The role of the subelytral cavity in respiration in a tenebrionid beetle, *Onymacris multistriata* (Tenebrionidae: Adesmiini)

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Abstract

This study measured the respiratory patterns in the tenebrionid beetle, *Onymacris multistriata*, using flow-through respirometry to measure carbon dioxide emission from the mesothoracic spiracles separately and simultaneously with that from around the elytral case. 96% of the total CO2 emitted was via the mesothoracic spiracles. These spiracles used a discontinuous gas exchange cycle similar to that measured from other tenebrionid beetles. Although the circadian rhythm of the beetles resulted in changes to the period durations and cycle frequencies in the discontinuous gas exchange cycles, the mesothoracic spiracle remained the major site for gas exchange. Thus the subelytral cavity plays a different role in respiration other than the elimination of CO2 build-up. It is expected that other arid dwelling flightless beetles will also be shown to use the mesothoracic spiracle as the major route for CO2 emission.

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1. Introduction

Recently it has been reported that a flightless scarab beetle, *Circellium bacchus*, uses only one mesothoracic spiracle for gas exchange (Duncan and Byrne, 2002). Previous studies on respiration in flightless beetles, mainly tenebrionid beetles, hypothesized that air enters through the mesothoracic spiracles and is expelled from the body into the subelytral cavity through the elytral spiracles opening into this cavity (Ahearn, 1970; Nicolson et al., 1984). Thus there is a unidirectional flow of air through the body. The mesothoracic spiracles in tenebrionid beetles are two large spiracles situated laterally on the thorax in the membrane between the pro- and mesothoracic segments where they open to the exterior. The other pairs of spiracles (metathoracic and several pairs of abdominal spiracles) referred to as the elytral spiracles, open into the subelytral cavity, an airtight space formed by the fusion of the elytra (Draney, 1993).

The carbon dioxide rich air which has accumulated in the subelytral cavity finally leaves through an aperture over the anus (Nicolson et al., 1984). Duncan and Byrne (2002), however, found that in the flightless dung beetle air flow was not retrograde as suggested by Ahearn (1970), and described above, but rather anterograde or tidal and carbon dioxide was mainly expelled through the mesothoracic spiracle. Their study raises the question as to whether all beetles which possess a subelytral cavity use the mesothoracic spiracles only for gas exchange, or the subelytral cavity is used by some beetles to reduce respiratory water loss in the manner described by Ahearn (1970).

For the flightless dung beetle Duncan and Byrne (2002) postulated that the beetle reduces respiratory water loss by using discontinuous gas exchange cycles, the spiracles open and close intermittently, through one mesothoracic spiracle thus limiting the area of the respiratory system exposed to the dry external air (Duncan, 2002). Diurnally active tenebrionid beetles have been shown to use the discontinuous gas exchange cycle of respiration whereas the nocturnal beetles use a form of continuous respiration, the spiracles remain open (Bartholomew et al., 1985; Lighton, 1991; Duncan and
Dickman, 2001 and Duncan et al., 2002). The diurnal tenebrionid beetles’ discontinuous gas exchange cycle pattern has a closed period where the spiracles are closed for approximately 20% of the duration of the cycle (Lighton, 1991; Duncan et al., 2002). This is followed by a flutter period in which discrete bursts of CO₂ (miniature openings) are produced. Miniature openings within the flutter period limit the time for opening of the spiracles, and have been only been found in tenebrionid beetles and a species of locust (Hustert, 1975). A long and conspicuous flutter period is characteristic of arid dwelling insects (Lighton and Wehner, 1993). The burst period is the rapid unloading of CO₂ which minimises the time the spiracles are open and therefore reduces the loss of water vapour. Thus, all three periods (closed, flutter and burst) which make up the discontinuous gas exchange cycle, are important for reducing water loss (see Miller 1981; Kestler, 1985 and Lighton, 1996 for more detailed descriptions of these three periods).

In this study I measured the respiratory patterns in the tenebrionid beetle, Onymacris multistriata Haag (Tenebrionidae: Adesmiini) by measuring the carbon dioxide emission from the mesothoracic spiracles separately and simultaneously with that from around the elytral case. Hence the function of the subelytral cavity in the respiration in this adesmine tenebrionid beetle was determined. Beetles belonging to the tribe Adesmiini are xerophilous and diurnally active (Koch, 1961). Onymacris multistriata lives in sand dunes where it can be seen running from grass tussock to grass tussock in search of food during the day (pers. obs.), and take thermal refuge within grass tussocks where they remain alert (Louw and Seely, 1982). Few insects are active during the activity periods of these beetles thus predators are a threat. A further question based on their behaviour pattern is how does the respiratory pattern measured from these beetles change according to their circadian rhythm?

2. Materials and methods

2.1. Insects

Onymacris multistriata Haag were collected from the Northern Cape Province in South Africa (N = 8, mean mass = 0.448 g). These beetles are found in an isolated community on the sand dunes in this region. They were collected during the morning while they were actively running on the dunes. The beetles were housed in 10 l bins, half-filled with soil collected from the dunes, in an insectary at 23 °C with a 14 h:10 h light:dark cycle. Extra heat and light was provided by a lamp which was on the same cycle. The beetles were fed cabbage leaves and oats. They survived well for several months under laboratory conditions.

2.2. Respirometry

A flow-through respirometry system was used to measure gas exchange in the anterior (head and mesothorax) and posterior (elytral case; which covers the metathorax and abdominal section) halves of a live, intact beetle (for details see Duncan, 2002). Briefly, 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). 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 seal between the prothorax and the abdomen. This seal was made airtight by glueing the latex sheet to the beetle using rubber cement. The rubber cement could be peeled from the beetle at the end of the measurements. 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 chamber, giving each section a volume of approximately 100 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.

Carbon dioxide emissions in each section of the chamber were measured separately and simultaneously using flow-through respirometry (Fig. 1). Each compartment had its own source of air, scrubbed of CO₂ and H₂O vapour by a Drierite/Ascarite column, which was drawn through the compartment at 50 ml min⁻¹ (controlled by its own calibrated Supelco flow meter) and individual Licor CO₂ analyser (either LI-6251 or LI-6262) with a resolution of 0.1 ppm. The distance of the tubes connecting each compartment to its respective analyser was kept to the minimum and was identical. Using manometers the pressure within each compartment was tested and found to be identical. Thus differences in the CO₂ measurements from each chamber are not due to pressure differences on two sides of the latex sheet. Readings of the volume of CO₂ emitted were taken every 5 s and recorded using a computerised data acquisition software (Datacan V, Sable Systems). Measurements were made on individual beetles that were weighed to ± 0.1mg (Precisa 160A balance). All beetles were measured for a minimum of 6 h in dim red light during the night. 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 23 ± 1 °C. To test for any circadian rhythm effects three beetles were measured during the day in dim red light, and observed to note when they were stationary.

Baseline drift of the analysers was corrected during analysis from measurements at the beginning and end of each trial with the respirometry chamber empty. All
Fig. 1. Diagram of the experimental set-up, showing the beetle divided into an anterior part (A) and posterior part (P). Each chamber has its own flow-through respirometry system.

measurements were corrected to standard temperature and pressure (STP). The CO₂ recordings were converted to rate of CO₂ production VCO₂ in ml h⁻¹. The discontinuous gas exchange cycle (DGC) characteristics (as given in Table 2) were calculated as follows: the DGC frequency (= burst frequency) is calculated by determining the number of peaks of CO₂ per sec, and the DGC duration is one complete cycle. The mean rate of CO₂ emission is the mean value over several complete DGC cycles and to obtain the emission volume, integration of the area under the curve against hours was performed.

2.3. Statistical methods

Data are represented as mean ± standard deviation. Sample size (N) is indicated in the text as either representing individual beetles or in the case of gas exchange characteristics, 10–20 discontinuous gas exchange cycles per beetle. Unless otherwise noted, statistical comparisons were either with the Student’s t-test or paired t-test. Regression analysis was done by the least squares method.

3. Results

3.1. Mesothoracic versus elytral cavity gas exchange

The trace of CO₂ emission from the same beetle (mass = 0.408 g) with separate recordings of the mesothoracic spiracles and elytral case is shown in Fig. 2. Only one pair of spiracles, mesothoracic spiracles occur in the anterior part while seven pairs of spiracles open below the elytral case (into the subelytral cavity). All eight beetles produced a similar CO₂ emission pattern and the results are summarised in Table 1.

The recordings from the mesothoracic spiracles show distinct discontinuous gas exchange cycles with a flutter period consisting of several discrete bursts of CO₂, a subsequent burst period and a definite closed period. A far weaker cyclic CO₂ emission was recorded from the elytral case, and the three periods of the discontinuous gas exchange cycle could not be determined (Fig. 2). In most cases, but not all, the small increments of CO₂ emission from the elytral case corresponded with the burst period of the mesothoracic spiracles. The rate of CO₂ emission from the elytral case was significantly lower than that from the mesothoracic spiracles (t₀.₀₅,₇ = 6.79, p < 0.001). Of the total CO₂ emitted, 95.6 ± 5.0 % (N = 8) was expelled through the mesothoracic spiracles.

A large difference in the absolute magnitude of CO₂ emission rates from the mesothoracic spiracles and the elytral case was observed (Fig. 2). The CO₂ emission from the mesothoracic spiracles and elytral case as a function of the total CO₂ emission, averaged over several discontinuous gas exchange cycles for each beetle, showed that the mesothoracic spiral rate of CO₂ emission increased as the metabolic rate increased (Fig. 3).
Fig. 2. Recording of carbon dioxide emission from a 0.408 g *Onymacris multistriata* at 23 °C from (A) the anterior mesothoracic spiracles, where the majority of CO₂ is emitted using discontinuous gas exchange cycles, and (B) the posterior elytral case, which contribute less to CO₂ emission. Note the different scales on the y-axis.

Table 1
Comparison of the rates of CO₂ emission from the mesothoracic spiracles and from the elytral case of *Onymacris multistriata*

<table>
<thead>
<tr>
<th>Beetle number</th>
<th>Mass (g)</th>
<th>Rate of CO₂ emission (µl h⁻¹)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mesothoracic spiracles</td>
<td>Elytral case</td>
</tr>
<tr>
<td>1</td>
<td>0.482</td>
<td>21.87</td>
<td>4.17</td>
</tr>
<tr>
<td>2</td>
<td>0.457</td>
<td>44.20</td>
<td>0.19</td>
</tr>
<tr>
<td>3</td>
<td>0.408</td>
<td>54.29</td>
<td>0.62</td>
</tr>
<tr>
<td>4</td>
<td>0.542</td>
<td>36.94</td>
<td>1.21</td>
</tr>
<tr>
<td>5</td>
<td>0.318</td>
<td>26.50</td>
<td>1.45</td>
</tr>
<tr>
<td>6</td>
<td>0.566</td>
<td>35.8</td>
<td>0.28</td>
</tr>
<tr>
<td>7</td>
<td>0.318</td>
<td>26.65</td>
<td>1.56</td>
</tr>
<tr>
<td>8</td>
<td>0.49</td>
<td>68.2</td>
<td>1.91</td>
</tr>
</tbody>
</table>

*a* Readings from eight individuals are shown to illustrate that the use of the mesothoracic spiracles for gas exchange is typical.

Fig. 3. The CO₂ emission rates from the mesothoracic spiracles and elytral case as a function of total CO₂ emission in *Onymacris multistriata* (*N* = 8 beetles, mean mass = 0.448 g). The regression equations are:

\[ m = -2.4 + 1.02 t, \quad r^2 = 0.99, \quad p = 0.0001; \]
\[ el = 2.4 - 0.02 t, \quad r^2 = 0.008, \quad p = 0.5; \]

where \( m \) = mesothoracic spiracular \( V_{\text{CO}_2} \), \( el \) = elytral case \( V_{\text{CO}_2} \) and \( t \) is total \( V_{\text{CO}_2} \).

However the elytral case contribution to the total rate of CO₂ emission remained unchanged. Thus the mesothoracic spiracles seem to be the main site of gas exchange in this tenebrionid beetle.

### 3.2. Mesothoracic spiracle: discontinuous gas exchange cycle

The discontinuous gas exchange pattern measured from the mesothoracic spiracle (Fig. 2a, Table 2) is similar to that measured from the Namib Desert tenebrionid beetles (Lighton, 1991) and Negev Desert tenebrionid beetles (Duncan et al., 2002). All these diurnally active tenebrionid beetles produced a flutter period consisting of several discrete bursts of CO₂ emission with no CO₂ emitted between these bursts. These regular mini-CO₂ bursts may be due to miniature openings. The number of miniature openings during each flutter period ranged from 2–11 with 4–5 occurring most often. The frequency...
of CO₂ bursts within the flutter period was similar to that found in Negev Desert tenebrionid beetles (Duncan et al., 2002), as the flutter period increased in length there was a corresponding increase in number of miniature openings ($r^2 = 0.66$, $p < 0.05$). During the flutter period, 18% of total CO₂ release from the mesothoracic spiracles occurred (Flutter : Burst period volume ratio = 0.18 ± 0.04, $N = 8$). The flutter period makes up 47.4% of the cycle length.

The flutter period is followed by a burst period during which the spiracles are open with no evidence of active ventilation. The length of the burst period is 31.2% ($N = 8$) of the total cycle length. Due to the slight amounts of CO₂ emitted during the closed period, I can assume that the spiracles remained closed during this time. Lighton et al. (1993) have shown that accurate interpretations of spiracular movements could be made from CO₂ emissions.

3.3. Effect of circadian rhythm

Three beetles measured during the day remained in a resting state for part of the measurements. Although the beetles could not be continuously monitored activity disrupted the CO₂ emission trace (Fig. 4). In all three beetles 17 times more CO₂ was emitted through the mesothoracic spiracles than from the elytral case (Fig. 4). In two of the three beetles the rate of CO₂ emission during the day was significantly higher than that measured at night (Table 3). However the volume of CO₂ emitted during each burst period was significantly less in each case, thus the increase in metabolic rate is due to the increase in frequency of cycles (Table 3). The average duration of the discontinuous gas exchange cycle measured during the day is 4.1 ± 1.9 min ($N = 3$) compared to 9.8 ± 2.1 min ($N = 8$) measured during the night. Thus an increase in the rate of gas exchange cycles would increase the rate of CO₂ emission or maintain the same value. In two beetles a flutter period was observed, and miniature openings occurred infrequently. The flutter period mostly consisted of a gradual increase in the CO₂ emission before the spiracles opened fully to release the accumulated CO₂. In one beetle the flutter period during the night was 1.2 times longer, while in the other it was six times longer. Thus the discontinuous gas exchange cycles that were measured from beetles during the day gave rise to four changes: diminished to no flutter period; a diminished to same burst period duration (with lower CO₂ emission); shorter closed period; and an increase in cycle frequency.

4. Discussion

My findings confirm the idea that the function of the subelytral cavity in respiration needs to be re-evaluated. It seems increasingly likely that beetles which possess a sealed subelytral cavity use the mesothoracic spiracle as the major site for gas exchange. Thus respiratory airflow does not occur from anterior to posterior, but is either tidal or anterograde. Cockroaches and locusts pass inspired air from the anterior spiracles, through longitudinal trunks that connect the tracheae and out through the more posterior abdominal spiracles (Miller, 1982). Retrograde respiratory airflow is presumed to be typical for all insects (Hadley, 1994; Lighton, 1996).

This adesmine tenebrionid beetle showed a similar discontinuous gas exchange cycle to the other adesmine tenebrionid beetles previously measured by Lighton (1991), but was found to be limited to the mesothoracic spiracles. Onymacris multistriata have no close phylogenetic links with the Scarabaenidae dung beetle.
Fig. 4. Recordings of CO\textsubscript{2} emission from a 0.332 g *Onymacris multistriata* measured during (A) the day, and (B) the night. Note in both cases very little CO\textsubscript{2} leaves from the elytral case.

Table 3
Comparison of CO\textsubscript{2} emission rates from individual *Onymacris multistriata* beetles measured during the day and during the night. (Used the paired *t*-test for comparison)

<table>
<thead>
<tr>
<th>Beetle number</th>
<th>Mass (g)</th>
<th>Total CO\textsubscript{2} emission (µl h\textsuperscript{-1})</th>
<th><em>t</em>-value</th>
<th>DCG frequency (mHz)</th>
<th><em>t</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.457</td>
<td>60.6±5.4</td>
<td>Day</td>
<td>3.33\textsuperscript{a}</td>
<td>2.7±0.3</td>
</tr>
<tr>
<td>6</td>
<td>0.566</td>
<td>133.3±6.5</td>
<td>Night</td>
<td>23.8\textsuperscript{b}</td>
<td>6.5±0.6</td>
</tr>
<tr>
<td>7</td>
<td>0.318</td>
<td>30.3±1.6</td>
<td></td>
<td>1.35</td>
<td>5.3±1.5</td>
</tr>
</tbody>
</table>

\textsuperscript{a} *p < 0.01.\textsuperscript{b} *p < 0.001.

(Circellium bacchus), so the spiracle use is not due to phylogenetic connection. Both groups of beetles have the morphological feature of a sealed subelytral cavity in common, and both inhabit arid environments. These features could have influenced the use of the mesothoracic spiracle for gas exchange. Unfortunately, due to the small size of *O. multistriata*, I was not able to test whether both or only one of the mesothoracic spiracle pair was being used for respiration. As observed in *C. bacchus*, the mesothoracic spiracles are conspicuously larger than the rest, and in a similar position. The spiracles in *O. multistriata* are not scleritised and the elytral spiracles are situated more dorsally on the abdominal segments than in *C. bacchus*.

These results show that the mesothoracic spiracles are opening and closing on an intermittent basis, and there is no periodic opening of the subelytral cavity. *Bartholomew et al.* (1985) cut a fenestra in the elytra of the tenebrionid beetle *Onymacris plana* and measured the same discontinuous pattern of respiration as previously measured from an intact beetle. They concluded that the respiratory pattern depended on ventilatory movements and spiracular openings rather than on periodic opening of the subelytral cavity. However, the theory that the elytral spiracles are the sites for expelling air remained. Periodic opening of the subelytral cavity cleft would result in CO\textsubscript{2} peaks in the trace from the elytral case. *Fig. 2b* shows that very little CO\textsubscript{2} was measured from outside the elytral case which gives support to the suggestion that the subelytral cavity is hermetically sealed (Draney, 1993). *Gorb* (1998) has shown that the subelytral cavity of an arid adapted tenebrionid beetle is tightly sealed by microtrichia.

### 4.1. Water balance

The ability of arid dwelling tenebrionid beetles to avoid water loss may be the most important factor in favouring water balance in these beetles (Edney, 1971). Although respiratory water loss contributes a small amount to overall water loss in the insects known to use discontinuous gas exchange cycles (Hadley, 1994), even this small reduction would increase their survival chances. In some arid adapted insects with low cuticular
permeabilities, respiratory water loss could be a major route for water loss (Zachariassen, 1991). Restricting gas exchange to one pair of spiracles will help minimise potential water loss (Lehmann, 2001), as only a small area of the respiratory passages are exposed to desiccation. The subelytral cavity is maintained at a high humidity (Zachariassen, 1991) thus periodic opening of the elytral cleft to expel large volumes of accumulated CO₂ would also result in loss of water. This loss could potentially be greater than that from the individual spiracles.

The discontinuous gas exchange pattern of CO₂ emission measured from the mesothoracic spiracles in *O. multistriata* is similar to that found by Lighton (1991) in ten species of tenebrionid beetles from the Namib Desert, and Duncan et al. (2002) in two species of tenebrionid beetles from the Negev Desert. These beetle species are the only beetles which have been shown to produce discrete bursts of CO₂ (miniature openings) during the flutter period. The frequency of these bursts within the flutter period was similar for all the tenebrionid species. The only other insect in which miniature openings have been observed, is the locust, *Locusta migratoria migratorioides* (Hustert, 1975). This distinct flutter period could assist in reducing the respiratory water loss as the diffusive loss of CO₂ and water vapour is restricted to discrete events (Miller, 1981). Furthermore, during the flutter period the spiracles are not opened completely (Kestler, 1971) thus the beetles could be limiting water loss by matching spiracle opening with their instantaneous metabolic demands (Lehmann, 2001). The accumulation of CO₂ in the respiring tissues will finally cause the mesothoracic spiracles to open widely and release the CO₂.

The importance of the flutter period in reducing respiratory water loss has been described by Lighton and Garrigan (1995). Long and distinct flutter periods appear to be common in arid adapted arthropods, e.g. the ant species *Cataglyphus* (Lighton and Wehner, 1993) and a trogid beetle (Bosch et al., 2000). In *O. multistriata* the flutter period had the longest duration of the discontinuous gas exchange cycle.

### 4.2. Circadian effects on respiratory pattern

A variety of stressors can change the discontinuous gas exchange cycle patterns in arthropods (Kestler, 1991; Fielden and Lighton, 1996; Tartes et al., 1999). During visual observations on a few beetles, I have noted that even slight movements were accompanied by noticeable changes in the respiratory pattern. Most studies on beetle respiration have measured beetles during the diel period when the beetle is most likely to be inactive (e.g. Davis et al., 1999; Bosch et al., 2000), yet the effects of circadian rhythm have not been tested. The tenebrionid beetle in this study showed a similar behaviour to that reported for Namib Desert tenebrionid beetles (Koch, 1961), viz. active running during the morning until the temperatures increased and then later in the afternoon. During periods of high temperature the beetles used thermal refuges in the sand or under bushes. Seely et al. (1988) found that the Namib *Onymacris* species had an unusually high preferred body temperature. They suggested that due to thermal refuges, the beetles could maintain a high body temperature, so as to limit the reduction in reaction time. Slower reaction time would leave the beetle subjected to predation. Thus during their activity period the beetles remained alert.

*Onymacris multistriata* measured during the day showed an increase in metabolic rate, indicating that they were not fully quiescent. Although there was an increase in metabolic rate, the beetles continued to use the discontinuous gas exchange cycles. During the day the beetles can replace lost water through feeding or drinking (Nicolson, 1990). However, to maintain water balance the beetle should ensure that water lost during the inactive periods is kept to a minimum. Thus a more pronounced flutter period was measured when the beetles were at their most quiescent. Bartholomew et al. (1985) found that intermittent respiration was still apparent when the tenebrionid beetles were running on a treadmill.

The alternative hypothesis to the theory that discontinuous gas exchange cycles evolved as a water conserving mechanism (Kestler, 1985) is that these respiratory cycles facilitate respiration in the hypercapnic and hypoxic environments experienced by arthropods living underground (chthonic hypothesis; Lighton, 1998). *O. multistriata* bury themselves in the dune sand (laboratory observations) during the night, and sometimes during the day. As the beetles bury just below the surface I do not expect that they experience conditions of hypercapnia or hypoxia. Louw et al. (1986) found that CO₂ diffused very rapidly through a 5 cm column of dune sand, the depth to which tenebrionid beetles are found. As *Onymacris* species are detritus feeders with very limited access to water and active during hot temperatures (Louw and Seely, 1982), maintaining water would be an important strategy. Thus from this study there is more support for the hygic hypothesis.

Regardless of the time of day that the respiratory measurements took place, all the beetles used the mesothoracic spiracles for gas exchange. Even when the beetles were active within the chamber the volume of CO₂ emitted through the mesothoracic spiracles was greater than that measured from the elytral case (Fig. 4).

*Onymacris multistriata* has a subelytral cavity yet uses the mesothoracic spiracles for gas exchange during quiescent and alert periods. This lends support to the theory that the subelytral cavity does not act as a respiratory water saving device in the manner previously thought. It is expected that other flightless beetles will
also be shown to use the mesothoracic spiracles as the major route for CO₂ emission.

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