

Discontinuous gas exchange and the significance of respiratory water loss in scarabaeine beetles

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Summary

Respiratory water loss in insects is a controversial topic. Whilst earlier studies considered respiratory transpiration a significant component of overall water loss, to the extent that it was thought to be responsible not only for the evolution of discontinuous gas exchange cycles (DGCs) but also for variation in DGC patterns, later work repeatedly questioned its importance. In particular, investigations of the proportional contribution of respiratory transpiration to total water loss in species showing DGCs suggested that respiratory transpiration was unlikely to be important in these species. In turn, these studies have been criticized on analytical grounds. In this study we investigated variation in cuticular and respiratory water loss rates in five *Scarabaeus* dung beetle species, all of which show discontinuous gas exchange cycles, to ascertain the significance of respiratory water loss using modern analytical techniques. In particular, we determined whether there is variation in water loss rates amongst these beetles, whether both respiratory and cuticular water loss rates contribute significantly to variation in the former, and whether metabolic rate variation and variation in the duration of the DGC periods contribute

significantly to variation in respiratory water loss rate. Total water loss rate varied such that species from arid areas had the lowest rates of water loss, and both cuticular and spiracular transpiration contributed significantly to variation in overall water loss rate. Moreover, variation in metabolic rate and in the duration of the DGC periods contributed significantly to variation in respiratory water loss rate. By contrast, examination of proportional water loss revealed little other than that it varies between 6.5% and 21%, depending on the species and the temperature at which it was examined. Cuticular water loss scaled as $\text{mass}^{0.721}$, but did not differ from that expected from geometric considerations alone. By contrast, respiratory water loss scaled as $\text{mass}^{0.531}$, suggesting that gas exchange takes place by diffusion and convection. Our results provide direct evidence that respiratory water loss forms a significant component of water balance, and that changes in both metabolic rate and DGC characteristics contribute to modulation of respiratory water loss.

Key words: gas exchange, desiccation resistance, metabolic rate, scaling, water loss, dung beetle, *Scarabaeus*.

Introduction

The significance of the contribution of respiratory transpiration to total water loss in insects is controversial. Early studies not only suggested that respiratory water loss forms an important component of total transpiration (Loveridge, 1968; Edney, 1977), but also argued that the need for reduction of respiratory transpiration has been a major factor underlying the evolution of discontinuous gas exchange (Levy and Schneiderman, 1966; Burkett and Schneiderman, 1974; Lighton, 1994). Discontinuous gas exchange cycles (DGCs) are usually confined to times when insects (or other arthropods; see Lighton, 1998; Klok et al., 2002) are at rest, and involve the alteration of three major periods: a Closed (C) period, during which the spiracles are tightly shut and there is no respiratory water loss, a Flutter (F) period, when convective flow of air into the tracheal system is thought to reduce water

loss, and an Open (O) period, during which most water loss takes place because the spiracles are held open (Lighton, 1994, 1996). Because respiratory transpiration is mostly restricted to a short O-period, it has long seemed obvious that discontinuous gas exchange has evolved to reduce water loss (for reviews see Kestler, 1985; Lighton, 1994, 1996). Moreover, several studies have reported considerable variation in the characteristics (especially the duration) of the C-, F- and O-periods among species from different habitats, suggesting that modulating the characteristics of the periods is a significant way in which respiratory water loss might be altered (Lighton, 1988a, 1990, 1991; Lighton et al., 1993; Davis et al., 1999; Bosch et al., 2000; Duncan and Dickman, 2001; Duncan et al., 2002a).

Several other avenues of investigation seem to confirm the significance of the contribution of respiratory transpiration to

total water loss. Experimental manipulations in which spiracles are held open artificially demonstrated that water loss rates increase dramatically if the spiracles are kept open (Bursell, 1957; Edney, 1977, see also Lighton et al., 1993). Based on comparative data and on laboratory selection experiments, numerous studies have also shown or argued that metabolic rates in species or populations from dry environments are lower than those in species or populations from more mesic environments, thus reducing water loss under the xeric conditions (Juliano, 1986; Hoffmann and Parsons, 1989a; Gibbs et al., 1997; Chown and Gaston, 1999; Davis et al., 2000; Gibbs, 2002a; Gibbs et al., 2003). In addition, large-scale comparative studies by Zachariassen and his colleagues (Zachariassen et al., 1987; Zachariassen, 1996), strongly suggest that respiratory transpiration must account for a significant proportion of water lost by arid-environment insects during dehydration, and have led these authors to conclude that modification of this loss could represent a significant fitness benefit.

By contrast, in those studies that have examined the proportional contribution of respiratory transpiration to total water loss, the general conclusion has been that respiratory water loss is low, contributing 3–15% of the total, and with little relationship to the environment (mesic or xeric) occupied by the species in question (Hadley and Quinlan, 1993; Lighton et al., 1993; Quinlan and Hadley, 1993; Quinlan and Lighton, 1999). Indeed, Hadley (1994a,b) pointed out that in the majority of species examined, respiratory water loss constitutes such a small proportion of total water loss that it ‘...is difficult to see how even major changes in its relative contribution would affect the water status of these animals’. The importance of respiratory water loss has been questioned by findings that insects subjected to desiccation stress often abandon discontinuous gas exchange (Hadley and Quinlan, 1993; Quinlan and Hadley, 1993; Chappell and Rogowitz, 2000; Rourke, 2000), and that some species from xeric areas apparently do not exhibit this gas exchange pattern at all (Lighton and Berrigan, 1995; Lighton, 1996). Moreover, several studies have shown that neither a change in gas exchange pattern nor a reduction in metabolic rate are accompanied by a reduction in water loss (Djawdan et al., 1997; Williams and Bradley, 1998; Williams et al., 1998; Bradley et al., 1999; Rourke, 2000; Shelton and Appel, 2001a,b), and that even substantial changes in metabolic rate might not markedly improve survival time *via* water conservation (Bosch et al., 2000).

Nevertheless, the view that respiratory water loss forms an important component of total transpiration continues to permeate the modern literature. For example, in revisiting the comparative analysis of water loss undertaken by Zachariassen and his colleagues, Addo-Bediako et al. (2001) concluded that respiratory water loss is of considerable importance in xeric insect species, although they did not find support for reduced metabolic rate as a means by which a reduction in transpiration might be effected. Similarly, in a comparative analysis of water balance in *Drosophila* species

from mesic and xeric habitats, Gibbs et al. (2003) concluded that lower rates of water loss in the xeric species are achieved primarily by reduction in respiratory losses associated with a reduction in metabolic rate and activity levels, and improved spiracular control. Based on investigations of discontinuous gas exchange in beetles, Duncan and Dickman (2001) and Duncan et al. (2002a) supported the water conservation hypothesis for the DGC because the other hypotheses seemed not to apply to the species they investigated (see also Vogt and Appel, 2000). Indeed, Duncan (2003) went on to conclude that every facet of the DGC can be altered (presumably in an adaptive fashion) to effect respiratory water savings. Thus, it is clear that at present there is little consensus regarding the significance of respiratory water loss in insect water balance. Whilst many arguments have been raised against its likely significance, it continues to engender support. This state of affairs was perhaps best summed up by Quinlan and Lighton (1999) who argued that the ‘...interrelationships between gas exchange and water balance are still largely a matter of conjecture’.

Whilst reviewing respiratory water loss in insects, Chown (2002) proposed several ways in which the current polarization of findings might be overcome. Amongst these, the most significant included a move away from the use of proportions for expressing respiratory water loss (see also Packard and Boardman, 1988, 1999), the more frequent use of comparative analyses that involve work on species in which respiratory and cuticular transpiration can be clearly distinguished, and clear *a priori* statements of the null expectation. In the latter case, arguments regarding the proportional contribution of respiratory loss to total transpiration, the relationship between metabolic rate and respiratory water loss rate, and the contribution of variation in components of the DGC to water conservation can be restated as: (1) variation in respiratory water loss is unrelated to variation in total water loss, (2) variation in metabolic rate is unrelated to variation in respiratory water loss rate and (3) there is no covariation in DGC period characteristics and respiratory water loss. In general, the alternatives to these null expectations are straightforward, although in the case of DGC period characteristics there are two, non-exclusive alternative hypotheses. These are that covariation between C- and/or F-period duration and water loss rate is negative (Lighton, 1990; Davis et al., 1999), and/or that covariation between O-period duration and water loss rate is positive (Lighton, 1990; Duncan et al., 2002a).

In this study, we examine these three major hypotheses in an analysis of water loss in five species of *Scarabaeus* dung beetles that exhibit discontinuous gas exchange (Davis et al., 1999). We determine whether there is variation in total water loss rate across the individuals in our sample, whether respiratory water loss contributes significantly to this variation, whether metabolic rate and water loss rate covary, and whether DGC period durations covary with water loss rate. In doing so our aim is not only to investigate patterns of water loss in these species, but also to provide an example of the type of analysis

that could be useful for overcoming the stalemate that currently reigns in the field of respiratory water loss in insects.

Materials and methods

Study material

The five, diurnally active species of *Scarabaeus* (Coleoptera, Scarabaeidae, Scarabaeinae) comprised two flightless species, with fused elytra, that were collected on the arid west coast of South Africa, and three volant species that were collected from more mesic eastern habitats in the country (Table 1). Both of the west coastal species were collected in shrubland on sandveld near Kleinsee. *Scarabaeus gariepinus* was collected on very coarse-grained dune sands and shows a distribution northwards into the Namib Desert (Davis et al., 2000), whereas *Scarabaeus striatus* was collected closer to the coast on less coarse-grained sands and shows a distribution centred in Namaqualand, north-west South Africa. Both species feed on dry dung pellets that are rehydrated after burial (Scholtz, 1989). Of the three north-easterly species, *S. galenus* was collected in open woodland on sandy soils in the hot dry lowlands near Crocodile Bridge in the Kruger National Park, *S. rusticus* was collected at moderate altitude in grassland on sandy loam from Rustenburg Nature Reserve, which is situated on the cooler rocky hillsides of the Magaliesberg, and *S. westwoodi* was collected from grassland on finer-grained soils at high altitude along the Sani Pass on the cool, wet lower slopes of the Drakensberg. For further details of geographical distribution, climatic influences and diet of the species, see Davis et al. (1999, 2000).

Experimental design

Individuals were held at 25°C under a 12 h:12 h L:D photoperiod for at least 5 days before respirometry commenced. Individual beetles were starved for 24–48 h before a trial to avoid excretion during the experiment. Each individual was weighed prior to and after a trial using a Sartorius R 200 D digital balance (Epsom, UK), and mean mass was used in data analyses. A single beetle was placed in the cuvette during the morning ($\pm 10:00$ h) to allow it to settle

before data measurement over the following night, when diurnal Scarabaeini are relatively inactive (Davis, 1996, 2002). The cuvette was placed inside a water jacket connected to a Grant LTD20 water bath (Cambridge, UK) that controlled the selected temperature to within $\pm 0.2^\circ\text{C}$. The laboratory was maintained in darkness throughout each trial.

Flow-through respirometry based on a Sable Systems (Henderson, Nevada, USA) turnkey system was used to measure both CO₂ and H₂O production (fully described in Davis et al., 1999, 2000). Air was passed through columns of Drierite and soda lime to remove both water vapour and CO₂. The scrubbed air was then passed through an automated baselining system, the 340 ml cuvette containing the beetle, and a Li-Cor CO₂/H₂O Analyzer Model Li 6262 at a flow rate of 150 ml min⁻¹. Sable Systems DATACAN V software was used for data capture and analysis, and all measurements were corrected to standard temperature and pressure.

In each of the five *Scarabaeus* species, temperature-modulated changes in the amounts of both CO₂ and H₂O production were recorded at 4°C intervals across a temperature range from 16°C to 32°C (with the exception of *S. striatus* – see Table 2). Whereas 10 or more individuals were measured for *S. westwoodi* and *S. rusticus*, fewer individuals were available for the other three species (Table 2). For each individual, measurements of CO₂ and H₂O release were made at 5 s intervals during an overnight period of 9–10 h commencing at dusk (18:00–19:00 h). Periodic observations during initial respirometry work revealed that DGCs were indicative of immobile individuals (Davis et al., 1999), and therefore provided standardized data for intra- and interspecific comparisons. Where possible, DGC measurements were only entered into the analysis for data recorded after the first 4 h, and for each DGC, measurements of both CO₂ and H₂O were made over the same range of samples. Because the DGC periods were less readily discerned in the water vapour trace than in the CO₂ recording, the water vapour data were divided into O-period and combined Closed/Flutter (CF) period. C-period emission rate of CO₂ was very close to the baseline (see also Davis et al., 1999), suggesting that there is minimal escape of gasses during the C-period, indicating that CO₂ is lost

Table 1. Mean annual rainfall, altitude and position of the collection localities of the five *Scarabaeus* dung beetle species examined in this study

Species	Approximate locality	Grid reference	Altitude (m)	Rainfall (mm)
<i>S. gariepinus</i> (Ferreira)	Kleinsee	29°34.090'S 17°17.000'E	190	70
<i>S. striatus</i> (Castelnau)	Kleinsee	29°46.511'S 17°22.555'E	300	105
<i>S. galenus</i> (Westwood)	Crocodile Bridge	25°22.623'S 31°46.739'E	182	554
<i>S. rusticus</i> (Boheman)	Rustenburg	25°35.674'S 27°11.372'E	1260	617
<i>S. westwoodi</i> Harold	Sani Pass	29°36.297'S 29°21.191'E	1900	1185

Table 2. Summary statistics for mass, \dot{V}_{CO_2} , CF-period duration, O-period duration, cuticular and spiracular water loss rates and volumes, and percentage of water lost through the spiracles in five species of Scarabaeus dung beetles

Species/ Temperature (°C)	N	Mass (g)	\dot{V}_{CO_2} (ml h ⁻¹)	CF duration (s)	O duration (s)	\dot{V}_{H_2O} (mg h ⁻¹)		Volume (mg)		Spiracular water loss (%)
						Cuticular	Spiracular	Cuticular	Spiracular	
<i>S. garipepinus</i>										
16	4	1.186±0.069	0.0180±0.0007	4798±2282	3312±838	1.275±0.089	0.250±0.029	2.984±1.126	0.443±0.159	13.0±1.2
20	4	1.158±0.064	0.0241±0.0017	6170±2077	3073±601	0.999±0.054	0.370±0.054	2.505±0.712	0.569±0.174	17.9±1.6
24	4	1.130±0.062	0.0372±0.0011	3055±780	3152±186	1.026±0.056	0.408±0.045	1.729±0.158	0.482±0.069	21.4±1.1
28	4	1.097±0.059	0.0527±0.0025	1612±459	1426±93	1.695±0.065	0.374±0.031	1.413±0.185	0.208±0.036	12.7±0.5
32	4	1.055±0.058	0.0733±0.0040	770±123	1257±111	2.095±0.065	0.399±0.019	1.182±0.150	0.161±0.016	12.2±0.3
<i>S. striatus</i>										
16	3	0.885±0.178	0.0167±0.0021	3712±923	2628±58	1.880±0.367	0.273±0.064	3.289±0.778	0.339±0.079	9.8±2.2
20	7	0.753±0.044	0.0322±0.0025	2170±359	1671±258	2.379±0.180	0.267±0.015	2.499±0.389	0.202±0.030	7.6±0.6
28	4	0.684±0.046	0.0484±0.0029	519±141	940±211	3.108±0.511	0.327±0.044	1.186±0.235	0.105±0.023	8.3±2.0
<i>S. galenus</i>										
16	1	1.256	0.0319	4778	2450	2.218	0.205	4.463	0.333	6.8
20	6	1.596±0.068	0.0744±0.0067	1670±716	1019±89	2.440±0.173	0.291±0.057	1.866±0.598	0.146±0.049	7.0±0.4
24	4	1.681±0.057	0.1225±0.0201	948±447	746±156	3.124±0.365	0.436±0.010	1.324±0.243	0.124±0.028	8.3±0.5
28	4	1.398±0.128	0.1079±0.0155	2014±612	763±139	4.107±0.114	0.656±0.129	3.042±0.792	0.227±0.069	6.8±0.8
32	2	1.406±0.131	0.1461±0.0197	2203±1739	1000±118	4.271±0.375	0.925±0.210	3.610±1.872	0.372±0.181	9.6±0.4
<i>S. rusticus</i>										
16	10	1.057±0.054	0.0368±0.0024	4793±619	1763±78	1.721±0.148	0.401±0.040	3.164±0.465	0.384±0.047	11.2±0.9
20	10	1.050±0.049	0.0581±0.0038	3380±394	1806±108	2.189±0.191	0.480±0.043	3.153±0.384	0.393±0.049	11.7±1.5
24	10	1.061±0.051	0.0906±0.0078	1905±348	1516±57	2.656±0.242	0.587±0.068	2.667±0.546	0.316±0.036	12.1±1.8
28	10	1.081±0.049	0.1379±0.0114	1177±253	1365±127	3.269±0.266	0.703±0.076	2.435±0.482	0.316±0.044	12.9±1.8
32	10	1.073±0.044	0.1807±0.0128	539±115	1026±87	4.308±0.306	0.708±0.080	1.941±0.321	0.221±0.028	10.7±1.2
<i>S. westwoodi</i>										
16	10	1.905±0.100	0.0865±0.0067	2615±370	2304±147	3.909±0.182	0.579±0.043	5.308±0.489	0.488±0.031	8.7±0.4
20	10	1.741±0.105	0.1160±0.0077	1991±235	1873±136	5.612±0.507	0.647±0.068	5.941±0.643	0.449±0.064	7.2±0.8
24	10	1.758±0.086	0.1692±0.0100	962±126	1308±135	6.951±0.808	0.715±0.076	4.383±0.701	0.332±0.059	7.1±0.8
28	11	1.689±0.096	0.2325±0.0119	688±93	1027±104	8.598±0.657	0.819±0.062	3.980±0.394	0.270±0.028	6.5±0.6
32	10	1.890±0.112	0.3197±0.0154	376±39	935±63	10.154±0.517	1.082±0.123	3.655±0.262	0.299±0.035	7.6±0.8

primarily through the spiracles. However, CF-period \dot{V}_{H_2O} emission rate was well above the baseline, representing primarily cuticular water loss.

For each individual, at each temperature, mean \dot{V}_{CO_2} , CF-period duration, O-period duration, \dot{V}_{CO_2} for the CF and O periods, CO_2 volume for the CF and O periods, the nadir of \dot{V}_{H_2O} for the CF period (cuticular water loss rate), \dot{V}_{H_2O} for the O-period (cuticular plus respiratory water loss rate), respiratory water loss rate (O-period only), total water loss rate (\dot{V}_{H_2O}), volume of cuticular water loss, volume of respiratory water loss, total water loss volume, and proportional contribution of respiratory water loss were calculated. The data for each individual were generally derived from the mean data for at least four DGCs (though in some instances fewer DGCs were used), and in all subsequent analyses these mean values for each individual were used as the primary data. Although some authors use values from each cycle in a discontinuous gas exchange of a single individual as primary, independent data points (e.g. Duncan et al., 2002a,b), we consider this pseudoreplication and did not do so.

Data analysis

Generalized linear modelling (see McCullagh and Nelder, 1989; Quinn and Keough, 2002) was used to obtain best-fit models for total \dot{V}_{H_2O} , respiratory \dot{V}_{H_2O} and cuticular \dot{V}_{H_2O} . The independent variables included in these models were: (1) treatment temperature and species identity for total \dot{V}_{H_2O} , to determine if there is variation in total water loss rate amongst species; (2) treatment temperature, $\log_{10}(\text{mass})$, $\log_{10}(\text{cuticular water loss rate})$, and $\log_{10}(\text{spiracular water loss rate})$ for total \dot{V}_{H_2O} , (3) treatment temperature, $\log_{10}(\text{mass})$, $\log_{10}(\text{CF-period duration})$, $\log_{10}(\text{O-period duration})$, and $\log_{10}(\text{mean } \dot{V}_{CO_2})$ for respiratory \dot{V}_{H_2O} ; (4) treatment temperature, and $\log_{10}(\text{mass})$ for $\log_{10}(\text{cuticular } \dot{V}_{H_2O})$.

In all cases a normal distribution and identity link function were specified, and the Akaike Information Criterion (AIC) was used to identify the best subset of explanatory variables. In cases where the AIC was similar for different subsets of variables, the model with the fewest variables was chosen (Quinn and Keough, 2002). These models were run again and Type III likelihood tests were used to confirm the significance of each variable. Because Davis et al. (2000) found that wing status (flying vs. flightless) explained much of the variation in \dot{V}_{CO_2} in these *Scarabaeus* species (see also Reinhold, 1999; Addo-Bediako et al., 2002), and because flightlessness is reputedly a means by which beetles are able to effect a water savings (Draney, 1993; Chown et al., 1998), the latter (three) analyses were repeated including a dummy variable for wing status.

Results

In the case of total water loss rate, treatment temperature ($\chi^2_{(1)}=153.5$, $P<0.0001$) and species identity ($\chi^2_{(4)}=285.4$,

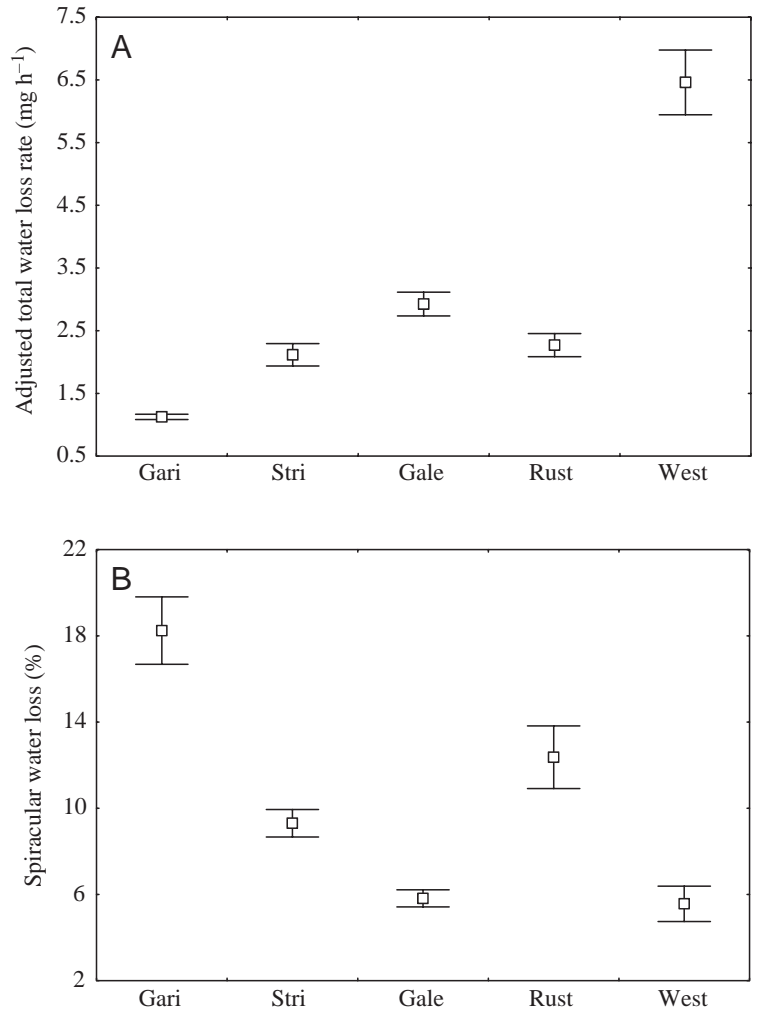


Fig. 1. (A) Water loss rate (mg h^{-1}) adjusted for body mass, and (B) proportional contribution of spiracular to total water loss (%), adjusted for body mass, at 20°C in the five *Scarabaeus* species investigated in this study. Variation in rate of water loss is in the direction expected from the mean annual rainfall of the area from which the species was collected. Values are means \pm S.E.M. Gari, *S. gariepinus*; Stri, *S. striatus*; Gale, *S. galenus*; Rust, *S. rusticus*; West, *S. westwoodi*.

$P<0.0001$) contributed significantly to the model, verifying that there is indeed considerable variation amongst species in the total rate of water loss (Fig. 1). Mean rates of water loss for each species covaried significantly with the mean annual rainfall of the area in which each species was collected ($r_s=0.9$, $P=0.037$). Summary statistics for the DGC and water loss characteristics also revealed substantial variation amongst species in these parameters (Table 2). The best-fit model for total water loss rate included only cuticular water loss rate and spiracular water loss rate (full model AIC=441.4, with just these terms AIC=438.1) (Table 3). Therefore, both cuticular water loss and spiracular water loss contribute to variation in total water loss rate (each of the latter are affected by temperature, which has an effect on cuticular transpiration and DGC frequency, but temperature tends not to enter the models

Table 3. Generalized linear model of the explanatory variables on total water loss rate

Explanatory variables	Parameter estimate (d.f.)	Type III log-likelihood (deviance)	χ^2 (deviance/d.f.)	P
Excluding wing status				
\log_{10} (Cuticular \dot{V}_{H_2O})	8.621	-325.7	219.3	0.0001
\log_{10} (Spiracular \dot{V}_{H_2O})	2.277	-225.5	18.83	0.0001
	(151)	(149.15)	(0.987)	
Including wing status				
\log_{10} (Cuticular \dot{V}_{H_2O})	9.240	-323.0	229.9	0.0001
\log_{10} (Spiracular \dot{V}_{H_2O})	2.427	-219.7	23.2	0.0001
Wing status		-216.1	15.9	0.0001
	(150)	(134.44)	(0.896)	

significantly when forced in initially using a Type I approach – data not shown). When wing status was incorporated, the best-fit model included wing status, cuticular water loss rate and spiracular water loss rate, with a lower AIC (424.12) than the model excluding wing status. However, Quinn and Keough (2002) have pointed out that the AIC should be interpreted with caution when both categorical and continuous predictors are included in the model. In this context the reduction in the deviance/d.f. value (Table 3) compared with the model not including wing status suggests that there is little difference between the two models.

The best-fit model for spiracular water loss rate included CF-period duration, O-period duration, and \dot{V}_{CO_2} (AIC=-187.3, compared to -184.8 for the full model) (Table 4). When wing status was considered, the best-fit model included CF-period duration, O-period duration, \dot{V}_{CO_2} and wing status (AIC=-189.2). However, CF-period duration was not significant ($\chi^2=2.995$, $P=0.08$). Therefore, the best fit model included wing status, O-period duration and \dot{V}_{CO_2} (Table 4). One explanation for the exclusion of CF-period duration from the model including wing status is the particularly prolonged duration of the CF period in *S. garipepinus*, and to a lesser extent in *S. striatus* (see Table 2).

In the case of cuticular water loss, mass and treatment temperature contributed significantly to the model, as did wing status (Table 5). To obtain an indication of the scaling of cuticular and respiratory water loss rate, the relationship between \log_{10} (mass) and \log_{10} (cuticular water loss), and

\log_{10} (mass) and \log_{10} (spiracular water loss) was investigated for measurements made at 20°C (for which most data were available) using generalized linear models. Cuticular water loss scaled significantly ($P=0.002$) as $\text{mass}^{0.721 \pm 0.234}$, which is not significantly different from a value expected from geometric considerations alone ($\text{mass}^{0.667}$, $t_{(37)}=0.231$, $P>0.5$). Spiracular water loss scaled significantly ($P=0.0009$) as $\text{mass}^{0.531 \pm 0.160}$, which is also not significantly different from a value expected from geometric considerations alone ($\text{mass}^{0.667}$, $t_{(37)}=-0.85$, $P>0.4$). By contrast, \dot{V}_{CO_2} scaled significantly ($P=0.0001$) as $\text{mass}^{1.284 \pm 0.160}$.

Discussion

Although there was considerable variation among the *Scarabaeus* species in total water loss rate and the components thereof, in a direction that might be expected based on the habitats they occupy (see also Davis et al., 2000, 2002; Davis, 2002), the variation in the proportional contribution of respiratory water loss to the total was less clear (Fig. 1). Indeed, the proportional contributions were all within the range of (or close to in the case of *S. garipepinus*), the low values reported previously in the literature, e.g. 15% for the grasshopper *Melanoplus sanguinipes* (Rourke, 2000), 2% for the ant *Camponotus vicinus* (Lighton, 1992), and 5% for the dung beetle *Aphodius fossor* (Chown and Holter, 2000). Moreover, unlike total water loss rate, there was no relationship between rainfall of the site at which the species

Table 4. Generalized linear model of the explanatory variables on spiracular water loss rate

Explanatory variables	Parameter estimate (d.f.)	Type III log-likelihood (deviance)	χ^2 (deviance/d.f.)	P
Excluding wing status				
\log_{10} (CF duration)	-0.138	92.5	10.3	0.0014
\log_{10} (O duration)	0.387	85.0	25.2	0.0001
\log_{10} (\dot{V}_{CO_2})	0.496	47.0	101.2	0.0001
	(150)	(2.537)	(0.017)	
Including wing status				
\log_{10} (O duration)	0.263	89.1	18.0	0.0001
\log_{10} (\dot{V}_{CO_2})	0.654	31.3	133.6	0.0001
Wing status		92.5	11.1	0.0009
	(150)	(2.522)	(0.017)	

Table 5. Generalized linear model of the explanatory variables on cuticular water loss rate

Explanatory variables	Parameter estimate (d.f.)	Type III log-likelihood (deviance)	χ^2 (deviance/d.f.)	P
Temperature	0.216	-347.4	46.3	0.0001
Log ₁₀ (mass)	9.184	-345.8	43.0	0.0001
Wing status		-327.5	6.14	0.013
	(150)	(608.3)	(4.05)	

were collected and proportional contribution of respiratory water loss to the total ($r_s = -0.7$, $P = 0.18$). These findings might easily have resulted in a conclusion similar to that reached by Hadley (1994a,b), and many other recent workers (see Introduction), i.e. that respiratory water loss is largely unimportant in insects. However, based on a simple theoretical argument, Chown (2002) argued that analyses of the proportional contribution of respiratory transpiration to water loss are more likely to obscure than to clarify investigations of respiratory water loss. In particular, he argued that if selection were to reduce cuticular and respiratory transpiration in concert, then changes in the proportional respiratory transpiration would not be detected at all. Likewise, depending on other life history requirements, such as those associated with flightless as opposed to volant species (Zera and Denno, 1997; Reinhold, 1999), modulation of respiratory and cuticular water loss might occur independently. Therefore, instead of concluding that respiratory water loss is probably insignificant in these species, we chose to further explore the contributions of cuticular and respiratory transpiration to total water loss.

Perhaps unsurprisingly, both cuticular and respiratory water loss contributed significantly to total water loss rate, in keeping with much of the comparative literature on water balance (for reviews, see Edney, 1977; Wharton, 1985; Hadley, 1994b; Addo-Bediako et al., 2001). In other words, the first of our null hypotheses was falsified. In these beetles, variation in respiratory water loss was related to variation in total water loss.

Variation in spiracular water loss rate was best explained by a combination of CF-period duration, O-period duration and \dot{V}_{CO_2} . The estimates for these parameters indicated that decreasing rates of water loss are associated with an increase in the duration of the CF-period, a decline in the duration of the O-period, and a decrease in metabolic rate (Table 4). These results provide strong support for the hypothesis that alteration of metabolic rate at rest can be used to effect a change in water loss, as has previously been suggested by several authors (Barnhart and McMahon, 1987; Zachariassen et al., 1987, 1988; Lighton and Bartholomew, 1988; Hoffmann and Parsons, 1989b; Chown and Gaston, 1999). Therefore, the second of our null hypotheses can be rejected.

In the context of the relationship between metabolic rate and total water loss rate, it is important to note that total rates of water loss measured here differed from gravimetric estimates made for two of the species for which data are available (see Klok, 1994). This suggests that comparisons between studies using short-term water loss rate measurements and those

undertaken over longer-term periods (e.g. Zachariassen et al., 1987, 1988; Zachariassen, 1996; Addo-Bediako et al., 2001) must be made with caution. Nonetheless, in the context of these kinds of comparative analysis of water loss and metabolic rate, it is important to note that in these *Scarabaeus* species, the relationship between the residuals of each of the log(rate)-log(mass) relationships (see Addo-Bediako et al., 2001) was positive ($r^2 = 0.97$, $P = 0.014$). This finding provides evidence that the relationship between log(metabolic rate) and log(water loss rate) was not simply a function of the covariation of both variables with body mass. The same result was obtained in a multiple regression with water loss rate and both mass and metabolic rate as independent variables, as recommended by Freckleton (2002). That is, metabolic rate was retained as a significant term (partial correlation $t = 4.3$, $P < 0.05$). Indeed, although larger body size has frequently been identified as an important means by which insects in general (Schoener and Janzen, 1968; Rimmert, 1981; Lighton et al., 1994; Le Lagadec et al., 1998; but see also Gibbs and Matzkin, 2001; Chown and Klok, 2003; Gibbs et al., 2003), and dung beetles in particular (Chown et al., 1995), might alter their responses to environmental water availability, it did not enter most of our models as a significant term. Clearly, both cuticular and spiracular water loss rates scale with body mass, but, especially in the latter case, body mass is much less important in explaining variation than are other factors. Moreover, it should be kept in mind that body size can affect desiccation resistance not only *via* variation in water loss rates, but also *via* variation in water content (Lighton et al., 1994; Chown et al., 1995).

The inclusion of CF-period and O-period durations in the best-fit model for spiracular water loss also suggests that modifications in the pattern of gas exchange might be important for altering water loss rates. That is, our third hypothesis has also been rejected. Whilst it has long been suspected that there is covariation between gas exchange patterns and water loss rates (Lighton, 1988a, 1990, 1991; Lighton et al., 1993; Davis et al., 1999; Bosch et al., 2000; Duncan and Dickman, 2001; Duncan et al., 2002a), few studies have examined the relationships between DGC characteristics and spiracular water loss (for exceptions, see Lighton, 1992; Lighton et al., 1993; Quinlan and Lighton, 1999). For the most part, inferences concerning the importance of modulation of either CF-period or O-period duration for altering spiracular water loss are based on measurements of \dot{V}_{CO_2} only (e.g. Bosch et al., 2000; Duncan, 2003), and then rarely involving investigations of more than one or two species (though for

exceptions, see Lighton, 1991; Davis et al., 1999; Duncan and Byrne, 2000). By contrast, our results provide explicit support for the idea that modulation of DGC characteristics and metabolic rate can be used to alter water loss rate. Moreover, these changes are in a direction that is consistent with a response to changes in environmental water availability. That is, species from more arid areas have lower metabolic rates, shorter O-periods and longer CF-periods. Whilst we cannot conclude that these changes are adaptive, mostly because the two wingless species are more closely related to each other than they are to any of the other species (for further discussion, see Davis et al., 2000), our results suggest that this is likely to be the case. In other words, the data provide support for the proposition that by reducing the period for which spiracles remain open, and by prolonging the duration of the closed and flutter periods, species showing DGC can reduce respiratory water loss. Although the contribution of the F-period to water savings hinges on whether gas exchange takes place predominantly by convection or diffusion (Lighton, 1988b; Lighton and Garrigan, 1995; Lighton, 1996), it seems likely that there would be substantial convective airflow in the F-period in these species, because this has been found in other dung beetle species (Chown and Holter, 2000; Duncan and Byrne, 2000). Nonetheless, the exclusion of CF-period duration from the model including wing status suggests that alteration of CF-period duration might only be a response to very arid conditions, rather than one found in all species, whereas all species might modulate water loss *via* changes in O-period duration. Prolonged CF-period durations in other beetle species from extremely arid areas (Bosch et al., 2000; Duncan and Byrne, 2000; Duncan et al., 2002a) provide support for this contention.

Although body mass was excluded from the model used to explain respiratory water loss, we undertook two additional analyses to investigate the scaling of both cuticular and spiracular water loss, because scaling of these variables has long been of interest in water balance physiology (Kestler, 1985; Nagy and Peterson, 1988; Lighton and Feener Jr, 1989; Lighton et al., 1994; Lehmann et al., 2000). Although several estimates of the scaling of overall water loss have been made, ranging from 0.484 to 0.943 (Nagy and Peterson, 1988; Zachariassen et al., 1988; Chown, 1993; Lighton et al., 1994), consensus scaling equations for respiratory and cuticular water loss in insects have not been derived (Edney, 1977; Arlian and Veselica, 1979; Peters, 1983; Wharton, 1985; Hadley, 1994b). In part, this must be a consequence of the difficulty of distinguishing respiratory and cuticular water loss, and the problems of catabolism when water loss is measured gravimetrically (for a discussion, see Nicolson, 1980; Edney, 1982; Addo-Bediako et al., 2001). By contrast, the measurements of respiratory and cuticular transpiration made here are not confounded by these problems. The scaling exponent of 0.721 for cuticular water loss did not differ from an expectation of 0.67 based on geometric considerations alone (as is the case for overall water flux in the *Drosophila* species examined by Lehmann et al., 2000). This finding does not

provide support for Kestler's hypothesis (Kestler, 1985) that cuticular water loss should scale as $\text{mass}^{0.33}$. Kestler (1985) argued that because cuticular thickness scales as $\text{mass}^{0.33}$, cuticular water loss should scale similarly. The fact that our findings and those of Lehmann et al. (2000) do not support this hypothesis is perhaps not unexpected given that it is not only cuticular thickness that determines water loss rates (Gibbs et al., 1991; Gibbs, 1998, 2002b; Rourke, 2000). Spiracular water loss had a somewhat lower scaling exponent (0.531), although it did not differ from 0.67 either. According to Kestler (1985), in a purely diffusion-based system, respiratory water loss should scale as $\text{mass}^{0.33}$, whereas in a convection based system it should scale as $\text{mass}^{1.0}$. The intermediate value obtained here suggests that both diffusive and convective water loss take place in these species.

In conclusion, we have rejected all three of our null hypotheses, thereby providing considerable support for previous contentions that modulation of respiratory water loss is important for water balance in insects. Moreover, our results also provide direct, comparative evidence that in species with discontinuous gas exchange cycles, alterations in both metabolic rate and gas exchange pattern contribute to changes in respiratory water loss. Therefore, our work not only provides direct evidence for several theoretically appealing but empirically poorly supported ideas, but also joins a growing body of evidence (e.g. Lehmann, 2001; Gibbs et al., 2003) demonstrating that respiratory water loss cannot be discounted in investigations of insect water balance. In the context of discontinuous gas exchange cycles it also suggests that hypotheses for the origin and maintenance of these cycles, which are predicated on water savings (the hygric and chthonic genesis hypotheses; see Lighton and Berrigan, 1995; Lighton, 1996, 1998), should not be discarded just yet.

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