The Journal of Experimental Biology 215, 3388-3393

doi:10.1242/jeb.072769

RESEARCH ARTICLE

Allometric scaling of discontinuous gas exchange patterns in the locust *Locusta migratoria* throughout ontogeny

Edward P. Snelling[1,*], Philip G. D. Matthews[2] and Roger S. Seymour[1]

[1]School of Earth and Environmental Sciences, University of Adelaide, South Australia 5005, Australia and [2]School of Biological Sciences, University of Queensland, St Lucia, Queensland 4072, Australia

*Author for correspondence (edward.snelling@adelaide.edu.au)

SUMMARY

The discontinuous gas exchange cycle (DGC) is a three-phase breathing pattern displayed by many insects at rest. The pattern consists of an extended breath-hold period (closed phase), followed by a sequence of rapid gas exchange pulses (flutter phase), and then a period in which respiratory gases move freely between insect and environment (open phase). This study measured CO_2 emission in resting locusts *Locusta migratoria* throughout ontogeny, in normoxia (21 kPa P_{O_2}), hypoxia (7 kPa P_{O_2}) and hyperoxia (40 kPa P_{O_2}), to determine whether body mass and ambient O_2 affect DGC phase duration. In normoxia, mean CO_2 production rate ($\dot{M}_{CO_2}$; μmol h^{-1}) scales with body mass (M_b; g) according to the allometric power equation $\dot{M}_{CO_2}=9.9M_b^{0.95\pm0.09}$, closed phase duration (C; min) scales with body mass according to the equation $C=18.0M_b^{0.38\pm0.29}$, closed+flutter period (C+F; min) scales with body mass according to the equation $C+F=26.6M_b^{0.20\pm0.25}$ and open phase duration (O; min) scales with body mass according to the equation $O=13.3M_b^{0.23\pm0.18}$. Hypoxia results in a shorter C phase and longer O phase across all life stages, whereas hyperoxia elicits shorter C, C+F and O phases across all life stages. The tendency for larger locusts to exhibit longer C and C+F phases might arise if the positive allometric scaling of locust tracheal volume prolongs the time taken to reach the minimum O_2 and maximum CO_2 set-points that determine the duration of these respective periods, whereas an increasingly protracted O phase could reflect the additional time required for larger locusts to expel CO_2 through a relatively longer tracheal pathway. Observed changes in phase duration under hypoxia possibly serve to maximise O_2 uptake from the environment, whereas the response of the DGC to hyperoxia is difficult to explain, but could be affected by elevated levels of reactive oxygen species.

Key words: allometry, discontinuous gas exchange, insect, locust, respiration.

Received 19 March 2012: Accepted 6 June 2012

INTRODUCTION

The discontinuous gas exchange cycle (DGC) is a breathing pattern known to occur in quiescent insects from at least five orders (Chown, 2011; Marais et al., 2005). It is characterised by the sequential repetition of three phases: a closed (C) phase, where the spiracles are occluded, preventing the exchange of O_2 and CO_2 between the insect and the atmosphere; a flutter (F) phase, where the spiracles open and close repetitively, admitting O_2 while releasing limited amounts of CO_2; and an open (O) phase, where the spiracles open completely, allowing O_2 and CO_2 to move freely along their partial pressure gradients.

The adaptive significance of the DGC is an ongoing source of controversy (Chown, 2011; Chown et al., 2006; Quinlan and Gibbs, 2006). The hygric hypothesis posits that the tightly occluded spiracles during the C phase and the inward convective movement of air that can occur early during the F phase function to reduce respiratory water loss (Buck et al., 1953; Levy and Schneiderman, 1966). Alternatively, the oxidative damage hypothesis proposes that the C and F phases of the DGC function to limit O_2 influx into the tracheal system, thereby preventing cell damage caused by oxygen-derived free radicals (Bradley, 2000; Hetz and Bradley, 2005). More recently, the neural hypothesis suggests that the DGC provides no direct adaptive advantage, but instead arises as a consequence of the downregulation of the nervous system, affecting respiratory control (Matthews and White, 2011a).

Although there is little consensus over which of these competing hypotheses best accounts for the evolution of DGCs within the Insecta, the respiratory cues controlling the breathing pattern are often assumed to be consistent between species (but see Harrison et al., 2006), based on early work on cecropia silk moth *Hyalophora cecropia* pupae (Burkett and Schneiderman, 1974; Levy and Schneiderman, 1966), and more recent work on atlas moth *Attacus atlas* pupae (Förster and Hetz, 2010) and adult cockroaches *Nauphoeta cinerea* (Matthews and White, 2011b). The end of the C phase, and thus initiation of the F phase, is apparently triggered by a decline in internal O_2 to some minimum set-point that presumably corresponds to the minimum partial pressure gradient required to satisfy the insect's resting aerobic needs. Over the course of the F phase, enough O_2 is admitted into the tracheal system to maintain or even increase internal concentrations of the gas, whereas only small amounts of CO_2 are released to the atmosphere, which otherwise continues to accumulate within the insect's intracellular fluids and haemolymph (the insect's body fluids) (Bridges and Scheid, 1982; Harrison et al., 1995). The build-up of CO_2 over the C+F phases eventually reaches a maximum set-point, and this triggers the O phase during which internal O_2 levels increase rapidly because of the high partial pressure difference between the

atmosphere and the tracheal system (~15–18 kPa). In contrast, the efflux of accumulated CO_2 takes longer because a large fraction of CO_2 is bound-up as bicarbonate in the body fluids, resulting in a relatively modest outward partial pressure difference (~2 kPa). Precisely what determines the duration of the O phase is, at present, still unresolved (Matthews and White, 2011b).

Given that internal O_2 and CO_2 levels appear to trigger transition between phases of the DGC, it is likely that the durations of the different phases might vary depending on factors such as metabolic rate, tracheal volume, body fluid volume and ambient O_2 levels. For instance, the duration of the C phase could be longer in insects with relatively large tracheal volumes and low mass-specific metabolic rates, and in hyperoxic atmospheres, as these factors would likely extend the time required for internal O_2 levels to reach the minimum O_2 set-point. Likewise, the combined C+F period, when CO_2 accumulates mostly in the insect's body fluids, might be longer in individuals with low mass-specific metabolic rates and relatively large body fluid volumes, as this should extend the time taken to reach the maximum CO_2 set-point.

Because factors such as an insect's metabolic rate, tracheal volume and body fluid volume inevitably vary with body mass, the phases of the DGC that are influenced by these variables are also likely to vary with body mass, and these differences can be investigated using allometry. Allometric equations take the form $y=aM_b^b$, where y is the variable of interest, a is the coefficient (elevation), b is the exponent (slope) and M_b is body mass. The principle of allometric cancellation can be applied in the analysis (Calder, 1996). If y is equal to the product of two other variables, then the sum of their individual scaling exponents will equal the exponent derived for y, and if y is equal to the division of two other variables, then the difference between their scaling exponents will equal the exponent for y. For example, if C phase duration is directly proportional to tracheal volume, but inversely proportional to O_2 consumption rate, and if we know that locust *Schistocerca americana* tracheal volume scales throughout ontogeny with an exponent of $M_b^{1.30}$ (Lease et al., 2006) and resting O_2 consumption rate scales as $M_b^{0.80}$ (Harrison et al., 2005), then C phase duration should scale with an exponent of $M_b^{0.50}$, assuming the minimum O_2 set-point does not vary significantly with locust body mass, which seems reasonable for an intraspecific analysis. If C phase duration is sensitive to ambient O_2 levels, then this will be expressed in the coefficient value of the allometric equation, provided that all life stages are affected to the same relative extent. For example, if hypoxia causes a 50% reduction in the duration of the C phase across all body masses, then the coefficient value will halve and the exponent will remain unchanged. However, if the duration of the C+F phase is directly proportional to the insect's body fluid volume, which likely scales as $M_b^{1.00}$, but inversely proportional to resting CO_2 production rate, which likely scales in parallel with O_2 consumption as $M_b^{0.80}$, then the C+F duration should scale with an exponent of $M_b^{0.20}$. Once again, this assumes the maximum CO_2 set-point scales independent of locust body mass, although even if it did change throughout ontogeny, one could factor this into the model. And lastly, if the duration of the O phase reflects the time required to expel enough CO_2 from the body to restore acid–base balance, and if larger insects can adequately compensate for longer CO_2 diffusion distances using convective abdominal pumping (Hamilton, 1964; Kestler, 1985; Kestler, 1991), then O phase duration might not vary significantly with body mass, $M_b^{0.00}$.

To date, few studies have investigated the allometric scaling of the DGC, and those that exist are all interspecific comparisons, half of them utilise a less than ideal body mass range, and their findings are inconsistent. An early investigation showed that DGC frequency, and thus DGC duration, is conserved in tenebrionid beetles across a ~40-fold body mass range (Lighton, 1991). Some support for this finding was offered by a subsequent study that found no significant effect of body mass on DGC frequency, DGC duration or the duration of the C+F and O phases in cerambycid beetles, but this was only over an approximately sixfold range in body mass (Chappell and Rogowitz, 2000). Another study that analysed gas exchange patterns in scarab beetles found that the C, F and O phase durations all scale invariantly with body mass, but that total DGC duration is significantly shorter in larger beetles, and thus DGC frequency increases with body mass, scaling with an exponent of $M_b^{0.56}$, but this was only over an approximately fourfold range in body mass (Davis et al., 1999). And contradicting all these studies, a recent meta-analysis of 49 insect species found that larger insects tend to have longer DGCs, and thus DGC frequency decreases with body mass scaling with an exponent of $M_b^{-0.20}$ (Terblanche et al., 2008).

The largely conflicting results offered by the four previous studies into the allometric scaling of the DGC means we are little wiser about the potential effects body mass may have on the gas exchange pattern. Certainly the narrow body mass range analysed in two of these studies limits the likelihood of detecting statistical significance, and probably reflects the difficulty in obtaining different sized insects that will readily engage in DGCs. A preliminary investigation on locusts *Locusta migratoria* detected DGCs at all six stages of the life cycle, from first instar to the adult, during which time body mass increases more than 50-fold. Thus, the aim of the present study was to record resting CO_2 emission patterns throughout locust ontogeny under normoxic, hypoxic and hyperoxic atmospheres, and then to allometrically analyse the frequency and the duration of the DGC and its phases. Allometric scaling patterns during ontogenetic development are often different from those observed across species – this study is the first to determine the characteristics of the DGC throughout the life cycle of an insect.

MATERIALS AND METHODS

Animals

Gregarious-phase locusts *Locusta migratoria* (Linnaeus 1758) were sourced from a breeding colony at the University of Adelaide, South Australia, where they were maintained in large plastic terraria at 33±1°C under a 12 h:12 h light:dark cycle, and fed wheatgrass and wheat germ *ad libitum*. Food was removed 24 h prior to all measurements.

Body fluid measurements

Whole-body fluid mass was estimated in a total of 21 locusts, consisting of individuals from each of the six life stages advanced throughout a complete life cycle, including adults. It was calculated as the difference in body mass before and after complete dehydration in a freeze-dryer (FD5, Dynavac, Melbourne, VIC, Australia). Body mass was measured to 0.1 mg on an analytical balance (AE163, Mettler, Greifensee, Switzerland).

DGC and CO_2 measurements

A flow-through respirometry system recorded resting CO_2 emission in a total of 30 locusts, once again with individuals from each life stage, under three ambient O_2 partial pressures: 21, 40 and 7 kPa. Briefly, 21 kPa P_{O_2} was generated using an air compressor (AT-250A, Sparmax, Taipei, Taiwan), which pumped outside air through a 5 l buffer cylinder into a series of Drierite (W. A. Hammond

Drierite Co., Xenia, OH, USA), soda lime and Drierite columns, which removed H_2O vapour and CO_2. Flow rate was then regulated with a mass flow controller (Mass-Trak 810C, 0–1000 ml min^{-1}, Sierra Instruments, Monterey, CA, USA; calibrated with a Gilibrator bubble flow meter, Sensidyne, Clearwater, FL, USA) before entering a 22–23°C temperature-controlled cabinet where the air stream was directed through a syringe-type metabolic chamber that contained the resting locust in semi-darkness. In first and second instars, the chamber volume was 0.3 and 0.6 ml, respectively, through which air was pushed at a rate of 150 ml min^{-1} standard temperature and pressure, dry (STPD); in third and fourth instars, the chamber volume was 1.2 and 4.4 ml, respectively, and the flow rate was 250 ml min^{-1} STPD; and in fifth instar and adult locusts, the chamber volume was 6.0 and 8.0 ml, respectively, and the flow rate was 350 ml min^{-1} STPD. Excurrent air from the metabolic chamber was passed through a small Drierite column before entering a CO_2 gas analyser (LI-820, LI-COR Biosciences, Lincoln, NE, USA), and a bypass line around the chamber allowed for baseline measurements of CO_2 concentration between gas treatments.

To generate 40 and 7 kPa P_{O_2}, cylinders of compressed O_2 and N_2 (BOC Gases, Adelaide, SA, Australia) were connected to a custom-built gas-mixing apparatus consisting of two mass flow controllers (GFC-171, Aalborg Instruments and Controls, Orangeburg, NY, USA; verified with a calibrated Oxzilla FC-2 O_2 gas analyser, Sable Systems, Las Vegas, NV, USA), regulated by a computer running control software through a digital–analogue converter (ProfessorDAQ and PowerDAQ PD2-AO, United Electronic Industries, Walpole, MA, USA). To ensure adequate mixing of the dry O_2 and N_2, the generated gas stream was passed through a 5 l convective mixing chamber that contained a 12 V built-in fan. From here, the gas stream was then connected to the existing respirometry system just prior to the mass flow controller regulating flow into the metabolic chamber.

Each locust was initially acclimated for 1 h in the metabolic chamber while it was ventilated with air. The three ambient gas treatments were then sequentially introduced into the metabolic chamber in a random order. Each gas treatment involved an initial 15 min washout period followed by 2 h of CO_2 measurements. Immediately after each respirometry session, body mass was recorded to 0.1 mg on the AE163 Mettler analytical balance. The analog outputs from the mass flow controller and CO_2 gas analyser were recorded to a computer at 1 s intervals with a PowerLab data acquisition system and LabChart software (ADInstruments, Bella Vista, NSW, Australia). CO_2 emission rates were calculated as the product of the incurrent flow rate and the fraction of CO_2 in air exiting the metabolic chamber following the removal of H_2O vapour (Withers, 2001). An instantaneous correction was applied using an empirical technique previously described (Seymour et al., 1998; Snelling et al., 2011), and then instantaneous CO_2 emission rates were plotted with time for each gas treatment in each locust. Overall mean CO_2 production rate was calculated over several complete DGCs for each gas treatment in each locust, and then a visual analysis of the time series was used to determine the duration of the O, F and C phases in locusts that displayed charactistic DGCs. The large burst of CO_2 during the O phase meant that it could be clearly differentiated from the C and F phases; however, it was more difficult to identify the exact transition point of the C and F phases because both are associated with relatively low CO_2 release rates. To help overcome this problem, and to make the analysis more consistent between individuals and treatments, a line was plotted across the time series equivalent to 30% of mean CO_2 production rate, and when a series of small CO_2 spikes broke the line, we used this as an indication that the C phase had ended and the F phase had begun.

All mean values and allometric power equations include 95% confidence intervals. Allometric data were transformed into the $\log_{10}$ base before statistical analysis using ordinary least-squares regressions. ANCOVA comparisons of regressions (Zar, 1998) and *F*-tests to determine whether slopes differ significantly from zero were carried out with GraphPad Prism 5 statistical software (GraphPad Software, La Jolla, CA, USA).

RESULTS

Body fluid mass

Locusts used for body fluid measurements varied 74-fold in body mass, from 0.0130 to 0.9567 g. Total body fluid mass (M_f; g) increases with body mass throughout locust development, following the allometric power equation $M_f=0.72M_b^{0.97\pm0.01}$ ($r^2=1.00$). Although the exponent is close to isometry, younger locusts have a slightly larger relative body fluid mass compared with older locusts. Averaged over all locusts, body fluid accounts for approximately 78.6±2.1% of wet body mass.

Effect of body mass on the DGC

Of the 30 locusts used for respirometry, 10 individuals exhibited characteristic closed–flutter–open DGC patterns during rest under normoxic conditions (Fig. 1). These 10 locusts are the focus of the present investigation, and cover a 56-fold body mass range, from 0.0166 to 0.9216 g. In 21 kPa P_{O_2}, resting CO_2 production rate is proportional to body mass raised to the power of 0.95±0.09, and thus mass-specific CO_2 production is independent of body mass, scaling with an exponent that is not significantly different from zero, –0.05±0.09 (*F*-test, $P>0.05$; Fig. 2, Table 1). Despite this, the total duration of the DGC increases with body mass, from 18.4±3.3 min in first instars to 33.2±1.4 min in adults, scaling with body mass with an exponent of 0.22±0.17. The increase in total DGC duration with body mass is due to longer C and O phases in older locusts:

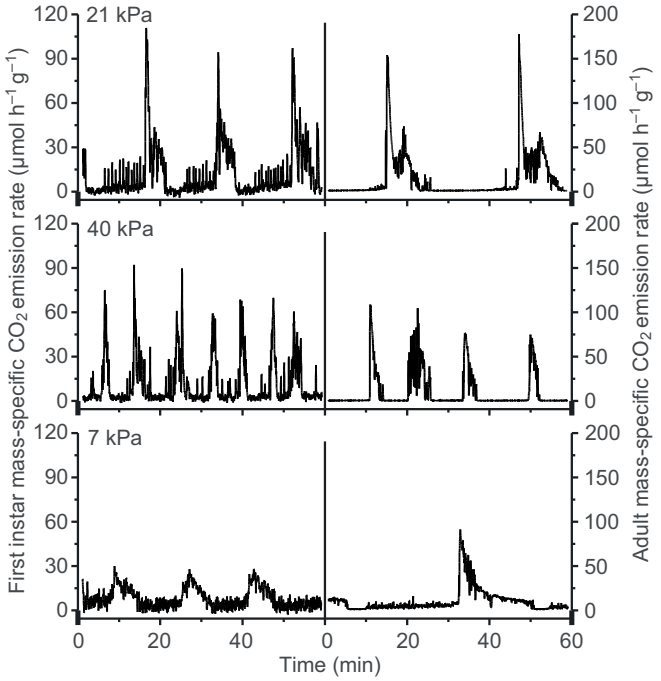

Fig. 1. CO_2 emission patterns in a 16.6 mg first instar (left) and a 922 mg adult (right) locust under 21, 40 and 7 kPa ambient O_2 partial pressures.

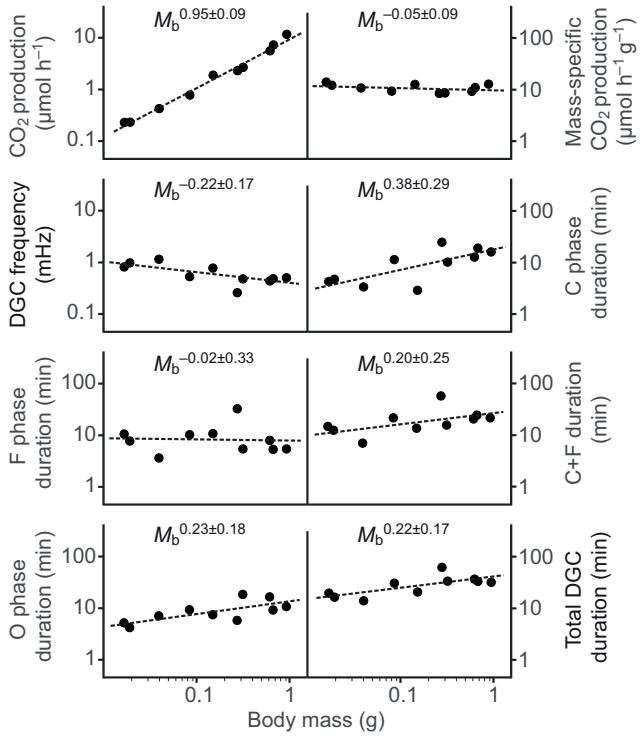

Fig. 2. Allometric relationship between body mass and CO_2 production rate, discontinuous gas exchange cycle (DGC) frequency, and the duration of the DGC and its phase components under 21 kPa ambient O_2 partial pressure (N=10 individuals). See Table 1 for power equations and statistical analyses.

C phase duration increases from 4.6±0.4 min in first instars to 17.8±3.0 min in adults, and scales with body mass raised to the power of 0.38±0.29, and O phase duration increases from 4.7±1.0 min in first instars to 10.1±1.5 min in adults, and scales with body mass raised to the power of 0.23±0.18. Statistical analysis confirms that C phase, O phase and total DGC duration all scale with body mass with exponents significantly greater than zero (F-test, P<0.05). F phase duration, in contrast, is approximately 9.9±5.2 min regardless of life stage, and thus scales independent of body mass, with an exponent of –0.02±0.33.

Effect of ambient oxygen pressure on the DGC

The allometric scaling of CO_2 production rate did not change under different ambient O_2 partial pressures (Table 1). However, total DGC duration in 40 kPa P_{O_2} was approximately one-third of that exhibited under normoxia (ANCOVA, P<0.05) across all life stages. The shorter DGC duration in 40 kPa P_{O_2} was due to a significant reduction in all three phases of the DGC (ANCOVA, P<0.05), especially the F phase, which was almost completely eliminated. In 7 kPa P_{O_2}, the allometric scaling of total DGC duration is statistically indistinguishable from that reported for normoxia (ANCOVA, P>0.05); however, the duration of the C phase is approximately one-quarter of that recorded under normoxic conditions (ANCOVA, P<0.05) across all life stages, while the O phase is approximately twice as long (ANCOVA, P<0.05) across all life stages. F phase duration also increased in 7 kPa P_{O_2}, although not significantly compared with normoxia (ANCOVA, P>0.05). The decrease in C phase and increase in F phase duration counterbalanced one another such that the combined C+F duration was similar and statistically indistinguishable from that recorded under normoxic conditions (ANCOVA, P>0.05).

DISCUSSION

Effect of body mass on the DGC

An important finding of this study is that DGCs are exhibited by all locust life stages, from 20 mg first instars to 1 g adults (Fig. 1). The ontogenetic occurrence of the DGC provides the opportunity to analyse whether and how the breathing pattern varies with increasing body mass. Using an allometric approach, we found that despite overall CO_2 production rate scaling near-isometrically with body mass, 0.95±0.09, the duration of the DGC cycle is significantly longer in older locusts, scaling with body mass raised to the power of 0.22±0.17 in normoxia (Fig. 2, Table 1). Thus, the scaling of locust DGC frequency is –0.22±0.17, which aligns closely with the meta-analysis that derived an exponent of –0.20 across 49 insect species (Terblanche et al., 2008).

The increase in DGC duration with locust body mass arises partly because the C phase duration scales with a rather steep exponent of 0.38±0.29. The increase in C phase duration with body mass could occur if larger individuals take longer to exhaust their tracheal O_2 stores, which is conceivable given that locust tracheal volume, at least in the related species *S. americana*, scales with body mass raised to the power of 1.30 throughout ontogeny (Lease et al., 2006), whereas the mean rate at which O_2 is extracted from tracheae during the C phase should scale in parallel with mean CO_2 production rate, with an exponent of 0.95. In fact, the relationship between tracheal volume, O_2 consumption and C phase duration could be directly related: $M_b^{1.30}/M_b^{0.95}\approx M_b^{0.38}$.

Unlike the duration of the C phase, the duration of the F phase is invariant with locust body mass, scaling with an exponent of

Table 1. Allometric power equations for CO_2 production rate (μmol h^{-1}), mass-specific CO_2 production rate (μmol h^{-1} g^{-1}), discontinuous gas exchange cycle (DGC) frequency (mHz), and closed, flutter, closed + flutter, open and total DGC duration (min) under 21, 40 and 7 kPa ambient O_2 partial pressures (N=10 individuals)

	21 kPa	40 kPa	7 kPa
CO_2 production	$9.9M_b^{0.95\pm0.09}$ (r^2=0.99)	$9.6M_b^{0.93\pm0.07}$ (r^2=0.99)$^{n.s.}$	$11.3M_b^{0.97\pm0.09}$ (r^2=0.99)$^{n.s.}$
CO_2 production (mass-specific)	$9.9M_b^{-0.05\pm0.09}$ (r^2=0.15)	$9.6M_b^{-0.07\pm0.07}$ (r^2=0.42)$^{n.s.}$	$11.3M_b^{-0.03\pm0.09}$ (r^2=0.05)$^{n.s.}$
DGC frequency	$0.40M_b^{-0.22\pm0.17}$ (r^2=0.52)	$1.3M_b^{-0.16\pm0.23}$ (r^2=0.25)$^{n.s.,*}$	$0.30M_b^{-0.20\pm0.27}$ (r^2=0.27)$^{n.s.}$
Closed duration	$18.0M_b^{0.38\pm0.29}$ (r^2=0.54)	$6.9M_b^{0.24\pm0.34}$ (r^2=0.24)$^{n.s.,*}$	$4.9M_b^{0.57\pm0.47}$ (r^2=0.49)$^{n.s.,*}$
Flutter duration	$7.8M_b^{-0.02\pm0.33}$ (r^2=0.00)	$0.20M_b^{-0.40\pm0.79}$ (r^2=0.14)$^{n.s.,*}$	$17.6M_b^{0.12\pm0.53}$ (r^2=0.03)$^{n.s.}$
Closed + flutter duration	$26.6M_b^{0.20\pm0.25}$ (r^2=0.29)	$7.1M_b^{0.16\pm0.38}$ (r^2=0.11)$^{n.s.,*}$	$22.9M_b^{0.16\pm0.48}$ (r^2=0.07)$^{n.s.}$
Open duration	$13.3M_b^{0.23\pm0.18}$ (r^2=0.51)	$4.6M_b^{0.11\pm0.21}$ (r^2=0.16)$^{n.s.,*}$	$26.3M_b^{0.27\pm0.22}$ (r^2=0.49)$^{n.s.,*}$
Total DGC duration	$42.1M_b^{0.22\pm0.17}$ (r^2=0.52)	$12.9M_b^{0.16\pm0.23}$ (r^2=0.25)$^{n.s.,*}$	$54.7M_b^{0.20\pm0.27}$ (r^2=0.27)$^{n.s.}$

Equations are in the form $y=aM_b^b$, where y is the variable of interest, a is the coefficient (elevation), b is the exponent (slope) and M_b is body mass (g). n.s., no significant difference in slope (ANCOVA, P>0.05) compared with 21 kPa P_{O_2}; *, significant difference in elevation (P<0.05) compared with 21 kPa P_{O_2}.

–0.02±0.33 in normoxia (Fig. 2, Table 1). If the C and F phase durations are combined, the resulting body mass exponent for the C+F period is 0.20±0.25, which is also not significantly different from zero. The apparent independent scaling of the C+F period with body mass conforms to our prediction that C+F duration is directly proportional to the insect's total body fluid volume, 0.97 [assuming body fluid buffer value is independent of mass (Bridges and Scheid, 1982; Harrison et al., 1995)], but inversely proportional to CO_2 production rate, 0.95, and can therefore be calculated as: $M_b^{0.97}/M_b^{0.95}=M_b^{0.02}$. However, we cannot ignore the fact that the C+F body mass exponent of 0.20±0.25 appears to trend upwards, and we may have failed to detect a significant difference from an exponent of zero because of variation. If C+F duration does lengthen in older individuals, then it could once again be due to the disproportionately large tracheal volume of older locusts, which might provide an increasingly important sink for CO_2 during this period of the DGC.

The duration of the O phase also increases in larger locusts, scaling with body mass raised to the power of 0.23±0.18 in normoxia (Fig. 2, Table 1). This might arise because the diffusion pathway for respiratory gases between the locust's tissues and the atmosphere is longer in larger individuals. If larger locusts lack relatively larger tracheal diameters and tracheole surface areas, then this could further reduce diffusive capacity. To some extent, diffusion limitations are overcome by active ventilation in older locusts, which appear to engage readily in abdominal pumping during the O phase (Hamilton, 1964; Kestler, 1985; Kestler, 1991).

Effect of ambient oxygen pressure on the DGC

Manipulating ambient O_2 partial pressure had a significant effect on the DGC. In hyperoxia, the F phase was almost completely eliminated and the C and O phases contracted significantly, increasing DGC frequency threefold compared with normoxia (Table 1). Given that the F phase is initiated when tracheal O_2 levels reach a minimum threshold (Förster and Hetz, 2010; Matthews and White, 2011b), it is perhaps unsurprising that it is eliminated under hyperoxic conditions. However, it is harder to explain the shorter C phase duration, because in hyperoxia this should depend on the time taken for internal CO_2 levels to reach a maximum set-point, and thus should be of similar duration to the C+F period observed in normoxia. Of course, no insect would ever experience hyperoxia in nature and so the response cannot be considered adaptive, and instead may arise as a consequence of altering the feedback mechanisms that dictate phase characteristics of the DGC. Recent research indicates that moth pupae displaying DGCs show an increase in reactive oxygen species (ROS) during the C and F phases where they may serve as a hypoxia indicator (Boardman et al., 2012). However, minimum ROS levels were also found to increase in hyperoxia, and so the possibility exists that artificially increased levels of ROS in hyperoxia may elicit a similar response to what would be expected in hypoxia (i.e. a contracted C phase). It is also interesting that hyperoxia significantly contracted the duration of the O phase, which might arise simply because of the small amount of CO_2 that would have accumulated over the short, preceding C phase. Thus, it would almost appear to be a self-perpetuating cycle whereby a contracted C phase brings about a contracted O phase, and *vice versa*.

Exposure to hypoxic conditions brought about a significant reduction in the duration of the C phase, whereas the F phase tended to increase, such that the combined C+F duration did not change significantly compared with normoxia (Table 1). The contracted C phase is consistent with a lack of internal O_2 initiating the early onset of the F phase (Förster and Hetz, 2010; Matthews and White, 2011b). The F phase then tends to be slightly longer probably because CO_2 levels must still accumulate to the same final threshold before the O phase is initiated (Harrison et al., 1995). Interestingly, hypoxia also elicited a longer O phase, which is unusual given that this component of the DGC is primarily thought to function in off-loading CO_2 accumulated over the C+F period. This probably indicates that an interaction exists between the O_2 and CO_2 set-points, such that the O phase responds to the physiological need to eliminate CO_2, as well as the need to admit sufficient O_2 into the tracheal system.

A comparison with earlier studies reveals significant variation among insect species in the response of the DGC to altered ambient O_2 partial pressures (for a review, see Harrison et al., 2006). For instance, hypoxia increases F phase duration in carpenter ants *Camponotus vicinus* (Lighton and Garrigan, 1995), cecropia moth pupae (Schneiderman, 1960), and to some extent locusts (Table 1), but in adult dung beetles *Aphodius fossor*, hypoxia decreases F phase duration (Chown and Holter, 2000). Similarly, hypoxia increases O phase duration in the locusts and decreases it in the dung beetles, whereas it has no apparent effect in the carpenter ants. And as for the effect of hyperoxia, the decrease in F phase duration in locusts is consistent with that reported for the moth pupae, whereas the decrease in C phase duration is the exact opposite to what occurs in the moth pupae. The only clear pattern to emerge from these studies is that hypoxia elicits a contracted C phase, which, as previously discussed, is consistent with a lack of internal O_2 initiating the early onset of the F phase. The apparent variation among insect species suggests that the physiological and anatomical factors that determine minimum O_2 set-points and maximum CO_2 set-points, and perhaps the interaction between the O_2 and CO_2 set-points, might vary significantly between insect species.

Conclusions

For the first time we show the occurrence of the DGC at each stage of an insect's development. Using an allometric approach, we find significant effects of body mass on the DGC, where older, larger locusts have significantly longer C and O phase durations, whereas the duration of the F phase appears unaffected by body mass, lasting for approximately 10 min (at 22–23°C) at all stages of the life cycle. An acute response to ambient O_2 partial pressure also occurs, with hyperoxia eliciting a reduction in the duration of all three phases of the DGC, and hypoxia eliciting a reduction in the C phase, but an increase in the duration of the F and O phases.

The significant variation observed in DGC phase durations between individuals cannot solely be due to variation in body mass. Potentially, much of this variation can be attributed to likely changes in the ratio between body fluid volume and tracheal lumen volume that occur within the life-span of an individual instar, during which time the tissue mass grows and the air sacs are displaced (Greenlee and Harrison, 2004). Conceivably, a diminishing air sac volume within an instar life-span could result in a contracted C phase because of the reduced O_2 storage capacity, and it could also limit tracheal ventilation and CO_2 off-loading, thus increasing O phase duration. Depending on the relative importance of the tracheal lumen compared with the insect's body fluids in providing a sink for CO_2, the combined duration of the C+F period may also be affected. Testing this hypothesis remains an important area for future research into the factors that influence the phase characteristics of the insect DGC.

LIST OF ABBREVIATIONS

C	closed phase of the DGC
C+F	combined closed and flutter phases of the DGC
DGC	discontinuous gas exchange cycle
F	flutter phase of the DGC
M_b	body mass
$\dot{M}_{CO_2}$	CO_2 production rate
M_f	body fluid mass
O	open phase of the DGC
ROS	reactive oxygen species

ACKNOWLEDGEMENTS

The authors thank Professor Stephen Simpson and Mr Tim Dodgson from the University of Sydney for providing the founding locust colony. We also appreciate technical assistance supplied by Dr Cameron Barr from the University of Adelaide. Two anonymous reviewers provided valuable feedback that greatly improved the manuscript.

FUNDING

This research was supported by the Australian Research Council, project no. DP0879605.

REFERENCES

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. (2000). The discontinuous gas exchange cycle in insects may serve to reduce oxygen supply to the tissues. *Am. Zool.* **40**, 952.

Bridges, C. R. and Scheid, P. (1982). Buffering and CO_2 dissociation of body fluids in the pupa of the silkworm moth, *Hyalophora cecropia*. *Respir. Physiol.* **48**, 183-197.

Buck, J., Keister, M. and Specht, H. (1953). Discontinuous respiration in diapausing *Agapema* pupae. *Anat. Rec.* **117**, 541.

Burkett, B. N. and Schneiderman, H. A. (1974). Roles of oxygen and carbon dioxide in the control of spiracular function in *cecropia* pupae. *Biol. Bull.* **147**, 274-293.

Calder, W. A., III (1996). *Size, Function, and Life History*. New York, NY: Dover Publications.

Chappell, M. A. and Rogowitz, G. L. (2000). Mass, temperature and metabolic effects on discontinuous gas exchange cycles in eucalyptus-boring beetles (Coleoptera: Cerambycidae). *J. Exp. Biol.* **203**, 3809-3820.

Chown, S. L. (2011). Discontinuous gas exchange: new perspectives on evolutionary origins and ecological implications. *Funct. Ecol.* **25**, 1163-1168.

Chown, S. L. and Holter, P. (2000). Discontinuous gas exchange cycles in *Aphodius fossor* (Scarabaeidae): a test of hypotheses concerning origins and mechanisms. *J. Exp. Biol.* **203**, 397-403.

Chown, S. L., Gibbs, A. G., Hetz, S. K., Klok, C. J., Lighton, J. R. B. and Marais, E. (2006). Discontinuous gas exchange in insects: a clarification of hypotheses and approaches. *Physiol. Biochem. Zool.* **79**, 333-343.

Davis, A. L. V., Chown, S. L. and Scholtz, C. H. (1999). Discontinuous gas-exchange cycles in *Scarabaeus* dung beetles (Coleoptera: Scarabaeidae): mass-scaling and temperature dependence. *Physiol. Biochem. Zool.* **72**, 555-565.

Förster, T. D. and Hetz, S. K. (2010). Spiracle activity in moth pupae—the role of oxygen and carbon dioxide revisited. *J. Insect Physiol.* **56**, 492-501.

Greenlee, K. J. and Harrison, J. F. (2004). Development of respiratory function in the American locust *Schistocerca americana*. II. Within-instar effects. *J. Exp. Biol.* **207**, 509-517.

Hamilton, A. G. (1964). The occurrence of periodic or continuous discharge of carbon dioxide by male desert locusts (*Schistocerca gregaria* Forskal) measured by an infra-red gas analyser. *Proc. Biol. Sci.* **160**, 373-395.

Harrison, J. F., Hadley, N. F. and Quinlan, M. C. (1995). Acid-base status and spiracular control during discontinuous ventilation in grasshoppers. *J. Exp. Biol.* **198**, 1755-1763.

Harrison, J. F., Lafreniere, J. J. and Greenlee, K. J. (2005). Ontogeny of tracheal dimensions and gas exchange capacities in the grasshopper, *Schistocerca americana*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **141**, 372-380.

Harrison, J. F., Frazier, M. R., Henry, J. R., Kaiser, A., Klok, C. J. and Rascón, B. (2006). Responses of terrestrial insects to hypoxia or hyperoxia. *Respir. Physiol. Neurobiol.* **154**, 4-17.

Hetz, S. K. and Bradley, T. J. (2005). Insects breathe discontinuously to avoid oxygen toxicity. *Nature* **433**, 516-519.

Kestler, P. (1985). Respiration and respiratory water loss. In *Environmental Physiology and Biochemistry of Insects* (ed. K. H. Hoffmann), pp. 137-183. Berlin, Germany: Springer-Verlag.

Kestler, P. (1991). Cyclic CO_2 release as a physiological stress indicator in insects. *Comp. Biochem. Physiol. C* **100**, 207-211.

Lease, H. M., Wolf, B. O. and Harrison, J. F. (2006). Intraspecific variation in tracheal volume in the American locust, *Schistocerca americana*, measured by a new inert gas method. *J. Exp. Biol.* **209**, 3476-3483.

Levy, R. I. and Schneiderman, H. A. (1966). Discontinuous respiration in insects. II. The direct measurement and significance of changes in tracheal gas composition during the respiratory cycle of silkworm pupae. *J. Insect Physiol.* **12**, 83-104.

Lighton, J. R. B. (1991). Ventilation in Namib Desert tenebrionid beetles: mass scaling and evidence of a novel quantized flutter-phase. *J. Exp. Biol.* **159**, 249-268.

Lighton, J. R. B. and Garrigan, D. (1995). Ant breathing: testing regulation and mechanism hypotheses with hypoxia. *J. Exp. Biol.* **198**, 1613-1620.

Marais, E., Klok, 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.

Quinlan, M. C. and Gibbs, A. G. (2006). Discontinuous gas exchange in insects. *Respir. Physiol. Neurobiol.* **154**, 18-29.

Schneiderman, H. A. (1960). Discontinuous respiration in insects: role of the spiracles. *Biol. Bull.* **119**, 494-528.

Seymour, R. S., Withers, P. C. and Weathers, W. W. (1998). Energetics of burrowing, running, and free-living in the Namib Desert golden mole (*Eremitalpa namibensis*). *J. Zool.* **244**, 107-117.

Snelling, E. P., Seymour, R. S., Matthews, P. G. D., Runciman, S. and White, C. R. (2011). Scaling of resting and maximum hopping metabolic rate throughout the life cycle of the locust *Locusta migratoria*. *J. Exp. Biol.* **214**, 3218-3224.

Terblanche, J. S., White, C. R., Blackburn, T. M., Marais, E. and Chown, S. L. (2008). Scaling of gas exchange cycle frequency in insects. *Biol. Lett.* **4**, 127-129.

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

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