The hygric hypothesis does not hold water: abolition of discontinuous gas exchange cycles does not affect water loss in the ant *Camponotus vicinus*

John R. B. Lighton[1,2,*] and Robbin J. Turner[2]

[1]Department of Biological Sciences, University of Nevada at Las Vegas, Las Vegas, NV 89154-4004, USA and [2]Sable Systems International, 6340 S. Sandhill Road, Suite 4, Las Vegas, NV 89120, USA

*Author for correspondence (e-mail: john@johnlighton.org)

SUMMARY

The discontinuous gas exchange cycle (DGC) of insects and other tracheate arthropods temporally decouples oxygen uptake and carbon dioxide emission and generates powerful concentration gradients for both gas species between the outside world and the tracheal system. Although the DGC is considered an adaptation to reduce respiratory water loss (RWL) – the 'hygric hypothesis' – it is absent from many taxa, including xeric ones. The 'chthonic hypothesis' states that the DGC originated as an adaptation to gas exchange in hypoxic and hypercapnic, i.e. underground, environments. If that is the case then the DGC is not the ancestral condition, and its expression is not necessarily a requirement for reducing RWL. Here we report a study of water loss rate in the ant *Camponotus vicinus*, measured while its DGC was slowly eliminated by gradual hypoxia (hypoxic ramp de-DGCing). Metabolic rate remained constant. The DGC ceased at a mean P_{O_2} of 8.4 kPa. RWL in the absence of DGCs was not affected until P_{O_2} declined below 3.9 kPa. Below that value, non-DGC spiracular regulation failed, accompanied by a large increase in RWL. Thus, the spiracular control strategy of the DGC is not required for low RWL, even in animals that normally express the DGC.

Key words: discontinuous gas exchange, DGC, insect respiration, respiratory water loss.

INTRODUCTION

The DGC, or discontinuous gas exchange cycle, is a remarkable, low-frequency decoupling between oxygen uptake and CO_2 emission that occurs in several orders of tracheate arthropods (for reviews, see Miller, 1981; Kestler, 1985; Lighton, 1996; Lighton, 1998; Chown et al., 2006). DGC duration is not significantly dependent on body mass under about 1 g (Lighton, 1991) and lasts about 5–10 min in typical animals, although in some taxa, such as ticks, it may last for $\geqslant$1 h (Lighton and Fielden, 1995). A DGC consists of three phases delineated by different actions of the spiracles. Spiracles are tightly controlled valves that control access to the tracheal system, which transports respiratory gases directly to and from the tissues. During the C (constricted-spiracles) phase, external gas exchange is minimal, endotracheal partial pressure of oxygen (P_{O_2}) falls, and CO_2 is mostly buffered. At a critical P_{O_2} of ~4 kPa, the F (fluttering-spiracles) phase begins, during which O_2 enters the insect principally by diffusion at a rate sufficient to meet mitochondrial requirements, while CO_2 continues to be buffered by internal tissues and diffuses out far more slowly than its rate of production. Finally, the O (open-spiracle) phase begins and the CO_2 escapes, whereupon the cycle repeats.

The DGC is generally considered an evolutionary adaptation to reduce respiratory water loss (RWL) rates (Buck et al., 1953). However, in recent years this hypothesis has become more controversial (Lighton and Berrigan, 1995; Lighton, 1996; Lighton, 1998; Hetz and Bradley, 2005; Chown et al., 2006). Comparing animals that express the DGC with those that do not is difficult. It requires extensive phylogenetic and environmental information that is not always available and that may be vulnerable to outlier effects (White et al., 2007). A direct assessment of the water loss correlates of the DGC in a single animal would be useful. However attempts to 'turn off' the DGC of an animal that normally utilizes it and to directly measure the effect of this manipulation on water loss have been problematic. Gibbs and Johnson report that in the *Pogonomyrmex* ants they studied, RWL was equivalent whether or not the DGC was expressed (Gibbs and Johnson, 2004), but the interpretation of this finding is difficult because the ants that did not express the DGC also displayed a higher metabolic rate. Lighton et al. could cause the harvester ant *Pogonomyrmex californicus* to abandon the DGC (Lighton et al., 2004), which it normally expressed (Quinlan and Lighton, 1999), by raising its temperature to 40°C. Respiratory water loss could then be measured by briefly exposing the ant to pure O_2, causing it to briefly close its spiracles, while monitoring its rate of water loss in real time. After compensation for the increase in temperature, no significant increase in RWL during continuous gas exchange was found relative to the DGC condition. However, this too was a less than satisfactory test, because the measurements were indirect (in the case of continuous gas exchange) and required compensation for the very large effect of temperature on both the ant's metabolic rate and the water vapor pressure saturation deficit to which it was exposed.

Here we report a method for assessing RWL while progressively disabling the DGC of an insect, the carpenter ant *Camponotus vicinus*. The ant is taken from complete expression of a conventional DGC to complete abolition of the DGC by gradual ramped hypoxia. This technique (hypoxic ramp de-DGCing) should be universally applicable for animals expressing the DGC, and it allows accurate quantification of the DGC's water loss correlates. We tested the null hypothesis that abolition of the DGC would cause no significant increase in water loss rates (WLRs).

MATERIALS AND METHODS
Animals

We collected *Camponotus vicinus* Mayr ants from dead tree stumps at Mount Potosi, Clark County, NV, USA in the Boreal fall. The ants were kept at ambient temperatures (26±2°C) and an ambient L/D cycle in shallow polyethylene containers and were given water and 20% w/v sucrose solution *ad libitum*. They were used within two weeks of capture and remained in apparently good condition for at least a further two months. We used exclusively female alates (the winged reproductive caste) for these experiments.

Respirometry

We created a gradual hypoxic ramp by mixing air and nitrogen, using two Tylan FC-260 mass flow control valves driven by an MFC-4 gas mixing unit [Sable Systems International (SSI), Las vegas, NV, USA]. The MFC-4 was programmed to generate a gradual downward ramp of 19 to 1.5 kPa P_{O_2} at the local barometric pressure over a time-span of 90 min. This was achieved by progressively bleeding nitrogen into a dry, CO_2-scrubbed airstream while maintaining a constant total flow rate of 100 ml min^{-1}. Simultaneously, the CO_2 production of the ant was measured with an SSI TR-2 respirometry system, with WLR measured using a SSI RH-100 water vapor analyzer. The P_{O_2} in the airstream was continuously checked using a SSI FC-1b O_2 analyzer. The system was automatically baselined at the beginning and end of each recording, which typically lasted ~3.5 h. Ant activity was monitored using a SSI AD-2 optical activity detector. Data were acquired at 1 Hz with digital filtration using a SSI UI-2 16-bit A/D converter and SSI ExpeData software. Ants were weighed to 0.01 mg at the beginning and end of each run using a Mettler AG245 balance.

Conversion of the recorded data from p.p.m. CO_2 and kPa water vapor pressure to μl h^{-1} CO_2 production and mg h^{-1} water loss, and response correction to correct washout kinetics (Bartholomew et al., 1981) were carried out under batch macro control in ExpeData as described elsewhere (Lighton and Turner, 2004). The CO_2, O_2 and water vapor traces were lag-corrected into synchrony, and, within each trace, each DGC was analyzed to yield data on (C+F) phase duration, CO_2 emission and WLRs plus the equivalent data for the O phase, together with mean kPa P_{O_2} during each complete DGC. From these data, an ExpeData spreadsheet macro calculated data on DGC frequency, plus overall DGC CO_2 production and WLRs.

Statistics

All means are accompanied by sample numbers (*N*) and standard deviations (s.d.). Means were compared using analysis of variance (ANOVA). Significance was set at $P<0.05$. Regression analysis was by least squares, with axis transformation where noted, with significance testing by ANOVA. Statistical tests were carried out with ExpeData's RudeStat facility, the algorithms of which have been validated against Systat IV.

RESULTS

Results are reported for 17 *Camponotus vicinus* female alates. A typical hypoxic ramp is shown in Fig. 1. The descriptive statistics are summarized in Table 1. All animals reacted to the hypoxic ramp in a similar way. A steady increase in the time interval between DGCs was apparent as the hypoxia progressed. Finally, below ~8.4 kPa P_{O_2}, the DGC was completely abolished and gas exchange became continuous. As hypoxia progressed, a critical point was reached at ~3.9 kPa P_{O_2} at which spiracular control was lost and RWL increased rapidly. At that stage the ant became active, showing escape behavior. Finally, after P_{O_2} declined below ~2.3 kPa, the ant's spiracles opened fully and WLR reached a high plateau level. The ant rapidly restored spiracular control when oxygen levels were restored to normal. However, only one ant expressed the DGC again during the approximately 30 min of normoxic recovery at the end of each run.

Rate of CO_2 emission in normoxia did not scale significantly with body mass over the relatively narrow 65–80 mg body mass range of the alate ants (log-transformed axes; $F_{1,15}=1.97$, $P>0.1$) and was identical to the value predicted for an arthropod of the same mean mass at 26°C [0.020 ml h^{-1} assuming respiratory quotient (RQ)=1] (Lighton et al., 2001). Curiously, a later allometric equation by Chown et al. (Chown et al., 2007) overestimates the rate of CO_2 uptake ($\dot{V}_{CO_2}$) of *C. vicinus* by twofold. To analyze the effects of P_{O_2} on rate of CO_2 emission and water loss, the DGC data were pooled by P_{O_2} in 1 kPa bins over the range 9–19 kPa. Oxygen partial pressure over the range 19–9 kPa did not affect CO_2 production rate during the period when the ant expressed the DGC ($r^2=0.01$, $F_{1,9}=0.09$, $P=0.4$).
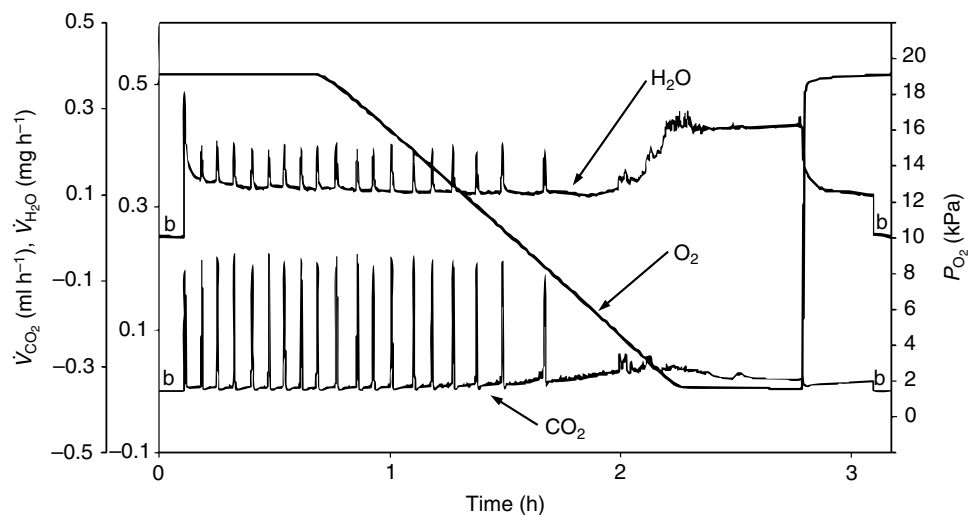

Fig. 1. The effect of gradual hypoxia on an alate *Camponotus vicinus* ant, mass 79.5 mg. Baselines for H_2O and CO_2 are equal to zero and are denoted by 'b'. As hypoxia progresses, the interval between O phases (sharp upward spikes) increases until the discontinuous gas exchange cycle (DGC) ceases altogether below ~8 kPa P_{O_2}. Rate of CO_2 production ($\dot{V}_{CO_2}$) is the innermost of the two left-hand axis scales.

Table 1. Effects of graded hypoxia in *Camponotus vicinus*

Body mass	73.31±6.77 mg
Total $\dot{V}_{CO_2}$	0.0196±0.0027 ml h^{-1}
Total WLR	0.593±0.160 mg h^{-1}
C+F phase WLR	0.525±0.149 mg h^{-1}
DGC cut-off	8.44±1.05 kPa O_2
WLR at cut-off	0.548±0.133 mg h^{-1}
WLR at 6.5 kPa	0.559±0.157 mg h^{-1}
WLR at 5.5 kPa	0.609±0.160 mg h^{-1}
Critical kPa	3.89±0.61 kPa O_2
WLR critical kPa	0.785±198 mg h^{-1}
Plateau start kPa	2.30±0.37 kPa O_2
WLR plateau kPa	1.485±0.228 mg h^{-1}

The table shows the rate of CO_2 production (total $\dot{V}_{CO_2}$), water loss rate (total WLR) and WLR during the (C+F) phases, during the time the discontinuous gas exchange cycle (DGC) was expressed (see text); the WLR measured immediately after the last DGC; the WLR measured at successively lower oxygen partial pressures (P_{O_2}s) (6.5 and 5.5 kPa); the P_{O_2} at which spiracular control begins to break down and WLR starts to increase (critical kPa O_2); and the P_{O_2} at which WLR reaches a high, plateau level, corresponding to a complete breakdown of spiracular control. All measurements were made at 26±2°C. Means and standard deviations are shown. *N*=17 female alate *Camponotus vicinus* on all rows.

As previously shown (Lighton and Garrigan, 1995), hypoxia caused spiracular area to increase during the F phase to compensate for the reduced O_2 gradient across the spiracles, causing more CO_2 to emerge during the F phase (Fig. 2) and thus delaying the hypercapnic triggering of the O phase, causing DGC frequency to fall (Fig. 3). If the respiratory exchange ratio (RER) during the F phase is estimated by assuming an RQ of 1.0, and thus a steady-state rate of O_2 consumption equal to the rate of CO_2 production, the RER increases from a reasonable F phase value of 0.22 in normoxia [see Lighton (Lighton, 1998) for a discussion on RER during the F phase] to 0.66 below 9 kPa P_{O_2}. Also as previously reported (Lighton and Garrigan, 1995), WLRs during the O phase of the DGC increased slightly but significantly as P_{O_2} dropped (r^2=0.782, $F_{1,9}$=32.25, P=0.0003). An increase in exposed tracheolar area as P_{O_2} fell may have caused this effect, which had a low slope of only –0.00968 mg h^{-1} kPa^{-1}. O phase duration was not affected by hypoxia ($F_{1,9}$=0.007, P=0.4). Peak WLR during the highest 10 s of the last five O phases prior to loss of the DGC was only 70.0±7.9% of peak steady-state WLR below 2.3 kPa (which presumably corresponds to maximal spiracular area). However, comparing these rates is problematic because of the dynamic nature of WLR during the O phase and the resulting 'blunting' effect of analyzer response time and water vapor adsorption to tubing, which cannot be completely eliminated by response correction.

Given that the rate of CO_2 emission increased during the F phase (but not over the entire DGC) during hypoxia, it is reasonable to expect a similar increase in whole-DGC rate of water loss. It is noteworthy that this did not occur; indeed, the opposite occurred. As P_{O_2} declined from 19 to 9 kPa, the WLR measured over the entire DGC cycle (C+F+O) actually declined significantly (Fig. 4; and see Discussion). Within individual DGCs, in the water vapor traces no distinction between the C and F phases was discernible, although the C and F phases were clearly distinguished in the CO_2 traces (Fig. 1). We therefore conclude that the vast majority of the water loss measured during C and F phases, and thus during the entire DGC with its brief O phases (Fig. 4), was cuticular in origin (see also Lighton and Garrigan, 1995).

To assay the effect on RWL of completely eliminating the DGC, which ended below 8.4 kPa on average, the overall WLR was
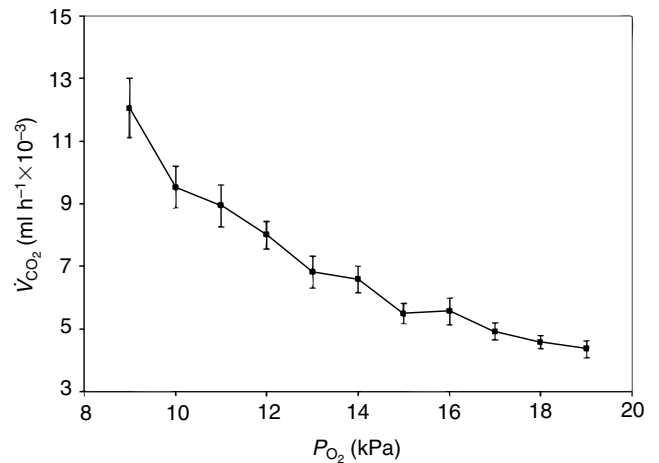

Fig. 2. The effect of hypoxia on the rate of CO_2 production ($\dot{V}_{CO_2}$) in the F phase of the discontinuous gas exchange cycle (DGC) in *Camponotus vicinus*. As oxygen partial pressure (P_{O_2}) declines, spiracular area increases to compensate for the reduced trans-spiracular O_2 concentration gradient, elevating CO_2 output in the F phase as a byproduct. Consequently, the time taken to reach the hypercapnic threshold for the next O phase is increased, reducing DGC frequency (see Fig. 3). P_{O_2} explains 91% of the variance in F phase $\dot{V}_{CO_2}$ ($F_{1,9}$=90.1, P<10^{-5}). Overall $\dot{V}_{CO_2}$ of the entire DGC remains constant across P_{O_2}s (see text). Error bars in this and subsequent graphs denote standard errors.
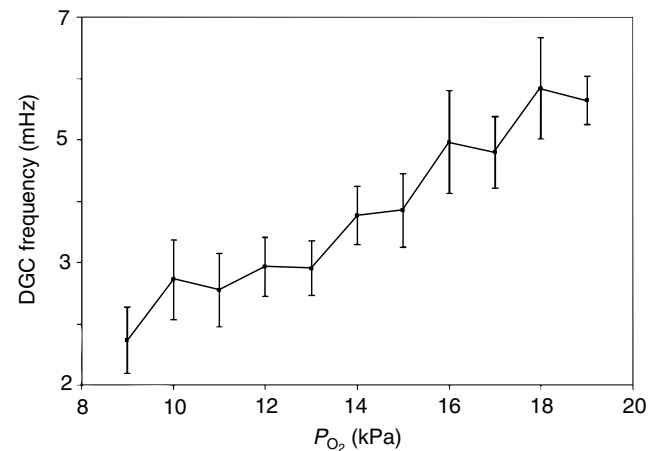

Fig. 3. The effect of hypoxia on the frequency of the discontinuous gas exchange cycle (DGC) in *Camponotus vicinus*. Hypoxia delays the initiation of the O phase (see Fig. 2), lowering DGC frequency. Partial pressure of O_2 (P_{O_2}) explains 94% of the variance in DGC frequency ($F_{1,9}$=150.4, P<10^{-6}).

averaged for 1 min around the 6.5 and 5.5 kPa O_2 hypoxic ramp points, which are two and three standard deviations, respectively, below the DGC cut-off point (see Fig. 4). Gas exchange was continuous during these periods. Four data sets were then compared using ANOVA: (1) the mean WLR over the entire period during which the DGC was expressed for each ant; (2) the WLR averaged for 1 min immediately after the last DGC for each ant; and the abovementioned WLRs averaged at the (3) 6.5 and (4) 5.5 kPa O_2 hypoxic ramp points for each ant. No significant differences were found [$F_{3,64}$=0.53, P=0.34; grand mean=0.572±0.072 mg h^{-1} (s.e.m.)] (see also Fig. 4).
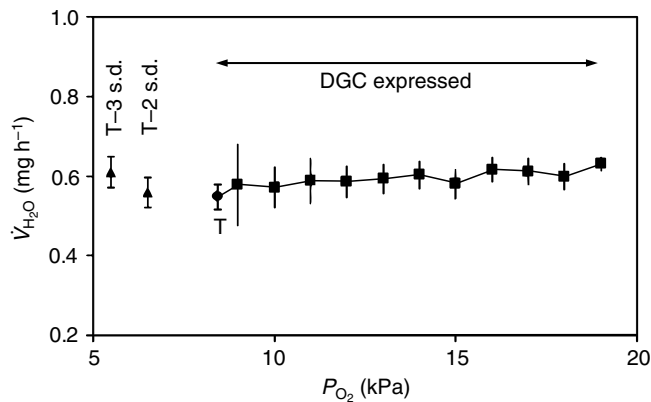
Fig. 4. The effect of hypoxia on water loss rate in the ant *Camponotus vicinus*. While the discontinuous gas exchange cycle (DGC) was expressed during the hypoxic ramp, overall water loss rate decreased significantly (see text); r^2=0.673, $F_{1,9}$=18.54, P=0.002. T = termination of the DGC at 8.44 kPa (Table 1). T−2s.d. = two standard deviations below the termination point. T−3s.d. = three standard deviations below the termination point. Hypoxia has no significant effect on water loss rate (WLR) over the indicated range, even when the DGC is not expressed (see text).

Thus, the null hypothesis that abolition of the DGC would cause no significant increase in WLRs was not disproved.

DISCUSSION

At least in *Camponotus vicinus* alates, gradual abolition of the DGC did not impact overall WLRs. This statement should, however, be treated with a degree of caution. This is because total WLRs over each cycle of the whole DGC were measured, and these are a combination of cuticular and respiratory WLRs. Cuticular WLRs dominate overall WLRs in ants by a large margin [see Lighton et al. (Lighton et al., 2004) and references therein]. In addition, overall WLRs are not constant over time; after initial exposure to dry air, WLR stabilizes after a period of 2–3 h, prior to which insects lose water slightly more rapidly [see Lighton and Feener (Lighton and Feener, 1989) for a discussion of this effect and its possible causes].

A modest reduction in WLR during the course of a 3 h run was therefore to be expected, and did indeed occur, explaining the slight but significant decrease in WLR during the downward hypoxic ramp (Fig. 4). Thus, in our recordings, elapsed time (which is linear against P_{O_2}) (see Fig. 1) rather than P_{O_2} was the dominant influence on overall WLR. However, this does not affect our findings because each DGC can be said to contain something approximating its own baseline for respiratory gas exchange. This is the C phase, during which very little (if any) respiratory gas exchange takes place. If P_{O_2} affected respiratory WLR during the F phase, then an increasingly apparent disparity in WLR between the C and F phases of each DGC should have emerged as P_{O_2} declined. No such effect was apparent.

Spiracular control did, however, break down below ~3.9 kPa. After that point, RWL increased to a plateau nearly threefold higher than normal levels of cuticular plus respiratory water loss, even higher than peak values of WLR during the O phase (see Results and Fig. 1).

It is noteworthy that the breakdown of spiracular control over RWL commenced at an external P_{O_2} of ~3.9 kPa, close to the regulated level of endotracheal P_{O_2} in the F phase of the DGC, which is generally estimated to be 3–5 kPa (Levy and Schneiderman, 1966; Hetz and Bradley, 2005). At an external P_{O_2}

of 3.9 kPa, the O_2 concentration gradient across the spiracles was evidently too weak to sustain the type of continuous gas exchange that minimizes RWL and, as a consequence, RWL increased suddenly, dramatically and unambiguously. It is reasonable to assume that at this point a change occurred from primarily diffusive to convective gas exchange, although we were unable to confirm this because the ants' escape behavior at that point overwhelmed any ventilation signals we might otherwise have measured with the activity detector. In any event, from the moment following the last O phase until spiracular control was finally lost, gas exchange was continuous but no significant change in total WLR was evident (Fig. 4). We consider it unlikely that cuticular permeability is a function of P_{O_2}, so we infer from this that RWL did not change significantly until spiracular control was lost. For more on the controversies regarding diffusion *vs* convection in the DGC, see Lighton (Lighton, 1996; Lighton, 1998) and Chown et al. (Chown et al., 2006).

Loss of the DGC should therefore not be confused with loss of stringent spiracular control and minimization of RWL. Strict spiracular control is obviously required to minimize RWL. There is no doubt that increasing an insect's spiracular conductance to extreme levels causes a large increase in RWL (Mellanby, 1935) (Fig. 1 below 3.9 kPa P_{O_2}). However, it does not follow from this that selective pressure to reduce RWL through strict spiracular control necessarily points causation's arrow towards the DGC. Other strategies of spiracular control may be equally or more efficient, though at present they are under-explored and under-understood. This is emphasized by the absence of the DGC among many xeric arthropods (Lighton, 1996; Lighton, 1998; Schilman et al., 2005; Schilman et al., 2007) and especially by its secondary loss in certain hyperxeric insects (Lighton and Berrigan, 1995). Gibbs and Johnson (Gibbs and Johnson, 2004) also found that expressing the DGC did not reduce RWL; however, they regarded this as disproof of the chthonic, not the hygric, hypothesis. As Chown et al. (Chown et al., 2006) explained, "*the actual hypothesis being tested (see Gibbs and Johnson, 2004) was the hygric hypothesis, not the chthonic hypothesis, because it was a test of the water-retention properties of the DGC under normoxic and acapnic conditions*." It might be said that Gibbs and Johnson (Gibbs and Johnson, 2004) drove another nail into the hygric hypothesis' coffin without realizing at the time whose coffin it was.

Returning to the present investigation, it is reasonable to object that if the DGC was indeed an adaptation to increase the efficiency of gas exchange in hypoxia, then it is curious that severe hypoxia abolishes it (see also Lighton and Garrigan, 1995). However, this ignores the probable co-occurrence of both hypoxia and hypercapnia in chthonic environments. It is possible that hypercapnia is the primary driving force behind the evolution of the DGC, especially in an environment where pulsatile emission of CO_2 (which, after its release, will diffuse away from the animal in the inter-pulse interval) further aids in the establishment of a maximal trans-spiracular CO_2 concentration gradient. It is in any event unlikely that natural P_{O_2}s fall below 8 kPa in most underground environments, although more measurements are needed to ascertain whether or not this assertion is valid.

Evidence is steadily mounting that the evolutionary origin of the DGC may be only distantly related to selective pressures involving RWL. Rather, its origin may reflect the requirement for generating large, temporally decoupled concentration gradients to facilitate O_2 uptake and especially CO_2 emission in hypoxic and hypercapnic environments, i.e. the chthonic hypothesis (Lighton, 1996; Lighton, 1998; Chown et al., 2006). Other explanations are also possible

(Hetz and Bradley, 2005; Chown et al., 2006). So far, the detailed measurements of microenvironments, combined with the detailed phylogenies required to evaluate the chthonic hypothesis, are at best minimal and patchily distributed. Early attempts to evaluate the competing hypotheses of the evolutionary genesis of the DGC by inter-comparing DGCers (White et al., 2007) rather than by comparing DGCers with non-DGCers, are necessarily inconclusive. As ever, more data are needed, but the notion that the DGC *per se* is required for low RWL – even in animals that normally express the DGC – is now a candidate for honorable interment. Which of the other evolutionary hypothesis attending the wake will first accompany it is up for debate.

REFERENCES

Bartholomew, G. A., Vleck, D. and Vleck, C. M. (1981). Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *J. Exp. Biol.* **90**, 17-32.

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

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.* **75**, 345-349.

Chown, S. L., Marais, E., Terblanche, J. S., Klok, C. J., Lighton, J. R. B. and Blackburn, T. M. (2007). Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. *Funct. Ecol.* **21**, 282-290.

Gibbs, A. G. and Johnson, R. A. (2004). The role of discontinuous gas exchange in insects: the chthonic hypothesis does not hold water. *J. Exp. Biol.* **207**, 3477-3482.

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. 137-186. Berlin: Springer.

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. (1996). Discontinuous gas exchange in insects. *Annu. Rev. Entomol.* **41**, 309-324.

Lighton, J. R. B. (1998). Notes from underground: towards ultimate hypotheses of cyclic, discontinuous gas exchange in tracheate arthropods. *Am. Zool.* **38**, 483-491.

Lighton, J. R. B. and Berrigan, D. (1995). Questioning paradigms: caste-specific ventilation in harvester ants, *Messor pergandei* and *M. julianus* (Hymenoptera: Formicidae). *J. Exp. Biol.* **198**, 521-530.

Lighton, J. R. B. and Feener, D. H. (1989). Water loss and cuticular permeability in foragers of the desert ant, *Pogonomyrmex rugosus. Physiol. Zool.* **62**, 1232-1256.

Lighton, J. R. B. and Fielden, L. J. (1995). Mass scaling of standard metabolism in ticks: a valid case of low metabolic rates in sit-and-wait strategists. *Physiol. Zool.* **68**, 43-62.

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

Lighton, J. R. B. and Turner, R. J. (2004). Thermolimit respirometry: an objective assessment of critical thermal maxima in two sympatric desert harvester ants, *Pogonomyrmex rugosus* and *P. californicus. J. Exp. Biol.* **207**, 1903-1913.

Lighton, J. R. B., Brownell, P. H., Joos, B. and Turner, R. J. (2001). Low metabolic rate in scorpions: implications for population biomass and cannibalism. *J. Exp. Biol.* **204**, 607-613.

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.

Mellanby, K. (1935). The evaporation of water from insects. *Biol. Rev.* **10**, 317-333.

Miller, P. L. (1981). Ventilation in active and inactive insects. In *Locomotion and Energetics in Arthropods* (ed. C. F. Herreid and C. R. Fourtner), pp. 367-390. New York: Plenum Press.

Quinlan, M. C. and Lighton, J. R. B. (1999). Respiratory physiology and water relations of three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). *Physiol. Entomol.* **24**, 293-302.

Schilman, P. E., Lighton, J. R. B. and Holway, D. A. (2005). Respiratory and cuticular water loss in insects with continuous gas exchange: comparison across five ant species. *J. Insect Physiol.* **51**, 1295-1305.

Schilman, P. E., Kaiser, A. and Lighton, J. R. B. (2007). Breathe softly, beetle: continuous gas exchange, water loss and the role of the subelytral space in the tenebrionid beetle, *Eleodes obscura. J. Insect Physiol.* doi: 10.1016/j.jinsphys.2007.09.001.

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**, 8356-8361.