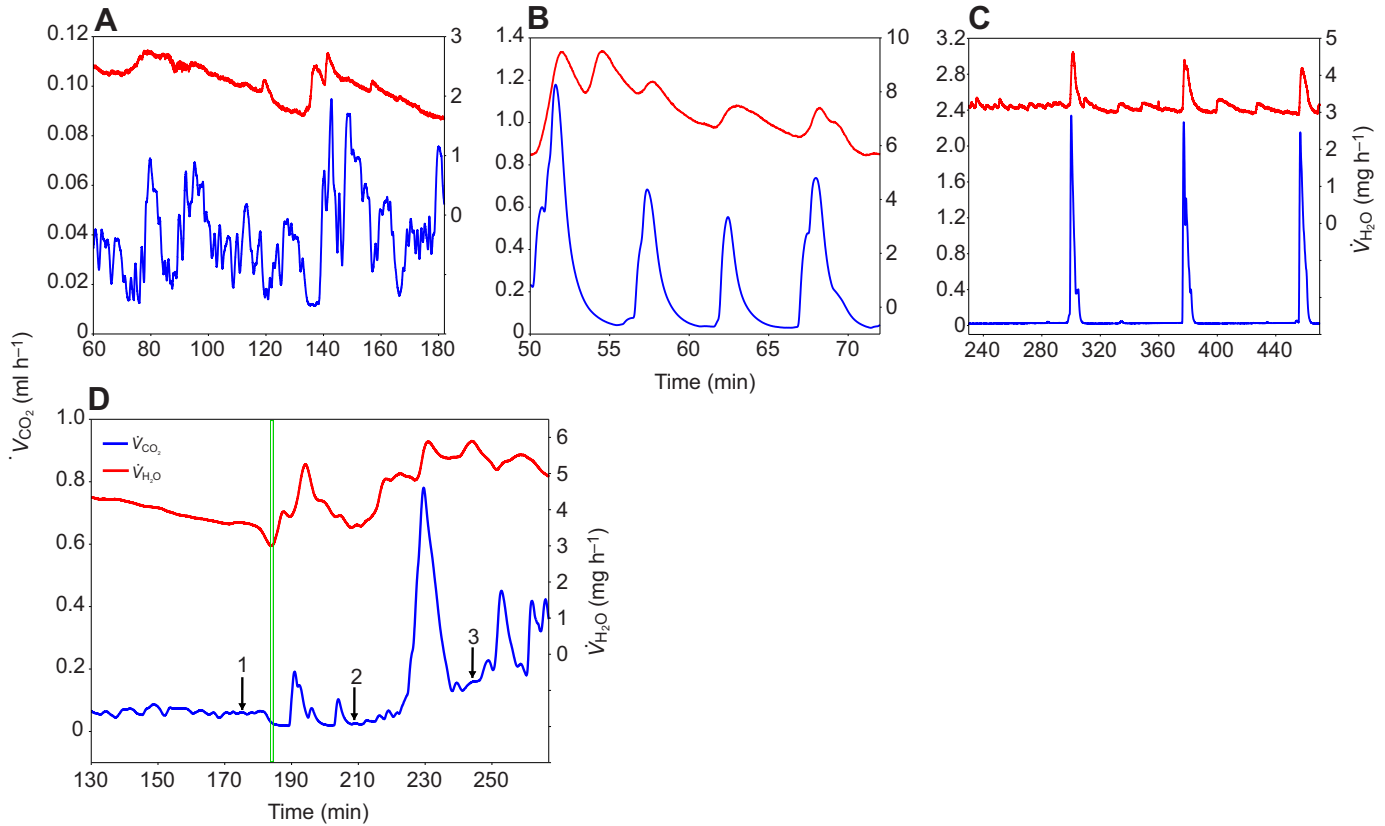


Fig. 1. Representative continuous, cyclic and discontinuous gas exchange patterns for *Aptera fusca*. Measurements were made at 15°C and a flow rate of 200 ml min⁻¹. Examples of the different gas exchange patterns: (A) continuous gas exchange (mass: 1.927 g); (B) cyclic gas exchange (mass: 1.861 g); and (C) discontinuous gas exchange (mass: 1.892 g). For a discussion regarding how the different gas exchange patterns were defined, see Materials and methods. (D) A typical recording of an *A. fusca* individual (mass: 2.179 g) for the hyperoxic switch method for estimation of respiratory water loss. The different gas switches are indicated on the graph: (1) hyperoxic switch (100% O₂); (2) anoxic switch (100% N₂); and (3) switch back to normoxia. A large dip in \dot{V}_{H_2O} (rate of H₂O release) is clearly visible following the switch to hyperoxic gas. A dip in \dot{V}_{CO_2} (rate of CO₂ release) that coincides with the dip in \dot{V}_{H_2O} is also visible. When the gas is switched to nitrogen, a rise in both \dot{V}_{CO_2} and \dot{V}_{H_2O} occurs. The period marked with the green bar indicates the part of the data trace that was used for the calculation of respiratory water loss.

tests for each temperature separately (15, 20, 25 and 30°C). Cyclic gas exchange was combined together with DGE for this analysis because there were only three cases of cyclic gas exchange out of 40 trials. Next, Friedman's ANOVAs were performed across the four temperatures to compare mean overall, CF phase and O phase of DGE values for the following variables: duration, \dot{V}_{CO_2} , CO₂ emission volume, \dot{V}_{H_2O} , H₂O emission volume, and \dot{V}_{H_2O} per unit \dot{V}_{CO_2} . We then performed linear regressions of each gas exchange and water loss variable against temperature. Differences in slopes were compared with a general linear model homogeneity-of-slopes test in STATISTICA. Finally, we performed linear regressions of \dot{V}_{CO_2} with DGE cycle frequency and O-phase CO₂ emission volume to determine whether the observed increase in MR with temperature was due to increased burst frequency or increased emission volume (see Klok and Chown, 2005).

Interspecific comparison

Mean \dot{V}_{CO_2} at 25°C, taken from the temperature experiments ($N=9$, because one individual was a significant outlier), was converted from ml CO₂ h⁻¹ to μ W by first converting to units of ml O₂ h⁻¹ and then using the oxyjoule conversion factor of $(16+5.164 \times RQ)$ J ml⁻¹ (Lighton et al., 1987), where RQ is the respiratory quotient and is assumed to be 0.84 (Lighton, 2008).

A data set including gas uptake rate (mol O₂ day⁻¹) and WLR (mol H₂O day⁻¹) for 30 insect species was compiled by Woods

and Smith (Woods and Smith, 2010). To compare the variation in \dot{V}_{H_2O} with gas exchange rate for *A. fusca* with that of the universal model for insects, we added 10 non-independent data points for *A. fusca* to the Woods and Smith (Woods and Smith, 2010) data set: means from gas exchange at 15, 20, 25 and 30°C; mean of pooled individuals showing DGE/cyclic patterns at 15°C; mean of individuals showing CGE at 15°C; maximum steady state rates obtained during the anoxic burst; mean hypC-phase rates; and finally the mean CF- and O-phase rates obtained at 15°C in individuals showing DGE. Although it could be argued that comparison of slopes from intraspecific and interspecific regressions may be of limited value (Heusner, 1991), we reasoned that a reduction in \dot{V}_{CO_2} associated with a transition from CGE to cyclic and/or to DGE, if it were useful for saving water, should result in a decrease in the orthogonal distance to the interspecific relationship, and therefore included a comparison with the intraspecific relationship.

RESULTS

Of the 39 total repetitions, almost two-thirds of all individuals exhibited CGE (62% of trials), while cyclic gas exchange was the rarest pattern (5% of trials; Table 1). Four individuals consistently had the same gas exchange pattern during every trial, and only one of these exhibited DGE consistently. Within the hyperoxic switch trials, almost two-thirds exhibited a hypC phase (63% of trials), and

Table 1. Summary of the number of observations of each gas exchange pattern exhibited by each of the 13 recorded *Aptera fusca* individuals, for each of the trials (three trials per individual)

Individual	Mass (g)	Normoxia			Hyperoxic switch		
		CGE	DGE	Cyclic	Mean cycles	hypC phases	Anoxic bursts
1	2.30±0.09	0	3	0	2.3		
2	1.96±0.07	2	1	0	3.0	3	3
3	1.94±0.04	1	2	0	4.5	3	3
4	1.81±0.07	2	1	0	5.0	2	2
5	2.10±0.09	2	0	1	3.0	3	3
6	2.34±0.07	3	0	0		1	2
7	2.57±0.08	3	0	0			
8	2.09±0.18	2	1	0	2.0	2	3
9	2.37±0.12	1	2	0	3.5	2	2
10	2.52±0.05	3	0	0		1	1
11	2.61±0.08	1	2	0	3.0		
12	2.33±0.09	2	0	1	4.0	0	1
13	2.78±0.17	2	1	0	3.0		
Total		24 (62%)	13 (33%)	2 (5%)		17 (63%)	20 (74%)

The mean number of cycles (where cyclic gas exchange was present), as well as the number of hypC-phases and anoxic bursts that were observed during the hyperoxic switch, are also indicated. Mass data are means ± s.e.m.

CGE, continuous gas exchange; DGE, discontinuous gas exchange; hypC phase, hyperoxic C phase.

in 74% of the trials an anoxic burst was visible in the H₂O trace (Table 1).

None of the measured metabolic or water loss variables differed significantly across gas exchange pattern types when measured over 22 h at 15°C (Table 2). CWL, RWL and %RWL estimates differed significantly among water loss partitioning methods when compared only among individuals that performed DGE and had a hypC phase (Table 2). CWL, as estimated by the hyperoxic switch method, was significantly lower than when measured by the other two methods ($\chi^2=9.00$, d.f.=2, $P=0.011$). RWL and %RWL were significantly higher when calculated by the hyperoxic switch method than when calculated with the other two methods ($\chi^2=9.00$, d.f.=2, $P=0.011$ and $\chi^2=9.00$, d.f.=2, $P=0.011$, respectively). RWL as measured by the hyperoxic switch method was significantly higher in individuals that performed DGE prior to the gas switch than those that performed CGE (REML ANOVA, $F=7.63$, d.f.=1, 6, $P=0.033$). Repeatability was low across the tested range of criteria, except for the anoxic H₂O burst, which was significantly highly repeatable ($F=39.28$, d.f.=8, 11, $P<0.0001$; Table 3).

Temperature effects on \dot{V}_{CO_2} and \dot{V}_{H_2O}

There was no significant association between gas exchange pattern and temperature ($\chi^2=4.31$, d.f.=6, $P=0.63$). \dot{V}_{CO_2} , \dot{V}_{H_2O} and \dot{V}_{H_2O} per unit \dot{V}_{CO_2} did not differ significantly among CGE and DGE/cyclic gas exchange at any of the four temperatures. However, at 30°C, CO₂ emission volumes were significantly higher during CGE than during DGE (Table 4). Mean \dot{V}_{CO_2} and \dot{V}_{H_2O} increased significantly with an increase in temperature (Fig. 2A, supplementary material Table S1), while \dot{V}_{H_2O} per unit \dot{V}_{CO_2} significantly decreased with increasing temperature. DGE CF-phase \dot{V}_{CO_2} was significantly higher at 25°C than at the other temperatures used in the trials (Fig. 2B, supplementary material Table S1). DGE CF-phase CO₂ emission volume significantly decreased with increasing temperature (Fig. 2B). Closed/flutter and O-phase duration decreased significantly, although at different rates, with an increase in temperature (homogeneity-of-slopes test: $F=18.56$, d.f.=1, $P<0.001$; Fig. 3, supplementary material Table S2).

In regression analysis we found a significant positive relationship between trial temperature and log \dot{V}_{CO_2} , and a significant negative

Table 2. Comparison of gas exchange and water loss variables among different gas exchange pattern types and methods of partitioning water loss in *A. fusca*

	Gas exchange pattern ^a				Water loss partitioning method ^b				Hyperoxic switch method ^c		
	DGE	CGE	Cyclic	<i>P</i>	Hyperoxic switch			<i>P</i>	DGE	CGE	<i>P</i>
					Traditional	Regression	<i>P</i>				
\dot{V}_{CO_2} (ml h ⁻¹)	0.09±0.01	0.12±0.01	0.08±0.03	0.084							
\dot{V}_{H_2O} (mg h ⁻¹)	2.94±0.43	3.06±0.45	2.46±0.64	0.498							
\dot{V}_{H_2O} per unit \dot{V}_{CO_2}	32.64±3.82	24.95±2.48	36.94±19.83	0.129							
Slope	1.62±0.61	1.75±0.28	1.85±1.58	0.892							
CWL \dot{V}_{H_2O} (mg h ⁻¹)	2.72±0.36	2.80±0.42	2.10±0.49	0.321	6.29±0.97	2.47±1.20	6.06±1.08	0.011	2.47±1.20	2.66±0.49	0.873
RWL \dot{V}_{H_2O} (mg h ⁻¹)	0.22±0.10	0.26±0.05	0.36±0.14	0.767	0.15±0.11	3.74±0.47	0.38±0.22	0.011	3.74±0.47	2.06±0.36	0.033
%RWL of TWL	6.49±1.97	8.91±1.34	13.98±2.21	0.255	1.71±1.03	67.81±13.08	6.14±3.50	0.011	67.81±13.08	45.67±7.19	0.204

Trials were run at 15°C (values are means ± s.e.m.). DGE, discontinuous gas exchange; CGE, continuous gas exchange; \dot{V}_{CO_2} , rate of CO₂ release; \dot{V}_{H_2O} , rate of H₂O release; CWL, cuticular water loss; RWL, respiratory water loss; TWL, total water loss. Statistically significant differences are highlighted in bold.

^a $N=39$ trials on 13 cockroaches; P -values are from REML ANOVA. CWL and RWL were calculated using the regression method as this method is applicable to all gas exchange patterns.

^b $N=6$ trials on 4 cockroaches; P -values are from Friedman's ANOVA. Only experimental trials that exhibited both DGE and a hyperoxic C phase were utilized for this analysis.

^c $N=16$ trials on 8 cockroaches; P -values are from REML ANOVA. All experimental trials that exhibited a true hyperoxic C phase were utilized for this analysis, regardless of gas exchange pattern, but there were no instances of cyclic gas exchange which fit these criteria.

Table 3. Repeatability values for mean \dot{V}_{CO_2} , mean $\dot{V}_{\text{H}_2\text{O}}$, CWL, RWL, anoxic burst, and slope of the regression method

	F-ratio	d.f.	Repeatability	Lower CI	Upper CI	n_o
Mean \dot{V}_{CO_2}	1.71	12, 26	0.191±0.10	-0.12	0.58	3.0
Mean $\dot{V}_{\text{H}_2\text{O}}$	2.01	12, 26	0.252±0.11	-0.07	0.63	3.0
CWL						
Traditional method	1.72	7, 5	0.312±0.45	-0.90	0.84	1.6
Hyperoxic method	1.23	7, 9	0.101±0.35	-0.51	0.70	2.1
Regression method	2.03	12, 26	0.256±0.19	-0.07	0.63	3.0
RWL						
Traditional method	2.32	7, 5	0.454±0.38	-0.72	0.88	1.6
Hyperoxic method	1.16	7, 9	0.071±0.36	-0.53	0.69	2.1
Regression method	1.08	12, 26	0.025±0.17	-0.23	0.43	3.0
Anoxic H ₂ O burst	39.28*	8, 11	0.946±0.04	0.82	0.99	2.2
Slope (regression method)	0.98	12, 26	-0.008±0.17	-0.25	0.39	3.0

Cuticular water loss (CWL) and respiratory water loss (RWL) were calculated by using the three different methods.

n_o is a weighted mean representing the number of data points used per individual in the ANOVA out of a maximum of three. If all three experimental runs were used in analysis for all individuals (balanced design), then $n_o=3$ (Lessells and Boag, 1987).

* $P<0.0001$. Statistically significant repeatability is highlighted in bold. \dot{V}_{CO_2} , rate of CO₂ release; $\dot{V}_{\text{H}_2\text{O}}$, rate of H₂O release.

relationship between trial temperature and log $\dot{V}_{\text{H}_2\text{O}}$ per unit \dot{V}_{CO_2} (supplementary material Table S2). Additionally, frequency of DGE cycles, CF-phase \dot{V}_{CO_2} and $\dot{V}_{\text{H}_2\text{O}}$, and O-phase $\dot{V}_{\text{H}_2\text{O}}$ all increased significantly with elevated temperature (supplementary material Table S2). \dot{V}_{CO_2} was positively and significantly correlated with frequency, but not with O-phase CO₂ emission volume. This indicates that increased \dot{V}_{CO_2} with increased temperature is a function of increased frequency of bursts, and not increased burst volume (supplementary material Table S2).

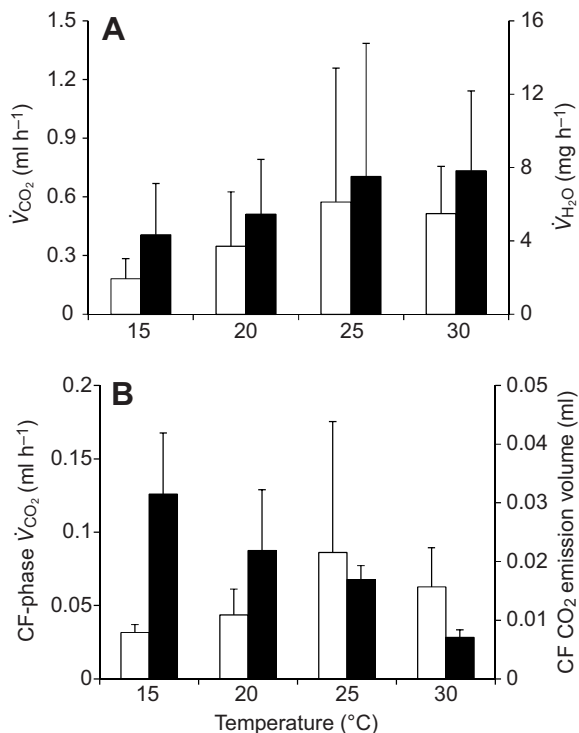


Fig. 2. Results of Friedman's ANOVA to compare means of \dot{V}_{CO_2} , $\dot{V}_{\text{H}_2\text{O}}$ and closed/flutter (CF)-phase CO₂ emission volume among temperature trials (A) averaged across several discontinuous gas exchange (DGE) cycles, and (B) averaged for a number of CF phases of the DGE cycle. Open bars correspond to the left y-axis, and solid bars correspond to the right y-axis. Data are means \pm s.d.

Relationship of water loss to metabolic rate

Mean mass-specific MR for *A. fusca* at 25°C was 144.017 $\mu\text{l CO}_2\text{g}^{-1}\text{h}^{-1}$, and observed WLR was 5.085 mg h^{-1} (data from temperature switch trials). The ratio of log MR to log WLR was not significantly different between DGE and CGE (mean \pm s.e.m.=1.62 \pm 0.02, 1.57 \pm 0.03, respectively; one-way ANOVA, $F=0.55$, d.f.=2, $P>0.05$). WLRs of *A. fusca* were higher than those expected based on MR of any of the other insect species included in the Woods and Smith (Woods and Smith, 2010) data set. Further, most WLR values that were measured under a range of experimental conditions and states for *A. fusca* fall outside of the 95% prediction interval of the WLR–gas-uptake-rate relationship for all insect species. Only three measured WLR values (obtained from maximum \dot{V}_{CO_2} during anoxic burst, mean O-phase \dot{V}_{CO_2} at 15°C and mean \dot{V}_{CO_2} at 25°C) fell within the 95% prediction interval (Fig. 4).

DISCUSSION

From this study we have established a basic understanding of gas exchange variation and concomitant water loss for *A. fusca*. In addition to adding new data to a growing global data set of gas exchange characteristics of diverse terrestrial organisms (see White et al., 2007; Terblanche et al., 2008; Woods and Smith, 2010), this baseline understanding allows us to contribute to the debate

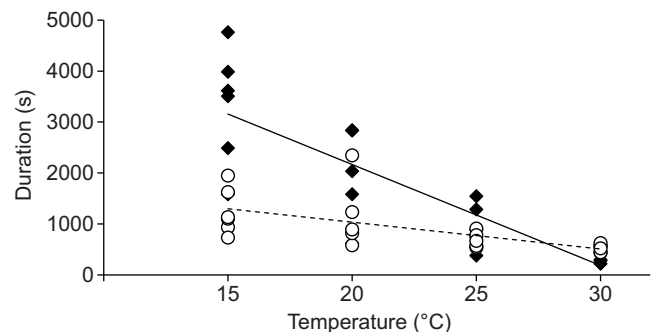


Fig. 3. Linear regressions of closed/flutter (CF)-phase duration (filled diamonds, solid line) and open (O)-phase duration (open circles, dashed line) with temperature. Regression equation for CF phase duration: $y=-198.48x+6136.308$; $r^2=0.699$; $P<0.0001$. Regression equation for O phase duration: $y=-52.689x+2087.951$; $r^2=0.369$; $P<0.0035$.

Table 4. Comparison of \dot{V}_{CO_2} , \dot{V}_{H_2O} and emission volume at different temperatures within different gas exchange patterns (results from Mann–Whitney U-test)

Temperature (°C)	\dot{V}_{CO_2} (ml h ⁻¹)				\dot{V}_{H_2O} per unit \dot{V}_{CO_2}				CO ₂ emission volume (ml)				H ₂ O emission volume (mg)							
	CGE	N	DGE*	N	CGE	N	DGE*	N	CGE	N	DGE*	N	CGE	N	DGE*	N				
15	0.22±0.07	4	0.15±0.02	6	5.65±1.75	4	3.15±1.00	4	27.01±2.48	4	25.60±8.28	4	0.29±0.07	4	0.19±0.03	6	7.49±1.44	4	3.90±1.32	4
20	0.26±0.07	5	0.40±0.16	5	5.21±1.52	5	6.71±1.98	3	19.14±3.10	5	24.85±11.01	3	0.47±0.20	5	0.27±0.06	5	8.97±3.69	5	5.91±1.78	3
25	0.29±0.11	3	0.69±0.30	7	3.64±1.50	3	9.62±3.85	5	12.39±3.97	3	16.55±4.39	5	0.74±0.42	3	0.35±0.10	7	8.15±5.27	3	5.48±1.07	5
30	0.41±0.09	4	0.58±0.11	6	5.34±0.90	4	10.30±2.47	4	13.82±2.20	4	21.67±4.44	4	0.81±0.17	4	0.20±0.09	6	10.54±1.71	4	4.06±1.75	4

Values are means ± s.e.m. Bold type indicates significant difference at the 95% level ($P=0.04$).

*DGE includes DGE and cyclic gas exchange patterns. \dot{V}_{CO_2} , rate of CO₂ release; \dot{V}_{H_2O} , rate of H₂O release; CGE, continuous gas exchange; DGE, discontinuous gas exchange.

regarding the evolutionary origins and potential benefits of gas exchange pattern variation, and in particular DGE, in the context of water savings.

Gas exchange pattern

For *A. fusca*, gas exchange pattern is highly variable across individuals, and even over time within a single individual. Only four out of the total of 13 recorded individuals showed the same gas exchange pattern during every trial (Table 1). This variability in pattern type within and between individuals has also been observed in other cockroach species (Miller, 1973; Marais and Chown, 2003; Gray and Chown, 2008; but see Schimpf et al., 2012). Of the total of 39 trials that were run, DGE was exhibited in 33% of the trials, CGE in 62% and cyclic gas exchange in 5% (Table 1).

Comparing methods for partitioning water loss

Calculating CWL using the traditional method is precise (Chown et al., 2006a; Gray and Chown, 2008) and can be used as a benchmark to compare the precision of the newer, alternative methods. For the hyperoxic switch method, all variables estimated had low repeatability, except for the anoxic burst. A true hypC phase and anoxic burst occurred in 63 and 74% of the runs, respectively, although complete spiracle opening and closure were still not achieved in all runs. This may indicate an overall insensitivity of the species to oxygen variation, which may be of further interest itself in light of the oxidative damage hypothesis of DGE (e.g. Hetz and Bradley, 2005; Boardman et al., 2012; Matthews et al., 2012).

Gray and Chown (Gray and Chown, 2008) found the regression method to be the least accurate and obtained negative values for RWL in some cases. We found that the results obtained from this method were highly reliant upon appropriate data selection. Once the data were suitably extracted, however, when comparing the absolute and percentage values of CWL and RWL obtained across the three different methods, the regression method resulted in values similar to those of the traditional method. The values obtained from the hyperoxic switch method were higher, although not always significantly so, for RWL and %RWL (relative to TWL), and significantly lower for CWL. In the case of %RWL, the estimate obtained from the hyperoxic switch method was very different from that of the traditional and regression methods. This result is largely in keeping with other studies. For example, Gray and Chown (Gray and Chown, 2008) found that the hyperoxic switch method yielded significantly higher estimates for %RWL than the other two methods. However, the difference in their estimates was smaller than that found in this study [present study: 45–67% RWL in hyperoxic switch *versus* 2–14% RWL in traditional and regression methods, respectively; Gray and Chown (Gray and Chown, 2008): 21% RWL in hyperoxic switch *versus* 13–14% RWL in traditional and regression methods, respectively] and may be a consequence of the fact that the hyperoxic switch method was developed for estimation of RWL on continuous, rather than discontinuous, gas exchange patterns (Lighton et al., 2004). Mellanby (Mellanby, 1934) found that if an insect's spiracles are forced open (because of anoxia or hypercapnia), WLR increases at a rate that is independent of MR. This increased water loss because of forced spiracular opening in our trials could therefore have contributed to the different values that were obtained by the hyperoxic switch method in comparison with values obtained from the other two methods. This indicates the need for additional comparisons of these three methods under varying experimental conditions and in different taxa, although

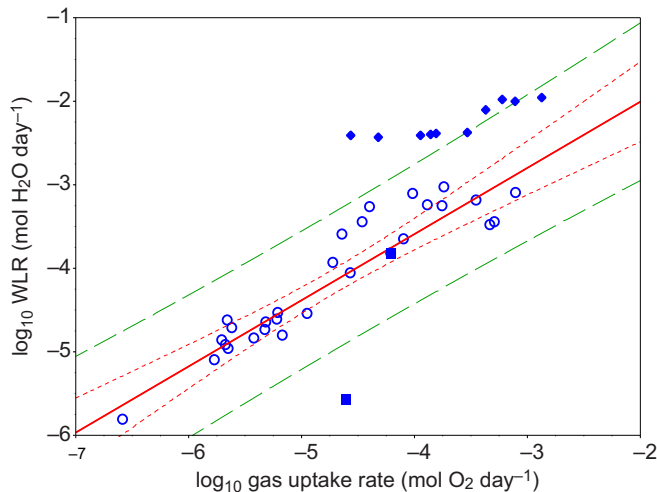


Fig. 4. Water loss rate (WLR) as a function of oxygen uptake rate (MR, metabolic rate) for 31 species of insects. Results obtained from our study for *Aptera fusca* are included with data for 30 species obtained from Woods and Smith (Woods and Smith, 2010). Regression equation for all insects ($N=30$): $\log_{10} \text{WLR} = 0.792 \times \log_{10} \text{MR} - 0.421$ (indicated by solid line; red stippled lines indicate 95% confidence intervals; green dashed lines indicate 95% prediction intervals). Open circles indicate values for 28 non-cockroach insect species and solid squares indicate values for two cockroach species from Woods and Smith (Woods and Smith, 2010). Solid diamonds indicate values obtained in this study for *A. fusca* under a range of experimental conditions and states measured in our experiments (see Materials and methods for details).

clearly gas exchange pattern type and the use of forced spiracular opening may be major factors influencing the outcomes of the three methods.

DGE as a water saving strategy

There was no significant difference in any gas exchange or water loss variables among the three gas exchange patterns at 15°C, suggesting limited support for pattern variation being associated with a RWL reduction (cf. Williams et al., 2010). At 15°C, we found %RWL (relative to TWL) to be 6.5% for DGE, 8.9% for CGE, and 14.0% for cyclic gas exchange, although this variation was not significant and did not follow the expected rank order (DGE < cyclic < CGE). The factors underlying this lack of difference are unclear. On the one hand, this may be a consequence of unusually high cuticular WLR. The cuticular permeability for *A. fusca* was calculated as being $12.3 \mu\text{g h}^{-1} \text{cm}^{-2} \text{Torr}^{-1}$. Compared with the cuticular permeabilities for other mesic [e.g. *Periplaneta americana*, $55 \mu\text{g h}^{-1} \text{cm}^{-2} \text{Torr}^{-1}$; *P. fuliginosa*, 57; *Diploptera punctata*, 20.9; *Pycnoscelus surinamensis*, 38.7; *Blatta orientalis*, 48 (Edney, 1977; Hadley, 1994)] and xeric cockroach species [e.g. *Arenivaga investigata*, $30 \mu\text{g h}^{-1} \text{cm}^{-2} \text{Torr}^{-1}$; *Arenivaga apache*, 80.6 (Edney, 1977; Hadley, 1994)] this value is relatively low, although these measurements from the literature were made under a range of different, typically hotter, conditions. However, cuticular permeability estimated for *Blattella germanica* [$3.42 \mu\text{g h}^{-1} \text{cm}^{-2} \text{Torr}^{-1}$ (Dingha et al., 2005)] and *Perisphaeria* sp. [$3.88 \mu\text{g h}^{-1} \text{cm}^{-2} \text{Torr}^{-1}$ (Gray and Chown, 2008)] is much lower.

On the other hand, the species may have an unusually high MR relative to what might be expected for its size. However, this was also unlikely to be the case for *A. fusca*. On a mass-specific basis, MR for *A. fusca* is similar to that of another cockroach, *Perisphaeria* sp., from South Africa [MR for all gas exchange patterns:

Perisphaeria, $73.9 \mu\text{l CO}_2 \text{g}^{-1} \text{h}^{-1}$ versus *A. fusca*, $107.1 \mu\text{l CO}_2 \text{g}^{-1} \text{h}^{-1}$ (our data from temperature switch trials at 20°C)] (Marais and Chown, 2003). The temperature coefficient (Q_{10}) for MR of this species is 2.1, which is also close to the assumed consensus value of 2.0 (Lighton, 2008), and influenced all pattern types similarly (supplementary material Table S1). For DGE, we found that this increase was due to an increase in cycle frequency and not an increase in burst volume, as was found in weevils (Klok and Chown, 2005) and grasshoppers (Chappell et al., 2009). Mean $\dot{V}_{\text{H}_2\text{O}}$ increased with higher temperature for DGE, but not for CGE (Table 4). At 30°C, however, the emission volume of H_2O was higher during CGE than during DGE, although not significantly so ($P=0.06$; Table 4). It may be argued that this outcome lends some support to the hygric hypothesis, as water loss is expected to be lower during DGE than during other gas exchange patterns, especially under more desiccating conditions (higher temperatures/lower humidity) (Schimpf et al., 2009). However, given the mesic habitat occupied by the species, one would have expected a shift between pattern types at all temperatures, and an overall reduction in $\dot{V}_{\text{H}_2\text{O}}$ wherever possible, especially if atmospheric moisture was low, as was the case in all our trials.

Although individual phases of the DGE cycle may in some cases be altered for improved water conservation (e.g. longer C and shorter O phases, Terblanche et al., 2010) or to ensure oxygen supply meets demand (e.g. Contreras and Bradley, 2011), DGE may have originally evolved in association with reduced energy consumption states (Lighton, 1996; Matthews and White, 2011a; see Chown, 2011). Indeed, low MR is characteristic of DGE relative to other patterns (Marais and Chown, 2003; Gibbs and Johnson, 2004), and is typically observed in quiescent individuals only. It is therefore possible that DGE arises at low MR, and once the central nervous system relinquishes control to the pattern generators in the peripheral nervous system (Matthews and White, 2011a) in order to save energy. It is also possible that the interaction between P_{O_2} and P_{CO_2} set-points determine the opening and closing of the spiracles (Chown, 2011). In *A. fusca*, as temperature increased both CF- and O-phase duration decreased significantly, while burst frequency increased (supplementary material Tables S1, S2). This alteration in phase duration could be due to the increased metabolic demand (due to increasing temperature), leading to modified spiracular opening/closing, and simultaneously avoiding acidic pH accumulation in the haemolymph.

On the basis of the hygric cost of gas exchange (Woods and Smith, 2010), however, *A. fusca* is unusual because it shows a high WLR per unit energy consumption (expressed as mol O_2 uptake) compared with the mean predicted for other insects, which in turn is higher than the universal model prediction for all organisms. This suggests that *A. fusca* loses more water than expected for its MR, indicating that it is likely under considerable pressure to conserve water, especially if exposed to xeric conditions. It is well established that insects can sense changes in their environment (e.g. variation in atmospheric RH and temperature), and may respond by means of a range of behavioural and physiological changes (reviewed in Chown et al., 2011). For *A. fusca*, however, changes in the pattern of gas exchange do not seem to be one of these responses, as desiccating conditions did not result in the increased presence of DGE, nor an alteration in gas exchange pattern more generally. One potential reason why *A. fusca* does not respond to low RH by altering its gas exchange pattern might be because, when compared with other insects or cockroach species, the relationship of WLR to MR for *A. fusca* does not differ with gas exchange pattern, a result that is inconsistent with several other studies that suggest DGE is useful

for conserving water (e.g. Chown and Davis, 2003; Schimpf et al., 2009; Williams et al., 2010). This observation is further supported by the fact that depression of \dot{V}_{CO_2} (evidenced by our temperature or gas switching experiments) (Fig. 4) does not allow *A. fusca* to reduce its WLR in a manner that brings it closer to the interspecific relationship of the universal model (i.e. the orthogonal distance is not reduced). Indeed, the slope of the interspecific relationship is significantly shallower than the slope of the interspecific relationship for other organisms (*t*-test of slopes: $t=5.78$, *d.f.*=29, $P<0.0001$), indicating that variation in gas exchange pattern, or a reduction in MR in particular, in this insect may ultimately not be capable of serving a water conservation function. This outcome stands in stark contrast to other work on gas exchange and water loss in cockroaches published to date (e.g. Schimpf et al., 2009; Schimpf et al., 2012) and therefore warrants further investigation.

LIST OF SYMBOLS AND ABBREVIATIONS

C phase	closed phase
CF phase	closed/flutter phase
CGE	continuous gas exchange
CWL	cuticular water loss
DGE	discontinuous gas exchange
F phase	flutter phase
hypC phase	hyperoxic C phase
MR	metabolic rate
O phase	open phase
RH	relative humidity
RQ	respiratory quotient
RWL	respiratory water loss
TWL	total water loss
\dot{V}_{CO_2}	rate of CO ₂ release
\dot{V}_{H_2O}	rate of H ₂ O release
WLR	water loss rate

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AUTHOR CONTRIBUTIONS

J.S.T., C.P.P. and C.S.B. designed the research. B.G., C.S.B. and C.P.P. executed the research. This work served as part of C.P.P.'s Honour's project. B.G. and C.S.B. undertook additional experimental work, data extraction and analyses. All authors contributed to analyses and writing.

COMPETING INTERESTS

No competing interests declared.

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REFERENCES

- Becker, W. A. (1984). *Manual of Quantitative Genetics*. Pullman, WA: Academic Enterprises.
- Benoit, J. B. and Denlinger, D. L. (2007). Suppression of water loss during adult diapause in the northern house mosquito, *Culex pipiens*. *J. Exp. Biol.* **210**, 217-226.
- Benoit, J. B. and Denlinger, D. L. (2010). Meeting the challenges of on-host and off-host water balance in blood-feeding arthropods. *J. Insect Physiol.* **56**, 1366-1376.
- Boardman, L., Terblanche, J. S., Hetz, S. K., Marais, E. and Chown, S. L. (2012). Reactive oxygen species production and discontinuous gas exchange in insects. *Proc. Biol. Sci.* **279**, 893-901.
- Bradley, T. J. (2007). Control of the respiratory pattern in insects. *Adv. Exp. Med. Biol.* **618**, 211-220.
- Chappell, M. A., Bailey, N. W., Redak, R. A., Antolin, M. and Zuk, M. (2009). Metabolic similarity despite striking behavioral divergence: aerobic performance in low- and high-density forms of the Mormon cricket. *Physiol. Biochem. Zool.* **82**, 405-418.
- Chown, S. L. (2002). Respiratory water loss in insects. *Comp. Biochem. Physiol.* **133A**, 791-804.
- Chown, S. L. (2011). Discontinuous gas exchange: new perspectives on evolutionary origins and ecological implications. *Funct. Ecol.* **25**, 1163-1168.
- Chown, S. L. and Davis, A. L. V. (2003). Discontinuous gas exchange and the significance of respiratory water loss in Scarabaeine beetles. *J. Exp. Biol.* **206**, 3547-3556.
- Chown, S. L., Gibbs, A. G., Hetz, S. K., Kloke, C. J., Lighton, J. R. B. and Marais, E. (2006a). Discontinuous gas exchange in insects: a clarification of hypotheses and approaches. *Physiol. Biochem. Zool.* **79**, 333-343.
- Chown, S. L., Marais, E., Picker, M. D. and Terblanche, J. S. (2006b). Gas exchange characteristics, metabolic rate and water loss of the heelwalker, *Karoophasma biedouwensis* (Mantophasmatodea: Austrophasmatidae). *J. Insect Physiol.* **52**, 442-449.
- Chown, S. L., Sørensen, J. G. and Terblanche, J. S. (2011). Water loss in insects: an environmental change perspective. *J. Insect Physiol.* **57**, 1070-1084.
- Clusella-Trullas, S., van Wyk, J. H. and Spotila, J. R. (2009). Thermal benefits of melanism in cordylid lizards: a theoretical and field test. *Ecology* **90**, 2297-2312.
- Contreras, H. L. and Bradley, T. J. (2011). The effect of ambient humidity and metabolic rate on the gas-exchange pattern of the semi-aquatic insect *Aquarius remigis*. *J. Exp. Biol.* **214**, 1086-1091.
- Dingha, B. N., Appel, A. G. and Eubanks, M. D. (2005). Discontinuous carbon dioxide release in the German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae), and its effect on respiratory transpiration. *J. Insect Physiol.* **51**, 825-836.
- Duncan, F. D., Krasnov, B. and McMaster, M. (2002). Novel case of a tenebrionid beetle using discontinuous gas exchange cycle when dehydrated. *Physiol. Entomol.* **27**, 79-83.
- Edney, E. B. (1977). *Water Balance in Land Arthropods*. Berlin: Springer-Verlag.
- Gefen, E. (2011). The relative importance of respiratory water loss in scorpions is correlated with species habitat type and activity pattern. *Physiol. Biochem. Zool.* **84**, 68-76.
- Gibbs, A. G. and Johnson, R. A. (2004). The role of the chthonic hypothesis does not hold water. *J. Exp. Biol.* **207**, 3477-3482.
- Gibbs, A. G., Fukuzato, F. and Matzkin, L. M. (2003). Evolution of water conservation mechanisms in *Drosophila*. *J. Exp. Biol.* **206**, 1183-1192.
- Gray, E. M. and Bradley, T. J. (2006). Evidence from mosquitoes suggests that cyclic gas exchange and discontinuous gas exchange are two manifestations of a single respiratory pattern. *J. Exp. Biol.* **209**, 1603-1611.
- Gray, E. M. and Chown, S. L. (2008). Bias, precision and accuracy in the estimation of cuticular and respiratory water loss: a case study from a highly variable cockroach, *Perisphaeria* sp. *J. Insect Physiol.* **54**, 169-179.
- Groenewald, B., Hetz, S. K., Chown, S. L. and Terblanche, J. S. (2012). Respiratory dynamics of discontinuous gas exchange in the tracheal system of the desert locust, *Schistocerca gregaria*. *J. Exp. Biol.* **215**, 2301-2307.
- Hadley, N. F. (1994). *Water Relations of Terrestrial Arthropods*. San Diego, CA: Academic Press.
- Hadley, N. F. and Quinlan, M. C. (1993). Discontinuous carbon dioxide release in the eastern lubber grasshopper *Romalea guttata* and its effect on respiratory transpiration. *J. Exp. Biol.* **177**, 169-180.
- Hetz, S. K. (2007). The role of the spiracles in gas exchange during development of *Samia cyathia* (Lepidoptera, Saturniidae). *Comp. Biochem. Physiol.* **148A**, 743-754.
- Hetz, S. K. and Bradley, T. J. (2005). Insects breathe discontinuously to avoid oxygen toxicity. *Nature* **433**, 516-519.
- Heusner, A. A. (1991). Size and power in mammals. *J. Exp. Biol.* **160**, 25-54.
- Johnson, R. A., Kaiser, A., Quinlan, M. and Sharp, W. (2011). Effect of cuticular abrasion and recovery on water loss rates in queens of the desert harvester ant *Messor pergandei*. *J. Exp. Biol.* **214**, 3495-3506.
- Kestler, P. (1985). Respiration and respiratory water loss. In *Environmental Physiology and Biochemistry of Insects* (ed. K. H. Hoffmann), pp. 137-186. Berlin: Springer.
- Kloke, C. J. and Chown, S. L. (2005). Temperature- and body mass-related variation in cyclic gas exchange characteristics and metabolic rate of seven weevil species: broader implications. *J. Insect Physiol.* **51**, 789-801.
- Krebs, C. J. (1999). *Ecological Methodology*, 2nd edn. Menlo Park, CA: Benjamin Cummings.
- Lessells, C. M. and Boag, P. T. (1987). Unrepeatable repeatabilities: a common mistake. *Auk* **104**, 116-121.
- Lighton, J. R. B. (1992). Direct measurement of mass loss during discontinuous ventilation in two species of ants. *J. Exp. Biol.* **173**, 289-293.
- Lighton, J. R. B. (1994). Discontinuous ventilation in terrestrial insects. *Physiol. Zool.* **67**, 142-162.
- Lighton, J. R. B. (1996). Discontinuous gas exchange in insects. *Annu. Rev. Entomol.* **41**, 309-324.
- Lighton, J. R. B. (2008). *Measuring Metabolic Rates: a Manual for Scientists*. Oxford: Oxford University Press.
- Lighton, J. R. B. and Turner, R. J. (2008). The hygric hypothesis does not hold water: abolition of discontinuous gas exchange cycles does not affect water loss in the ant *Camponotus vicinus*. *J. Exp. Biol.* **211**, 563-567.
- Lighton, J. R. B., Bartholomew, G. A. and Feener, D. H. (1987). Energetics of locomotion and load carriage and a model of the energy cost of foraging in the leaf-cutting ant *Atta colombica* Guer. *Physiol. Zool.* **60**, 524-537.
- Lighton, J. R. B., Schilman, P. E. and Holway, D. A. (2004). The hyperoxic switch: assessing respiratory water loss rates in tracheate arthropods with continuous gas exchange. *J. Exp. Biol.* **207**, 4463-4471.
- Loveridge, J. P. (1968). The control of water loss in *Locusta migratoria migratorioides* R. & F. *J. Exp. Biol.* **49**, 15-29.
- Marais, E. and Chown, S. L. (2003). Repeatability of standard metabolic rate and gas exchange characteristics in a highly variable cockroach, *Perisphaeria* sp. *J. Exp. Biol.* **206**, 4565-4574.
- Marais, E., Kloke, C. J., Terblanche, J. S. and Chown, S. L. (2005). Insect gas exchange patterns: a phylogenetic perspective. *J. Exp. Biol.* **208**, 4495-4507.

- Matthews, P. G. D. and White, C. R.** (2011a). Discontinuous gas exchange in insects: is it all in their heads? *Am. Nat.* **177**, 130-134.
- Matthews, P. G. D. and White, C. R.** (2011b). Regulation of gas exchange and haemolymph pH in the cockroach *Nauphoeta cinerea*. *J. Exp. Biol.* **214**, 3062-3073.
- Matthews, P. G. D., Snelling, E. P., Seymour, R. S. and White, C. R.** (2012). A test of the oxidative damage hypothesis for discontinuous gas exchange in the locust *Locusta migratoria*. *Biol. Lett.* **8**, 682-684.
- Mellanby, K.** (1934). The site of loss of water from insects. *Proc. R. Soc. Lond. B* **116**, 139-149.
- Miller, P. L.** (1973). Spatial and temporal changes in the coupling of cockroach spiracles to ventilation. *J. Exp. Biol.* **59**, 137-148.
- Quinlan, M. C. and Hadley, N. F.** (1993). Gas exchange, ventilatory patterns, and water loss in two lubber grasshoppers: quantifying cuticular and respiratory transpiration. *Physiol. Zool.* **66**, 628-642.
- Schimpf, N. G., Matthews, P. G. D., Wilson, R. S. and White, C. R.** (2009). Cockroaches breathe discontinuously to reduce respiratory water loss. *J. Exp. Biol.* **212**, 2773-2780.
- Schimpf, N. G., Matthews, P. G. D. and White, C. R.** (2012). Cockroaches that exchange respiratory gases discontinuously survive food and water restriction. *Evolution* **66**, 597-604.
- Schneiderman, H. A.** (1960). Discontinuous respiration in insects: role of the spiracles. *Biol. Bull.* **119**, 494-528.
- Schneiderman, H. A. and Williams, C. M.** (1955). An experimental analysis of the discontinuous respiration of the *Cecropia* silkworm. *Biol. Bull.* **109**, 123-143.
- Terblanche, J. S., Marais, E., Hetz, S. K. and Chown, S. L.** (2008). Control of discontinuous gas exchange in *Samia cynthia*: effects of atmospheric oxygen, carbon dioxide and moisture. *J. Exp. Biol.* **211**, 3272-3280.
- Terblanche, J. S., Clusella-Trullas, S. and Chown, S. L.** (2010). Phenotypic plasticity of gas exchange pattern and water loss in *Scarabaeus spretus* (Coleoptera: Scarabaeidae): deconstructing the basis for metabolic rate variation. *J. Exp. Biol.* **213**, 2940-2949.
- Weldon, C. W., Daniels, S. R., Clusella-Trullas, S. and Chown, S. L.** (2013). Metabolic and water loss rates of two cryptic species in the African velvet worm genus *Opisthopatus* (Onychophora). *J. Comp. Physiol. B* **183**, 323-332.
- White, C. R., Blackburn, T. M., Terblanche, J. S., Marais, E., Gibernau, M. and Chown, S. L.** (2007). Evolutionary responses of discontinuous gas exchange in insects. *Proc. Natl. Acad. Sci. USA* **104**, 8357-8361.
- Williams, C. M., Pelini, S. L., Hellmann, J. J. and Sinclair, B. J.** (2010). Intra-individual variation allows an explicit test of the hygric hypothesis for discontinuous gas exchange in insects. *Biol. Lett.* **6**, 274-277.
- Wobschall, A. and Hetz, S. K.** (2004). Oxygen uptake by convection and diffusion in diapausing moth pupae (*Attacus atlas*). *Int. Congr. Ser.* **1275**, 157-164.
- Woods, H. A. and Smith, J. N.** (2010). Universal model for water costs of gas exchange by animals and plants. *Proc. Natl. Acad. Sci. USA* **107**, 8469-8474.