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Respiratory airflow in a wingless dung beetle

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Summary

The sealed subelytral cavity of many flightless beetle species is widely acknowledged to be an adaptation to water saving in arid-habitat species. However, this hypothesis relies on the acceptance of two largely untested assumptions: (i) that the movement of respiratory gases is unidirectional from anterior to posterior and (ii) that the coordinated action of the spiracles directs this flow. We tested these assumptions by simultaneously measuring CO_2 and O_2 exchange at the anterior mesothorax, independently of gas exchange in the posterior body, which included the subelytral cavity, of a large apterous beetle, *Circellium bacchus*. Flow-through respirometry revealed a marked discontinuous gas-exchange cycle (DGC) pattern from the anterior half of the body. Very little CO_2 was released from the posterior body, where the DGC was not apparent. Labelled air was shown to flow forwards from the posterior to the anterior body. Individual sampling from the mesothoracic spiracles revealed that the right mesothoracic spiracle, lying outside the elytral cavity, is the primary route for respiratory gas exchange in *C. bacchus* at rest. This discovery necessitates a reassessment of the currently assumed role of the subelytral cavity in water conservation and is, to our knowledge, the first demonstration of forward airflow associated with the unilateral use of a single thoracic spiracle for respiration in an insect.

Key words: *Circellium bacchus*, discontinuous gas exchange cycle, respiration, spiracle, Scarabaeidae, dung beetle, subelytral cavity.

Introduction

The terrestrial dominance of insects is attributed mainly to their ability, amongst other adaptations, to resist desiccation through their respiratory systems (Villani et al., 1999). However, despite many years of investigation (Wigglesworth, 1965), there is still uncertaintity as to exactly how gas and water move through this system of spiracular openings and tracheae (Wasserthal, 1996). Cockroaches and locusts pass inspired air from the anterior spiracles, through longitudinal trunks that connect the segmental tracheae and out through the more posterior abdominal spiracles, generating a flow of fresh air through the body (Miller, 1982). Lepidopteran pupae show similar patterns of respiration (Schneiderman, 1960), and some species may be able to control the actions of individual spiracles (Slama, 1988, 1999).

Retrograde respiratory airflow is presumed to be typical for all insects (Hadley, 1994; Lighton, 1996), and expelling air into a sealed space below the elytra is assumed to be an adaptation, found in many species of wingless beetle (Cloudsley-Thompson, 1964; Ahearn, 1970; Draney, 1993), to an arid habitat. Because the cavity maintains its air at a high humidity, water loss during respiration should be reduced because the tracheae will not be exposed to dry air. However, the validity of this hypothesis requires an anterior-to-posterior flow of respiratory gases through the body and differential control of the spiracles, neither of which has previously been demonstrated in these insects (Hadley, 1994).

Spiracular control appears to be most precise in insects that inhabit dry environments, where they limit water loss by opening their spiracles for only limited periods during a discontinuous gas-exchange cycle (DGC) (for reviews, see Kestler, 1985; Lighton, 1994, 1996; Wasserthal, 1996). The DGC is a cyclic discontinuity in external gas exchange that typically consists of three periods (Miller, 1981; Kestler, 1985). There is a closed (C) period, during which the spiracles are shut, preventing both respiratory water loss and gas exchange. Oxygen levels in the tracheae drop, while CO_2 is largely buffered in the tissues and haemolymph. This is followed by the flutter (F) period, during which slight, intermittent opening of the spiracles allows some normoxic O_2 uptake through the spiracles by diffusion and convection, but little CO_2 or water vapour is lost. The final period, the CO_2 burst (B) period, is triggered when the accumulation of CO_2 from respiring tissues causes some or all of the spiracles to open widely. Rapid unloading of CO_2 should minimise the time that the spiracles are open and therefore reduce water vapour loss. However, the role of the DGC as a water-saving

mechanism remains controversial (Hadley, 1994; Shelton and Appel, 2000; Williams and Bradley, 1998), and some authors propose that it is more likely to be a response to a high-[CO_2] atmosphere than to water stress (Lighton, 1996). We have demonstrated a relationship between the DGC and habitat aridity that is independent of phylogeny in five species of African dung beetle (Duncan and Byrne, 2000) and that most water loss occurs during the burst period of the DGC in *Circellium bacchus* (Duncan, 2002).

To investigate the route of external gas exchange, we chose a large apterous beetle that would allow us simultaneously to measure CO_2 and O_2 exchange at the mesothorax, independently of exchange from rest of the body, and in particular the subelytral cavity. *Circellium bacchus* is a ball-rolling dung beetle that feeds on the dung of large herbivores (elephant, buffalo and black rhinoceros) and is now restricted to a few populations in the eastern Cape of South Africa, apparently because of competition with heterothermic, winged dung beetles (Chown et al., 1995). Although the beetle is locally abundant, it is considered to be endangered because it occurs at only seven widely separated localities in the Cape Province of South Africa (Coles, 1993). A permit was obtained to collect 10 beetles from Addo Elephant Park, which has a low annual rainfall of approximately 400 mm, spread throughout the year (mean 34±7.1 mm per month, mean ± S.D.; range 23–48 mm) (Sutherst and Maywald, 1985). *Circellium bacchus* live for more than 2 years and spend dry periods underground in sandy soil (Tribe, 1976). Females may spend more than 4 months underground, tending a single brood ball, during which time it is not known whether they feed (Coles, 1993). Given the dry habitat and the lifestyle of this species, it is assumed to be under selection pressure to reduce water loss. *C. bacchus* exhibits an extreme example of the DGC (Duncan and Byrne, 2000) and is a strict ectotherm, so metabolic measurements are not compromised by heterothermy (Nicolson, 1987).

C. bacchus has eight pairs of spiracles along its body (Fig. 1), of which the single pair of large, ventral mesothoracic spiracles occurs anteriorly in the membrane connecting the prothorax and mesothorax, behind the coxal cavities of the prothoracic legs. Here, they open into the space created by the constriction between the prothorax and the mesothorax. The posterior half of the body has a single pair of smaller metathoracic spiracles and six pairs of even smaller abdominal spiracles, all of which open dorsally into the subelytral cavity. Sealing a latex skirt between the two body sections permitted measurement of gas exchange from each half of a live beetle enclosed in a respiratory chamber (Fig. 2; Duncan, 2002). Sampling gas from the anterior and posterior body allowed us to address three questions with regard to the role of the subelytral cavity in respiration. First, from which body half does the majority of gas exchange take place? Second, in which direction do respiratory gases move through the beetle? Third, which spiracles are involved in this gas flow?

Materials and methods

Animals

Adult *Circellium bacchus* (Fabricius) (Scarabaeidae, Scarabaeinae) were collected from the Addo Elephant National Park (33°30′S, 25°41′E) in the Cape Province, South Africa. 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. They were fed fresh cow dung weekly and survived well for several months under laboratory conditions.

Respirometry

A flow-through respirometry system was used to measure gas exchange in the anterior and posterior halves of a live, intact beetle (for details, see Duncan, 2002). The beetle to be tested was placed in a Perspex respirometry chamber divided crosswise by a sheet of latex (dental dam, 0.02 mm thick)

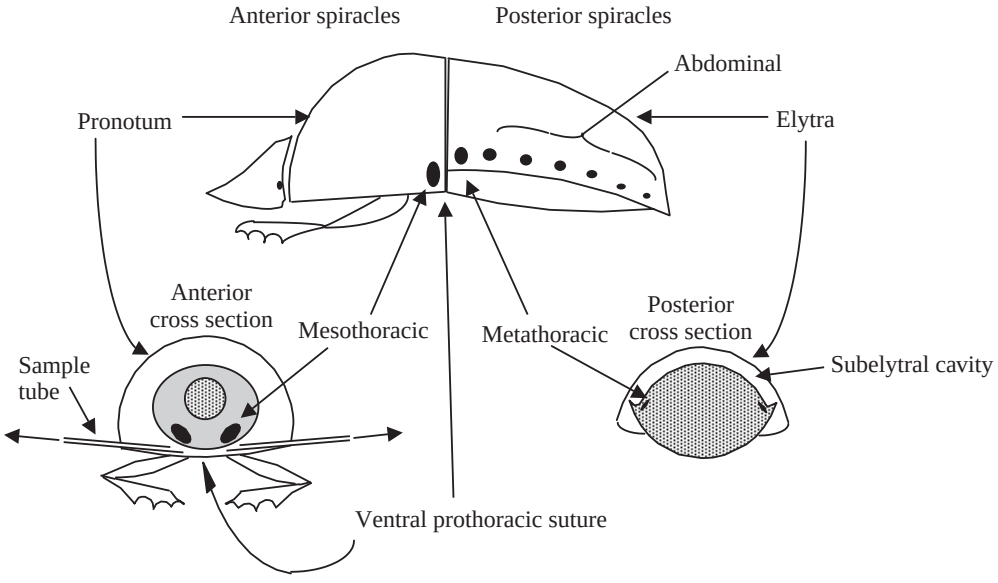

Fig. 1. A schematic representation of the position of the anterior and posterior spiracles in *Circellium bacchus*. The position of the tubes, which were attached to the ventral prothoracic suture and used to sample each mesothoracic spiracle separately, is shown.

(Fig. 2). 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 a tight seal between the prothorax and abdomen. The posterior section of the chamber was then bolted to the anterior section. The latex sheet sealed the joint between the two sections of the apparatus, giving each section a volume of approximately 250 ml. One inlet and one outlet pipe served each section of the chamber. The entire configuration was tested for leaks, and the latex sheet was renewed for each trial.

Gas emissions in each section of the chamber were measured separately and simultaneously using flow-through respirometry, with each section having its own source of air, flow controller and gas analysers. Briefly, air scrubbed of CO_2 and H_2O was drawn into each section at a flow rate of 50 ml min^{-1}. The outlet from each section was led to a separate Licor CO_2 analyser (LI-6262 and LI-6251; 0.1 p.p.m.) and then to the O_2 sensor (Qubit Systems gas-phase O_2 sensor, model S-102, with a resolution of 0.1 %) with its own flow control (calibrated Supelco flow meter). Readings of the concentration of CO_2 and O_2 in the separate chambers were taken every 5 s and recorded using a computerised data-acquisition software (Datacan V, Sable Systems). Recordings were made on individuals weighed to ±0.1 mg (Precisa 160A balance). The respiration pattern of each beetle was measured for a minimum of 6 h in dim red light. The beetles were occasionally observed to ensure that they remained quiescent during sampling. Only measurements from stationary beetles were used in the analysis. After measurements, the beetles were reweighed. Experiments were conducted in an air-conditioned laboratory at a temperature of approximately 23±1 °C.

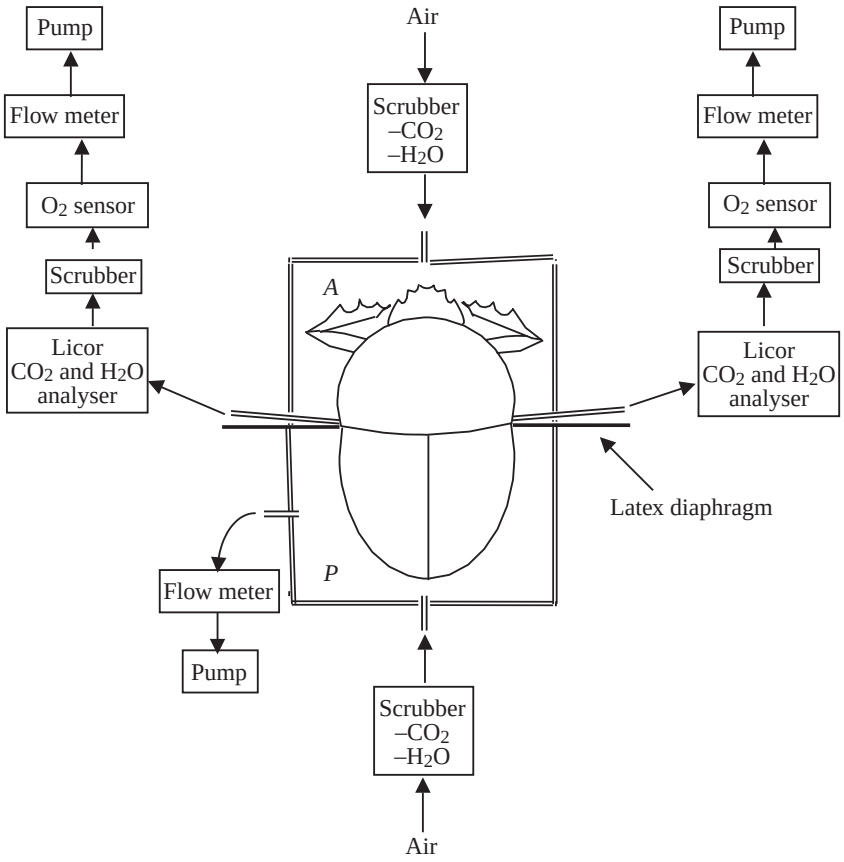

Fig. 2. The flow-through respirometry apparatus used to sample respiratory emissions individually from the mesothoracic spiracles of *Circellium bacchus*. *A*, anterior section of the respirometry chamber, *P*; posterior section of the respirometry chamber.

Baseline drift of the analysers was corrected during analysis from measurements taken at the beginning and end of each trial with the respirometry chamber empty. All measurements were corrected to standard temperature and pressure (STP). The CO_2 recordings were converted to the rate of CO_2 production ($\dot{V}_{CO_2}$; in ml h^{-1}), and O_2 data were converted to the rate of O_2 consumption (in ml h^{-1}). To measure O_2 consumption within the flow-through system, an H_2O/CO_2 scrubber was placed between the CO_2 analyser and the O_2 analyser, which was upstream of the flow meter (Withers, 1977). This allowed us to interpret a large drop in O_2 concentration as O_2 consumption. This assumption was checked by injecting small volumes of CO_2 and N_2 into the system.

The DGC characteristics were calculated as follows. The DGC frequency (=burst frequency) was calculated by determining the number of peaks of CO_2 emission per second, and the DGC duration was taken as one complete cycle (i.e. closed, flutter and burst periods). The mean rate of CO_2 emission was calculated as the mean value over several complete discontinuous gas-exchange cycles. To measure the emission volume, the area under the curve was integrated against time.

Each experiment was repeated with 2–6 individuals. To ensure that the results obtained were not sampling artefacts, between trials beetles were rotated within the chamber and the chamber within the apparatus. This removed any chance of inverse of air flow being due to slight pressure differences on the two sides of the latex sheet. The same apparatus, with slight modifications, was used for each experiment.

Direction of airflow

To determine the direction of gas movement in the beetle, O_2-enriched air containing 30 % O_2 was used as the tracer gas. The gas mixture was prepared using a Columbus Instruments gas mixer and was stored in a Douglas bag. The gas mixture was first drawn into the posterior section of the respirometer, and both sides of the chamber were monitored for O_2 and CO_2 emissions, using the method described above. The gas mixture was then drawn through the anterior section while monitoring both ends of the chamber. The experimental apparatus was otherwise as described above. The 30 % O_2 gas mixture

allowed the beetle to continue to respire normally (compare Figs 3 and 5).

Monitoring individual spiracles

Gas exchange was measured separately at each of the two mesothoracic spiracles. Because these spiracles are situated in the soft intersegmental membrane, it was not possible to seal tubes around each individual spiracle. Instead, two tubes (1 mm diameter) were glued to the ventral prothoracic suture such that each single spiracle opened directly into one tube (Figs 1, 2). The beetle was placed in the respirometer as described above except that only the gas from the anterior section was sampled by the flow-through system. This section had an additional outlet so that the tube from each mesothoracic spiracle led directly to its own CO_2 and O_2 analyser and was controlled by an individual flow meter. To prevent an accumulation of CO_2 in the posterior section of the respirometer altering the normal resting respiratory pattern, air scrubbed of CO_2 and H_2O was drawn through the posterior section at a flow rate of 50 ml min^{-1}. Beetles were rotated within the chamber to ensure that the two mesothoracic spiracles were being sampled independently. A small amount of cross-sampling did occur, which confirmed that both tubes were collecting gas emissions, but was so small that we were able to conclude that each spiracle was acting independently.

Statistical analyses

Data are represented as means ± standard deviation (S.D.). Sample size (*N*) is indicated in the text as representing individual beetles or, in the case of gas-exchange characteristics, 3–10 discontinuous gas-exchange cycles per beetle. Statistical comparisons were made using Student's *t*-tests. Regression analysis was performed using the least-squares method.

Results

Anterior versus *posterior spiracular gas exchange*

It was not possible for beetle activity to be continuously monitored during measurements. However, visual observations of the beetles while recording gas exchange revealed that even slight movements were accompanied by obvious changes in the pattern and volume of CO_2 emission. Any measurements suggesting activity were therefore excluded from the following analysis. Total CO_2 emissions measured from the combined sections of the respirometer were not significantly different from those obtained from the whole animal in a previous study (Duncan and Byrne, 2000) (321±134 µl CO_2 h^{-1}, N=6, this study, compared with 407±204 µl CO_2 h^{-1}, N=7, t=0.88 P>0.05).

We recorded a DGC respiratory pattern from the anterior body half (Fig. 3A), which showed a burst of CO_2 emission approximately once per hour, lasting on average for 37±5 min (N=6), resulting in a mean DGC duration of 70±16 min (N=6) (Table 1). Lighton et al. (1993) showed that accurate interpretations of spiracular movements could be made from CO_2 emissions, allowing us to conclude that the anterior mesothoracic spiracles remained closed during the rest of the DGC. As with whole-animal recordings, the closed and flutter periods could not be separated and are thus referred to as the interburst period. Oxygen uptake occurred at the beginning of the CO_2 burst period (Fig. 3A). The peak of O_2 uptake lasted 15±10.7 % (N=6) of the duration of the CO_2 burst period, and no corresponding increase in the period of O_2 uptake occurred with an increase in CO_2 burst duration.

Weaker cyclic CO_2 emission was recorded from the posterior spiracles, but a DGC was not apparent (Fig. 3B). No distinct periods could be seen. In most cases, but not all, the small increments of CO_2 emission from the posterior spiracles occurred during the corresponding burst period of the anterior

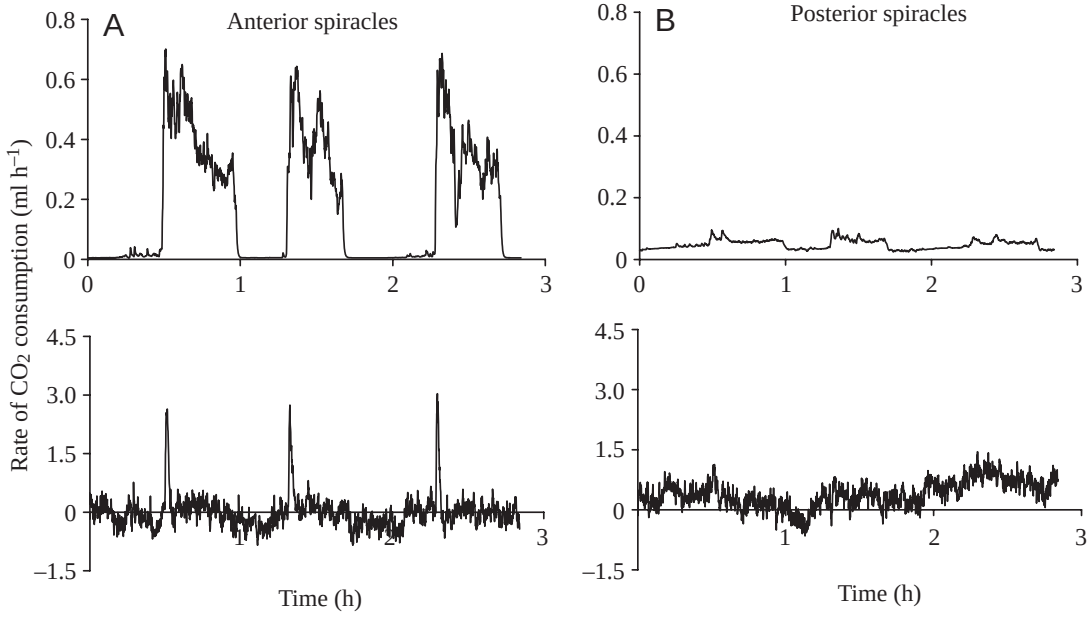

Fig. 3. CO_2 and O_2 exchange of a *Circellium bacchus* (mass 8.153 g) from (A) the anterior mesothoracic spiracles, where the majority of exchange occurs, and (B) the posterior metathoracic and abdominal spiracles, which contribute less to respiratory exchange. The readings are from one individual and are matched in time. Negative values of O_2 consumption are due to baseline drift.

Table 1. *Characteristics of the discontinuous gas-exchange cycle from the anterior mesothoracic spiracles of* Circellium bacchus

Variable	Mean	S.D.
Body mass (g)	7.41	1.86
DGC		
Frequency (mHz)	0.26	0.05
Duration (min)	69.6	16.0
CO_2 emission		
Burst CO_2 volume (μl)	252.01	81.86
Burst duration (min)	37.2	5.4
Interburst CO_2 volume (μl)	3.91	0.9
Interburst duration (min)	30.9	15.3
O_2 uptake		
Volume (μl)	82.27	24.71
Duration (min)	5.2	2.2

DGC, discontinuous gas-exchange cycle.
N=six beetles; total DGCs=63, approximately 10 DGCs per beetle.

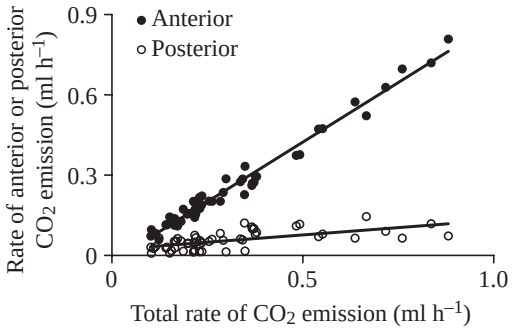

Fig. 4. The relationship between anterior and posterior CO_2 emission rates during the discontinuous gas-exchange cycle (DGC) in *Circellium bacchus* (*N*=53 DGCs in six beetles, mean mass 7.427 g). The regression equations are: a=–0.022+0.891t, r^2=0.98, P=0.001; p=0.022+0.109t, r^2=0.4, P=0.001, where a is anterior spiracular $\dot{V}_{CO_2}$, p is posterior spiracular $\dot{V}_{CO_2}$ and t is total $\dot{V}_{CO_2}$. When the mean values for each beetle (*N*=6) are used, the regression equations are: a=–0.021+0.866t, r^2=0.98, P=0.002; p=0.021+0.134t, r^2=0.5, P=0.11.

spiracles. The rate of CO_2 emission was 1.5–7 times lower than that from the anterior spiracles ($t_{0.05,10}$=3.36, P<0.01) (Table 2). Of the total CO_2 emitted, 79.4±4.48 % (*N*=6) was expelled through the anterior mesothoracic spiracles. We assume that there is little movement of the posterior spiracles, which open into the subelytral cavity, because only a small coefficient of variation of CO_2 emission was seen; 0.5±0.15 compared with 1.3±0.32 for the anterior spiracles (t=5.47, P<0.001). No measurable O_2 uptake was recorded for the posterior body half, and fluctuations in the rate of O_2 consumption can be attributed to baseline drift rather than to actual consumption.

A large difference in the absolute magnitude of CO_2 emission rates from the anterior and posterior spiracles was noted (Fig. 3). The length of the DGC period of the anterior spiracular emissions was used to calculate the CO_2 output from both anterior and posterior spiracles. The anterior spiracular rate of CO_2 emission was 77.5±0.07 % (*N*=6) of total CO_2 output, while the rate of CO_2 emission from the posterior spiracles was 22.5±0.07 % (*N*=6) of total CO_2 output. The anterior and abdominal spiracle rates of CO_2 emission, averaged over the entire measurement (i.e. several DGCs) for each beetle, showed that the anterior rate of CO_2 emission as a proportion of the total rate of CO_2 emission increased as the metabolic rate rose (Fig. 4), with the posterior spiracular contribution declining from 53 to 4 % of the anterior spiracular output.

Therefore, from the above results, the anterior spiracles appear to be the main site of gas exchange during respiration at rest. Little gas exchange was seen to take place *via* the subelytral cavity, and the anterior-to-posterior airflow expected in apterous beetles was not observed in *C. bacchus*.

Table 2. *Comparison of the rates of CO_2 emission from the anterior mesothoracic spiracles and from the posterior metathoracic and abdominal spiracles, which open into the subelytral cavity, of* Circellium bacchus

Beetle number	Mass (g)	Absolute rate of CO_2 emission (μl h^{-1})		Relative rate of CO_2 emission (μl h^{-1} g^{-1})	
		Anterior	Posterior	Anterior	Posterior
1	5.509	131.76	39.57	23.77	7.12
2	9.352	455.08	66.82	48.66	7.13
3	9.204	155.61	36.78	16.66	4.28
4	8.153	215.57	56.10	26.44	6.88
5	7.429	298.16	83.89	40.13	11.29
6	4.913	293.2	102.89	59.68	20.94

Readings from six individuals are shown to illustrate that the use of the anterior spiracles for gas exchange is typical.

Direction of airflow

Drawing air containing 30 % O_2 over the posterior body half revealed that, during the early part of the burst phase, O_2 was withdrawn from this section of the respirometer and then expelled into the anterior chamber by the mesothoracic spiracles when they opened to release CO_2 (Fig. 5A). When the same gas mixture was drawn into the anterior respiratory chamber, we detected no airflow from the thorax to the abdomen (Fig. 5B). The elevated O_2 levels had no noticeable effect on the respiration patterns of the beetles other than slightly increasing the period of the DGC when used in the anterior section of the respirometer (Fig. 5A,B). The metabolic rates of these beetles (372.16 μl h^{-1}, *N*=2) showed that they were within the range of metabolic rates recorded in the rest of the study (322.57 μl h^{-1}, *N*=6). CO_2 emission rates from the anterior and posterior regions did not indicate an altered metabolic rate, and the emission from the posterior spiracles was within the normal pattern seen in all the other trials. The prolonged drop in the O_2 concentration at the anterior spiracles

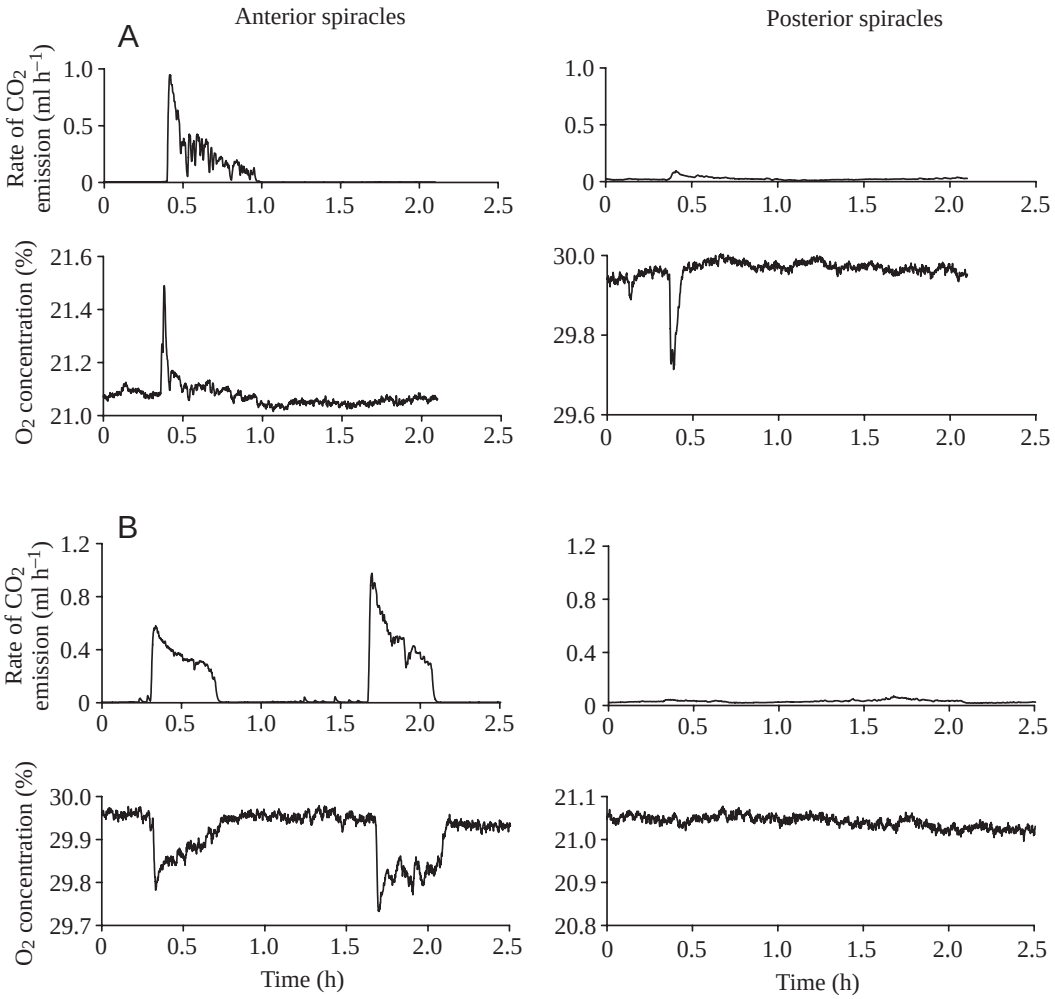

Fig. 5. Recordings of CO_2 and O_2 exchange of *Circellium bacchus* (mass 5.790 g) from the posterior and anterior spiracles when a gas mixture containing 30 % O_2 was drawn over the elytral cavity (A). O_2-enriched air entered through these spiracles because it could be seen to exit *via* the anterior mesothoracic pair of spiracles. When the 30 % O_2-enriched air mixture was drawn over the anterior mesothoracic spiracles (B), no high-O_2-content air was seen to leave *via* the posterior spiracles opening into the subelytral cavity. Comparison of Figs 3 and 5 reveals no change in the breathing pattern due to the elevated O_2 concentration in the gas mixture.

(Fig. 5B) is assumed to be a result of the elevated levels of O_2 taking longer to reach equilibrium than when at normal concentrations (cf. Fig. 3A). Why this should be so is unclear, and this phenomenon warrants further experimental investigation.

From Fig. 5A, it appears that O_2 can diffuse rapidly through the beetle, in through the posterior spiracles and out through the anterior spiracles, along its concentration gradient. The anterior and posterior spiracles may be opening in a coordinated fashion because the O_2 consumption peak corresponds closely with the start of the CO_2 burst phase. Airflow in this experiment is from posterior to anterior, contrary to the assumption of CO_2 emission from the posterior spiracles into the subelytral cavity.

Carbon dioxide emission rates from individual spiracles

Measuring gas exchange separately at individual mesothoracic spiracles revealed that the right spiracle was the main route for CO_2 emission and O_2 uptake (Fig. 6). Sampling tubes were successfully attached to three beetles, all of which respired through the right mesothoracic spiracle. From these individuals, we calculated that 89.2±12.0 % (N=3) of the total CO_2 output from the anterior body half, which amounts to 76 % of the total body output, occurred through the right mesothoracic spiracle (Table 3).

Discussion

From these findings, we conclude that, in *C. bacchus* at least, respiratory air flow does not occur in only one direction from anterior to posterior and that gas exchange is mainly *via* just one of the two anterior mesothoracic spiracles when at rest. The evidence we have gathered suggests that ventilation may be tidal and, if *C. bacchus* is typical of apterous beetles in general, then the function of the subelytral cavity in reducing respiratory water loss needs re-examination. Most authors working on flightless beetles have assumed that the direction of air flow is retrograde and that expired air leaves through the subelytral cavity *via* an aperture above the anus (e.g. Nicholson et al., 1984). This should create a flow of air from the thorax to the abdomen (Bartholomew et al., 1985). Our recordings undermine the accepted explanation that reduced rates of respiratory water loss in flightless beetles will result from air being drawn in through the thoracic spiracles and CO_2 being expelled through the abdominal spiracles and out through the subelytral cavity.

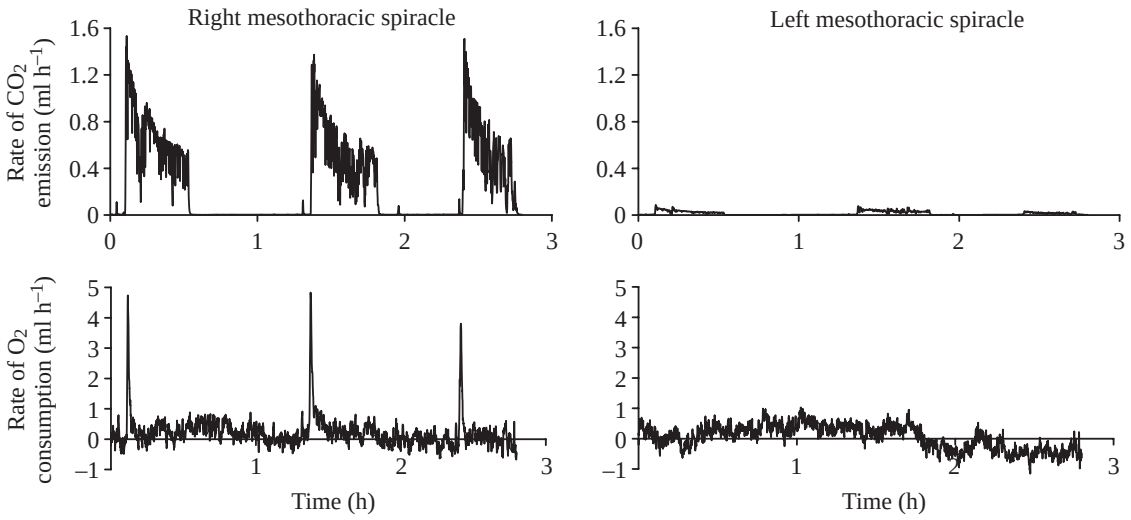

Fig. 6. Simultaneous recordings of CO_2 and O_2 exchange from the right and left mesothoracic spiracles of an individual *Circellium bacchus* (mass 8.455 g). The small amount of CO_2 detected from left spiracle is probably due to slight cross-sampling of the right spiracle.

Airflow

Several lines of evidence support the hypothesis that ventilation in *C. bacchus* at rest involves tidal or anterograde air flow. Respiration during activity is a more complex subject, and our results may or may not be applicable to it.

First, the mesothoracic spiracles are responsible for approximately 80 % of the CO_2 emission and most of the O_2 uptake that we measured in *C. bacchus* at rest in a normal atmosphere. Oxygen in an enriched atmosphere was seen to be consumed from the posterior chamber, which could be due to diffusion into the body *via* the abdominal spiracles under these circumstances. The anterior thoracic spiracles are visibly larger than their posterior counterparts in *C. bacchus*, and in many other insects (Richter, 1969; Lighton et al., 1993; Zachiariassen, 1991), and have been shown to be responsible for 73 % of CO_2 exchange in *Cataglyphis bicolor* (Lighton et al., 1993) and for 70 % of water loss in a flightless, arid-habitat tenebrionid, *Phrynocolus petrosus* (Zachiariassen, 1991). Permanent apertures cut into the elytra of *Onymacris plana*, a desert tenebrionid, caused no change in the cyclic respiratory pattern (Bartholomew et al., 1985), which is now explicable if the majority of respiration takes place through the mesothoracic spiracles in that species.

Second, the proportion of CO_2 emission from the anterior spiracles rises as the total rate of CO_2 emission increases (Fig. 4), indicating that the contribution of the mesothoracic spiracles becomes more important in respiratory exchange as $\dot{V}_{CO_2}$ increases; notably, the DGC is maintained. Virtually no increase in the outflow of CO_2 from the abdominal spiracles is seen, despite the presence of seven additional pairs of spiracles under the elytra, which are open during at least part of the DGC, when they are presumably available for gas exchange, as shown by the small amounts of CO_2 released into the posterior chamber and the ingress of O_2 from an O_2-enriched atmosphere.

Third, when O_2-enriched air was flooded over the posterior half of the body, an anterograde flow of oxygen was recorded during the burst phase of the DGC. Opening of the anterior spiracles and the subsequent loss of water vapour from the tracheae, coupled with opening of the subelytral spiracles, could allow bulk flow of O_2 into the body and diffusion through the longitudinal tracheal trunks, which then exits through the wide-open anterior spiracles. However, no CO_2 burst was seen from the abdomen during either this trial or normal respiration, which suggests that the subelytral spiracles remain closed during at least part of the DGC or that their

Table 3. *Comparison of the discontinuous gas-exchange cycle burst volume from the right and left mesothoracic spiracles of* Circellium bacchus

Beetle number	Mass (g)	*N*	Right mesothoracic spiracle		Left mesothoracic spiracle	
			CO_2 burst volume (µl)	O_2 burst volume (µl)	CO_2 burst volume (µl)	O_2 burst volume (µl)
3	11.228	7	524±182	89.4±23.1	28.3±19.4	–
4	8.119	11	505±567	72.9±22.4	32±34.2	–
6	4.636	4	129±32	23.8±16.0	38.6±36.7	–

Readings from three beetles are shown to illustrate that the use of the right mesothoracic spiracle for gas exchange at rest appears to be typical.

N, number of peaks analysed.

Values are means ± S.D.

– denotes that oxygen readings were indistinguishable from the baseline recording.

exchange capacity, in combination with that of the elytral cavity, is limited. Recordings of the action of the spiracles and abdominal pumping movements will be required to resolve this paradox. The rigid ventral surface of the abdomen reveals no movements during respiration, but strong pumping actions of the dorsal surface have been noted. These are certainly important under stress and may be responsible for the peaks of CO_2 emission seen during the burst phase of the DGC. Nevertheless, we conclude from the above that the majority of air flow in and out of *C. bacchus* is through the anterior mesothoracic spiracles and that the subelytral spiracles contribute little to CO_2 output at rest. Our results do not preclude the possibility that air enters *via* the subelytral cavity, where it will become saturated before entering the posterior spiracles. However, as the air leaves *via* the anterior spiracles, water will have to be retrieved from it or be lost.

Use of a single spiracle

A surprising finding was that the right mesothoracic spiracle is the main site of CO_2 emission and oxygen uptake. The dextral preference of the three specimens examined is probably be due to chance because beetles were collected on an *ad hoc* basis from a large population and are unlikely to be close relatives since the species wander long distances in search of dung and have a low reproductive rate (Coles, 1993). Given the identical appearance of the left-hand spiracle, we would expect other individuals preferentially to use this spiracle. Unilateral dominance of abdominal spiracles has been observed in blaberid cockroaches under various conditions, but never involving the thoracic spiracles or reversal of flow through them (Miller, 1982). The right mesothoracic spiracle looks identical to its left-side partner but, with a surface area of 3.73 mm^2, it is more than four times larger than any of the posterior spiracles. Spiracle size, position and number are highly variable within the Scarabaeoidea, which is considered to be a reflection of the varied environmental pressures experienced by the different groups (Richter, 1969; Browne and Scholtz, 1999). Our own observations within the Scarabaeidae show *C. bacchus* to have unusually large thoracic spiracles. Activity, or an increase in metabolic rate, would presumably result in opening of the left spiracle, doubling the diffusive capacity available for respiration. A single, permanently opened mesothoracic spiracle was found to be sufficient for the gas-exchange needs of an inactive ant, *Cataglyphis bicolor*, which can potentially increase gas-exchange rates sevenfold by using its full complement of thoracic spiracles (Lighton et al., 1993). Walking beetles would probably benefit from autoventilation, but ball-making in a hypercapnic dung heap, or in the hot sun, might necessitate active ventilation. Abdominal pumping *versus* convection for ventilation warrants further investigation in this species.

The role of the abdominal spiracles of *C. bacchus* in respiration could be masked by the presence of the subelytral cavity, which will dampen small oscillations in gas exchange taking place under the elytra. Although a flutter phase was not seen, it may still be taking place in the abdominal spiracles. Duncan and Dickman (2001) recorded a flutter phase from a flightless carabid *Cerotalis* sp. and *Carenum* sp. from the Simpson desert, and Duncan and Byrne (2000) demonstrated flutter phases in winged dung beetles from mesic habitats, which would be expected not to have such tightly sealed elytra as *C. bacchus*. Lighton et al. (1993) found that the abdominal spiracles in *Cataglyphis bicolor* play a major role during the early flutter phase of the DGC, when they allow bulk flow of oxygen into the spiracles with minimal water loss of water because of their limited diffusive capacity. Allowing for the possibility of temporal coordination of the subelytral and mesothoracic spiracles in *C. bacchus*, the hypoxic trigger point for opening of the abdominal spiracles could be reached earlier in the subelytral cavity if it were to become hypoxic more rapidly than the mesothorax. The flutter phase would then be initiated in the subelytra when the negative pressure gradient is large, aiding the bulk flow of O_2, which was clearly seen to be taken up from an O_2-enriched atmosphere by the posterior spiracles. Ingress of O_2 would then inhibit the flutter phase in the subelytral spiracles, and CO_2 accumulation would eventually stimulate the anterior spiracles to open. Restricting the CO_2 output to one large exit point may save water by reducing the area of the system exposed to the atmosphere.

Considerations for respiratory water saving in insects

Two hypotheses arise from these results, both of which probably reflect on water-saving adaptations in insects. First, the use of a single spiracle for respiration at rest could reduce water loss by restricting exchange to a single site. Consequently, only a small area of the respiratory passages would be open to the atmosphere for short periods during the DGC, as has been found in quiescent cockroaches (Miller, 1982) and moth pupae that do not have access to free water (Levy and Schneiderman, 1958). Second, the mesothoracic spiracles of *C. bacchus* are large, as they are in desert tenebrionids (Draney, 1993), and have a well-developed sieve plate across their opening. If the sieve plate functions as a water-retention mechanism (Schneiderman, 1960; Schmitz and Wasserthal, 1999), then a tidal airflow through a single opening, as found in mammals (Schmidt-Nielsen, 1997), into and out of a humid subelytral cavity could also contribute to reduced water loss. Many beetles, including *C. bacchus*, have a highly developed system of large air sacs attached to the tracheae which, coupled with abdominal pumping, could be involved in moving air back and forth through the body through longitudinal tracheal tracts (Wasserthal, 1996). Surface activity of *C. bacchus* is closely tied to sunny periods after rainfall (Coles, 1993), which will expose beetles to variable humidities during extreme activity. The rest of their time is spent underground in sandy soils, either brooding a single offspring or simply waiting for rain. The humidity experienced underground is unknown, but humidity recordings for Addo Elephant Park show an annual mean maximum and minimum relative humidity of 79±6.1 and 48±2.9 % respectively (means ± S.D., N=30 years) (Sutherst and

Maywald, 1985). A mechanism to reduce water loss under these circumstances would be selectively advantageous and give *C. bacchus* an advantage over other large flighted dung beetles, which are conspicuously absent from this habitat.

The role of the DGC as a water-saving adaptation remains controversial (Lighton, 1996; Davis et al., 1999) and inconclusive (Hadley, 1994; Zachariassen, 1991; Ahearn, 1970). However, the extreme DGC in *C. bacchus* described here, coupled with its large body size and low metabolic rate, correlate strongly with its dry habitat distribution (Duncan and Byrne, 2000) and can be interpreted as a survival trade-off against limited competitiveness in more mesic environments (Chown and Gaston, 1999). Our results indicate that, although many insect species may show a typical DGC respiration pattern at rest, the spiracles involved in gas exchange may differ, as will the resultant effect on water conservation. Forward airflow through the respiratory system of apterous beetles will force insect physiologists to rethink the way in which the subelytral cavity could function to reduce respiratory water loss.

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