MASS, TEMPERATURE AND METABOLIC EFFECTS ON DISCONTINUOUS GAS EXCHANGE CYCLES IN EUCALYPTUS-BORING BEETLES (COLEOPTERA: CERAMBYCIDAE)

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Summary

Ventilatory accommodation of changing metabolic rates is a relatively little-studied aspect of the discontinuous gas exchange cycles (DGCs) that occur in a wide variety of terrestrial arthropods. We used correlation analysis of resting metabolic rate (RMR, measured as the rate of CO_2 emission; $\dot{V}_{CO_2}$) and several components of the DGC to examine accommodation to both temperature-induced changes and individual variation in RMR in two wood-boring beetles (*Phorocantha recurva* and *P. semipunctata*; Coleoptera: Cerambycidae).

At low to moderate ambient temperatures (T_a; 10–20 °C), *Phorocantha* spp. displayed a characteristic DGC with relatively brief but pronounced open (O) phase bursts of CO_2 emission separated by longer periods of low $\dot{V}_{CO_2}$, the flutter (F) phase. However, the $\dot{V}_{CO_2}$ never fell to zero, and we could not reliably differentiate a typical closed (C) phase from the F phase. Accordingly, we pooled the C and F phases for analysis as the C+F phase. At higher T_a (30 °C), the duration of the combined C+F phase was greatly reduced. There were no differences between the two species or between males and females in either RMR or characteristics of the DGC. We found large variation in the major DGC components (cycle frequency, durations and emission volumes of the O and C+F phases); much of this variation was significantly repeatable. Accommodation of temperature-induced RMR changes was almost entirely due to changes in frequency (primarily in the C+F phase), as has been found in several other discontinuously ventilating arthropods. Frequency changes also contributed to accommodation at constant T_a, but modulation of emission volumes (during both O and C+F phases) played a larger role in this case.

The DGC is often viewed as a water conservation mechanism, on the basis that respiratory evaporation is minimal during the C and F phases. This hypothesis assumes that the F phase is primarily convective (because of a reduction in tracheal P_{O_2} and total intratracheal pressure during the C phase). To test this, we measured the DGC in beetles subjected to varying degrees of hypoxia in addition to normoxia. As predicted for a largely diffusive F phase, we found an increase in the volume of CO_2 emitted during the C+F phase in hypoxic conditions (10.4 % oxygen). This finding, together with a reduced tendency to utilize a DGC at high T_a (when water stress is greatest) and a natural history in which water availability is probably not limiting for any life stage, suggests that a reduction of respiratory evaporation may not have been critical in the evolution of the DGC of *Phorocantha* spp. Instead, selection may have favored discontinuous ventilation because it facilitates gas exchange in the hypercapnic and hypoxic environments commonly encountered by animals (such as *Phorocantha* spp.) that live in confined spaces.

Key words: discontinuous gas exchange, ventilation, metabolic rate, beetle, scaling, repeatability, hypoxia, *Phorocantha recurva*, *Phorocantha semipunctata*, Cerambycidae.

Introduction

Discontinuous gas exchange cycles (DGCs) are widespread in terrestrial arthropods. First described in lepidopteran pupae, DGCs have been reported in a number of insect orders and in several other tracheate groups, including ticks, solphugids, pseudoscorpions and harvestmen (for reviews, see Lighton, 1994, 1996). A sequence of three distinct phases of spiracle behavior occurs in a 'classical' DGC. The spiracles are closed and gas exchange is negligible during the closed (C) phase.

During this time, O_2 is removed from the tracheal spaces to support tissue respiration, but this is not replaced volume-for-volume by CO_2 (which largely remains dissolved in the tissues and hemolymph); in consequence, intratracheal pressures fall. The C phase is followed by a flutter (F) phase, in which the spiracles 'flutter' (open minimally and transiently) and net gas flux is low and largely inwardly convective (e.g. Levy and Schneiderman, 1966; Kestler, 1985; Lighton et al., 1993; but

see Lighton and Garrigan, 1995). Subsequent to the F phase is a period (usually fairly brief) when the spiracles open fully and gas exchange is rapid (O phase); this is sometimes accompanied by active muscle-assisted ventilation. In some species, the interval between successive O phases may be many hours, and animals breathing in this manner can display long periods of C phase with little or no exchange of respiratory gas and with consequent large fluctuations in internal P_{O_2} and P_{CO_2} (Levy and Schneiderman, 1966; Lighton, 1994, 1996).

It was originally proposed that the DGC evolved primarily from selection as a water conservation mechanism, since respiratory evaporation (presumably the major water loss route in animals with water-impermeable cuticles) should be low during the C and inwardly convective F phases. However, several authors have questioned this concept (e.g. Hadley and Quinlan, 1993; Hadley, 1994). A more recent view is that the DGC evolved under selection to enhance the efficacy of gas exchange in hypoxic and/or hypercapnic environments (Lighton and Berrigan, 1995; Lighton, 1996).

In either of these functional and evolutionary scenarios, an important question is how the DGC adjusts to allow for changing rates of gas exchange. This is particularly interesting in view of the fluctuations in internal environment (P_{O_2}, P_{CO_2}, etc.) imposed by the DGC and because ectothermic arthropods exposed to variation in environmental temperature undergo substantial concomitant changes in metabolic rate (Q_{10} effect) even in the absence of activity. There are relatively few data on patterns of ventilatory (DGC) accommodation, and these have come from a limited number of taxa. Several studies of ants (Lighton, 1988, 1996; Lighton and Wehner, 1993), tenebrionids (Lighton, 1991) and scarab beetles (Davis et al., 1999) have examined DGC accommodation of temperature-induced changes in resting metabolic rate. This approach can provide many useful insights into ventilatory expansibility, given the typical metabolic Q_{10} of 2–2.5. However, it is intrinsically limited in that it does not differentiate the effects of changes in metabolic rate on the DGC from possible direct influences of temperature that are independent of metabolism (e.g. changes in the solubility and buffering of CO_2). One way to circumvent this limitation is to make use of natural variation in both resting metabolic rates and the DGC. If sufficient variation is present, correlation analysis can reveal associations between metabolic and ventilatory variables exclusive of thermal effects (e.g. Bennett, 1987; Hayes and Shonkwiler, 1996).

We used this approach in a study of discontinuous ventilation in two species of wood-boring beetles in the genus *Phorocantha* (Coleoptera: Cerambycidae). A previous examination of exercise metabolism in these beetles (Rogowitz and Chappell, 2000) revealed a strong and highly variable DGC across a range of ambient temperatures. In the present study, we addressed three questions. Our primary interest was how the DGC of *Phorocantha* spp. accommodates different rates of respiratory gas exchange, both across a range of temperatures and at constant temperature. Since these analyses were based largely on statistical interpretation of phenotypic variation, we also tested the individual consistency over time (repeatability) of both metabolic rate and DGC variables. Finally, we explored the effects of hypoxia on the metabolic rate and ventilation of *Phorocantha* since adaptation to low P_{O_2} has been proposed as a primary factor in the evolution of the DGC (Lighton and Berrigan, 1995) and because *Phorocantha* of all life stages spend considerable time in tunnels, crevices or other confined spaces that might be expected to be relatively hypoxic.

Materials and methods
Animals

Long-horned eucalyptus borers (*Phorocantha semipunctata* and *P. recurva*) are native to Australia. They were accidentally introduced into California, where their host plants (various species of eucalyptus trees) are commonly used as ornamentals. The two *Phorocantha* species have similar natural histories. Larval stages burrow into and eat cambium tissues, and pupation occurs in chambers in sapwood. Adults feed on nectar and pollen; they are nocturnally active and rest in bark crevices and other natural cavities during the day. Courtship, mating and oviposition take place on the trunks and branches of eucalyptus (both sexes fly at night and are particularly attracted to injured trees; Hanks et al., 1995, 1996a,b).

We obtained *P. semipunctata* and *P. recurva* from a laboratory colony managed by Dr Timothy Paine at the University of California, Riverside. The colony was initially stocked with free-living beetles captured in the Riverside area after they had been attracted to freshly cut eucalyptus wood (T. Paine, personal communication). Beetles were housed in small screen-wire cages containing a paper shelter and *ad libitum* water and food (20 % sucrose solution and eucalyptus pollen). The cages were maintained at room temperature (22–24 °C).

Respirometry

We used a continuous-flow (open system) respirometer to measure rates of CO_2 emission ($\dot{V}_{CO_2}$; ml h^{-1}). The CO_2 analyzer (LiCor 6251; Lincoln, Nebraska, USA) was capable of resolving differences of 0.2 p.p.m. of CO_2 in air. It was calibrated weekly against a precision gas mixture; there was almost no drift between calibrations. The metabolic chamber was a modified 10 ml syringe barrel (internal volume in our tests was approximately 9 ml); it was perfused with dry, CO_2-free air (Dryerite and soda lime) at flow rates of 80–120 ml min^{-1} (depending on animal size; all but a few animals were tested at 103–108 ml min^{-1}) maintained (±1 %) by a Tylan mass flow controller. The combination of high flow rate and low chamber volume ensured rapid flushing and accurate resolution of short-term fluctuations in gas exchange. Because of low downstream resistance, the internal pressure build-up in the chamber was small (<0.133 kPa above ambient atmospheric pressure at the highest flow rates, measured with a water manometer). Outputs from the CO_2 analyzer and flow

controller, together with ambient temperature (T_a; measured with copper–constantan thermocouples connected to a Sable Systems TC-1000 thermometer), were recorded on a Macintosh computer equipped with a National Instruments A/D converter and custom-designed software for data acquisition and analysis (Warthog Systems; written by M. A. Chappell and available at www.warthog.ucr.edu). Excurrent air was dried with magnesium perchlorate prior to entering the CO_2 analyzer.

We calculated $\dot{V}_{CO_2}$ as:

$$\dot{V}_{CO_2} = \dot{V}(F_{ECO_2} - F_{ICO_2})/(1 - F_{ECO_2}[1 - (1/RQ)]), \quad (1)$$

where $\dot{V}$ is the flow rate corrected to standard temperature and pressure (STP; $0\,°C$ and $101.1\,kPa$), F_{ICO_2} and F_{ECO_2} are the initial and final fractional concentrations of CO_2, respectively, and RQ is the respiratory quotient. Since F_{ICO_2} was zero because incurrent air was scrubbed of CO_2, the equation simplifies to:

$$\dot{V}_{CO_2} = \dot{V} \times F_{ECO_2}/(1 - F_{ECO_2}[1 - (1/RQ)]), \quad (2)$$

We used an RQ of 0.88 based on published values for *Phorocantha* maintained in similar conditions (Rogowitz and Chappell, 2000). Modest changes in RQ had a negligible effect on calculated $\dot{V}_{CO_2}$ (given the low values of F_{ECO_2} during our measurements, the denominator of equation 2 was always very close to 1.0). We checked the system for leaks by measuring gas exchange in the absence of a beetle; the calculated $\dot{V}_{CO_2}$ in these tests did not differ from zero.

Measurement procedures and resting metabolic rate

Most animals were tested at one of three T_a values (10, 20 or 30 °C), although a small number of measurements were performed at other T_a values (12–16 °C, 22–26 °C and 32–35.7 °C). Some individuals were tested at more than one T_a, with the order chosen randomly. Regression analysis indicated little within-individual consistency between T_a values (after correction for body mass). Accordingly, samples from the same individual at different T_a were treated as independent. Beetles were weighed (±0.1 mg), placed in the metabolic chamber and allowed to recover from handling for at least 30 min before measurements commenced. Most animals ceased exploratory movements within 5 min after insertion into the chamber and subsequently remained immobile. When a DGC was present, we restricted analyses to periods during which the pattern of ventilation (frequency and amplitude) was stable. Measurements lasted for a minimum of 20 min at 30–35.7 °C, 60 min at 20–26 °C and 150 min at 10–15 °C, with at least 30 min allowed for adjustment after each change in T_a. We recorded CO_2 concentrations in reference gas for several minutes before and after measurements to account for any analyzer drift, with linear interpolation between reference points (in all cases, drift was negligible). Resting metabolic rates (RMRs) were calculated as the mean $\dot{V}_{CO_2}$ during intervals of at least 20 min in which beetles were not active (at T_a below 30 °C, averaging intervals were usually considerably longer). Most individuals exhibited discontinuous ventilation (except at

high T_a), and in these cases we averaged metabolic rate over an integral number of ventilation cycles ($N \geqslant 3$; Lighton, 1991) to avoid biasing RMR estimates.

Components of the DGC

Although *Phorocantha* exhibited clearly identifiable O and F phases (Figs 1, 2), we were unable unambiguously to resolve a C phase (i.e. with no CO_2 emission) and to differentiate it from the F phase, especially at high T_a. Accordingly, we focused our analyses on contrasts between the O phase and the rest of the DGC (the combined C+F phases).

For all recordings of discontinuous ventilation that contained at least four sequential O phases, we calculated nine variables of the ventilatory cycle (Fig. 1). Frequency is the cycle rate (mHz; measured between bursts of CO_2 emission). O phase amplitude is the highest instantaneous $\dot{V}_{CO_2}$ (ml h^{-1}) attained during a burst. O phase duration is the time (s) from the beginning of a burst until $\dot{V}_{CO_2}$ returns to stable levels. C+F phase duration is the time (s) for the remainder of a cycle. O phase volume is the quantity of CO_2 (μl) released during the O phase. C+F phase volume is the quantity of CO_2 released during the C+F phase. O phase fractional duration ($f_{O,Time}$) is the fraction of the cycle duration occupied by the O phase. O phase fractional RMR ($f_{O,RMR}$) is the fraction of total CO_2 volume emitted during bursts (=O phase volume/total CO_2 volume emitted during the cycle). O phase gain is the ratio of mean $\dot{V}_{CO_2}$ during the O phase relative to during the rest of the DGC; i.e. the factorial increase in $\dot{V}_{CO_2}$ during the O phase ($f_{O,RMR}/f_{O,Time}$).

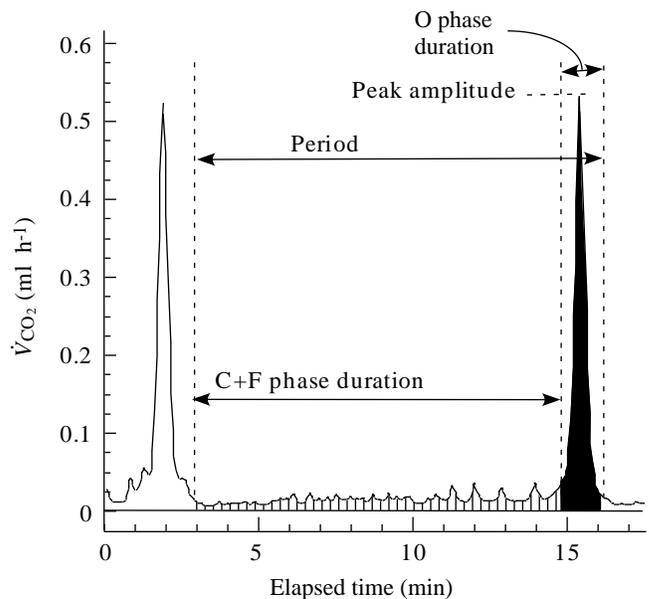We attempted to use computer algorithms to identify the

Fig. 1. Representative example of a single ventilatory cycle from a 0.316 g *Phorocantha recurva* at 20 °C, showing the measured components of the discontinuous gas exchange cycle. The dark area is the open (O) phase emission volume and the hatched area is the closed+flutter (C+F) phase emission volume. Note the absence of a completely closed phase (see also Fig. 3).

beginning and end of the O phase, and for most DGC bouts a procedure that searched for peaks exceeding a particular value (interactively specified on the computer screen by the user) proved satisfactory in identifying and describing O phase bursts. In a few instances of noisy data, this approach failed and we made those determinations by visual inspection.

Differences in flow rate, and the dependence of gas diffusion coefficients on temperature, could affect the response characteristics of the system to brief events, such as O phase bursts, irrespective of changes in physiology or behavior. The degree of distortion should be inversely related to event duration. We attempted to eliminate these effects by rapid flushing of the chamber (in essence, by providing a sufficiently strong convective flow to minimize the influences of diffusion and mixing). We tested the response characteristics by injecting CO_2 in pulses simulating O phase bursts (3 ml of 0.25 % CO_2 in dry air was injected into the chamber over a 15 s interval). Recorded data were converted into *faux* $\dot{V}_{CO_2}$ (to account for flow rate differences). The results indicated that the different flow rates ($80–120\,\text{ml}\,\text{min}^{-1}$) and T_a values ($10–35\,°C$) had little influence on pulse height and duration, presumably because the time constant for the chamber was considerably less than the duration of O phase events.

Effects of oxygen concentration

To examine metabolic rate and the DGC in hypoxic conditions, we blended dry nitrogen gas (N_2) into the incurrent air stream at rates that provided oxygen concentrations (P_{O_2}) of $15.7\pm0.2\,\%$, $10.4\pm0.2\,\%$ and $6.0\pm0.5\,\%$. The flow of N_2 was regulated by a second mass flow controller (Applied Materials), and P_{O_2} was monitored ($\pm0.002\,\%$) by an Ametek S-3A analyzer. As a control, we also tested all hypoxia-exposed individuals in air ($20.95\,\%$ O_2). Different combinations of P_{O_2} and T_a were presented in random order; other procedures for hypoxia testing were as described above.

Repeatability

We tested the repeatability of RMR and ventilation variables for a subset of individuals measured twice over intervals of 48–72 h. Repeatability was evaluated by residuals analysis (Hayes and Shonkwiler, 1996), using standardized residuals obtained from multiple regression of RMR against body mass and T_a (we converted mass and RMR to $\log_{10}$ values to linearize them prior to analysis).

Statistical analyses

We report data as means $\pm$ S.D. unless noted otherwise. To compensate for the effects of body mass and T_a on RMR and ventilation, we used analysis of covariance (ANCOVA) with these factors as covariates. For all tests, mass and RMR were linearized by conversion to $\log_{10}$ values; bounded fractions ($f_{O,RMR}$ and $f_{O,Time}$) were arcsine-square-root-transformed. $P<0.05$ was considered to be significant; we used a sequential Bonferroni procedure to correct for Type I errors in multiple simultaneous tests (Rice, 1989). Analyses were performed using Statistica/Mac (StatSoft), a statistics package for the Macintosh.

Results

Metabolic rates

As expected, RMR was strongly influenced by both body mass and T_a, but it was not affected by either species or sex (ANCOVA; $P=0.52$ for species and $P=0.72$ for sex; $N=102$ measurements of 19 female and 26 male *P. semipunctata* and 10 female and 18 male *P. recurva*). Within the range of body mass (0.0956–0.582 g; mean 0.303±0.104 g) and T_a (°C) in our entire data set (including 45 animals that were not sexed), RMR ($\text{ml}\,CO_2\,\text{h}^{-1}$) varied as $m^{0.853}$, where m is body mass (g), with a Q_{10} of 2.49:

$$\log\text{RMR} = -1.345 + 0.853\log m + 0.0395 T_a \qquad (3)$$

($r^2=0.70$, $P<0.000001$ for both mass and T_a; $N=182$ measurements on 118 individuals). The predicted RMR at 20 °C for an individual of average mass is $0.1009\,\text{ml}\,CO_2\,\text{h}^{-1}$. This value is slightly higher than our previous RMR value from *Phorocantha* spp. (Rogowitz and Chappell, 2000).

After correcting for body mass and temperature effects, there was considerable between-individual variation in RMR (Fig. 2). The average absolute difference between observed and predicted RMR was 43 % (approximately 55 % of individuals had an RMR between 0.57 times and 1.43 times the predicted value). To account for this variation in analyses of DGC components, we used mass+T_a residuals (termed RMR_{Resid}).

Characteristics of the DGC

We did not monitor activity continuously during most of our measurements. However, during visual observations on a
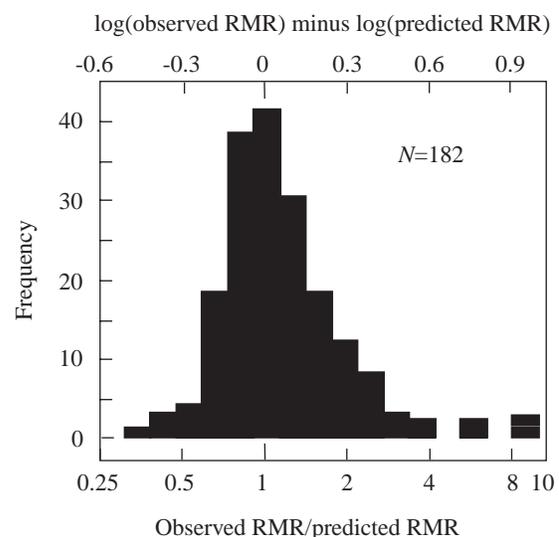

Fig. 2. Variation in the resting metabolic rate (RMR) of *Phorocantha* spp. The plot shows the distribution of observed RMR in relation to predicted RMR values based on a regression of RMR against body mass and ambient temperature ($N=182$ measurements from 118 individuals, mass range 0.0956–0.582 g; temperature range 10–35.7 °C. The average difference from predicted values was approximately 43 %.
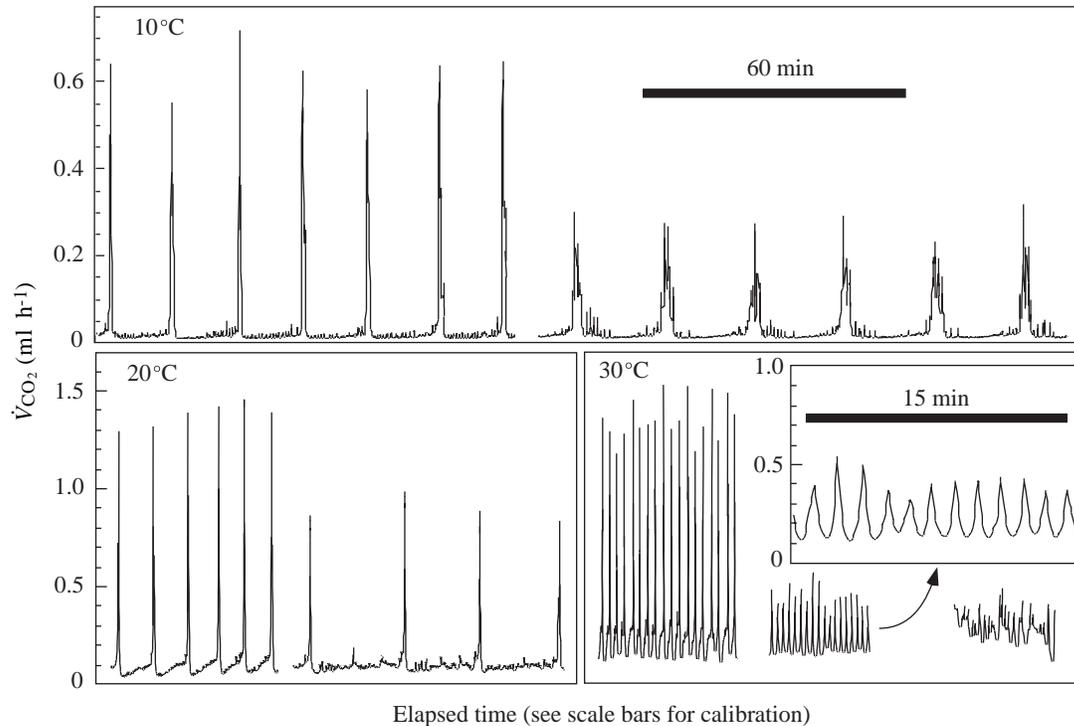
Fig. 3. Examples of discontinuous gas exchange cycles from *Phorocantha semipunctata* at three ambient temperatures (T_a), showing variation within and between T_a values. The inset in the 30 °C panel shows a fourfold expansion of the time scale, revealing the decrease in the duration of the closed+flutter (C+F) phases in relation to the open (O) phase that often occurred at this T_a. Animal mass values: 10 °C, left, 0.371 g; right, 0.2705 g; 20 °C, left, 0.424 g, right, 0.371 g; 30 °C, left, 0.371 g, middle, 0.2812 g, right, 0.3374 g.

subset of the test animals, we only observed DGCs in resting animals, and activity, even slight movements, was accompanied by readily identifiable changes in the pattern of CO_2 emission. We assume, therefore, that all DGC values in our data set came from inactive beetles.

The DGC in both *P. recurva* and *P. semipunctata* varied greatly with temperature and among individuals (Figs 3, 4). At high T_a, the ventilation pattern often resembled a 'sawtooth' with little or no period of stable, low $\dot{V}_{CO_2}$ (C+F phase) between peaks. At lower T_a values, the bursts of CO_2 emission usually occurred in a more typical DGC pattern of large, abrupt and sharply defined peaks (O phase) separated by substantial intervals of low $\dot{V}_{CO_2}$. Although $\dot{V}_{CO_2}$ frequently fell to low levels immediately after peaks (Fig. 1), there were no instances of a classical closed phase with $\dot{V}_{CO_2}$ falling to zero (Levy and Schneiderman, 1966; Kestler, 1985; Lighton, 1994, 1996).

The probability that beetles would ventilate discontinuously varied with T_a. At both 10 and 20 °C, approximately 67 % of individuals showed an unambiguous DGC (N=30 of 45 animals at 10 °C; N=31 of 46 animals at 20 °C). Only 37.5 % of beetles tested at 30 °C showed a DGC (N=15 of 40 animals), and this was a significantly lower fraction than at cooler T_a (χ^2=7.23, P=0.0072). Of four animals tested at a higher T_a (32–35.7 °C), two exhibited a DGC, but both shifted to continuous ventilation within 20 min.

None of the nine DGC components was significantly affected by either sex or species (ANCOVA; Table 1). However, T_a, body mass and RMR_{Resid} all had substantial effects on various aspects of the DGC (Table 2). Multiple regression analysis revealed that body mass was strongly correlated to O phase amplitude and to O phase and C+F phase volumes, but did not affect other DGC variables. Ambient temperature affected all DGC variables except $f_{O,RMR}$ and O phase and C+F phase volumes (Fig. 4). RMR_{Resid} significantly affected O phase amplitude, $f_{O,Time}$, frequency, C+F phase duration, O phase volume, $f_{O,RMR}$ and O phase gain (Table 2, Fig. 5); these effects were consistent when data for each T_a (10, 20 and 30 °C) were analyzed separately.

Our results indicate that changes in frequency, O phase amplitude and C+F phase duration accompany all changes in RMR, whether these are due to temperature changes (Q_{10} effects; Fig. 4) or to variation in RMR that is independent of mass and T_a (RMR_{Resid}; Fig. 5). In brief, increased RMR is accommodated by increased O phase amplitude and frequency. The increase in frequency results from declines in the duration of both the C+F and O phases, but the former changes much more than the latter except at high T_a (Fig. 6). These adjustments in phase duration have concomitant effects on $f_{O,Time}$ and O phase gain. In contrast to the effects of Q_{10} on RMR (which strongly influence O phase duration but not volume), individual variation in metabolic rate produces large changes in volume and relatively minor adjustments in duration (Fig. 7). Consequently, frequency modulation plays a
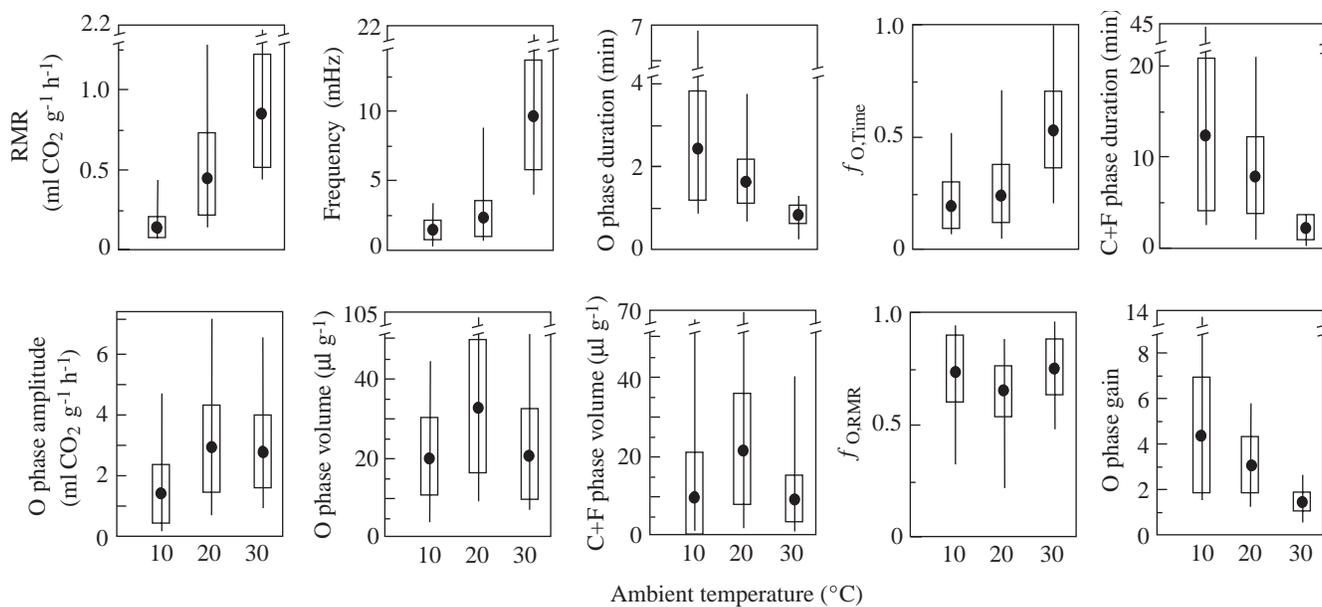
Fig. 4. Effects of ambient temperature on metabolic rate and components of the discontinuous gas exchange cycle in *Phorocantha* spp. Data are shown as means (filled circles), standard deviations (boxes) and ranges (vertical lines). RMR, resting metabolic rate; $f_{O,RMR}$, fractional O phase resting metabolic rate; $f_{O,Time}$, fractional O phase duration; O phase, open phase; C+F phase, closed+flutter phase. N=42 (10 °C), 75 (20 °C) and 59 (30 °C) measurements from 118 individuals (0.0956–0.582 g).

much larger role in accommodating Q_{10} effects than in accommodating differences in RMR$_{Resid}$.

Repeatability

Correlations between initial and final measurements were positive for RMR and for all components of the DGC (r=0.26–0.57) but, because of the relatively small sample size

(23 animals tested twice), only frequency, O phase amplitude, C+F phase duration and O phase volume were significantly repeatable (Table 3).

Table 1. *Effects of species and sex on ventilation variables in* Phorocantha *expressed as* P *values from an analysis of covariance design*

	Sex	Species	Interaction
Frequency	0.72	0.52	0.22
O phase amplitude	0.97	0.045	0.083
C+F phase duration	0.62	0.60	0.18
O phase duration	0.0071	0.028	0.39
O phase volume	0.45	0.31	0.37
C+F phase volume	0.023	0.99	0.94
$f_{O,RMR}$	0.25	0.018	0.72
$f_{O,Time}$	0.055	0.51	0.40
O phase gain	0.39	0.0054	0.10

N=49 *P. recurva* and *N*=79 *P. semipunctata*.

To control for mass and temperature effects, we used these variables as covariates; to control for effects of individual differences in resting metabolic rate (RMR), we used mass+T_a residuals of RMR as a third covariate (log values of mass and RMR were used in the analyses).

No *P* values remained significant after a sequential Bonferroni correction for multiple simultaneous tests (Rice, 1989).

$f_{O,RMR}$, O phase fractional RMR; $f_{O,Time}$, O phase fractional duration; O phase, open phase; C+F phase, closed+flutter phase.
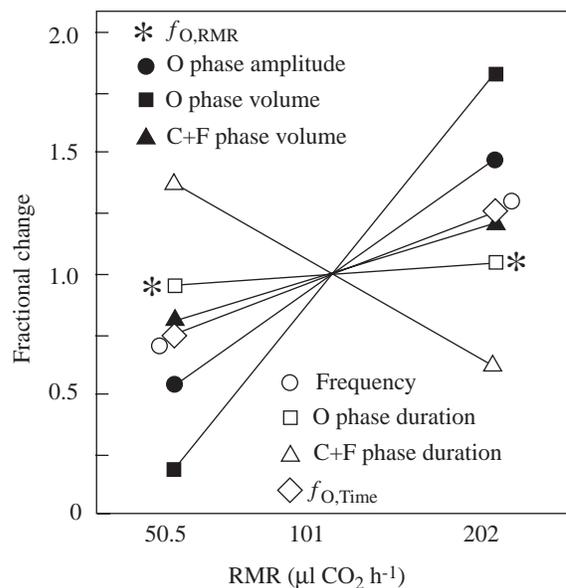

Fig. 5. Predicted changes in discontinuous gas exchange cycle components across a fourfold range of resting metabolic rate (RMR) for a 0.303 g *Phorocantha* spp. at 20 °C. Data are shown as factorial changes from mean values (at RMR=101 μl CO$_2$ h^{-1}) computed from multiple regressions based on body mass, temperature and individual RMR residuals. $f_{O,RMR}$, fractional O phase resting metabolic rate; $f_{O,Time}$, fractional O phase duration; O phase, open phase; C+F phase, closed+flutter phase.

Table 2. *Multiple regressions of the effects of temperature, mass and resting metabolic rate on ventilation variables in* Phorocantha

| | | | Partial correlations | | |
| | | | Mass | T_a | RMR_{Resid} |
	r	P $F_{3,172}$	P	P	P
Frequency	−0.703	<0.000001 (56.1)	−0.011 (0.88)	0.579 (<0.000001)	0.297 (0.000069)
O phase amplitude	0.605	<0.000001 (33.1)	0.415 (<0.000001)	0.264 (0.00043)	0.501 (<0.000001)
C+F phase duration	0.679	<0.000001 (49.0)	-0.017 (0.82)	−0.647 (<0.000001)	−0.32 (0.000016)
O phase duration	0.628	<0.000001 (37.3)	−0.039 (0.61)	−0.623 (<0.000001)	0.10 (0.19)
O phase volume	0.690	<0.000001 (52.5)	0.369 (0.000005)	−0.055 (0.47)	0.658 (<0.000001)
C+F phase volume	0.360	0.000021 (8.71)	0.338 (0.000052)	0.034 (0.65)	0.134 (0.079)
$f_{O,Time}$	0.665	<0.000001 (45.5)	−0.046 (0.54)	0.626 (<0.000001)	0.328 (0.000010)
$f_{O,RMR}$	0.279	0.0029 (4.86)	−0.11 (0.15)	0.049 (0.52)	0.250 (0.00089)
O phase gain	0.656	<0.000001 (43.3)	0.0008 (0.94)	−0.619 (<0.000001)	−0.325 (0.000012)

N=176 measurements on 118 individuals.

Resting metabolic rate is expressed as mass+T_a residuals (RMR_{Resid}); log values of mass and metabolic rate were used in the analysis.

All P values <0.005 are significant after a sequential Bonferroni correction for multiple simultaneous tests (Rice, 1989).

$f_{O,Time}$, fractional O phase duration; $f_{O,RMR}$, fractional O phase resting metabolic rate; O phase, open phase; C+F phase, closed+flutter phase.

Table 3. *Repeatability of metabolic rate and discontinuous ventilation cycle variables in* Phorocantha

Variable	r	F	P
Resting metabolic rate	0.38	3.60	0.072
Frequency	0.56	9.62	0.0054*
O phase amplitude	0.57	10.2	0.0043*
C+F phase duration	0.57	9.88	0.0049*
O phase duration	0.47	5.81	0.025
O phase volume	0.57	9.93	0.0048*
C+F phase volume	0.26	1.49	0.24
$f_{O,RMR}$	0.30	2.14	0.16
$f_{O,Time}$	0.49	6.58	0.018
O phase gain	0.46	5.73	0.026

Repeatability (r) was calculated from residuals of regressions against log(body mass) and ambient temperature (Hayes and Shonkwiler, 1996).

N=23 individuals (0.194–0.476 g) tested over intervals of 48–72 h at temperatures of 10, 20 and 30 °C (some animals did not exhibit discontinuous ventilation at every temperature).

P values with asterisks (*) are significant after a sequential Bonferroni correction for multiple simultaneous tests (Rice, 1989).

$f_{O,RMR}$, fractional O phase resting metabolic rate; $f_{O,Time}$, fractional O phase duration; O phase, open phase; C+F phase, closed+flutter phase.

Effects of hypoxia

We observed little change in either metabolic rate or the characteristics of the DGC during exposure to moderately hypoxic conditions. The only significant effects were an increase in C+F phase volume and a corresponding slight decline in $f_{O,RMR}$ at low P_{O_2} (Table 4). Over a twofold range of P_{O_2} (20.95 % to 10.4 %), the change in $f_{O,RMR}$ was approximately 41 % and the change in C+F phase volume was approximately 46 %.

In more severe hypoxia (5.5–6.5 % O_2), 12 of 13 animals ventilated continuously; one animal exhibited a DGC for approximately 30 min and then shifted to continuous ventilation (these tests were run at 20 °C only). Thus, the minimum oxygen concentration permitting a sustained DGC is probably between 7 and 10 %. Of the 13 animals tested in severe hypoxia, 12 showed a typical DGC in normoxic conditions.

Discussion
Environmental conditions

When considering the characteristics and physiological function of the DGC of *Phorocantha*, it is important to compare the test conditions used in our study with the natural environments experienced by these beetles. As larvae and

Fig. 6. Contribution of open (O) phase duration (open symbols) and closed+flutter (C+F) phase duration (filled symbols) to the total cycle duration of the discontinuous gas exchange cycle (DGC) across a range of temperatures and body sizes. The main figure is an expanded view of data for cycle durations of less than 1000 s; the complete range of data is shown in the inset. The solid lines and the lower dashed line in the main figure show the non-significant relationship of O phase duration to DGC period at 10 °C and 20 °C, respectively. The same relationship was significant at 30 °C (O phase duration=45.2+ 0.117 DGC period, r^2=0.13, $F_{1,57}$=9.6, P=0.003) but the regression line is not shown. The slanted dashed lines show the relationship between C+F phase duration on DGC period at 10 °C (short dashes; C+F phase duration=1.002 DGC period– 136.5, r^2=0.99, $F_{1,40}$=7874, P<0.000001) and 20 °C (long dashes; C+F phase duration= 0.997 DGC period–100.6, r^2=0.99, $F_{1,73}$=6095, P<0.000001). The regression at 30 °C was significant but is not shown (C+F phase duration=0.9883 DGC period–45.2, r^2=0.90, $F_{1,57}$=550, P<0.000001). N=176 measurements from 118 individuals (0.0956–0.582 g).
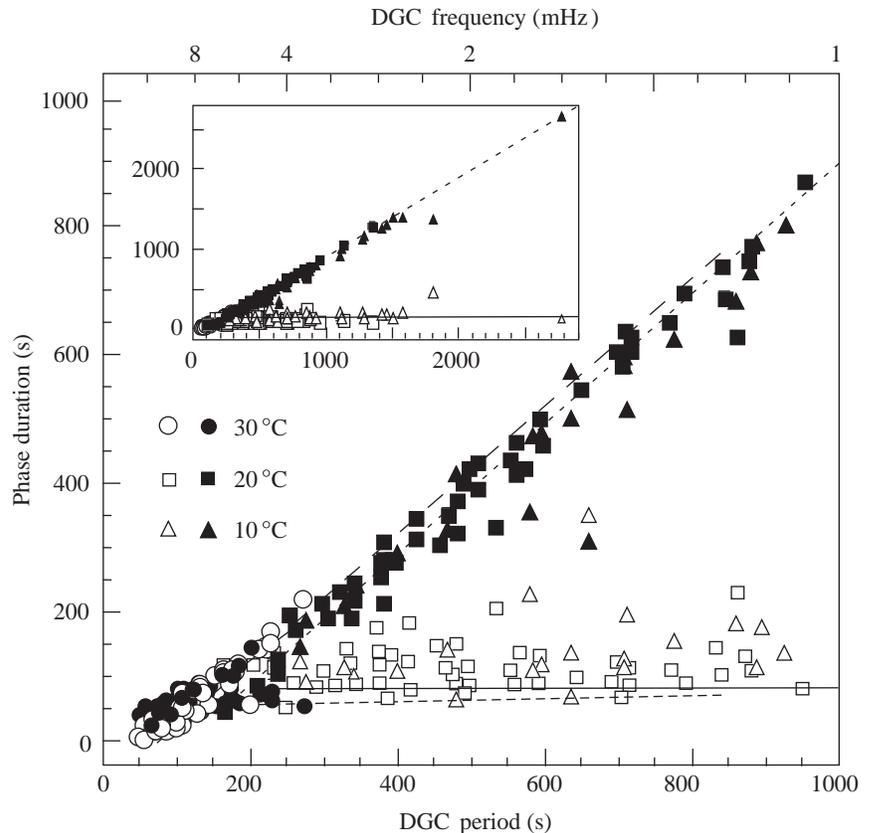

Table 4. *Effect of* P_{O_2} *on metabolic rate and discontinuous ventilation cycle variables in* Phorocantha

Variable	$F_{2,59}$	P	Adjusted means for pO2 values of		
			20.95 %	15.7 %	10.4 %
Resting metabolic rate (ml CO_2 h^{-1})	3.08	0.051	0.118	0.120	0.0930
Frequency (mHz)	1.86	0.16	1.70	1.66	1.16
O phase amplitude (ml CO_2 h^{-1})	1.43	0.25	1.13	1.43	1.01
C+F phase duration (s)	2.22	0.12	493	523	792
O phase duration (sec)	4.81	0.012	70.3	79.2	94.1
O phase volume (μl CO_2)	0.62	0.54	10.8	10.4	9.27
C+F phase volume (μl CO_2)	8.62	0.00052*	4.97	8.04	12.0
$f_{O,RMR}$	18.5	<0.000001*	0.686	0.571	0.470
$f_{O,Time}$	2.69	0.076	0.294	0.253	0.165
O phase gain	1.56	0.22	3.85	3.83	5.05

Animals (N=16) were tested in ambient air (20.95 % O_2), in 15.7 % O_2 and in 10.4 % O_2 at temperatures of 10, 20 and 30 °C (some individuals did not exhibit discontinuous ventilation at every combination of temperature and P_{O_2}.

Barometric pressure was 97.1–97.8 kPa for all measurements.

The analysis was an analysis of covariance with log(mass) and ambient temperature as covariates.

Adjusted means are for a mass of 0.355 g at 20 °C.

P values with asterisks (*) are significant after a sequential Bonferroni correction for multiple simultaneous tests (Rice, 1989).

$f_{O,RMR}$, O phase fractional resting metabolic rate; $f_{O,Time}$, O phase fractional duration; O phase, open phase; C+F phase, closed+flutter phase.

pupae, *Phorocantha* spp. inhabit the vascular tissues and sapwood of eucalyptus trees, and therefore experience consistently humid environments that are probably buffered from the extremes of ambient temperature variation and seem likely to become hypoxic or hypercapnic. Adults are exposed to a considerably greater range of thermal and hygric

conditions. In southern California, *Phorocantha* spp. tend to emerge from pupation in late winter or spring (Hanks et al., 1995, 1996a), so the adult stage is mainly present in the warm, dry spring and summer months. Although nocturnal, active adults experience air temperatures comparable with those used in this study (10–30 °C over the months of April–August;
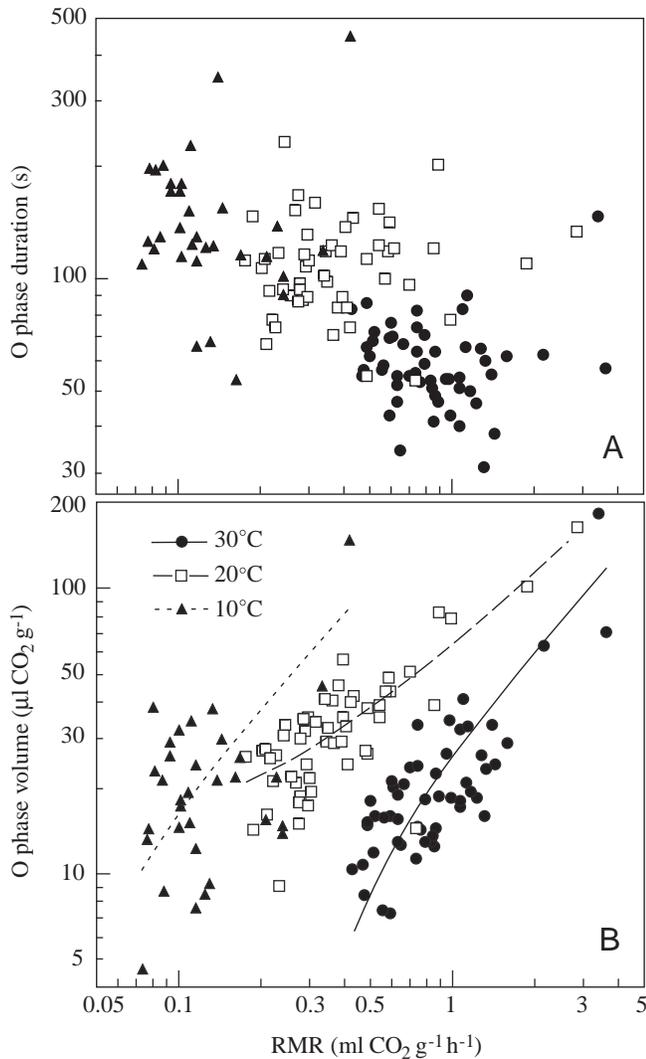
Fig. 7. (A) Relationship between resting metabolic rate (RMR) and open (O) phase duration at three different temperatures. Data are shown on log–log scales for clarity. O phase duration decreases as RMR increases across temperatures (r^2=0.09, $F_{1,138}$=13.3, P=0.00038), but correlations within each temperature are not significant. (B) Relationship between RMR and O phase volume at three different temperatures. Data are shown on log–log scales for clarity. There is essentially complete overlap of O phase volumes at the three temperatures, but significantly different within-temperature correlations between RMR and O phase volume (*V*). The regression equations are: 10 °C, V=−0.0053+0.209RMR, r^2=0.46, $F_{1,30}$=25.0, P=0.000025; 20 °C, V=0.012+0.0521RMR, r^2=0.84, $F_{1,52}$=265, P<0.000001; 30 °C, V=−0.0086+0.0338RMR, r^2=0.66, $F_{1,51}$=98, P<0.000001. All comparisons between temperatures are significant. The slopes of the regressions differ for 10 °C *versus* 20 °C (P=0.000005) and for 10 °C *versus* 30 °C (P=0.0000023). The slopes are similar for the 20 °C *versus* 30 °C comparison (P=0.45) but the intercepts differ (P<0.000001)

M. A. Chappell, unpublished data; Hanks et al., 1996a). Temperatures in the diurnal refugia used by adults (crevices under loose bark) are fairly similar but can exceed 35 °C on particularly hot days (M. A. Chappell, unpublished data).

Night-time humidity during the active season, although variable, is often is quite low, particularly in summer. Humidities in diurnal refugia tend to be higher than in open areas (M. A. Chappell, unpublished data). Although we lack data on gas concentrations in under-bark cavities, we speculate that these confined and poorly ventilated spaces, which can be shared by several beetles, may become somewhat hypoxic and hypercapnic. Thus, most of our various test conditions were a reasonably close match to the general environmental milieu experienced by adult *Phorocantha* spp.

Metabolic rate

Resting metabolic rates of *Phorocantha* averaged approximately 2.9 times higher than predicted for flightless insects of similar mass (Lighton and Fielden, 1995, 1996), but are within the expected range for volant species. The RMR measured in the present study averaged approximately 30 % higher than values previously reported for these beetles (Rogowitz and Chappell, 2000), and we did not find the rather small interspecific and intersexual differences noted in our earlier paper (in that study, gender divergences were much larger for maximal $\dot{V}_{CO_2}$ during running exercise than for RMR). The reason for the difference is not clear, but the overall relationship between mass and RMR was very similar in the two studies. Across a sixfold range of body mass, *Phorocantha* RMR scaled with $mass^{0.853}$, a value similar to the $mass^{0.825}$ scaling demonstrated for insects and spiders (across a much larger mass range) by Lighton and Fielden (1995).

In the context of investigating DGC accommodation of changing metabolic rates, our most salient finding about RMR is the extent of individual variation, which covered more than an order of magnitude even after correction for mass and temperature effects (Fig. 2). This is considerably larger than the typical individual variation reported for RMR or basal metabolic rate in vertebrates (e.g. Chappell and Bachman, 1994; Chappell et al., 1996, 1999; Bech et al., 1999). At least in part, the wide range may reflect individual differences in digestive status or reproductive condition (in their holding cages, the beetles had access to both food and mates). Whatever the cause of the metabolic variation, *Phorocantha* spp. exhibited a pronounced DGC across the entire observed range of RMR.

Repeatability

Although the statistical power of our tests was constrained by the relatively small sample size, we found significant repeatability in several primary determinants of the DGC (including frequency, O phase amplitude and O phase volume), together with positive *r* values in all other variables (Table 3). These results indicate considerable individual consistency in the DGC and provide validation for our correlative approach to studying DGC accommodation of changing RMR. We are aware of no comparable studies of the consistency of discontinuous ventilation, but repeatabilities for the DGC of *Phorocantha* are comparable with those reported for resting or maximal aerobic metabolic rate in vertebrates (Hayes and

Chappell, 1990; Chappell et al., 1995, 1996; Hayes et al., 1998; Bech et al., 1999) and in *Phorocantha* (Rogowitz and Chappell, 2000).

General characteristics of the DGC in Phorocantha

Most *Phorocantha* tested at low to moderate temperatures (10–20 °C) breathed discontinuously. In these individuals, the O phase was fairly brief (averaging 20–25 % of the DGC cycle) and its duration and volume were relatively constant across a wide range of RMRs (Figs 3, 4, 6). In contrast, the C+F phase comprised most of the DGC, and C+F duration was closely tied to overall DGC frequency (Fig. 6). Although the O phase occupied only a small portion of the DGC, the fraction of RMR emitted during this phase ($f_{O,RMR}$) and the relative O phase CO_2 emission rate (O phase gain) were high. Except for the absence of a clearly defined C phase (also noted in lubber grasshoppers; Hadley and Quinlan, 1993), these patterns resemble the general characteristics of the DGC described in a variety of terrestrial arthropods (Lighton, 1994, 1996; Lighton et al., 1993; Lighton and Wehner, 1993; Davis et al., 1999). We could not observe spiracle behavior directly during our measurements. We do not know, therefore, whether the spiracles remained partially open at all times or whether they did in fact close during the C phase but measurable quantities of CO_2 were emitted through other pathways.

That pattern changed markedly when *Phorocantha* were tested at 30 °C or above. Relatively few individuals showed a DGC at these T_a, values and, when it did occur, the O phase occupied a much larger fraction of the ventilatory cycle (Figs 3, 4); in some cases, there was little or no discernible period of stable, low $\dot{V}_{CO_2}$ between O phase bursts. In consequence, O phase duration was more closely coupled to DGC frequency than at lower T_a values (Fig. 6). Nevertheless, O phase volume remained relatively constant across the temperature range 10–30 °C, and O phase amplitude changed only slightly (Table 2; Fig. 4). Despite the larger $f_{O,Time}$ at high T_a values, $f_{O,RMR}$ changed little, and the relative importance of the O phase in the overall ventilation cycle (O phase gain) declined considerably compared with cooler T_a values (Fig. 4).

Water balance and hypoxia

Following its characterization in lepidopteran pupae, the DGC was generally assumed to have evolved as a mechanism to enhance water conservation. This dogma was based on the assumption that the F phase is largely convective, with an inward air flow generated by low intratracheal pressure (largely blocking outward diffusion of water vapor). Thus, there should be little or no respiratory water loss during two of the three phases of the DGC (C and F); since these phases comprise perhaps 80–90 % of the cycle duration, considerable water savings could result (for a review, see Lighton, 1996). In this scenario, the importance of the DGC in water conservation is obviously linked to ambient temperature. As temperature rises, saturation vapor pressures in the tracheae climb rapidly, and metabolic rate increases because of the Q_{10} effect. It follows that rates of respiratory water loss should be maximal at high

T_a, because of a combination of the high water vapor content of tracheal gas and the high ventilation rates to support elevated RMR. Accordingly, if the DGC is a functional or evolutionary response to water stress, it should be most pronounced at high T_a. This prediction does not appear to be true for adult *Phorocantha*, even though they are often exposed to warm temperatures and low humidities when active. At high T_a, we found a decrease in the proportion of animals that utilized a DGC and, in those individuals that did ventilate discontinuously, a substantial reduction (or elimination) of the C+F phase (i.e. the elements of the DGC that can effectively reduce respiratory water loss). These findings are not consistent with the hypothesis that the DGC of *Phorocantha* evolved as a water conservation mechanism ('hygric genesis'; Lighton, 1996).

The main alternative hypothesis is that the DGC evolved as a result of selection to facilitate gas exchange in the hypercapnic and hypoxic environments experienced by burrowing or cavity-dwelling arthropods ('chthonic genesis'; Lighton, 1996). In such conditions, a DGC is beneficial because it provides increased animal-to-environment concentration gradients for both O_2 and CO_2, thereby increasing rates of diffusional gas flux. In this model, water conservation can follow secondarily because the higher flux rates for O_2 and CO_2 reduce the time the spiracles need to be open. The genus *Phorocantha* appears to fit that model because larvae, pupae and adults extensively or exclusively utilize cavities with limited air movement or exchange (larvae burrow into and feed on *Eucalyptus* cambium tissue, pupation occurs in chambers excavated in sapwood and adults spend the daylight period resting in crevices or cracks in bark). Moreover, all three life stages probably have access to water. Larvae are in moist environments, pupation chambers are likely to be at least moderately humid and adults feed largely on nectar. As a caveat, it should be emphasized that active adults routinely experience strongly desiccating conditions (high temperatures and low humidities) for hours at a time in summer, and it seems likely that their daytime refugia are considerably drier than the microhabitats of the larvae and pupae.

Since chthonic challenges may be an important factor in the ecology of *Phorocantha* spp., their response to experimental hypoxia is of interest from both functional and evolutionary perspectives. Lighton (1996) and Lighton and Garrigan (1995) suggest that a DGC based on an inwardly convective F phase driven by low intratracheal pressures (a fundamental assumption in the 'hygric genesis' model) would respond to hypoxia by decreasing the duration of both the F phase and the DGC itself. Such a response is seen in lepidopteran pupae (Burkett and Schneiderman, 1974) and the scarab beetle *Aphodius fossor* (Chown and Holter, 2000). In contrast, a largely diffusive F phase is expected to respond to hypoxia by an increase in F phase duration. The latter prediction was verified in a headless ant preparation (*Camponotus vicinus*; Lighton and Garrigan, 1995). Our results with *Phorocantha* spp. are not conclusive, but favor the diffusive F phase model.

The duration of the DGC and the C+F phases tended to increase in animals exposed to 10.4 % oxygen (although neither change was significant; Table 4). However, at that P_{O_2}, we found a significant increase in C+F phase emission volume (2.4 times the value during normoxia) and a corresponding decrease in $f_{O,RMR}$ (47 % of RMR compared with 68.5 % during normoxia), as predicted by the diffusive F phase model. Increased C+F phase emission volumes at low P_{O_2} also occurred in *A. fossor* despite a decrease in C+F duration (Chown and Holder, 2000). This diversity of response patterns (even within the order Coleoptera), coupled with the small number of species tested, makes it difficult to confirm any evolutionary scenario and emphasizes the need for additional comparative data.

Mass scaling of the DGC

Lighton (1991, 1996) suggested that DGC frequency in insects is independent of mass and that the ubiquitous mass$^{0.6–0.9}$ scaling of metabolic rate is accommodated by a corresponding scaling in CO_2 emission volumes (primarily during the O phase). This was largely confirmed in a recent examination of a group of 1–2 g scarab beetles (Davis et al., 1999) and, across a somewhat wider range of body mass, by the present study. In *Phorocantha*, body mass strongly influenced emission volumes in both the O and C+F phases, but had no effect on DGC frequency, O or C+F phase duration, $f_{O,RMR}$, $f_{O,Time}$ or O phase gain. The O phase volume of *Phorocantha* scaled with mass$^{0.834}$, which is statistically indistinguishable from the scaling to mass$^{0.853}$ for metabolic rate.

The DGC frequency in *Phorocantha* spp. averaged approximately 2.8 mHz at 20 °C, or 4.6 mHz at 25 °C by multiple regression (equivalent to 10.1 and 16.6 cycles h^{-1}, respectively). In comparison with DGC frequencies of other insects tested at 24–25 °C, the value for these species of *Phorocantha* is similar to that of a tenebrionid weighing approximately 1 g (Lighton, 1991) but substantially higher than DGC frequencies in 1–2 g scarabs (approximately 0.4 mHz; Davis et al., 1999), a 0.74 g tenebrionid *Onymacris unguicularis* (approximately 0.62 mHz) and 2.9 g lubber grasshoppers (approximately 0.7 mHz; Hadley and Quinlan, 1993).

Accommodation of changing metabolic rate

In theory, species with discontinuous ventilation could accommodate changing rates of gas exchange by adjusting DGC frequency, the duration of the various phases of the DGC, the volumes (or rates) of gas emitted in the different phases or a combination of these responses. In all species studied to date, frequency modulation is a major factor in accommodation. Temperature-induced changes in RMR were accompanied by linear changes in DGC frequency in Namib Desert tenebrionid beetles (Lighton, 1991), ants (Lighton, 1988; Lighton and Wehner, 1993; Lighton and Berrigan, 1995), grasshoppers (Hadley and Quinlan, 1993) and dung beetles (Davis et al., 1999). However, other details, especially phase-specific

emissions, differ markedly. In the ant *Camponotus vicinus*, both DGC frequency and O phase volume were adjusted to accommodate changing RMR. The Q_{10} for frequency was 3.05; in combination with a Q_{10} of 0.61 for O phase volume, this large frequency change is matched to a Q_{10} of 1.86 for RMR (Lighton, 1988). In contrast, in another ant species (*Cataglyphus bicolor*; Lighton and Wehner, 1993) and several South African dung beetles (*Scarabaeus* spp.; Davis et al., 1999), accommodation to changing RMR is almost entirely due to frequency modulation, with little or no change in O phase volume. *Phorocantha* spp. have a similar response; their DGC accommodates temperature-induced shifts in RMR almost exclusively by changing frequency, mainly by a modulation of C+F phase duration (Fig. 6).

That picture changes somewhat when the accommodation of *Phorocantha* spp. at constant T_a is considered. In contrast to temperature-induced RMR changes, accommodation at constant T_a relies primarily on substantial changes in emission volume with relatively moderate (but nonetheless significant) changes in frequency (Fig. 5). Most of the change in emission volume occurred in the O phase, which maintained a constant duration but showed a dramatically increased amplitude (and hence emission rate) with increasing RMR (Figs 5, 7). However, the C+F phase was also affected significantly, with increased volume but decreased duration (i.e. higher emission rates) at high RMR (Fig. 5).

There are few comparative data on accommodation of variable RMR at constant T_a. Like *Phorocantha*, fasting ticks (*Amblyomma marmoreum*; the only other discontinuously ventilating arthropods for which within-temperature accommodation has been examined across a wide range of RMR), CO_2 emission during the O phase was modulated in proportion to RMR (Lighton et al., 1993). In the ant *Camponotus vicinus*, discontinuous ventilation persists with moderate activity; active individuals have a higher $\dot{V}_{CO_2}$ than resting ants at the same T_a, and this is accommodated largely by increased DGC frequency (Lighton, 1988). In different castes of two other ants (*Messor julianus* and *M. pergandei*; Lighton and Berrigan, 1995), variation in RMR was significantly, but not strongly, correlated with frequency and burst volume. These ants use a combination of both factors in accommodating variation in RMR (at least within the fairly narrow range of RMR encompassed by the study), but not in a consistent pattern.

The reason for the difference in accommodation within and between temperatures in *Phorocantha* spp. is unclear, but variability in the solubility and buffering of CO_2 may be partly responsible. Both factors change dramatically with temperature, but are presumably constant if T_a is stable. Hence, a simple model for accommodation of changing RMR at constant T_a can be proposed. As RMR increases, CO_2 is produced at higher rates from tissue respiration and, hence, accumulates more rapidly in the tissues and hemolymph. This will shorten the time necessary to reach a hypercapnic set point that initiates the O phase, thereby increasing DGC frequency. Higher rates of CO_2 production (with concomitant increases in internal P_{CO_2}) should

also lead to elevated CO_2 emission rates during a primarily diffusive F phase and during the diffusive (and possibly convective) O phase. Validation of this speculation, and clarification of many other functional and evolutionary questions about the DGC, requires considerable additional experimental work, together with more comparative data.

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