The Journal of Experimental Biology 201, 71–80 (1998)
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JEB1104

EFFECTS OF TEMPERATURE ON CUTICULAR LIPIDS AND WATER BALANCE IN A DESERT *DROSOPHILA*: IS THERMAL ACCLIMATION BENEFICIAL?

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Accepted 16 October 1997; published on WWW 9 December 1997

Summary

The desert fruit fly *Drosophila mojavensis* experiences environmental conditions of high temperature and low humidity. To understand the physiological mechanisms allowing these small insects to survive in such stressful conditions, we studied the effects of thermal acclimation on cuticular lipids and rates of water loss of adult *D. mojavensis*. Mean hydrocarbon chain length increased at higher temperatures, but cuticular lipid melting temperature (T_m) did not. Lipid quantity doubled in the first 14 days of adult life, but was unaffected by acclimation temperature. Despite these changes in cuticular properties, organismal rates of water loss were unaffected by either acclimation temperature or age. Owing to the smaller body size of warm-acclimated flies, *D. mojavensis* reared for 14 days at 33 °C lost water more rapidly on a mass-specific basis than flies acclimated to 25 °C or 17 °C. Thus, apparently adaptive changes in cuticular lipids do not necessarily result in reduced rates of water loss. Avoidance of high temperatures and desiccating conditions is more likely to contribute to survival in nature than changes in water balance mediated by surface lipids.

Key words: acclimation, cuticular lipids, desert fruit fly, *Drosophila mojavensis*, pheromone, temperature, water loss.

Introduction

Temperature is the dominant environmental variable affecting the distribution and abundance of organisms. Ecologically relevant changes in temperature have profound effects on physiological processes (Johnston and Bennett, 1996). Acclimatory responses to temperature are well-documented (e.g. changes in membrane lipid saturation and fluidity, expression of heat-shock proteins). Thermal acclimation comprises a subset of the range of organismal responses to the environment known as phenotypic plasticity (Huey and Berrigan, 1996). An important question to physiologists, ecologists and evolutionary biologists alike is whether phenotypic responses are beneficial, i.e. do they improve the performance or fitness of the organism in its new environment (Leroi *et al.* 1994*a*,*b*; Hoffmann, 1995; Huey and Berrigan, 1996)?

Rates of water loss from insects and other terrestrial arthropods are very dependent upon temperature (Hadley, 1994*a*), and water conservation problems at high temperatures are compounded by the increasing gradient for transcuticular water loss (Gelman *et al.* 1988). Fruit flies in the genus *Drosophila* are smaller than many arthropods commonly studied by physiologists, and so cuticular water loss may pose an especially severe problem. Despite this, *Drosophila* have invaded deserts around the world (Barker and Starmer, 1982; David *et al.* 1983). The cactophilic species of the *repleta* group, including *D. mojavensis*, have been well studied by evolutionary biologists interested in speciation processes (e.g. Zouros and d'Entremont, 1980; Etges, 1990; Ruiz *et al.* 1990; Markow, 1991), but the physiological mechanisms by which these small insects survive under desert conditions are poorly understood.

One of the major factors allowing insects to succeed in deserts and other terrestrial environments is a thin layer of lipids on the surface of the cuticle, which forms the primary barrier to cuticular transpiration (Hadley, 1994*a*). Surface lipids of *Drosophila* are composed almost entirely of long-chain hydrocarbons, especially alkenes (Blomquist *et al.* 1985; Bartelt *et al.* 1986; Jackson and Bartelt, 1986). Those of *D. mojavensis*, which is endemic to the Sonoran desert, are dominated by hydrocarbons with chain lengths of 29–39 carbons (Markow and Toolson, 1990; Toolson *et al.* 1990; Stennett and Etges, 1997), whereas cuticular lipids of *D. melanogaster* and other mesic species may consist mainly of compounds less than 30 carbon atoms in length (Toolson, 1982; Cobb and Jallon, 1990). An adaptive explanation for these differences is that longer chain-length hydrocarbons provide a better barrier to transpiration, as a result of their higher melting temperatures (Lockey, 1988). Because of their small size, desert *Drosophila* should be isothermal to their environment. Air temperatures in the Sonoran desert can reach

49 °C (C. Breitmeyer, personal communication), whereas surface lipids of *D. melanogaster* melt at 30–35 °C (Gibbs *et al.* 1997). Thus, environmental temperatures are high enough to melt the surface lipids of non-desert *Drosophila* and increase their cuticular permeability.

The composition of cuticular lipids is affected by temperature, diet and other environmental factors (Howard, 1993; Howard *et al.* 1995). Individuals acclimated to higher temperatures tend to have longer chain-length surface lipids as well as reduced levels of unsaturation and methyl branching. These changes have been correlated with reduced rates of water loss in *D. pseudoobscura* (Toolson, 1982; Toolson and Kuper-Simbrón, 1989) and other arthropods (Hadley, 1977; Toolson and Hadley, 1979). In geographic and inter-specific comparisons, *Drosophila* from xeric habitats lose water less rapidly and are more desiccation-tolerant than mesic species or populations (Stanley *et al.* 1980; Eckstrand and Richardson, 1980, 1981). These traits should be beneficial to desert flies, which may need to disperse over large distances where water is unavailable (Johnston and Heed, 1975; Coyne *et al.* 1982, 1987; Breitmeyer and Markow, 1997). However, the importance of surface lipids in allowing *Drosophila* to survive in desert habitats is not known.

Drosophila mojavensis would seem to be an ideal organism in which to investigate the effects of thermal acclimation on surface lipids and water loss. It encounters high and variable temperatures on diurnal and seasonal time scales (C. M. Breitmeyer, personal communication), and it is the most temperature-tolerant of the four Sonoran-endemic *Drosophila* species (R. Stratmann and T. A. Markow, in preparation). Previous studies have shown that its surface lipids change with acclimation temperature in an apparently adaptive manner, with longer chain-length hydrocarbons increasing in abundance at higher temperatures (Markow and Toolson, 1990). To investigate the physiological effects of acclimation to temperature on desert *Drosophila*, we measured rates of water loss from *D. mojavensis* acclimated to 17, 25 and 33 °C during the first 14 days of adult life. We analyzed cuticular lipids in two ways. Lipid quantity and composition were investigated using gas chromatography, and lipid physical properties were studied using infrared spectroscopy. Our results indicate that the relationships between cuticular lipid composition, lipid physical properties and rates of water loss are not as straightforward as generally believed. Our data do not support the hypothesis that temperature-related changes in cuticular lipids aid in the conservation of water.

Materials and methods

Insect collection and maintenance

The study population was descended from several hundred *D. mojavensis* collected from rotting cacti near San Carlos, Sonora, Mexico, in May 1994. They were maintained on banana medium at 25 °C with constant illumination, under the following conditions. Adults were held in a population cage at population sizes of over 2000. Eggs were collected from banana food plates containing a dab of yeast–acetic acid paste, and groups of approximately 50 eggs each were placed in 35 ml vials containing 10 ml of banana medium. Most flies required 17–21 days for development from the egg stage to eclosion, and 7–10 days of adult life were allowed before egg collection for the next generation.

For thermal acclimation experiments, larvae were allowed to develop, pupate and eclose at 25 °C. Vials were checked daily at 13:00 h local time, and emergent adults were transferred to fresh vials containing banana food, at densities of 30 flies per vial. These vials were held at 17, 25 or 33 °C, and flies were assayed at 2, 5, 8 and 14 days. Flies were transferred to fresh food every 2–3 days. Cuticular lipid analyses were performed on the third laboratory generation after collection, and water loss and body size measurements were performed using generations 13–15. Subsequent experiments at generation 25 revealed no apparent differences in either water loss rates or surface lipids, suggesting that adaptation to laboratory culture did not affect these characters.

Cuticular lipid analyses

Surface lipids were analyzed in two ways. For analyses of the physical properties of the lipids, ten flies of a given gender, age and temperature treatment were placed on a silica gel column in a Pasteur pipet (Toolson, 1982). Cuticular hydrocarbons were eluted with 8 ml of hexane. The volume of the eluant was reduced by evaporation under nitrogen, and samples were stored at −20 °C until analysis. Three extracts of 10 flies each were assayed for each treatment.

Lipid melting temperatures were determined using a Perkin-Elmer Systems 2000 Fourier transform infrared (FTIR) spectrometer. The frequency of $–CH_2–$ symmetrical stretching vibrations increases as hydrocarbons melt and can be used as an index of lipid melting (Gibbs and Crowe, 1991). Surface lipid samples were dissolved in hexane and applied to a CaF_2 window. After the solvent had evaporated, the window was placed in a Peltier-device temperature controller. The sample temperature was increased from 15 °C to 60 °C in approximately 2 °C increments. Plots of $–CH_2–$ vibrational frequency against temperature were sigmoidal. The midpoint of the phase transition (T_m) was calculated from a fitted logistic equation.

For analyses of cuticular lipid quantity and composition, surface lipids were isolated as described above, except that 2.5 μg of *n*-docosane was applied to the silica gel column along with the flies. Eluted hydrocarbons were analyzed using a 30 m×0.32 μm DB-1 or DB-5 capillary column (J&W Scientific, Sacramento, California, USA) in a Hewlett-Packard 5890A gas chromatograph. Lipids were quantified by comparison with the peak area of the docosane standard.

Water loss rates

Rates of water loss were determined using a TR-2 flow-through respirometer (Sable Systems, Henderson, Nevada, USA; Gibbs *et al.* 1997). This system allowed sequential measurements on eight chambers (six experimental and two

empty controls). Dry air was pumped through the chambers at a flow rate of 25 ml min^{-1}. Water lost from flies was measured using a Li-Cor LI6262 humidity sensor (Li-Cor Instruments, Lincoln, Nebraska, USA). To minimize problems with water absorbed to surfaces, the respirometer and fly chambers were purged continuously with dry air. The sensor was calibrated by injecting 0.5–3 µl drops of water into the airstream (Gibbs *et al.* 1997).

All measurements were performed at 25 °C. We chose this intermediate temperature because it matched one of our treatments and the routine maintenance temperature for the population and would not be expected to induce stress responses such as the synthesis of heat-shock proteins (Huey and Bennett, 1990; Feder, 1996; Krebs and Feder, 1997). Rates of water loss were measured using groups of 20 flies (males and females assayed separately) in 5 ml glass and aluminum chambers (Gibbs *et al.* 1997). To minimize variation associated with acclimation to the chambers or progressive dehydration stress, placement of flies in their chambers was staggered so that each group had been in its chamber for 3 h before measurement. Preliminary experiments indicated that flies lost water rapidly during the first 2 h in the chamber, but loss rates stabilized at a lower value after that. Rates of water loss were measured for a 1 h period. To minimize potential wash-out artefacts due to switching chambers, only data from the last 30 min of the measurement period were used.

Whenever possible, measurements on a given day included flies of the same adult age (0, 2, 5, 8 or 14 days post-eclosion). One group of males and one group of females from each of the acclimation temperatures were assayed. Sufficient flies for each treatment were sometimes unavailable, owing to low numbers eclosing on a particular day. These treatments were then assayed in the following generation, so that at least three determinations of water loss rate were obtained for each combination of gender, age and temperature.

Body size

Groups of five flies (genders assayed separately) were dried overnight at 50 °C. Each group was weighed on a tared piece of aluminum foil to a precision of 0.1 µg using a Cahn microbalance. Six groups of five flies were measured for each combination of gender, age and acclimation temperature. The mean mass for each treatment group was used to calculate mass-specific rates of water loss for identically treated flies from the same generation.

Statistical analyses

Data were analyzed by analysis of variance (ANOVA) using Minitab software. Acclimation temperature, age and gender were treated as fixed effects, and all interaction terms were included in the model. Tukey *post-hoc* tests were performed when an ANOVA indicated a statistically significant main effect. For measurements of water loss and body size, data were collected for day 0 (newly eclosed) flies. Because only one treatment group existed for this age class (i.e. 25 °C pre-adult rearing temperature), these data were omitted from statistical analyses. Day 0 data have been included in the figures when available, as an indicator of the status of newly emerged flies at the beginning of the acclimation period.

Results

Cuticular lipids

Surface lipids of *D. mojavensis* consisted primarily of hydrocarbons containing 29–37 carbon atoms (odd numbers only), although small quantities of 39-carbon species appeared on most chromatograms. On the basis of previous studies of this species, large 29- and 31-carbon peaks were tentatively identified as 2-methyloctacosane and 2-methyltriacontane, respectively (Toolson *et al.* 1990). The 33-carbon group included several peaks. The 35- and 37-carbon groups were dominated by two large peaks each, which presumably represented the novel symmetrical alkadienes described by Toolson *et al.* (1990). Because some peaks could not be resolved consistently in our system, we lumped all peaks containing the same number of carbon atoms for statistical analyses (abbreviated C29–C37).

Hydrocarbon quantity increased significantly with age (ANOVA; $P<0.013$), but did not differ significantly among genders or acclimation temperatures (Fig. 1). The mean

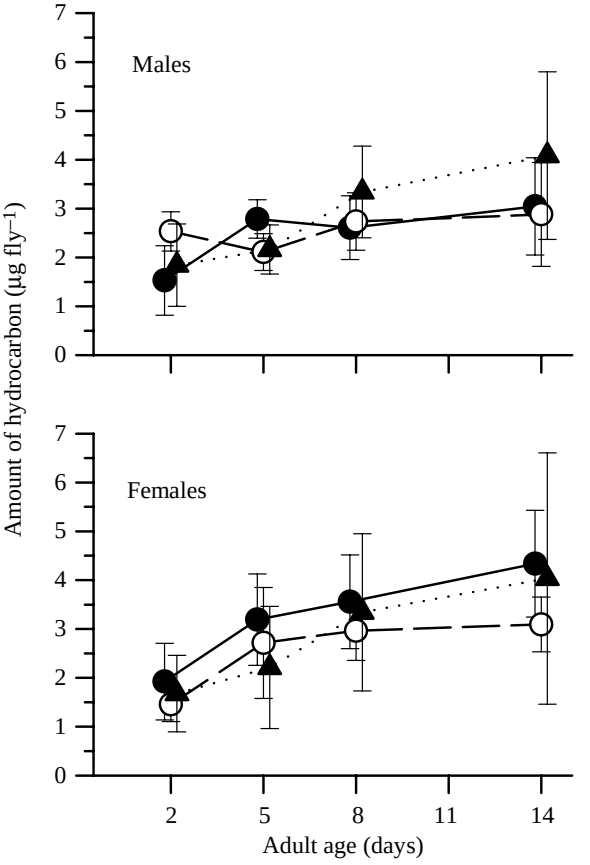

Fig. 1. Effects of age and acclimation temperature on cuticular lipid quantity in *Drosophila mojavensis*. Each point represents the mean (± S.E.M.) of three samples, using lipids extracted from 10 individuals per sample. Acclimation temperature: ● 17 °C; ○ 25 °C; ▲ 33 °C.

quantity doubled from 1.79 μg fly^{-1} at 2 days to 3.58 μg fly^{-1} at 14 days. The increased levels were due to greater amounts of hydrocarbons containing 33–37 carbon atoms in older flies (ANOVA, P<0.002 for each chain-length class). Temperature significantly affected the quantities of three chain-length classes. Amounts of C29 hydrocarbons decreased at higher temperatures, whereas amounts of the C33 and C37 groups increased with temperature.

Temperature- and age-related increases in the levels of longer-chain hydrocarbons resulted in significantly greater mean chain lengths in older and warm-acclimated flies (Fig. 2). After 2 days of adult acclimation, 33 °C-acclimated flies had diverged from the 17 and 25 °C treatments, and all three acclimation groups had diverged within 5 days. A statistically significant temperature-by-age interaction was detected (P<0.018), reflecting the increasing divergence between treatment groups as flies aged.

One would predict that longer chain-length hydrocarbons, as were found in older and warm-acclimated flies, should melt at higher temperatures. However, although temperature and age significantly affected T_m (P<0.003 and P<0.016, respectively), *post-hoc* tests revealed no patterns consistent with this hypothesis. Flies reared at 33 °C had T_m values significantly higher than those of 25 °C-reared flies, but lipids from 17 °C-reared flies melted at intermediate temperatures (Fig. 3). The only statistically significant difference in T_m between age groups was a significant difference between 2- and 8-day-old flies (Tukey *post-hoc* test).

Rates of water loss

Fig. 4 depicts a typical recording of water loss from *D. mojavensis*. Bursts of water loss were probably due to excretion events (Gibbs *et al.* 1997) and represented less than 10 % of total water loss. No significant effects of temperature, age or gender were detected for rates of water loss per individual (Fig. 5), although a marginally non-significant temperature-by-age interaction was observed (P<0.073). However, large differences in body mass were apparent among treatment groups (Fig. 6). Female flies weighed more than males, flies lost mass as they aged, and acclimation temperature was negatively associated with body mass (P<0.001 for each effect).

Fig. 7 depicts rates of water loss recalculated on a mass-specific basis, using data from Figs 5 and 6. When the data were expressed in this manner, temperature, age and

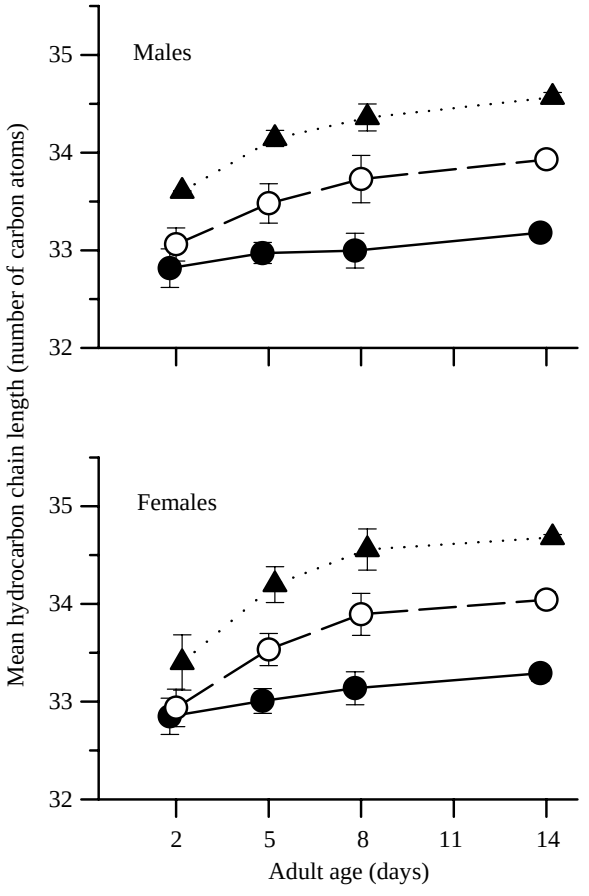

Fig. 2. Effects of age and acclimation temperature on mean hydrocarbon chain length. Each point represents the mean (± S.E.M.) of three samples, using lipids extracted from 10 individuals per sample. Acclimation temperature: ● 17 °C; ○ 25 °C; ▲ 33 °C.

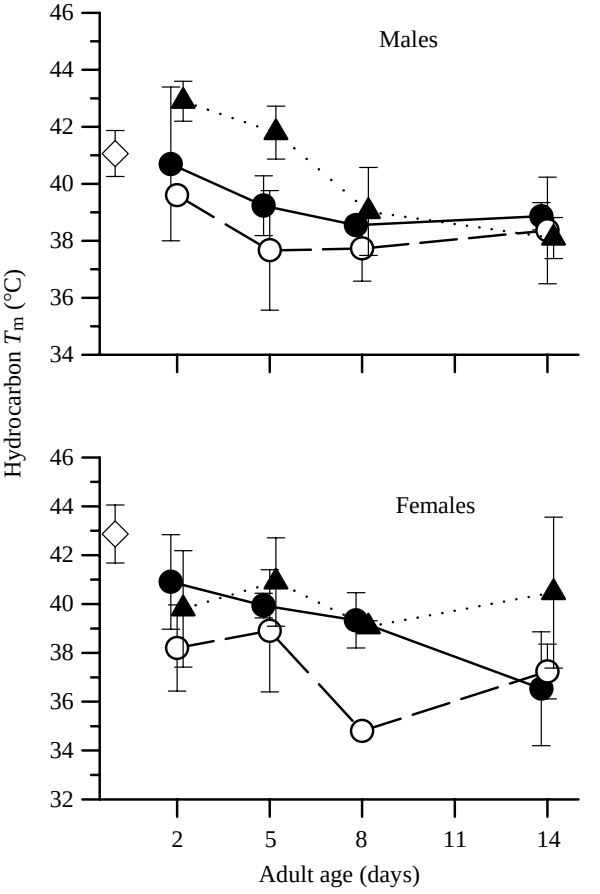

Fig. 3. Effects of age and acclimation temperature on cuticular lipid melting temperature (T_m) in *Drosophila mojavensis*. Each point represents the mean (± S.E.M.) of three samples, using lipids extracted from 10 individuals per sample. Acclimation temperature: ● 17 °C; ○ 25 °C; ▲ 33 °C. Diamonds indicate data for newly emerged adults.

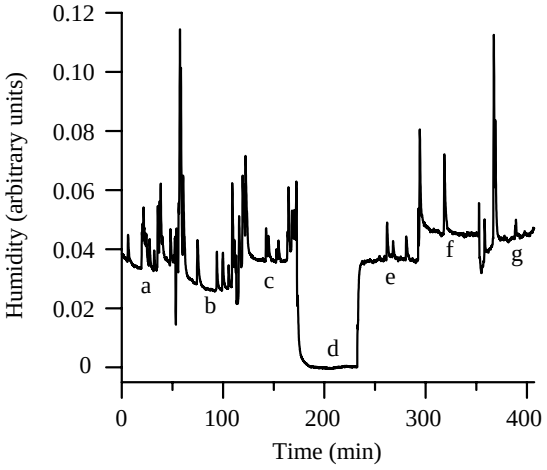

Fig. 4. Representative recording of water loss from *Drosophila mojavensis*. Water loss was recorded for 1 h for each group of 20 flies. Flies in this example were 5 days post-eclosion. From left to right, acclimation temperatures and genders for each 1 h recording period were: (a) 17 °C females, (b) 17 °C males, (c) 25 °C females, (d) empty chamber, (e) 25 °C males, (f) 33 °C females, (g) 33 °C males.

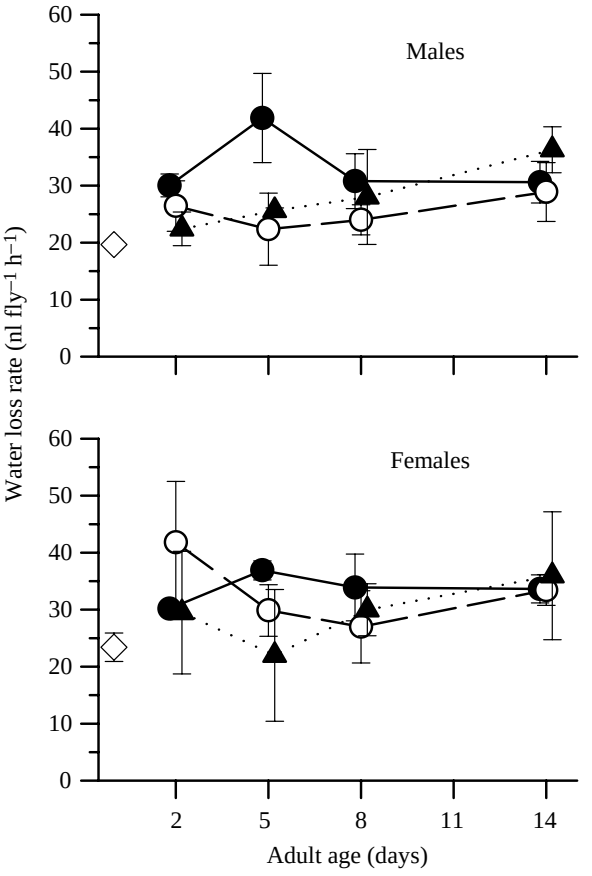

Fig. 5. Effects of age and acclimation temperature on water loss from *Drosophila mojavensis*. Each point represents the mean (± S.E.M.) of 3–4 measurements, using groups of 20 flies per sample. Acclimation temperature: ● 17 °C; ○ 25 °C; ▲ 33 °C. Diamonds indicate data for newly emerged adults.

temperature-by-age interactions significantly affected water loss (P<0.001 for each variable). *Post-hoc* tests indicated that these results were due to greater rates of water loss in flies acclimated to 33 °C for 14 days than in other treatment groups. Because males were smaller, but lost water as rapidly as females on an individual basis, males had higher mass-specific rates of water loss than females (P<0.016).

Discussion

Several studies have correlated acclimatory changes in cuticular lipid composition with differences in rates of water loss (Hadley, 1977; Toolson and Hadley, 1979; Toolson, 1982; Toolson and Kuper-Simbrón, 1989). The high surface area-to-volume ratio of *Drosophila* makes these insects particularly susceptible to water loss. High temperatures magnify the problems of water conservation, especially at the low humidities encountered in deserts. Thus, *D. mojavensis* should face particularly severe problems related to water conservation, and thermal acclimation of cuticular lipids should serve to reduce water loss. The unexpected results obtained in this study impact both upon specific questions regarding the relationship between surface lipids and water loss and upon general issues regarding the adaptive significance of thermal acclimation.

Relationship between cuticular lipids and rates of water loss

Two paradigms regarding the effects of cuticular lipids on insect water loss have gained wide acceptance: insects having greater amounts of cuticular lipids should lose water less rapidly (Hadley, 1994*a*), and differences in surface lipids that increase T_m should result in reduced cuticular water loss (Lockey, 1988; Noble-Nesbitt, 1991). Several studies of thermal acclimation have provided correlative evidence consistent with both of these hypotheses. Increased hydrocarbon chain length, and decreased levels of unsaturation and methyl branching, are correlated with reduced rates of water loss (Hadley, 1977; Toolson and Hadley, 1979; Toolson, 1982; Hadley and Schultz, 1987). These changes in lipid composition should increase T_m (Gibbs and Pomonis, 1995). These studies have provided the basis for adaptive explanations regarding inter-specific and geographic differences in lipid composition (e.g. Lockey and Oraha, 1990; Gibbs *et al.* 1991; Lockey, 1992).

Our results contradict these models for surface lipid function in several ways. Although we found a consistent pattern of increased hydrocarbon chain lengths in warm-acclimated *Drosophila* (Fig. 2), lipid melting temperatures exhibited no clear correlation with acclimation temperature. Chain lengths increased with age, but T_m decreased (Fig. 3). The unexpected inverse relationship between T_m and hydrocarbon chain length probably reflects structural differences between short- and long-chain species. The C29 and C31 species consisted primarily of terminally branched alkanes (Toolson *et al.* 1990;

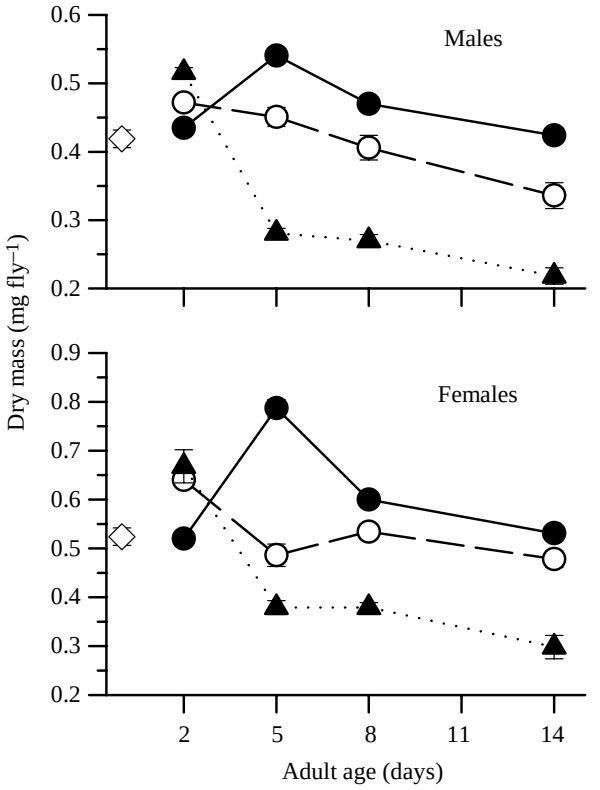

Fig. 6. Effects of age and acclimation temperature on body mass in *Drosophila mojavensis*. Each point represents the mean (± S.E.M.) of six samples, using five individuals per sample. Acclimation temperature: ● 17 °C; ○ 25 °C; ▲ 33 °C. Diamonds indicate data for newly emerged adults.

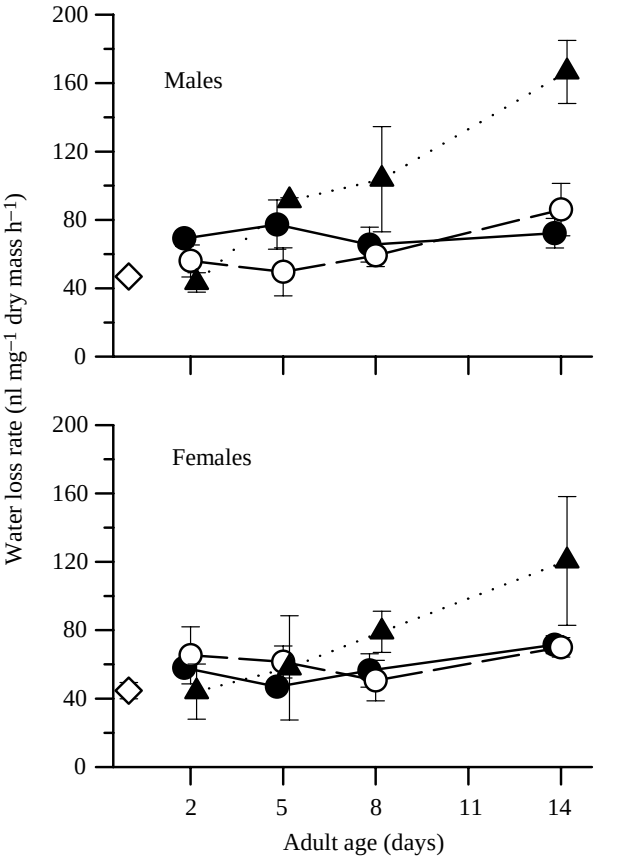

Fig. 7. Effects of age and acclimation temperature on mass-specific rates of water loss from *Drosophila mojavensis*. Data were generated by combining data from Figs 5 and 6. Acclimation temperature: ● 17 °C; ○ 25 °C; ▲ 33 °C. Diamonds indicate data for newly emerged adults.

Stennett and Etges, 1997), which would tend to melt at temperatures similar to *n*-alkanes of the same chain length (Gibbs and Pomonis, 1995). Longer-chain hydrocarbons were more unsaturated than the 29- and 31-carbon species, a difference that could tend to decrease the melting temperature to a greater extent than chain length would increase T_m (Gibbs and Pomonis, 1995). Thus, increases in chain length associated with thermal acclimation or age may be offset by the T_m-lowering effects of greater unsaturation.

Even in the absence of differences in surface lipid composition, one would predict that rates of water loss would be negatively correlated with surface-lipid density (Hadley, 1994*a*). However, the doubling of lipid quantity as flies aged did not affect rates of water loss (Figs 1, 5). An important factor to be considered is body size. Rates of water loss are often reported per unit of surface area, which is usually calculated from a general equation relating area and wet mass, rather than measured directly (e.g. Toolson, 1982; Toolson and Kuper-Simbrón, 1989). We chose not to do this, because our flies were all reared to adulthood under the same conditions. Thus, the quantity of cuticle laid down in the puparium should have been the same across treatment groups, despite the nearly threefold variation in dry mass related to acclimation temperature and adult age (Fig. 6). We note that if we had expressed our data in terms of the surface area calculated from the mass, our results would have been even less consistent with adaptive thermal acclimation. Flies reared at 33 °C would have had the smallest calculated surface areas and, therefore, the highest densities of cuticular lipids and the highest cuticular permeabilities.

Our results present an interesting comparison with those of laboratory selection experiments using *D. melanogaster* (Graves *et al.* 1992; Gibbs *et al.* 1997). In those studies, selection for desiccation resistance resulted in reductions of water-loss rates of approximately 40 %. Desiccation-selected and control populations had similar amounts of cuticular hydrocarbons, which exhibited only minor differences in chain length and T_m. In another study, rates of water loss of the *Parched* mutant of *D. melanogaster* were as high as those of dead wild-type flies whose surface lipids had been removed by hexane extraction, despite the fact that cuticular lipid quantity and composition in the mutant strain were similar to those of wild-type flies (Kimura *et al.* 1985). The physiological basis for high rates of water loss in the *Parched* flies is unknown and could be due to defects in water transport by the gut, but these studies demonstrate that significant differences in rates of

water loss can occur with little change in surface lipids; in the current study, significant differences in lipid composition had little or no effect on water loss rates. Thus, cuticular lipids and rates of water loss can vary independently.

One possible explanation for the differing results of this study and previous work is the techniques used to measure rates of water loss. Studies of larger arthropods have often used individuals whose respiratory and digestive openings have been sealed to prevent non-cuticular water loss (Toolson and Hadley, 1979; Hadley and Schultz, 1987). This procedure, which may result in inadvertent cuticular damage, may be unnecessary, since respiratory losses generally account for less than 10 % of total water loss (Hadley, 1994*b*). Rates of water loss in *Drosophila* have often been measured using dead flies, with water loss being measured gravimetrically in the first few hours after death (Toolson, 1982; Toolson and Kuper-Simbrón, 1989; Graves *et al.* 1992), whereas we used a flow-through system to measure rates of water loss in live *Drosophila*. I have found that rates of water loss, in various *Drosophila* species, are relatively high in the first 2 h in the respirometer, then stabilize at a constant level until flies begin to die from desiccation stress (A. G. Gibbs, unpublished observations). Also, dead flies lose water 50–100 % more rapidly than live *Drosophila*. Because we determined water loss in live flies, our experiments should provide a better measure of water loss under natural conditions.

Insects lose water *via* non-cuticular avenues, such as respiration and excretion, and it could be argued that temperature-related increases in these components may offset acclimatory reductions in cuticular transpiration. Aside from the fact that one must then explain why other components of water balance respond to temperature in a non-adaptive manner, several lines of evidence argue against this idea. Peaks of water loss shown in Fig. 4 probably represent excretion events (Gibbs *et al.* 1997). These accounted for less than 10 % of total water loss, suggesting that excretory losses constitute a relatively minor component of overall water balance.

Several recent studies indicate that respiratory water loss also accounts for less than 10 % of total losses (Quinlan and Hadley, 1993; Hadley, 1994*b*). *Drosophila* are smaller than most other insects studied and have higher mass-specific metabolic rates; thus, they may have a relatively high respiratory water loss. Desiccation-selected populations of *D. melanogaster* have evolved the capacity for discontinuous gas exchange (Williams *et al.* 1997), which may decrease respiratory transpiration (Lighton *et al.* 1993; Lighton, 1994). However, preliminary results suggest that respiratory water loss in these populations accounts for no more than 25 % of total water loss (A. E. Williams, in preparation). *Drosophila mojavensis* does not appear to exhibit discontinuous gas exchange (A. G. Gibbs, unpublished observations). We conclude that the flies in our study lost most of their water through the cuticle, so that our measurements of total water loss provide a good indication of cuticular transpiration.

We have provided several possible explanations for the unexpected results that neither cuticular lipid quantities nor physical properties appear to be correlated with rates of water loss. The null hypothesis is that the model itself is wrong. Certainly, rates of water loss are much higher when lipids are removed from the cuticle, but perhaps the mere presence of a lipid barrier is sufficient, and details of its chemical or biophysical make-up are inconsequential. The phenotypic response of cuticular lipids to the environment may not be adaptive, but instead may represent an epiphenomenon of the effects of temperature on other processes. For example, differential effects of temperature on biosynthetic enzymes could result in differences in surface lipid composition, in the absence of any regulatory responses to temperature (Gibbs and Mousseau, 1994; Gibbs, 1998).

The ecological relevance of our temperature treatments must also be considered. Little is known about the actual conditions experienced by fruit flies in nature. *Drosophila melanogaster* larvae experience temperatures up to 45 °C in rotting fruit (Feder, 1996), although mean developmental temperatures are approximately 21 °C (Jones *et al.* 1987). In the field, adult *D. mojavensis* remain in cactus rots during the day and emerge at dusk (T. A. Markow, personal communication). The retreat to cactus rots in the morning occurs when air temperatures reach 30 °C (A. G. Gibbs, unpublished observations). Inside the cactus rots, conditions are presumably very humid, but temperatures may exceed 40 °C (A. G. Gibbs, C. M. Breitmeyer and J. A. Alipaz, unpublished observations). When cactus rots become too warm, *D. mojavensis* leave the cactus and spend the day in nearby shade. In addition, rotting cacti are an ephemeral and non-abundant resource (Breitmeyer and Markow, 1997), and *Drosophila* have been shown to migrate over large distances in the desert (Coyne *et al.* 1987). These factors make it likely that *D. mojavensis* experiences both high temperatures and desiccating conditions in nature.

Cuticular pheromones in Drosophila mojavensis

A complicating factor in understanding water balance in *Drosophila* and many other insects is that some cuticular lipid components also serve roles in chemical communication (Howard, 1993). In *Drosophila*, hydrocarbons involved in sex recognition are major components of the surface lipids (Scott, 1994; Cobb and Jallon, 1990; Coyne *et al.* 1994). To the extent that pheromones affect surface lipid properties and cuticular transpiration, the need for effective communication may adversely affect water conservation. Markow and Toolson (1990) observed that male *D. mojavensis* acclimated to 34 °C had an altered surface lipid composition, including differences in putative pheromones, and a reduced mating success relative to 17 °C-acclimated males. Thus, an apparent trade-off between acclimation to high temperature and reproductive success was mediated by changes in cuticular lipid structure, which could also affect rates of water loss.

We have confirmed the acclimatory changes in cuticular hydrocarbons observed by Markow and Toolson (1990). However, as described above, rates of water loss were unaffected. Thus, we reject the hypothesis that the requirement for reduced water loss conflicts with male reproductive

success. We suggest that body size may also have affected male mating success in previous work. In our initial studies of cuticular lipids in the third laboratory generation, we noticed relatively high mortality in the 33 °C treatment groups, and flies looked 'shriveled' under a microscope. This apparent difference was confirmed by later measurements of dry mass, in which flies acclimated to 33 °C for 14 days weighed approximately half as much as 17 °C-acclimated flies (Fig. 6). Reduced body size at higher developmental (larval) temperatures has been correlated with reduced reproductive success in *Drosophila* spp. on numerous occasions (e.g. Huey *et al.* 1995; but see Zamudio *et al.* 1995). Thus, the reduced mating success of warm-acclimated *D. mojavensis* observed by Markow and Toolson (1990) may be due to the effects of temperature on courtship stimuli other than cuticular pheromones, to smaller body size or to the generally reduced vigor of males reared at 34 °C.

Is thermal acclimation beneficial?

Several authors have recently pointed out the relative lack of studies designed to test in a rigorous manner the beneficial effects of thermal acclimation (e.g. Leroi *et al.* 1994*a*,*b*; Hoffmann, 1995; Huey and Berrigan, 1996). Studies using *D. melanogaster* have shown that fitness components such as fecundity can be affected by developmental temperature (Huey *et al.* 1995; Zamudio *et al.* 1995). We measured a physiological index of organismal performance, water loss. The warm temperatures and low humidity of desert environments, coupled with the small size of *Drosophila*, should be important selective features for desert fruit flies. Although water loss is not a direct measure of fitness, it is clearly of importance to desert organisms and therefore should be an appropriate measure of organismal performance.

In *D. mojavensis*, apparently adaptive differences in cuticular lipids did not result in reduced rates of water loss. Twofold increases in surface lipid quantity with age did not affect rates of water loss, and increases in mean hydrocarbon chain length resulted in lower (not higher) T_m values. Rates of water loss per individual were unaffected by thermal acclimation. Differences possibly associated with sex-recognition pheromones were not correlated with water loss rates, so no apparent trade-off exists between the ability of *D. mojavensis* to conserve water and its reproductive success. These laboratory results need to be considered in the context of the actual environmental conditions experienced in nature, but they also provide another example of a trait for which an apparently beneficial phenotypic response to the environment does not actually appear to benefit the organism (Leroi *et al.* 1994*a*,*b*; Huey *et al.* 1995; Zamudian *et al.* 1995). These studies illustrate the need for rigorous evaluation of adaptive hypotheses for thermal acclimation of physiological characters.

This work was supported by NSF grant IBN-9317471 and a Faculty Research and Travel grant to A.G.G. We thank T. A. Markow for advice in general *Drosophila* biology and for letting us accompany her laboratory in the field, C. M. Breitmeyer for discussions and field assistance, J. A. Alipaz for assistance in field collections, and V. A. Pierce for help with Minitab. C. M. Breitmeyer and A. E. Williams shared interesting unpublished results and provided helpful comments on the manuscript.

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