Respiratory and cuticular water loss in insects with continuous gas exchange: Comparison across five ant species

Pablo E. Schilman[a],*, John R.B. Lighton[b], David A. Holway[a]

[a]Section of Ecology, Behavior and Evolution, Division of Biological Sciences, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116, USA
[b]Department of Biological Sciences, University of Nevada at Las Vegas, 4505 Maryland Parkway, Las Vegas, Nevada 89154-4004, USA

Abstract

Respiratory water loss (RWL) in insects showing continuous emission of CO_2 is poorly studied because few methodologies can measure it. Comparisons of RWL between insects showing continuous and discontinuous gas exchange cycles (DGC) are therefore difficult. We used two recently developed methodologies (the hyperoxic switch and correlation between water-loss and CO_2 emission rates) to compare cuticular permeabilities and rates of RWL in five species of ants, the Argentine ant (*Linepithema humile*) and four common native ant competitors. Our results showed that RWL in groups of ants with moderate levels of activity and continuous gas exchange were similar across the two measurement methods, and were similar to published values on insects showing the DGC. Furthermore, ants exposed to anoxia increased their total water loss rates by 50–150%. These results suggest that spiracular control under continuous gas exchange can be as effective as the DGC in reducing RWL. Finally, the mesic-adapted Argentine ant showed significantly higher rates of water loss and cuticular permeability compared to four ant species native to dry environments. Physiological limitations may therefore be responsible for restricting the distribution of this invasive species in seasonally dry environments.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Linepithema humile; Gas exchange; Water loss; Cuticular permeability; Respiratory water loss; Biological invasion

1. Introduction

Water is essential for life; animals contain a high proportion of water. Since terrestrial animals live in a medium (air) that contains thousands of times less water than themselves, they should possess effective mechanisms to prevent desiccation by passive loss of water. Because of their large body surface area to mass ratios, terrestrial arthropods are especially susceptible to dehydration (Chown, 2002; Edney, 1977; Hadley, 1994). This is particularly true for water loss through the cuticle of terrestrial insects, but other sources may also contribute to desiccation (e.g., water loss through the spiracles when insects exchange gases during respiration).

The importance of respiratory water loss (RWL) in insects is controversial. It has been claimed in the literature that the cuticle is the primary route of water loss in terrestrial arthropods, and that RWL is such a small component of total water loss that it can be ignored (Edney, 1977; Hadley, 1994). For example in four ant species expressing discontinuous gas exchange cycles (DGC), the RWL rate varied between 2–8% of total water loss (Lighton, 1992; Quinlan and Lighton, 1999). Moreover, data from more than 20 species of insects from four different orders show that RWL is less (and usually much less) than 20% of total water loss (see Table 1 from Chown, 2002). As a consequence, many authors have concluded that modulation of such a small contribution is unlikely to represent a fitness benefit

*Corresponding author. Tel.: +1 858 822 5206;
fax: +1 858 534 7108.
E-mail address: pschilman@yahoo.com (P.E. Schilman).

Table 1
Masses and catabolic flux rates

Ant species	Temp. (°C)	N	Individual mass (mg)	$V\,CO_2$ ($\mu l\,h^{-1}\,ant^{-1}$)	MR (μW)	SMR (μW)	MR vs. SMR Paired t-test	Q_{10}
Forelius mccooki	20	6	0.274 ± 0.052	0.26 ± 0.04	1.52 ± 0.21	0.61 ± 0.10	$t_5 = 14.488$, $P < 0.0001$	
	30	6	0.278 ± 0.014	0.59 ± 0.10	3.45 ± 0.57	1.24 ± 0.06	$t_5 = 9.168$, $P = 0.0003$	2.02
	40	6	0.266 ± 0.020	1.05 ± 0.05	6.13 ± 0.68	2.39 ± 0.16	$t_5 = 16.291$, $P < 0.0001$	
Linepithema humile	20	9	0.460 ± 0.036	0.55 ± 0.16	3.18 ± 0.95	0.96 ± 0.06	$t_8 = 6.740$, $P < 0.0001$	
	30	6	0.424 ± 0.008	0.66 ± 0.09	3.85 ± 0.53	1.79 ± 0.03	$t_5 = 9.424$, $P = 0.0002$	1.73
	40	6	0.428 ± 0.020	1.54 ± 0.07	8.96 ± 0.96	3.60 ± 0.14	$t_5 = 13.178$, $P < 0.0001$	
Crematogaster californica	20	5	1.435 ± 0.161	0.79 ± 0.08	4.63 ± 1.02	2.53 ± 0.24	$t_4 = 5.609$, $P = 0.0050$	
	30	7	1.360 ± 0.198	1.47 ± 0.38	8.57 ± 2.23	4.83 ± 0.60	$t_6 = 4.692$, $P = 0.0034$	1.82
	40	5	1.485 ± 0.209	2.66 ± 0.43	15.55 ± 2.53	10.43 ± 1.26	$t_4 = 5.940$, $P = 0.0040$	
Dorymyrmex insanus	20	5	0.613 ± 0.083	0.33 ± 0.02	1.91 ± 0.15	1.22 ± 0.14	$t_4 = 10.019$, $P = 0.0006$	
	30	6	0.579 ± 0.025	0.67 ± 0.06	3.94 ± 0.37	2.33 ± 0.09	$t_5 = 8.964$, $P = 0.0003$	2.25
	40	6	0.571 ± 0.046	1.57 ± 0.27	9.16 ± 1.55	4.60 ± 0.32	$t_5 = 7.036$, $P = 0.0009$	
Solenopsis xyloni	20	5	0.415 ± 0.023	0.32 ± 0.06	1.86 ± 0.37	0.88 ± 0.04	$t_4 = 6.009$, $P = 0.0039$	
	30	6	0.431 ± 0.012	0.76 ± 0.09	4.42 ± 0.51	1.81 ± 0.04	$t_5 = 13.094$, $P < 0.0001$	1.78
	40	6	0.648 ± 0.096	1.47 ± 0.15	8.56 ± 0.85	5.13 ± 0.66	$t_5 = 18.624$, $P < 0.0001$	

Data are reported as mean $\pm$ SD. N = sample size. MR = metabolic rate. SMR = standard metabolic rate predicted by the allometric equation for inactive tracheate arthropods, using a $Q_{10} = 2$ (Lighton et al., 2001).

(Lighton, 1994, 1998). However, we can speculate that RWL is a small component of total water loss just because it is under strong natural selection. This has been only partially demonstrated as changes of gas exchange patterns resulting from selection (Gibbs, 1999) for desiccation resistance in *Drosophila* in the laboratory (Williams et al., 1997, 1998). Selection to reduce RWL is also supported by the fine control of spiracle opening even under highly energetic activities such as flying (Lehmann, 2001). The two main pathways of water loss in insects, i.e., through the cuticle and the spiracles, may both have been minimized by natural selection, but the selective mechanisms evolved independently because mechanisms to reduce water loss are completely different in each case. To reduce water loss through the cuticle the thickness or the composition of waxes and other hydrocarbons can be modulated, while to reduce water loss through the spiracles the morphology of the spiracles may be altered or their degree of opening kept to the minimum that is strictly necessary to exchange gases, i.e., CO_2 and O_2, without imposing a needless RWL penalty (e.g., Lehmann, 2001).

It has been widely suggested that the DGC evolved to reduce RWL, although other possible explanations have recently been discussed (see Bradley, 2000; Hetz and Bradley, 2005; Lighton, 1996; Lighton and Berrigan, 1995; and also Chown et al., 2005 for review). Only a few comparative studies concentrate on the importance of RWL (e.g., Chown and Davis, 2003), but RWL in insects that show a pattern of continuous gas exchange remain largely uninvestigated. This is not because RWL

is thought to be unimportant in insects with continuous gas exchange, but until now distinguishing between cuticular and spiracular water loss rates (WLRs) in insects with continuous gas exchange was technically not feasible (but see Gibbs and Johnson, 2004; Lighton et al., 2004). The hyperoxic switch (Lighton et al., 2004) is a non-invasive technique that modulates spiracular opening by manipulating gas composition, and permits the measurement of RWL in insects with continuous gas exchange. This technique has the advantage that it can be used for groups of insects. This, in turn, allows investigators to improve the signal/noise ratio when making measurements of WLRs in insects, ants in this case, with a mass of an order of magnitude smaller than has been measured by open flow respirometry, with the concomitant advantages of this methodology, i.e., the high temporal resolution, which allows minimization of measurement errors and overestimates (for discussion see Lighton and Fielden, 1996; Lighton et al., 2004).

In this study we measured physiological characteristics of insect gas exchange such as metabolic rate, cuticular permeability (CP), RWL and maximal rates of RWL through diffusion (which could be an indicator of total respiratory throughput capacity) in groups of insects exhibiting continuous patterns of gas exchange. To measure these parameters we applied recently developed methodologies to measure RWL in insects with continuous gas exchange, i.e., the hyperoxic switch method (Lighton et al., 2004), and the correlation between water-loss and CO_2 emission rates (Gibbs and Johnson, 2004). We also compared these two

methodologies and addressed the ecological hypothesis that the mesophilic and exotic ant species *Linepithema humile* (Argentine ant) will lose water more readily when compared with four common species of xeric-adapted ants native to the southwestern US, which compete with the Argentine ant. We measured CO_2 production and water vapor emission in groups of Argentine ants, *L. humile*, and four common native ant species from coastal southern California, i.e., *Forelius mccooki, Dorymyrmex insanus, Crematogaster californica*, and *Solenopsis xyloni* at three different temperatures (20, 30 and 40 °C) using open flow respirometry.

2. Materials and methods

2.1. Animals

We collected all ants used in this study from San Diego Co., CA, USA. We collected a colony fragment of the Argentine ant, *L. humile,* from Los Peñasquitos Canyon Preserve. Workers of *D. insanus* were collected from urban edges in La Jolla. Colonies of *F. mccooki*, and workers of *C. californica* and *S. xyloni* were collected at the University of California Elliot Chaparral Reserve. The native ants selected are among the most common species found in xeric scrub habitats in San Diego, and all are commonly displaced by Argentine ants (Suarez et al., 1998; Holway, 2005).

All colonies or groups of workers were maintained in round polyethylene nest containers (diameter 28 cm) lined with Fluon® to prevent ants from escaping. Each nest container had three nest chambers (test tubes with water reservoirs backed by cotton wool and wrapped in aluminum foil to block ambient light). Colonies were maintained in a temperature-controlled room (23 ± 1 °C) with an ambient photoperiod for 2–4 weeks prior to testing. They were fed on crickets (once a week), ca. 25% sugar solution (three times per week) and water ad libitum.

2.2. Respirometry

We used flow-through respirometry to measure real time water vapor emission and CO_2 production in unrestrained ant workers. For all measurements we used the high-resolution TR-2 Sable System International (SSI; Las Vegas, Nevada, USA) flow-through respirometry system (Duncan and Lighton, 1994). Briefly, air free of CO_2 and H_2O was drawn through low-permeability, Bev-A-Line tubing (to minimize errors associated with water vapor absorbance) and a respirometry chamber also made of Bev-A-Line tubing with stainless steel mesh filters at a flow rate of 20 ml min^{-1} controlled by a Tylan FC-260 mass-flow control valve attached to a SSI two-channel mass-flow controller (TR-MFC1). The

water vapor and CO_2 produced by the ants were measured by a SSI RH-100 water vapor analyzer (0.1 Pa of resolution and 1% accuracy) and a Li-Cor CO_2 analyzer (resolution 0.1 ppm CO_2), respectively. Specimen temperatures were controlled to ± 0.1 °C by a SSI's Pelt-4 temperature controller and SSI's PTC-1 Peltier Effect cabinet with a volume of 8 l. The CO_2 analyzer was zeroed with CO_2-scrubbed outside-building air and spanned at 976 ppm with a certified span gas. The water vapor analyzer was zeroed with nitrogen and spanned by bubbling air through pure water at an accurately known temperature (measured by a thermocouple attached to a SSI TC1000 thermocouple meter, accuracy < 0.2 °C) ca. 5 °C lower than ambient. The RH-100 was set to its dewpoint mode, and adjusting it to read the correct water temperature, i.e., temperatures reading from the TC1000 and RH-100 matched. See Lighton et al. (2004) for a detailed explanation of the setup.

2.3. Experimental procedure

Each group of ants was weighed to the nearest 0.01 mg with a Mettler AG245 balance. Meanwhile, the start baselines for the carbon dioxide and water vapor analyzers were recorded. After weighing the ants, and pausing the recording, we aspirated ants directly into the Bev-A-Line respirometry chamber, which was immediately connected to the respirometry system. The recording continued until a plateau in WLR was reached. At that point the gas flowing through the system was changed to pure oxygen (which did not affect the water vapor or CO_2 analyzers), and the recording was restarted. After a further ca. 20 min, the oxygen was changed to pure nitrogen, and the recording continued until the ants' CO_2 production fell to near baseline levels. Finally, the recording was paused again, the ants were removed from the respirometry chamber, and the end baseline was recorded. Each recording lasted about 1.5–2 h, including initial and final baselines, and consisted of a variable number of data points taken at 2-s intervals from either a group of 3 *S. xyloni* or *C. californica* workers, or groups of 10 workers for the other species (i.e., *D. insanus, F. mccooki* and *L. humile*). We took 5–9 independent measurements for each species at each of three temperatures (20, 30 and 40 °C).

2.4. Measuring respiratory water loss: the hyperoxic switch method

Estimates of gross CP do not allow one to distinguish between respiratory and cuticular water loss. RWL is also difficult or impossible to measure in isolation. To distinguish between these two components of water loss, we developed a new and non-invasive technique that modulates spiracular opening by gas composition

(Lighton et al., 2004). After ants in a respirometry chamber reach a steady state of CO_2 and water vapor emission under normoxic conditions, we infuse pure O_2, which causes a temporary decrease in spiracular area and a coincident, transient drop in CO_2 output and H_2O vapor loss. We then measure RWL from the proportional decrease of H_2O vapor under hyperoxic conditions, and estimate CP by subtracting RWL from gross CP. We conservatively estimate RWL, as a percentage of total WLRs, with Eq. (1).

$$CRWL = 100(\Delta WL/WL), \tag{1}$$

where CRWL is the conservative estimate of RWL rate as a percentage of total WLR, ΔWL the change in WLR across the hyperoxic switch, and WL the total rate of water loss prior to the hyperoxic switch. In contrast, the upper limit of RWL rate can be expressed as follows:

$$URWL = CRWL/(\Delta CO_2/CO_2), \tag{2}$$

where URWL is the upper estimate of RWL rate as a percentage of total WLR, ΔCO_2 is the change in rate of CO_2 emission (VCO_2) across the hyperoxic switch, and CO_2 is VCO_2 prior to the hyperoxic switch. Eq. (2) corresponds to a transient reduction of spiracular conductance, and thus RWL, to zero. CO_2 is arguably a more reliable index of the degree of spiracular closure than H_2O vapor, because H_2O vapor has extremely sluggish kinetics compared to CO_2. It is trivial to modify Eq. (2) to allow for a less than complete reduction in spiracular conductance across the hyperoxic switch (equivalent to a unity numerator factor in Eq. (2)); this assumption simply allows us to set an upper limit to the contribution of RWL to total water loss.

2.5. Measuring respiratory water loss: the CO_2 emission vs. water loss regression method

We also analyzed the data with a new technique developed by Gibbs and Johnson (2004). Briefly, we plotted WLR against CO_2 release for each individual group of ants using the 2 s time-averaged values over 0.5–1 h of respiratory recording. Extrapolation to the intercept provides an estimate of corrected cuticular water loss, i.e., without the spiracular component. The slope of each regression line estimates the hygric cost of gas exchange for that recording, i.e., the incremental increase in water loss associated with CO_2 release. RWL is calculated with the equation

$$RWL_{reg} = RS*CO_2, \tag{3}$$

where RWL_{reg} is the RWL estimated by the regression method (Gibbs and Johnson, 2004), RS is the slope of the regression expressed in $mg\,H_2O\,h^{-1}\,\mu l\,CO_2\,h^{-1}$, and CO_2 is the VCO_2 in $\mu l\,CO_2\,h^{-1}$. For a detailed explanation of the method, see Gibbs and Johnson (2004).

The CO_2 and water vapor signals were lag corrected because they were slightly out of phase due to the experimental arrangement (CO_2 and water vapor detectors were arranged in series, i.e., a given bolus of air reached the water vapor analyzer before it reached the CO_2 analyzer). We excluded the first 3–5 min of the recording in order to avoid higher values of water vapor and CO_2 caused by wash-out effects in the system. The final peaks of CO_2 and water vapor after infusion of pure N_2 were also excluded from the analysis because the peak of CO_2 requires O_2 for its production, but O_2 was something the ants, in their case, no longer had. This contrasts with water vapor output, which in the short term is independent of oxygen availability. We also excluded all defined peaks of water vapor (e.g., produced by grooming or excretion) that were independent of the metabolic state of the insects.

2.6. Analysis and statistics

Data were stored in a laptop computer by Datacan V data acquisition software with its UI-2 16-bit interface (basic accuracy = 0.05%) and analyzed with ExpeData data analysis software (SSI). The following corrections and conversions were made from the recordings: (1) CO_2 and H_2O baselines were subtracted, (2) CO_2 in ppm was converted to $\mu l\,h^{-1}$ (see (Lighton, 1991) for formulae), (3) H_2O vapor pressure in Pa was converted to $mg\,H_2O\,ml^{-1}$ (by dividing by $T*461.5$, where $T =$ temperature of the water vapor sensor in kelvin, and (4) H_2O concentration in $mg\,ml^{-1}$ was converted to WLR in $mg\,h^{-1}$ (by multiplying by flow rate in $ml\,h^{-1}$).

After corrections and conversions were made, the following values were measured and analyzed from the recording: (1) mean values of CO_2 and H_2O plateaus (pre-O_2 treatment; see below), (2) magnitude of post-O_2 decline in CO_2, and mean post-O_2 decline in H_2O, (3) the mean over a 30 s peak of maximal CO_2 emission and WLR post-N_2, and (4) the mean of the post-N_2 water loss plateau. We saved these values in a spreadsheet for further data manipulations as the conversion of CO_2 emission rate to energy units of microwatts, assuming the dissipative oxidative catabolism of glucose (see Lighton, 1991). The spreadsheet also included the calculation of the water vapor saturation deficit from chamber temperature (formulae in Lighton and Feener, 1989), the ant surface area (Lighton and Feener, 1989) and, thence, gross CP (i.e., combined respiratory and cuticular water loss).

Means are accompanied by standard deviations, except when noted, and sample sizes, and they are compared by analysis of variance (ANOVA) or t-test. Regressions are by least squares, with axis transformations where noted, and are tested for statistical significance by analysis of variance. Regressions are compared by analysis of covariance (ANCOVA).

3. Results

3.1. Masses and catabolic flux rates

Our samples of ants weighed between 0.2 to 1.5 mg per ant (Table 1). The difference across species was highly significant ($F_{4,85} = 333.83$, $P < 0.0001$, One-way ANOVA). However, as expected, there were no significant differences in mass at different temperatures ($F_{2,87} = 0.109$, $P = 0.897$, One-way ANOVA).

As might be expected of groups of 3 or 10 ants in a novel environment, the ants were active during the recordings, except when knocked out by displacement of oxygen (in pure nitrogen). Thus each species demonstrated a significant increase in the catabolic flux rate compared with the predicted value for inactive insects, corrected for temperature assuming a Q_{10} of 2 (equation from Lighton et al., 2001; see Table 1 for comparison).

Are the catabolic flux rates of the five ant species different? To answer this question, we divided catabolic flux rates in µW by live mass in mg raised to the 0.856 power, which is the interspecific mass scaling exponent for tracheate arthropods (Lighton et al., 2001). This eliminates the mass dependence of catabolic flux rates, allowing the five species to be directly compared. By ANCOVA of log-transformed mass-independent catabolic flux rates (log-transformed metabolic rate) vs. temperature, the five species differed in the temperature sensitivity of the catabolic flux rate, or slope ($F_{4,80} = 3.49$, $P < 0.01$). The slopes correspond to Q_{10} from 1.73 to 2.25, which are close to the usually assumed value of 2.0 (see Table 1). For example, _F. mccooki_ had a slope of 0.0306, which corresponds to a Q_{10} of $10^{((10)(0.0306))}$ or 2.02. Because the mass-independent metabolic rates (MR) of the five species significantly differed in their sensitivity to temperature, differences between intercepts could not be tested.

3.2. Water loss rates and gross cuticular permeabilities

A typical run is shown in Fig. 1. The relationship between mass-independent WLRs for the five ant species in units of $(mg\,H_2O\,h^{-1}(mg\,ant)^{-1})$ and the three temperatures tested are shown in Table 2. At high temperatures, _L. humile_, per mg of ant, loses water at a far higher rate compared to the other ant species (Table 2). The result is a steeper slope of mass-independent WLR vs. temperature for _L. humile_ (ANCOVA: same slope: $F_{4,80} = 10.027$, $P < 0.0001$). At 40 °C, for example, _L. humile_ loses 0.263 ± 0.027 $(mg\,H_2O\,h^{-1}(mg\,ant)^{-1})$ in totally dry air. In contrast, under the same conditions and at the same temperature, the other species lost significantly smaller amounts of water (Fig. 2; One-way ANOVA: $F_{4,24} = 32.712$, $P < 0.0001$); per unit of mass, Argentine ants lose water between 19 to 158% more rapidly compared to the other ant species (Fig. 2).
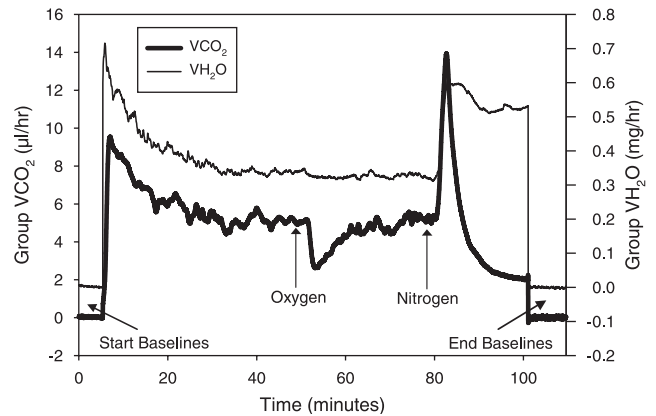

Fig. 1. Typical recording of 10 _L. humile_ workers at 20 °C. Switch from normal dry air to pure O_2 and N_2 indicated by arrows. Influx of pure O_2 causes a modulation of spiracular opening, resulting in a reduction in CO_2 and H_2O vapor emission, allowing us to distinguish respiratory and cuticular water loss rates. Influx of pure N_2 causes a sharp increase in CO_2 and H_2O vapor emission followed by a rapid decline of CO_2 to near baseline levels and a H_2O vapor plateau. Beginning and end of the recording are baselines.

Of course, these species are more fairly compared if their cuticular permeabilities are examined. This takes into account, the surface area of the ants, and over the temperature range tested, CP is independent of temperature. This is because the driving force for cuticular WLR is the water vapor pressure saturation deficit, which rises rapidly with temperature, and area-specific cuticular WLRs are divided by that figure. We confirmed the expected temperature independence of cuticular WLRs by ANCOVA (across species, no significant correlation between CP and temperature; $F_{1,84} = 2.195$, $P = 0.142$; Table 3). Using the "classic" units, the gross cuticular permeabilities, i.e., with the spiracular component included, for the different ant species range between $15.5 \pm 4.4\,\mu g\,cm^{-2}\,h^{-1}\,Torr^{-1}$ for _C. californica_ to $30.8 \pm 5.9\,\mu g\,cm^{-2}\,h^{-1}\,Torr^{-1}$ for _L. humile_. The difference across species is highly significant (Table 3; One-way ANOVA: $F_{4,85} = 28.44$, $P < 0.0001$); per unit surface area, Argentine ants lose water between 25% and 99% more rapidly compared to the other ant species (Table 3).

3.3. Respiratory water loss and corrected cuticular permeabilities

RWL is generally considered to be a small component of total water loss in most ant species, and it is also extremely difficult to measure (in particular with insects showing continuous gas exchange). The hyperoxic switch technique causes a transient drop in carbon dioxide output when normal air is replaced with pure O_2 in the respirometry chamber (Fig. 1). The percentage of this transient decline, relative to immediately pre-oxygen

Table 2
Linear regressions of water loss rates vs. temperature

Ant species	Slope	Intercept	N	r^2	ANOVA
Forelius mccooki	0.008164 ± 0.0008351 (0.006394–0.009935)	−0.1205 ± 0.02596 (−0.1755 to −0.06545)	18	0.8566	$F_{1,16} = 95.575$, $P < 0.0001$
Linepithema humile	0.009125 ± 0.0008584 (0.007328–0.01092)	−0.1228 ± 0.02555 (−0.1762 to −0.06930)	21	0.8560	$F_{1,19} = 112.98$, $P < 0.0001$
Crematogaster. californica	0.003906 ± 0.0005607 (0.002711–0.005101)	−0.06522 ± 0.01736 (−0.1022 to −0.02821)	17	0.7639	$F_{1,15} = 48.540$, $P < 0.0001$
Dorymyrmex insanus	0.004987 ± 0.0006710 (0.003557–0.006418)	−0.04809 ± 0.02122 (−0.09331 to −0.002859)	17	0.7865	$F_{1,15} = 55.244$, $P < 0.0001$
Solenopsis xyloni	0.004902 ± 0.0005369 (0.003757—0.006046)	−0.03896 ± 0.01698 (−0.07515 to −0.002770)	17	0.8475	$F_{1,15} = 83.346$, $P < 0.0001$

Relationship between temperature (°C) and mass-independent rate of water loss (mg $H_2O\,h^{-1}$ (mg ant)$^{-1}$) for the five species of ant studied. The regression equation is in the form $y = b*$temperature$ + a$. Slope and intercept values are reported as mean ± SE and lower and upper 95% confidence interval (in parentheses).

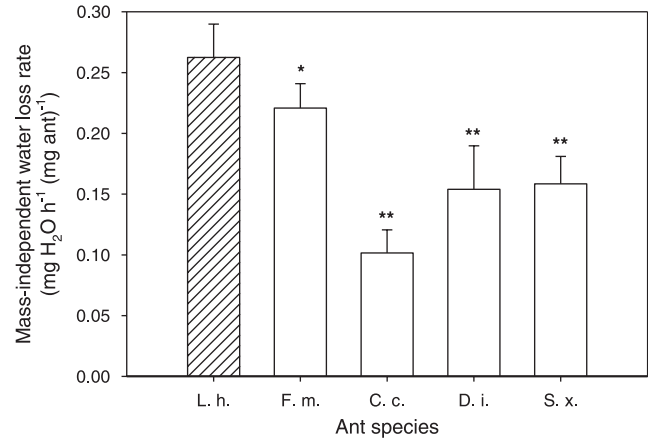Fig. 2. Mean (+1 SE) mass-independent rates of water loss (mg $H_2O\,h^{-1}$ (mg ant)$^{-1}$) at 40 °C and ca. 0% relative humidity for *Linepithema humile*: L.h. (hatched bar) and four native ant species (open bars): *Forelius mccooki* (F.m.), *Crematogaster californica* (C.c.), *Dorymyrmex insanus* (D.i.), and *Solenopsis xyloni* (S.x.). Posteriori (Dunnett's test after ANOVA, control group: *L. humile*): **$P < 0.01$, *$P < 0.05$.

plateau levels, was independent of species and temperature (ANCOVA for among-species comparisons of temperature vs. percentage CO_2 emission rate decline; same slopes: $F_{4,78} = 1.40$; $P = 0.24$; $F_{4,82} = 0.38$; same intercepts $P = 0.18$). On average, the transient decline in carbon dioxide emission expressed as a percentage varied from 60 ± 13% to 69 ± 18% for *F. mccooki* and *C. californica*, respectively (Table 3). The duration of this reduction was typically <5 min for all species assayed. The decline in carbon dioxide emission did cause a matching, temporary decrease in WLR, which was between 5.08 ± 2.67% and 8.04 ± 4.16% for *C. californica* and *L. humile*, respectively. If we assume that the oxygen effect reduced RWL rates by an amount proportional to the observed proportional reduction in carbon dioxide emission rates, then the upper limit to the RWL for the species showing the lowest drop, i.e., *C. californica,* would be approximately (5/0.7) = 7%. The difference of upper limit to the RWL across species is significant (Table 3; One-way-ANOVA: $F_{4,85} = 3.433$, $P < 0.02$).

Can the differences in RWL across the five ant species be explained by differences in their MR? To answer this question, we compared by ANCOVA the RWL (mg $H_2O\,h^{-1}$) vs. catabolic flux rates (µl $CO_2\,h^{-1}$). The five species did not differ in the MR sensitivity of the RWL, or slope ($F_{4,80} = 1.657$, $P = 0.168$). The regressions posses a common slope of 0.006196. However, they differed in their intercepts ($F_{4,84} = 4.147$, $P < 0.005$). Similar results were found when we use the area-independent RWL estimated by the regression method (data not shown).

With the estimated RWL we can correct the values of CP by subtracting the respiratory component of water

Table 3
Hyperoxic effects, respiratory and cuticular water loss components

Ant species	N	Reduction CO_2 (%) after O_2	Reduction WLR (%) after O_2	Upper limit RWL	Gross CP ($\mu g\,h^{-1}\,cm^{-2}\,Torr^{-1}$)	Corrected CP ($\mu g\,h^{-1}\,cm^{-2}\,Torr^{-1}$)	Peak WLR (%) after N_2	Peak CO_2 (%) after N_2	Plateau WLR (%) after N_2
Forelius mccooki	18	60.46±13.19	6.62±4.12 (2)	11.12±6.57 (2) a	21.59±4.31 c	19.14±3.84 c	146.64±57.66 c	142.72±59.26	38.37±14.50 a
Linepithema humile	21	61.64±26.27	8.04±4.16 (1)	15.93±13.24 (1) a	30.78±5.89 a	25.64±5.64 a	61.77±31.22 a	150.75±84.46	33.17±14.41 (5) a
Crematogaster californica	17	69.45±18.05 (2)	5.08±2.67 (4)	7.78±4.88 (4) c	15.48±4.39 c	14.32±4.27 c	98.94±32.46 c	268.63±60.65	70.23±28.11 c
Dorymyrmex insanus	17	67.86±15.74	5.65±1.88	8.97±4.02 b	24.29±3.69 c	22.10±3.41 b	46.39±22.25 a	250.93±94.85	28.69±21.40 a
Solenopsis xyloni	17	68.00±17.92 (3)	8.32±3.61 (1)	12.62±4.82 (3) a	24.55±3.36 c	21.48±3.28 b	61.67±15.94 a	149.39±38.84	27.95±15.82 a

Data are reported as mean±SD. N = sample size. WLR = water loss rate. CO_2 and WLR reductions are expressed as % below the normoxic steady-state condition. CP = cuticular permeability, either gross (cuticular + respiratory) or corrected by subtraction of RWL. CO_2 and WLR peaks as well as WLR plateau after N_2 are expressed as % above the normoxic steady-state condition. Note: numbers between parentheses indicate missing values, mostly due to a small reduction of CO_2 or WLR after infusion of pure O_2, or excretion events that corrupted WLR trace, or because we found no steady plateau of WLR after N_2. There are no missing values for corrected CP because mean values of RWL were used when needed (see text). Torr = 133.3 Pa. *Posteriori* (Dunnett test, control group: *L. humile*): a–b, $P < 0.05$; a–c, $P < 0.01$; same letter do not differ significantly.

loss from the gross CP. We replace missing values of RWL by the average for each species. Again like for the gross CP, the difference of corrected CP across species is highly significant (Table 3; One-way ANOVA: $F_{4,85} = 17.859$, $P < 0.0001$).

3.4. Response to anoxia

When insects are exposed to anoxia (by infusing pure N_2 in this case), we observed a huge peak of RWL when the spiracles are opened to their maximum extent (Fig. 1). Considered as a percentage increase, this figure is independent of temperature ($F_{1,84} = 2.459$, $P = 0.121$) but differs significantly across the species (ANOVA: $F_{4,85} = 23.747$, $P < 0.0001$; Table 3).

A large transient increase in CO_2 emission also followed exposure to nitrogen (Fig. 1). Using ANCOVA, we observed a significant correlation between the 30-s CO_2 peak increase and temperature ($F_{1,84} = 20.793$, $P < 0.0001$). The correlation was significantly different across species, with different slopes across species ($F_{4,80} = 4.426$, $P = 0.0028$; ANCOVA), and with a mean peak increase over normoxic, plateau levels ranging from 143% to 269% across species.

Following the initial brief peak of water loss, there is a lower plateau at an approximately steady state (Fig. 1). This plateau, expressed as a percentage increase over steady-state overall WLR in normoxia, was also independent of temperature ($F_{1,84} = 0.972$, $P = 0.3271$; ANCOVA), but highly significantly different across species (ANOVA: $F_{4,80} = 13.670$, $P < 0.0001$; Table 3).

3.5. Cuticular and respiratory components of water loss: Comparison of results by the hyperoxic and the regression method.

Fig. 3A shows the average values of corrected CP, i.e., without RWL, for the five different ant species estimated with two recently developed methods. Values of corrected CP smaller than $3\,\mu g\,cm^{-2}\,Torr^{-1}$ as measured by the method of Gibbs and Johnson (2004) were excluded from the analysis due to their doubtful biological meaning. A total of five such values, one from *C. californica* and two from *F. mccooki* and *L. humile*, were excluded. The regression method of Gibbs and Johnson (2004) yields values that tend to be similar or in some cases smaller compared to those obtained using the hyperoxic switch method (Fig. 3A). In general the regression method showed a higher variability on the results that could be related to the use of groups of ants. Using the coefficient of variation or coefficient of variability,

$$V = s/X, \tag{4}$$

where V is the coefficient of variation, which is a relative measure without units, s the standard deviation and X
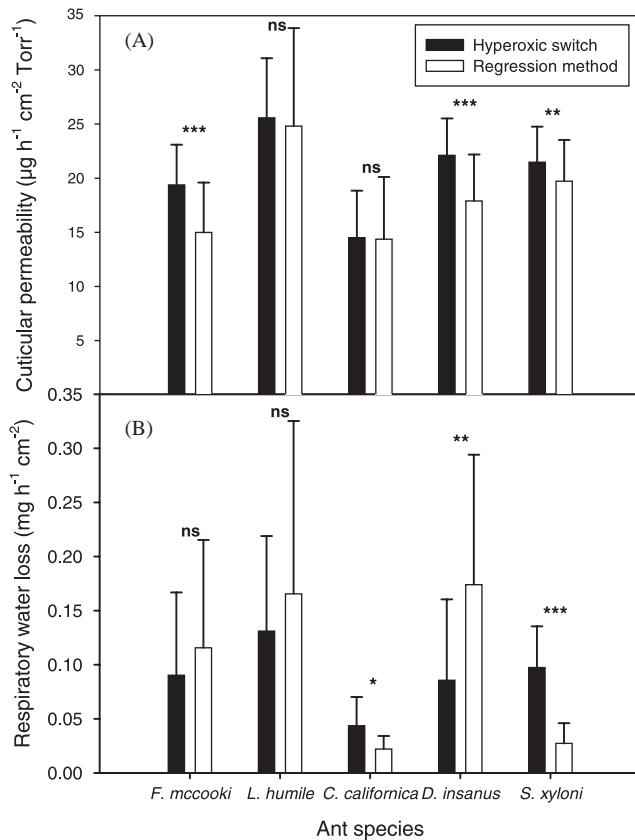
Fig. 3. Mean ($+1$ SD) of (A) corrected cuticular permeabilities ($\mu g\, cm^{-2}\, h^{-1}\, Torr^{-1}$), and (B) respiratory water loss ($mg\, h^{-1}\, cm^{-2}$) measured at 20, 30 and 40 °C and ca. 0% relative humidity for *Forelius mccooki*, *Linepithema humile*, *Crematogaster californica*, *Dorymyrmex insanus*, and *Solenopsis xyloni*. Comparison between the hyperoxic switch (black bars) and the regression method (white bars). ***$P < 0.0001$, **$P < 0.005$, *$P < 0.05$, ns = not significant (paired *t*-test).

the mean (Zar, 1984). We found that coefficients of variation for the regression vs. the hyperoxic method are: 0.31 vs. 0.19 for *F. mccooki*, 0.36 vs. 0.22 for *L. humile*, 0.40 vs. 0.30 for *C. californica*, 0.24 vs. 0.15 for *D. insanus* and 0.19 vs. 0.15 for *S. xyloni*.

Fig. 3B shows the average values of RWL for the five different ant species estimated with the same two methods. For all species, except for *C. californica*, an equivalent pairing resulted from a significant correlation using the regression method of Gibbs and Johnson (2004). Two values of RWL for *L. humile* were excluded from the analysis because they were negative (regression method), and thus they do not have any biological meaning.

However, when we take all the RWL data from the five species and compare the RWL estimated by the hyperoxic switch and regression method, we found by ANCOVA that the area-independent RWL rates vs. mass-independent catabolic flux rates did not differ between methods. Data extracted using the two methods

have a common slope or sensitivity of RWL to MR ($F_{1,152} = 0.0391$, $P = 0.4$), and they also share the same intercept ($F_{1,153} = 1.252$, $P = 0.265$). Thus, for all the data analyzed together the RWL estimated by the two methods did not differ significantly.

4. Discussion

Our study represents a unique attempt to measure and to compare MR and RWL in five species of insects showing continuous gas exchange during moderate levels of activity. We measured WLR in groups of ants showing a higher MR (ca. double) than the expected MR at rest or standard metabolic rate (SMR) according to their mass. Measuring MR and WLR in groups of ant workers is relevant since ants are social insects, which in natural conditions spend much of their time crowded in their nests. Previous studies have found no effect of group size on MR of the harvester ant *Pogonomyrmex rugosus* (Lighton and Bartholomew, 1988) or the carpenter ant *Camponotus fulvopilosus* (Lighton, 1989). The effect of group size on WLR remains to be tested.

As we discussed in a previous study (Lighton et al., 2004), the reduction in CO_2 release after the infusion of pure O_2 may not have reached 100% because we measured groups of insects with interindividual variation in spiracular opening. This idea is consistent with our results in which the species that displayed the largest decrease in CO_2 after O_2 infusion were *S. xyloni* and *C. californica* (where the measurements were made on groups of three individuals instead of ten) with 68% and more than 69%, respectively. Moreover, to date, Lighton et al. (2004) found that the largest decrease in CO_2 release after infusion of pure O_2 occurred in *Pogonomyrmex californicus* (almost 90%). It is interesting to note that these measurements were done on individuals, rather than with groups of insects (Lighton et al., 2004).

4.1. Respiratory water loss

The percentage of RWL relative to total water loss for ants with continuous gas exchange and that are moderately active (MR ca. two fold the SMR; Table 1) is similar to RWL values for insects exhibiting DGC (see Table 1 from Chown, 2002). Our results suggest that even with continuous gas exchange the control of the spiracles effectively retards RWL. Such control of spiracular opening was elegantly demonstrated for flying flies (Lehmann, 2001). Moreover, similar RWL rates were found for *P. californicus* workers that showed continuous and DGC (Lighton et al., 2004). However, this comparison should be made with caution since it was made with different individuals at different temperatures (for discussion see Lighton et al., 2004). Taken together, this evidence at least suggests that DGC is not

the only evolutionary strategy that can significantly reduce RWL.

Not surprisingly, there is a positive relation between the RWL rates and the MR of the five ant species tested (note that this is required in order to apply the regression method). A similar positive relation was previously found in species from two families of beetles; this correlation was stronger in species from dry than mesic environments (Zachariassen et al., 1987). Moreover, the increase in RWL with increasing metabolic rate supports the hypothesis that species adapted to xeric environments have a lower SMR compared to species adapted to mesic ones, as was first demonstrated for insects in a study on the harvester ant *P. rugosus* (Lighton and Bartholomew, 1988). It also indirectly supports the idea of RWL reduction in species with DGC. This is because although Gibbs and Johnson (2004) did not find a reduction of RWL per unit of CO_2 released with the DGC, they found a significant reduction of MR with that pattern of gas exchange. Insects with DGC gas exchange patterns had significantly lower MR (Gibbs and Johnson, 2004) with a concomitant reduction in total RWL.

4.2. Comparison of two methods to measure RWL in insects with continuous gas exchange

In relation to the comparison of the two new methodologies employed to measure RWL in insects with continuous gas exchange (Gibbs and Johnson, 2004; Lighton et al., 2004), both techniques proved to be useful although each one has advantages and disadvantages. For example, the regression method, proposed by Gibbs and Johnson (2004), permits comparison of RWL for insects with and without DGC, whereas the hyperoxic switch method, proposed by Lighton et al. (2004), can only handle the non-discontinuous case. On the other hand, the regression method has the disadvantage of higher variability of the results and sensitivity to the different flow-through kinetics of CO_2 and water vapor, while the hyperoxic switch method appears to be more accurate under the same circumstances. The greater variability of estimates of CP produced by the regression method is clearly evident from the coefficients of variation, which average almost 50% higher for the regression method. It also should be mentioned that some data were discarded from the regression method analysis because they had no biological meaning, e.g., negatives values of RWL. Discarded data were solely the result of the regression method, not the hyperoxic switch method. These errors could be explained in part by the fact that the data were acquired to utilize the hyperoxic switch method and later on, the same data were analyzed by the regression method. One might expect better results with the regression method on recordings with wide-ranging

values of MR, because this would allow a more statistically meaningful slope to be assigned to the relation of CO_2 and water vapor emission.

4.3. Response to anoxia

In response to anoxia, all species showed a large peak of CO_2 and RWL. These peaks are followed by a fast drop in CO_2 emission to levels near the baseline and a smaller drop in water vapor output until a steady-state plateau was reached. We used the peak of water vapor as an indirect measurement of aerobic scope because it corresponds to maximal spiracular opening and in the short term is independent of O_2 availability (see Lighton et al., 2004 for a detailed discussion). Clearly, *F. mccooki* is able to attain far higher rates of peak RWL than the other species. We assume that this is indicative of a higher capacity for aerobic throughput. This is, of course, a necessary correlate to this species' high maximum foraging temperature in the field and concomitantly far larger peak metabolic demands (Hölldobler and Wilson, 1990). Again, the prominent peak in RWL after anoxia found in all species suggests a stringent control of spiracular opening, presumably to avoid excessive loss of water. However, this novel non-invasive technique to estimate relative tracheolar surface areas should be verified by direct surface area measurement.

4.4. Ecological perspectives

As expected for a species adapted to mesic environments when compared with species adapted to xeric ones, and under conditions commonly encountered by ground-foraging ants, the exotic Argentine ant (*L. humile*) experiences a higher mass specific WLR (Fig. 2) and CP compared to ants adapted to dry environments (Table 3). The native ant species included in this study are common species that are all displaced by Argentine ants in southern California (Holway, 2005). The physiological limitation that differentiates Argentine ants from the native ants examined in this study, could explain the close association between soil moisture and Argentine ant abundance (Holway et al., 2002). This positive association may be accentuated by the Argentine ant's habit of constructing short-lived, shallow soil nests (Newell and Barber, 1913), which provide little refuge from heat and aridity compared to the deeper nests typically built by arid-adapted ants (Hölldobler and Wilson, 1990). Although interspecific competition from native ants might interact with abiotic factors to influence invasion success under some circumstances, no published study to date has reported any correlation (positive or negative) between native ant diversity and community susceptibility to invasion by the Argentine ant (Holway, 1998; Holway et al., 2002; Sanders et al., 2003). The higher WLR through both the

cuticle and the spiracles (Table 3) of the Argentine ants, relative to those of the native ants studied here, suggests that the physiological characteristics of *L. humile* limit its ability to invade dry environments. A more detailed study of the behavior and water relations in this system will further an understanding the mechanics of Argentine ant invasions.

4.5. Conclusions

Our modest contribution to the field of physiological ecology and water relations in insects could be described as follows: First, we showed a plausible mechanism by which the Argentine ant (an exotic ant species that excels as an invader) may be limited in its distribution and spread, i.e., higher WLR and CP compared with common native ant species. This is in agreement with the tendency towards lower CP in xeric-adapted insects compared to mesic-adapted ones (for review see Hadley, 1994). Second, we used two recently developed methodologies to estimate RWL in insects with continuous gas exchange (Gibbs and Johnson, 2004; Lighton et al., 2004). We discussed the advantages and disadvantages of these two methods. Finally, we found that RWL estimates for groups of insects with continuous gas exchange and moderate levels of activity are similar to published estimates of RWL for insects with DGC. This result suggests that DGC is not the only way to reduce RWL, and that the control of spiracular opening under continuous gas exchange could be as effective as the DGC in reducing RWL. However, at present the role of DGC is under scrutiny (see Chown et al., 2005 for a detailed discussion of this topic) and more work should be done in order to solve this long-standing problem.

Acknowledgments

References

Bradley, T.J., 2000. The discontinuous gas exchange cycle in insects may serve to reduce oxygen supply to the tissues. American Zoologist 40, 952.

Chown, S.L., 2002. Respiratory water loss in insects. Comparative Biochemistry and Physiology Part A 133, 791–804.

Chown, S.L., Davis, A.L.V., 2003. Discontinuous gas exchange and the significance of respiratory water loss in scarabaeine beetles. Journal of Experimental Biology 206, 3547–3556.

Chown, S.L., Gibbs, A.G., Hetz, S.K., Klok, C.J., Lighton, J.R.B., Marais, E., 2005. Discontinuous gas exchange in insects: a clarification of hypotheses and approaches. Comparative Biochemistry and Physiology Part A, in press.

Duncan, F.D., Lighton, J.R.B., 1994. Water relations in nocturnal and diurnal foragers of the desert honeypot ant *Myrmecocystus*: implications for colony-level selection. Journal of Experimental Zoology 270, 350–359.

Edney, E.B., 1977. Water Balance in Land Arthropods. Springer, Berlin, Heidelberg, New York.

Gibbs, A.G., 1999. Laboratory selection for the comparative physiologist. Journal of Experimental Biology 202, 2709–2718.

Gibbs, A.G., Johnson, R.A., 2004. The role of discontinuous gas exchange in insects: the chthonic hypothesis does not hold water. Journal of Experimental Biology 207, 3477–3482.

Hadley, N.F., 1994. Water Relations of Terresrial Arthropods. Academic Press, Inc., San Diego, CA, USA.

Hetz, S.K., Bradley, T.J., 2005. Insects breathe discontinuously to avoid oxygen toxicity. Nature 433, 516–519.

Hölldobler, B., Wilson, E.O., 1990. The Ants. Belknap Press, Harvard University Press, Cambridge.

Holway, D.A., 1998. Factors governing rate of invasion—a natural experiment using Argentine ants. Oecologia 115, 206–212.

Holway, D.A., 2005. Edge effects of an invasive species across a natural ecological boundary. Biological Conservation 121, 561–567.

Holway, D.A., Suarez, A.V., Case, T.J., 2002. Role of abiotic factors in governing susceptibility to invasion: a test with Argentine ants. Ecology 83, 1610–1619.

Lehmann, F.-O., 2001. Matching spiracle opening to metabolic need during flight in *Drosophila*. Science 294, 1926–1929.

Lighton, J.R.B., 1989. Individual and whole-colony respiration in an African formicine ant. Functional Ecology 3, 523–530.

Lighton, J.R.B., 1991. Measurements on insects. In: Payne, P.A. (Ed.), Concise Encyclopedia on Biological and Biomedical Measurements Systems. Pergamon Press, Oxford.

Lighton, J.R.B., 1992. Direct measurement of mass loss during discontinuous ventilation in two species of ants. Journal of Experimental Biology 173, 289–293.

Lighton, J.R.B., 1994. Discontinuous ventilation in terrestrial insects. Physiological Zoology 67, 142–162.

Lighton, J.R.B., 1996. Discontinuous gas exchange in insects. Annual Review of Entomology 41, 309–324.

Lighton, J.R.B., 1998. Notes from underground: towards ultimate hypotheses of cyclic discontinnous gas-exchange in tracheate arthropods. American Zoologist 38, 483–491.

Lighton, J.R.B., Bartholomew, G.A., 1988. Standard energy metabolism of a desert harvester ant, *Pogonomyrmex rugosus*: effects of temperature, body mass, group size, and humidity. Proceedings of the National Academy of Sciences USA 85, 4765–4769.

Lighton, J.R.B., Berrigan, D., 1995. Questioning paradigms: caste-specific ventilation in harvester ants, *Messor pergandei* and *M. julianus* (Hymenoptera: Formicidae). Journal of Experimental Biology 198, 521–530.

Lighton, J.R.B., Feener, D.H., 1989. Water-loss and cuticular permeability in foragers of the desert ant *Pogonomyrmex rugosus*. Physiological Zoology 62, 1232–1256.

Lighton, J.R.B., Fielden, L.J., 1996. Gas exchange in wind spiders (Arachnida, Solphugidae): independent evolution of convergent control strategies in Solphugids and insects. Journal of Insect Physiology 42, 347–357.

Lighton, J.R.B., Brownell, P.H., Joos, B., Turner, R.J., 2001. Low metabolic rate in scorpions: implications for population biomass and cannibalism. Journal of Experimental Biology 204, 607–613.

Lighton, J.R.B., Schilman, P.E., Holway, D.A., 2004. The hyperoxic switch: assessing respiratory water loss rates in tracheate arthropods with continuous gas exchange. Journal of Experimental Biology 207, 4463–4471.

Newell, W., Barber, T.C., 1913. The Argentine Ant. Bureau of Entomology Bulletin, Washington, DC, pp. 1–98.

Quinlan, M.C., Lighton, J.R.B., 1999. Respiratory physiology and water relations of three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). Physiological Entomology 24, 293–302.

Sanders, N.J., Gotelli, N.J., Heller, N.E., Gordon, D.M., 2003. Community disassembly by an invasive species. Proceedings of the National Academy of Sciences USA 100, 2474–2477.

Suarez, A.V., Bolger, D.T., Case, T.J., 1998. The effect of fragmentation and invasion on the native ant community in coastal southern California. Ecology 79, 2041–2056.

Williams, A.E., Rose, M.R., Bradley, T.J., 1997. CO_2 release patterns in *Drosophila melanogaster*: the effect of selection for desiccation resistence. Journal of Experimental Biology 200, 615–624.

Williams, A.E., Rose, M.R., Bradley, T.J., 1998. Using laboratory selection for desiccation resistance to examine the relationship between respiratory pattern and water loss in insects. Journal of Experimental Biology 201, 2945–2952.

Zachariassen, K.E., Andersen, J., Maloiy, G.M.O., Kamau, J.M.Z., 1987. Transpiratory water loss and metabolism of beetles from arid areas in east Africa. Comparative Biochemistry and Physiology 86A, 403–408.

Zar, J.H., 1984. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ.