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THE SCALING OF CARBON DIOXIDE RELEASE AND RESPIRATORY WATER LOSS IN FLYING FRUIT FLIES (*DROSOPHILA* SPP.)

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Summary

By simultaneously measuring carbon dioxide release, water loss and flight force in several species of fruit flies in the genus *Drosophila*, we have investigated respiration and respiratory transpiration during elevated locomotor activity. We presented tethered flying flies with moving visual stimuli in a virtual flight arena, which induced them to vary both flight force and energetic output. In response to the visual motion, the flies altered their energetic output as measured by changes in carbon dioxide release and concomitant changes in respiratory water loss. We examined the effect of absolute body size on respiration and transpiration by studying four different-sized species of fruit flies. In resting flies, body-mass-specific CO_2 release and water loss tend to decrease more rapidly with size than predicted according to simple allometric relationships. During flight, the mass-specific metabolic rate decreases with increasing body size with an allometric exponent of –0.22, which is slightly lower than the scaling exponents found in other flying insects. In contrast, the mass-specific rate of water loss appears to be proportionately greater in small animals than can be explained by a simple allometric model for spiracular transpiration. Because fractional water content does not change significantly with increasing body size, the smallest species face not only larger mass-specific energetic expenditures during flight but also a higher risk of desiccation than their larger relatives. Fruit flies lower their desiccation risk by replenishing up to 75 % of the lost bulk water by metabolic water production, which significantly lowers the risk of desiccation for animals flying under xeric environmental conditions.

Key words: carbon dioxide release, water loss, flight force, fruit fly, *Drosophila*.

Introduction

The elevated power output required for flapping flight places special demands and constraints on the respiratory system of insects. On the one hand, the respiratory system must permit the enormous flux of oxygen and carbon dioxide to and from flight muscles (Ellington, 1985; Gilmour and Ellington, 1993; Josephson, 1997; Lehmann and Dickinson, 1998). On the other hand, the structures that permit an exchange of respiratory gases leave an animal susceptible to the loss of water vapor, thus increasing the danger of desiccation.

At rest, many insects exhibit a discontinuous respiratory pattern, a behavior that is thought to limit water loss to prevent desiccation (Lighton, 1994). Resting rates of water loss and carbon dioxide release have been measured for a variety of insects under different environmental conditions (Croghan et al., 1995; Loveridge, 1968b; Nikam and Khole, 1989; Noble-Nesbitt et al., 1995; Williams et al., 1997). Locomotor activity normally disrupts the discontinuous breathing cycle, and most active insects open their spiracles continuously in order to match the increased requirement for oxygen (Lighton, 1988a,b).

Although numerous studies of water loss in insects exist in the literature, very few have directly measured water loss during flight when the oxygen demand is greatest. Observations in several species of insects, ranging from various beetles to a desert locust, indicate that during flight the second and third spiracles are held fully open to allow maximum gas exchange between the thorax and the ambient air (Loveridge, 1968a,b; Miller, 1960, 1966). In addition to opening their spiracles, these large insects augment gas exchange with active ventilatory pumping of the thoracic tracheal system during flight. In small insects such as fruit flies, however, diffusion alone is thought to be sufficient to match the oxygen requirements during flight, and no additional active ventilation of the tracheal system is needed (Krogh, 1920; Weis-Fogh, 1964). Even in the absence of active ventilation, pure diffusion could supply oxygen to the thorax flight muscles with a safety factor of 2–3 (Weis-Fogh, 1964).

The flux of any gas through the tracheal system is proportional to the difference between the tracheal and ambient partial pressure for that gas multiplied by a term that

characterizes the geometry over which the diffusion occurs. This term is given by the effective area of the tracheal system divided by its effective length (Kestler, 1985). Assuming that the tracheal system of *Drosophila* spp. scales isometrically with size, tracheal area and tracheal length should scale with body mass with exponents of 0.67 and 0.33, respectively. Thus, the geometric term that governs diffusion should scale with an allometric exponent of 0.33. However, in most invertebrates, resting metabolic rate increases with body mass with an allometric exponent of between 0.77 and 0.93 (Altman and Dittmer, 1968; Anderson, 1970; Anderson and Prestwich, 1982; Greenstone and Bennett, 1980; Lighton et al., 1993a; Lighton and Wehner, 1993). The difference in the scaling exponents between tracheal geometry and metabolic rate suggests that the partial pressure forces that drive O_2 and CO_2 in and out of the body might increase with increasing size with allometric exponents of between 0.44 and 0.60. In contrast, since the ends of the tracheal system are filled with liquid water in both small and large insects, the partial pressure driving force of water vapor should not vary with size (Beament, 1964; Edney, 1977). Under steady environmental conditions, the flux of water vapor through the spiracles depends solely on the geometry of the diffusive path and should increase with an exponent 0.33 with increasing body mass. Small flies should therefore face a higher risk of desiccation than their larger relatives. Additional water loss through the cuticle could reduce the survival time even more, because it depends on cuticular area and cuticular thickness, and smaller insects should possess a thinner integument than their large relatives (Kestler, 1985).

One difficulty with assessing the effects of scaling on respiration and transpiration is that changes in body size are often accompanied by changes in body shape. This problem can be partly circumvented by comparing closely related species of insects whose bodies are morphologically similar over a large size range. Fruit flies within the genus *Drosophila* fit this criterion and are thus well suited for studying the effects of body size on spiracular respiration and transpiration. Here, we investigate how small fruit flies (*Drosophila* spp.) cope with the problem of high water loss through their spiracles and integument during rest and flight. We present real-time recordings of carbon dioxide and water release while tethered flies varied the production of aerodynamic flight forces. We extended our analysis to flies of similar shape but with different body size to determine the effects of body size on respiration and respiratory transpiration in small insects.

Materials and methods

Animals

The flight data within this paper were collected from 2- to 5-day-old female fruit flies (N=62). The laboratory colonies were originally obtained from the Drosophila National Species Resource Center (Bowling Green, Ohio, USA). For 1–2 years, the selected animals had been maintained at room temperature (22 °C) and reared on commercial *Drosophila* medium (Carolina Biological) under standard laboratory conditions. We tested flies from four different species: *D. nikananu* Burly (N=11), *D. melanogaster* Meigen (N=27), *D. virilis* Sturtevant (N=10) and *D. mimica* Hardy (N=14), with mean wet body masses, m_{wet}, of 0.65±0.06, 1.05±0.13, 1.9±0.19 and 3.06±0.52 mg, respectively (means ± S.D.). Some of the force measurements and respirometry data presented here have been published previously in an analysis of muscle efficiency and aerodynamic flight performance (Lehmann and Dickinson, 1997, 1998). Unless stated otherwise, all reported values represent means ± S.D. Throughout the paper, we performed reduced major axis regression (model II) on species mean values as part of the statistical data analysis. Since the species are separated by millions of generations, we assume that each species can be treated as statistically independent. However, water loss rates and other physiological characters may evolve within a few tens of generations under laboratory conditions (Gibbs et al., 1997), which would justify a statistical analysis on the whole data set. Treating all tested animals as one large population, the statistical analysis produced exponents similar to those obtained using species mean values, but at P values consistently below 0.005 (except for Fig. 6C, P=0.29). This result gives confidence in the general conclusions we draw from our statistical analysis on four species mean values, despite the relatively high P values.

Measurement of flight force

We have previously provided a more detailed description of the experimental apparatus (Lehmann and Dickinson, 1997, 1998) and give only a brief outline here. The flies were tethered and flown in a flight arena in which stroke amplitude, stroke frequency and total flight force were simultaneously measured under closed-loop conditions. By changing the relative stroke amplitude of its two wings, each fly controls the angular (azimuth) velocity of a 30 ° wide vertical dark bar displayed in the arena. Under these conditions, flies actively modulate their wing kinematics to stabilize the stripe in the front region of their visual field. While the fly actively controlled the velocity of the vertical bar, we oscillated a superimposed pattern of diagonal stripes in the vertical direction. As the background pattern moves up and down, the fly modulates its total flight force in an attempt to stabilize the retinal slip.

Cold-anesthetized flies were glued to tungsten tethers using Crystal Clear adhesive (Loctite) and allowed to recover for at least 1 h before testing. Although some animals began flying spontaneously when positioned in the respirometry chamber, others were induced to fly using a short air puff from below. In most cases, we recorded two flight sequences from each animal, representing a mean flight time of 13±6 min. Throughout the paper, we will use the terms ‘hovering performance’ or ‘hovering conditions’ to describe the portions of the flight sequence during which the flies generated a flight force within ±1 % of their body weight. The terms ‘maximum performance’ and ‘minimum performance’ describe the 1 % of each flight sequence during which the flies produced maximum and minimum flight force, respectively.

Respirometry

We used a Licor 6562 for all respirometric measurements. Typical signal-to-noise ratios (SNRs) for water measurements during rest and hovering flight in *D. melanogaster* were 5 dB and 18 dB, respectively. In the same species, the SNRs for CO_2 measurements were typically 22 dB during rest and 41 dB during hovering flight. The CO_2 output signal of the Licor was calibrated using 99.7 p.p.m. span gas (Scott Specialty Gas) in nitrogen. To calibrate the water signal, we collected room air in a 2 l Douglas bag. We determined the relative humidity of this gas sample using a digital hygrometer probe (Davis Instruments) previously calibrated using $MgCl_2$ calibration salts (Cole Parmer) of 33 % and 75 % relative humidity. The air bag was then connected to the inlet of the 18 ml respirometry flight chamber, and the Licor was calibrated according to the reading of the hygrometer. To perform flight experiments, room air was scrubbed of water and CO_2 using a Drierite–Ascarite–Drierite column and pulled through the flight chamber at a flow rate of 200 ml min^{-1}, regulated by a mass-flow controller (Sierra). The data were subsequently sampled at 8.3 Hz using an Axotape data-acquisition system (Axon Instruments). Resting values recorded before each flight sequence were subtracted from the raw signal to yield mass-specific rates of carbon dioxide release, $\dot{V}^*_{CO_2}$, and water loss, $\dot{V}^*_{H_2O}$, for flight calculated in units of ml g^{-1} wet body mass h^{-1} and µl g^{-1} wet body mass h^{-1}, respectively. Data were corrected to 760 mmHg (101 kPa) standard pressure and also for slow drift in cases where the gas flux through the empty chamber was different before and after the experimental trial. The measured values of CO_2 release were transformed into metabolic rates assuming catabolism of sucrose, as described previously (21.4 J ml^{-1} CO_2; Lighton, 1991). The temperature within the respirometry chamber was 23–25 °C. The pressure inside the flight chamber during the use of flow-through respirometry did not differ significantly from the barometric pressure measured outside the chamber.

Water content

To provide suitable background data, we determined the total water content of 160 fruit flies. Equal numbers of males and females from each of the four species were removed from their colony and immediately killed using isoamylacetate fumes. After determining body length and mass, we placed the flies into small glass vials for desiccation. After incubating the animals for 3 weeks at 60 °C on a heating plate, we measured dry body mass. The total water content was calculated from the difference between wet and dry mass.

Water loss in resting animals

The slow baseline drift of the Licor output signal for water vapor and the low signal-to-noise ratio made it difficult to estimate resting levels of water loss rates in single individuals using the relatively large respirometry flight chamber. We therefore determined $\dot{V}_{H_2O}$ in unrestrained flies on the basis of mass loss at 0 % relative humidity. After measuring their initial mass, 10 females of each fly species were placed into small plastic polymerase chain reaction vials perforated with 50 small holes. The mean wet body masses of the four species were 0.49±0.2 mg (*D. nikananu*), 0.95±0.25 mg (*D. melanogaster*), 1.67±0.34 mg (*D. virilis*) and 2.44±0.55 mg (*D. mimica*). The vials were placed within a sealed container filled with Drierite to establish 0 % relative humidity. We determined the mass loss of each individual every 30 min until it died. The measured total rates of water loss represent the sum of fecal water loss, cuticular and spiracular water loss, water loss through the proboscis and the loss of hygroscopic water bonded to the cuticle (Loveridge, 1968a). These experiments were performed at an ambient temperature of 21–24 °C.

Results

Water content of fruit flies

To test whether total body water scales isometrically with body size, we determined the dry mass (m_{dry}) of 159 flies selected from the four species. When dry mass is plotted against wet mass (m_{wet}), the animals segregate into two species groups (Fig. 1A). The three smaller species, *D. nikananu*, *D. melanogaster* and *D. virilis*, fall roughly on the same regression line (m_{dry}=0.33m_{wet}–0.05, mean r^2=0.95, mean P<0.0001, N=119 flies), whereas the water content of *D. mimica* increases slightly differently with size (m_{dry}=0.31m_{wet}–0.23, r^2=0.80, P<0.0001, N=40 flies). The slopes of the two groups are statistically indistinguishable [ANOVA, two-tailed t-test, P(parallel slope)>0.2], whereas the intercepts of the regression lines are significantly different

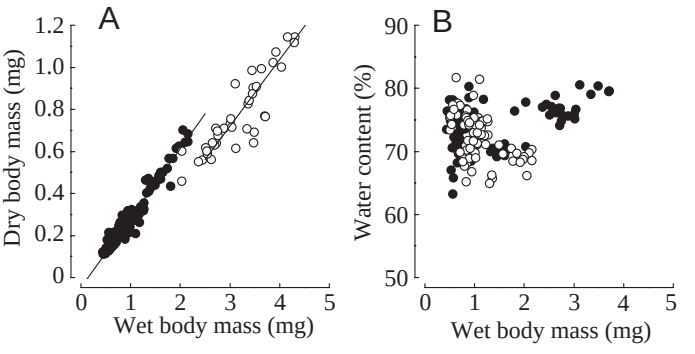

Fig. 1. Water content in four drosophilid species. (A) Regressions between wet and dry body mass for the three smaller species, *D. nikananu*, *D. melanogaster* and *D. virilis* (filled circles), and the largest species, *D. mimica* (open circles). (B) Mean water content within the drosophilid family is shown for 20 males (filled circles) and 20 females (open circles) of each fly species. Water content is 72±4 % in *D. nikananu*, 74±2 % in *D. melanogaster*, 72±4 % in *D. virilis* and 76±2 % in *D. mimica* (means ± S.D.).

Table 1. *Differences in mean body mass between males and females of the four tested species*

Fly species	Gender	*N*	m_{wet} (mg)	m_{rel} (%)
D. nikananu	Male	20	0.59±0.11	79.8
D. nikananu	Female	20	0.74±0.14***	
D. melanogaster	Male	20	0.73±0.07	65.4
D. melanogaster	Female	19	1.12±0.09***	
D. virilis	Male	20	1.18±0.40	75.4
D. virilis	Female	20	1.57±0.11***	
D. mimica	Male	22	2.84±0.46	77.5
D. mimica	Female	18	3.66±0.46***	

Asterisks indicate significant differences (P<0.001) in body mass between genders (two-tailed *t*-test).

m_{wet}, wet body mass; m_{rel}, mass ratio of male to female flies.

Values are means ± S.D.

[ANCOVA, two-tailed *t*-test, P(equal intercept)<0.0001]. The mean values of all four species fall on a regression line with a slope of 0.24±0.01 (m_{dry}=0.24m_{wet}+0.04, r^2=0.99, P=0.004, N=4 species), indicating that small animals possess the same fractional water content as their larger relatives. Water content, the ratio of wet to dry mass, is plotted against wet mass in Fig. 1B. Pooling the mean values for all species, water content amounts to approximately 75±6 % of the fly's body mass (N=4 species). We found a very small, but significant, difference in water content between the genders (difference 1.7±1.3 %, P<0.01, N=4 species), although males are 25.5±6.3 % smaller by mass than females (Table 1).

CO_2 release and water loss in resting flies

According to the experimental procedure described above, we determined $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$ in resting fruit flies using both flow-through respirometry and mass loss. Using mass loss to estimate resting water flux became necessary because the baseline measured using flow-through respirometry was not stable enough to yield satisfactory measurements of $\dot{V}_{H_2O}$ in individual flies. Fig. 2 shows the loss of body mass of 10 animals for each of the four species of fruit flies. Individuals died an average of 14.7±8.0 h after the onset of desiccation (N=4 species, Fig. 3C; Table 2). Survival times varied from 4.0 h in *D. nikananu* to 23.4 h in *D. virilis*, in rough proportion to body mass. By averaging the differences in body mass between successive data points in Fig. 2A–D, we determined a mean water loss rate of 39.1±10 nl h^{-1} (N=4 species). At the time of death, the animals had lost on average 40±9 % of their initial body mass (N=4 species). On the basis of an initial water content of 75 %, this indicates that fruit flies can lose 53±11 % of their bulk water reserves before succumbing to desiccation.

In contrast to $\dot{V}_{H_2O}$, it was possible to measure resting levels of $\dot{V}_{CO_2}$ for individual flies within the respirometry flight chamber. The mean $\dot{V}_{CO_2}$ of tethered fruit flies that were not flying was 5.09±1.91 µl h^{-1} (0.11±0.04 J h^{-1}, N=4 species; Table 2). To address the question of how resting $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$ scale with body size in resting flies, we fitted the mean values for each species to the standard allometric equation (Fig. 3). With increasing body size, mass-specific $\dot{V}_{H_2O}$ ($\dot{V}^*_{H_2O}$) shows a tendency to decrease in proportion to body mass with an exponent of −1.03±0.24 ($\dot{V}^*_{H_2O}$=35.8$m_{wet}^{-1.03}$, r^2=0.89, P=0.055, N=4 species; Fig. 3B), suggesting that in resting fruit

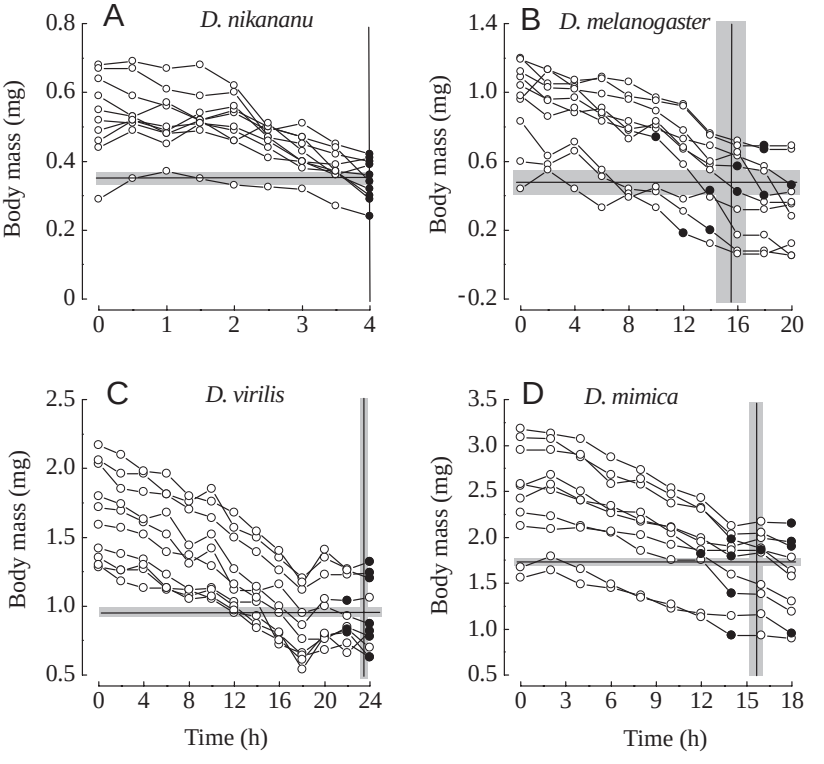

Fig. 2. Loss of body mass by fruit flies resting at 0 % relative humidity due to evaporation of water through their cuticle and spiracles. Ten individuals of each of the four drosophilid species were removed from their colonies and placed in small vials for desiccation. Open circles indicate the body mass of each fly; the time of death of each individual is indicated by a filled circle. For each species, mean body mass and mean time of death are indicated by black lines. The grey areas indicate the S.E.M. of the plotted mean values.

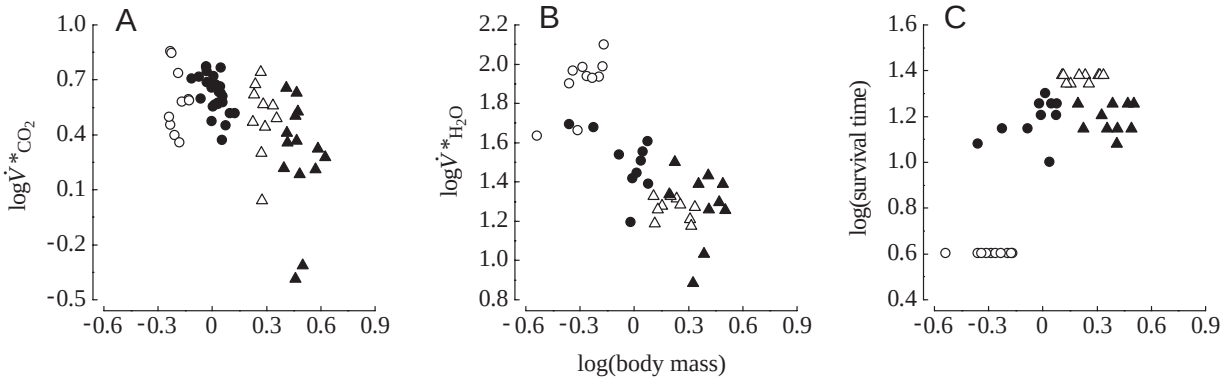

Fig. 3. Resting body-mass-specific rate of carbon dioxide release ($\dot{V}$*$_{CO_2}$, A; ml g^{-1} h^{-1}), resting body-mass-specific rate of water loss ($\dot{V}$*$_{H_2O}$, B; µl g^{-1} h^{-1}) and survival time (C; h) *versus* body mass (mg) in four species of fruit flies. Open circles, *D. nikananu* (*N*=11); filled circles, *D. melanogaster* (*N*=27); open triangles, *D. virilis* (*N*=10); filled triangles, *D. mimica* (*N*=14).

flies absolute water loss rate does not change with size. Resting $\dot{V}$*$_{CO_2}$ shows a tendency to decrease in proportion to body mass with an exponent of –0.52±0.17 ($\dot{V}$*$_{CO_2}$=3.7$m_{wet}^{-0.52}$, r^2=0.79, *P*=0.11, *N*=4 species, Fig. 3A).

CO₂ release and water loss during flight

Throughout all arena experiments, the flies fixated on a vertical black stripe by actively varying the amplitude difference between the left and the right wing stroke. To introduce a regular modulation of locomotory activity, we oscillated a superimposed pattern of diagonal stripes in the vertical direction. A typical response to these combined horizontal closed-loop and vertical open-loop conditions is shown in Fig. 4. As the background pattern moves up and down, the fly tries to follow the movement in order to minimize retinal slip in the vertical direction. Except for rare cases in which the animal ceased flying completely, we observed no attenuation in the behavioral response of the flies over the course of experiments lasting approximately 13 min. Flight force, $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$ during minimum, hovering and maximum performance are shown in Table 3. All values for gas release represent net rates calculated by subtracting resting values. The modulation of flight force was accompanied by regular changes in both carbon dioxide release and respiratory water loss. During hovering flight, fruit flies release 32.4±5.1 ml g^{-1} h^{-1} of CO_2 (0.69±0.11 kJ g^{-1} h^{-1}) and 67.3±36.9 µl g^{-1} h^{-1} of water, representing on average 9.7-fold (CO_2) and 2.4-fold (H_2O) increases over resting rates (*N*=4 species). The moving visual stimulus induced flight force modulation of 105±25 % peak-to-peak (*N*=4 species). In response to these changing power requirements, $\dot{V}$*$_{CO_2}$ varied on average by 30±18 % (from 25.1±5.2 to 34.2±5.6 ml g^{-1} h^{-1}), while $\dot{V}$*$_{H_2O}$ changed by 24±7 % (from 57.6±28.8 to 71.0±35.0 µl g^{-1} h^{-1}) of its mean value (*N*=4 species, Table 4). We found no significant difference in the modulation of $\dot{V}$*$_{H_2O}$ and $\dot{V}$*$_{CO_2}$ (*P*=0.5), suggesting that most of the changes in CO_2 release during flight can be explained by alterations in spiracle opening area rather than by changes in the tracheal partial pressure for CO_2. This aspect of insect respiration behavior will be addressed separately in a study of the control of spiracle opening area during flight in *D. melanogaster* (F.-O. Lehmann, unpublished data).

As shown in Fig. 5 for *D. nikananu*, a few of the flies tested exhibited large transient increases in the rate of water loss that were not accompanied by releases of CO_2. During these water spikes, $\dot{V}$*$_{H_2O}$ rose to 10 times the resting level. Each single $\dot{V}$*$_{H_2O}$ spike in Fig. 5 represents an average loss of 2.6±0.9 nl of water (*N*=7 spikes) corresponding to 0.5 % of the fly's water content. In most of the flight sequences, water spikes occurred during the initial stage of flight, soon after the fly had been placed into the respirometry chamber, and were often correlated with extensions of the proboscis. Release of feces,

Table 2. *Resting water loss, resting CO_2 release and survival time in the four drosophilid species at 0 % relative humidity*

Fly species	*N* for H_2O	*N* for CO_2	m_{wet} (mg)	$\dot{V}_{CO_2}$ (µl h^{-1})	$\dot{V}$*$_{CO_2}$ (ml g^{-1} h^{-1})	$\dot{V}_{H_2O}$ (nl h^{-1})	$\dot{V}$*$_{H_2O}$ (µl g^{-1} h^{-1})	T_s (h)	m_{death} (%)
D. nikananu	10	11	0.49±0.20	2.69±1.05	3.69±2.37	46.5±20.6	83.6±24.1	4.0±0	67±10
D. melanogaster	10	27	0.95±0.25	4.44±0.84	4.30±0.96	30.1±9.3	33.4±10.5	15.6±3.1	50±14
D. virilis	10	10	1.67±0.34	6.35±2.35	3.36±1.29	30.7±6.0	18.4±2.3	23.4±1.0	57±6
D. mimica	10	14	2.44±0.55	6.87±3.36	2.30±1.22	49.2±18.8	20.4±7.2	15.6±2.3	68±10

$\dot{V}$*$_{H_2O}$, body-mass-specific rate of water loss; $\dot{V}$*$_{CO_2}$, body-mass-specific rate of CO_2 release; T_s, survival time; m_{wet}, wet body mass, m_{death}, body mass at death in proportion to wet body mass.

Values are means ± S.D.

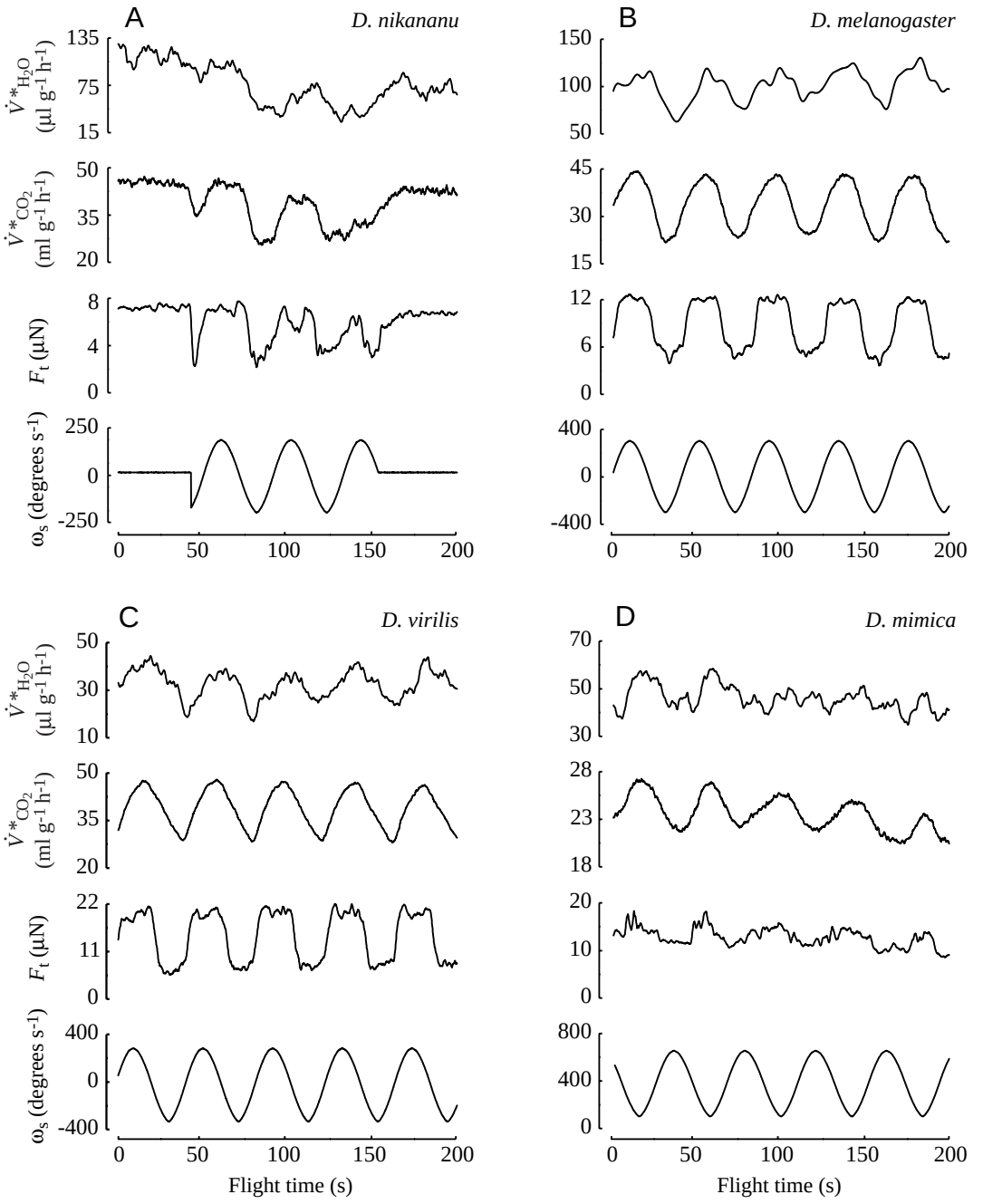

Fig. 4. Typical flight sequences for the four species of fruit flies in which the flies responded to the motion of the visual background pattern. In all flies, changes in the net rate of body-mass-specific CO_2 release ($\dot{V}^*_{CO_2}$) are accompanied by changes in the net rate of body-mass-specific water loss ($\dot{V}^*_{H_2O}$), which varies with aerodynamic flight force (F_t) production. ω_s, angular velocity of the moving background pattern.

verified by the presence of droppings in the flight chamber, typically resulted in higher rates of water loss than the water spikes shown in Fig. 5. Thus, we tentatively assign the water spikes to a loss of water through the proboscis.

The scaling of CO_2 release and water loss during flight

To study the effects of body size on respiration and transpiration during flight, we compared mean $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$ among the four *Drosophila* species. The total flux of CO_2 measured under hovering conditions increases in proportion to body mass with an allometric exponent 0.78±0.02 ($\dot{V}_{CO_2}=34.4m_{wet}^{0.78}$, $r^2=0.99$, $P<0.0005$, $N=4$ species, Fig. 6A). In comparison, the rate of body-mass-specific CO_2 release decreases in proportion to body mass with an exponent of –0.22±0.02 ($\dot{V}^*_{CO_2}=34.4m_{wet}^{-0.22}$, $r^2=0.99$, $P=0.006$, $N=4$ species, Fig. 6B), indicating that flight is relatively more costly in small insects. However, the variance within the data set measured for each species is quite large. In *D. melanogaster*,

Table 3. *Water loss and CO_2 release for minimum, hovering and maximum flight performance in the four drosophilid species*

Fly species	N	m_{wet} (mg)	Performance	F/R CO_2	F/R H_2O	$\dot{V}_{CO_2}$ ($\mu l\,h^{-1}$)	$\dot{V}^*_{CO_2}$ ($ml\,g^{-1}\,h^{-1}$)	$\dot{V}_{H_2O}$ ($nl\,h^{-1}$)	$\dot{V}^*_{H_2O}$ ($\mu l\,g^{-1}\,h^{-1}$)	$F_t/m_{wet}\boldsymbol{g}$ ($\mu N\,mg^{-1}$)
D. nikananu	6	0.64±0.06	Minimum	7.5	1.1	20.3±4.8	31.9±8.5	51.6±28.4	79.0±38.1	0.55±0.20
D. nikananu	4	0.64±0.08	Hover flight	9.1	1.4	24.5±1.1	38.9±4.3	63.8±24.8	98.5±28.1	1
D. nikananu	6	0.64±0.06	Maximum	8.6	1.4	23.2±6.9	36.4±11.6	65.8±24.3	102±34.3	1.2±0.30
D. melanogaster	25	1.06±0.12	Minimum	6.1	3.0	27.3±5.0	26.0±5.4	89.2±27.1	85.3±29.1	0.39±0.13
D. melanogaster	25	1.06±0.12	Hover flight	8.0	3.5	35.5±5.9	33.6±5.2	104±23.4	99.1±23.9	1
D. melanogaster	25	1.06±0.12	Maximum	8.6	3.5	38.0±6.2	36.2±6.3	105±23.0	100±25.6	1.35±0.21
D. virilis	6	1.9±0.21	Minimum	6.5	1.7	41.4±5.2	22.0±3.3	52.2±20.6	28.0±11.8	0.33±0.16
D. virilis	6	1.9±0.21	Hover flight	9.0	1.8	57.4±10.4	30.1±3.9	54.0±12.1	28.6±6.9	1
D. virilis	6	1.9±0.21	Maximum	11.4	2.2	72.7±12.1	38.2±4.6	66.8±16.0	35.1±7.9	1.7±0.28
D. mimica	13	3.07±0.54	Minimum	9.2	2.4	63.4±17.8	20.3±3.9	119±43.9	37.9±11.7	0.30±0.14
D. mimica	4	3.23±0.42	Hover flight	12.7	2.8	87.4±19.1	27.0±4.7	140±40.1	42.8±7.9	1
D. mimica	13	3.07±0.54	Maximum	11.7	3.0	80.3±20.8	25.8±4.7	147±41.5	46.7±12.6	0.90±0.20

F/R CO_2, ratio of CO_2 release during flight (*F*) to CO_2 release at rest (*R*); *F/R* H_2O, ratio of water loss during flight to water loss at rest; F_t, total flight force; $\boldsymbol{g}$, gravitational constant.

Other abbreviations are as in Table 2.

Some of the *D. nikananu* and *D. mimica* did not produce flight forces high enough to sustain hovering flight (compare *N* values in column 2). In these species, the rate of CO_2 release at maximum performance is therefore slightly smaller than during hovering.

Values are means ± S.D.

for example, the rate of body-mass-specific CO_2 release varies from 24 to 46 $ml\,g^{-1}\,h^{-1}$ and covers almost the whole range of values measured in all species.

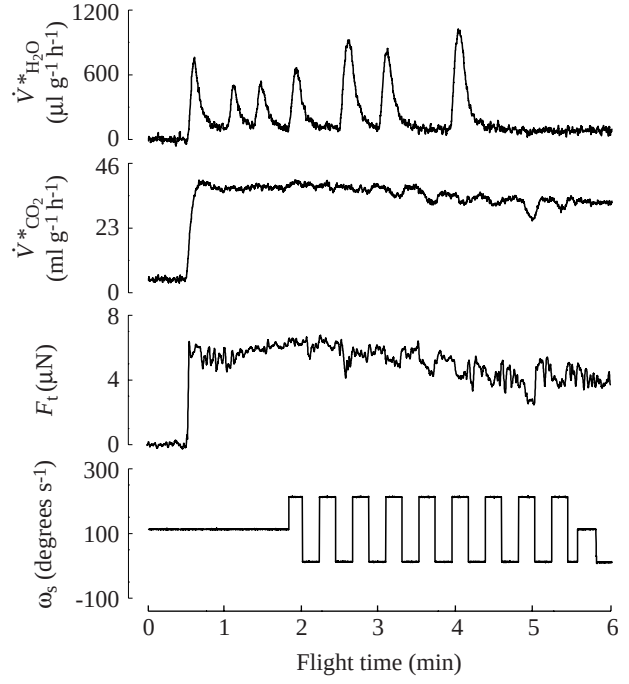

Fig. 5. Transient changes in of total water loss rate recorded during the flight of a single *Drosophila nikananu*. Peaks of high water loss are characterized by an up to 10-fold increase in water loss rate. Water spikes only occur during flight and are often correlated with the extension of the fly's proboscis. For abbreviations, see legend to Fig. 4.

During hovering, the rate of water loss shows a tendency to increase with increasing body size with an allometric exponent of 0.63±0.39 ($\dot{V}_{H_2O}=65.3m_{wet}^{0.63}$, $r^2=0.25$, $P=0.5$, $N=4$ species). This value is close to that measured for $\dot{V}_{CO_2}$ and not significantly different from the scaling value of 0.67 predicted according to the surface-to-volume ratio model for cuticular water loss ($P=0.5$). The low P value of the regression line is mainly due to the values for *D. virilis*, which appears to be particularly resistant to desiccation. As with $\dot{V}^*_{CO_2}$, $\dot{V}^*_{H_2O}$ shows a tendency to decrease with an exponent of −0.88±0.39 with increasing body size among all four species ($\dot{V}^*_{H_2O}=77.7m_{wet}^{-0.88}$, $r^2=0.62$, $P=0.22$, $N=4$ species, Fig. 6D). The allometric exponent −0.88 suggests that under hovering conditions mass-specific water loss scales out of proportion with body size in all flies. Thus, a small fly operates under the burden of both a high metabolic cost and a greater risk of desiccation during flight. Mean $\dot{V}^*_{H_2O}$ and $\dot{V}^*_{CO_2}$ at maximum

Table 4. *Peak-to-peak modulation of flight force, respiratory water loss and CO_2 release while a fly varied its total power output in response to vertical oscillation of the visual background pattern*

Fly species	N	$\Delta\dot{V}^*_{CO_2}$ (%)	$\Delta\dot{V}^*_{H_2O}$ (%)	ΔF_t (%)
D. nikananu	6	11±14	32±26	76±16
D. melanogaster	25	32±17	18±19	112±22
D. virilis	6	54±16	28±36	136±22
D. mimica	12	22±20	17±38	100±26

$\dot{V}^*_{CO_2}$, body-mass-specific rate of CO_2 release; $\dot{V}^*_{H_2O}$, body-mass-specific rate of water loss; F_t, total flight force.

Values are means ± S.D.

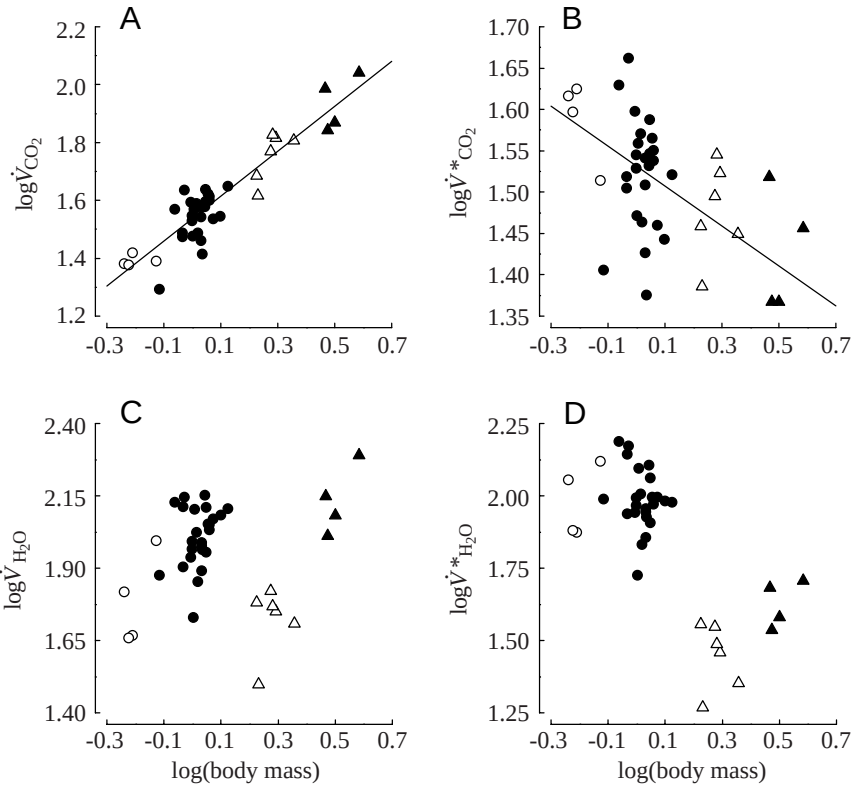

Fig. 6. Scaling relationships between rates of CO_2 release ($\dot{V}_{CO_2}$, A; µl h^{-1}) and water loss ($\dot{V}_{H_2O}$, C; nl h^{-1}) in four species of fruit flies during flight *versus* body mass (mg). Each data point represents the mean gas flux sampled from flight sequences during which the fly generated a flight force within ±1 % of its body weight. $\dot{V}^*_{CO_2}$ (B; ml g^{-1} h^{-1}) and $\dot{V}^*_{H_2O}$ (D; µl g^{-1} h^{-1}) indicate body-mass-specific values. Regressions were fitted for species mean values. Open circles, *D. nikananu* (*N*=4); filled circles, *D. melanogaster* (*N*=25); open triangles, *D. virilis* (*N*=6); filled triangles, *D. mimica* (*N*=4).

flight performance apparently scale very similarly to those during hovering with exponents of –0.79±0.31 ($\dot{V}^*_{H_2O}$=83.9$m_{wet}^{-0.79}$, r^2=0.69, *P*=0.17, *N*=4 species) and –0.27±0.14 ($\dot{V}^*_{CO_2}$=37.0$m_{wet}^{-0.27}$, r^2=0.46, *P*=0.32, *N*=4 species), respectively.

Discussion

This study has determined the scaling relationships between CO_2 release and water loss in four species of fruit flies in the genus *Drosophila* during rest and hovering flight. By using a virtual-reality flight arena, we have shown that flying fruit flies vary the rate of CO_2 release and water loss according to the actual power requirements for flight. Moreover, the data indicate that, in resting flies, the mass-specific rate of respiration tends to be greater than in warm-blooded vertebrates and that the mass-specific rate of respiratory transpiration apparently decreases faster with increasing body size than predicted according to simple allometric relationships. During flight, the scaling of mass-specific CO_2 release approximates the scaling values found in other flying insects, whereas mass-specific water loss seems to decrease faster with increasing size than can be predicted from spiracular transpiration (Kestler, 1985). Part of the additional water loss might be due to a possible increase in spiracle opening area that results from increased power requirements for flight in small animals. The following discussion provides a detailed assessment of these findings and tries to draw a more complete picture of total water balance in the genus *Drosophila*.

Water loss in resting flies

Rates of water loss in *Drosophila* spp. have previously been determined by measuring the mass loss of individuals at rest in a flow-through chamber (Gibbs et al., 1998; Williams et al., 1997). Gibbs et al. (1998) found that water loss rates were relatively high in *Drosophila mojavensis* within the first 2 h after placing the flies into the respirometry chamber and stabilized after the flies faced desiccation stress. Loveridge (1968a) has suggested that in *Locusta migratoria* this high initial water loss may be due to hygroscopic water bonded to the cuticle. Our data suggest that water loss rates do not change significantly over over the first 2 h after placing the flies into the respirometry chamber, remaining constant until the flies die from desiccation (Fig. 2A–D).

The rates of water loss measured in *D. melanogaster* and *D. virilis* (30.1 and 30.7 nl h^{-1}, respectively; Table 2) are low compared with the rates found for *D. melanogaster* in a previous study (46 nl h^{-1}), but similar to those found in females of the desert species *D. mojavensis* (30 nl h^{-1}) and females of desiccation-selected *D. melanogaster* (26 nl h^{-1}; Gibbs et al., 1997, 1998). The estimated mean survival time in *D. melanogaster* of approximately 15.6 h (Fig. 3C; Table 2) is significantly higher than the values of 9 h in flies selected for postponed senescence and 11 h in a control group (Williams et al., 1997). Although it is tempting to attribute this difference to genetic background, it is also possible that experimental techniques explain the longer survival time of our flies compared with previous studies. The flies used in our study faced desiccation stress in dry but still air, whereas Gibbs et al. (1997, 1998) and Williams et al. (1997) employed a flow-

through chamber, which could easily have enhanced water loss through the cuticle. Convection attenuates the concentration gradient of water vapor in the boundary layer around the insect body and thus causes water to evaporate more quickly from the cuticle surface (Denny, 1993). Alternatively, a convective air stream could also enhance water loss rate by passively ventilating the fly's tracheal system when the spiracles are open for gas exchange. In the large African cerambycid *Petrognatha gigas*, Miller (1966) reported that during flight air passed along the large metathoracic trunks, entering through spiracle 2 and leaving from spiracle 3. However, it remains uncertain whether convective flow inside the respirometry chamber can significantly enhance tracheal ventilation in *Drosophila* spp. given the low Reynolds number of roughly 0.007 for tracheal air flow (free air-stream velocity $2.4\times10^{-3}\,m\,s^{-1}$; tracheal diameter 43.7 μm; Manning and Krasnow, 1993).

Besides cuticular and spiracular water loss, fruit flies face a tremendous loss of body water through two additional distinct pathways: the proboscis and the digestive apparatus. Water loss through the feces can become quite large and even exceeds $\dot{V}_{H_2O}$ through the proboscis. Water loss through the proboscis is relatively high, and small flies may lose up to 3.5 % of their bulk water within a series of seven water spikes (Fig. 5). By way of comparison, this is the quantity that a resting fly would lose over a period of 17 min *via* water loss through the cuticle and spiracles. Thus, reducing fecal water content and reducing the loss of water through the proboscis must be considered as important strategies by which the animal can minimize the loss of bulk water.

The results shown in Fig. 6C suggest that during flight *D. virilis* may possess xeric adaptations that are not shared by the other three species. Such adaptations might reflect either the current ecological specializations of this species or, alternatively, its phylogenetic lineage. With respect to the function and morphology of the respirometry system, the species in this study may vary according to their phylogenetic relationships. Although it has a penchant for breweries, *D. virilis* is closely related to the *D. repleta* group in which many species are endemic to American deserts (Ashburner, 1989). However, several species of the *D. virilis* group reside in riparian habitats that should not necessarily maintain xeric adaptations. Without a more extensive phylogenetic analysis, the origin of the anti-desiccation performance in flying *D. virilis* remains unknown.

Metabolic water production

At 0 % relative humidity, fruit flies face an enormous desiccation stress because the partial pressure difference for water vapor is maximal. Insects have several pathways for replenishing lost water, including drinking, water absorption from atmospheric air and metabolic water production. Water absorption through the cuticle can be excluded if relative humidity drops below roughly 45 % (Beament, 1961; Hadley, 1994). However, to draw a complete picture of total water balance in our flies, we must estimate metabolic water production on the basis of food oxidation.

Within the Diptera, glycogen is the primary source of fuel during flight (for a review, see Ziegler, 1985). The amount of water formed during the oxidation of glycogen is $0.56\,mg\,H_2O\,mg^{-1}$ glycogen (Schmidt-Nielsen, 1997). We estimated metabolic water production during rest and flight on the basis of glycogen consumption from our CO_2 measurements (respiratory quotient, RQ=1) using a conversion factor of $1.19\,mg\,glycogen\,ml^{-1}\,CO_2$ (Schmidt-Nielsen, 1997). Under hovering conditions, fruit flies produce approximately $21.6\pm3.4\,\mu l\,metabolic\,H_2O\,g^{-1}\,body\,mass\,h^{-1}$ (N=4 species). Compared with the net rate at which the flies lose water during flight ($67.3\,\mu l\,g^{-1}\,h^{-1}$), metabolic water compensates for on average 41.7±22.2 % of total water loss (N=4 species, Fig. 7B). This value varies somewhat among the four species (*D. nikananu*, 28.3±9.7 %, N=4; *D. melanogaster*, 23.4±4.8 %, N=25; *D. virilis*, 72.8±13.7 %, N=6; *D. mimica*, 42.4±3.9 %, N=4), suggesting that flying *Drosophila* spp. replace a

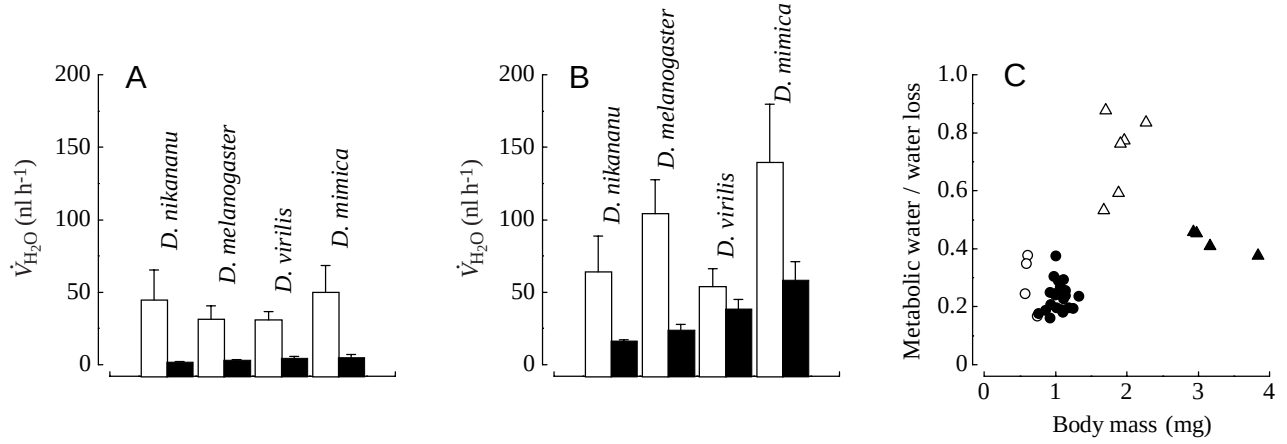

Fig. 7. Rates of water loss and metabolic water production (A) in resting fruit flies and (B) during hovering flight. Open columns, net rate of water loss; filled columns, net production of metabolic water calculated on the basis of carbohydrate combustion. Values are means + S.D. (C) Metabolic water production expressed as a fraction of water loss during flight. Symbols are as in Fig. 6.

significant amount of lost bulk water by metabolic water. For comparison, other insects can completely replace evaporative water loss by metabolic water production during flight under similar environmental conditions. Water-loaded honeybee (*Apis mellifera*) workers lose 79.7 µl water g^{-1} h^{-1} but produce 74.4 µl metabolic water g^{-1} h^{-1}, and flying bees (*Centris pallida*) completely replace 81.6 µl evaporative water g^{-1} h^{-1} by metabolic water at an ambient temperature of approximately 32 °C (Louw and Hadley, 1985; Roberts et al., 1998). In male bumblebees (*Bombus lucorum*) flying at an ambient temperature of 20 °C, metabolic water production is 12.4 µl g^{-1} h^{-1} greater than evaporative water loss (Bertsch, 1984). In resting fruit flies, metabolic water production has a small effect on total water balance. On the basis of the data shown in Fig. 3, metabolic water production compensates for only 8 % of total water loss (*N*=4 species, *D. nikananu*, 2.9 %; *D. melanogaster*, 8.6 %; *D. virilis*, 12.1 %; *D. mimica*, 7.5 %; Fig. 7A). The difference in the ratio of metabolic water production to water loss between resting and flying animals demonstrates that in resting flies spiracular water loss can only explain a small part of total water loss.

Because of metabolic water production, it has been suggested that the carbohydrate content of an insect facing desiccation stress might be considered as an important source of energy and water (Graves et al., 1992). Evidence for this hypothesis comes from selection studies in *D. melanogaster* in which the content of carbohydrates is significantly increased in desiccation-selected lines compared with unselected control lines (Graves et al., 1992; Djawdan et al., 1998). However, since total water content has also increased in these flies, a high level of carbohydrates might result from low locomotor activity rather than representing an active mechanism *per se* (Gibbs et al., 1997). Moreover, if it costs the fly more water to have its spiracles open to metabolize sugar than the animal gets back by metabolic water production, an increase in carbohydrate content cannot be considered as a strategy to increase tolerance to desiccation stress. Another potential role for glycogen in desiccation tolerance, however, is its ability to bind water. Glycogen typically binds 3–5 times its mass in water (Schmidt-Nielsen, 1997), and a high level of glycogen might thus serve as a mechanism by which fruit flies prevent water from being lost through the cuticle or other pathways.

Scaling of carbon dioxide release and water loss rate

Comparative studies on different-sized spiders, ticks and ants suggest scaling exponents for mass-specific resting metabolic rate in invertebrates ranging from –0.067 to –0.232 (Anderson, 1970; Anderson and Prestwich, 1982; Greenstone and Bennett, 1980; Lighton and Wehner, 1993). The difficulty with assessing the effects of scaling on respiration and transpiration between different groups of insects is that changes in body size are mostly accompanied by changes in the insect's respiratory system, including changes in ventilation pattern, spiracle control or other physiological and morphological modifications. This problem can be partly circumvented by comparing closely related species such as *Drosophila* spp., whose bodies are morphologically similar over a large size range (M. H. Dickinson, unpublished data). We assume here that the geometry of the tracheal system follows the outer body measures and scales in proportion to body size.

In resting fruit flies, mass-specific metabolic rate shows a tendency to decrease with decreasing body size with an allometric exponent –0.52 (Fig. 3A). This value is slightly smaller than the scaling exponents for other invertebrates mentioned above, but greater than the resting value of –0.25 obtained for warm-blooded vertebrates. In contrast, CO_2 release during flight, measured under hovering conditions, falls according to a mass-specific allometric exponent –0.22 (Fig. 6B). For comparison, Casey (1989) has summarized mass-specific energy expenditures in various taxa of freely hovering insects. Scaling exponents for mass-specific rates are scattered around –0.36 and are –0.20 in *Manduca sexta*, –0.31 in sphinx moths, –0.31 for saturniid moths and a variety of species from several different families, –0.38 for bumblebees, –0.44 for euglossine bees and –0.51 for *Hyles lineata*. These insects cover at least a 10- to 15-fold range in body mass. It is therefore surprising that the linear relationship between energy metabolism and body surface area was also found in our small subset of fruit flies that covers only a fivefold range of body mass. The difference between the scaling values for $\dot{V}^*_{CO_2}$ in resting and flying animals remains unclear, but it may imply different physiological states of the animals during rest and forced locomotion.

In resting insects, mass-specific transpiration rates tend to decrease with increasing body size. The cockroach *Periplaneta americana*, with a large body mass of 1.03 g, yields low mass-specific water loss rates ranging from 1.0 to 2.1 µl g^{-1} body mass h^{-1} (Kestler, 1985; Treherne and Willmer, 1975). Large desert locusts, *Locusta migratoria* and *Romalea guttata*, and the large ant *Pogonomyrmex rugosus* lose water at similar rates of approximately 5.5, 15.4 and 3.8 µl g^{-1} h^{-1}, respectively (Hadley and Quinlan, 1993; Lighton et al., 1993b; Loveridge, 1968a; Weis-Fogh, 1967). A 79.7 mg honeybee loses 19 µl water g^{-1} h^{-1} at rest (Louw and Hadley, 1985). Fig. 3B shows that the four *Drosophila* spp. follow this overall trend within a fivefold range of body size. Mass-specific resting $\dot{V}_{H_2O}$ ($\dot{V}^*_{H_2O}$) shows a tendency to decrease with a scaling exponent –1.03, suggesting that absolute water loss rate is independent of body size and that smaller flies therefore face desiccation stress sooner than do their larger relatives. This scaling exponent is larger than the values of –0.33 predicted for cuticular water loss according to the surface-to-volume ratio model and –0.67 predicted for spiracular water loss according to the isometric model of respiratory gas exchange by Kestler (1985), who suggests that high cuticular water loss rates in small animals might be explained by their thin integument. If small insects possess proportionally thinner cuticle than larger animals, an increase in water loss through the cuticle with decreasing body mass might partly explain the low allometric exponent found in the present study.

During flight, $\dot{V}^*_{H_2O}$ shows a tendency to decrease with

increasing body size with an exponent of −0.88, a value that is slightly smaller than the comparable exponent for resting values. In contrast to resting flies, cuticular water loss cannot explain the high scaling exponent, because in flying animals $\dot{V}^*_{H_2O}$ represents net rates calculated by subtracting resting values. Assuming that the fly loses most of its water through the open spiracles, the isometric model of spiracular transpiration predicts that $\dot{V}^*_{H_2O}$ should fall in proportion to body mass with an exponent of −0.67 (Kestler, 1985). The difference between the predicted exponent and the value found in the present study might result from the increase in power requirements for flight indicated by the scaling exponent of −0.22 for mass-specific CO_2 release (Fig. 6B). This exponent implies that small flies might increase their total spiracle opening area to match the geometry of the diffusive path to the increased requirements for oxygen during flight. On the one hand, this behavior augments exchange rates of respiratory gases; on the other hand, it also permits water to evaporate more quickly through the tracheal system. To test more explicitly whether an increase in spiracle opening area can explain the high scaling exponent for $\dot{V}^*_{H_2O}$ during flight, we have determined how the ratio of $\dot{V}^*_{H_2O}$ to $\dot{V}^*_{CO_2}$ scales with body mass. This ratio shows a tendency to decrease in proportion to body mass with an exponent of −0.73±0.39 ($\dot{V}^*_{H_2O}/\dot{V}^*_{CO_2}=2.3\times10^{-3}m_{wet}^{-0.73}$, $r^2=0.42$, $P=0.36$, $N=4$ species), which is close to the exponent of −0.67 according to the isometric model of spiracular transpiration.

In conclusion, this comparative study on insect respiration has provided new insights into how carbon dioxide release and water loss scale with body size. In contrast to their larger relatives, small flies face not only larger mass-specific energetic expenditures during flight but also a tremendous risk of desiccation. Besides cuticular and spiracular water loss, total water balance in fruit flies is further affected by loss of water through the proboscis or feces and by extensive metabolic water production as a result of carbohydrate combustion. It is clear, however, that fruit flies may use many other strategies to avoid desiccation, including habitat choice, alterations in locomotor activity pattern, changes in cuticle composition, more effective control of spiracle activity or changes in breathing behavior. To compare the strategies by which fruit flies limit their water loss during different types of locomotion, we are currently investigating possible changes in breathing pattern and water loss rates in four species of *Drosophila* during terrestrial locomotion.

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