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Discontinuous Gas Exchange, Water Loss, and Metabolism in *Protaetia cretica* (Cetoniinae, Scarabaeidae)

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ABSTRACT

Insects are at high risk of desiccation because of their small size, high surface-area-to-volume ratio, and air-filled tracheal system that ramifies throughout their bodies to transport O₂ and CO₂ to and from respiring cells. Although the tracheal system offers a high-conductance pathway for the movement of respiratory gases, it has the unintended consequence of allowing respiratory transpiration to the atmosphere. When resting, many species exchange respiratory gases discontinuously, and an early hypothesis for the origin of these discontinuous gas exchange cycles (DGCs) is that they serve to reduce respiratory water loss. In this study, we test this “hygric” hypothesis by comparing rates of CO₂ exchange and water loss among flower beetles *Protaetia cretica* (Cetoniinae, Scarabaeidae) breathing either continuously or discontinuously. We show that, consistent with the expectations of the hygric hypothesis, rates of total water loss are higher during continuous gas exchange than during discontinuous gas exchange and that the ratio of respiratory water loss to CO₂ exchange is lower during discontinuous gas exchange. This conclusion is in agreement with other studies of beetles and cockroaches that also support the hygric hypothesis. However, this result does not exclude other adaptive hypotheses supported by work on ants and moth pupae. This ambiguity may arise because there are multiple independent evolutionary origins of DGCs and no single adaptive function underlying their genesis. Alternatively, the observed reduction in water loss during DGCs may be a side effect of a nonadaptive gas exchange pattern that is elicited during periods of inactivity.

Introduction

Although all terrestrial animals lose water to the atmosphere, insects are particularly at risk of desiccation because of their small size and high surface-area-to-volume ratio (Edney 1977). Insects lose water via their excretory system, their cuticle, and their respiratory system. Although water loss through the first two avenues can be reduced to very low levels by increasing reabsorption of water before waste excretion or by decreasing the permeability of the cuticle (Hadley 1994a; Chown and Nicolson 2004), respiratory water loss (RWL) cannot be so easily prevented. This is because the respiratory system is a large, gas-permeable surface that is in intimate contact with the atmosphere. Because no biological membrane has a lower conductance for water than for CO₂ or O₂, the gas-permeable surface of the insect’s tracheal system is accordingly also highly permeable to water vapor. Thus, respiration is inseparable from water loss.

Insects regulate gas exchange between their tracheal system and the atmosphere by opening and closing their spiracles, the muscular valves that lie at the openings of the tracheal system. Spiracles open in response to intratracheal hypercapnia and hypoxia, thereby releasing CO₂ from the tracheal system and admitting O₂ when required (Miller 1960; Matthews and White 2011b). In doing so, they also inadvertently allow water vapor to transpire from the saturated tracheal surface to the atmosphere. Since RWL occurs as a consequence of the need to admit O₂ and release CO₂, it can be reduced in only one of two ways. First, it can be reduced in absolute terms by a decreasing metabolic rate (MR), because this reduces the insect’s demand for gas exchange and associated respiratory transpiration. However, this strategy is limited to periods of inactivity, as both spiracle conductance and water loss necessarily increase with the onset of physical activity and an increase in MR. Alternatively, RWL may be reduced in relative terms by decreasing the ratio of H₂O lost to CO₂ and O₂ exchanged. This latter option can be achieved by increasing CO₂ levels within the tracheal system relative to the intratracheal water vapor level, which is generally considered to be saturated at the insect’s body temperature (Lighton 1996). Because the water vapor partial pressure gradient driving RWL is constant, increasing intratracheal CO₂ levels allow for faster CO₂ efflux during shorter gas exchange periods, thereby limiting the time available for respiratory transpiration. The discontinuous gas exchange cycle (DGC) displayed by some insects has been put forward as a possible mechanism for reducing RWL by doing just this: generating periods of intratracheal hypercapnia during alternating cycles of apnoea and gas exchange (Chown et al. 2006). However, the situation regarding relative and absolute reduc-

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tions in RWL is not so straightforward, because the gas exchange pattern adopted by an insect is linked to its respiratory demand by its MR (Bradley 2007; Contreras and Bradley 2009, 2010). Thus, any discussion regarding RWL in insects involves issues of both gas exchange pattern and MR (Chown 2002; Contreras and Bradley 2010).

Gas Exchange Pattern

Insects display a wide variety of gas exchange patterns, which are typically classified according to the pattern of CO₂ release as being continuous, cyclic, or discontinuous. The DGC is characterized by periods in which the insect's spiracles are closed, preventing almost all gas exchange (the closed [C] phase), before the spiracles begin to rapidly flutter open and closed (the flutter [F] phase), permitting limited gas exchange. Finally, the spiracles open wide (the open [O] phase) immediately preceding the next C phase (Chown et al. 2006). By limiting the amount of time the spiracles are fully open as well as increasing intratracheal CO₂ levels during the C and F phases of the cycle, it has been proposed that DGCs reduce RWL, compared with continuous gas exchange patterns (Buck et al. 1953; Buck and Keister 1955; Levy and Schneiderman 1966a). In a few insects, the generation of negative intratracheal pressures during the C phase also allows for inwardly convective "suction ventilation" during the following F phase, further retarding the loss of water from the tracheal system (Lighton et al. 1993; Wobschall and Hetz 2004). Although there is a growing body of evidence to support the hypothesis that DGCs reduce water loss (a necessary precursor to proving the adaptive benefit of the DGC as per the hygric hypothesis; Schimpf et al. 2009; Williams et al. 2010), it is not incontrovertible, and several arguments opposing the importance of DGCs for limiting water loss have been put forward. These arguments cite the inconsistency with which DGCs are displayed by insects in arid environments and under desiccating conditions (Lighton 1998), with some species known to abandon DGCs when dehydrated (Hadley and Quinlan 1993; Fielden and Lighton 1996). Furthermore, the adaptive function of DGCs falls within a general disagreement over the importance of RWL as a driving force for the selection of respiratory patterns. It has been noted that, for many insects, RWL constitutes far less than 20% of their total water loss (TWL), with the majority of water being lost through the cuticle (Chown 2002). Therefore, it has been argued that the evolutionary importance of conserving such a small fraction of an insect's total water budget is likely to be minimal (Hadley 1994a, 1994b). When DGCs occur is also relevant to the issue of water loss, because this pattern appears to be displayed almost exclusively by resting insects (Hadley and Quinlan 1993; Chappell and Rogowitz 2000; Gibbs and Johnson 2004). A lower MR equates to a lower requirement for gas exchange and, therefore, to a lower rate of RWL, regardless of the gas exchange pattern employed. However, if RWL is lower during DGCs than during continuous gas exchange at the same MR, then this would provide additional support for the hygric hypothesis.

Thus, is water loss reduced by the gas exchange pattern or simply by the reduction in MR?

MR

The relationship between low MR and DGCs has long been remarked upon, with investigators realizing that this pattern is more likely to be displayed by quiescent insects with lower MRs, usually at low environmental temperatures (Kestler 1985; Bradley 2007; Contreras and Bradley 2009, 2010, 2011). This is relevant to RWL for two reasons. First, a low MR reduces the insect's respiratory demands and so reduces gas exchange and associated RWL. Second, the water vapor pressure within the tracheal system is considered to be close to saturated at the insect's body temperature (Lighton 1996). Therefore, low temperatures reduce the water vapor pressure within the tracheal system, lowering the partial pressure gradient driving respiratory transpiration and further reducing RWL. Taken in concert, these two factors suggest that respiratory gas exchange is a minor source of water loss when temperature and metabolism are low and also when DGCs are displayed. Nonetheless, studies have shown that, although ants are more likely to display DGCs when their MRs are low, independent of ambient temperature, breathing discontinuously does not decrease RWL relative to continuous or cyclic patterns of gas exchange (Gibbs and Johnson 2004). Conversely, studies involving quiescent moth larvae have found no difference between the MRs of individuals displaying either DGCs or continuous gas exchange patterns, but DGCs did reduce RWL (Williams et al. 2010). Clearly, how temperature and MR influence the occurrence of DGCs and the effect of DGCs on RWL require additional investigation.

In this study, we test the hypothesis that discontinuous gas exchange reduces RWL. To do so, we examine the effects of temperature and gas exchange pattern on MR and rates of water loss in flower beetles *Protaetia cretica* (Cetoniinae, Scarabaeidae).

Material and Methods

Animals

Flower beetles *Protaetia cretica* were collected from inflorescences of the dragon lily *Dracunculus vulgaris* growing in an olive orchard next to the village of Panormos in Crete (35°24'46.05"N, 24°41'6.03"E) in May 2008. Crete possesses a temperate Mediterranean climate characterized by warm, dry summers and cool, wet winters. Average temperatures for May range from 15.6° to 24.2°C, while the average relative humidity is 63.4% (Hellenic National Meteorological Service; <http://www.hnms.gr>). Beetles were taken to a field laboratory established within a local apartment and were kept in glass jars until required, usually within 2–3 d. Eight of the 10 beetles that exhibited DGCs were weighed to 2 mg on a 1210 portable balance (Tanita) before measurement (mean ± SD, 0.70 ± 0.13 g).

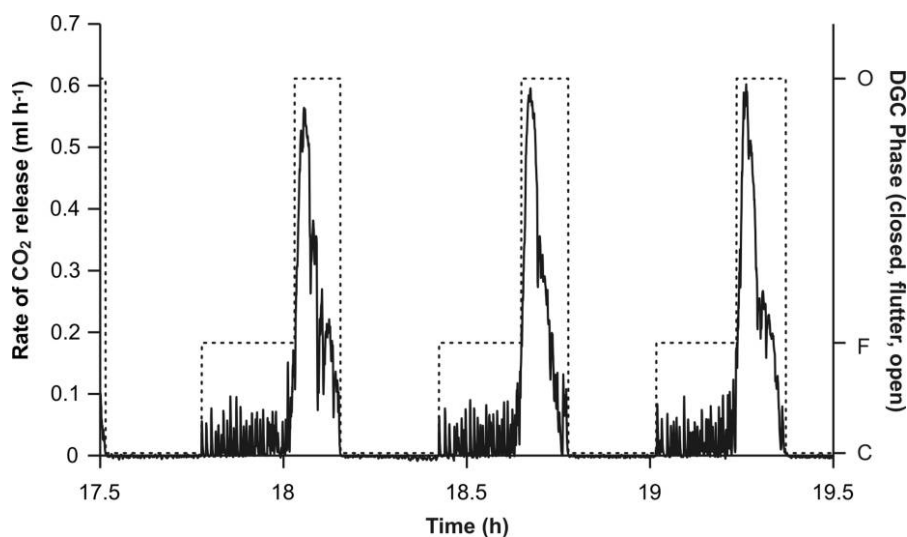


Figure 1. Example of a discontinuous gas exchange (DGC) exhibited by a beetle weighing 732 mg measured at 17.1°C. Solid lines indicate rate of CO₂ release. Dashed lines indicate the DGC phases determined automatically by an algorithm written in Excel.

Respirometry

Rates of CO₂ release (an indirect proxy for MR) and water vapor loss from individual *P. cretica* were measured using a flow-through respirometry system incorporating a Li-7000 CO₂/H₂O infrared gas analyzer (Li-COR) operating in differential mode. Atmospheric air was drawn from outside the building using an SS3 pump/flow meter (Sable Systems International) at 200–250 mL min⁻¹, dried by passing it through a column of Drierite (W. A. Hammond Drierite), through cell A of the Li-7000, before being passed through a 25-mL glass vial containing a beetle and finally through cell B of the infrared gas analyzer. Using the Li-7000 in differential mode allowed the beetle's respiration to be clearly separated from the atmospheric level of CO₂ present in the air stream. During measurements, the temperature of the respirometry chamber was maintained to within ±0.2°C using a miniature Peltier constant temperature cabinet controlled by a PID circuit. Beetles were measured at selected temperatures from 10° to 30°C. Temperatures were changed in a random order and maintained for a minimum of 4 h. A calibrated thermistor connected to a UI-2 A/D converter (Sable Systems International) was used to record the temperature within the respirometry chamber. The A/D converter was also connected to the voltage output from the flow meter as well as to the Li-7000's auxiliary voltage outputs, which were configured for ΔCO₂ (μmol CO₂ mol⁻¹) and ΔH₂O (mmol H₂O mol⁻¹). Data from the A/D converter were recorded at 1 Hz using Expedata software (Sable Systems International) running on a laptop personal computer, whereas Li-COR software was used to simultaneously log all infrared gas analyzer parameters.

Analysis

Respirometry traces were analyzed using a spreadsheet in Microsoft Excel. An algorithm was used to discriminate between the O, C, and F phases of the DGC according to threshold levels of CO₂ emission, which were chosen following a visual inspection of each data set (fig. 1). The correct identification of the phases was further aided using the explicit assumption that the phases of the DGC proceeded in the order C, F, O, C; thus, transient changes in CO₂ emission that occurred during a phase did not cause the spreadsheet to incorrectly identify a phase change. The total amount of CO₂ and H₂O lost during each phase as well as the phase's duration were then computed for each experimental temperature. Beetles periodically displayed periods of continuous respiration when the DGC was no longer displayed. Rates of cuticular water loss (CWL) were determined during the C period for those beetles that displayed a discontinuous pattern of respiration.

Data were analyzed in JMP software, version 8.0.2.2 (SAS Institute), using repeated-measures ANOVA with beetle identification number as a random factor. Unless otherwise stated, mean values for each beetle at each temperature were analyzed, and data are presented as mean ± SEM. Post hoc comparisons were undertaken using Student's *t*-tests (Quinn and Keough 2002).

Results

MR and Gas Exchange

Protaetia cretica displayed two distinct gas exchange patterns: a typical discontinuous pattern characterized by the sequential repetition of C, F, and O phases (fig. 1) and a continuous pattern in which CO₂ release never decreased to 0. The MR of

P. cretica increased with Q_{10} values that were significantly different between DGC and continuous respiration (3.1 and 3.8, respectively; $P = 0.0003$; fig. 2A). Durations of the C, F, and O phases all decreased with temperature ($P < 0.001$ in all cases), with Q_{10} values of 0.20, 0.33, and 0.56, respectively (fig. 3). As such, the ratio of the duration of the O to CF phases increased significantly with temperature ($Q_{10} = 2.44$; $P < 0.0001$; fig. 2C). The frequency of the DGC increased with temperature with a Q_{10} of 3.1, whereas the volume of CO_2 released during the O phase decreased significantly with a Q_{10} of 0.8 ($P < 0.001$; fig. 4). At 20°C, which was the temperature at which the largest number of measurements were made, rates of CO_2 exchange differed between all phases of discontinuous gas exchange as well as between these and continuous gas exchange ($P < 0.0001$; fig. 5A). The rate of CO_2 exchange during the C phase was not significantly different from 0, the rate of CO_2 exchange was 7.3-fold higher during the O phase than during the F phase, and the rate of CO_2 exchange was 1.8-fold higher during the O phase than during continuous gas exchange (table 1; fig. 5A).

Water Loss

Rates of TWL (mg h^{-1}) increased with temperature ($P = 0.0005$) with a Q_{10} of 3.31 that was not significantly different between DGC and continuous respiration ($P = 0.68$; fig. 2B). TWL was significantly higher during continuous gas exchange than during DGCs ($P = 0.009$; fig. 2B). The ratio of TWL to $\dot{V}\text{CO}_2$ was not related to temperature ($P = 0.67$) and was significantly higher during DGCs than during continuous gas exchange ($P = 0.03$; fig. 6). At 20°C, water loss differed significantly between phases of the DGC and continuous gas exchange ($P < 0.0001$). TWL was not significantly different between the C and F phases of the DGC and between the O phase of the DGC and continuous gas exchange (table 1; fig. 5B). Water loss was 67% higher during the O phase than during the C phase (table 1; fig. 5B).

Of the 21 beetles measured, only 10 displayed DGCs, and five of those 10 displayed periods of both continuous and discontinuous gas exchange. A direct comparison between rates of RWL during continuous gas exchange and DGCs could be made only for those individual beetles that displayed both patterns of respiration. RWL was quantified as the difference between CWL determined during the C phase of the DGC and the TWL. RWL increased with temperature with a Q_{10} of 3.65 that was not significantly different between continuous and discontinuous gas exchange ($P = 0.91$). RWL was significantly higher during continuous gas exchange than during discontinuous gas exchange ($P < 0.0001$) and, when averaged across all temperatures, accounted for $28.1\% \pm 3.2\%$ of TWL during continuous gas exchange and $15.6\% \pm 2.5\%$ of TWL during DGCs. The ratio of RWL to $\dot{V}\text{CO}_2$ was not related to temperature ($P = 0.93$) and was higher during continuous than during discontinuous gas exchange ($P = 0.001$; fig. 6).

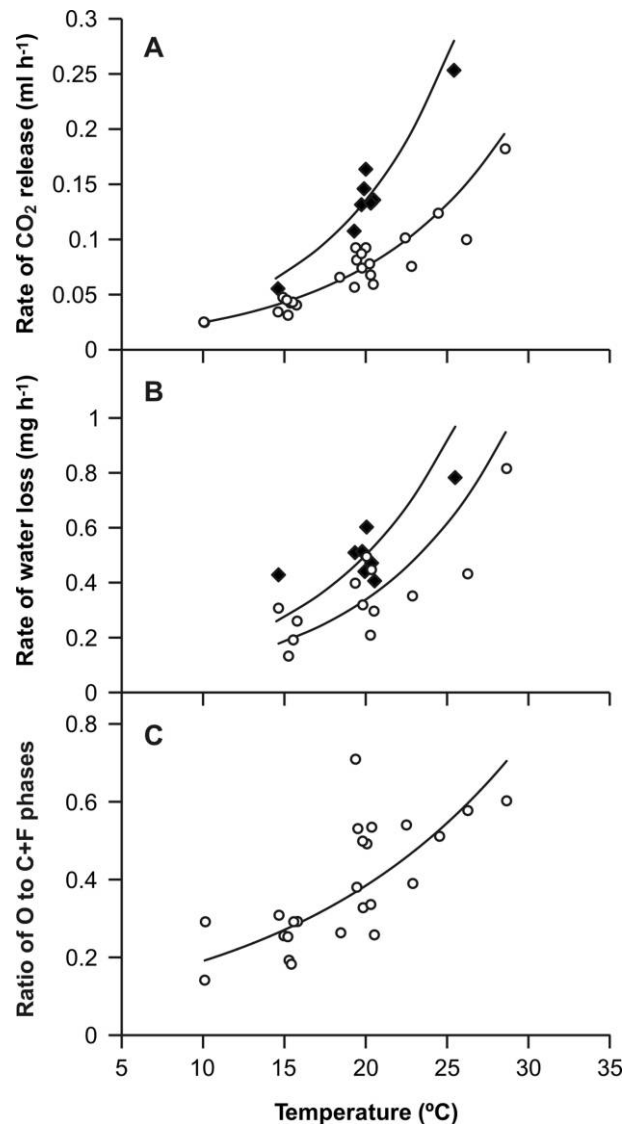


Figure 2. Effect of temperature on rates of CO_2 production (A), total water loss during continuous (filled diamonds) and discontinuous (open circles) gas exchange (B), and the ratio of the duration of the open (O) to closed and flutter (C+F) phases of the discontinuous gas exchange cycle (C).

Discussion

Gas Exchange Pattern and Water Loss

In this study, we tested the hypothesis that discontinuous gas exchange reduces water loss in the flower beetle *Protaetia cretica*. In support of this hypothesis, the rate of TWL was lower during discontinuous gas exchange than during continuous gas exchange at all temperatures (fig. 2B). This reduction in TWL can be attributed to the lower MRs (fig. 2A) and hence lower gas exchange requirements of those beetles displaying DGCs. However, in terms of absolute water efficiency, DGCs do not increase the ratio of CO_2 released to TWL, as might be expected according to the hygric hypothesis. This is attributable to two

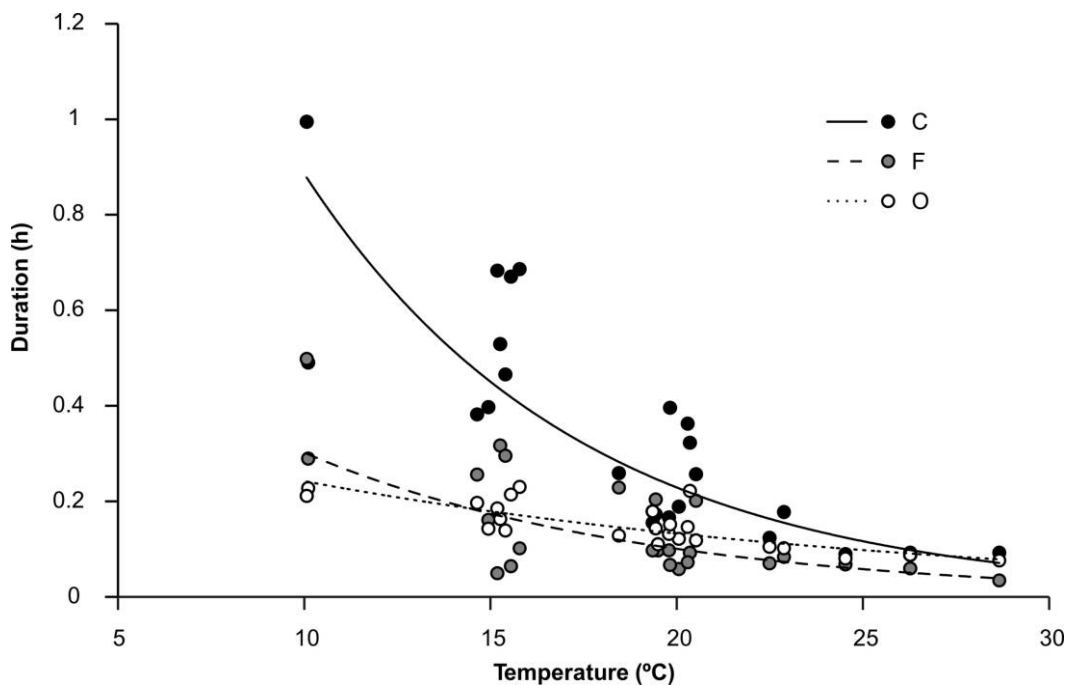


Figure 3. Effect of temperature on durations of the closed (C; black circles, solid line), flutter (F; gray circles, dashed line), and open (O; white circles, dotted line) phases of the discontinuous gas exchange cycle.

factors: first, beetles displaying DGCs had lower MRs than did those displaying a continuous pattern of gas exchange (fig. 2A) and, second, the major pathway for water loss in these beetles was through their cuticle (84% during DGCs and 59% during continuous gas exchange). Because CWL is not altered by the gas exchange pattern displayed, the lower mean rate of CO_2 release during DGCs serves only to reduce the ratio of CO_2 lost relative to the consistently high rate of CWL and, therefore, TWL (fig. 6). In contrast, if the ratio of CO_2 released to water lost is calculated using RWL only, then the opposite is true: DGCs lose less respiratory water relative to the amount of CO_2 released (fig. 6). The DGC achieves this by reducing RWL during the C and F phases (fig. 5B) while increasing the rate of CO_2 release during the O phase (fig. 5A). Because the rate of water loss through an open spiracle is the same regardless of whether it occurs during the O phase of a DGC or during continuous gas exchange (fig. 5B), the higher rate of CO_2 release during the O phase results in more CO_2 being expelled in relation to RWL. Thus, although DGCs appear to be no more effective than continuous gas exchange patterns in reducing the ratio of TWL : CO_2 released, DGCs can be considered to decrease the ratio of RWL : CO_2 released as well as being associated with lower absolute rates of TWL in concert with lower MR.

Two recent comparative analyses, mainly of beetles and ants (Marais et al. 2005; White et al. 2007), also support the hygric hypothesis, as have experimental studies of beetles (Chown and Davis 2003), cockroaches (Schimpf et al. 2009, 2011), termites (Sláma et al. 2007), moth larvae (Williams et al. 2010), and

arachnids (Sláma 1995). However, other studies question the importance of DGCs in reducing water loss (Gibbs and Johnson 2004) and demonstrate that some, but not all, species abandon DGCs when dehydrated (Hadley and Quinlan 1993; Sláma 1995; Fielden and Lighton 1996; Duncan et al. 2002) or when ambient temperatures, and thus desiccation stress, are highest (Rourke 2000). DGCs also continue in humid air when rates of water loss are greatly reduced (Contreras and Bradley 2009; Schimpf et al. 2009) and when CWL accounts for ~95% of TWL (Dingha et al. 2005). These findings call into question the general importance of limiting RWL in the origin of DGCs and contributed to the development of two additional adaptive hypotheses (reviewed by Chown et al. 2006; Quinlan and Gibbs 2006) and two nonadaptive hypotheses (Chown and Holter 2000; Matthews and White 2011a). The chthonic hypothesis suggests that DGCs enhance gas exchange in hypoxic or hypercapnic conditions that are characteristic of underground environments (Lighton and Berrigan 1995; Lighton 1998). The oxidative damage hypothesis suggests that DGCs provide adequate gas exchange while reducing oxidative damage to tissues at rest that would otherwise result from a tracheal system that has evolved to maximize oxygen availability during activity (Bradley 2000, 2006). Experimental studies of ants support the chthonic hypothesis (Lighton and Berrigan 1995; Lighton and Turner 2008), whereas studies of moth pupae support the oxidative damage hypothesis (Hetz and Bradley 2005; Terblanche et al. 2008). The lack of clear support for a single hypothesis across multiple groups may arise because DGCs have probably evolved independently in multiple groups of tracheated ar-

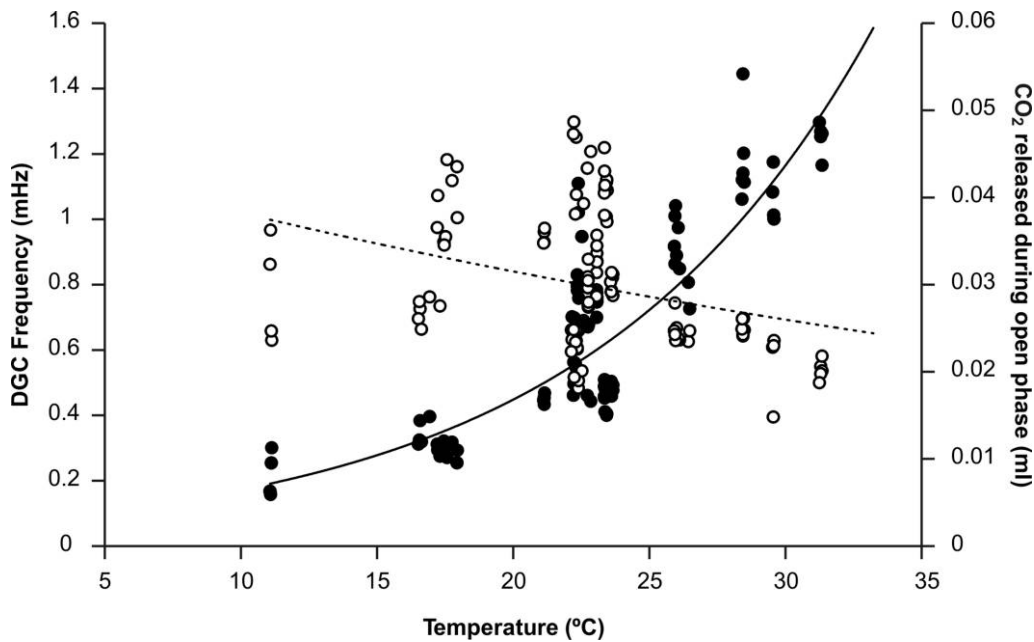


Figure 4. Effect of temperature on discontinuous gas exchange cycle (DGC) frequency (filled circles, solid line) and volume of CO₂ released during the open phase (open circles, dashed line).

thropods (Klok et al. 2002; Marais et al. 2005), so it is certainly possible that the adaptive significance in each species is as independent as its evolutionary origins (Matthews and White 2011a). Alternatively, DGCs may not be adaptive (Chown and Holter 2000) and may result simply from thoracic and abdominal ganglia regulating ventilation in response to hypoxic and hypercapnic stimuli in the absence of control from higher neural centers (Sláma 1988, 1994; Matthews and White 2011a). The emergence of DGCs then results from the interaction between O₂ and CO₂ “set points” and how these influence spiracular opening and closing (Burkett and Schneiderman 1967, 1974; Förster and Hetz 2009). Such a possibility does not preclude subsequent adaptive modification of the DGC pattern to, for example, restrict water loss (Buck et al. 1953; Schimpf et al. 2009), reduce oxidative damage (Bradley 2000; Hetz and Bradley 2005), or retain and release CO₂ to regulate hemolymph pH (Sláma 1994; Matthews and White 2009). At present, however, a reliance on acute experimental manipulation of individual insects, rather than a long-term experimental evolution approach, makes it difficult to determine which, if any, of these possibilities is the case. Additional work employing uniform methodologies across the multiple groups that show DGCs is required to establish whether there is a single adaptive explanation for the multiple independent evolutionary origins of DGCs.

DGC Frequency

Our results show that *P. cretica* accommodate increases in MR during DGCs by increasing the cycle’s frequency ($Q_{10} = 3.1$), whereas the volume of CO₂ released during each O phase shows

a slight decrease with increasing temperature ($Q_{10} = 0.8$; fig. 4). Although the decrease in CO₂ burst volume is significant ($P = 0.001$), the very low R^2 (0.09) of the linear regression indicates that temperature explains very little of this variation. The identical Q_{10} response of both DGC frequency and MR further supports the conclusion that DGC frequency is modulated by MR. This response has also been found for other scarabaeid (Davis et al. 1999; Terblanche et al. 2010), carabid (Duncan and Dickman 2001), cerambycid (Chappell and Rogowitz 2000), and curculionid (Klok and Chown 2005) beetles. This suggests that beetles, and indeed most other insects that display DGCs (including moth pupae and ants; Levy and Schneiderman 1966b; Lighton 1988a), modulate the frequency, rather than the O phase duration or “duty cycle,” of the DGC. This idea sits well with the hypothesis that the O phase of the DGC is initiated when intratracheal CO₂ crosses a threshold level and is then sustained until a fixed volume of CO₂ has been purged from the insect. However, the cause and maintenance of the subsequent C period is an enigma that remains to be solved (Förster and Hetz 2009).

DGCs and MR

As this study and others have shown, the likelihood of an insect displaying a DGC increases with decreasing MR (Gibbs and Johnson 2004; Bradley 2007; Contreras and Bradley 2009, 2010; Moerbitz and Hetz 2010). This has led to suggestions that it is low MR per se that causes the insect to display a DGC (Bradley 2007; Contreras and Bradley 2009). Although low MR is a necessary precondition for a long C phase during a DGC, Williams et al. (2010) found that the MR of moth larvae did

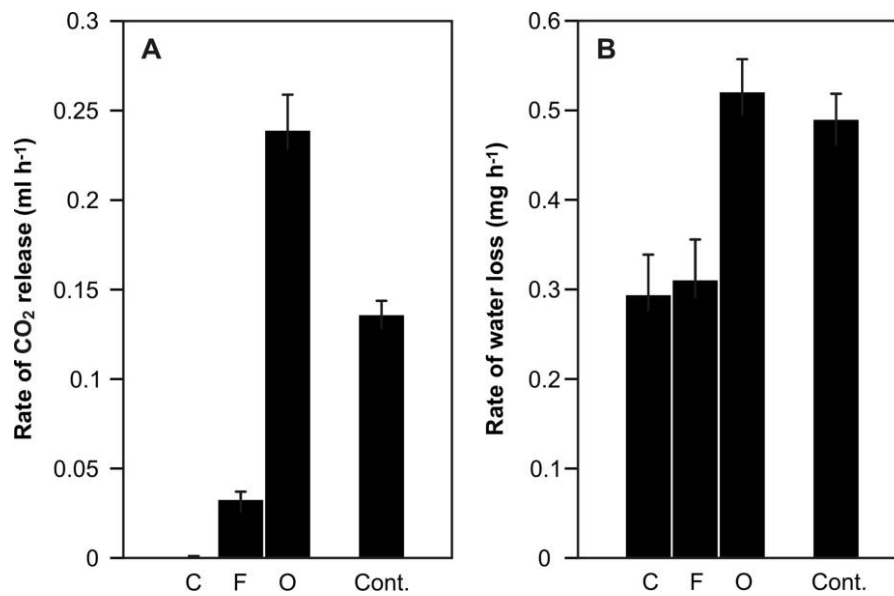


Figure 5. Variation in (A) the rate of CO₂ release ($n = 9$) and (B) the rate of water loss ($n = 6$) between phases of the discontinuous gas exchange cycle (C, closed; F, flutter; O, open) and continuous gas exchange (Cont.). Data are shown as mean \pm SEM.

not differ significantly between individuals with DGCs and those with continuous gas exchange patterns. The importance of MR in driving the occurrence of DGCs may also be called into question by examining the range of CO₂ release rates across which continuous and discontinuous gas exchange patterns were displayed by the flower beetles in this study. Although DGCs were undeniably more frequent at lower MRs, there is still overlap between the MRs of individuals displaying DGCs and continuous patterns of gas exchange (fig. 2A). Because temperature is the parameter most easily manipulated to control MR in insects, it is worth noting that temperature probably has other significant effects on the insect beyond a direct effect on MR. An insect experiencing high ambient temperatures will likely become physically active, either in response to the decreased cost of activity at higher ambient temperatures (Seymour et al. 2003) or in an attempt to move to more convivial thermal environments. Any increase in activity in response to increasing temperature would produce an increase in MR above that attributable to van 't Hoff or Q_{10} effects alone. This is one possible explanation for the higher Q_{10} of beetles displaying continuous gas exchange patterns relative to those that were breathing discontinuously (fig. 2A). Although activity was not recorded in these experiments, there is a large body of evidence that indicates DGCs are exhibited only by insects that are “resting” (Kestler 1985; Lighton 1988*b*, 1998; Hadley and Quinlan 1993; Chappell and Rogowitz 2000; Terblanche et al. 2010). If a resting state is a prerequisite for DGCs, then this would also explain the lack (but not absence) of individuals displaying DGCs at higher temperatures. These observations suggest that it might not be simply low MR that causes the emergence of DGCs but some other aspect of inactivity, possibly associated with changes in the neurological control of respiration (Mat-

thews and White 2011*a*). Future studies that seek to investigate the effects of temperature on MR and gas exchange pattern should record activity as a matter of course to allow periods of activity and quiescence to be distinguished and their effects on gas exchange pattern and MR to be determined.

Table 1: Gas exchange characteristics of *Protaetia cretica* at 20°C

Variable	Mean \pm SEM (sample size)
Discontinuous gas exchange:	
\dot{V}_{CO_2} (mL h ⁻¹)	.077 \pm .004 (9)
$\dot{M}_{\text{H}_2\text{O}}$ (mg h ⁻¹)	.361 \pm .043 (6)
Closed phase:	
Duration (h)	.237 \pm .034 (9)
\dot{V}_{CO_2} (mL h ⁻¹)	.000 \pm .001 (9)
$\dot{M}_{\text{H}_2\text{O}}$ (mg h ⁻¹)	.295 \pm .044 (6)
Flutter phase:	
Duration (h)	.110 \pm .018 (9)
\dot{V}_{CO_2} (mL h ⁻¹)	.033 \pm .004 (9)
$\dot{M}_{\text{H}_2\text{O}}$ (mg h ⁻¹)	.311 \pm .045 (6)
Open phase:	
Duration (h)	.147 \pm .012 (9)
\dot{V}_{CO_2} (mL h ⁻¹)	.239 \pm .020 (9)
$\dot{M}_{\text{H}_2\text{O}}$ (mg h ⁻¹)	.521 \pm .036 (6)
Continuous gas exchange:	
\dot{V}_{CO_2} (mL h ⁻¹)	.136 \pm .008 (6)
$\dot{M}_{\text{H}_2\text{O}}$ (mg h ⁻¹)	.491 \pm .028 (6)

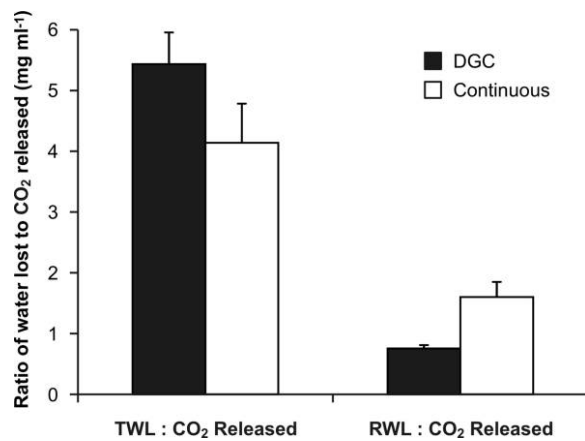


Figure 6. Effect of gas exchange pattern on the ratio of total water loss (TWL) and respiratory water loss (RWL) to CO₂ release. The difference between continuous and discontinuous gas exchange is significant for both total and respiratory water loss. Data are shown as mean \pm SEM ($n = 7$).

Acknowledgments

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