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Evolution of water conservation mechanisms in *Drosophila*

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Summary

Flies of the genus *Drosophila* inhabit a wide range of habitats, from the tropics to deserts to boreal forests. The primary physiological mechanism allowing *Drosophila* and other insects to survive in arid habitats is a reduction in rates of water loss. To understand mechanisms of water retention in greater detail, we investigated the three main routes by which *Drosophila* lose water: excretion, cuticular transpiration and respiratory loss through the spiracles. Excretory losses comprised <6% of total water flux and did not differ between xeric (cactophilic) and mesic species. No consistent relationship was observed between water-loss rates and the composition, physical properties or amounts of cuticular hydrocarbons, suggesting that cuticular transpiration did not differ among species from different habitats. Metabolic rates and water-loss rates were highly correlated. Cactophilic *Drosophila* were less active, and female cactophiles had lower metabolic rates than female mesic species of the same size. They were also more likely to exhibit a pattern of cyclic CO_2 release that may help to conserve water. We conclude that lower overall rates of water loss are achieved primarily by reduction of respiratory losses.

Key words: cuticular lipids, discontinuous ventilation, *Drosophila*, metabolic rate, water loss.

Introduction

Insects and other terrestrial arthropods are susceptible to water loss because of their small size, and these problems are exacerbated in the hot, dry conditions typical of deserts. In principle, desert arthropods can use one or more of three physiological mechanisms to survive water stress. First, desert insects may exhibit reduced rates of water loss relative to mesic species (Hadley, 1994a). Second, they can store large quantities of water in the form of bulk water or potential sources of metabolic water (glycogen and lipid; Gibbs et al., 1997). Third, they can tolerate the loss of a relatively large fraction of their body water; in the extreme, certain chironomid larvae can survive extended periods in an anhydrobiotic state (Hinton, 1960).

Comparative studies have found little consistent evidence that desert insects store more water or are able to tolerate lower water levels (Hadley, 1994a). The species with the highest water contents are mesic insects. Although some arid-adapted insects are able to tolerate loss of >50% of their water content (Zachariassen et al., 1987; Hadley, 1994a), they are not consistently better at surviving dehydration stress. Indeed, the species able to tolerate loss of the greatest fraction of its water is an aquatic beetle, *Peltodytes aquaticus* (Arlian and Staiger, 1979), although it should be noted that this species contains relatively large amounts of water to start with.

The most consistent difference between arid-adapted and mesic arthropods is that the former species lose water relatively slowly. This pattern has been documented for scorpions (Toolson and Hadley, 1977), spiders (Hadley et al., 1981), beetles (Hadley and Schultz, 1987; Zachariassen et al., 1987), ants (Hood and Tschinkel, 1990; Johnson, 2000) and fruitflies (Eckstrand and Richardson, 1980, 1981; Gibbs and Matzkin, 2001). A few water-profligate desert insects use evaporative cooling for thermoregulation, but these exceptional species have access to large quantities of water (e.g. xylem-feeding cicadas; Toolson, 1987).

Drosophila species occur in a wide range of habitats, including deserts, and differ in their ability to survive desiccation stress (van Herrewege and David, 1997; Hoffmann and Harshman, 1999; Gibbs and Markow, 2001; Gibbs and Matzkin, 2001). We have previously shown that enhanced desiccation resistance in cactophilic *Drosophila* from North American deserts is the result of reduced rates of water loss (Gibbs and Matzkin, 2001). In the present study, we examine the mechanistic basis for reduced water-loss rates. *Drosophila* lose water by three routes: excretion from the mouthparts and anus, transpiration through the cuticle, and respiratory losses by evaporation through open spiracles. (Females will also lose water in their eggs, but not under the conditions of our experiments.) We directly quantified total water loss and excretory water loss using flow-through respirometry.

Differences in cuticular lipids (composition, physical properties and amounts) were assayed as a proxy for cuticular transpiration. Metabolic rates, activity and ventilatory patterns were examined to assess the importance of respiratory water loss.

Materials and methods

Fruitfly species and maintenance

Fig. 1 depicts the phylogeny of the species used for measurements of metabolic rates, based on several sources (Russo et al., 1995; Pitnick et al., 1999; Durando et al., 2000; S. Perlman, personal communication). Collection information for most stocks is provided in Gibbs and Matzkin (2001). *Drosophila virilis* was provided by L. L. Jackson, and S. Perlman provided several mushroom-inhabiting species (see Table 1) collected in southeastern USA in 2001.

All flies were maintained on the laboratory photoperiod (approximately 12 h:12 h light:dark) at 24°C, which was the temperature used for all assays. Most species were reared in milk bottles on corn-meal medium, *Drosophila busckii* was reared on Wheeler–Clayton medium (Carson, 1987), and the cactophilic species were reared on banana medium containing powdered *Opuntia* cactus. A small piece of the host cactus was provided to cactophilic *Drosophila*, if necessary, to stimulate egg laying. Flies used in assays were collected and separated by sex within several hours of emergence to ensure that they were virgin. They were held in vials containing corn-meal medium and live yeast for 6–10 days, then the sexes were assayed separately.

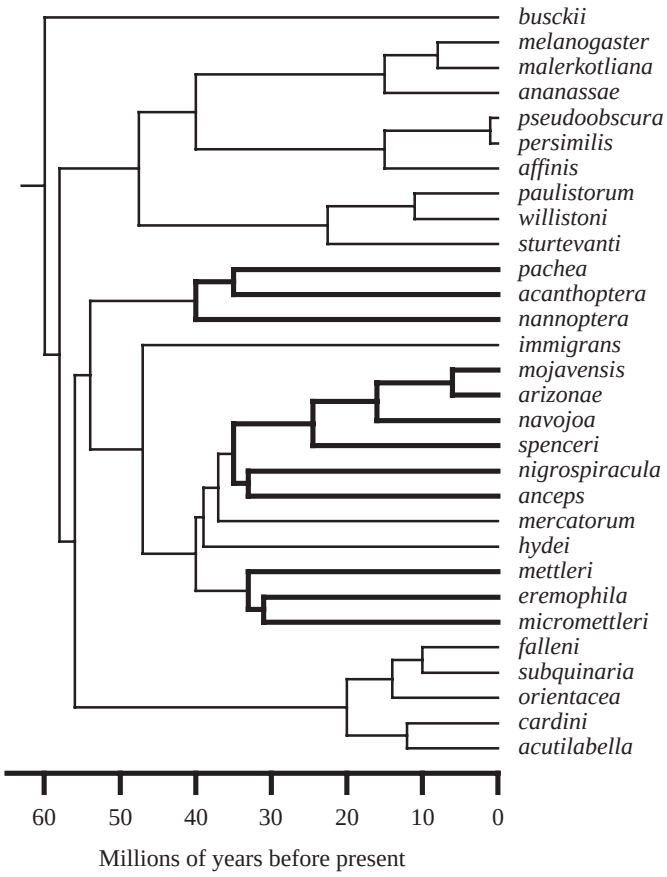

Fig. 1. Phylogeny of *Drosophila* species used for measurements of metabolic and water-loss rates. Cactophilic species are indicated by thick lines. Additional species were used in analyses of cuticular hydrocarbons (Table 1).

Respirometry

Metabolic rates and rates of water loss were measured using flow-through respirometry. Groups of 10–20 flies were placed in 5 ml glass–aluminum chambers, which were then placed in a Sable Systems (Las Vegas, NV, USA) TR-2 respirometer. Rates of carbon dioxide release and water loss were measured with a Li-Cor (Lincoln, NE, USA) LI-6262 infrared CO_2 and water-vapor sensor. Most data for total water-loss rates were reported previously (Gibbs and Matzkin, 2001); additional species are included here. Metabolic rates of individual flies were measured using the same conditions, except that the chambers were only 1 ml in volume.

Cuticular lipids

We isolated cuticular hydrocarbons (HCs) from 18 species and examined their composition and physical properties [melting points (T_m)]. Groups of 10–40 flies were placed on a silica gel column in a Pasteur pipette (Toolson, 1982), and HCs were eluted with approximately 7 ml of HPLC-grade hexane. The solvent was evaporated, and the lipid extracts were frozen until analysis. In some cases, we quantified lipid amounts by adding 2 µg of *n*-icosane in a small volume of hexane to the flies at the beginning of the column chromatography procedure, as an internal standard.

We measured lipid T_m using Fourier transform infrared (FTIR) spectroscopy (Gibbs and Crowe, 1991). Lipid extracts were dissolved in approximately 25 µl hexane and deposited on CaF_2 windows. After the solvent evaporated, samples were placed in a temperature-controlled cell holder in the spectrometer. The sample temperature was increased from <10°C to >50°C in 2°C increments. We followed the progress of lipid melting from the frequency of -CH_2- symmetric stretching vibrations, which increase from approximately 2849 cm^{-1} to approximately 2854 cm^{-1} as lipids melt. The midpoint of the phase transition (T_m) was calculated from logistic equations fitted to temperature-frequency plots.

We studied HC composition using a Hewlett-Packard (Palo Alto, CA, USA) 5890A gas chromatograph (GC) equipped with a DB-1 or DB-5 capillary column (30 m×0.32 mm i.d.; JW Scientific, Sacramento, CA, USA). Peaks were identified by comparison with the retention times of *n*-alkane standards and by reference to literature data on HC composition. For some species, we confirmed the identities of HCs by GC–mass spectrometry (MS) using a Hewlett-Packard 5988 GC–MS system at the Mass Spectroscopy Laboratory of the University of Arizona. Lipid amounts were determined by comparing peak areas with those for the *n*-icosane standard.

Activity

We used AD-1 activity meters from Sable Systems to assess the relative activity of individual flies. These use a near-infrared (900 nm) light source, which is reflected around the chamber to a detector. The chambers were covered with a thick dark cloth to minimize interference from room lighting. Movement was detected by fluctuations in the detector signal, with larger fluctuations generally corresponding to greater activity. Individual flies were placed in the same 5-ml chambers used for respirometry, with a flow rate of 100 ml min^{-1} dry air. Activity was recorded until at least 10 h after the mean desiccation survival time, as determined in previous experiments (Gibbs and Matzkin, 2001). With the exception of two cactophilic flies that survived for >48 h, all flies were dead at the end of the experimental runs. At least five individuals of each sex were assayed in each species.

Although the chambers were dark, a potential problem with these measurements is diurnal activity cycles. For each species, approximately half of the individuals were placed in the chambers at approximately 07.00 h local time, and the other half at approximately 18.00 h. Activity patterns were similar in both groups, suggesting that internal rhythms did not affect our results.

Data analysis

Statistical analyses were performed using Excel and JMP (SAS Institute) software. To control for phylogenetic relationships, we used the Phylogenetic Diversity Analysis Package (Garland et al., 1992) to implement Felsenstein's method of independent contrasts (Felsenstein, 1985). Because the species differed in body size, we regressed physiological variables (e.g. metabolic rate) against mass and used the residuals from these regressions in our phylogenetic analyses. For habitat comparisons, species were classified as either cactophilic (xeric) or mesic. Although not all cactophiles live in deserts, they and their host plants tend to inhabit dry regions. Mesic species can sometimes be found in deserts but are less desiccation resistant than cactophiles (Gibbs and Matzkin, 2001). Thus, the cactophile/non-cactophile distinction is reasonable, both ecologically and physiologically.

Results

Excretory water loss

Fig. 2 depicts a typical recording of water loss from a group of 16 female *Drosophila mercatorum*. These recordings revealed a baseline level of water loss, punctuated by intermittent bursts of water release. The bursts corresponded to excretory loss, probably by defecation. Total excretory losses were calculated by integrating the areas of these peaks. We counted the peaks to estimate how often flies defecated. Fig. 3 depicts the mean fecal water contents and defecation rates for 28 species. Flies from both mesic and arid environments defecated approximately once every 1–1.5 h. Surprisingly, xeric females tended to defecate more often than mesic females (*t*-tests, P=0.05 for females, P>0.5 for males),

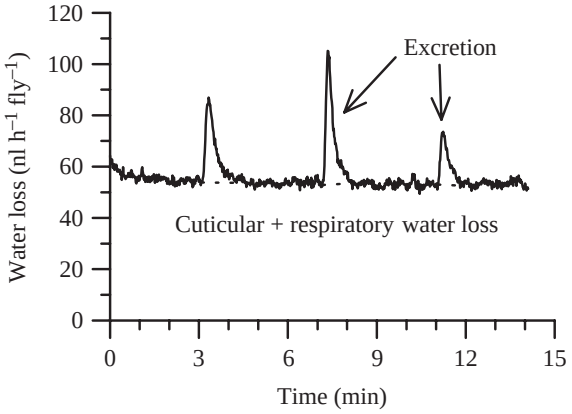

Fig. 2. Representative water-loss recording from a group of 16 female *Drosophila mercatorum*. Excretory losses were calculated by integrating the areas of intermittent water-loss peaks, which reflect defecation or oral losses.

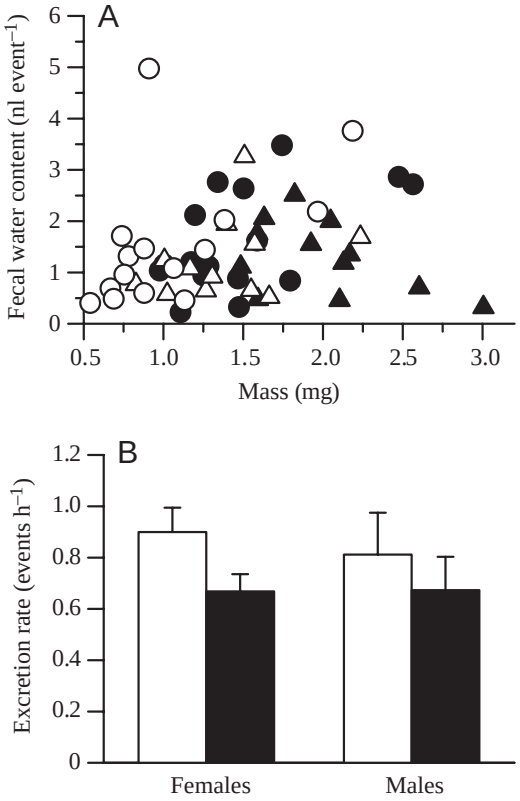

Fig. 3. Excretory water loss from *Drosophila* species. (A) Effects of body size on fecal water content. Filled symbols, females; open symbols, males. Circles, mesic species; triangles, cactophilic species. (B) Excretion rates of xeric (open bars; means ± S.E.M.) and mesic (filled bars) *Drosophila*.

but the total volume of water lost *via* excretion did not differ between these groups (ANCOVA, P>0.1 for both sexes). Most importantly, the percentage of water lost by excretion, relative to total water loss, averaged <6%. Because excretory water loss was so low and did not differ between desert and mesic species,

it could not have accounted for the large differences in total water-loss rates that we have reported previously (Gibbs and Matzkin, 2001).

Cuticular lipids

Melting points (T_m) of cuticular lipids are provided in Table 1. No differences between males and females were detected. The highest T_m occurred in mycophagous flies, which inhabit very moist forests. Species from the subgenus Sophophora tended to have the lowest T_m values, with cactophilic species being intermediate. Thus, a major determinant of T_m appeared to be phylogeny rather than habitat. To examine the relationship between T_m and water-loss rates, we first calculated residuals of water-loss rate as a function of mass for 11 species for which we also had T_m data. We then calculated phylogenetically independent contrasts (Felsenstein, 1985) for the residuals and T_m values. These were not significantly correlated (r^2=0.044, P=0.39), indicating that high T_m did not tend to reduce water loss.

Differences in T_m could result from changes in HC chain lengths, unsaturation or branching patterns. Alkenes are the major HC constituents of most *Drosophila* species studied to date, with branched alkanes being the next most abundant class (Bartelt et al., 1986; Jallon and David, 1987; Toolson et al., 1990). We did not obtain detailed structural information on HC composition, but Table 1 reveals that species with longer chain lengths tended to have higher T_m. With the exception of *D. virilis*, sophophorans had consistently shorter chain lengths than members of the subgenus Drosophila. In addition, a limited survey (seven species, including two cactophiles) indicated that flies from mesic and arid habitats had similar quantities of surface lipids for their size (not shown). Thus, desert *Drosophila* did not reduce cuticular permeability by increasing the thickness of the lipid barrier.

Table 1. *Melting points (T_m) and chain lengths of cuticular hydrocarbons isolated from* Drosophila *species*

Taxon	Habitat	T_m (N)	Chain length
Subgenus Sophophora			
melanogaster	Cosmopolitan	29.2±1.3 (17)	23–31
pseudoobscura	Temperate	24.7±1.1 (13)	25–31
subobscura	Temperate	27.3±1.3 (16)	23–31
willistoni	Tropical	34.4±1.7 (6)	ND
Subgenus Drosophila			
Virilis group			
virilis	Cosmopolitan	34.5±1.1 (6)	21–31
Repleta group			
arizonae	Cactophilic	32.8±1.1 (13)	29–39
hydei	Cosmopolitan	34.0±0.6 (12)	29–39
mercatorum	Mesic	32.2±0.5 (5)	ND
mojavensis	Cactophilic	36.0±1.0 (6)	29–39
nigrospiracula	Cactophilic	33.9 (2)	29–39
Cardini group			
acutilabella	Mycophilic	36.9±2.0 (6)	29–39
cardini	Mesic	37.0 (2)	ND
Quinaria group			
falleni	Mycophilic	40.4±3.0 (4)	27–37
guttifera	Mycophilic	41.2±2.6 (4)	29–37
recens	Mycophilic	40.7±1.8 (4)	27–33
subpalustris	Mesic	40.6±4.2 (3)	27–37
Testacea group			
neotestacea	Mycophilic	40.6±2.1 (4)	27–37
putrida	Mycophilic	41.8±2.3 (4)	29–37

Values are means ± S.E.M. No differences between males and females were detected, so T_m data for both sexes were pooled. ND, no data.

Metabolic rates

Fig. 4 depicts the effects of body size on metabolic rates of 30 *Drosophila* species. Metabolic rates of female flies were over 50% higher in mesic species than in cactophilic species (ANCOVA, P<0.012; adjusted least-squares means were 4.84 µl CO_2 h^{-1} for mesic species and 3.08 µl CO_2 h^{-1} for cactophiles). By contrast, metabolic rates of males did not differ as a function of habitat (ANCOVA, P>0.3). To examine further the relationship between metabolic rates and water-loss rates, both of which were positively correlated with body size [ANCOVA, P<0.04 for metabolic rates of both sexes; see Gibbs and Matzkin (2001) for analysis of water-loss rates], we first calculated the residuals of these parameters when plotted as a function of mass. The residuals were positively correlated

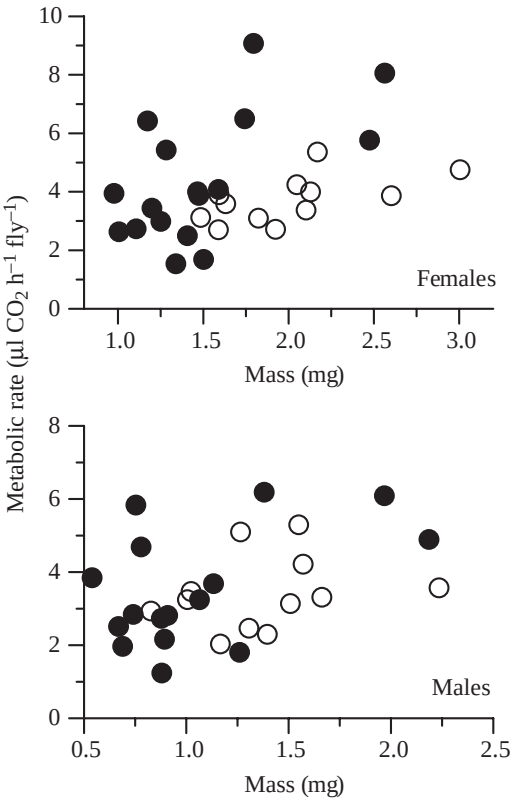

Fig. 4. Effects of size on metabolic rates of mesic and desert *Drosophila*. Filled symbols, mesic species; open symbols, cactophilic species.

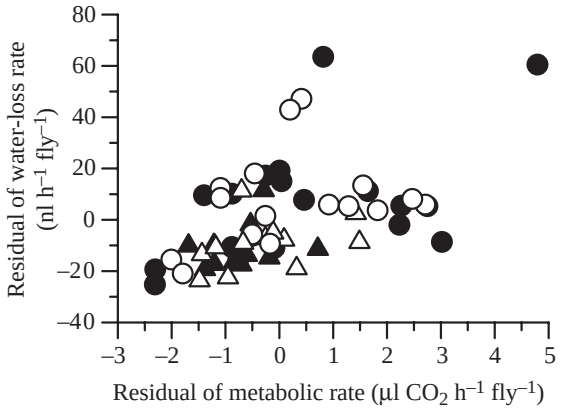

Fig. 5. Correlation between metabolic rates and water-loss rates of *Drosophila* species, after correction for body size. Filled symbols, females; open symbols, males. Circles, mesic species; triangles, cactophilic species.

in both sexes (Fig. 5; r=0.57, P<0.001 for females; r=0.39, P<0.04 for males).

Because the cactophilic species tended to be closely related (Fig. 1), we also analyzed the relationship between metabolic rate and water-loss rate using phylogenetically independent contrasts (Felsenstein, 1985). After controlling for phylogeny, positive correlations between residuals were still detected, albeit with reduced statistical significance (Fig. 6; P=0.008 for females, P=0.093 for males).

We also analyzed the relationship between metabolic rates and water-loss rates separately for cactophilic and mesic species, an approach similar to that taken previously (Zachariassen et al., 1987; Zachariassen, 1996; Addo-Bediako et al., 2001). Using simple ANCOVA, a marginally significant correlation was detected only for mesic females (P=0.073; P>0.25 for mesic males and for cactophilic flies of both sexes).

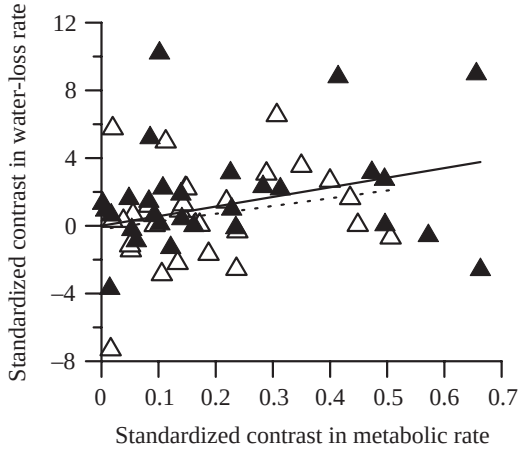

Fig. 6. Relationship between metabolic rates and water-loss rates of *Drosophila* species, after controlling for evolutionary history using phylogenetically independent contrasts (Felsenstein, 1985). Filled symbols and solid line, females (P=0.008); open symbols and broken line, males (P=0.093).

This correlation disappeared after phylogenetic relationships were taken into account using independent contrasts (P>0.2 for all four sex-habitat combinations).

Activity

Fig. 7 depicts representative activity recordings for a mesic fly (*Drosophila melanogaster*) and a desert fly (*Drosophila mojavensis*). The overall activity patterns for these two flies are shown in Fig. 8. The *D. melanogaster* individual was active almost continuously for 6 h, then exhibited no signs of activity thereafter. Presumably, this fly died at 6–7 h. The *D. mojavensis* individual was inactive for the first 12 h of the experiment, then became active for the next 14 h. The lack of activity after approximately 26 h suggests that the fly died of desiccation stress at this time.

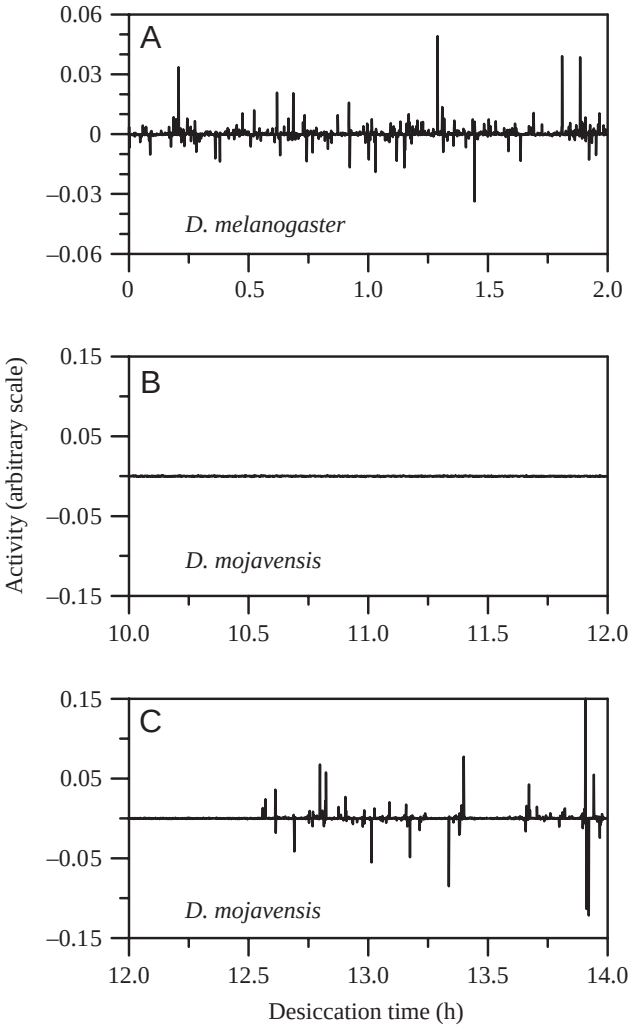

Fig. 7. Representative recordings of activity in individual *Drosophila*. Abscissa labels indicate the amount of time each fly had been in the activity chamber. Panels B and C depict consecutive recordings from the same individual. Note that activity recorders differ in their sensitivities, and their output may also be affected by the size of the fly. Thus, the scales only indicate relative activity and cannot be directly compared for different individuals.

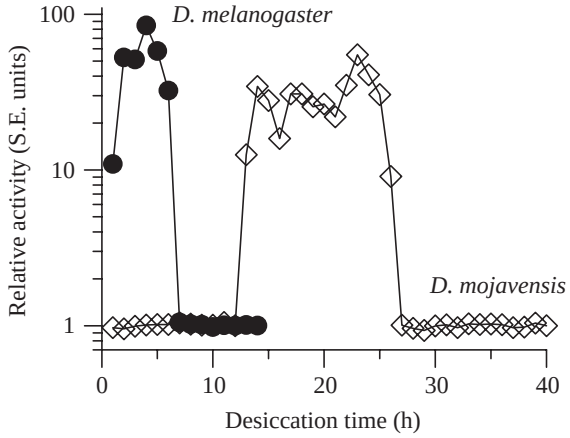

Fig. 8. Overall activity patterns for the same two flies as in Fig. 7. Values are standard errors of regression lines through the data, calculated for each hour of the recording. Values are expressed relative to the S.E.M. values calculated after the flies had ceased movement (i.e. relative to detector noise).

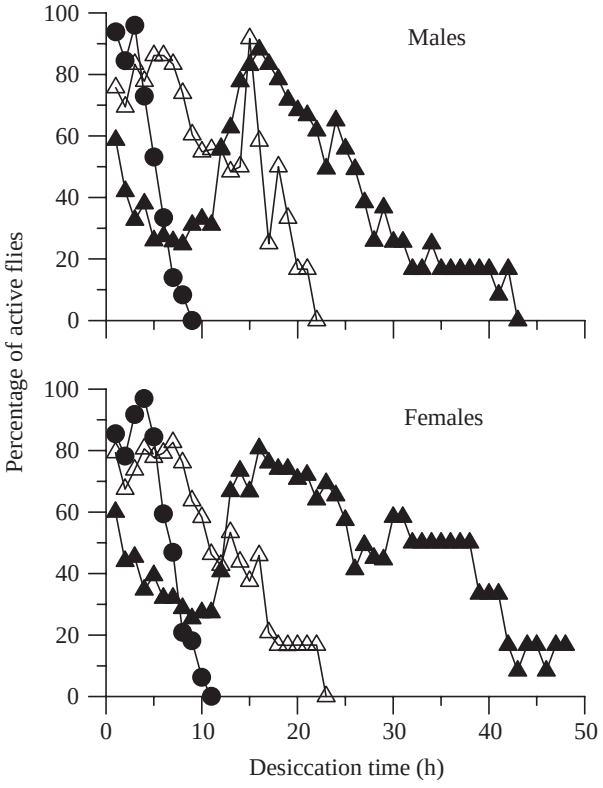

Fig. 9. Activity patterns for *Drosophila* species, grouped by their abilities to survive desiccation stress (circles, <10 h; open triangles, 10–24 h; filled triangles, >24 h). Individual flies were classified as either active or inactive for each hour of the recordings, and the percentage of active flies was calculated for each species. Flies were assumed dead and removed from the analysis after their last active period. Data are means of 4–6 species per category, with 5–10 individuals assayed for each sex from each species.

Similar activity patterns were observed in other species. In Fig. 9, we have placed 15 species into three groups, depending upon their ability to survive desiccation stress (Gibbs and Matzkin, 2001). Most individuals of the first group (four species with an average survival time of <10 h) were active almost continuously until their deaths. In the second group (six species that survived for 10–24 h), approximately 80% of flies were active up to approximately 8 h, then activity decreased as flies became dehydrated. The third group included four cactophilic species and *Drosophila cardini*, species for which the average survival was >24 h. Except for the first hour after being placed in their chambers, most of these flies were inactive for at least 10 h, then became active for ≥15 hours (Fig. 9). Although our activity meters could not quantify the actual amount of activity (e.g. distance traveled), these data indicate that lower initial activity levels were associated with greater desiccation resistance.

Ventilatory patterns

Discontinuous gas-exchange cycles have been observed in numerous arthropod taxa and can reduce respiratory water loss (Lighton, 1994, 1996). We examined CO_2-release patterns in at least 17 females each from six *Drosophila* species. We used

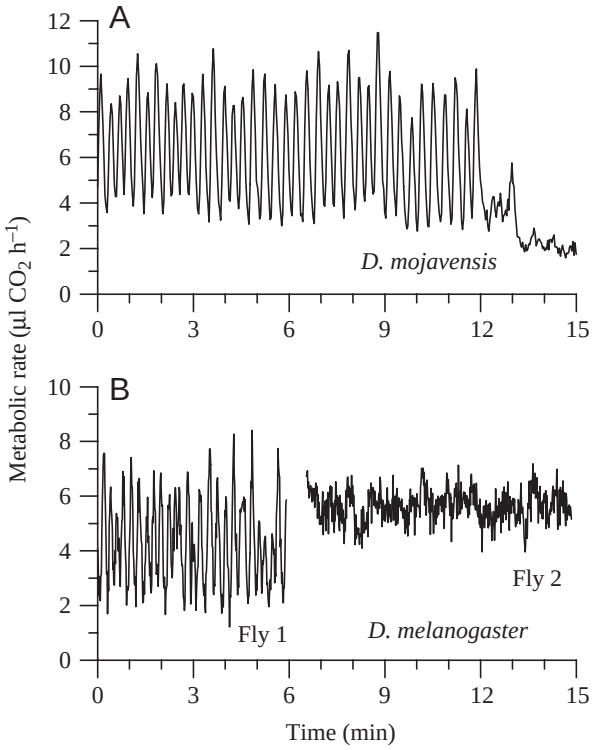

Fig. 10. Carbon dioxide release from individual *Drosophila*. (A) Cyclic CO_2 release by a single *Drosophila mojavensis*. This individual walked around its chamber during the first 12 min of the recording, then stopped moving for the last three minutes. (B) Representative recordings from two *Drosophila melanogaster*. The first individual performed cyclic CO_2 release, whereas the second one did not.

Table 2. *Cyclic ventilation in* Drosophila *species*

Species	Fraction performing cyclic ventilation
D. melanogaster	8/30
D. simulans	9/24
D. subobscura	2/18
D. willistoni	6/27
D. mojavensis	19/21
D. arizonae	15/17

females because they are larger than males and have higher metabolic rates. In most cases, CO_2 was released continuously, with no evidence of spiracular control. A striking exception was provided by the two cactophilic species examined; *Drosophila arizonae* and *D. mojavensis*. Approximately 90% of individuals from both species often released CO_2 in cyclic bursts (Fig. 10). Although we observed periods of cyclic CO_2 release in other species, these were relatively rare and brief (Table 2).

The pattern of cyclic ventilation also appeared to differ between mesic and cactophilic species. In mesic species, metabolic rates during cyclic and aperiodic periods were similar for a given individual. By contrast, cyclic ventilation in cactophilic *Drosophila* was associated with a marked increase in metabolic rate (Fig. 10A). Direct observations indicated that cyclic release occurred only when desert flies were active, whereas no such pattern was apparent for mesic species. It should be noted that CO_2 release never fell to zero, suggesting that the spiracles were never completely closed or that at least one remained open at all times. Individuals from all species were observed to switch back and forth between cyclic and acyclic CO_2 release.

Discussion

Desert organisms can survive desiccating conditions by one or more of three mechanisms: water storage, water conservation and dehydration tolerance. *Drosophila* from different habitats have similar water contents and are equally tolerant of low water content (van Herrewege and David, 1997; Gibbs and Matzkin, 2001). Thus, cactophilic species are more resistant to desiccation than are mesic species solely because they lose water less rapidly.

A potential confounding factor in our experiments is adaptation to laboratory culture. The stress resistance of *Drosophila* populations may change over time (Hoffmann et al., 2001) as a result of drift or relaxed selection. In our case, we performed most of our experiments within 2–3 years of collection, when the populations would have had little time to change. We also note that, even after 15 years in culture, the cactophilic species *Drosophila navojoa* remains more resistant to desiccation than non-cactophilic species (Gibbs and Matzkin, 2001). Thus, differences between mesic and xeric species appear to be robust.

Drosophila lose water by three major routes (excretion, cuticular transpiration and *via* the spiracles). Excretory water loss accounted for <6% of total losses in both mesic and desert species and did not differ between these groups (Fig. 3). Thus, reduced excretion cannot account for the greater desiccation resistance of desert species. This leaves cuticular and respiratory routes as the foci of desiccation resistance.

Cuticular lipids

Distinguishing between cuticular and respiratory water losses is difficult in any insect but particularly so in those as small as *Drosophila*. We therefore must use indirect evidence. Surface lipids provide the primary barrier to cuticular transpiration, so differences in the composition or amounts of these should affect rates of water loss. Lipid melting points (T_m) provide one indicator of water-proofing ability, as solid-phase lipids are less permeable than melted ones (Rourke and Gibbs, 1999). Thus, one would predict an inverse relationship between water loss and T_m. Structural differences that increase T_m, such as longer chain lengths and reduced unsaturation or methylbranching, should also be more prevalent in desert species.

Our GC analyses (Table 1), in combination with literature data on HC composition (Table 3), indicate no consistent relationship between chain length and habitat. Cactophilic and mycophilic members of the subgenus Drosophila had the longest-chain surface lipids (29–39 carbons), despite living in habitats that are the most distinct from each other. Other members of Drosophila, and all members of the subgenus Sophophora, had relatively short-chain surface lipids (23–33 carbons). Thus, phylogeny was a major factor affecting HC composition.

Chain lengths are not the only factors determining HC melting points; indeed, unsaturation and methylbranching have much greater effects on T_m (Gibbs and Pomonis, 1995). We did not perform detailed analyses of HC composition but were able to measure T_m values in species from a variety of habitats (Table 1). Lipids of species in the subgenus Drosophila, including mycophilic species from cool moist forests, had longer chain lengths and melted above 32°C. By contrast, sophophorans generally had short-chain, low-T_m HCs. Neither T_m nor lipid amounts were correlated with habitat or water-loss rates. It must be noted, however, that cuticular HCs also have a second important function as contact sex-recognition pheromones (Antony and Jallon, 1982; Markow and Toolson, 1990; Tompkins et al., 1993; Nemoto et al., 1994). Selection for reproductive success may have limited the evolution of better cuticular waterproofing.

In summary, none of the expected relationships between water-loss rates and cuticular lipids was detected. Similar conclusions have been obtained using thermally acclimated *D. mojavensis* (Gibbs et al., 1998) and desiccation-selected populations of *D. melanogaster* (Gibbs et al., 1997). Although lipid analyses can provide only an indirect indication, desert *Drosophila* did not appear to have reduced cuticular permeability relative to mesic species. This is not to say that cuticular transpiration was a minor component of the flies'

Table 3. *Literature data for cuticular lipids of* Drosophila *species*

Taxon	Chain length	References
Subgenus Sophophora		
Melanogaster subgroup		
melanogaster	23–33	1–3
melanogaster	23–29	4
Subgroup (7 species)		
erecta	23–33	4
Ananassae subgroup		
pallidosa	27–33	5
Obscura group		
persimilis	25–27	6
pseudoobscura	25–31	6–9
Subgenus Drosophila		
Repleta group		
arizonae	29–37	10,11
mettleri	29–37	10
mojavensis	29–37	10–12
nigrospiracula	27–37	10
Nannoptera group		
pachea	29–37	10
Virilis group		
virilis	21–31	13
11 species	21–31	14
Hawaiian picture-winged species		
adiastola	23–30	15
peniculipedis	21–27	15
setoimentum	21–27	15

Members of the Repleta and Nannoptera groups are cactophilic; other species are from mesic habitats.

References: 1, Jackson et al. (1981); 2, Antony and Jallon (1982); 3, Schaner et al. (1989); 4, Jallon and David (1987); 5, Nemoto et al. (1994); 6, Noor and Coyne (1996); 7, Toolson (1982); 8, Blomquist et al. (1985); 9, Toolson and Kuper-Simbron (1989); 10, Markow and Toolson (1990); 11, Stennett and Etges (1997); 12, Toolson et al. (1990); 13, Jackson and Bartelt (1986); 14, Bartelt et al. (1986); 15, Tompkins et al. (1993).

water budgets, only that it did not differ in any systematic manner among species from arid and mesic habitats. By elimination, the main mechanism by which desert fruitflies have reduced total water loss must have been by lowering respiratory losses through the spiracles.

Respiration and water loss

The significance of respiratory water loss in insects has become controversial in recent years (Hadley, 1994b; Lighton, 1996; Slama, 1999; Chown, 2002). Comparative studies have found a positive correlation between metabolic rates and water-loss rates in xeric, but not mesic, species (Zachariassen et al., 1987; Addo-Bediako et al., 2001). Reducing metabolic rates will help to conserve water by reducing the need for gas exchange, but this is not a sufficient condition; insects must also be able to regulate spiracular opening. Water-loss rates will remain high if the spiracles are open continuously, no matter how low the metabolic rate is. Unfortunately, spiracular regulation has been studied in only a limited set of species (Lighton, 1996). Several studies have compared water-loss rates when the spiracles are open and when they are closed. Respiratory water loss typically accounts for <10% of total losses (Quinlan and Hadley, 1993; Williams and Bradley, 1998), implying that cuticular transpiration must be the major loss route.

Other authors have argued that the relative importance of respiratory water loss is greater in xeric species because of greatly reduced cuticular permeability (Lighton et al., 1993; Zachariassen, 1996). Thus, a correlation between metabolic rate and water loss may be observed only in insects from arid environments, even when their metabolic rates are similar to those of mesic species (Zachariassen et al., 1987; Addo-Bediako et al., 2001). One difference between this work and ours is that our *Drosophila* species spanned only a fivefold range in body size, whereas Zachariassen et al. (1987) studied beetles differing by more than two orders of magnitude, and Addo-Bediako et al. (2001) used literature data spanning an even larger size range. We were therefore limited to a relatively narrow range of metabolic rates, which would have reduced our ability to detect habitat-related differences.

Another important difference between our experiments and previous work is that we used groups of flies and were unable to control for activity. We measured metabolic rates 2–5 h after flies had been placed in their respirometry chambers, at a time when cactophilic species tended to be much less active (Fig. 9). Our observations of individual flies suggested that metabolic rates increased more than twofold as a result of activity (Fig. 10). Thus, differences in locomotor activity can fully account for inter-specific differences in metabolic rates, and greater tracheal ventilation could have increased water loss. By contrast, Zachariassen et al. (1987) studied individual, inactive beetles, and most of the studies surveyed by Addo-Bediako et al. (2001) measured standard (resting) metabolic rates, so that activity-related water loss should not have been a factor.

Selection experiments have suggested that reduced locomotor activity and metabolic rate contribute to desiccation resistance (Hoffmann and Parsons, 1993). Direct measurements from individual *Drosophila* reveal that total water loss more than doubles as a consequence of tracheal ventilation during flight (Lehmann et al., 2000; Lehmann, 2001). Thus, respiratory losses may be very high in active flies. Unfortunately, water loss from individual flies in our experiments was so low that we could not measure it reliably against the background noise and drift in our respirometry system. In some cases, we noted that flies appeared to lose water more rapidly when they became active, but we were unable to quantify the increase with any confidence. Carbon dioxide readings never reached zero, indicating that at least one spiracle remained open at all times, which would also reduce our ability to distinguish respiratory losses. Thus, we were unable to reliably detect increases in water loss caused by

spiracular opening and therefore could not determine whether water loss from individual flies increased significantly when they became active.

We note that the cyclic CO_2 release we observed in desert *Drosophila* represents an unusual ventilatory pattern in insects. Discontinuous gas exchange in other species generally occurs when insects are quiescent (Lighton, 1996). The spiracles close completely on each cycle, as indicated by the cessation of CO_2 release. Carbon dioxide release in our experiments was cyclic but continuous. In addition, the minimal rate of CO_2 release during cyclic ventilation was often greater than that observed in inactive flies (Fig. 10). In these cases, cyclic CO_2 release may reflect abdominal pumping to aid convective gas exchange and oxygen delivery when demand is high (Weis-Fogh, 1964; Lehmann, 2001).

Summary

Water conservation is critical to the ecological success of desert *Drosophila*. We have been able to exclude one route for water loss – excretion – as a major component of overall water balance. We were unable to distinguish between cuticular transpiration and respiration as well as we would like, so our conclusions regarding these remain tentative. None of the expected differences in cuticular lipids was detected, whereas three parameters associated with respiratory water loss (metabolic rate, activity and spiracular control) differed between desert and mesic species. Thus, the overall evidence indicates that desert *Drosophila* conserve water by reducing its loss from the tracheal system.

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