Metabolic rate and respiratory gas-exchange patterns in tenebrionid beetles from the Negev Highlands, Israel

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Summary

This study correlates the pattern of external gas exchange with the diel activity of nine species of tenebrionid beetle from the Negev Desert, Israel. The study species are active throughout the summer months when daytime temperatures are high and no rain falls. There were no differences in standard metabolic rate, determined by flow-through respirometry, among the nine species. All the nocturnally active beetles exhibited a form of continuous respiration, whereas the two diurnally active and one crepuscular species exhibited a cyclic form of respiration referred to as the discontinuous gas-exchange cycle (DGC). The DGCs recorded have a long flutter period consisting of miniature ventilations, and 29–48 % of the total CO_2 output occurred during this period. In this study, the flutter period played an important role in the modulation of metabolic rate, in contrast to other studies in which the burst period has been shown to be important. We suggest that the long flutter period is important in reducing respiratory water loss in arid-dwelling arthropods. This study lends support to the hypothesis that discontinuous gas exchange is important in reducing respiratory water loss from beetles that need to minimise dessication because of the high water vapour pressure gradient they experience. If the use of underground burrows were responsible for the evolution of discontinuous gas exchange, then we would expect all nine tenebrionid species to use DGCs since both the nocturnally and diurnally active species bury in the sand during periods of inactivity. We conclude that the activity patterns of the beetles are more important than their habitat associations in designating the type of respiration used.

Key words: discontinuous gas-exchange cycle, diel activity, habitat association, Tenebrionidae, desert.

Introduction

Beetles belonging to the family Tenebrionidae represent an important part of the invertebrate fauna in deserts. They are able survive under even the harshest conditions as a result of physiological and behavioural adaptations and occupy certain ecological niches in desert habitats. The ecology of tenebrionid beetles living in the Ramon erosion cirque, at the southern boundary of the Negev Desert Highlands, which is a dry area that contains a sharp precipitation gradient with a mean annual precipitation from 100 mm in the northwest to 56 mm in the southeast, all occurring in winter, has been studied by Krasnov and Shenbrot (1996, 1997). They found that soil texture, productivity and vegetation architecture, but not topography, had profound effects on the spatial structure of the tenebrionid community.

In addition, they observed that there were two distinct spatial guilds of tenebrionid species. One guild was composed by species that preferred sandy-gravel soils and more 'xeric' habitats, and the other guild consisted of species that preferred clay soils and more 'mesic' habitats. Furthermore, some species occurred only in 'xeric' habitats, others only in 'mesic' habitats, although there were also species that occurred in both 'xeric' and 'mesic' habitats (see Table 1). There are four temporal phases of the beetle community, with the diversity of beetle communities being maximal in summer when the mean temperature is 34 °C (Krasnov and Ayal, 1995). Most of these summer-active species are crepuscular and nocturnal and use burrows, the undersides of stones, rock fissures and plant cover to hide during the day (Ayal and Merkl, 1994). Only two summer species, both belonging to the genus *Zophosis*, were exclusively active during the day.

In contrast to the Negev Desert, many Namib tenebrionid beetles are diurnal during the summer months. The aim of this study was to investigate the respiratory patterns and metabolic rates of the Negev Desert tenebrionid beetles and to compare them with those of the Namib Desert tenebrionid beetles. This will help to explain what physiological adaptations enable

Table 1. *Phylogenetic order, based on Kaszab (1982), and ecological characteristics of the Negev Desert tenebrionid beetles considered in this study*

Species	Tribe	Diel activity	Habitat	Occurrence
Zophosis complanata Solier	Zophosini	Diurnal	Mesic, xeric	Common
Zophosis punctata Brulle	Zophosini	Diurnal	Mesic	Common
Akis goryi Solier	Akidini	Nocturnal	Mesic	Common
Scaurus puncticollis Solier	Scaurini	Nocturnal	Mesic, xeric	Rare
Trachyderma philistina Reiche & Saulcy	Pimeliini	Nocturnal	Xeric	Common
Trachyderma hispida (Forskal)	Pimeliini	Nocturnal	Mesic	Uncommon
Pimelia canescens Klug	Pimeliini	Crepuscular, nocturnal	Xeric, mesic	Common
Pimelia grandis Klug	Pimeliini	Nocturnal	Mesic	Common
Blaps sulcata Laporte de Castelnau	Blaptini	Nocturnal	Mesic	Common

See text for a description of the habitat.

some species to be active during the day and cause others to adjust their activity to prevent dessication. Seely and Mitchell (1987) and Krasnov and Ayal (1995) have suggested that the activity patterns of tenebrionid beetles are influenced by factors, e.g. physiological and reproductive adaptations, other than simple avoidance of high temperatures.

A physiological adaptation that would enable beetles to forage at higher temperatures would be to reduce their rate of dessication. In a hyper-arid desert such as the Negev, there is a significant selection pressure on water economy. The diet of tenebrionid beetles, all kinds of litter and dead arthropods, is generally low in water, so these beetles employ various strategies to limit desiccation, e.g. physiological resistance (for reviews, see Edney, 1977; Hadley, 1994a), burying below the surface (Seely et al., 1988) or adjusting their surface activity (Edney, 1971).

An important means of physiological resistance to desiccation is to reduce the rate of water loss. There are two major components of water loss: the transcuticular component that takes place across the cuticle and the respiratory component that accompanies the exchange of respiratory gases through the spiracles. Although few studies have been performed in which respiratory water loss rates can be separated from overall water loss rates, this route of water loss is in some cases an important component of total water loss (Hadley, 1994b). From a detailed study of the tenebrionid beetle *Phrynocolus petrosus*, occurring in an arid area in East Africa, Zachariassen (1991) concluded that respiratory water loss was the dominant component of water loss.

Insects may be able to reduce respiratory water loss by using a cyclic form of respiration referred to as the discontinuous gas-exchange cycle (DGC) (for a review, see Kestler, 1985; Lighton, 1994, 1996; Wasserthal, 1996). Several beetle species have been shown to exhibit this form of respiration, e.g. carabid beetles (Punt et al., 1957; Kestler, 1971), Namib Desert tenebrionid beetles (Bartholomew et al., 1985; Louw et al., 1986; Lighton, 1991), Scarabaeidae dung beetles (Davis et al., 1999; Duncan and Byrne, 2000) and a trogid beetle (Bosch et al., 2000). Most, but not all, of these species occur in arid habitats. The importance of the DGC for arid-dwelling arthropods is under debate (see Kestler, 1985; Hadley, 1994b).

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 and which reduces 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 opening of the spiracles on an intermittent basis allows some normoxic O_2 uptake through the spiracles by diffusion and convection, but little loss of CO_2 or water vapour. These rapid spiracular movements ('fluttering') and short micro-openings are characteristic of the flutter period. Because of bulk movement of air into the insect, the minimised opening area and the low partial pressure differences, diffusive loss of CO_2 and water vapour is reduced. 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 in closed–flutter–open cycles or triggers ventilation as a bout of pumping movements in closed–flutter–ventilation cycles. The rapid unloading of CO_2 should minimise the time the spiracles are open and therefore reduce loss of water vapour. Thus, all periods are considered important in reducing respiratory water loss.

A study of the respiratory patterns of summer-active Negev Desert tenebrionid beetles with different diel activity patterns would enable us to determine whether there was any physiological adaptation that enables a few species to be active during the hot midday hours. Table 1 shows the habitat associations and ecological characteristics of the study species. Although all these species live under desert conditions, some are able to use the more xeric areas whereas others inhabit the more mesic areas. The study species are distributed between ancient and more modern tribes in the classification of Kaszab (1982). A comparison with the respiration patterns of the Namib Desert tenebrionid beetles (Louw et al., 1986; Lighton, 1991), of which several species are diurnal, would further our knowledge of the taxonomic scope of the occurrence of DGCs

within the family Tenebrionidae and help in explaining the mechanistic basis of the DGC.

Materials and methods

The nine beetle species (Table 1) were collected in Mitzpe Ramon, Israel, during July 2000. The nocturnally active beetles were collected by means of pitfall traps, the two diurnally active species were collected by hand while they were running about and the crepuscular/nocturnal species was collected from the hillocks around bushes. The beetles were housed in the laboratory in 10 l fish tanks with a layer of sand. They were fed dog food, and the occasional piece of apple and water was provided. The beetles were used in experiments within 4 weeks of collection and were released at their collection site after measurements.

A flow-through respirometry system was used to measure CO_2 emission in inactive beetles at 25 °C. Room air scrubbed of CO_2 and H_2O vapour, by a Drierite/Ascarite column, was pumped at 100 ml min^{-1} through a respirometry system consisting of a respirometry chamber (20 ml), a calibrated CO_2 analyser (Licor LI6262) and a mass-flow controller (Tylan). To ensure an accurate and constant airflow, a calibrated Supelco flow meter was also used. Readings of the amount of CO_2 produced were taken at 2 s intervals and recorded using computerised data-acquisition software (Datacan V, Sable Systems). Measurements were made on individual beetles weighed to ±0.1 mg using a Mettler balance (Toledo AB54). All beetle respiration was measured in the dark in a water bath maintained at 25 °C and verified using a mercury thermometer. To compensate for any circadian rhythm effect, the respiration of the nocturnally active beetles was measured during the day for 2–6 h per beetle. The diurnally active beetles were measured at night for 10–12 h, with samples taken at 5 s intervals. We noted no change in the respiratory pattern if the measurement of these beetles continued during the morning and the beetle remained inactive. All beetles were occasionally observed to ensure that they remained stationary during sampling.

To convert $\dot{V}_{CO_2}$ to metabolic rate (measured in energy units of W kg^{-1}), the respiratory quotient (RQ) was assumed to be 0.8, as determined previously for *Psammodes striatus* (Coleoptera: Tenebrionidae) (Lighton, 1988), which seems a reasonable assumption for the beetles. An RQ of 0.8 gives an energy equivalent of 24.5 J ml^{-1} CO_2 (Schmidt-Nielsen, 1980). This value was used to express metabolic rate in terms of energy.

The characteristics of the discontinuous gas-exchange cycle (DGC) (as given in Table 3) were calculated as follows. The mean rate of CO_2 emission in each period ($\dot{V}_{CO_2}$ in µl g^{-1} h^{-1}) is the volume of CO_2 emitted during that period, obtained by calculating the area under the CO_2 peak and then expressed as a mass-specific value and divided by the period duration. The period duration is the time taken for CO_2 to be emitted during that period. The DGC frequency (=burst frequency) is calculated by determining the number of peaks of CO_2 emission per second, and the period is the duration of one DGC (i.e. a closed, flutter and burst period; C, F and B, respectively, in Fig. 2).

At least three beetles were used from the common species to measure body water content and water loss tolerance. The beetles were kept in a desiccator (0 % relative humidity), which was then placed in an incubator at 28 °C. The beetles were individually weighed before placement in the incubator and observed on a daily basis until loss of coordination, when they were re-weighed. To obtain the dry mass, the beetles were placed in an oven at 110 °C until they reached a constant mass (Zachariassen et al., 1988).
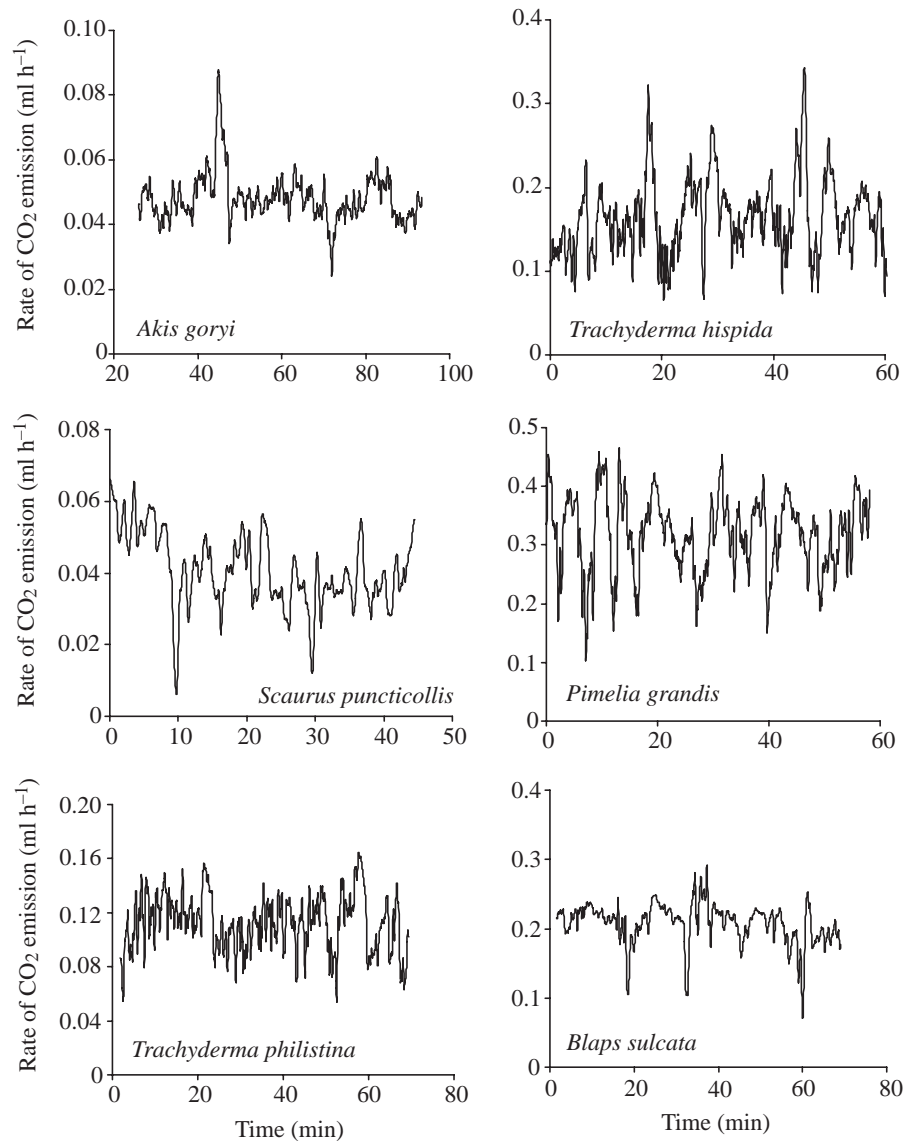
Unless stated otherwise, data are presented as means ± standard deviation (S.D.). Sample size (*N*) is indicated in the text either as representing individual beetles or, in the case of gas-exchange characteristics, for 5–90 discontinuous gas-exchange cycles per beetle. Unless noted otherwise, statistical comparisons were made either with Student's *t*-tests or by analysis of variance (ANOVA). Significant ANOVAs were followed with the Newman–Keuls multiple-range test. The significance level was set at $P<0.05$.

Table 2. *Rate of CO_2 emission and metabolic rate of Negev Desert tenebrionid beetles at 25 °C*

Species	N	Mass (g)	Rate of CO_2 emission (ml h^{-1})	Rate of CO_2 emission (ml g^{-1} h^{-1})	Metabolic rate (W kg^{-1})	CV
Zophosis complanata	9	0.112±0.03	0.02±0.01	0.172±0.049	1.17±0.33	2.12
Z. punctata	11	0.07±0.016	0.014±0.008	0.195±0.076	1.32±0.52	1.89
Akis goryi	6	0.478±0.114	0.092±0.058	0.202±0.123	1.37±0.84	0.4
Scaurus puncticollis	5	0.282±0.048	0.037±0.033	0.132±0.116	0.90±0.79	0.27
Trachyderma philistina	6	0.859±0.361	0.24±0.184	0.264±0.114	1.8±0.77	0.3
T. hispida	8	1.033±0.322	0.292±0.232	0.26±0.139	1.77±0.95	0.36
Pimelia canescens	2	0.312	0.036	0.114	0.78	1.07
P. grandis	6	2.098±0.377	0.535±0.356	0.243±0.141	1.65±0.96	0.26
Blaps sulcata	9	0.799±0.082	0.124±0.057	0.153±0.063	1.04±0.43	1.82

Values are means ± S.D. (where *N*>2).
CV, coefficient of variation.

Fig. 1. Recordings of the rate of CO_2 emission by *Akis goryi* (mass 0.443 g), *Scaurus puncticollis* (mass 0.362 g), *Trachyderma philistina* (mass 0.541 g), *T. hispida* (mass 0.851 g), *Pimelia grandis* (mass 1.334 g) and *Blaps sulcata* (mass 0.919 g).

observe or measure abdominal pumping in this study. The sudden reductions in CO_2 emission may be due to spiracular closing reflexes in response to vibrations in the laboratory, as described by Kestler (1971).

The two diurnally active *Zophosis* species showed a distinct DGC pattern (Fig. 2) similar to that measured in the Namib Desert tenebrionid beetles (Lighton, 1991). In both these studies, the tenebrionid beetles produced a flutter period consisting of several discrete bursts of CO_2 emission with little or no CO_2 output between these emissions. Because of their regularity, these minibursts may be due to miniature ventilations. The subsequent CO_2 burst period is possibly due to complete opening of the spiracles or to active ventilation. There was no evidence in the CO_2 emission trace (Fig. 2) to suggest that active ventilation was occurring during the burst period. The crepuscular/nocturnal species *Pimelia canescens* had a distinct DGC but with 0–7 typical micro-openings during the flutter period.

The DGC characteristics of the two *Zophosis* species were very similar (Table 3). Unfortunately, only two specimens of *P. canescens* were obtained, so it was not possible to make statistical comparisons among all three species. From the data in Table 3, *P. canescens* seemed to have longer burst period and closed period durations, resulting in a DGC of longer duration than that in the *Zophosis* species. There were fewer miniature ventilations during the flutter period. In all cases, DGC frequency was independent of beetle mass, as anticipated (see Lighton, 1991).

Results

Respiratory pattern

It was not possible for activity to be monitored continuously during the measurements. However, during visual observations on a few beetles, we noted that even slight movements were accompanied by noticeable changes in the pattern of CO_2 emission. Thus, we are confident that our measurements are from inactive beetles.

The nocturnally active beetles exhibited an irregular form of continuous ventilation (Fig. 1): they displayed a constant rate of CO_2 emission with little variation from its mean and a coefficient of variation (CV) less than 1 (Table 2). The only exception was *Blaps sulcata*, which had a CV of 1.82 and exhibited a form of cyclic respiration without the distinct periods that characterise DGCs. The oscillations in CO_2 emission could be due to abdominal pumping (ventilation), which is known to occur in beetles (Kestler, 1971; Bartholomew et al., 1985). We were unable, however, to

Zophosis *species: discontinuous gas-exchange cycle*

The duration of the flutter period was tightly controlled relative to the DGC duration. In both species, a significant positive correlation was found between flutter period duration and DGC duration. The flutter 'ventilation' period coefficient (the ratio of slopes of the linear regression of flutter period duration on DGC duration) was 0.80 ± 0.16 ($r^2=0.77$, $P=0.02$) for *Z. complanata* and 0.84 ± 0.08 ($r^2=0.94$, $P<0.001$) for *Z. punctata* (means ± S.E.M.). As the duration of the flutter period increased, the number of miniature ventilations within each flutter period also increased, and a similar distribution of the

Table 3. *Characteristics of the discontinuous gas-exchange cycle in tenebrionid beetles*

Species	*Zophosis complanata*	*Zophosis punctata*	*Pimelia canescens*
Mass (g)	0.112±0.03	0.069±0.016	0.312
N	9	10	2
Flutter period			
$\dot{V}_{CO_2}$ (µl g^{-1})	10.26±3.9	7.19±5.2	2.92
$\dot{V}_{CO_2}$ (µl g^{-1} h^{-1})	77.86±29.14	64.82±33.88	32.25
Duration (s)	501.01±189.56	398.99±190.19	42.6
Flutter duration (% of DGC duration)	73.82±16.74	62.82±11.63	42.6
Number of miniature ventilations	9.4±3.4	8.29±3.8	4.0
Burst period			
$\dot{V}_{CO_2}$ (µl g^{-1})	21.4±4.1	23.21±9.48	21.56
$\dot{V}_{CO_2}$ (µl g^{-1} h^{-1})	610.6±109.9	568.06±231.68	190.06
Duration (s)	128.67±19.44	150.62±37.68	442.36
Burst duration (% of DGC duration)	20.21±5.69	26.0±11.2	49.58
Closed period			
$\dot{V}_{CO_2}$ (µl g^{-1})	0.28±0.10	0.19±0.14	0.10
$\dot{V}_{CO_2}$ (µl g^{-1} h^{-1})	11.24±5.87	9.19±5.46	3.27
Duration (s)	108.66±45.71	88.84±61.35	145.6
Closed duration (% of DGC duration)	16.03±5.84	15.19±6.2	17.07
Discontinuous gas-exchange cycle			
Frequency (mHz)	1.59±0.41	1.86±0.77	1.2
Period (min)	11.27±3.46	10.35±3.86	14.64

Values are means ± S.D. for the *Zophosis* species and means for the two specimens of *Pimelia canescens*.
The sample size is the number of beetles measured, each representing the mean of 4–90 measurements.
DGC, discontinuous gas-exchange cycle.

number of miniature ventilations was found in the two species (Fig. 3). The frequency of CO_2 bursts within the flutter period was similar: *Z. complanata* had a frequency of 19.4±5.7 mHz (408 cycles) and *Z. punctata* had a frequency of 23.4±6.3 mHz (381 cycles) A significant positive correlation was found between $\dot{V}_{CO_2}$ (ml CO_2 h^{-1}) and the rate of flutter period CO_2 emission (µl g^{-1} h^{-1}) for both species. A large proportion of total CO_2 was emitted during the flutter period. The flutter period CO_2 volume/burst period CO_2 volume ratio was 0.48±0.17 in *Z. complanata* and 0.29±0.14 in *Z. punctata.*

The duration of the burst period was shorter than that of the flutter period and more similar to the duration of the closed period (Table 3) in both species. The burst period duration, however, was independent of DGC duration. A significant positive correlation was found between $\dot{V}_{CO_2}$ (ml CO_2 h^{-1}) and mass-specific volume of CO_2 emitted during the burst period.

Very small amounts of CO_2 were emitted during the closed period (Table 3). Only in *Z. complanata* was there a positive correlation between the duration of the closed period and DGC duration.

Metabolic rate

The standard metabolic rate for each beetle species was calculated from the mean $\dot{V}_{CO_2}$ (ml h^{-1}) during stable continuous gas exchange or while the beetle exhibited a regular discontinuous gas-exchange pattern (in the case of *Zophosis* species and *P. canescens*). All the beetle species had a similar metabolic rate (Table 2), implying a common resting state.

Grouping all the species data, we found that $\dot{V}_{CO_2}$ (ml CO_2 h^{-1}) scaled allometrically with mass, $\dot{V}_{CO_2}=0.179M^{1.06}$, where M is mass in g ($r^2=0.8$, $P<0.001$). For the three species exhibiting DGCs, the flutter period and burst period CO_2 emission volumes showed a significant relationship with body mass within a species and at the interspecific level. Grouping the data for these three species, the burst period CO_2 emission volume scaled with $M^{1.20}$ and the flutter period CO_2 emission volume scaled with $M^{1.44}$.

Discussion
Standard metabolic rate

To determine whether these tenebrionid species have lowered their metabolic rate to survive in more arid environments, we compared our values with those obtained by Lighton and Fielden (1995) for a large data set of arthropods. Our data for the individual beetles from nine species gave the allometric relationship $MR=1216M^{1.06}$ (where M is mass in g and MR is metabolic rate in µW) compared with the equation $MR=906M^{0.825}$ obtained by Lighton and Fielden (1995). This suggests that metabolic rates do not seem to have been reduced in the more arid environment. The differences between the two data sets could be due to unrecognised activity effects causing higher metabolic rates and because Lighton and Fielden (1995) used a Q_{10} value of 2.5 to correct metabolic rate to a temperature of 25 °C. This is not always a valid assumption, as has been
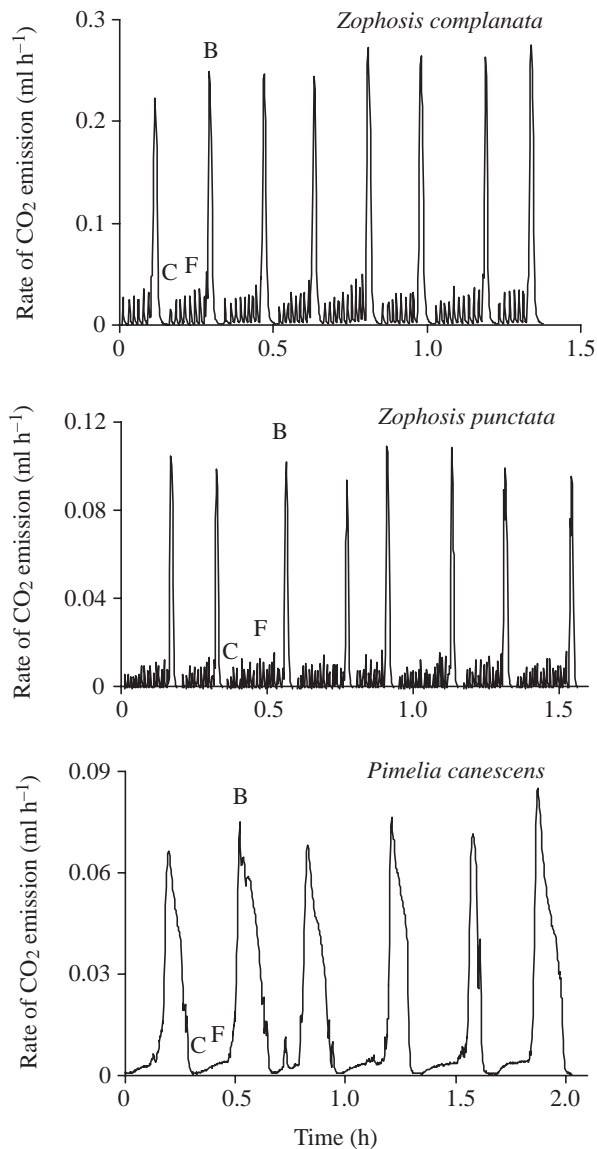
Fig. 2. Recordings of the rate of CO_2 emission by *Zophosis complanata* (mass 0.089 g), *Z. punctata* (mass 0.061 g) and *Pimelia canescens* (mass 0.273g). C marks the closed period, F marks the flutter period and B marks the burst period of a single discontinuous gas-exchange cycle.
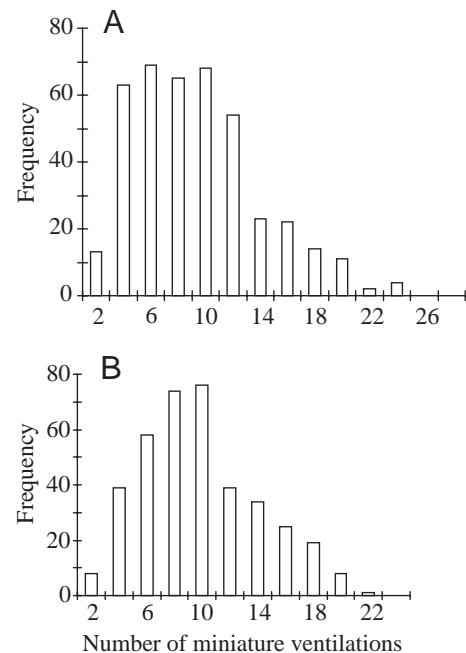

Fig. 3. Histograms showing the distribution of the number of miniature ventilations in each flutter period in (A) *Zophosis complanata* and (B) *Z. punctata*.

shown by Duncan and Dickman (2001). Without a larger data set of insect metabolic rates, we can only suggest that the Negev Desert beetles may have elected to be active when the water vapour pressure gradient was reduced, i.e. at night, or to use other adaptations to enable them to be active at high temperatures rather than lower their metabolic rates.

The discontinuous gas-exchange cycle in Zophosis *species*

An important finding of this study is that only the diurnally active Negev Desert tenebrionid beetles used DGCs and had a gas-exchange pattern similar to that found by Lighton (1991) in 10 species of tenebrionid beetle from the Namib Desert. These tenebrionid beetles are the only beetle species measured

to date to produce discrete bursts of CO_2 (miniature ventilations) during the flutter period. Miniature ventilations have previously been observed in the locust *Locusta migratoria migratorioides* (Hustert, 1975). In all the other beetle species, e.g. carabid beetles (Punt et al., 1957), Scarabaeidae dung beetles (Davis et al., 1999; Duncan and Byrne, 2000), a trogid beetle (Bosch et al., 2000) and wood-boring beetles (Chappell and Rogowitz, 2000), the flutter period consists of a gradual increase in the amount of CO_2 emitted that results in a large release of CO_2, the burst period (see Fig. 2, DGC in *P. canescens*). In many of the above investigations, the flutter and closed periods were indistinguishable.

The reduction in respiratory water loss is thought to result from the diffusive loss of CO_2 and water vapour being restricted to discrete cyclic events (Miller, 1981). Thus, the burst period (high respiratory water loss) of the DGC should be short relative to the closed and flutter periods (low respiratory water loss). In *Zophosis* species, the duration of the burst period is less than a quarter of the duration of a complete gas-exchange cycle (Table 3). For a desert species, we would have expected the closed period to be longer than the burst period, as found in the Namib Desert tenebrionid beetles (Lighton, 1991). Yet, in our study, the flutter period was 3–5 times longer than the closed period (Table 3), whereas it is only twice as long in the Namib Desert tenebrionids. In *Z. complanata*, 48 % of total CO_2 output occurred during the flutter period (29 % for *Z. punctata*), similar to the value found by Lighton (1991) for nine tenebrionid species, in which 24 % of total CO_2 release occurred during the flutter period (47 % for *Epiphysa arenicola*).

In the *Zophosis* species, the flutter period plays an important

Table 4. *Water balance characteristics of the tenebrionid beetles*

Species	N	Body water content (%)	Water loss tolerance (%)	Critical hydration level (%)	Days
Zophosis complanata	3	71.4±2.2	70.9±4.8	20.7±2.8	18±7
Z. punctata	3	70.1±3.4	69.7±17.0	21.3±12.5	14±4
Akis goryi	3	59.6±11.7	62.7±19.1	20.8±5.9	14±6
Trachyderma philistina	3	70.0±1.7	58.7±3.7	28.9±2.4	27±15
T. hispida	4	67.9±3.6	60.0±4.8	27.1±3.2	15±4
Pimelia grandis	3	74.9±2.3	66.5±4.8	25.0±3.4	17±2
Blaps sulcata	3	66.4±1.4	68.2±0.9	21.1±1.0	13±2

Values are means ± S.D.

Body water content=(initial mass minus dry mass)/initial mass; water loss tolerance=(initial mass minus mass at loss of coordination) / (initial mass minus dry mass); critical hydration level=(mass at loss of coordination minus dry mass)/initial mass; days=number of days the beetle spent without food and water at 28 °C and 0 % relative humidity before loss of coordination.

role in the modulation of metabolic rate with body size. The increase in metabolic rate was accommodated by increasing the rate at which CO_2 was emitted during the flutter period. Long and conspicuous flutter periods appear to be common in arid-adapted arthropods, e.g. the ant species *Cataglyphis* (Lighton and Wehner, 1993) and a trogid beetle (Bosch et al., 2000). The flutter period is, therefore, an important component of the gas-exchange cycle in arid-adapted insects and, in this study, the flutter period duration was found to be tightly coupled with DGC duration.

The importance of the flutter period in reducing respiratory water loss has been advocated by Lighton and Garrigan (1995). In the ant *Camponotus vicinus*, they found that the rate of water loss relative to CO_2 emission during the burst period increased approximately fourfold compared with flutter period levels. This was explained by suggesting that, as a result of spiracular fluttering, less water would be lost during the flutter period than during the burst period, when there is no spiracular control, even though the molar rates of CO_2 and water loss during the flutter period are equivalent. The miniature ventilations during the flutter period should also reduce water loss mainly because the respiratory passages are not continuously exposed to the external atmosphere. The DGCs recorded from *P. canescens* do not show numerous miniature ventilations during the flutter period (Fig. 2). Thus, respiratory water loss may be greater in these beetles, compelling them to change their activity pattern throughout the year. These beetles are diurnal during the winter months and become nocturnal during the summer months.

Ventilatory pattern and diel activity

From the above data, we can draw some conclusions about the function of the DGC in relation to diel activity. The activity patterns of the beetles are more important than their habitat associations in designating the type of respiration used. Thus, we propose that, in some beetle species, discontinuous ventilation has been used to limit respiratory water loss, enabling them to be active during the hot daytime hours. Being active during the day may enable the small *Zophosis* species to reduce competition for limited food resources. All the

tenebrionid species in this study are well-adapted to desert conditions. The values in Table 4 for body water content, critical hydration levels and water loss tolerance are typical for arid-adapted insects (Hadley, 1994b). It is possible that, because of their diel activity and small size, respiratory water loss is an important component of the water budget of diurnally active species.

Seely and Mitchell (1987) postulated that, if beetles need to minimise dessication by minimising the vapour pressure gradient between themselves and their surroundings then, in a typical desert area where there has been no recent rain, they would elect to be on the surface throughout the night. Six of the nine study species are active only at night. They may either not have a need to use discontinuous ventilation or discontinuous ventilation may not be the ancestral condition. It is interesting to note that the most ancient species in this group (Kaszab, 1982) do exhibit DGCs. Until a detailed phylogenetic analysis is performed and the respiratory patterns of more species of tenebrionid beetle are measured, we cannot be sure whether discontinuous ventilation was the ancestral condition or is a more-derived condition. In his study of the Namib Desert tenebrionid beetles, Lighton (1991) found DGCs in the one nocturnal beetle (*Epiphysa arenicola*), which inhabited a particularly harsh environment within the desert. If discontinuous ventilation is the ancestral condition, then factors other than being unable to reduce respiratory water loss through the use of DGCs may have induced beetles to be active at night.

Traditionally, it was proposed that the DGC evolved as a mechanism to enhance water conservation (Kestler, 1985; Lighton, 1996). The main alternative hypothesis is that the DGC evolved to facilitate respiration in the hypercapnic and hypoxic environments experienced by arthropods living underground (chthonic hypothesis) (Lighton, 1996). In our study, both the nocturnally active and the diurnally active beetles bury themselves in the soil, preferably into a small sandy hill close to a shrub, where the soil is less compact (B. Krasnov, personal observations). From the study of Louw et al. (1986) in the Namib Desert, we would be surprised if the

tenebrionid beetles experienced conditions of hypercapnia and hypoxia. Louw et al. (1986) found that CO_2 diffused very rapidly through a 5 cm column of dune sand, the depth at which the tenebrionid beetles are commonly found. None of our study species inhabits deep burrows where they might experience hypoxic or hypercapnic conditions. If the chthonic hypothesis is valid, there should be no difference in the respiratory patterns between the species since they all burrow into the sand. Furthermore, we would expect the nocturnally active beetles to bury deeper to escape the extreme day time temperatures.

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