

## DISCONTINUOUS CO<sub>2</sub> EMISSION IN A SMALL INSECT, THE FORMICINE ANT *CAMPONOTUS VICINUS*

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### SUMMARY

Standard rates of oxygen consumption ( $\dot{V}_{O_2}$ ) and CO<sub>2</sub> production ( $\dot{V}_{CO_2}$ ) were measured by constant-volume respirometry in the formicine ant, *Camponotus vicinus* Mayr, at temperatures ranging from 10 to 40°C. Over this range, the Q<sub>10</sub> with regard to  $\dot{V}_{O_2}$  was 1.79, and with regard to  $\dot{V}_{CO_2}$ , 1.84. Multiple regression equations relating  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$  of inactive ants to mass (0.016–0.088 g) and temperature were calculated. Periodic CO<sub>2</sub> emissions ('bursts') were monitored with flow-through respirometry. Burst frequency increased exponentially with temperature (Q<sub>10</sub> = 3.05), from 8.14 h<sup>-1</sup> at 15°C to 81.4 h<sup>-1</sup> at 35°C, and was not significantly correlated with body mass over the mass range (0.041–0.086 g) investigated. Burst volume, which could be accurately measured in one ant, decreased with temperature (Q<sub>10</sub> = 0.61), thus yielding the observed  $\dot{V}_{CO_2}$  Q<sub>10</sub> of 1.84.

### INTRODUCTION

The dynamics of external gas exchange in insects has important implications in the measurement of insect metabolic rates; it also provides insights into the functioning of a respiratory system that is complex, efficient, and unique to insects and a few other arthropods. One of the most striking aspects of external gas exchange in insects is its discontinuous, or intermittent, nature. Reports of periodic emissions, or bursts, of CO<sub>2</sub> from large insects have been present in the literature for many years (Schneiderman, 1953; Punt, Parser & Kuchlein, 1957; Hamilton, 1964), and such reports have now become commonplace (see reviews by Miller, 1981; Kaars, 1981). In addition, O<sub>2</sub> uptake is intermittent (Punt *et al.* 1957; Bartholomew, Lighton & Louw, 1984; Bartholomew & Lighton, 1985; Lighton, 1987). The early observations were incorporated, in the course of investigations of moth pupae (Schneiderman, 1960; Levy & Schneiderman, 1966c), into the passive suction ventilation or PSV model of insect ventilation.

The PSV model is widely accepted. Briefly, it states that the insect ventilation cycle (from one CO<sub>2</sub> burst to the next) falls into three stages. Immediately after the

Key words: discontinuous ventilation, insect ventilation, intermittent CO<sub>2</sub> emission.

CO<sub>2</sub> burst, the insect closes its spiracles ('closed phase'), and very little external gas exchange takes place. Then, as O<sub>2</sub> is consumed and CO<sub>2</sub> accumulates in the haemolymph, the endotracheal P<sub>O<sub>2</sub></sub> of the insect begins to fall, causing a negative endotracheal pressure to develop. The low endotracheal P<sub>O<sub>2</sub></sub> finally causes the spiracles to open slightly on an intermittent basis, allowing a slow ingress of O<sub>2</sub>, but little egress of CO<sub>2</sub> ('flutter phase'). The low egress of CO<sub>2</sub> during the flutter phase is largely caused by the negative endotracheal pressure. Finally, triggered by rising P<sub>CO<sub>2</sub></sub>, the spiracles open completely and the accumulated CO<sub>2</sub> escapes rapidly by diffusion, augmented in large insects by abdominal pumping ('open phase'). In general, the model accords reasonably with observation and with predictions derived from it (see, for example, Brockway & Schneiderman, 1967; Crozier, 1979; Snyder, Ungerman & Breed, 1980; Edwards & Miller, 1986; Lighton, 1987).

Most studies of intermittent external gas exchange in insects have concentrated on large insects (>0.5 g body mass). These can be studied conveniently by electromyography, and do not require particularly sensitive gas analysis. The gas exchange patterns of small insects are therefore little known. Some indirect evidence suggests that PSV phenomena do occur in small insects. For example, intermittent spiracle opening has been reported in mosquitoes (Krafsur & Graham, 1970), small flies (Case, 1956) and fleas (Wigglesworth, 1953), and abdominal pumping has been observed in small phorid flies (Miller, 1979). However, none of these insects was studied in terms of gas exchange. The extent to which continuous diffusion through open spiracles may in practice replace or alternate with PSV in small insects, owing to their small size, has not been determined. A knowledge of the patterns of gas exchange in small insects is not only of intrinsic comparative interest, it is also required if their metabolic rates are to be measured appropriately. As many small insects, such as ants, are of considerable ecological importance, the lack of data in this area is unfortunate.

I report here the first measurements of discontinuous CO<sub>2</sub> emission patterns in individual ants, *Camponotus vicinus* (hymenoptera: formicidae), mean body mass 0.056 g, together with an analysis of the relationship between discontinuous CO<sub>2</sub> emission, metabolic rate and temperature in that species.

## MATERIALS AND METHODS

### *Animals*

Subcolonies (approx. 150 ants) of *Camponotus vicinus* were collected in July and August 1986 from dead oak trees on the James Reserve of the University of California, at an elevation of 1640 m in the San Jacinto mountains in southern California. Their chief food source is honeydew, supplemented by opportunistic scavenging (R. Snelling, personal communication). The subcolonies were housed in 2-l glass jars, half-filled with detritus collected from dead oak stumps, and kept in an air-conditioned laboratory at an ambient temperature of 23 ± 2°C. Food and water in

the form of a 20% w/v solution of sucrose in water was always available. Measurements on ants were made within 2 weeks of capture.

### *Respirometry*

Standard O<sub>2</sub> consumption rates ( $s\dot{V}_{O_2}$ ), standard CO<sub>2</sub> production rates ( $s\dot{V}_{CO_2}$ ) and respiratory quotients (RQ) of inactive ants were measured using a modification of a constant volume technique described by Lighton, Bartholomew & Feener (1987). Eight 20 ml syringes equipped with three-way valves were prepared by drilling a 2 mm diameter hole through each syringe barrel approximately 15 mm from the open end. Eight ants were weighed individually to 0.1 mg, and each ant was introduced into a syringe. Rubber-tipped plungers were inserted about 5 mm into the syringes so that the ants could not escape, but air could still flow through the 2 mm holes. Each syringe was flushed with outdoor air through its three-way valve at a rate of 100 ml min<sup>-1</sup>.

The syringes were placed in a computer-controlled temperature cabinet that was initially set to 10°C for 90 min. At the end of this period the syringe plungers were inserted to the 20 ml calibration marks, thus occluding the 2 mm holes, and the three-way valves were closed. After an interval (typically 0.5–2 h, depending on the temperature), 17 ml of the air inside each syringe was transferred to sample syringes, and outside air was pumped through the syringes again. The  $s\dot{V}_{O_2}$  and  $s\dot{V}_{CO_2}$  of the ants were determined from the O<sub>2</sub> and CO<sub>2</sub> concentration in the air samples. An Anarad AR-50 CO<sub>2</sub> analyser and an Applied Electrochemistry S-3A O<sub>2</sub> analyser measured gas concentrations in conjunction with a computerized data acquisition system (Lighton, 1985). Similar measurements of  $s\dot{V}_{O_2}$  and  $s\dot{V}_{CO_2}$  were made at 2–5°C increments from 10 to 40°C. The ants were allowed to equilibrate at each temperature for 45 min, and were visually monitored frequently before and during measurements. Data from ants that moved vigorously or tried to escape were not used. Temperatures throughout this study were measured with 24-gauge copper–constantan thermocouples attached to Bailey Bat-12 thermocouple thermometers or AD595 thermocouple preamplifier chips. Both were calibrated to 0.1°C against a mercury thermometer traceable to the US Bureau of Standards. All values of  $s\dot{V}_{O_2}$  and  $s\dot{V}_{CO_2}$  were converted to STP.

The frequency of CO<sub>2</sub> emission was monitored in real time with a computerized flow-through respirometry system. The CO<sub>2</sub> analyser (Anarad AR-50, range 0–0.05% CO<sub>2</sub>) yielded a resolution of 0.0001% when utilized with computerized noise reduction techniques (Lighton, 1985). To minimize dead space in the flow-through respirometry system, a 2 ml glass syringe barrel was employed as the respirometer chamber (Fig. 1). An ant was weighed to 0.1 mg, placed in the barrel, and a rubber plunger perforated by a hole 1 mm in diameter was inserted to the 1 ml calibration mark. Outdoor air was pumped at 100 ml min<sup>-1</sup> through a H<sub>2</sub>O scrubber and into the open end of the syringe barrel. This outdoor air, the CO<sub>2</sub> concentration of which remained constant at approximately 0.03%, constituted the baseline of the

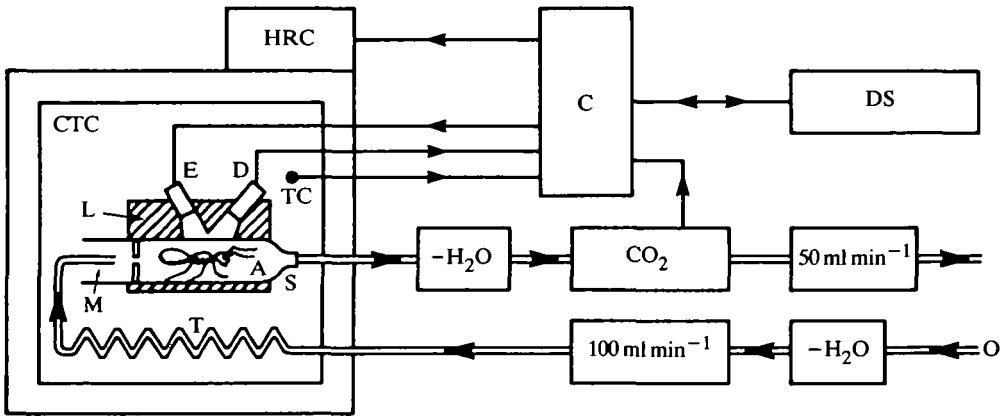


Fig. 1. Block diagram of the flowthrough respirometry system (not to scale). Double lines denote gas tubing; single lines denote electrical connections. Arrows denote direction of air flow (tubing) or direction relative to the computer (electrical connections). A box enclosing a flow rate denotes a combination of pump and flowmeter adjusted to give the indicated flow rate.  $-H_2O$  denotes a Drierite water scrubber. A, ant; C, computer;  $CO_2$ ,  $CO_2$  analyser; CTC, constant temperature cabinet; D, NIR detector; DS, disc storage; E, NIR emitter; HRC, heating and refrigeration control; L, Lucite support; M, pressure reduction manifold; O, source of outside air; S, syringe; T, thermal equilibration coil; TC, thermocouple.

system. Air was pulled at  $50 \text{ ml min}^{-1}$  from the respirometer chamber through a small  $H_2O$  scrubber, then through the  $CO_2$  analyser. Flow rates were measured with Brooks or Gilmont rotameters calibrated against a computerized bubble flowmeter (Bartholomew & Lighton, 1986). The temperature of the cabinet containing the respirometer system was controlled by computer (see below).

Because the  $CO_2$  emission patterns of inactive ants were susceptible to disturbance by activity (see Results), a method of monitoring ant movement was necessary. Specular near-infrared (NIR) reflection, utilizing NIR radiation from a light-emitting diode driven at 10 mW and detected by an NIR photodarlington transistor at an angle of  $45^\circ$  to the source, gave good results. The source and detector were positioned about 6 mm from the ant, outside the respirometer syringe, supported in a Lucite housing (Fig. 1). Movements of the ant that were barely visible to the naked eye resulted in large fluctuations in the output of the detector. Lack of movement corresponded to a fixed output at a level determined by the precise orientation of the ant relative to the NIR source and detector. As information on ant movement was confined to changes in detector output, the squared first derivative of the detector output was employed as an operational index of ant activity.

$CO_2$  concentration, ambient temperature and ant activity were monitored by a computer at 2.5 s intervals and stored on floppy disks at the end of each recording. Data were also graphed in real time on the computer monitor. Each recording (typical duration; 26 min) consisted of 640 data points on each of three channels. Between and during recordings, the computer controlled the temperature of the

cabinet containing the respirometer chamber to within 0.5°C of a programmed setpoint. Recordings commenced after 90 min equilibration at 15°C. At the end of each subsequent recording, the setpoint was ramped upwards by 5°C over a period of about 30 min, and the ant was equilibrated at the new temperature for a further 45 min before a new recording took place. After a recording at 35°C, the ramping direction was reversed until 15°C was reached, after which the temperature was ramped upwards again. A typical series of recordings of an individual ant consisted of 2–4 complete temperature cycles.

During analysis, each set of recordings was inspected for ant movement. Data from recordings during which the ant was active were not used. A peak-finding algorithm located each CO<sub>2</sub> emission peak (burst) and measured its height relative to the inter-burst sections, yielding the mean inter-burst period (in s) and the mean burst amplitude (in percent CO<sub>2</sub>). The mean temperature over the same period, which was always within 0.5°C and usually within 0.05°C of the setpoint, was also calculated.

To yield adequate temporal resolution of the CO<sub>2</sub> emission bursts, a comparatively high rate of air flow past the ant was required. This flow rate necessarily reduced the amplitude of the bursts, typically to approximately 0.0025% CO<sub>2</sub>. Because slow, random instrument drift often occurred during recordings, accurate linear baselines (i.e. stable to within 0.00005%) could not be reliably achieved. This did not affect data on burst frequency, but complicated determination of burst volumes, except in the case of very large ants (see Results). As a high rate of flow relative to the respirometer volume was used, and as each CO<sub>2</sub> burst was a discrete event that did not significantly overlap its neighbouring bursts, the application of washout-compensation equations was not necessary.

### Statistics

All means are accompanied by standard deviations and sample sizes. Differences between means were tested with Student's *t*-test, the paired *t*-test, or with the approximate *t*-test if variances differed significantly. Single and multiple regression analyses were carried out by the least squares method. Differences between regressions were tested by analysis of covariance. Results were considered significant at  $P < 0.05$ .

## RESULTS

### Standard metabolic rate

The standard metabolic rate of *Camponotus vicinus* was strongly dependent on temperature and body mass. Over the investigated range of temperatures (10–40°C) and body masses (0.016–0.088 g), the relationship between  $s\dot{V}_{O_2}$ , body mass and temperature was determined by multiple regression analysis to be:

$$\log s\dot{V}_{O_2} = -1.980 + 0.481(\log M) + 0.0254T, \quad (1)$$

where  $s\dot{V}_{O_2}$  is in  $\text{ml h}^{-1}$ ,  $M$  is body mass in g and  $T$  is temperature in  $^{\circ}\text{C}$ . The coefficient of multiple correlation was 0.936 ( $N = 89$  observations on 18 ants;  $P < 0.001$ ). There was a similar relationship between  $s\dot{V}_{CO_2}$ , body mass and temperature:

$$\log s\dot{V}_{CO_2} = -2.087 + 0.485(\log M) + 0.0264T, \quad (2)$$

where  $s\dot{V}_{CO_2}$  is in  $\text{ml h}^{-1}$  (coefficient of multiple correlation = 0.911;  $N = 89$  observations on 18 ants;  $P < 0.001$ ). Use of the above equations allows the  $s\dot{V}_{O_2}$  and  $s\dot{V}_{CO_2}$  of individual *Camponotus vicinus* to be predicted with good accuracy from body mass and ambient temperature.

The RQ of inactive ants was  $0.828 \pm 0.128$  ( $N = 89$  observations on 18 ants). The  $Q_{10}$  of *Camponotus vicinus* was 1.79 with respect to  $s\dot{V}_{O_2}$ ; when measured with respect to  $s\dot{V}_{CO_2}$ , the  $Q_{10}$  increased slightly to 1.84. This increase was not sufficient to cause a significant dependence of measured RQ on temperature.

#### *CO<sub>2</sub> emission frequency*

Ants in the body mass range 0.041–0.086 g (mean mass  $0.057 \pm 0.012$  g,  $N = 13$ ) invariably showed periodic  $\text{CO}_2$  emissions when quiescent (Fig. 2). The 45 min equilibration period that followed the approximately 30 min adjustment of the temperature to its new setpoint, was sufficient to stabilize burst frequency. Mean burst frequencies at a given temperature were not significantly affected by the temperature ramping direction (paired  $t$ -test on a sample of 12 pairs of burst

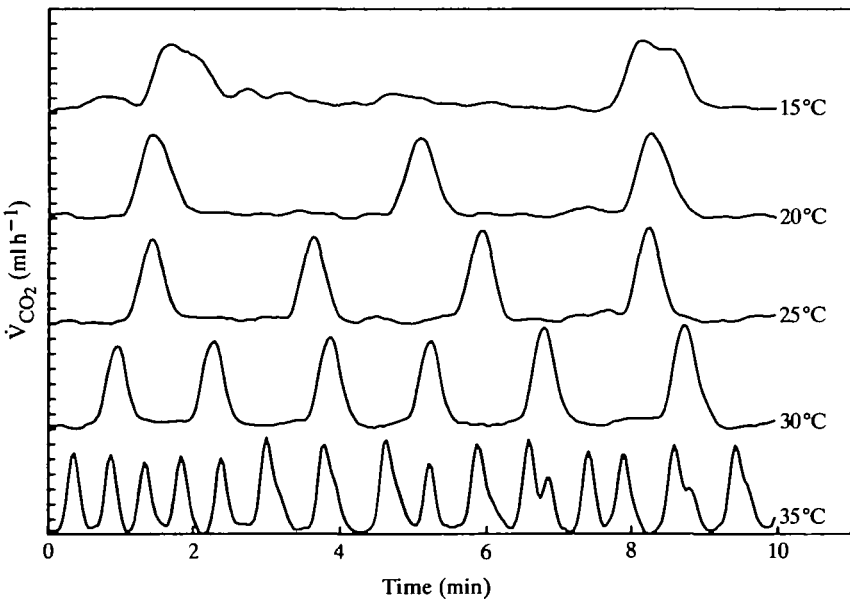


Fig. 2. Periodic  $\text{CO}_2$  emission from an individual ant, *Camponotus vicinus* (body mass 0.086 g) at five temperatures. Each mark on the y-axis represents  $0.01 \text{ ml h}^{-1}$  of  $\text{CO}_2$  emission.

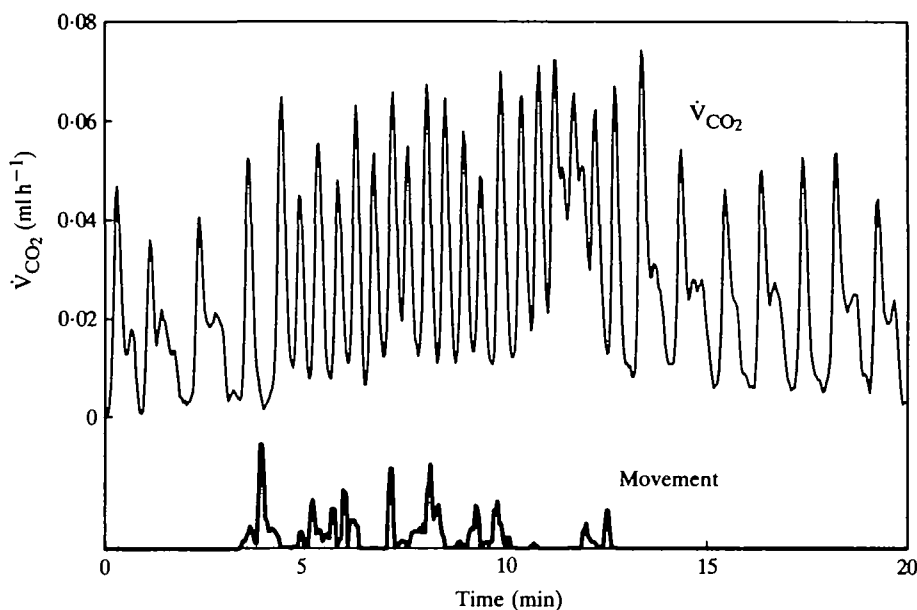


Fig. 3. The effect of movement (lower, thicker trace) on CO<sub>2</sub> emission patterns (upper, thinner trace) of an individual ant, body mass 0.047 g, at 30°C. Burst frequency while quiescent = 16.9 mHz (60.8 h<sup>-1</sup>); while active, 34.5 mHz (124.2 h<sup>-1</sup>).

frequencies with one positive and one negative ramping direction per pair = 0.66;  $P > 0.3$ ). The ants were exposed to a stream of dry air for fairly protracted periods, and it is possible that this affected their ventilation parameters. As conditions for yielding quantifiable and reproducible results conflicted with probable field conditions, the exact nature of the ants' ventilation parameters in still, moist air is therefore unknown, but it is most unlikely, in view of the relative consistency of their responses, that it differs fundamentally from that described here.

The burst frequency changed, and was sometimes completely disrupted, if the ant was active (Fig. 3). Insect ventilation is generally sensitive to changes in behaviour (Weber & Caillere, 1978; Lighton, 1985, 1987). Ants of smaller body mass also showed periodic CO<sub>2</sub> emissions, but remained consistently active in the respirometer chamber. As data on CO<sub>2</sub> emission from these ants could not be interpreted reliably, they were not used.

The frequency of CO<sub>2</sub> emission, or burst frequency, was strongly dependent on temperature (Fig. 2), but was also somewhat variable, both within and between individuals. Owing to this variability, burst frequency could not be demonstrably linked with body mass over the two-fold mass range investigated. Compared with explaining burst frequency variation on the basis of temperature alone, the use of multiple regression analysis incorporating both body mass and temperature increased the percentage of explained burst frequency variation by only 0.1% (from 85.5 to 85.6%). In addition, the scaling exponent of body mass was very low (0.1).

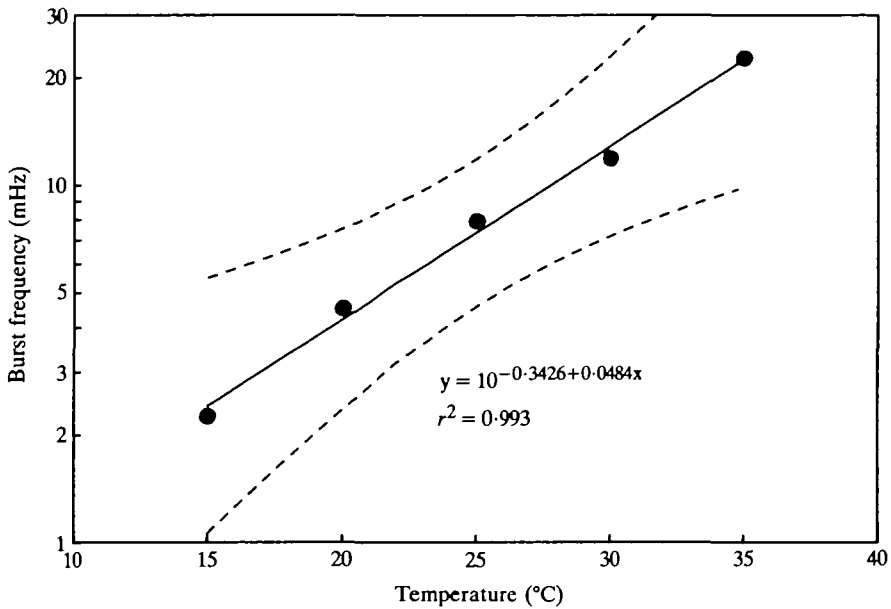


Fig. 4. The relationship between burst frequency and temperature.  $N = 53$  observations on 13 ants (see text). The dashed curves enclose the 95% confidence limits of the line.

Means of burst frequencies at each temperature were therefore calculated and analysed in terms of temperature alone.

Burst frequency increased exponentially from  $2.26 \pm 0.70$  mHz,  $N = 7$  ( $8.14 \text{ h}^{-1}$ ) at  $15^\circ\text{C}$  to  $22.61 \pm 5.16$  mHz,  $N = 8$  ( $81.4 \text{ h}^{-1}$ ) at  $35^\circ\text{C}$  (Fig. 4). The slope of the relationship between log-transformed burst frequency and temperature (0.0484) corresponds to a  $Q_{10}$  of 3.05. This is considerably higher than the observed  $Q_{10}$  of the relationship between log-transformed  $\dot{V}_{\text{CO}_2}$  and temperature (1.84). Unlike burst frequency, burst amplitude did not vary significantly with temperature (Fig. 5).

## DISCUSSION

### *Standard metabolic rate*

Considering the diversity and ecological importance of ants, their standard metabolic rates remain largely underexplored (Jensen, 1978). In addition, published accounts show little standardization of measurement conditions. The behavioural state of ants in respirometers ranges from activity (Mackay, 1982) to anaesthetic-induced immobility (Holm-Jensen, Jensen & Nielsen, 1980). The condition and feeding state of the ants has varied widely, probably with consequences on metabolic rates (Keister & Buck, 1964). Groups of ants or individual ants have been measured interchangeably by different investigators, raising the question of 'group effects' on standard metabolism, which can be negative or positive (Galle, 1978; Torossian, 1978; G. A. Bartholomew, J. R. B. Lighton & D. H. Feener, in preparation). In



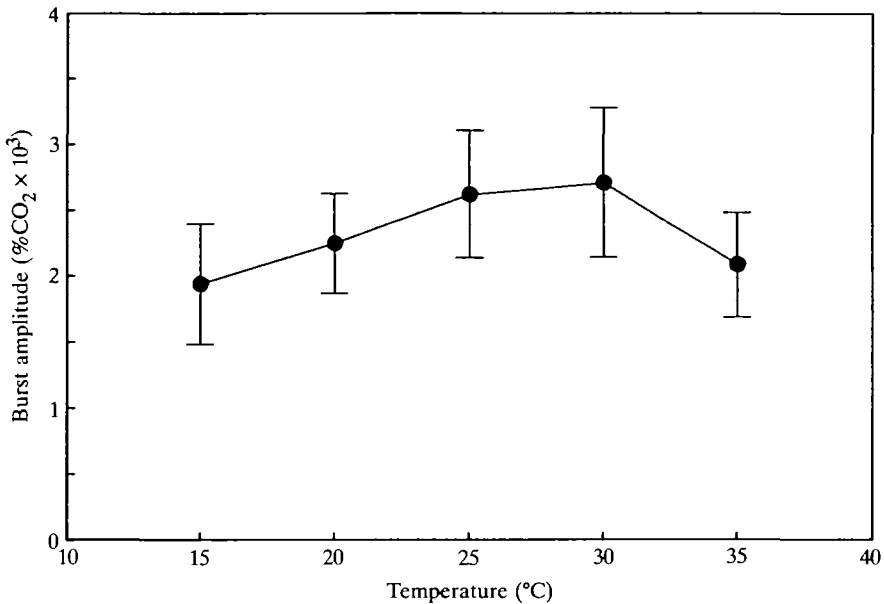


Fig. 5. The relationship between burst amplitude and temperature. The bars are  $\pm 1$  S.D.  $N = 53$  observations on 13 ants (see text). Differences between means are not significant.

addition, although many ant species are markedly polymorphic, most investigators have only published species means of body masses and standard metabolic rates. Allometric regressions derived from species means (e.g. Jensen & Nielsen, 1975; Jensen, 1978; Lighton *et al.* 1987) yield a larger scaling exponent than those derived from intraspecific data; interspecific regressions of  $\dot{V}_{O_2}$  on mass in invertebrates generally yield a scaling exponent of  $> 0.8$ , while most intraspecific regressions yield a lower exponent (approx. 0.67; see Heusner, 1985, for a discussion). Use of interspecifically derived regressions in an intraspecific context can therefore be inaccurate and misleading, especially in the case of ants very different in body mass from the species mean. The effect of temperature on metabolic rate is in similar confusion, with many investigators using arithmetic rather than exponential regressions to analyse their data, in spite of the marked curvilinearity of the  $\dot{V}_{O_2}$ -temperature relationships. These problems make the  $s\dot{V}_{O_2}$  data on *Camponotus vicinus* difficult to compare with those of other ant species.

Until a more standardized way of measuring and estimating ant standard metabolic rates is found, accurate prediction across the body mass range of a given species at various temperatures requires the use of multiple regression equations such as equation 1 on a species by species basis. Interestingly, equation 1 and the allometric equation of Lighton *et al.* (1987) yield identical results at 20°C and a body mass near 0.020 g, which is close to the mean species body mass of *Camponotus vicinus*. During collection of the subcolonies for the present study, large ants were selectively captured.

The mass scaling exponent of *Camponotus vicinus*  $s\dot{V}_{O_2}$  is unusually low (0.481). This implies that the relative proportion of metabolically active tissues is smaller in major workers than in minor workers (see Heusner, 1985). It is possible that the larger ants act as a fat or water depot, in addition to their role in colony defence. A similarly low scaling exponent of 0.545 is present in a congener of similar size from a completely different habitat, the Namib Desert gravel plains 'balbyter' ant *Camponotus fulvopilosus* (J. R. B. Lighton, unpublished data), in which a similar role for the major workers is possible. The  $Q_{10}$  of *Camponotus fulvopilosus* (1.90) is also similar to that of *Camponotus vicinus*, and considerably lower than most ant  $Q_{10}$  values in the literature; e.g. 3.10 (five North European species; Jensen & Nielsen, 1975) and 2.52 (*Formica rufa*; Holm-Jensen *et al.* 1980).

#### Intermittent CO<sub>2</sub> emission

##### $Q_{10}$ effects

Several different hypotheses can be advanced to explain the difference in  $Q_{10}$  between  $s\dot{V}_{CO_2}$  and burst frequency. Two of these hypotheses, which are not mutually exclusive, are readily testable. First, the volume of CO<sub>2</sub> emitted with each burst may decline with increasing temperature, thus compensating for the high  $Q_{10}$  of burst frequency. If CO<sub>2</sub> emission between bursts is negligible, then the volume of CO<sub>2</sub> emitted during a given time interval (e.g. 1 h) is equal to the mean volume of bursts over that interval, multiplied by burst frequency (e.g. in bursts h<sup>-1</sup>). From this it follows that burst volume at a given temperature can be predicted from allometrically calculated  $\dot{V}_{CO_2}$  (equation 2) divided by calculated burst frequency (equation in Fig. 4, scaled from mHz to h<sup>-1</sup> by multiplying by 3.6).

This first hypothesis can be tested in two ways. Firstly, the slope of log-transformed burst volumes, regressed against temperature, should yield a  $Q_{10}$  of 1.84/3.05, or 0.60. Second, log-transformed  $\dot{V}_{CO_2}$ , predicted on the basis of measured burst volumes and predicted burst frequency, and regressed against temperature, should yield a slope corresponding to a  $Q_{10}$  of 1.84. If either measured  $Q_{10}$  differs significantly from its predicted value, then the first hypothesis can be falsified. These tests, which are best carried out by analysis of covariance, are not sensitive to the presence of interburst CO<sub>2</sub> emission with a  $Q_{10}$  of approx. 1.84; any such CO<sub>2</sub> emission offsets the measured values from the predicted values, causing intercepts to differ, but does not affect slopes ( $Q_{10}$  values).

The second hypothesis assumes the existence of substantial CO<sub>2</sub> emission between bursts, the magnitude of which is dependent on temperature ( $Q_{10}$  = approx 1.84). This will result in a temperature-dependent discrepancy between measured and predicted burst volumes, and between measured and predicted  $s\dot{V}_{CO_2}$  values. If such a discrepancy is present, and if the first hypothesis is not disproved, the predicted values of  $s\dot{V}_{CO_2}$  will be higher than the measured values, leading to a constant offset of log-transformed burst volume and of log-transformed  $s\dot{V}_{CO_2}$  relative to predicted values (see above), when regressed against temperature. If no such offset exists, then the second hypothesis is falsified.

Because accurate measurement of burst volumes requires a very stable baseline, such measurement was not practical on most recordings. However, one series of CO<sub>2</sub> emission recordings of a large ant (0.086 g) was characterized by unusually stable baselines. CO<sub>2</sub> emission bursts (2–20 per recording, depending on temperature; three recordings per temperature) were integrated to yield volumes of CO<sub>2</sub> in cm<sup>3</sup> per burst. The baseline for integration was the mean of the lowest readings within 10 s to either side of each burst. The mean burst volumes were then regressed against temperature (Fig. 6), and compared to the predicted burst volumes of an ant of body mass 0.086 g.

When regressed against temperature, the slopes of predicted and measured log-transformed burst volumes are statistically indistinguishable (common slope =  $-0.0212$ ;  $F = 0.91$ ;  $df = 1, 6$ ;  $P = 0.4$ ), corresponding to a  $Q_{10}$  of 0.61, which is very similar to the predicted value of 0.60. Similarly, the predicted and measured log-transformed  $s\dot{V}_{CO_2}$  values, when regressed against temperature, have statistically indistinguishable slopes (common slope =  $0.0268$ ;  $F = 0.91$ ;  $df = 1, 6$ ;  $P = 0.4$ ), corresponding to a  $Q_{10}$  of 1.85, which is very similar to the predicted value of 1.84. Burst volumes therefore decreased with increasing temperature as predicted by the first hypothesis.

In both of the above regressions, however, the measured intercepts differed significantly from the predicted intercepts ( $F = 50$ ;  $df = 1, 7$ ;  $P < 0.001$ ). This can be explained by a temperature-dependent interburst emission of CO<sub>2</sub> equal to about 20% of total  $s\dot{V}_{CO_2}$ , which is in accordance with the second hypothesis (but see below).

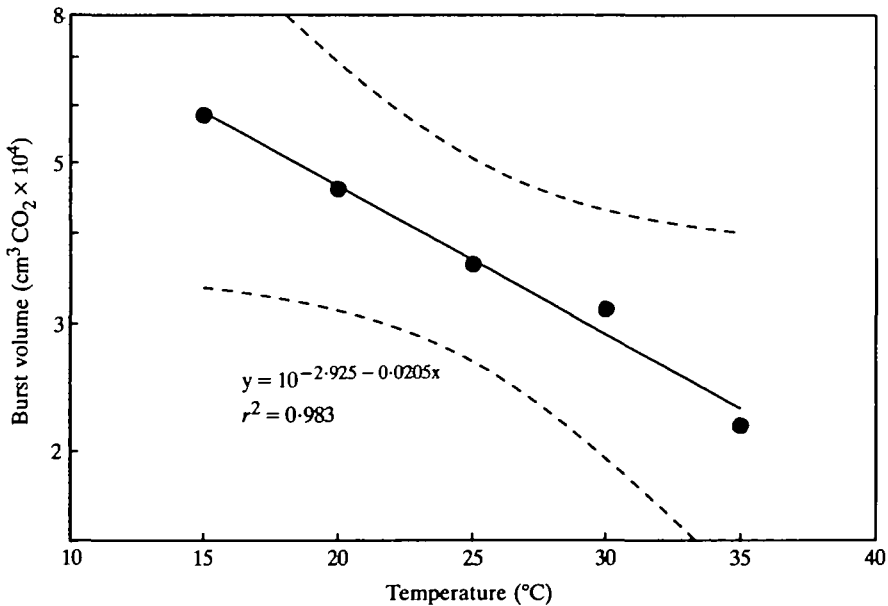


Fig. 6. The relationship between burst volume and temperature. See text. The dashed curves denote the 95% confidence limits of the line.

*General comments*

The patterns of intermittent CO<sub>2</sub> emission in *Camponotus vicinus* are compatible with the theory of PSV ventilation, and in general resemble those described in larger insects, except that the emissions occur at a higher frequency. Thus, in this species of small insect, at least at rest, PSV ventilation has not been replaced by or augmented with continuous diffusion through open spiracles. The chief selective advantage of PSV in small insects probably lies in a reduction of respiratory water loss rates, as a consequence of prolonged apnoea or incurrent-only air movement (see Miller, 1964; Loveridge, 1968; Krafur, 1971). The emission of CO<sub>2</sub> in *Camponotus vicinus* is presumably effected by diffusion rather than by active ventilation. In spite of the sensitivity of the motion detection system, no trace of movements that synchronized with CO<sub>2</sub> bursts was detected.

Although burst frequencies in *Camponotus vicinus* are generally higher than in larger insects at similar temperatures, the frequencies are low enough at low temperatures to cause significant inaccuracy in constant-volume or constant-pressure respirometry if periods of measurement are too short, or if pressure readings are taken immediately after a CO<sub>2</sub> burst, particularly if CO<sub>2</sub> absorption is slow. In addition, the effect of anaesthetics on the ventilation cycle should be investigated by workers who employ such drugs when attempting to measure 'basal' metabolism in ants (Holm-Jensen *et al.* 1980; Jensen & Holm-Jensen, 1980).

The significance of temperature-dependent inter-burst CO<sub>2</sub> production is difficult to assess. In a flowthrough system at a flow rate of 50 ml min<sup>-1</sup>, for example, the magnitude of this component at 15°C is equal to an offset of 0.000033 % CO<sub>2</sub>, which rises to 0.00011 % CO<sub>2</sub> at 35°C. The latter is just above the noise floor of the CO<sub>2</sub> analyser; the former is not. The 'continuous' component alluded to above may therefore be an artefact. However, such a temperature-dependent component has been described in saturniid pupae (Buck & Keister, 1955), and seems reasonable on theoretical grounds. As temperature increases, metabolic rate increases and the solubility of CO<sub>2</sub> decreases, which will lead to a higher endotracheal P<sub>CO<sub>2</sub></sub>. This should lead, in turn, to a higher interburst CO<sub>2</sub> emission rate, assuming that the efficiency of CO<sub>2</sub> containment does not change with temperature.

The well-defined reduction of burst volume with temperature, noted above, is somewhat puzzling. It appears that the central hypoxic and peripheral hypercapnic controls of the PSV cycle (see Kaars, 1981, for a review) may not modulate linearly with metabolic rate. Similar reductions of burst volume with increasing temperature were noted by Buck & Keister (1955) and Schneiderman & Williams (1955) in moth pupae. To my knowledge, these are the first such data on adult insects. The phenomenon runs counter to expectations, as there is a direct relationship between temperature and the P<sub>CO<sub>2</sub></sub> that triggers the burst phase (Levy & Schneiderman, 1966b). In addition, if low temperatures decrease the P<sub>CO<sub>2</sub></sub> threshold at which spiracular fluttering maintains a constant endotracheal P<sub>O<sub>2</sub></sub> that matches the insect's s $\dot{V}$ <sub>O<sub>2</sub></sub> (Levy & Schneiderman, 1966a), then this lower P<sub>O<sub>2</sub></sub> will also cause a decrease in the CO<sub>2</sub> burst threshold (Schneiderman, 1960), further reducing the likelihood of

larger burst volumes at lower temperatures. The most feasible explanation of the inverse relationship between temperature and burst volume is a decrease in haemolymph CO<sub>2</sub> buffering capacity with increasing temperature, of sufficient magnitude to counter the other effects. A component of interburst CO<sub>2</sub> emission that increases with temperature will also lead to a reduction of burst volumes with increasing temperature. Other factors may, in combination, contribute to the observed effect. However, the direct effects of temperature may be less important than changes in metabolic rate *per se*. For example, an increase in metabolic rate owing to activity at a constant temperature can also increase burst frequency (Fig. 3). It could therefore be hypothesized that a larger P<sub>CO<sub>2</sub></sub> in the haemolymph and endotracheal space may be tolerated when the rate of CO<sub>2</sub> production is low than when CO<sub>2</sub> production rate rises as a consequence of activity or an increase in temperature.

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