

# The role of the mesothoracic spiracles in respiration in flighted and flightless dung beetles

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## Summary

The relative role of the mesothoracic and abdominal spiracles in respiration was examined using flow-through respirometry in four dung beetle species from different habitats. Two species of flightless beetles, *Scarabaeus (Pachysoma) gariepinus* and *Scarabaeus (Pachysoma) striatum*, from the arid western region of southern Africa and a large flighted species, *Pachylomerus femoralis*, from a more mesic habitat were compared with *Circellium bacchus*, a flightless beetle from a low rainfall eastern area. All species showed a form of the discontinuous gas exchange pattern at rest. The mesic flighted species used a closed, flutter, open, cycle (CFO) while those species from more arid habitats used a closed, ventilation, cycle (CV) or

a closed, burst cycle (CB). The relative importance of the mesothoracic spiracles in CO<sub>2</sub> emission varied between the species, even between those from the same genus and habitat. *C. bacchus* and *P. femoralis* represent extremes of CO<sub>2</sub> emission from the mesothoracic spiracles; from almost total to almost none, respectively. Overall, mesothoracic CO<sub>2</sub> emission and convection were more pronounced in the dry habitat species, supporting the hypothesis that both strategies aid in the reduction of water loss.

Key words: Scarabaeini, discontinuous gas exchange cycle, arid habitat, subelytral cavity.

## Introduction

The discovery of anterograde airflow in a flightless dung beetle has brought into question the accepted role of the mesothoracic spiracles, abdominal spiracles and subelytral cavity in respiration in beetles (Duncan and Byrne, 2002). At rest, *Circellium bacchus* (Fabricius), a large flightless scarab from the East coast of South Africa, releases CO<sub>2</sub> and H<sub>2</sub>O from the abdominal spiracles into the subelytral cavity where they are held for an extended period before being released through the more anterior mesothoracic spiracles (Byrne and Duncan, 2003). Tenebrionid beetles from other arid regions of the world also use the mesothoracic spiracles for CO<sub>2</sub> emission (Lighton, 1991; Duncan, 2003), supporting the assumption that this respiratory pattern, coupled with flightlessness and the possession of a sealed subelytral cavity are adaptations to an arid habitat, although other interpretations of its function have been made (Cloudsley-Thompson, 1964, 2001; Ahearn 1970; Nicolson et al., 1984; Draney, 1993). The two sets of spiracles are neatly separated by the waist which is formed by the narrow passage between the thorax and the abdomen of the beetle (Fig. 1A) and allow separate experimental examination of gas exchange at the front and back of a live specimen (Duncan and Byrne, 2002; Duncan, 2003).

We tested the assumption that other flightless dung beetles

would emit proportionally more CO<sub>2</sub> from the anterior mesothoracic spiracles than the posterior subelytral spiracles, as evidence that they were using the subelytral cavity in the same manner as *C. bacchus*. We compared the relative contribution of both spiracle sets in two species of flightless dung beetles *Scarabaeus (Pachysoma) gariepinus* Ferreira, and *Scarabaeus (Pachysoma) striatum* Castelnau (Scarabaeini) from arid habitats, with that of a flighted species *Pachylomerus femoralis* (Kirby) (Scarabaeini), from a more mesic habitat. Our expectation was that the winged *P. femoralis* would be unable to seal its subelytral cavity as tightly as the flightless species and would therefore emit CO<sub>2</sub> from both the anterior and posterior body halves. All three of the above species belong to the tribe Scarabaeini. These results were compared with a previous study on the arid dwelling flightless dung beetle *C. bacchus* from the tribe Canthonini, which is an extreme example of anterograde CO<sub>2</sub> emission from the mesothoracic spiracles (Duncan and Byrne, 2002). The Scarabaeini have been shown to be a monophyletic tribe and have a sister relationship with the Canthonini (Philips et al., 2004), both tribes are in the same subfamily, Scarabaeinae.

Many species of beetles lack wings and, therefore, potentially possess an empty subelytral cavity. The function of

this space in respiration and water saving is under debate. Klok (1994) found that flightless dung beetles were more resistant to desiccation than winged species, although Chown et al. (1995), concluded that these differences were insignificant when adjusted for body mass. Nevertheless, in their experiments *C. bacchus* individuals were able to survive twice as long under desiccating conditions than similar mass *P. femoralis*. The air inside the subelytral cavity of flightless beetles is known to have a high water content, and by keeping this cavity closed an area of high humidity is created over the posterior spiracles (Zachariassen, 1991; Byrne and Duncan, 2003). The relative importance of respiratory water loss compared with cuticular transpiration has been in dispute (Zachariassen et al., 1987; Lighton 1994, 1998) but if there is selection pressure to conserve water it is likely to act on all routes for water loss, which includes the respiratory routes. Thus xeric insects should have reduced respiratory and

cuticular water loss. Gibbs (2002) showed that the structure of cuticular lipids in *Drosophila* did not correspond to interspecific differences in water loss rates and therefore concluded that respiratory adaptations, along with a decrease in metabolic rate, coupled with a reduction in locomotor activity, resulted in improved desiccation resistance in desert species.

In addition to the subelytral cavity, a discontinuous gas exchange cycle (DGC), seen during respiration at rest, is characteristic of many beetle species from several sub-orders and differing habitats (Lighton, 1991; Davis et al., 1999; Duncan and Dickman, 2001; Duncan et al., 2002; Duncan, 2003). The respiratory patterns of these species are characterised by CO<sub>2</sub> volleys during an extended burst phase (B) of the cycle, which increase in prevalence as one considers beetles from increasingly arid habitats (Duncan and Byrne, 2000). Similar patterns have been found in centipedes from three orders and different habitat types (Klok et al., 2002). This has been used as support for the hypothesis that the DGC is an additional water saving strategy (Kestler, 2003), and is bolstered by theoretical calculations that convective (active/forced) ventilation loses less water than passive diffusion (Kestler, 1985; P. Kestler, personal communication). Conversely, loss of the DGC at high temperatures (Chappell and Rogowitz, 2000) or hypoxia (Chown and Holter, 2000) has been used to question its value in respiratory water-saving. However, the gradual change from DGC to continuous CO<sub>2</sub> release with increasing metabolic needs or lack of oxygen, shows that the DGC persists as long as possible in situations of conflicting needs. The match of spiracular opening to metabolic demands in flying *Drosophila*, allowing a 23% reduction in water loss (Lehman, 2001), offers further support for the adaptive significance of spiracular control in diminishing desiccation stress.

The discontinuous gas exchange cycle has three distinct periods; closed (C), flutter (F) and burst (B), each contributing to reducing water loss (Kestler, 1985, 2003; and P. Kestler personal communication). The closed period, when all the spiracles are shut, prevents any gas exchange with the atmosphere (Bridges et al., 1980). Any closing saves water (the 'closing strategy'), as only CO<sub>2</sub> and O<sub>2</sub> are exchanged rapidly at the next opening; which is not the case for water vapour (the 'partial pressure

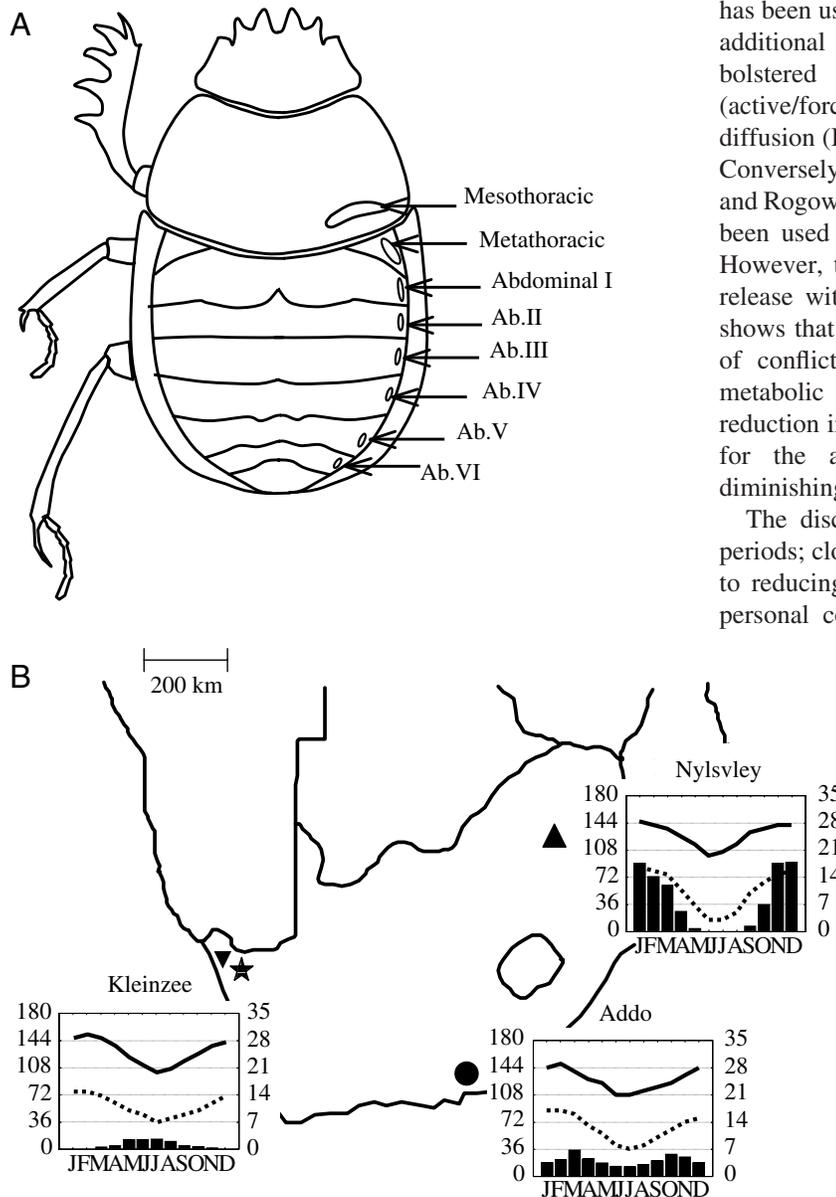


Fig. 1. (A). Relative positions of the anterior and posterior spiracles in dung beetles. (B) Collection localities of the four species of dung beetles used in this study indicating their separated distributions on the subcontinent. ▲ *Pachylomerus femoralis*; ▼ *Scarabaeus gariepinus*; ★ *S. striatum*; ● *Circellium bacchus*. Climate graphs indicate rainfall in mm (y1 axis, solid bars), and temperature in °C (y2 axis, mean maximum, unbroken line; mean minimum, broken line). Ab., abdominal segment.

strategy'; Kestler, 2003). The closed period is followed by the F period, during which the spiracles open, either repetitively in micro-openings (Kestler, 1985) or in more regular miniature inspirations as found in locust (Hustert, 1975) and tenebrionid beetles (Lighton, 1991; Duncan, 2003), or by a more or less wide opening with vibrating flutter movements according to the O<sub>2</sub> demand. Minimising the opening frequency minimises water loss by the closing strategy in combination with the partial pressure strategy. Simultaneous diffusion and convection during expiratory openings causes CO<sub>2</sub> to be released faster than H<sub>2</sub>O is lost (Kestler, 1985).

Four species, from two closely related tribes (Philips et al., 2004) and in the same subfamily, that have widely separate distributions (Fig. 1B) and different flight abilities, were chosen to examine the role of the mesothoracic spiracles in respiration. *Circellium bacchus* is a large, flightless, slow-moving beetle that has a restricted distribution in the xeric Valley Bushveld (Acocks, 1988) of the Eastern Cape Province of South Africa (Coles, 1993). It has the ability to roll dung balls for food caching, but this behaviour is largely restricted to immature 'teneral' adults, while mature adults feed at the middens of large herbivores (C. H. Scholtz, personal communication). Beetles of the subgenus *Pachysoma* are small to medium flightless species, found on the opposite coast, in a narrow sandy strip of the Western Cape Province, where the rainfall is very low (Fig. 1B), but the relative humidity varies between 79% to 88%. In addition, beetles forage on dry rodent pellets with which they provision a chamber below the moisture line in the sand where the dung rehydrates, providing food for about a week underground (Scholtz, 1989). Finally, *P. femoralis* is a widespread species, occurring across the central summer rainfall region of southern Africa. It is a large, powerful flier and competes strongly for dung, which it either rolls or butts away from the pat (Byrne et al., 2003). All four species have eight pairs of spiracles, one pair that open under the mesothorax, and seven under the elytra (one pair of metathoracic and six pairs of abdominal) (Fig. 1A). All species are diurnal.

Given the general ecological pattern of changing DGC patterns, from CFO cycles to CV cycles, found in dung beetle species adapted to arid habitats (Duncan and Byrne, 2000) and, specifically, the extreme example found in *C. bacchus*, coupled to an anterograde respiratory airflow, both of which are assumed to be water saving adaptations (Duncan and Byrne, 2002; Duncan, 2002), we would predict that different dung beetle species, from different tribes and different regions that are nevertheless both arid, are very likely to share similar respiratory adaptations.

### Materials and methods

*Pachylomerus femoralis* (Kirby) were collected from the central highveld region near Nylsvley Game Reserve (24°38'S, 28°45'E), Limpopo Province; *S. gariepinus* (Ferreira) and *S. striatum* (Castelnau) were collected from Kleinsee on the West coast of South Africa (29°34'S 17°17'E) and *C. bacchus*

(Fabricius) were collected from the Addo Elephant National Park in the Eastern Cape Province (33°30'S, 25°41'E). The beetles were housed in 10 l bins, half filled with soil, in an insectary at 25°C with a 14 h:10 h light:dark cycle. *Pachylomerus femoralis* and *C. bacchus* were fed fresh cow dung twice weekly, while the other two species were fed fresh or dry goat or rodent dung. All species survived for at least 6 months under laboratory conditions.

To measure the relative amounts of CO<sub>2</sub> released from the mesothoracic and subelytral spiracles, beetles were placed in a perspex respirometry chamber, divided crosswise by a sheet of latex. The head and prothorax of the beetle were pushed into the anterior section through a small hole in the centre of the latex sheet, which made an airtight seal between the prothorax and the abdomen. (For evidence that the seal was airtight see fig. 5 in Duncan and Byrne, 2002. For all beetles measured, the seal was tested by pumping a different gas concentration through one chamber and measuring it from both.) Each section of the chamber had a volume of approximately 100 ml. A flow-through respirometry system was used to measure CO<sub>2</sub> emission from an inactive beetle at room temperature (25±2°C), with simultaneous sampling from the anterior body, including the head and the mesothoracic spiracles, and the posterior body comprising the elytral case; which covers the metathoracic and abdominal spiracles (for details see Duncan, 2002, 2003; Duncan and Byrne, 2002). One inlet and one outlet served each compartment of the chamber. The air pressure in each compartment was monitored by manometers to ensure that there was no difference in pressure between the chambers throughout the experiment. The latex sheet was renewed for each trial.

Both compartments had independent air sources, scrubbed of CO<sub>2</sub> and H<sub>2</sub>O vapour by a Drierite/Ascarite column, which were drawn through at 50 ml min<sup>-1</sup> (controlled by separate calibrated Supelco flow meters; Bellefonte, PA, USA) and into individual Licor CO<sub>2</sub> analysers (a differential non-dispersive gas analyser, LI-6262, resolution 0.1 ppm; Licor, Lincoln, NE, USA). The length of the tubes to each analyser was identical and was kept to a minimum. Samples of the volume of CO<sub>2</sub> emitted were taken every 5 s and recorded using computerised data acquisition software (Datacan V, Sable Systems, Henderson, NV, USA). Measurements were made on individual beetles that had been weighed to ±0.1 mg (Precisa 160A balance; Instrulab, Midrand, South Africa). The beetles' respiration patterns were recorded for a minimum of 6 h during the night, with the same conditions being used for all the species. Beetles were re-weighed after the respiratory measurements.

Baseline drift of the analysers during recording was corrected from measurements taken at the beginning and end of each trial with the respirometry chambers empty. The zero drift over the time of the experiment was a continuous function, not cyclic. All measurements were corrected to standard temperature and pressure (STPD). The CO<sub>2</sub> recordings were converted to rate of CO<sub>2</sub> emission ( $\dot{V}_{CO_2}$ ) in ml h<sup>-1</sup>. The DGC characteristics were calculated as follows: the DGC frequency (= burst frequency) was calculated by determining the number

of peaks of CO<sub>2</sub> per second, and the DGC duration was considered as one complete cycle (closed, flutter, burst). The mean rate of CO<sub>2</sub> emission was taken as the mean value over several complete DGC cycles and the emission volume of CO<sub>2</sub> was obtained by integration of the area under the curve against duration in hours.

Data are presented as means  $\pm$  s.d. Sample size (*N*) is indicated in the text as either representing individual beetles or in the case of gas exchange characteristics, ten to twenty discontinuous gas exchange cycles per beetle. Unless otherwise noted, statistical comparisons were made either with

the Student's *t*-test or analysis of variance (ANOVA). Significant ANOVAs were followed with the Newman-Keuls multiple range test. Regression analysis was done by the least squares method and regression lines were compared using ANCOVA.

## Results

### *Anterior and posterior spiracle use*

The basic CO<sub>2</sub> release pattern for each of the species studied is different. *Pachylomerus femoralis*, *S. gariepinus* and *S.*

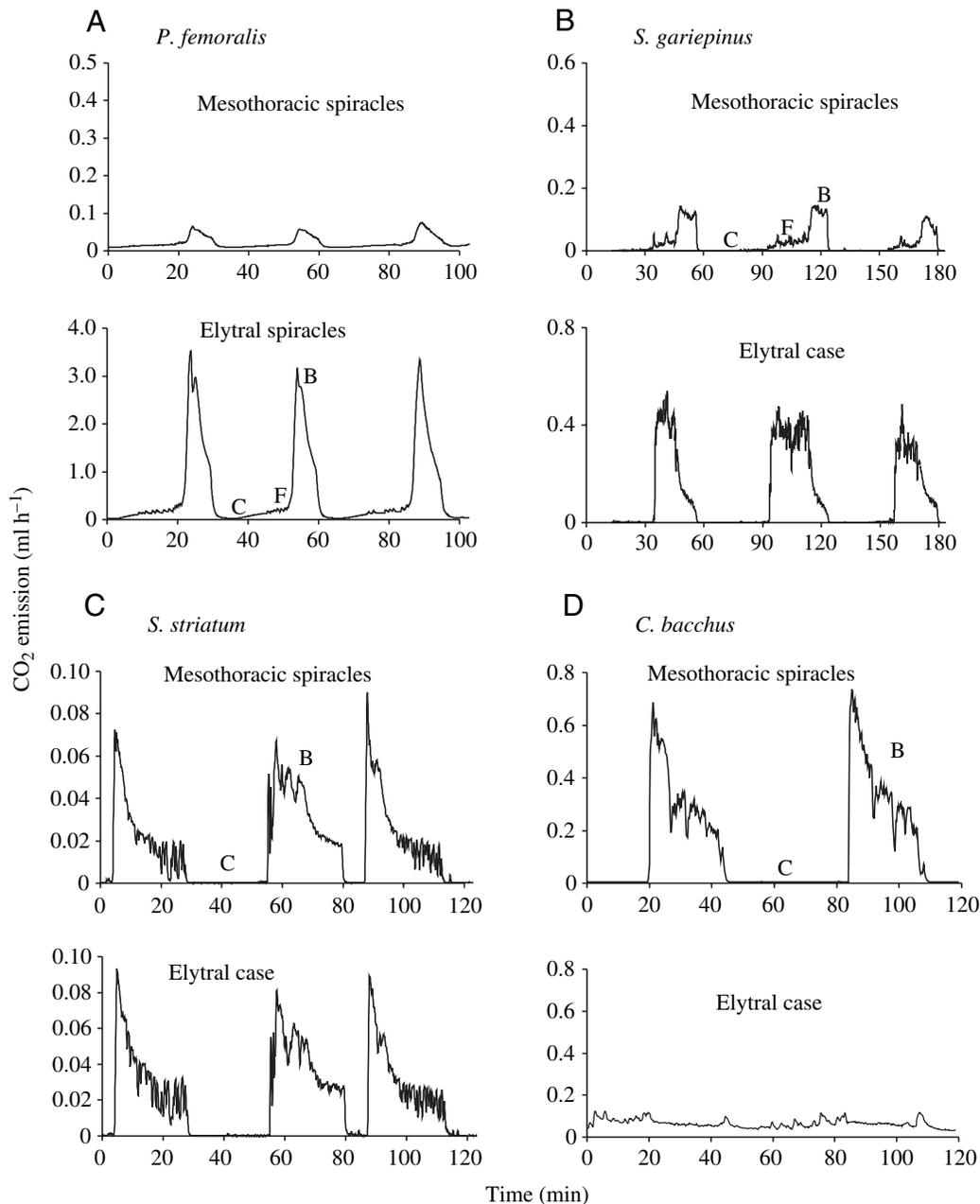


Fig. 2. Recordings of CO<sub>2</sub> emission from the anterior mesothoracic spiracles and the posterior elytral case in four species of dung beetles. (A) *Pachylomerus femoralis* (5.53 g), (B) *Scarabaeus gariepinus* (1.475 g), (C) *S. striatum* (0.487 g) and (D) *Circellium bacchus* (4.859 g). Note the different scales on the axes. C, closed period; F, flutter period; B, burst period. These are explained further in the text.

*striatum* all use a combination of both the anterior mesothoracic spiracles and the posterior subelytral spiracles for CO<sub>2</sub> emission, while *C. bacchus* uses the anterior spiracles almost exclusively (Fig. 2). However, the relative contribution of the posterior spiracles forms a series from *C. bacchus* followed by the other two wingless species, and finally *P. femoralis* (Table 1). The bulk of CO<sub>2</sub> emission takes place from the seven pairs of posterior spiracles under the elytra of *P. femoralis*, representing approximately 87.5% (7/8) of the total output. The CO<sub>2</sub> volume measured from the single pair of anterior spiracles does not differ significantly from 12.5% (1/8) of the total CO<sub>2</sub> emitted from the total, eight pairs of spiracles ( $t_{14,0.05}=0.59$ ,  $P=0.612$ ). A greater proportion (20.1%) of the total CO<sub>2</sub> volume leaves through the mesothoracic spiracles of *S. garipepinus*, which increases further in *S. striatum* to 46.2% of total volume, which is similar to the amount leaving from the elytral case ( $t_{10,0.05}=0.096$ ,  $P=0.93$ ), while *C. bacchus* releases fully 79.4% of its CO<sub>2</sub> from the anterior mesothoracic spiracles.

In *P. femoralis* and *S. garipepinus* the rate of CO<sub>2</sub> emission from the subelytral spiracles increased with rising metabolic rate (Fig. 3A,B) and the slopes were identical, with the common slope being 0.86. Conversely, in *C. bacchus* and *S. striatum* the mesothoracic emission rate increased with increasing metabolic rate (Fig. 3C,D), with both species having a common slope of 0.87 (ANCOVA).

#### Metabolic rates

The metabolic rate from the four beetle species formed a series with *C. bacchus* having the lowest, followed by the other

two wingless species and *P. femoralis* having the highest (Table 1). The mass specific metabolic rates showed a significant difference between the flighted *P. femoralis* and flightless *C. bacchus* (Table 1).

#### Respiratory patterns

The CO<sub>2</sub> emission trace for *P. femoralis* (Fig. 2A) shows the three periods of the DGC in a closed, flutter, open (CFO) cycle, as compared with the closed, flutter, ventilation (CFV) cycle seen in the other three species (Fig. 2B–D). The burst periods differs in that *P. femoralis* shows a smooth trace of CO<sub>2</sub> release without CO<sub>2</sub> volleys, while the other three species reveal strong CO<sub>2</sub> volleys (Fig. 2).

The frequency of DGC is significantly faster in *P. femoralis* than in the flightless species (Table 1). In *P. femoralis* and *S. garipepinus* the posterior spiracles contribute more to the emission of CO<sub>2</sub> in all the DGC periods, but in *S. striatum* there is no significant difference between the contribution of the mesothoracic spiracles and elytral case (Table 2). *Scarabaeus garipepinus* and *C. bacchus* have the longest closed period, occupying almost half of the DGC duration. In all the flightless species ventilative bursts, which are seen as volleys of CO<sub>2</sub> release within the burst period, are present.

#### Discussion

The patterns of breathing revealed by this study support the hypothesis that flightless dung beetles from different regions and tribes have similar respiratory patterns involving increased use of the mesothoracic spiracles for CO<sub>2</sub> emission. However,

Table 1. Comparison of the mean rate of CO<sub>2</sub> emission from the mesothoracic spiracles and elytral case in four species of dung beetles

Species	<i>P. femoralis</i>	<i>S. garipepinus</i>	<i>S. striatum</i>	<i>C. bacchus</i> *
N	5	7	6	6
Mass (g)	5.117±0.62 <sup>a</sup>	1.716±0.35 <sup>b</sup>	0.742±0.20 <sup>b</sup>	7.427±1.86 <sup>c</sup>
Rate of CO <sub>2</sub> emission (µl h <sup>-1</sup> )				
Mesothoracic spiracles	35.53±28.7 <sup>a</sup>	24.88±19.2 <sup>a</sup>	27.68±19.7 <sup>a</sup>	258.2±118.2 <sup>b</sup>
Posterior spiracles	468.39±97.13 <sup>a</sup>	121.45±75.3 <sup>b</sup>	28.56±11.0 <sup>b</sup>	119.5±123.6 <sup>b</sup>
Ratio of CO <sub>2</sub> emission				
Mesothoracic:elytral case	0.083±0.07 (range 0.019–0.22)	0.31±0.3 (0.11–1.06)	1.56±2.1 (0.22–5.9)	3.47±2.0 (0.42–6.81)
% total CO <sub>2</sub> emitted through mesothoracic spiracles	7.31±6.5	20.07±15.05	46.23±22.9	79.4±4.48
Frequency (mHz)	0.73±0.3 <sup>a</sup>	0.33±0.2 <sup>b</sup>	0.42±0.2 <sup>b</sup>	0.26±0.05 <sup>b</sup>
DGC cycles per hour	2.6±1.1 <sup>a</sup>	1.2±0.6 <sup>b</sup>	1.5±0.8 <sup>b</sup>	0.94±0.2 <sup>b</sup>
Total rate of CO <sub>2</sub> emission				
(µl h <sup>-1</sup> )	503.92±87.5 <sup>a</sup>	146.03±87.2 <sup>b</sup>	56.24±11.5 <sup>b</sup>	377.74±138.7 <sup>c</sup>
(µl h <sup>-1</sup> g <sup>-1</sup> )	98.66±13.2 <sup>a</sup>	80.53±39.6 <sup>a,b</sup>	80.19±25.4 <sup>a,b</sup>	51.52±18.12 <sup>b</sup>

Comparison of the mean rate of CO<sub>2</sub> emission from the mesothoracic spiracles and elytral case in four species of dung beetles (mean ± s.d.). N, number beetles measured. Means in the same row, followed by the same letter are not significantly different ( $P<0.05$ ; ANOVA with LSD range test). \*From Duncan and Byrne (2002).

a graded, rather than all-or-nothing, use of the anterior spiracles suggests that this adaptation is closely tailored to the demands of specific habitats. Many more species will need to be tested before a definite conclusion about this arid adaptation can be made.

#### Anterior and posterior spiracle use

Direct observation of spiracular movements and abdominal pumping are needed to interpret the respiratory strategies used by these beetles more precisely. These are difficult to view in a live specimen because of the tight elytra and spiracular sieve plates, but a reasonable interpretation of their movements can be made by proxy through their patterns of CO<sub>2</sub> release in a divided through-flow respirometry system. What is revealed is a pattern of breathing in which the flightless species are more similar to each other, despite being members of different tribes, but still remain specifically different from each other, even within the same genus. Each species remains a unique expression of the sum of adaptations which characterise it as a species.

*Pachylomerus femoralis* appears to be a predominantly diffusive burster of CO<sub>2</sub>, using outward diffusion mainly through the posterior spiracles, under relatively unsealed elytra, whereas *C. bacchus* restricts resting CO<sub>2</sub> emission to the anterior mesothoracic spiracles. Intermediate states of these two extremes are shown by the *Scarabaeus* species, where *S. garipepinus* directs 20% of its CO<sub>2</sub> output to the mesothoracic

spiracles, and *S. striatum* increases this to 46% of the total production. This progressive replacement of diffusion by a convective forward flow, and a replacement of diffusive outflow from the subelytral cavity by active emission through the mesothoracic spiracles is an anterograde version of the active ventilation strategy proposed by Kestler (2003). That it has not been absolutely adopted by all of the flightless species tested here, suggests that there may be a trade-off involving reduction of activity levels associated with a decline in metabolic rate.

#### Metabolic rates

Low metabolic rates have been previously reported for flightless dung beetles (Davis et al., 1999), arid dwelling carabids and tenebrionids (Zachariassen et al., 1987) and arid adapted *Drosophila* species (Gibbs et al., 2003). *Pachylomerus femoralis* is a pugnacious competitor, described as a facultative endotherm (Chown et al., 1995). Given the high energy requirements of competing for and rolling dung balls (Bartholomew and Heinrich, 1978), coupled with the energetic demands of long distance foraging flights (Yborrondo and Heinrich, 1996), this is predictable. *Circellium bacchus* is a slow moving species that has been shown to be strictly ectothermic (Nicolson, 1987), and may have been restricted to the arid bush of the Eastern Cape through competition with Scarabaeini such as *P. femoralis* in more open moist habitats (Chown et al., 1995). By comparison, it is reasonable to

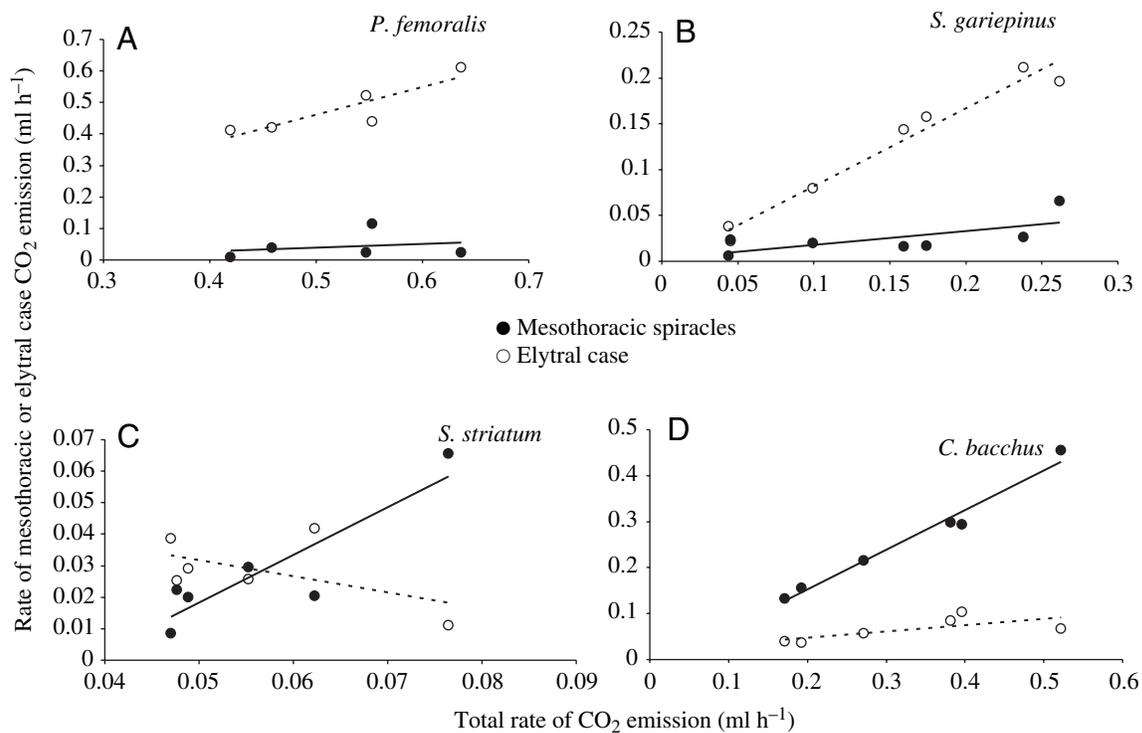


Fig. 3. The CO<sub>2</sub> emission rates from the mesothoracic spiracles and elytral case as a function of total CO<sub>2</sub> emission in four dung beetle species. The regression equations, for which there is a significant correlation, are given for each species, where: *m*, mesothoracic spiracular  $\dot{V}_{CO_2}$ ; *el*, elytral case  $\dot{V}_{CO_2}$ ; and *t*, total  $\dot{V}_{CO_2}$ . For *P. femoralis*:  $el=0.022+0.88t$ ,  $r^2=0.78$ ,  $P=0.048$ . For *S. garipepinus*:  $el=-0.003+0.85t$ ,  $r^2=0.96$ ,  $P=0$ . For *S. striatum*:  $m=-0.058+1.52t$ ,  $r^2=0.78$ ,  $P=0.02$ . For *C. bacchus*:  $m=-0.021+0.86t$ ,  $r^2=0.98$ ,  $P=0.002$ .

assume that given their small size, both *Scarabaeus* species are ectothermic, but after initially basking flat on the sand with their legs outstretched, they run swiftly while foraging for dung pellets on the surface (Scholtz, 1989). Their metabolic rates are intermediate between the two larger species and may reflect a combination of lower metabolic demands of flightlessness and behavioural thermoregulation in an open, sunny habitat.

Energetic constraints have been suggested to limit habitat use in Afrotropical dung beetle guilds (Krell et al., 2003). Even though the *Scarabaeus* species live in an apparently more water-stressed habitat than *C. bacchus*, they probably have access to a more predictable water supply in the daily fog that sweeps the area (Seely, 1978), the high air humidity, and moisture deep in the sand (Seely, 1978) where they rehydrate their dung supplies (Scholtz, 1989). On the sand surface, rodent pellets would be expected to persist in the absence of other competing species making their food supply reasonably stable. By contrast, *C. bacchus* can only utilise fresh dung and lives in a region of unpredictable rainfall with low soil moisture. The metabolic rates of the experimental species are, therefore, explicable in terms of their respective habitats and lifestyles and may indirectly contribute to a reduction in water loss as lower needs for gas exchange allow tactically longer closing periods between expiratory openings (Kestler, 2003).

#### Respiratory patterns

The CFO type of discontinuous gas exchange cycles seen in the mesic *P. femoralis* suggest a loss of the ventilation period. The CFV form of the discontinuous gas exchange cycle is typical for most adult insects except ants (Lighton, 1996). However, over-hydrated *Periplaneta americana* show a loss of pumping in the ventilation period (Kestler, 1985). Diffusion leads to higher water loss than pure convection as water molecules diffuse faster than both O<sub>2</sub> and CO<sub>2</sub> (Kestler, 1985). *Pachylomerus femoralis* therefore could have lost the active ventilation strategy to save energy at the expense of increased water loss. By contrast, all three arid-adapted species use both strategies of water retention, despite potential energy costs. From the 6 h of measurement the total mass loss per hour was significantly greater in *P. femoralis* (0.034±0.015 g h<sup>-1</sup>) than in either *S. garipepinus* (0.005±0.004 g h<sup>-1</sup>) or *S. striatum* (0.004±0.003 g h<sup>-1</sup>) ( $F_{(2,12)}=16.4$ ,  $P>0.05$ ), indicating a higher water loss rate for the mesic species. P. Kestler (personal communication) has shown that a combination of diffusion and convection saves more water than diffusion alone, but less than pure convection, which is not possible in insects due to the short spiracular diffusion path that leads to a rapid outward diffusion of CO<sub>2</sub> during expiration.

In conclusion, the closely related dung beetle species in this study show limited physiological variations in their respiratory patterns. Nevertheless, the arid-dwelling species from different tribes and dissimilar habitats on opposite coasts of the continent, show increasing use of the anterior mesothoracic spiracles for CO<sub>2</sub> expiration (which could be anterograde), coupled with more convection than diffusion in the discontinuous gas exchange cycle, than the centrally

Table 2. Characteristics of the discontinuous gas exchange cycle from the anterior and posterior spiracles of four dung beetle species

Species	<i>P. femoralis</i>			<i>S. garipepinus</i>			<i>S. striatum</i>			<i>C. bacchus</i> *		
	Mesothoracic spiracles	Posterior spiracles	Mesothoracic spiracles	Elytral case	Mesothoracic spiracles	Elytral case	Mesothoracic spiracles	Elytral case	Mesothoracic spiracles	Elytral case	Mesothoracic spiracles	Elytral case
Closed period												
sV̇CO <sub>2</sub> (μl g <sup>-1</sup> )	0.16±0.06	0.44±0.24	0.70±0.6	2.64±3.2	6.33±8.8†	1.91±1.4†	0.57±0.22†	—	1858.3±917.1†	—	—	—
Duration (s)	307.2±102.5		1620.8±1265.3		604.7±187.6†							
%DGC duration	20.7±5.5		42.7±16.4		34.2±11.9†							
Flutter period												
sV̇CO <sub>2</sub> (μl g <sup>-1</sup> )	0.60±0.4	4.44±2.6	1.01±0.9	—								
Duration (s)	496.4±290.1		534.7±353.0									
%DGC duration	29.0±9.8		17.5±12.4									
Burst period												
sV̇CO <sub>2</sub> (μl g <sup>-1</sup> )	2.40±2.3	35.86±13.7	7.47±3.9	44.26±16.83	14.89±12.5	17.62±12.9	36.1±15.5	—	2230.4±325.1	—	—	—
Duration (s)	785.7±288.6		1301±455.4	1836±533.2	1194.7±331.2							
%DGC duration	50.3±5.6		17.5±12.4	57.2±17.5	66.6±11.2							

\*From Duncan and Byrne (2002). †Note that these are the measurements for combined closed and flutter periods. '—' Denotes a negligible amount. Data shown are mean ± s.d.

distributed mesic species. The patterns discovered represent a continuum from extreme anterior mesothoracic spiracle respiration to expiration from all the spiracles, rather than a strict dichotomy between the two, suggesting that control of breathing in these insects is closely adapted to the demands of their respective habitats.

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