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SPIRACULAR CONTROL OF RESPIRATORY WATER LOSS IN FEMALE ALATES OF THE HARVESTER ANT *POGONOMYRMEX RUGOSUS*

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Summary

It has been suggested that the discontinuous ventilation cycle (DVC) observed in many insects, including all ants described to date, is an adaptation to reduce respiratory water loss. To test this hypothesis, it is necessary to measure respiratory water loss as a percentage of total water loss and to estimate what sustained rates of water loss would be in the absence of spiracular control. We used two independent techniques to measure real-time water loss rates in female alates of *Pogonomyrmex rugosus*. The first measured water vapor emission and CO_2 production simultaneously using dual-wavelength infrared absorbance analysis (DWIRAA). The second measured water loss gravimetrically. Real-time measurement allowed the separation of cuticular water loss rates (interburst) from water loss rates during the ventilation phase (burst) of the DVC. Cuticular permeability of *P. rugosus* female alates was only 27ng h^{-1} cm^{-2} Pa^{-1}, one-third of that reported for workers of the same species and the lowest yet reported for ants. Partly because of this low cuticular permeability, respiratory water loss represented a greater percentage of overall water loss (13%) than has generally been reported for other insects. The DWIRAA and gravimetric techniques gave equivalent results. Peak rates of water loss during the burst phase were 2.8-fold higher than cuticular water loss rates alone (7.68mg g^{-1} h^{-1} *versus* 2.77mg g^{-1} h^{-1} at 25°C). This is a conservative estimate of water loss rates in the absence of spiracular control. Contrary to findings in certain other insects that suggest a negligible role for respiratory water loss, we find that, in an insect that employs the DVC and has low cuticular permeability, overall water loss rates rise several-fold in the absence of direct spiracular control. Our findings lend strong support to the water conservation hypothesis for the role of the DVC. In at least some insects, respiratory water loss rates can reach magnitudes significant enough, relative to other routes of water loss, for strong selective pressure to act on them.

Introduction

A common feature of all ant ventilation described to date is its discontinuity (Lighton,

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1988*a*, 1990, 1992; Lighton and Wehner, 1993; Lighton *et al*. 1993). Contrary to the general model of small-insect ventilation *via* pure diffusion, championed so convincingly by Krogh (1941) in his classic work on *Cossus* larvae, many small adult insects ventilate discontinuously, restricting most of their overall gas exchange (and almost all of their CO_2 release) to events spaced several minutes apart, a characteristic thought until recently to hold true only for diapausing insect pupae (see Miller, 1981; Kestler, 1985; Slama, 1988, for reviews).

Even at the risk of embracing the Panglossian paradigm (Gould and Lewontin, 1979), it is difficult to resist suggesting an obvious adaptive value for this rather extreme ventilatory behavior. The adaptive significance follows naturally from the first observed occurrence of the discontinuous ventilation cycle (DVC) in diapausing pupae. Plainly, where respiratory water loss rates must be minimized, directional selection should drive the duration of spiracular opening, during which respiratory water loss is concentrated (Kestler, 1985; Lighton, 1988*b*, 1992), to a minimum consistent with acceptable gas exchange rates. The widespread, if not universal, occurrence of the DVC among ants, which in many cases are exposed to extreme hygric stress, provides further supporting evidence, as does the presence of the DVC (albeit modified) in certain desert beetles (Lighton, 1991).

In fact the situation is not so simple. To explain a high intensity of selective pressure on insect ventilation and respiratory water loss rates, respiratory water loss must play a significant role in the overall water loss budget. Although numerous studies of water loss rates in insects exist in the literature, very few have directly addressed the problem of respiratory water loss rates in a rigorous manner and only three published accounts contain explicitly quantitative data in this area (Machin *et al*. 1991; Lighton, 1992; Hadley and Quinlan, 1993). The last two in particular clearly demonstrate that respiratory water loss rates constitute a small fraction (approximately 2–8%) of total water loss *via* all avenues, most notably the cuticle. But is this merely evidence for an adaptive reduction of water loss rates by means of discontinuous ventilation?

In other words, the DVC may be so efficient at reducing respiratory water loss rates in some insects that, paradoxically, those rates may be dismissed as insignificant, so that respiratory water loss and the DVC itself are considered to be unimportant. It follows that further progress in assessing the water-loss correlates of the DVC is contingent on delineating upper as well as lower limits of respiratory contributions to overall water loss rates and on doing so in a way that clearly partitions respiratory and cuticular water loss rates. If water loss rates can be followed in ‘real time’, separation of respiratory and cuticular components of water loss during discontinuous ventilation becomes practicable (Lighton, 1992) and, more importantly, peak rates of water loss during the open-spiracle phase of the DVC can be quantified, allowing an approximate estimation of water loss rates in the absence of spiracular control. It is this latter measure which, in the authors’ opinion, should be of central importance in evaluating the adaptive significance of the DVC.

We chose as our experimental animal female alates (the female reproductive caste) of the southwestern United States harvester ant *Pogonomyrmex rugosus*. Female alates are of particular interest because their fitness represents a ‘bottleneck’ through which all

colony formation must pass (S. W. Rissing, personal communication). *P. rugosus* alates, like most ant alates, undergo a 'claustral' phase after insemination, during which they consume no food or water for several weeks until their first brood has been laid, hatched, fed, pupated and matured to the point where they can forage. At this stage the alates may be under intense selective pressure to minimize all routes of water loss. Their lowest rates of respiratory water loss therefore probably represent a genuine nadir for the species, against which their peak rates can be usefully compared.

Materials and methods

Animals

Pogonomyrmex rugosus Emery alates were collected near Phoenix, Arizona, after monsoon rains and transported by air freight to the University of Utah, where they were kept in glass test-tubes with a moistened plug of cotton-wool at the far end. They were fed on sliced mealworms and oats, and housed at an ambient temperature of 25±2°C on a 12h:12h L:D cycle. All measurements took place in an air-conditioned laboratory at 25±1°C.

Respirometry and water loss

We used a modified Sable Systems TR-2 respirometry system (Sable Systems, 476 E South Temple, Salt Lake City, UT 84111) to monitor discontinuous ventilation and water loss rates, using flow-through respirometry. Dry, CO_2-free air at room O_2 concentration was pulled through the respirometer chamber (glass, 5cm^3 internal volume, connected to the gas analyzer cuvette by a short length of Bev-A-Line low-permeability tubing) at a rate of 100$cm^3\,min^{-1}$ STPD, controlled by a Tylan mass flow controller. In addition to measuring CO_2 concentration in the excurrent airstream, using infrared absorbance at a wavelength of 4.26 μm, filtered by a narrow-bandpass filter and detected by a three-stage Peltier-effect-cooled detector, we employed a second alternately selected bandpass filter centered at 2.59 μm to measure infrared absorbance by H_2O (dual-wavelength infrared absorbance analysis or DWIRAA). By utilizing the same feedback-stabilized emitter and detector for both wavelengths, drift was practically eliminated and the CO_2 and H_2O signals were synchronous. After temperature correction and software linearization to account for Beer–Lambert deviations in the absorbance characteristics of CO_2 and H_2O, digitally filtered data were stored at 1s intervals. CO_2 records were then baseline-corrected and converted to rate of CO_2 production ($\dot{V}_{CO_2}$) in $ml\,h^{-1}$; H_2O data were also baseline-corrected and converted to water loss rate in $mg\,h^{-1}$.

Mass loss measurement

We measured mass loss directly using a Cahn C-32 ultramicrobalance, modified by suspending an extension hang-down of heat-treated Nichrome wire from its balance loop. This extension hang-down passed through a laser-aligned passage into a flow-through chamber (volume 50cm^3) and was attached to a free-hanging nichrome/aluminum stirrup on which the ant was placed. The flow-through chamber was suspended by short spacers from the ceiling of a larger container (volume 1000cm^3) equipped with a rubber-

insulated door. Air could be pushed into the larger container through tubing connected to a 50mm diameter cylinder, through which were drilled 500 0.5mm diameter holes, and could be pulled from the small flow-through chamber through a similar number of 0.3mm diameter holes into a Lucite collar. This arrangement minimized large, irregular air currents that otherwise added significant noise to the signal from the balance. The entire apparatus was surrounded by an open-floored wooden cabinet and rested on rubber blocks directly on a concrete floor.

In use, a 250mlmin^{-1} airstream, scrubbed of H_2O and CO_2, was pumped into the large cabinet. A sample of 10mlmin^{-1} was pulled from the small flow-through chamber, ensuring that the ant within was exposed to dry air. The output of the ultramicrobalance was sampled by a computer at approximately 3Hz to 18-bit or 0.1 μg resolution and averages (resolution 20–50ng) were stored every 5s. Differentiating the resulting trace (in mg) against time in hours yielded mass loss rate in mg h^{-1}. Assuming a respiratory quotient (RQ) of 0.727, the molar ratio of O_2/CO_2 and a value that is appropriate for this species (Lighton and Bartholomew, 1988), we could assume that the measured mass loss was equivalent to water loss (see Lighton, 1993, for details). The mass loss measurements could only be made on decapitated ants (Lighton, 1993) because even tiny movements caused massive disruption of the mass-loss recordings. However, decapitated ants are generally very similar in terms of ventilation patterns to intact ants (Lighton, 1992; Lighton *et al.* 1993). The mass loss and direct water loss techniques thus served as independent checks on our respiratory water loss measurements.

Statistics

Means are accompanied by standard deviations and sample sizes and are compared using Student's *t*-test. Regression is by least squares, with significance testing by analysis of variance. Regressions are compared using analysis of covariance (ANCOVA).

Results

Ventilation characteristics and water loss rates: flow-through measurements

Without exception, female *Pogonomyrmex rugosus* alates ventilated discontinuously. We were able to measure CO_2 emission and water loss rates simultaneously with good accuracy and resolution (Fig. 1).

To characterize ventilation and water loss characteristics, we analyzed 227 DVCs by 11 ants (mean mass 32.18±3.90mg), with approximately equal sample sizes for each ant. Summarized results are presented in Table 1.

As found in other xeric ant species (Lighton and Wehner, 1993), DVC frequency increased to accommodate elevated $\dot{V}_{CO_2}$ (Fig. 2), but burst CO_2 volume did not change with DVC frequency (r^2=0.05; P>0.4). Burst CO_2 volume and burst H_2O loss were, however, tightly correlated (Fig. 3), with burst CO_2 volume explaining nearly half of burst H_2O loss variance.

Interestingly, we found little evidence of a clear fluttering-spiracle (F) phase in any of the ants we examined. This is in contrast to other xeric ants (Lighton, 1990; Lighton and Wehner, 1993). Of course, flow-through respirometry is not able to detect bulk air flow

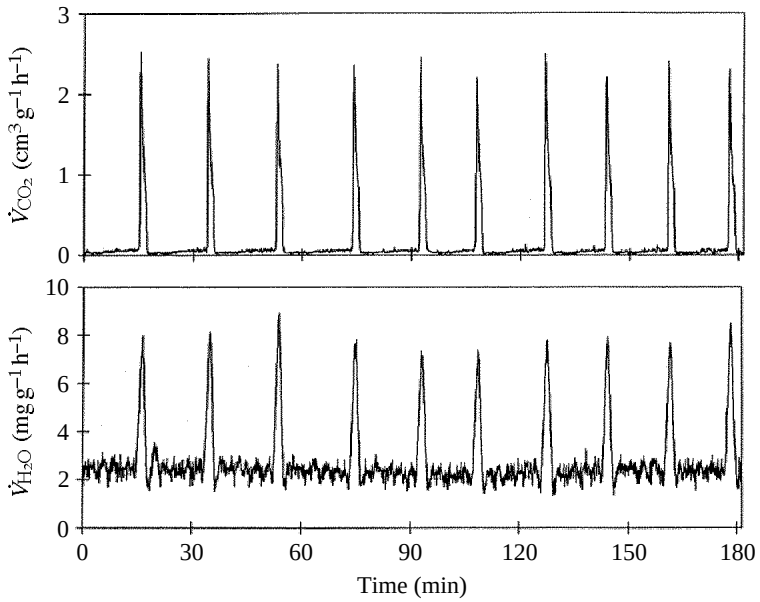

Fig. 1. Discontinuous release of CO_2 (upper trace: $cm^3 g^{-1} h^{-1}$) and water (lower trace: $mg\,g^{-1} h^{-1}$) by a female *Pogonomyrmex rugosus* alate, mass 31.4mg, at 25°C. Mean $\dot{V}_{CO_2}$ is $0.167 \pm 0.411 cm^3 g^{-1} h^{-1}$. The high standard deviation is caused by discontinuous ventilation (frequency 0.925mHz). In the water loss trace (mean $2.79 mg\,g^{-1} h^{-1}$), the H_2O loss rate in the interburst phase is readily distinguished from the much higher rate during the burst phase. Peak burst rate of H_2O loss yields a conservative estimate of H_2O loss rate in the absence of spiracular control. Note that the random interburst fluctuations in the water loss rate record are instrument noise.

Table 1. *Chief variables of the DVC in female alates of* Pogonomyrmex rugosus *at 25±1°C (utilizing flow-through respirometry,* N*=11, total DVCs=227, approximately 20 DVCs per ant*

Variable	Mean	S.D.	Units
Body mass	32.2	3.9	mg
DVC frequency	1.07	0.52	mHz
$\dot{V}_{CO_2}$	0.159	0.069	$cm^3 g^{-1} h^{-1}$
Burst CO_2 volume	36.6	6.1	$\mu l\, CO_2\, g^{-1}$
Burst H_2O loss	117.6	18.5	$\mu g H_2O\ g^{-1}$
H_2O/CO_2 ratio	3.25	0.41	$\mu g\, \mu l^{-1}$
Respiratory H_2O total loss (DVC)	13.0	5.4	%
Interburst H_2O loss	2.77	0.56	$mg\ g^{-1} h^{-1}$
Peak burst H_2O loss increase	4.91	0.91	$mg\,g^{-1} h^{-1}$
Respiratory H_2O loss (estimated for no DVC)	63.8	7.0	%

The 'peak burst H_2O loss increase' refers to the increase above interburst or cuticular water loss levels. This peak rate yields a conservative estimate of respiratory water loss rates in the absence of spiracular control (estimated for no DVC).

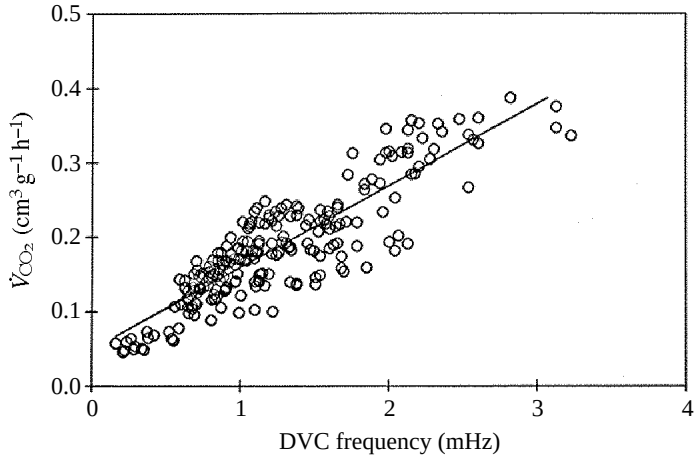

Fig. 2. The relationship between DVC frequency and $\dot{V}_{CO_2}$ in 227 DVCs by 11 ants. $\dot{V}_{CO_2}$=0.049+0.11DVCF, where $\dot{V}_{CO_2}$ is in cm^3 g^{-1} h^{-1} and DVC frequency (DVCF) is in mHz (r^2=0.77, P<0.0001). The intercept does not differ significantly from zero.

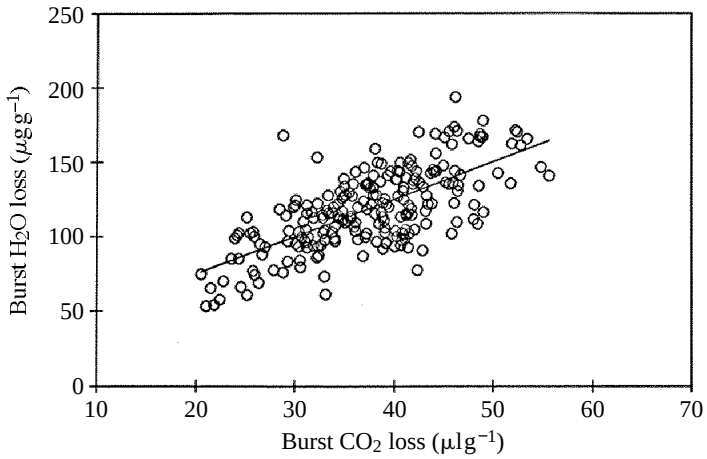

Fig. 3. The relationship between burst CO_2 volume and burst H_2O loss in 227 DVCs by 11 ants. B$\dot{V}_{H_2O}$=24.85+2.51B$\dot{V}_{CO_2}$, where B$\dot{V}_{H_2O}$ is burst water loss in μg g^{-1} and B$\dot{V}_{CO_2}$ is burst CO_2 loss in μl g^{-1} (r^2=0.47, P<0.0001). The intercept does not differ significantly from zero.

during the F phase; it is sensitive only to the diffusive component of the F phase. This component may be quite small, although generally detectable. Part of our inability to discern the F phase may have been the result of a trade-off between instrument noise and the capacity to measure both CO_2 and H_2O. Alternatively, the F phase may be highly efficient in *Pogonomyrmex rugosus* alates, with a negligible diffusive component. In view of the lack of a definitely measurable F phase in these ants, for analysis purposes we considered the intervals between peak CO_2 emissions (bursts) to be constricted-spiracle (C) or, more generally, interburst phases. We defined cuticular water loss rates as those measured during the interburst phase.

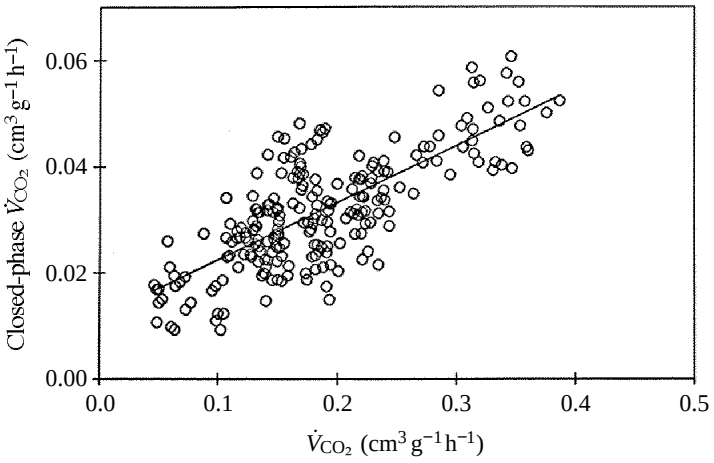

Fig. 4. Interburst CO_2 emission rate increases with $\dot{V}_{CO_2}$. IB$\dot{V}_{CO_2}$=0.012+0.108$\dot{V}_{CO_2}$, where IB$\dot{V}_{CO_2}$ is interburst rate of CO_2 emission in cm^3 g^{-1} h^{-1} and $\dot{V}_{CO_2}$ is in the same units (N=227 DVCs in 11 ants, r^2=0.56, P<0.0001).

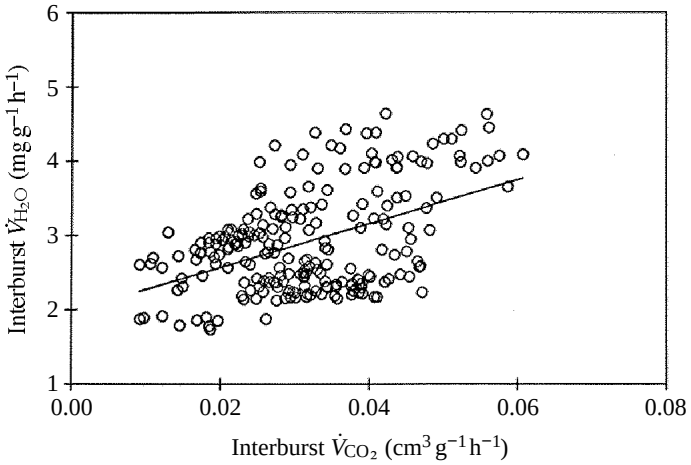

Fig. 5. Cuticular (i.e. interburst) water loss rate increases with interburst CO_2 emission rate. C$\dot{V}_{H_2O}$=1.98+29.47C$\dot{V}_{CO_2}$, where C$\dot{V}_{H_2O}$ is cuticular H_2O loss rate in mg g^{-1} h^{-1} and C$\dot{V}_{CO_2}$ is interburst cuticular CO_2 emission rate in cm^3 g^{-1} h^{-1} (N=227 DVCs in 11 ants, r^2=0.21, P<0.001).

During the interburst phase, CO_2 was emitted at a continuous very low rate that was tightly correlated with overall $\dot{V}_{CO_2}$ (Fig. 4). From the slope of this relationship, CO_2 was lost during the interburst period at 11.6±5.6% of total $\dot{V}_{CO_2}$, which is slightly less than the 14% of overall CO_2 lost by the arid-adapted *Cataglyphis bicolor* during its true C phase (Lighton and Wehner, 1993). This suggests that the F phase is indeed minimal in *Pogonomyrmex rugosus*.

Table 2. *DVC variables for decapitated female* Pogonomyrmex rugosus *alates measured by mass loss alone, at 25±1°C (*N*=7, total DVCs=384)*

Variable	Mean	S.D.	Units	Significance
Body mass	30.9	3.5	mg	NS
DVC frequency	2.14	0.66	mHz	*
Burst mass loss	126.2	22.4	$\mu g g^{-1}$	NS
Interburst H_2O loss	5.11	1.35	$mg\,g^{-1}h^{-1}$	*

Significance, significance test for difference from the same variables measured on intact ants using flow-through respirometry (Table 1); NS, $P>0.05$; $*P\leqslant 0.05$.

Mass loss rates are approximately equivalent to water loss rates at a respiratory quotient of 0.727 (see text).

The interburst H_2O loss rate was strongly correlated with the rate of CO_2 emission during the interburst period (Fig. 5).

Water loss rates: mass loss measurements

Table 2 summarizes the mass loss measurements. Note that DVC frequency was significantly greater in the decapitated ants, an effect of decapitation, presumably reflecting increased $\dot{V}_{CO_2}$ (Fig. 2) not observed in decapitated *Cataglyphis bicolor* (Lighton *et al.* 1993). As with intact ants, however, the volume of water lost in the burst did not change with DVC frequency ($P>0.3$), and so can be directly compared to burst water loss volumes obtained by water vapor measurement in the DWIRAA system; the two values do not differ significantly ($t=0.75$; $P>0.2$). This cross-validation suggests that gravimetric and direct water loss measurements can be used interchangeably for the measurement of respiratory water loss rates in insects, subject to the stringent activity limitations of the former technique.

Comparison of interburst water loss rates is complicated by the greater DVC frequency of the decapitated ants, which, as in intact ants, significantly affected interburst mass loss rates (Fig. 6). However, the relationship between DVC frequency and interburst mass loss rates (Fig. 6 legend) can be used to predict interburst water loss rates given the mean DVC frequency of the flow-through-system ants. This value ($3.02 mg\,g^{-1}h^{-1}$ at 1.07mHz) does not differ from the mean value obtained from the flow-through-system ants ($2.77\pm0.56 mg\,g^{-1}h^{-1}$, Table 1; $t=0.19$, $P>0.3$). This suggests that a high cuticular water loss rate in decapitated ants is a side-effect of elevated $\dot{V}_{CO_2}$ and DVC frequency. Whether this effect is causative or correlative remains unclear.

Cuticular permeability

The mean absolute cuticular water loss rate of our sample of intact *Pogonomyrmex rugosus* female alates (Table 1) is $0.089 mg\,h^{-1}$. Assuming a cuticular surface area of $1.04 cm^2$ (Lighton and Feener, 1989, equation 4), the cuticular permeability of female *P. rugosus* alates is $89\,\mu g\,h^{-1}\,1.04 cm^{-2}\,3173 Pa^{-1}$ of water vapor saturation deficit at 25°C=$27 ng\,H_2O\,cm^{-2}\,Pa^{-1}$. This is just 31% of the cuticular permeability of *P. rugosus* workers ($87 ng\,H_2O\,cm^{-2}\,Pa^{-1}$; Lighton and Feener, 1989).

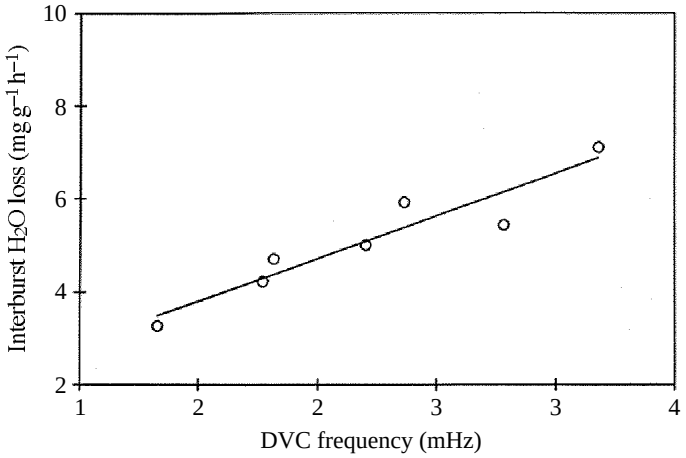

Fig. 6. In the direct mass-loss measurements, cuticular (interburst) H_2O loss rates increase with DVC frequency [correlated with $\dot{V}_{CO_2}$ (Fig. 1), which was not measured]. $C\dot{V}_{H_2O}$=1.06+1.83DVCF, where $C\dot{V}_{H_2O}$ is cuticular H_2O loss rate in mg g^{-1} h^{-1} and DVCF is DVC frequency in mHz (*N*=mean by ant of 384 DVCs in seven ants, r^2=0.89, *P*<0.005).

Discussion

Cuticular water loss rates: comparison with conspecific workers

The cuticular permeability of female *P. rugosus* alates is the lowest yet reported for any ant and, indeed, is among the lowest reported for any insect (see Edney, 1977; Machin *et al.* 1991). It is more than three times lower than that of worker ants of the same species, which already show the low cuticular permeabilities associated with desert arthropods (Lighton and Feener, 1989). The striking difference between alate and worker cuticular permeability in *P. rugosus* suggests that the phenomenon may be widespread in other ant species, especially among claustral colony founders. By extension, it is likely that such differences may be reduced or eliminated in ant species that do not undergo such rigorous isolation or do so under more favorable circumstances, for example in water-saturated soil.

Modulation of cuticular water loss rates

Our finding that $\dot{V}_{CO_2}$ and cuticular water loss rates are correlated is somewhat surprising. This relationship is too marked to be explained by H_2O escaping in concert with CO_2 from the constricted spiracles or through the cuticle. The relationship between H_2O and CO_2 release volumes during the burst phase (Fig. 3) has a slope of 2.5±0.2 $\mu g H_2O\,\mu l^{-1}\,CO_2$ while the relationship between rates of H_2O loss and CO_2 emission during the interburst phase has a slope of 29.5±3.8 $\mu g H_2O\,\mu l^{-1}\,CO_2$ (Fig. 5). This strongly suggests that overall $\dot{V}_{CO_2}$ directly or indirectly modulates cuticular H_2O permeability, because changes in the interburst CO_2 emission rate (and hence overall $\dot{V}_{CO_2}$) affect interburst H_2O loss rates tenfold more than equivalent changes while the spiracles are open, when it can be assumed that the H_2O/CO_2 loss ratio is at a maximum. Of course, the relationship between $\dot{V}_{CO_2}$ and cuticular permeability may be correlative

rather than causal. Other, unmeasured factors may simultaneously affect metabolic rate (and hence $\dot{V}_{CO_2}$) and cuticular water loss rates. Certainly, cuticular permeability has long been regarded as at least potentially labile (Treherne and Willmer, 1975; Noble-Nesbitt and Al-Shukur, 1987; but see Machin *et al.* 1986, 1991). Hadley and Quinlan (1993) also found evidence for alterations in cuticular permeability. Like all work in this area, our findings do not conclusively prove that cuticular permeability is under active control, but they certainly do not disprove the hypothesis. Investigating the correlation (if any) between interburst or CF water loss rates (*sensu* Machin *et al.* 1991) and metabolic rate in cockroaches may be instructive in this regard.

Respiratory water loss rates (comparative)

P. rugosus alates lose a greater percentage of total H_2O loss *via* respiration than either *Romalea guttata* grasshoppers (2–4%; Hadley and Quinlan, 1993) or other ants for which data are available (2–8%; Lighton, 1993). However, as alluded to above, the cuticular permeability of *P. rugosus* alates is extremely low. This greatly elevates the relative contribution of respiratory water loss to the overall water loss budget. The same may hold true of the relatively large respiratory water loss percentages reported by Machin *et al.* (1991; but see Hadley and Quinlan, 1993). It follows that the lower the cuticular permeability of an insect, the greater the potential selective pressure placed on its respiratory water loss characteristics. Thus, in xerophilic insects, it may be a general principle that the higher their respiratory water loss rates are in proportion to total water loss rates, the lower are their overall rates of water loss.

In certain respects, the ventilation and respiratory water loss rate characteristics of *P. rugosus* female alates resemble those of the xerophilic formicine ant *Cataglyphis bicolor* (Lighton, 1993). For example, DVC frequencies are similar (1.07±0.52 *vs* 1.15±0.85mHz), as are burst volumes of CO_2 (36.6±6.1 *vs* 35.2±4.91 $\mu l\,g^{-1}$). However, burst H_2O loss is lower in *P. rugosus* (117.6±18.5 *vs* 187.2±81.7 $\mu g\,g^{-1}$), and so is the ratio of H_2O lost to CO_2 emitted during the burst phase (3.25±0.41 *vs* 4.79±1.73 $\mu g\,H_2O\,\mu l^{-1}\,CO_2$). Furthermore, the interburst (cuticular) water loss rate is far lower (2.77±0.56 *vs* 6.34±5.03mg $g^{-1}\,h^{-1}$). In general, most of the characteristics of *P. rugosus* appear to be better optimized to reduce both cuticular and respiratory water loss rates than are those of *C. bicolor* workers. This is consistent with the selective pressures that presumably operate on ant queens during claustral colony foundation.

But some data suggest a more complicated situation. Notably, the mesic ant *Camponotus vicinus* loses far less H_2O per burst (55.3±16.1 $\mu g\,g^{-1}$; Lighton, 1993) than does either *Cataglyphis bicolor* or *P. rugosus* and its CO_2 emission efficiency is greater (2.58±0.35 $\mu g\,H_2O\,\mu l^{-1}\,CO_2$). Why, in these critical areas, is a mesic ant from montane North America paradoxically better able (in a simplistic sense at least) to reduce respiratory water loss than either of two far more xerophilic species? The requirement for more comparative data is obvious.

Rates of respiratory water loss in the absence of spiracular control

It has been convincingly argued (Hadley and Quinlan, 1993) that the usual reason put forward for the evolution of the DVC, namely its role in water conservation, is

problematic. As they point out, their experimental organism, the mesic Eastern lubber grasshopper *Romalea guttata*, counterintuitively appears to ventilate discontinuously only when fully hydrated and under circumstances when the risk of desiccation is already low, such as during its inactive scotophase. Even during active ventilation, it loses only about 7% of total water loss *via* its respiratory system. This may, however, reflect not so much the general unimportance of the DVC or respiratory water loss, as a reduction of selective pressure on respiratory water loss caused by already high cuticular water loss rates. As Hadley and Quinlan (1993) point out, in *Romalea guttata* at room temperature 'respiratory water loss is so small that it can be neglected'.

We do not consider, however, that these findings are necessarily valid for all insects. As mentioned above, respiratory water loss only becomes relevant if cuticular water loss rates are low. Our present data are unusual in that they allow quantification of both normal and maximal or near-maximal rates of respiratory water loss. This allows implicit comparison of respiratory water loss in the presence and absence of active spiracular control. (Note that non-discontinuous spiracular control regimes may entail the continuous modulation of spiracular opening near minimal levels. The water-loss correlates of such control regimes remain unclear.)

Our technique for placing an upper limit to respiratory water loss rates is very conservative. It assumes that the maximum measured H_2O emission rate during the burst phase equates to the sustained H_2O output rate that would occur if the spiracles were simply held open, i.e. if the DVC were abolished. However, the measured peak H_2O emission rate is certainly less than the sustained rate. Although H_2O is lost *via* diffusion at an approximately constant rate during the burst phase in ants (J. R. B. Lighton, in preparation), instrumental limitations precluded following its exact time course in either of the analytical systems employed here, in one case because of wash-out effects and in the other because of feedback time constants. Therefore, our measured rates of respiratory water loss were still pre-asymptotic when they reached their observed maximum by the end of the burst phase.

A clear picture emerges nevertheless. Cuticular H_2O loss was $2.77 mg\,g^{-1}\,h^{-1}$ (Table 1) and the increase at measured peak respiratory H_2O loss was $4.91 mg\,g^{-1}\,h^{-1}$, yielding a total water loss rate in the absence of the DVC of $7.68 mg\,g^{-1}\,h^{-1}$, a 2.8-fold increase over non-respiratory water loss rates. Since our estimate of respiratory water loss rate is conservative, it cannot be doubted that it is under strong selective pressure in arid environments, especially in insects with low cuticular permeability.

In conclusion, our findings strongly support the water-conservation hypothesis for the role of the discontinuous ventilation cycle in insects. However, this does not mean that stringent discontinuous ventilation regimes are necessarily the norm, especially where rates of cuticular water loss are high relative to respiratory water loss rates. It is nevertheless significant that, even in such cases, the capacity for discontinuous ventilation still exists (Hadley and Quinlan, 1993), implying that it is highly conserved even when it has little demonstrable effect. The widespread occurrence of the DVC in ants strongly suggests that their cuticular water loss rates have been reduced to the point where respiratory water loss rates are under significant selective pressure.

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References

EDNEY, E. B.(1977). *Water Balance in Land Arthropods.* Berlin: Springer Verlag.

GOULD, S. J. AND LEWONTIN, R. C. (1979). The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. *Proc. R. Soc. Lond.* **205**, 581–598.

HADLEY, N. F. AND QUINLAN, M. C. (1993). Discontinuous carbon dioxide release in the Eastern lubber grasshopper *Romalea guttata* and its effect on respiratory transpiration. *J. exp. Biol.* **177**, 169–180.

KESTLER, A. (1985). Respiration and respiratory water loss. In *Environmental Physiology and Biochemistry of Insects* (ed. K. H. Hoffmann), pp. 137–183. Berlin: Springer Verlag.

KROGH, A. (1941). *The Comparative Physiology of Respiratory Mechanisms.* Chapter 8, pp. 114–143. Philadelphia, PA: University of Pennsylvania Press.

LIGHTON, J. R. B. (1988*a*). Discontinuous carbon dioxide emission in a small insect, the formicine ant *Camponotus vicinus. J. exp. Biol.* **134**, 363–376.

LIGHTON, J. R. B. (1988*b*). Simultaneous measurement of oxygen uptake and carbon dioxide emission during discontinuous ventilation in the tok-tok beetle, *Psammodes striatus*. *J. Insect Physiol.* **34**, 361–367.

LIGHTON, J. R. B. (1990). Slow discontinuous ventilation in the Namib dune-sea ant, *Camponotus detritus* (Hymenoptera, Formicidae). *J. exp. Biol.* **151**, 71–82.

LIGHTON, J. R. B.(1991). Ventilation in Namib Desert tenebrionid beetles: mass scaling and evidence of a novel quantized flutter phase. *J. exp. Biol.* **159**, 249–268.

LIGHTON, J. R. B. (1992). Direct measurement of mass loss during discontinuous ventilation in two species of ants. *J. exp. Biol.* **173**, 289–293.

LIGHTON, J. R. B. AND BARTHOLOMEW, G. A. (1988). Standard energy metabolism of a desert harvester ant, *Pogonomyrmex rugosus*: Effects of humidity, temperature, body mass and group size. *Proc. natn. Acad. Sci. U.S.A.* **85**, 4765–4769.

LIGHTON, J. R. B. AND FEENER, D. H. (1989). Water loss and cuticular permeability in foragers of the desert ant, *Pogonomyrmex rugosus. Physiol. Zool.* **62**, 1232–1256.

LIGHTON, J. R. B., FUKUSHI, T. AND WEHNER, R.(1993). Ventilation in *Cataglyphis bicolor*: regulation of CO_2 release from the thoracic and abdominal spiracles. *J. Insect Physiol.* (in press).

LIGHTON, J. R. B. AND WEHNER, R. (1993). Ventilation and respiratory metabolism in the thermophilic desert ant, *Cataglyphis bicolor* (Hymenoptera, Formicidae). *J. comp. Physiol.* (in press).

MACHIN, J., KESTLER, P. AND LAMPERT, G. J. (1991). Simultaneous measurements of spiracular and cuticular mass losses in *Periplaneta americana*: implications for whole-animal mass loss studies. *J. exp. Biol.* **161**, 439–453.

MACHIN, J., O'DONNELL, M. J. AND KESTLER, P. (1986). Evidence against hormonal control of integumentary water loss in *Periplaneta americana. J. exp. Biol.* **121**, 339–348.

MILLER, P. L. (1981). Ventilation in active and in inactive insects. In *Locomotion and Energetics in Arthropods* (ed. C. F. Herreid II), pp. 367–390. New York: Plenum.

NOBLE-NESBITT, J. AND AL-SHUKUR, M. (1987). Effects of desiccation, water-stress and decapitation on integumentary water loss in the cockroach, *Periplaneta americana. J. exp. Biol.* **131**, 289–300.

SLAMA, K. (1988). A new look at insect respiration. *Biol. Bull. mar. biol. Lab., Woods Hole* **175**, 289–300.

TREHERNE, J. E. AND WILLMER, P. G. (1975). Hormonal control of integumentary water loss: evidence for a novel neuroendocrine system in an insect (*Periplaneta americana*). *J. exp. Biol.* **63**, 143–159.