# DISCONTINUOUS GAS EXCHANGE CYCLES IN *APHODIUS FOSSOR* (SCARABAEIDAE): A TEST OF HYPOTHESES CONCERNING ORIGINS AND MECHANISMS

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## Summary

It has been suggested that discontinuous gas exchange cycles (DGCs) in insects evolved in response to hypoxic and/or hypercapnic environments, and that the flutter phase of the DGC is predominantly diffusive. We tested these hypotheses by examining the rate of $CO_2$ release at a declining series of oxygen partial pressures in adults of *Aphodius fossor*, an inhabitant of moist to wet dung pats that can have low oxygen and high carbon dioxide concentrations. This mesic species showed a pronounced DGC, but progressively abandoned it when exposed to declining oxygen concentrations. That is, in response to a reduction in oxygen concentration, the closed phase declined in length and eventually disappeared, the flutter phase declined in length, while the flutter phase rate of $CO_2$ release increased, the open phase peak rate of $CO_2$ release declined and DGC frequency increased. Nonetheless, overall $CO_2$ release rate remained virtually unchanged. Thus, it appears that the flutter phase in *A. fossor* has a significant convective component. In addition, in response to declining oxygen concentrations, the spiracles are opened for prolonged periods to increase gas exchange. This behaviour is unlikely to incur a significant water loss penalty in the moist habitats in which *A. fossor* lives. Given the switch to continuous ventilation by *A. fossor* under hypoxic conditions, we conclude that current adaptive explanations for the DGC are inadequate. Rather, the DGC may be the default state of a system regulated by two interacting feedback loops when demands are absent.

Key words: discontinuous gas exchange, ventilation, water loss, hypoxia, beetle, $CO_2$ release, *Aphodius fossor*.

## Introduction

Discontinuous gas exchange cycles (DGCs) are known from a variety of insects and other arthropods. The DGC was first thought to have originated primarily as a means of reducing water loss because respiratory water loss rates would be zero during the closed (C) phase, and much reduced if the flutter (F) phase were solely or predominantly convective, as has been demonstrated in moth pupae, cockroaches and some ants (Levy and Schneiderman, 1966; Kestler, 1985; Lighton et al., 1993a). Such a restriction of respiratory transpiration rates would be particularly important where cuticular water loss rates had already evolved to low levels (for further discussion, see Hadley, 1994; Lighton, 1994, 1996). However, more recently, this idea has been questioned (see Hadley and Quinlan, 1993; Quinlan and Hadley, 1993; Hadley, 1994; Lighton, 1994, 1996; Lighton and Garrigan, 1995).

Lighton and Berrigan (1995) have proposed that the DGC may originally have evolved to enhance gas exchange efficacy, without the excessive water loss penalty associated with continuous gas exchange (for details, see Lighton, 1996), under hypoxic and hypercapnic circumstances. These are especially likely to be encountered by psammophilous beetles and subterranean ants. Lighton (1996) argued that although the DGC could certainly be co-opted as a strategy to combat water loss (an exaptation; for a discussion, see Endler, 1986), especially where the F phase is predominantly convective (as in lepidopteran pupae), large fluctuations in an insect's internal environment associated with a DGC would impose physiological costs that species might seek to avoid. Experimental work by Lighton and Berrigan (1995) and a broad comparative analysis by Lighton (1996) provide some support for this chthonic origination hypothesis. Nonetheless, Lighton (1996) concluded that considerably more descriptive and experimental work, on a broader range of taxa, is required if the evolutionary genesis of the DGC is to be unravelled successfully.

Much of this work will undoubtedly have to focus on the factors that modulate the duration of the three phases of the DGC and the nature of the dominant gas exchange modality during the F phase (see above and Lighton, 1996). Lighton (1988, 1994, 1996), Lighton and Berrigan (1995) and Lighton

and Garrigan (1995) have concluded that at least in some tenebrionid beetles and in some ants, especially *Camponotus vicinus*, the F phase is primarily diffusive. They demonstrated this using simultaneous $CO_2$ and $O_2$ flow-through respirometry in the case of beetles (Lighton, 1988) and by exposing ants to various oxygen concentrations (Lighton and Garrigan, 1995). In the latter case, Lighton and Garrigan (1995) argued that because ambient $O_2$ levels determine the maximum molar concentration of oxygen available at the onset of the C phase, hypoxia should trigger an early switch to the F phase. Similarly, if convection predominates during the F phase, increasing levels of nitrogen will impede negative endotracheal pressure generation, thus restricting the inward movement of oxygen, which reduces F phase duration. In contrast, if the F phase is predominantly diffusion-based, then hypoxia must elevate $CO_2$ emission levels, causing a delay in the $CO_2$-mediated switch to the open (O) phase, thus increasing F phase duration. Lighton and Garrigan (1995) produced experimental support for the latter scenario and concluded that, in this case, the F phase is predominantly diffusive.

The aim of the present study is therefore to address both the evolutionary genesis and F phase gas exchange modality debates. To achieve this, we have focused our attention on *Aphodius fossor* (L.) (Scarabaeidae, Aphodiinae), an inhabitant of fresh cattle dung pats in the Holarctic (Dellacasa, 1983), a species ideally suited to address these questions. In Denmark, the adults of *A. fossor* are mostly active in the late spring/early summer (May–June). They fly mostly in the evening and at night, and occasionally during the day, and hence mostly at high ambient humidities (Landin, 1961, 1968; P. Holter, personal observations) (at Sjælsmark meteorological station approximately 12 km south-east of the site where *A. fossor* was collected for the present study, relative humidity ranges from 39 to 70 %, and mean temperatures from 9.0 to 17.6 °C; 10 year means, 1988–1997). Therefore, this species can be thought of as mesic. In addition, the adult beetles spend most of their lives in cattle dung pats that have water contents in excess of 80 % (Holter, 1982). Within the dung pat, they are not confined to well-defined zones (Holter, 1982), and in wet pats, where microbial activity is high, may consequently be exposed to oxygen concentrations as low as 2 % and $CO_2$ levels in excess of 10 % (Holter, 1991). Indeed, laboratory trials have indicated that adult *A. fossor* can maintain unvarying oxygen uptake rates from normoxic conditions down to 1 % $O_2$ (Holter and Spangenberg, 1997).

On these grounds, we set out to test two major predictions. First, if the DGC originated primarily in response to hypoxic and/or hypercapnic environments, rather than as a strategy to reduce water loss, *A. fossor* should show a pronounced DGC, especially under hypoxic conditions. It lives in profoundly hypoxic/hypercapnic environments, but is unlikely to encounter dry conditions and, even then, it has ready access to a water supply. Second, because it possesses flaccid, membranous abdominal tergites (as does the tenebrionid *Psammodes striatus*), modulation of the DGC in response to

hypoxia should reveal a largely diffusion-based F phase (see Lighton, 1988, 1996).

## Materials and methods

Adults of *Aphodius fossor* (L.) were collected from dung pats in the Strødam Nature Reserve (55°58′N, 12°16′E), 35 km to the north west of Copenhagen, Denmark. Beetles were returned to the laboratory in Copenhagen within 4 h of collection and transferred individually to 185 ml plastic containers containing moist potting soil. The beetles were kept for a minimum of 3 days at 15±1 °C, the temperature at which all subsequent work was performed.

Following this period, $CO_2$ release was measured and gas exchange characteristics were examined using a Sable Systems (Henderson, Nevada, USA) respirometry system. Air was passed through Drierite and soda-lime columns to remove water vapour and $CO_2$. It was then passed through an automated baselining system, through the 10 ml cuvette containing the beetle, and finally through a Li-Cor $CO_2/H_2O$ analyzer (model Li 6262; 1–2 p.p.m. accuracy for both channels at 350 p.p.m.) at a flow rate of 100 ml min$^{-1}$. Datacan V software was used for data capture and analysis. All measurements were corrected to standard temperature and pressure and are expressed as ml $CO_2$ g$^{-1}$ h$^{-1}$ or mg $H_2O$ g$^{-1}$ h$^{-1}$. Respiration rate and gas exchange characteristics were first examined using ambient air. Thereafter, $O_2$ concentrations of approximately 15, 10 and 5 % in nitrogen (scrubbed of all water) were used. These concentrations were obtained by mixing $O_2$ and $N_2$ delivered by two Sierra Instruments mass-flow meters, both controlled by a Mikrolab Aarhus flow controller. Oxygen concentrations were subsequently assessed using a gas chromatograph (Mikrolab Aarhus) with thermal conductivity detector at 120 °C and a Molesieve 5A column (1.8 m×2 mm). The treatments were randomized by day, and five beetle specimens were examined per day. Within treatments, the order of examination of beetles was also randomized. Following this, beetles were finally examined at 2 % $O_2$ in nitrogen, again with the order of examination randomized within this treatment.

Individual beetles were weighed (using a Mettler AJ100 electronic microbalance sensitive to 0.1 mg), placed in the darkened cuvette 1 h before the treatment, after which each treatment/measurement was undertaken for 2 h in the absence of light, excluding the time required for automatic baseline determination (a further 30 min). On a variety of occasions, beetles were inspected for activity in the cuvette using a dim red light. In most instances, beetles were quiescent. In those cases in which they were active, this was also clearly visible on the DGC recording, and that particular measurement was discarded. After the measurements, beetles were reweighed. A final trial consisted of exposure of a further two beetles to a serial decline in $O_2$ concentration, over a 6 h period, from normoxic levels to approximately 2 %. Washout time, for each reduction in $O_2$ concentration, was determined prior to these

trials using the Li-Cor analyser with no beetle present in the cuvette, and was found to be approximately 6 min.

All calculations of $CO_2$ and $H_2O$ release rates (on a mass-specific basis) and examinations of gas exchange characteristics were carried out using Sable Systems Datacan V software. For each individual, the mean $CO_2$ release rate ($ml\,g^{-1}\,h^{-1}$), the frequency of the DGC, measured from O phase peak to O phase peak (mHz), and the mean height of these O phase peaks ($ml\,g^{-1}\,h^{-1}$) were measured over the entire recording. Thereafter, mean $CO_2$ release rate and phase duration (s) were measured for each of the phases. Where a measure of emission volume ($ml\,g^{-1}$) was required, integration of the area under the curve against time (h) was undertaken. The resulting data were then used as input to Statistica for calculation of means and standard errors, for the determination of the percentage contribution of F phase $CO_2$ release rate to total $CO_2$ release rate and for investigating the relationships between phase characteristics and $O_2$ concentration using least-squares regression.

To compare our data on *A. fossor* with those obtained in a previous study by Holter and Spangenberg (1997), we used the rate of $CO_2$ release over the entire recording to estimate $\dot{V}_{CO_2}$, and converted this to µW (see Lighton and Fielden, 1995), assuming a respiratory quotient between 0.7 and 0.9.

To enable comparisons between water balance characteristics of these beetles and those determined previously for other Scarabaeidae (e.g. Zachariassen et al., 1987; Chown et al., 1995), the gravimetric protocol of Chown et al. (1995) was followed. Beetles were acclimated on moist filter paper for 2 days with no access to food. They were then placed individually into mesh-covered, open-ended plastic tubes (approximately 2 ml volume), which in turn were placed into 185 ml vials containing 100 ml of silica gel (providing a relative humidity of approximately 5 %). Beetles were weighed at approximately 8 h intervals until they died. The mass recorded in the interval prior to death was used to calculate water loss rate, maximum water loss tolerated and time until death. No defecation was observed during these trials. Water loss rates obtained in this manner were compared with those calculated from the decrease in mass associated with 2 h exposures to dry air flowing over beetles at $100\,ml\,min^{-1}$ in the respirometry experiments. Because of the noise associated with infrared measurements, C phase and F
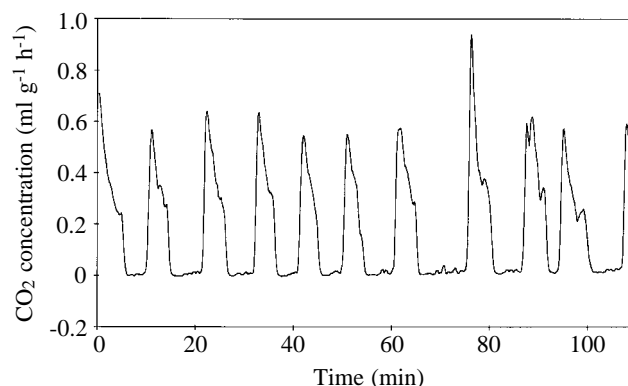


Fig. 1. Typical recording of discontinuous gas exchange in *Aphodius fossor* measured over 2 h at 15 °C under normoxia.

phase rates of $H_2O$ release could not be adequately distinguished using data collected by the Li-Cor 6262. Hence, we were unable to characterize water loss during these phases. Nonetheless, we regarded the lowest value during the combined C/F phase as a measure of cuticular water loss, and subtracted this from O phase water loss to obtain an estimate of respiratory as opposed to cuticular water loss. This was performed for normoxic conditions only.

For all analyses, the significance level was set at $P<0.05$, and values reported are means ± S.E.M. throughout.

## Results

Under normoxic conditions, gas exchange in *Aphodius fossor* (mean body mass 0.1213±0.0034 g, $N$=10) was characterized by typical, discontinuous gas exchange cycles (Fig. 1). Under these conditions, standard metabolic rate, measured as mean $CO_2$ release rate over the entire recording was 0.1649±0.0065 $ml\,g^{-1}\,h^{-1}$. As ambient oxygen concentrations declined, mean $CO_2$ release rate remained constant, except at the lowest level (measured as 2.84 % $O_2$), at which $CO_2$ release rate increased significantly and by approximately 23 % over the mean value for $\dot{V}_{CO_2}$ combined over all the higher $O_2$ concentrations (Table 1). Nonetheless, with declining ambient $O_2$ concentration, DGC frequency increased (duration declined) (DGC

Table 1. $\dot{V}_{CO_2}$, *discontinuous gas exchange cycle (DGC) duration and frequency, and open phase peak height in* Aphodius fossor *under normoxic and declining ambient $O_2$ concentrations*

| $O_2$ concentration (%) | $N$ | $\dot{V}_{CO_2}$ ($ml\,g^{-1}\,h^{-1}$) | DGC period (s) | DGC frequency (mHz) | Open phase peak height ($ml\,g^{-1}\,h^{-1}$) |
|---|---|---|---|---|---|
| 20.47 | 10 | 0.165±0.007 | 999.4±115.4 | 1.146±0.144 | 0.673±0.028 |
| 16.55 | 9 | 0.203±0.015 | 514.6±69.9 | 2.271±0.312 | 0.659±0.041 |
| 11.28 | 10 | 0.195±0.014 | 285.0±16.7 | 3.615±0.208 | 0.468±0.032 |
| 6.13 | 10 | 0.203±0.016 | 218.4±21.9 | 5.080±0.568 | 0.406±0.031 |
| 2.84 | 10/3[a] | 0.236±0.013* | 216.2±49.8 | 5.275±0.974 | 0.307±0.024 |

[a]Sample size at 2.84 % was 10 for $\dot{V}_{CO_2}$ and 3 for the other variables.

An asterisk denotes a significant difference from all other values at the 5 % level using a Tukey HSD test ($F_{2,42}$=4.171, $P$=0.006).
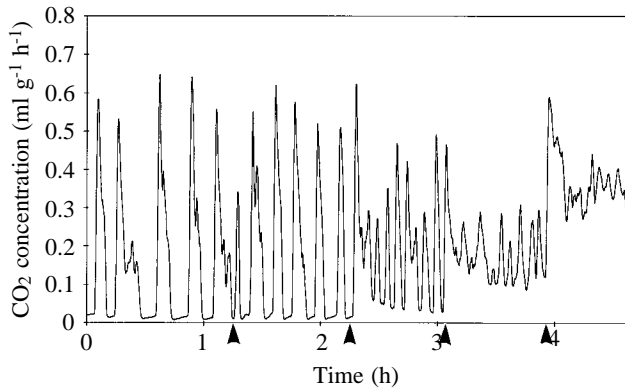
Fig. 2. Changes in the characteristics of the discontinuous gas exchange cycle in *Aphodius fossor* during a sequential decline in oxygen concentration. Virtually instantaneous changes in $O_2$ concentration were made at the points indicated by the arrowheads. These are, from left to right: 20.47 % to 16.55 %, 16.55 % to 11.28 %, 11.28 % to 6.13 %, and 6.13 % to 2.84 %.

frequency=$-0.275[O_2]+6.78$, $F_{2,39}=99.63$, $P=0.00001$, $r^2=0.714$) and O phase peak height declined (O phase peak $CO_2$ release=$0.022[O_2]+0.255$, $F_{2,39}=65.76$, $P=0.00001$, $r^2=0.622$) significantly (Table 1). Because a randomized experimental design for collection of the data was used to eliminate ageing effects, this alteration in DGC characteristics is best illustrated using one of the experiments in which $O_2$ concentration was systematically reduced (Fig. 2).

The characteristics of the DGC phases also changed considerably with declining ambient oxygen concentration. C
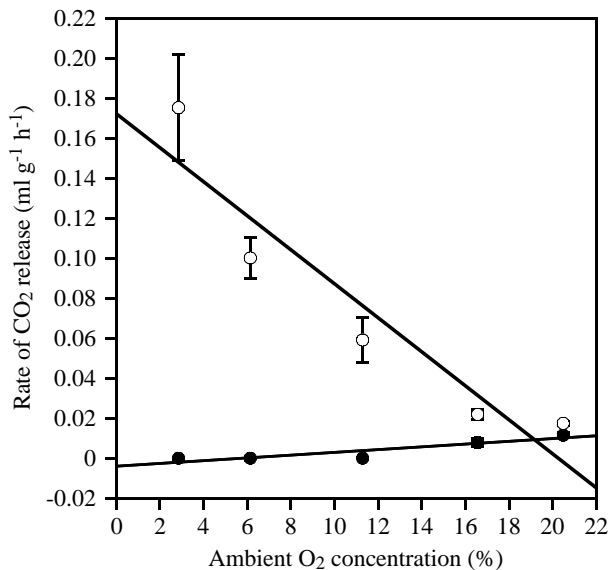


Fig. 3. The effects of ambient oxygen concentration on closed (C) phase (filled circles) and flutter (F) phase (open circles) rates of $CO_2$ release. Values are means ± S.E.M. ($N=42$). The regression lines are based on the individual data points: C phase $CO_2$ release rate=$0.0008[O_2]-0.006$ ($F_{2,39}=63.9$, $P=0.00001$, $r^2=0.615$); F phase $CO_2$ release rate=$-0.0072[O_2]+0.151$ ($F_{2,39}=86.24$, $P=0.00001$, $r^2=0.683$).
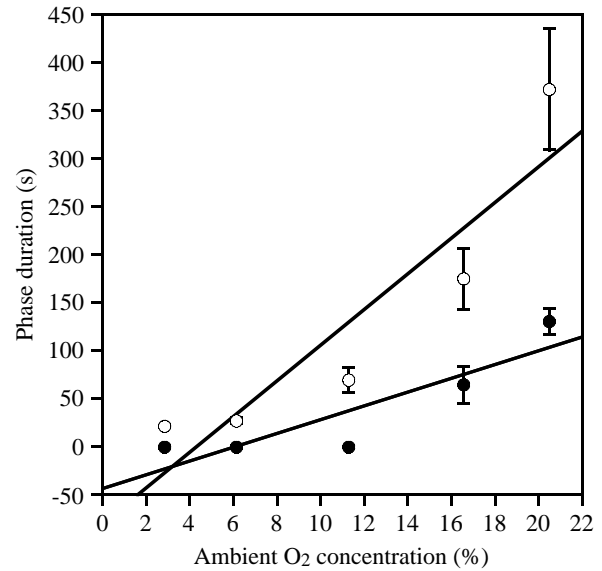


Fig. 4. The effects of ambient oxygen concentration on closed (C) phase (filled circles) and flutter (F) phase (open circles) duration. Values are means ± S.E.M. ($N=42$). The regression lines are based on the individual data points: C phase duration=$8.29[O_2]-60.81$ ($F_{2,39}=63.67$, $P=0.00001$, $r^2=0.614$); F phase duration=$21.04[O_2]-117.9$ ($F_{2,39}=47.16$, $P=0.0001$, $r^2=0.541$).

phase $CO_2$ release rate and duration declined significantly with declining oxygen concentration, and it appeared that this phase was eliminated entirely at oxygen concentrations less than 16 % (Figs 2–4). F phase duration also decreased significantly with declining $O_2$ concentration (Fig. 4), although the rate of F phase $CO_2$ release showed the opposite trend (Fig. 3), and the proportion of the total rate of $CO_2$ release attributed to F phase $CO_2$ release increased significantly with declining oxygen concentration (Fig. 5). With declining $O_2$ concentration, the rate of O phase $CO_2$ release declined (O phase $CO_2$ release rate=$0.0066[O_2]+0.186$, $F_{2,39}=30.08$, $P=0.00001$, $r^2=0.429$), as did O phase duration (O phase duration=$17.96[O_2]+58.76$, $F_{2,39}=59.55$, $P=0.00001$, $r^2=0.598$) and O phase $CO_2$ emission volume (O phase $CO_2$ emission volume=$0.0018[O_2]-0.0013$, $F_{2,39}=87.96$, $P=0.00001$, $r^2=0.687$).

During normoxia, cuticular water loss prior to death (estimated as $0.319\pm0.069$ mg, but with a small F phase component) contributed approximately 95 % to the total water loss, whereas respiratory water loss ($0.013\pm0.003$ mg) accounted for 5 %. Overall water loss rates in normoxic moving air ($1.328$ mg h$^{-1}$) were considerably higher than those obtained using gravimetric protocols ($0.633$ mg h$^{-1}$).

## Discussion

Assuming a respiratory quotient (RQ) of between 0.7 and 0.9, a value that may be reasonable for animals feeding on dung and its microflora (Landin, 1961, 1968; Holter, 1982; P. Holter, unpublished results), the standard metabolic rate (SMR) of
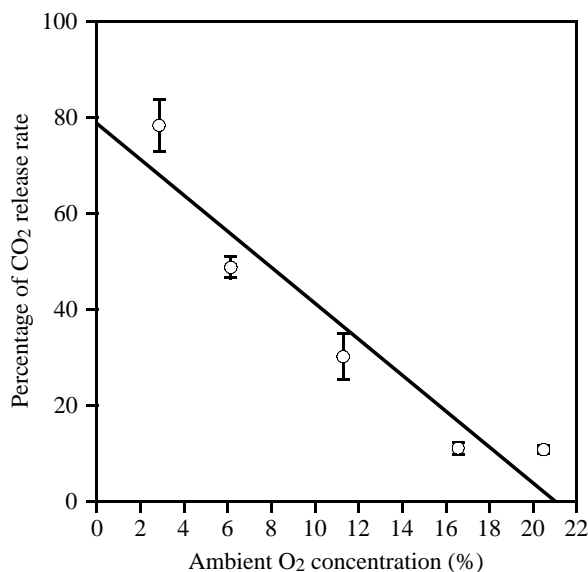
Fig. 5. The effects of ambient oxygen concentration on the percentage contribution of the flutter (F) phase rate of $CO_2$ release to the total rate of $CO_2$ release. Values are means ± S.E.M. (*N*=42). The regression line is based on the individual data points: percentage F phase $\dot{V}_{CO_2}=-3.249[O_2]+70.76$ ($F_{2,39}=134.06$, *P*=0.00001, $r^2=0.770$).
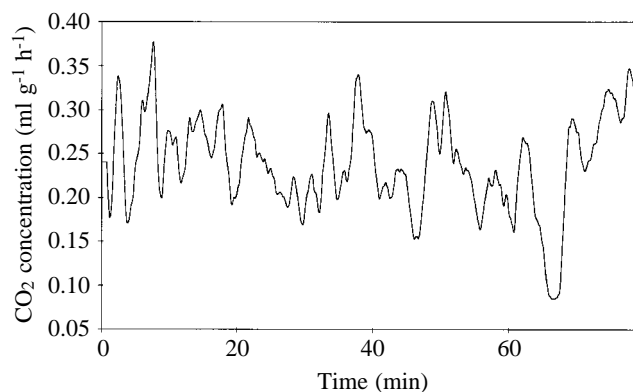


Fig. 6. Typical recording of discontinuous gas exchange in an individual *Aphodius fossor*, desiccated to approximately half its maximum tolerable water loss, at 15 °C (mean $\dot{V}_{CO_2}=0.231\,ml\,g^{-1}\,h^{-1}$).

*Aphodius fossor*, estimated from the $CO_2$ release rate, is 124–140 µW at 15 °C. This is approximately 65 % lower than the values obtained by Holter and Spangenberg (1997) for the same species using different methods. Those authors did not control for the activity of the beetles, as we did here using flow-through methods and observations, and they conducted their study at a temperature 5 °C higher than used in the present study, which may account for the differences in the two estimates of SMR (see also Lighton and Fielden, 1995).

Nonetheless, in terms of maintenance of SMR, we found almost the same response of *A. fossor* to declining oxygen concentrations as was found by Holter and Spangenberg (1997). $CO_2$ release rate did not differ significantly with decreasing [O₂] to approximately 6 % O₂, and the increase in metabolic rate observed at approximately 3 % O₂ was small relative to the overall mean $CO_2$ release rate at all higher O₂ concentrations. The absence of a short-term response of metabolic rate to declining oxygen concentrations is not unusual in insects, which can generally maintain constant oxygen uptake rates down to at least 5 % O₂ (Schneiderman and Williams, 1955; Keister and Buck, 1974; Herreid, 1980; Loudon, 1988). At lower O₂ molar fractions, insects can be characterized either as conformers, which generally respond with a decline in metabolic rate (e.g. Loudon, 1988), or as regulators, which often increase their ventilation rate in response to declining $P_{O_2}$ (Herreid, 1980). The regulatory response was shown by *A. fossor*; the increase in rate of $CO_2$ release was probably due to a small increase in the cost of convective ventilation caused by active muscular contractions at the lowest ambient oxygen concentration (see below).

In response to declining oxygen concentrations, the duration of the C phase declined, and it appeared to be entirely absent at ambient O₂ concentrations below 10–16 % (Figs 2, 4). This provides further evidence in support of declining O₂ tension acting as the major trigger for the termination of this phase (Levy and Schneiderman, 1966; Lighton, 1996). However, it is of considerable interest that this probably represents an endotracheal $P_{O_2}$ setpoint of approximately 10–15 kPa, although this was not measured in the current study. This is considerably higher than the widely accepted value of 5 kPa reported by Levy and Schneiderman (1966) (see also Lighton, 1994, 1996).

In contrast to Lighton and Garrigan's (1995) findings for the ant *Camponotus vicinus*, F phase duration in *A. fossor* declined significantly with declining ambient O₂ concentration (Fig. 4), although F phase $CO_2$ release (Fig. 3) and the proportional contribution of F phase $CO_2$ release rate to the overall rate of $CO_2$ release (Fig. 5) increased with declining oxygen concentration. Following Lighton and Garrigan's (1995) arguments (see above; see also Snyder et al., 1995), we suggest that in *A. fossor* the F phase was at least partially convective. Thus, as ambient oxygen concentrations decline, the absence of a significant negative endotracheal pressure must force premature termination of this phase, hence causing the observed increase in DGC frequency. The apparent absence of air sacs in this species (S. L. Chown and P. Holter, personal observations) and the curious semi-telescoping movements of the sternites and tergites that can be observed at very low oxygen concentrations when the elytra and wings are removed lend additional support to the suggestion that a significant negative endotracheal pressure cannot be produced. Indeed, it appears that, at the lowest oxygen concentrations, active ventilation using muscular contractions is required to ensure that metabolic demands for O₂ can be met.

Nonetheless, the increase in F phase rate of $CO_2$ release suggests that there was also a significant diffusive component to this phase. This increase in spiracular conductance is

probably brought about by an increase in the effective diameter of the spiracles (i.e. the extent to which they open, see Lighton and Garrigan, 1995), an assumption supported by the disappearance of the C phase with declining $O_2$ concentration. That is, at the lower oxygen concentrations, the spiracles do not close at all.

Therefore, it appears that, in response to declining ambient oxygen concentrations, *A. fossor* switches from a pattern of discontinuous gas exchange to one of continuous diffusion/convection (see also Schneiderman and Williams, 1955). $CO_2$ release rate during the O phase declines in importance as a consequence of declining O phase volume and duration, while the rate of F phase $CO_2$ release increases despite a declining F phase duration. In the light of these dramatic changes, the existence of an F phase in the conventional sense may well be disputed (Fig. 2). However, Lighton and Berrigan's (1995) proposition that, in response to a hypoxic/hypercapnic environment, insects can either open their spiracles maximally for long periods or sequester $CO_2$ and deplete $O_2$ until an adequate partial pressure gradient can be established is intriguing in this context. Indeed, it appears that the former response is exactly the one shown by *A. fossor*. Although it is likely to be associated with elevated water loss rates (Lighton et al., 1993b; Lighton and Berrigan, 1995), the penalty imposed on *A. fossor* by continuous convection and diffusion is unlikely to be high for two reasons. First, *A. fossor* loses the vast majority of water through cuticular transpiration and, by the standards of Zachariassen et al. (1987) it is a typically mesic species in this regard. Second, such high water loss rates, even if compounded by an increase in water loss associated with continuous ventilation, are unlikely to be significant in a species that spends the greater part of its adult and larval existence in a microhabitat with an 80 % water content and that is characterized by high humidities and relatively low temperatures, at least when the adults are active (see above; see also Landin, 1961, 1968; Holter, 1982).

If conservation of water is relatively unimportant to adult *A. fossor*, if the DGC is not used to enhance gas exchange under hypoxic conditions (and presumably hypercapnic conditions because the two are usually encountered simultaneously in this species, see Holter, 1991, 1994), and if the DGC is eliminated in water-stressed adults (Fig. 6), then why should this species exhibit a DGC at all? Lighton and Berrigan (1995) suggested that a DGC may not be necessary under normoxic conditions in insects because partial pressure gradients are sufficient to allow both diffusive and convective gas exchange, and Quinlan and Hadley (1993) have demonstrated that, even if partially water-stressed, insects are likely to abandon the DGC because of the reduced $CO_2$ buffering capacity of the haemolymph. One explanation for the presence of the DGC in *A. fossor* may be that, during diapause or quiescence in adults, water conservation is likely to be at a premium. Although a sound argument, there is no evidence for a period of quiescence in the summer activity period of this species, and it is the larval stage that overwinters (Landin, 1961). An alternative explanation might be that at some point in the evolutionary

history of this species water conservation was indeed at a premium, and that the DGC has been retained as a plesiotypic character state (but see Coope, 1995, for an argument that beetles, including those in the genus *Aphodius*, tracked their favoured climates). Both scenarios are plausible, and both would lend support to the argument that the DGC is of adaptive value to terrestrial insects. However, both call for a *deus ex machina* that the available data prevent us from employing.

Thus, neither of the adaptive scenarios proposed for the genesis of the DGC appears to be suitable in the case of *A. fossor*, and general dissatisfaction with the water conservation hypothesis is also widespread (e.g. Hadley, 1994; Lighton, 1996; Williams and Bradley, 1998). Interpreted in the light of suggestions that the distribution and characteristics of the DGC are somewhat idiosyncratic with regard to taxa from mesic and xeric environments (Lighton, 1996), and indications that the DGC is likely to be polyphyletic (Lighton and Fielden, 1996), our findings suggest that the DGC may not be adaptive in a strict sense. Rather, the regular periodic nature of the DGC may be an epiphenomenon of the interaction of two feedback systems regulating a single function faced with minimal demand. Recently, it has been found that, in the absence of any deterministic driving variables, interacting feedback systems can show a variety of behaviours ranging from a single steady state to ordered cyclic behaviour (Kauffman, 1993). The periodic nature of a variety of physiological phenomena is thought to be the consequence of such an interaction (Glass and Mackey, 1988) but, when demand is placed on such a system, the emergent behaviour may give way to more tightly regulated function. This may be the case with the DGC, although admittedly we have little positive evidence to support this idea. However, given the difficulty of confirming adaptive explanations for the DGC, we suggest that this possibility be given consideration.

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