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SHORT COMMUNICATION

DIRECT MEASUREMENT OF MASS LOSS DURING DISCONTINUOUS VENTILATION IN TWO SPECIES OF ANTS

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A central controversy in the field of insect ventilation is the effect of the discontinuous ventilation cycle (DVC) on respiratory water loss rates. An accurate method of measuring water loss rates during the ventilation cycle would provide a direct means of resolving the controversy. It would also create opportunities for the comparative study of respiratory water loss rates in insects that employ different ventilation strategies and occupy different habitats.

Investigators have measured respiratory water loss in insects using alumina hygrometer chips (Hadley and Quinlan, 1982), dewpoint hygrometers (Lighton, 1988) and continuous measurement of mass loss (Kestler, 1985; Machin *et al.* 1991). These systems have their strengths and weaknesses: alumina-chip hygrometers respond relatively slowly (though this may not always be so) and require frequent and very careful calibration, while dewpoint hygrometers, which in general do not require frequent calibration, are not sensitive enough to track tiny humidity changes accurately; in addition they are slow-responding and prone to tracking artifacts. The primary advantage of the mass loss technique is its appealing directness. However, it requires knowledge of CO_2 emission and O_2 uptake volumes, or compensation for these volumes determined by separate weighing in water-saturated air, and may additionally be affected to some extent by weight changes (*sensu stricto*) owing to O_2 and N_2 balance and volume and density changes (see Kestler, 1985, for a discussion). It cannot be used with active insects. Further, it would seem that sensitivity limitations must militate against its use with second-by-second temporal resolution in small insects that may lose, for example, 100 μg h^{-1} of both cuticular and respiratory H_2O.

I report here the use of mass loss measurements to estimate respiratory water loss rates during the ventilation (V) phase of small insects (two species of formicine ants; *Camponotus vicinus* (Mayr), common throughout arid to mesic areas of the Southwestern United States, and *Cataglyphis bicolor* (Fab.), a common xeric thermophilic scavenger from Tunisia).

I solved the central problem of activity, which disrupts both ventilation and mass loss measurements, by decapitating the ants and carefully sealing the resulting wound with a

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low-melting-point wax. After allowing about 2 h for recovery, the ants were profoundly passive and ventilated with clearly discernable closed, flutter (F) and ventilation (V) phases (see below; see also J. R. B. Lighton, T. Fukushi and R. Wehner, in preparation). Prior to measurement, I immobilized the ants by affixing the tips of their tarsi to a small square of paper, so that they stood in a normal posture, because decapitated ants could stand and walk, though undirectedly, and some restraint was necessary to prevent them from escaping from the apparatus. The effect, if any, of decapitation on cuticular water loss rates has not been resolved (Noble-Nesbitt and Al-Shukur, 1987; Machin, *et al.* 1986; Machin *et al.* 1991) but is unlikely, based on current evidence, to alter the conclusions of this study significantly.

I conducted the experiments at 25±1 °C. First, I weighed, beheaded and sealed an ant, allowed it to recover for 2 h, and measured its ventilation characteristics by flow-through CO_2 respirometry for 2–3 h in dry air as described elsewhere (Lighton, 1990). I then transferred it to the weighing pan of a Cahn C-32 ultramicrobalance (tared to its 25 mg range with 0.1 μg resolution) and recorded its mass, using a sampling interval of 2 s with approximately 10 subsamples averaged for each data point recorded. The weighing chamber was desiccated to near 0 % relative humidity by an open container of Drierite, and static-induced artifacts were reduced by a low-level source of ionizing radiation. I used DATACAN V software (Sable Systems, Salt Lake City, Utah) for all data acquisition and analysis.

The mass loss events are clearly visible after differentiation of the mass loss trace to yield mass loss rate in mg h^{-1} (Fig. 1). Their periodicity is statistically equivalent to that of the observed ventilation phases (Table 1). A continuous basic mass loss component

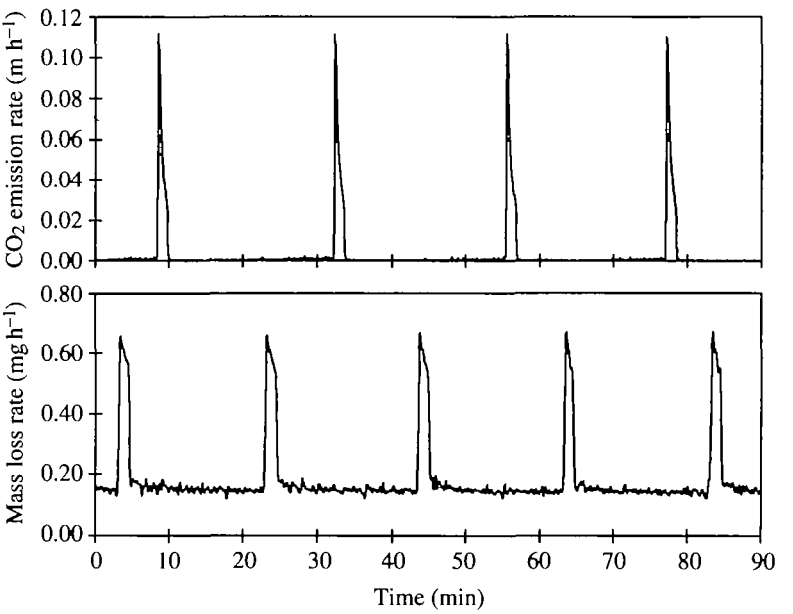

Fig. 1. CO_2 emission and mass loss rates measured successively at 25 °C on the same ant, a decapitated *Cataglyphis bicolor* worker; original body mass 31.6 mg. DVC frequency was approximately 0.8 mHz in each case.

Table 1. *A comparison of DVC frequency, CO_2 emission, and V phase mass loss (=estimated H_2O loss at a respiratory quotient of 0.727; see text) in two species of ants at 25±1 °C*

	Camponotus vicinus	*Cataglyphis bicolor*	Significance
Body mass (mg)	35.15±4.91	24.50±7.75	***
$v\dot{V}_{CO_2}$ ($ml\,g^{-1}\,h^{-1}$)	0.237±0.094	0.145±0.067	**
Burst volume ($\mu l\,CO_2\,g^{-1}$)	21.42±7.24	39.06±6.78	***
Burst frequency (mHz, CO_2)	3.133±1.068	1.152±0.847	***
Burst frequency (mHz, mass)	2.611±1.097	1.391±1.104	**
Burst mass loss ($\mu g\,g^{-1}$)	55.32±16.14	187.2±81.75	***
Interburst mass loss ($mg\,h^{-1}\,g^{-1}$)	6.651±3.85	6.337±5.03	ns
$\mu g\,H_2O\,\mu l^{-1}\,CO_2$	2.583±0.347	4.792±1.731	***

N=6 for each species.

Mass is total original body mass. $v\dot{V}_{CO_2}$, rate of CO_2 loss *during the ventilation phase of the DVC only*.

The burst frequencies, measured first in terms of CO_2 production and then in terms of differentiated mass loss, do not differ significantly within species (t-test $P > 0.2$).

Interburst mass loss rates are measured between V phases. Student's t-tests were used to test the statistical significance of differences between the means: ** $P<0.01$; *** $P<0.001$; NS, not significant, $P>0.05$.

Values are mean±S.D.

was always evident, with the respiratory events rising above that level. I calculated total mass loss over each ventilation event by integration, using the basic (cuticular) mass loss rates as the baseline.

To estimate water loss rates from the measured mass loss rates, I made the simplifying assumption that mass changes owing to O_2 uptake and CO_2 loss were equivalent, thus assuming a respiratory quotient (RQ) of 0.727, equivalent to the molar ratio of O_2:CO_2. O_2 uptake was assumed to be constant throughout the DVC (Schneiderman and Williams, 1953). Weight changes caused by shifts in internal N_2 concentration and volume were assumed to be negligible. This approach is similar to that taken by Machin *et al.* (1991), except that I was able to measure CO_2 emission volumes during the V phase in separate measurements and could thus calculate the ratio of H_2O lost per unit of CO_2 emitted.

Some informative differences between the two ant species emerge (Table 1). In particular, *Cataglyphis bicolor*, the more xeric of the two species, has a far lower DVC frequency than does *Camponotus vicinus*, a tendency noted in another arid-adapted ant species, *Camponotus detritus* (Lighton, 1990). Reflecting this tendency is *Cataglyphis bicolor*'s far larger V phase CO_2 emission volume – in spite of which, it has a far lower $\dot{V}_{CO_2}$, as estimated from V phase CO_2 emission volume and frequency, than does *Camponotus vicinus*. Its lower DVC frequency and larger F phase contribution to total CO_2 loss (Lighton and Wehner, 1993) presumably cause this effect.

Most surprisingly, the estimated respiratory water loss per V phase in *Cataglyphis bicolor*, a xerophilic ant, is over threefold higher than in the mesic *Camponotus vicinus* (Table 1). This is only partly explained by *C. bicolor*'s larger V phase CO_2 emission volume; *Cataglyphis bicolor* also loses more H_2O per unit of CO_2. The possibility that the

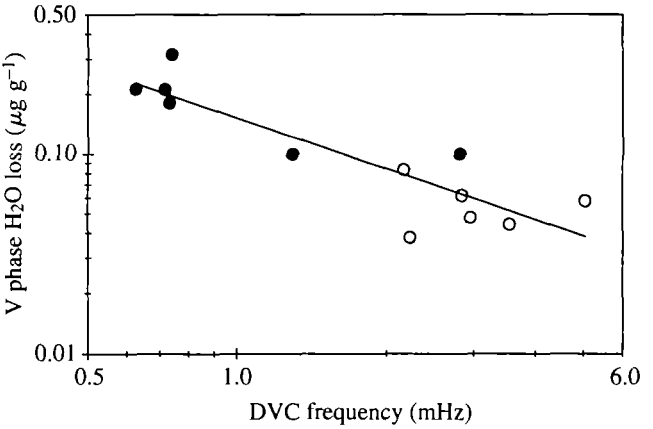

Fig. 2. The relationship between DVC frequency and estimated respiratory water loss rates during the V phase in two species of ants; *Camponotus vicinus* (open circles) and *Cataglyphis bicolor* (filled circles). For the entire data set, estimated V phase H_2O loss in $\mu g\,g^{-1}$ is $0.152F^{-0.851}$ where F is DVC frequency in mHz (r^2=0.78; P=0.0001).

two species react differently to the experimental protocol cannot be ruled out. However, the two species appear to occupy overlapping areas of a continuum relating respiratory water loss rate and ventilation frequency (Fig. 2).

It should be noted that boundary layer effects and stratification in the weighing cell may have elevated the relative humidity at the ant's surface, thereby depressing water loss rates relative to rates measured in a regime that employed flowing air.

Given that tiny changes in mass in small insects can now be resolved on a 2-s time scale as actual rates (rather than inferring rates from chart-recorder slopes), further technical refinements in instrumentation, data acquisition and modelling of the detailed mass budget promise to yield a wealth of comparative data on the detailed time course of water loss during the insect discontinuous ventilation cycle.

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