

QUESTIONING PARADIGMS: CASTE-SPECIFIC VENTILATION IN HARVESTER ANTS, *MESSOR PERGANDEI* AND *M. JULIANUS* (HYMENOPTERA: FORMICIDAE)

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Summary

Do developmental constraints in ant colonies limit gas exchange strategies to those displayed by female alates (presumptive queens)? In the xeric harvester ant genus *Messor*, we found that *M. pergandei* and *M. julianus* female alates ventilated highly discontinuously, as predicted, but *M. julianus* workers ventilated less discontinuously and *M. pergandei* workers (which occur in more xeric habitats) ventilated continuously. We present the salient characteristics of the discontinuous ventilation cycles of the species and the manner in which they are modulated by CO₂ emission rates at a single temperature (24 °C). We demonstrate that, in *M. julianus* workers, open-spiracle phase CO₂ emission rate only slightly exceeds overall CO₂ emission rate, making discontinuous ventilation marginal, a state extrapolated in *M. pergandei* to continuous ventilation. However, workers are plainly capable of far

greater rates of CO₂ emission than when inactive at 24 °C, so the lack of discontinuous ventilation in *M. pergandei* under normoxic conditions is not likely to be imposed by physiological constraints and may, in fact, be a response to its xeric environment. We hypothesize – aside from phylogenetic effects – that discontinuous ventilation occurs primarily in insects that may experience hypoxic and hypercapnic conditions, such as ant queens during claustral colony foundation and perhaps workers within the nest environment; that discontinuous ventilation is not necessarily essential to reduce respiratory water loss; and that it will not necessarily occur in castes or species routinely exposed to xeric but normoxic conditions.

Key words: ventilation, ant, *Messor pergandei*, *Messor julianus*, gas exchange.

Introduction

Discontinuous ventilation, in the sense of discontinuous external gas exchange, is widely distributed in adult insects (see reviews by Miller, 1981; Kestler, 1985; Slama, 1988; Lighton, 1994). Both the existence and some aspects of the control mechanisms of the discontinuous ventilation cycle or DVC have been studied in cockroaches (Kestler, 1980, 1985; Machin *et al.* 1991), grasshoppers (Hadley and Quinlan, 1993), beetles (Lighton, 1988a, 1991a) and ants (Lighton, 1988b, 1990, 1992; Lighton and Wehner, 1993; Lighton *et al.* 1993a,b). These studies may, however, give the erroneous impression that discontinuous ventilation is universal. Researchers naturally tend to study a phenomenon in its more pronounced and quantifiable forms, so exceptions that test the implied rule tend to be under-represented in the literature. For example, it is quite reasonably assumed that mealworm larvae (*Tenebrio molitor*) ventilate discontinuously (Snyder *et al.* 1995; Loudon, 1989), and it may be equally reasonably concluded in the light of evidence from African xeric tenebrionid beetles (Lighton, 1991a) that American xeric tenebrionid beetles such as *Eleodes* spp do so as well. Neither is in fact the case (Lighton, 1994; M. C. Quinlan and J. R. B. Lighton, in preparation). Far from being universal,

discontinuous ventilation is, in fact, patchily distributed among insects (see also Hadley and Quinlan, 1993). Ants are unusual in this respect; adults of every species so far studied have ventilated discontinuously.

Why do adult ants ventilate discontinuously? The unusual life-history of most ant species is reflected in their respiratory physiology. During the claustral phase of colony establishment, the founding queen excavates a burrow, seals the entrance, lays eggs, tends and feeds larvae, and feeds the new workers prior to the initiation of foraging, all from her internal reserves (Hölldobler and Wilson, 1990). If – as is presumably the case – the claustral chamber becomes significantly hypercapnic and hypoxic, discontinuous gas exchange will be the most practical gas exchange strategy because it maximizes partial pressure gradients and minimizes durations of spiracular opening (see Discussion).

Given the indisputable evidence for discontinuous ventilation in workers of primitive ant genera (*Pogonomyrmex*, Lighton *et al.* 1993b; M. C. Quinlan and J. R. B. Lighton, in preparation; *Leptogenys*, Duncan and Crewe, 1994) and somewhat more derived genera (*Camponotus*, Lighton, 1988b, 1990, 1992; *Cataglyphis*, Lighton and Wehner, 1993; Lighton

et al. 1993a), and given the necessity for discontinuous ventilation in the claustral stage and thus in the queen, discontinuous ventilation in the workers may be a correlated developmental character rather than a necessary characteristic. Accordingly, we hypothesized that, if a selective advantage accrued to abandoning discontinuous ventilation (cf. *Tenebrio* and *Eleodes*), then loss of discontinuous ventilation would be found in workers rather than queens. Further, such a loss would most probably arise in highly derived species with an extreme tolerance for xeric environments because, in such species, cuticular permeability is low enough to elevate the selective significance of respiratory water loss (Lighton *et al.* 1993b; Lighton, 1994) and decoupling of correlated characters in the worker caste is more likely to have occurred than in more primitive and conservative genera, such as *Pogonomyrmex*.

An excellent example of a highly derived, xeric ant species is *Messor pergandei*. This member of the myrmicine subfamily is among the most specialized xeric granivores of all ants. In the words of Hölldobler and Wilson (1990), they 'flourish in the deserts of southwestern Arizona, southern California and Baja California. In Death Valley, one of the driest and hottest places in North America, the *Messor pergandei* are the most abundant ants; they have a biomass approximately equal to that of the total rodent population in the same area (Went *et al.* 1972). The toughness of the species in the face of harsh conditions is legendary... [In one study] colonies survived even after twelve years of severe drought (Tevis, 1958).' We therefore selected workers and alates of *M. pergandei* as our primary study animal in this investigation. To control, to at least some extent, for phylogenetic effects, we also studied worker and alate ventilation in a congener, *Messor julianus*, that thrives in the more mesic and productive environment of the Baja peninsula in Mexico.

Materials and methods

Animals

Alates and workers from *M. pergandei* (Mayr) and *M. julianus* (Pergande) colonies were collected during the February mating flight season from a site 15 km south of Phoenix, Arizona, USA, and from Baja California, Mexico, respectively, and transported by air to our laboratory at the University of Utah shortly after collection. We maintained the ants in small plastic containers at an ambient temperature of $24 \pm 0.5^\circ\text{C}$ and supplied them with water and oat flakes *ad libitum*. Our experiments were performed within 2 weeks of capture.

Respirometry

Our respirometric techniques are described in full elsewhere (Lighton, 1991b; Lighton *et al.* 1993b). Briefly, we utilized a Sable Systems TR-3 high-resolution, high-sensitivity flow-through respirometry system (Sable Systems, 476 E. South Temple, Salt Lake City, UT 84112) to detect, with a temporal resolution of 1 s, the CO_2 added by a single worker or alate ant to a CO_2 -free airstream. From CO_2 concentration and flow rate,

controlled at 50 ml min^{-1} by a mass flow controller, we calculated the rate of CO_2 output (\dot{V}_{CO_2}) according to Lighton (1991b). The three phases of the discontinuous ventilation cycle (DVC) are easily distinguishable in high-resolution records of \dot{V}_{CO_2} , and the interpretations used in this paper have been verified in other species of ants by direct visual observation of spiracular activity (Lighton *et al.* 1993a). All measurements took place at ambient temperature in an air-conditioned laboratory regulated at $24 \pm 0.5^\circ\text{C}$. Computerized data acquisition and analysis were performed using DATACAN V high-resolution A/D hardware and software (Sable Systems).

Statistics

Means are accompanied by standard deviations and sample sizes. Means are compared with Student's *t*-test, utilizing the square root of arcsine-transformed data in the case of ratios and percentages. Least-squares regressions, with axis transformation where appropriate, were used. The significance level of regressions was evaluated with analysis of variance (ANOVA), and regressions were compared with analysis of covariance (ANCOVA). The significance level was set at $P < 0.05$.

Results

Standard metabolic rate

The standard metabolic rates (SMRs) of the workers and alates of *M. pergandei* and *M. julianus* are shown in Tables 1 and 2 with sample sizes. All castes and species displayed SMRs typical for ants of their body masses (Fig. 1). We

Table 1. *Metabolic and ventilatory parameters of Messor pergandei workers, male and female alates at an ambient temperature of 24°C*

Parameter	Workers (<i>N</i> =38)	Male alates (<i>N</i> =26)	Female alates (<i>N</i> =25)
Live body mass (mg)	7.19 \pm 3.53	16.47 \pm 1.63	39.40 \pm 2.11
\dot{V}_{CO_2} ($\mu\text{l h}^{-1}$)	1.95 \pm 1.45	4.98 \pm 1.59	6.46 \pm 2.01
F \dot{V}_{CO_2} ($\mu\text{l h}^{-1}$)	–	0.60 \pm 0.13	1.46 \pm 0.65
Burst CO_2 (μl)	–	0.259 \pm 0.081	0.362 \pm 0.118
Burst frequency (mHz)	–	5.28 \pm 2.08	4.75 \pm 1.67
Burst duration (s)	–	95.0 \pm 22.5	103.4 \pm 22.8
CV (%)	28.3 \pm 15.8	118.7 \pm 38.1	102.5 \pm 22.1

Parameters are the mean of three discontinuous ventilation cycles for each animal, where appropriate.

CV, mean coefficient of variation (standard deviation of each recording divided by its mean; not equivalent to overall standard deviation divided by overall mean). The CV figure increases with increasing ventilatory discontinuity. Flutter phase data (prefixed with F) were collected from three male alates; the remaining males did not display an identifiable flutter phase.

Dashed lines denote no data, because we did not observe consistent discontinuous ventilation in any worker of this species (see text).

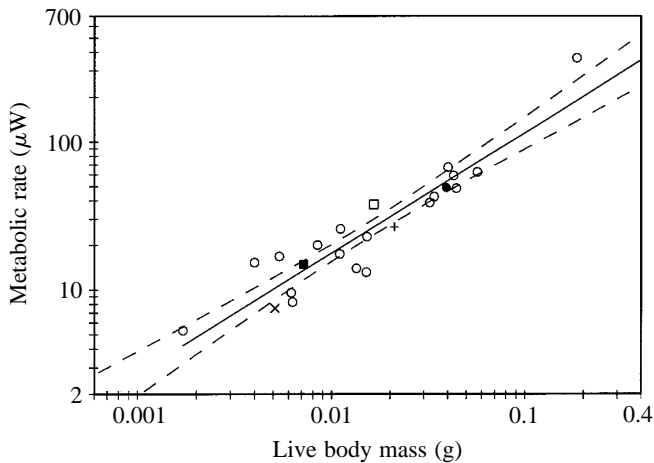


Fig. 1. The relationship between live body mass and metabolic rate in 23 species of ants (open circles), including *Messor pergandei* female alates (filled circle), male alates (open square) and workers (filled square), and *M. julianus* queens (+) and workers (×). All data are corrected from measurement temperature (23–30 °C) to 25 °C assuming a Q_{10} of 2.5 (Withers, 1992); references to the other ant data are in Lighton and Fielden (1994). The equation relating metabolic rate (MR) in μW to live body mass (M) in g is $\text{MR} = 743.0M^{0.812}$ [$r^2 = 0.87$; $F(1,21) = 140$; $P < 0.0001$].

emphasize this point here because some of our ventilation results (below) differ significantly from those previously reported in ants. These differences are not caused by significantly deviant SMRs.

The discontinuous ventilation cycle

Occurrence

When inactive, all alates of both species ventilated discontinuously (Figs 2, 3). In *M. julianus*, all inactive workers also ventilated discontinuously (Fig. 3). However, we

Table 2. Metabolic and ventilatory parameters of *Messor julianus* workers and female alates at an ambient temperature of 24 °C

Parameter	Workers (N=39)	Female alates (N=50)
Live body mass (mg)	5.09±0.38	21.11±0.77
\dot{V}_{CO_2} ($\mu\text{l h}^{-1}$)	0.99±0.37	3.50±0.60
$F\dot{V}_{\text{CO}_2}$ ($\mu\text{l h}^{-1}$)	0.22±0.03	0.53±0.21
Burst CO_2 (μl)	0.027±0.010	0.295±0.077
Burst frequency (mHz)	9.73±3.47	3.26±1.02
Burst duration (s)	61.5±14.0	115.1±24.7
CV (%)	68.1±21.8	146.3±37.5

CV, See Table 1 legend.

Parameters are the mean of three discontinuous ventilation cycles for each animal.

Flutter phase data (prefixed with F) were collected from three workers and 48 female alates; the remaining individuals did not display an identifiable flutter phase. No male alates of this species were collected.

observed no such tendency in *M. pergandei* workers (Fig. 2). In a total of 38 *M. pergandei* workers examined, we found no evidence of consistent or unambiguous discontinuous ventilation. Most displayed a constant \dot{V}_{CO_2} with very little variation from its mean value (Fig. 2). This was not caused by an activity-induced disruption of discontinuous ventilation (Lighton, 1988b). We observed the ants standing motionless in the respirometer vials for long periods while ventilating continuously and the SMR measured was characteristic of motionless ants of their body mass (Fig. 1). In another investigation, carried out in Zzyzx, California, on *Messor pergandei* locomotion energetics (J. R. B. Lighton and F. D. Duncan, in preparation), no evidence of ventilatory

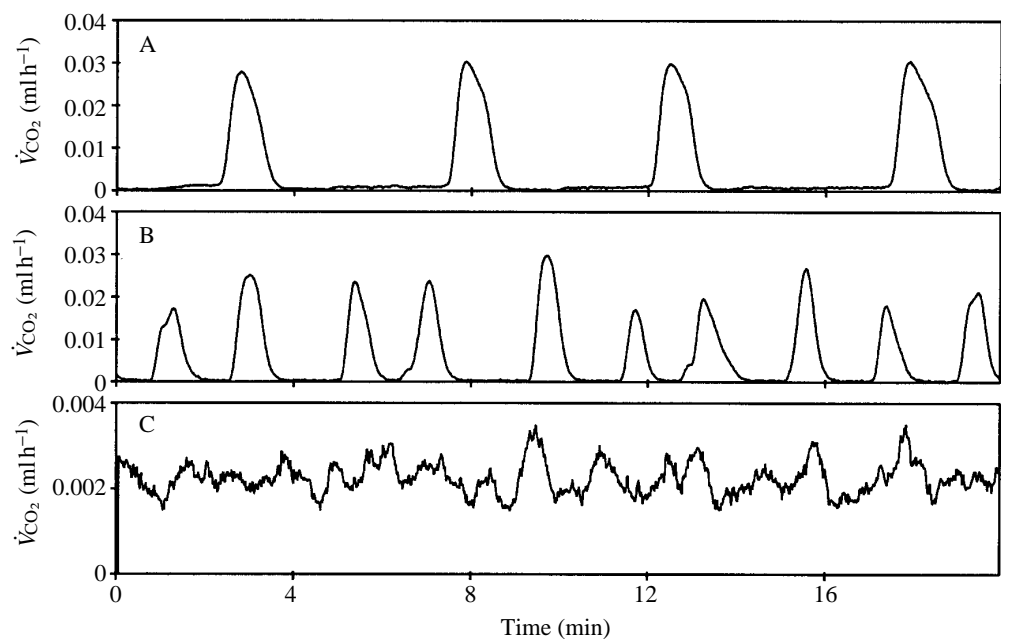


Fig. 2. Ventilation, as externally measured rates of CO_2 emission (\dot{V}_{CO_2} in ml h^{-1}), of a typical individual *Messor pergandei* female alate (A), male alate (B) and worker (C). Note scale differences on the y-axes. Live body masses were 41.7, 18.6 and 6.6 mg respectively.

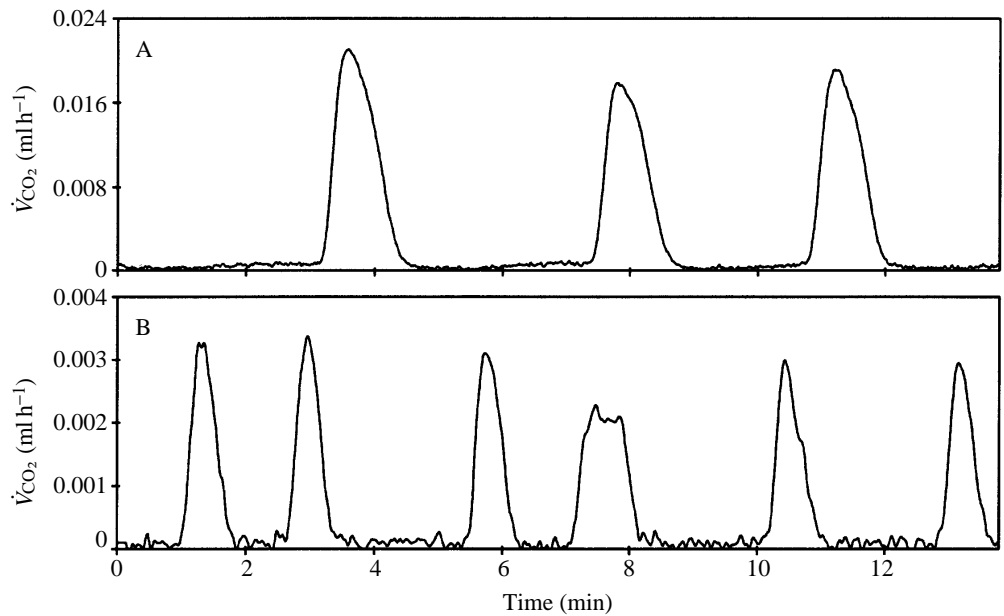


Fig. 3. Ventilation, as externally measured rates of CO_2 emission (\dot{V}_{CO_2} in ml h^{-1}), of a typical individual *Messor julianus* female alate (A) and worker (B). Variation in \dot{V}_{CO_2} between open (O) phases in the worker is caused by instrument noise (note scale differences on the y-axes). Live body masses were 22.6 and 5.5 mg respectively.

discontinuity was found in any of over 100 recordings of motionless workers at a wide range of temperatures. In one unusually large *M. pergandei* worker, in the present investigation, we did observe a brief episode of discontinuous ventilation on one occasion (data not shown) so we cannot conclusively state that *M. pergandei* workers never, under any circumstances, ventilate discontinuously. We are confident, however, that these events are exceedingly rare, at least under the normoxic and normocapnic (or acapnic) conditions of our experiments.

Salient values of the DVC in *M. pergandei* and *M. julianus* are listed in Tables 1 and 2.

The distinction between discontinuous and non-discontinuous ventilation is qualitatively obvious (Figs 2, 3), but can also be quantified by using the coefficient of variation (CV; standard deviation/mean; Tables 1, 2). Utilizing the arcsine of square-root-transformed CVs for testing purposes (to normalize CV distribution), *M. pergandei* workers were highly significantly less variable in their ventilation than male or female alates of their own species or the workers or female alates of *M. julianus* ($t > 5.8$; $P < 0.00001$). Interestingly, the workers of *M. julianus*, although they were still able to ventilate discontinuously, had significantly lower CVs than the female alates (Table 2; $P < 0.001$).

Phases and timing

The ventilation phase coefficient is a measure of the incremental increase in ventilatory phase duration as overall DVC duration increases (Lighton, 1990, 1991a). The durations of the closed (C), flutter (F) and burst (O) phases as a function of total DVC duration in both species are shown in Figs 4, 5 and 6, respectively. In *M. pergandei* female alates, the C ventilation phase coefficient was $14.1 \pm 4.4\%$ (S.E.), while the F ventilation phase coefficient was $61.7 \pm 5.4\%$ and the O ventilation phase coefficient was $19.1 \pm 5.4\%$. Male alates differed chiefly in not displaying a consistent F phase, which

was found in only three of our sample of 26 ants. Their C ventilation phase coefficient was therefore larger, at $86.7 \pm 5.1\%$ (Fig. 4). In *M. julianus* female alates, the C ventilation phase coefficient was $16.1 \pm 2.2\%$, the F ventilation phase coefficient was $63.3 \pm 3.6\%$ and the O ventilation phase coefficient was $16.9 \pm 2.6\%$. No workers displayed an unambiguous F phase; consequently, their C ventilation phase coefficient was greater, at $76.4 \pm 5.0\%$. Their O ventilation phase coefficient was $16.9 \pm 4.1\%$ (Fig. 6).

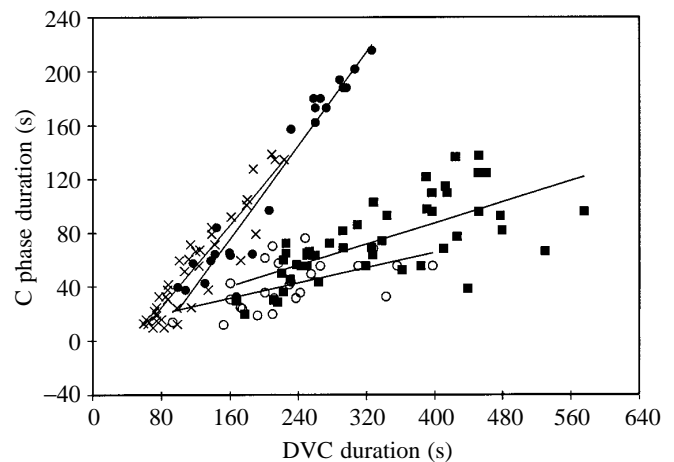


Fig. 4. The relationship between closed (C) phase duration and total DVC duration in discontinuously ventilating *M. pergandei* and *M. julianus*. *M. pergandei* workers did not ventilate discontinuously and are not shown. *M. pergandei* female alates are open circles, male alates are filled circles; *M. julianus* female alates are filled squares and workers are crosses. Individual regression lines are shown. Male alates seldom, and workers never, displayed a flutter (F) phase (three male alates that did display an F phase are excluded from this graph); their C phase therefore occupies a larger proportion of their total DVC duration than in female alates of either species (ANCOVA: shared slope 70.5% ; $P > 0.4$). The female alates shared a common slope or C ventilation phase coefficient of 18.5% (ANCOVA: $P > 0.4$).

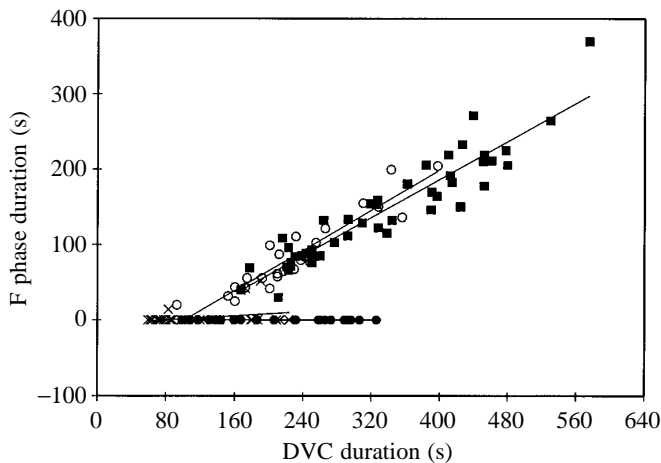


Fig. 5. The relationship between flutter (F) phase duration and total DVC duration in discontinuously ventilating *M. pergandei* and *M. julianus*. *M. pergandei* workers did not ventilate discontinuously and are not shown. *M. pergandei* female alates are open circles, male alates are filled circles; *M. julianus* female alates are filled squares and workers are crosses. Individual regression lines are shown. Male alates seldom, and workers never, displayed an F phase (three male alates that did display an F phase are excluded from this graph); their F phase durations therefore lie along the horizontal line $y=0$. The female alates shared a common slope or F ventilation phase coefficient of 64.0% (ANCOVA: $P>0.2$).

In the case of the O phase, ventilation phase coefficients can be easily misinterpreted owing to their incremental nature. If O phase duration is expressed instead as a percentage of total DVC duration, a more intuitive measure of the relative duration of the O phase emerges. In *M. pergandei*, O phase

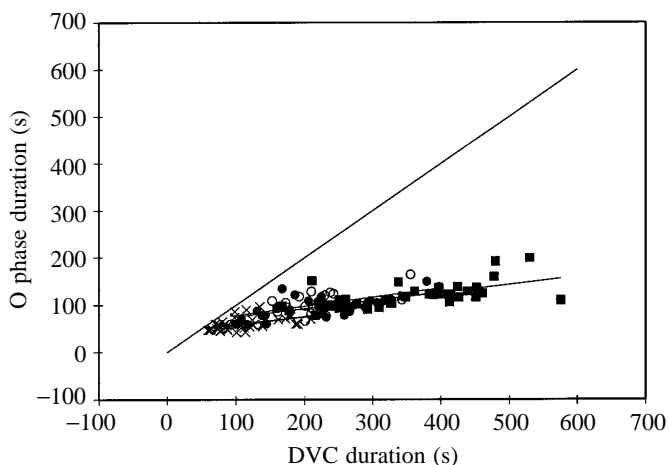


Fig. 6. The relationship between burst (O) phase duration and total DVC duration in discontinuously ventilating *M. pergandei* and *M. julianus*. *M. pergandei* workers did not ventilate discontinuously and are not shown. *M. pergandei* female alates are open circles, male alates are filled circles; *M. julianus* female alates are filled squares and workers are crosses. Individual regression lines are shown. The line of equality between O phase and total DVC durations is also shown (see Discussion). All species and castes shared a common slope or O ventilation phase coefficient of 17.3% (ANCOVA: $P>0.4$).

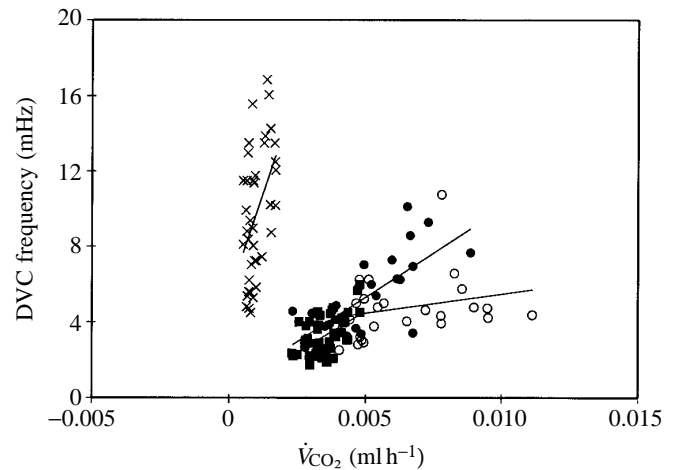


Fig. 7. The relationship between overall \dot{V}_{CO_2} and DVC frequency in discontinuously ventilating *M. pergandei* and *M. julianus*. *M. pergandei* workers did not ventilate discontinuously and are not shown. *M. pergandei* female alates are open circles, male alates are filled circles; *M. julianus* female alates are filled squares and workers are crosses. Individual regression lines are shown. They do not share common slopes (ANCOVA: $P<0.001$; see text). Where y is DVC frequency in mHz and x is \dot{V}_{CO_2} , in *M. pergandei* female alates, $y=3.43+202.9x$ ($r^2=0.06$, $P>0.2$) and in male alates, $y=-0.61+938.0x$ ($r^2=0.52$, $P<0.001$). In *M. julianus* female alates, $y=0.24+862.2x$ ($r^2=0.26$, $P<0.001$) and in workers, $y=5.43+4354.5x$ ($r^2=0.26$, $P=0.003$).

duration is $47.0\pm 11.8\%$ of the DVC in female alates and a statistically equivalent $47.4\pm 14.0\%$ in male alates. In female alates of *M. julianus*, relative O phase duration was significantly less at $35.9\pm 8.6\%$ ($P<0.01$), reflecting the longer DVCs in this species (Tables 1, 2). In workers of *M. julianus*, however, O phase duration was long indeed, occupying over half of each DVC ($57.1\pm 17.0\%$), significantly more than in male or female alates of either species ($P<0.001$).

Modulation

In *M. pergandei* alates, ventilation frequency increased with \dot{V}_{CO_2} (Fig. 7), but the effect was only modestly significant, with \dot{V}_{CO_2} explaining less than 15% of DVC frequency variation. Males displayed a slight, but significant, trend towards higher DVC frequencies at high \dot{V}_{CO_2} (analysis of covariance, ANCOVA: $P<0.05$). Burst volume, or the volume of CO_2 emitted during the O or burst phase, also increased with \dot{V}_{CO_2} (Fig. 8), but less than 40% of burst volume variance was explained by \dot{V}_{CO_2} , and male and female alates did not differ significantly in the modulation of burst volume by \dot{V}_{CO_2} (ANCOVA: $P>0.1$). Under these circumstances, it is reasonable to hypothesize that alates displaying high burst frequencies will also display low burst volumes, and *vice versa*. This hypothesis can be tested by examining the relationship between unexplained variances in DVC frequency and burst volume *versus* \dot{V}_{CO_2} . If this is done by analysis of the residuals of data in Figs 7 and 8, this hypothesis cannot be disproved (Fig. 9). In spite of slightly higher DVC frequencies

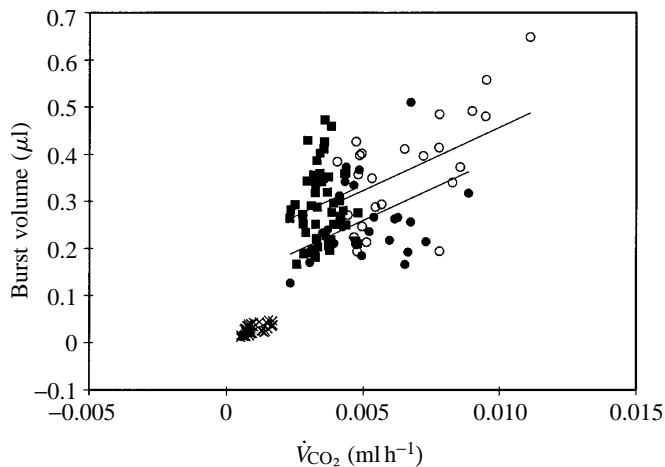


Fig. 8. The relationship between overall \dot{V}_{CO_2} and open (O phase) CO_2 emission volume in discontinuously ventilating *M. pergandei* and *M. julianus*. *M. pergandei* workers did not ventilate discontinuously and are not shown. *M. pergandei* female alates are open circles, male alates are filled circles; *M. julianus* female alates are filled squares and workers are crosses. Individual regression lines are shown. They share a common slope of 26.79 [ANCOVA: $F(3,132)=2.54$; $P=0.06$; see text]. The intercepts of the alates are statistically equivalent, but the worker intercept is significantly smaller (see text for further details).

in males, the inverse co-modulation of DVC frequency and burst volume in male and female alates is identical [ANCOVA on residuals in Fig. 9: $F(1,47)=0.02$; $P(\text{same slope})=0.4$; $F(1,48)=0$; $P(\text{same intercept})=0.4$]. Equivalently, multiple regression analysis, using \dot{V}_{CO_2} as the dependent variable and

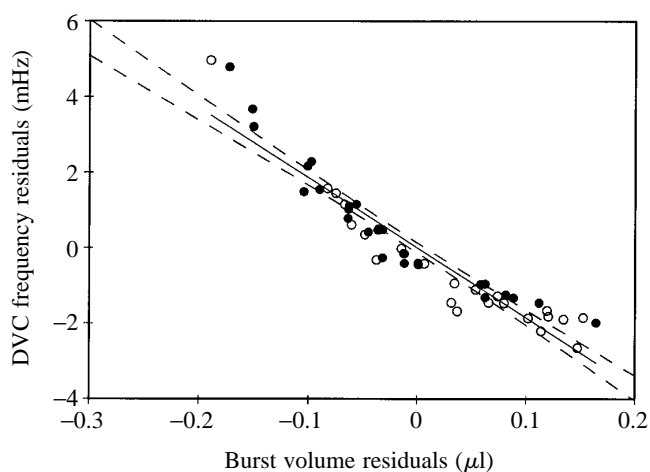


Fig. 9. The relationship between unexplained variance in the relationship between DVC frequency and \dot{V}_{CO_2} O-phase (Fig. 7) and the unexplained variance in the relationship between burst volume and \dot{V}_{CO_2} (Fig. 8) in *M. pergandei* female alates (open circles) and male alates (filled circles), showing that a strong inverse co-modulation of DVC frequency and O-phase volume explains 90% of the previously unexplained variance of these parameters as a function of \dot{V}_{CO_2} . Where x is burst volume residuals and y is DVC frequency residuals, $y = -18.56x$ ($r^2=0.90$, $P<0.001$). See text for further details.

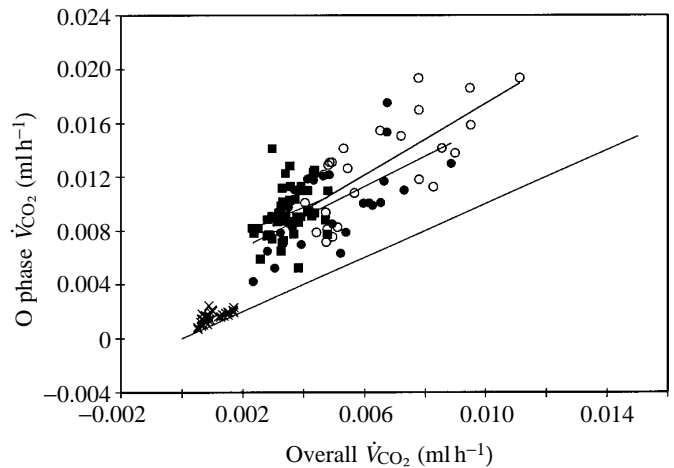


Fig. 10. The relationship between overall \dot{V}_{CO_2} and open (O phase) CO_2 emission rate in discontinuously ventilating *M. pergandei* and *M. julianus*. *M. pergandei* workers did not ventilate discontinuously and are not shown. *M. pergandei* female alates are open circles, male alates are filled circles; *M. julianus* female alates are filled squares and workers are crosses. Individual regression lines are shown; note that the lower line is not a regression line but is drawn at equality between O phase \dot{V}_{CO_2} and overall \dot{V}_{CO_2} (see text). The fitted lines share a common slope of 1.21 (ANCOVA; $P=0.3$; see text). The intercepts of the alates are statistically equivalent, but the worker intercept is significantly (tenfold) smaller (see text for further details).

DVC frequency and burst volume as the independent variables, yields a coefficient of multiple correlation of 0.964. Thus, 93% of the variance of CO_2 is explained by the equation:

$$\dot{V}_{\text{CO}_2} = 0.000866\text{DVCF} + 0.0172\text{BV}, \quad (1)$$

where \dot{V}_{CO_2} is in ml h^{-1} , DVCF is discontinuous ventilation frequency in mHz , and BV is burst volume in μl (combined male and female alate data).

Changes in burst volumes as a function of \dot{V}_{CO_2} were not mediated by changes in O phase duration ($r^2=0.03$; $P>0.3$). Rather, an increase in the rate of CO_2 emission during the O phase was responsible for the increase in burst volume. This increase in O phase \dot{V}_{CO_2} was strongly correlated with overall \dot{V}_{CO_2} ($r^2=0.53$; $P<0.001$), with O phase \dot{V}_{CO_2} increasing $33 \pm 18\%$ (S.E.M.) faster than overall \dot{V}_{CO_2} in both male and female alates (Fig. 10).

The situation is broadly equivalent in *M. julianus* female alates and workers, but alates differ from workers in the details of DVC frequency and burst volume modulation. In both castes, DVC frequency increased with increasing \dot{V}_{CO_2} (Fig. 7), but significantly more rapidly in the case of the workers (ANCOVA: $P<0.01$). The power of \dot{V}_{CO_2} in predicting DVC frequency was, however, poor ($<26\%$; Fig. 7). Female alates did not modulate burst volume in accordance with \dot{V}_{CO_2} ; burst volume varied over a threefold range, apparently at random (Fig. 8; $r^2=0.003$; $P=0.4$). Workers, in contrast, increased burst volumes significantly with increased \dot{V}_{CO_2} , although the relationship was still fairly weak ($r^2=0.33$; Fig. 8). Analysis of the residuals for *M.*

julianus in Figs 7 and 8 revealed, however, inverse co-modulation of DVC frequency and burst volume broadly equivalent to that of *M. pergandei* (Fig. 11). However, workers and female alates differed highly significantly in the details of the modulation. In terms of variance unexplained by \dot{V}_{CO_2} , workers modulated DVC frequency *versus* burst volume some 30 times more steeply than female alates (Fig. 11).

By multiple regression analysis, in female alates of *M. julianus*:

$$\dot{V}_{CO_2} = 0.000907DVCF + 0.009999BV \quad (2)$$

(coefficient of multiple correlation 0.916, 83 % of variance in \dot{V}_{CO_2} explained), while in workers of *M. julianus*:

$$\dot{V}_{CO_2} = 0.0000907DVCF + 0.0338BV \quad (3)$$

(coefficient of multiple correlation 0.965, 93 % of variance in \dot{V}_{CO_2} explained).

As with *M. pergandei*, an increase in O phase \dot{V}_{CO_2} was responsible for increasing burst volume as \dot{V}_{CO_2} increased; O phase duration remained unchanged as a function of \dot{V}_{CO_2} ($P>0.2$). By ANCOVA, the relationship between O phase \dot{V}_{CO_2} and overall \dot{V}_{CO_2} in all discontinuously ventilating species and castes shared a common slope of 1.2 (ANCOVA; $P>0.2$; Fig. 10), but the O phase \dot{V}_{CO_2} of the workers was significantly lower, corresponding to a significantly lower intercept (10 % of the female alate value; ANCOVA: $P<0.001$; Fig. 10).

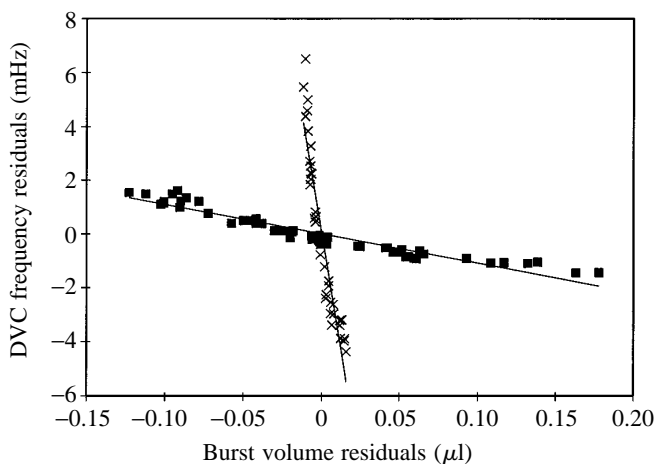


Fig. 11. The relationship between unexplained variance in the relationship between DVC frequency and \dot{V}_{CO_2} (Fig. 7) and the unexplained variance in the relationship between burst volume and \dot{V}_{CO_2} (Fig. 8) in *M. julianus* female alates (filled squares) and workers (crosses). In both cases, a strong inverse co-modulation of DVC frequency and burst volume explains more than 90 % of the previously unexplained variance of these parameters as a function of \dot{V}_{CO_2} . Where x is burst volume residuals and y is DVC frequency residuals, in female alates $y = -10.94x$ ($r^2 = 0.92$, $P < 0.001$), and in workers, $y = -349.7x$ ($r^2 = 0.90$, $P < 0.001$). The slope is significantly greater in the workers. This means that workers modulate DVC frequency approximately 30-fold more than burst volume as a function of \dot{V}_{CO_2} (see text and equation 3 for further details).

Discussion

Discontinuous ventilation

Modulation

In all discontinuously ventilating insects studied so far, changes in \dot{V}_{CO_2} are accommodated by orderly, linear changes in DVC frequency (Lighton, 1988b; Lighton and Wehner, 1993). In xeric ants, such as the Saharan thief ant *Cataglyphis bicolor*, burst volumes are fixed at a maximal level that is independent of \dot{V}_{CO_2} (Lighton and Wehner, 1993), while in more mesic ants, such as *Camponotus vicinus*, burst volumes decline linearly with increasing \dot{V}_{CO_2} and increasing DVC frequency (Lighton, 1988b). Thus, in *Cataglyphis*, only DVC frequency is modulated by \dot{V}_{CO_2} , while in *Camponotus* both DVC frequency and burst volume are modulated, but in a linear, predictable fashion. The alates of *M. pergandei* and *M. julianus* are therefore atypical. They modulate burst frequency and burst volume in an apparently capricious fashion, quite unlike the more stereotypical modulation observed in the other species examined to date. Neither burst frequency nor burst volume change with \dot{V}_{CO_2} in ways that suggest tight regulation of one parameter or the other.

Direct comparisons of our ventilation modulation data with those presented in the papers cited above are complicated, however, by the relatively small range of \dot{V}_{CO_2} shown by our ants, which were investigated at a single temperature. In the other investigations, \dot{V}_{CO_2} was changed over a much wider range by altering the ambient temperature. It is possible (but speculative as yet) that DVC modulation by \dot{V}_{CO_2} may differ if it is measured at single temperatures and/or over narrow ranges *versus* a wide range of temperatures and \dot{V}_{CO_2} values.

Nevertheless, some informative principles emerge from our data. The modulation characteristics of the alates of both species were generally similar (Figs 7–11). However, as a function of overall \dot{V}_{CO_2} , the workers of *M. julianus* modulated DVC frequency tenfold more, and burst volumes 3.4-fold less, than did *M. julianus* alates (Fig. 11; equation 3). The low O phase \dot{V}_{CO_2} of the *M. julianus* workers explains their reliance on the modulation of burst frequency, rather than burst volume, with changing \dot{V}_{CO_2} ; this is very clearly shown in Fig. 10. This aspect of their respiratory physiology is particularly informative when considered in the light of the apparent inability of workers in the congeneric species *M. pergandei* to ventilate discontinuously under the conditions of our experiments.

Caste effects

We have demonstrated that the alates of both *Messor* species examined here ventilate discontinuously, while the workers of *M. julianus* do so rather poorly – with over 50 % of each DVC spent in the O phase – and the workers of *M. pergandei* do so extremely rarely. What is the basis of this difference between alates and workers? The picture becomes clearer if we consider a *reductio ad absurdum*, namely diffusive gas exchange by an insect that emits CO_2 during the O phase at a rate equal to mitochondrial formation of CO_2 or ‘overall \dot{V}_{CO_2} ’. Such an insect is incapable of discontinuous ventilation. Its ‘O phase’ is

a continuous process of diffusion. At the other extreme, an insect that emits CO₂ very rapidly during its O phase, and therefore minimizes the relative duration of its O phase, must ventilate highly discontinuously. Between these two extremes is a broad continuum in which O phase rates of CO₂ release exceed steady-state, mitochondrial-level \dot{V}_{CO_2} by variable margins. Obviously, the closer an insect approaches the line of equality between O phase \dot{V}_{CO_2} and overall \dot{V}_{CO_2} , the more marginal is its capacity for discontinuous ventilation. In this respect, Fig. 10 is highly informative. Because the O phase \dot{V}_{CO_2} of the *M. julianus* workers, as a function of their overall \dot{V}_{CO_2} , is tenfold lower than that of the female alates, the workers are barely above the line of equality between O phase \dot{V}_{CO_2} and overall \dot{V}_{CO_2} at which the DVC must break down. In addition, *M. julianus* workers devote a large enough proportion of their DVC to the O phase to draw them close to the line of equality at which the DVC becomes a continuous 'O phase' (Fig. 6). Note that burst CO₂ emission volumes scale as body mass to the power 1.67 in *M. julianus* (data from Table 2). Thus, in the O phase, female alates of *M. julianus* release 11 times more CO₂, or 2.6 times more CO₂ per unit body mass, than do workers of that species.

It follows that *M. julianus* workers barely utilize the strategy of discontinuous ventilation, although the alates of both species do so to a very marked extent. Further, *M. pergandei* workers do not utilize discontinuous ventilation at all, but are situated on the lines of equality in Figs 6 and 10, a position in no way predictive of poor adaptation to xeric environments, as they themselves (quite apart from the examples of *Eleodes* and *Tenebrio*) prove. Is this lack of discontinuous ventilation dictated by physiological necessity, i.e. limited maximal gas exchange rates?

In this context, it is important to realize that, in the case of *M. julianus* workers at least, the O phase \dot{V}_{CO_2} data in Fig. 10 are by no means indicative of maximal \dot{V}_{CO_2} . We infer this from the observation that *M. julianus* workers (like *M. pergandei* workers) are active at temperatures more than 15 °C above those utilized in the present investigation, corresponding to a threefold increase in \dot{V}_{CO_2} without even taking the effects of activity into account. Unless three- to tenfold changes in transpiracular diffusion gradients occur, which is unlikely, any increase in overall \dot{V}_{CO_2} must be accompanied by decreased occlusion of the spiracles and/or by active ventilation.

Thus, the rather mediocre performance of *M. julianus* workers as discontinuous ventilators and the failure of *M. pergandei* workers to ventilate discontinuously do not reflect physiological constraints imposed by low maximal rates of CO₂ output. In addition, their ventilation strategy does not appear to exact a water loss penalty. Certainly, in the case of *M. pergandei* workers, failure to ventilate discontinuously has not elevated their overall water loss rates to unusual levels; on the contrary, the species thrives in arid areas (see Introduction) and the water loss rates of workers are low and typical of xeric arthropods (Edney, 1977; Lighton *et al.* 1995).

Given the appropriate stimulus, it is therefore likely that more pronounced discontinuous ventilation can be elicited from *M. julianus* workers and that *M. pergandei* workers can

be induced to ventilate discontinuously. This stimulus is likely to be hypoxia and/or hypercapnia of the degree (currently unknown) to which workers are exposed within the nest itself. This hypothesis is readily testable. The advantage (if any) that accrues to maintaining continuous ventilation in normoxic, dry air remains problematic.

Is discontinuous ventilation necessary?

A cautiously comparative approach yields a working hypothesis concerning the puzzlingly patchy distribution of discontinuous ventilation in adult insects. We note that discontinuous ventilation is common in insects likely to encounter high concentrations of CO₂ (or low concentrations of O₂) in their natural environments. For example, subterranean ant nests are generally assumed to be hypoxic and hypercapnic, and in the case of cockroaches, the burrows beneath stones in which they generally live in nature are poorly ventilated. Many of the Namib Desert tenebrionids known to ventilate discontinuously spend most of their time buried in sand, where because of slow diffusion their immediate environment is likewise likely to be hypoxic and hypercapnic. In contrast, their confamiliar American representatives generally do not burrow or sand-swim, but seek shelter by creeping under vegetation where conditions are close to normoxic and normocapnic, as is also presumably the case with the natural haunts of *Tenebrio*.

The trend is suggestive, although as yet we lack the broad base of comparative data required to progress beyond speculation. And, as always, phylogeny may introduce unwelcome if potentially heuristic complications, such as normoxic insects that ventilate discontinuously on a somewhat capricious basis (see, for example, Hadley and Quinlan, 1993). A clear mechanistic determinant of the trend towards continuous ventilation in normoxic environments is, however, obvious. It is impossible to initiate a net flux of CO₂ or O₂ from areas of low to high partial pressures. The endotracheal volume of an insect must, of necessity, be hypoxic and hypercapnic relative to its environment or metabolically useful diffusion-driven flux cannot occur. An insect can solve the conundrum of diffusive gas exchange in significantly hypoxic and hypercapnic environments in two distinct ways. First, it may open its spiracles maximally for long periods, thus increasing net diffusive flux and compensating to some extent for a shallow partial pressure gradient. However, unless the insect's environment is perfectly water-saturated, this strategy may impose an unacceptable water loss penalty; overall water loss rates can increase several-fold while the spiracles are open (Lighton *et al.* 1993b). The second alternative is to sequester CO₂ and deplete O₂ within the tracheal system for as long as physiologically feasible. In this case, the partial pressure gradient will increase until a brief period of spiracular opening can effect the required net flux. Hence, discontinuous ventilation.

In normoxic and normocapnic environments, the DVC may be considered a somewhat intemperate strategy because of the far steeper partial pressure gradients driving net fluxes of O₂ into, and CO₂ out of, the insect's tracheal system, and it may not, in fact, be necessary. We infer this from the observed

absence of discontinuous ventilation in some successful denizens of xeric habitats (*Tenebrio* and *Eleodes*). The advantages, if any, accruing to this more sedate ventilatory strategy are problematic, particularly because the partial pressure gradient driving the diffusive uptake of O₂ is approximately 18 kPa, while that driving the diffusive release of CO₂ is only about 4 kPa (Levy and Schneiderman, 1966). This explains the short-term respiratory exchange ratio of approximately 0.2 during the steady-state, diffusive F phase of those insects in which it has been measured (Lighton, 1988a; J. R. B. Lighton, in preparation). Obviously, the F phase is not an indefinitely sustainable gas-exchange strategy. For continuous diffusion-driven gas exchange to be practical, O₂ and CO₂ partial pressure gradients need to be similar, which can be accomplished by lowering O₂ partial pressure gradients (at the expense of increasing spiracular diffusion rates and presumably entailing a water loss penalty) or by elevating endotracheal CO₂ partial pressures approximately fivefold (with problematic, if not calamitous, effects on pH regulation). From this we deduce that some form of continuous active, convective ventilation is likely to accompany any shift away from discontinuous gas exchange. If the correct balance between active ventilation and passive diffusion is found, however, one potential benefit of continuous gas exchange may be a more constant internal environment (see Bridges and Scheid, 1982). The detailed nature of this ventilatory strategy awaits investigation, but it is not unduly Panglossian to infer that some selective advantage must accompany the trend away from discontinuous gas exchange in some highly xeric insects. It is, in any event, clear that the respiratory physiology of adult insects may be more subtle, sophisticated and varied than is generally supposed.

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