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Review

## Water balance in desert *Drosophila*: lessons from non-charismatic microfauna<sup>☆</sup>

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### Abstract

Water stress is a particularly important problem for insects and other small organisms in arid environments. Cactophilic fruit flies in the genus *Drosophila* have invaded deserts on numerous occasions, including multiple independent invasions of North American deserts. Because the evolutionary history of this genus is so well studied, we can investigate the mechanisms of adaptation in a rigorous phylogenetic context. As expected, desert fruit flies lose water less rapidly than their mesic congeners. They are also able to tolerate the loss of a greater percentage of body water, but this difference is mainly due to phylogenetic history, and does not represent an adaptation specifically to desert habitats. A laboratory analogue of desert *Drosophila* is provided by populations of *D. melanogaster* that have been subjected to selection for desiccation resistance. Selected populations resemble desert species in that they lose water slowly, relative to control populations, and are not more tolerant of dehydration stress. They differ, however, in having much higher water contents and different behavioral responses to desiccating conditions. Our comparisons of laboratory and natural populations reveal that not all possible adaptive mechanisms evolve in stressful environments. Different physiological and behavioral strategies may evolve depending upon the particular options available in the environment.

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### 1. Introduction

The desert is a hot, dry place. Physiologists have devoted great effort to understand how desert organisms survive and thrive in such an extreme environment. Larger animals have two important size-related advantages. Their surface area:volume ratio is lower, so they are less prone to evaporative

water loss from their surface, and their size provides thermal inertia that can reduce the danger of overheating. A third advantage is their greater mobility, generally, so that many desert mammals and birds simply leave the area when conditions become too severe.

Insects and other arthropods do not have these advantages, yet they are the most diverse and successful desert animal taxa. Studies of desert arthropods have emphasized their small size, but most of the species examined (e.g. tenebrionid beetles, scorpions, grasshoppers) are actually quite large in relation to typical arthropods. Smaller, less well studied arthropods face even greater physiological challenges than these 'charismatic megafauna'.

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## 2. *Drosophila* as a model for desert adaptation

My focus here is on the fruit fly genus *Drosophila*. Although the human commensal *D. melanogaster* is the most widely used animal model for genetics and evolution, comparative physiologists have only recently begun to appreciate the potential of *Drosophila* as a study system (Huey et al., 1991; Feder, 1996; Gibbs et al., 1997; Marden et al., 1997). *Drosophila* species have invaded a wide range of terrestrial habitats, from boreal and tropical forests to deserts around the world. They differ greatly in their physiological tolerance limits (Stanley et al., 1980; Parsons, 1982; van Herrewege and David, 1997; Gibbs and Matzkin, 2001), suggesting that fruit flies can provide a good model with which to study adaptation to stressful environments.

Several problems arise with any attempt to relate environmental conditions to differences in organismal physiology, and *Drosophila* are no exception. Natural habitats are complex, and it can be difficult to identify those factors that are the most important selective features. Although heat and lack of water appear to be important factors for desert organisms, many species can escape these stresses by behavioral means, such as migration, burrowing or estivation, and therefore may not experience them. Evolutionary history must also be considered. Do differences between desert and mesic species really reflect adaptation to their habitats, or do some species retain ancestral characteristics that only appear to be adaptive? To address this question one requires knowledge of phylogenetic relationships, but very few studies of desert animals have incorporated this information.

The evolutionary relationships of *Drosophila* are well studied, so that differences between species can be studied within a rigorous phylogenetic context. In the deserts of southwestern North America, numerous *Drosophila* species, particularly those of the *repleta* group, inhabit the necrotic tissues of columnar cacti. These species have provided a widely studied model system for speciation processes (Barker and Starmer, 1982; Ruiz et al., 1990; Markow and Hocutt, 1998; Etges et al., 1999), so a good deal is known about their evolution and natural history.

Other researchers have created 'artificial deserts' in the laboratory, by subjecting *D. melanogaster* and other species to selection for resistance to desiccation or high temperatures (e.g. Kiliias and

Alahiotis, 1985; Hoffmann and Parsons, 1989, 1993; Huey et al., 1991; Rose et al., 1992; Loeschke and Krebs, 1996). The ease with which *Drosophila* can be reared in the laboratory allows environmental conditions to be rigorously controlled, and the short generation times allow these selection experiments to be carried out within only a few months or years. In addition, populations can be replicated to provide statistical power, and unselected control populations can be maintained for direct comparison to the ancestral condition (Rose et al., 1996; Gibbs, 1999). Selected populations can provide a useful comparison to natural populations of *Drosophila*. If both laboratory-selected flies and desert species exhibit similar differences from control populations and mesic species, then we gain support for the selective advantage of these differences. If, however, flies from laboratory and natural deserts differ in their apparent mechanisms of adaptation, then our understanding of one study system or the other must be lacking.

The major goal of this review is to systematically compare physiological differences between flies from arid and mesic habitats, using both natural and laboratory populations. Differences between the two systems allow us to generate hypotheses about the conditions faced by flies in nature, which we can then test in nature or in the lab. I will concentrate on water stress, but it should be noted that desert *Drosophila* are more tolerant of heat stress than mesic species (Stratman and Markow, 1998; Krebs, 1999), and that populations of *D. melanogaster* can evolve increased heat tolerance in response to laboratory selection (Kiliias and Alahiotis, 1985; Huey et al., 1991; Loeschke and Krebs, 1996). Thus, similar comparisons between the lab and the field can be made in regard to heat stress as well.

## 3. Climatic conditions

The conditions experienced by desiccation-selected flies in the laboratory are straightforward; they have abundant food and water except during the imposition of selection, when they have neither. The temperature is constant and non-stressful (~25 °C). In nature, both humidity and temperature vary in time and space, and the highest temperatures and lowest humidities tend to co-occur. Because of their size, however, desert *Drosophila* could potentially avoid extreme conditions

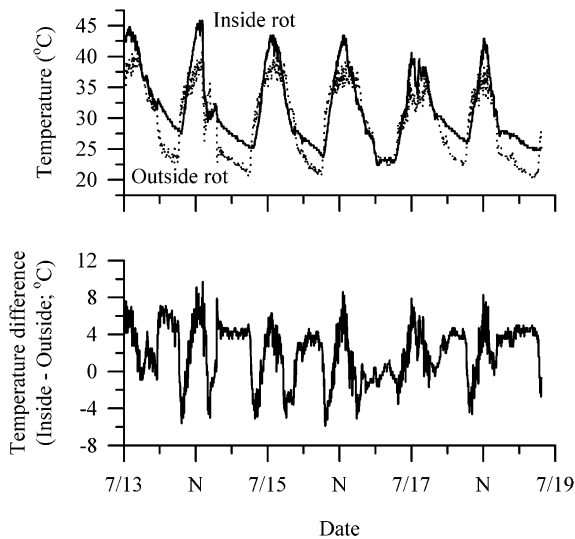


Fig. 1. Environmental temperatures experienced by desert *Drosophila* over a 6-day period in Tucson, Arizona (July, 2001). 'N' corresponds to noon on even-numbered days. (a) Recordings from thermistor probes placed inside an air pocket of a rotting saguaro cactus, and in shaded air nearby. (b) Difference in temperature between the air inside and outside of the rot pocket.

altogether by occupying relatively cool and moist microhabitats.

Flies in the Sonoran Desert are found in and around necrotic cacti, their primary source of food and water, at all times of the day. Because water is readily available inside shaded air pockets that form inside necroses, rotting cacti appear to be an excellent refuge from macroclimatic stress. However, field data suggest necroses are not particularly benign. Temperatures inside air pockets of necroses are just as warm as those outside, and sometimes warmer (Fig. 1). The air inside is more humid, but internal humidities can be <20% RH during the warmest part of the afternoon (Gibbs et al., unpublished). Vapor-pressure deficit values (the driving force for transcuticular water loss) can exceed 6 kPa inside rots (and 12 kPa outside). Thus, desert *Drosophila* are exposed to highly desiccating conditions in nature, and we can infer that desiccation stress has been an important selective factor in their evolution. In accordance with this idea, cactophilic *Drosophila* survive much longer under desiccating conditions than their mesic relatives (Gibbs and Matzkin, 2001).

#### 4. Mechanisms of desiccation resistance: water balance

For any desert animal, water acquisition and water conservation are both critical components of the overall water budget. Survival under desiccating conditions can be achieved by storing greater amounts of water (as either bulk water or metabolic water stores), by decreasing the rate at which water is lost, or by being more tolerant of low water content. In the extreme case of the latter, anhydrobiotic organisms can survive in a nearly completely dry state (Crowe et al., 1992).

Several comparative studies of water balance in *Drosophila* have been performed (Hoffmann and Harshman, 1999); unfortunately these have generally not controlled for phylogeny. This may be an important issue; apparent differences in dehydration tolerance between cactophilic and mesic species disappear when phylogenetic relationships are taken into account (Gibbs and Matzkin, 2001; Fig. 2). Desert species also do not exhibit high water contents; in fact, males of cactophilic species tend to have relatively low water contents, the opposite of the expected pattern (Gibbs and Matzkin, 2001). Thus, water conservation remains as the only mechanism by which to increase desiccation survival. Only a few comparative studies have measured water-loss rates of *Drosophila*. As predicted, species from arid habitats lose water more slowly than their mesic congeners (Eckstrand and Richardson, 1980, 1981; Gibbs and Matzkin, 2001; Fig. 3).

In the laboratory, *Drosophila* populations respond rapidly to selection, so that increased desiccation resistance can be detected within ten generations (Hoffmann and Parsons, 1989, 1993; Blows and Hoffmann, 1993; Archer, 1999). Selected lines lose water less rapidly than their controls, but they and their controls are equally tolerant of low water content (Ringo and Wood, 1984; Hoffmann and Parsons, 1993; Gibbs et al., 1997). These results parallel those of comparative studies. On the other hand, long-term selection lines contain much more water than their controls (Gibbs et al., 1997; Chippindale et al., 1998), but other, short-term selection lines do not appear to accumulate water (Hoffmann and Parsons, 1993).

In summary, water budgets of desiccation-selected *Drosophila* and natural populations exhibit both similarities and differences. In both cases, arid-adapted flies lose water less rapidly, but are no

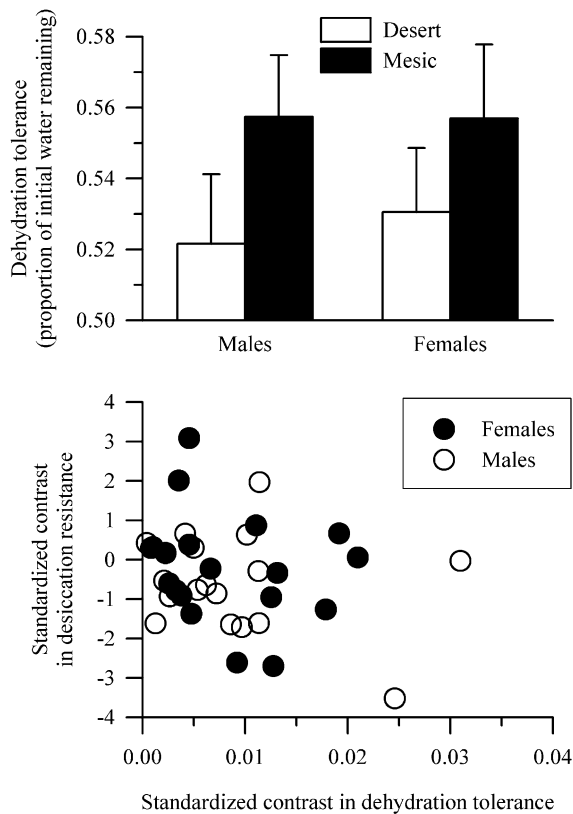


Fig. 2. Dehydration tolerance of desert (cactophilic) and non-desert *Drosophila* species. Upper panel: Fraction of initial water content remaining when the flies died from desiccation stress ( $P < 0.05$  for males;  $P < 0.07$  for females). Lower panel: Relationship between dehydration tolerance and desiccation resistance (hours survived in dry air at 25 °C), after controlling for phylogenetic relatedness using independent contrasts (Felsenstein, 1985). No significant correlation was found for either sex ( $P > 0.3$ ). Data from Gibbs and Matzkin (2001).

more tolerant of low water content (Gibbs et al., 1997; Hoffmann and Harshman, 1999; Harshman and Hoffmann, 2000; Gibbs and Matzkin, 2001). Only in the laboratory, however, has increased water content evolved, and then only in certain populations. Despite the apparent similarity in selective regimes, physiological evolution has taken different paths. An additional important point is that not all expected mechanisms of desiccation survival have evolved, in either case. Increased ability to tolerate dehydration stress would certainly increase survival, but it has not evolved even under strong directional selection for >120 laboratory generations (Gibbs et al., 1997). Desert species could improve their survival by carrying a

'canteen' of extra water, but they do not (Gibbs and Matzkin, 2001).

## 5. Mechanisms of water conservation

Both laboratory and natural populations have evolved improved water conservation—have they done so in the same way? Water can be lost through the spiracles during respiration, by transpiration through the cuticle, or by excretion from the mouthparts or feces. (Females can also lose substantial amounts of water in their eggs.) Reductions in any or all of these routes could be responsible for lower overall water-loss rates.

Excretory water loss accounts for a small fraction, no more than 6%, of total losses in either laboratory or natural populations (Gibbs et al., 1997; Gibbs et al., unpublished data). This leaves respiratory and cuticular water losses as the main sites of water conservation. It is difficult to distinguish between these, especially in insects as small as *Drosophila*, so we must use indirect evidence to try to determine which, or both, is involved.

The main barrier to cuticular water loss is the epicuticular lipids, so evidence of reduced cuticular permeability should be apparent by greater amounts of surface lipid or differences in lipid composition. In particular, compositional changes that increase the melting point of the lipids (longer hydrocarbon chain lengths, reduced saturation or methyl branching) are expected to reduce cuticular water loss (Gibbs, 1998; Rourke and Gibbs, 1999).

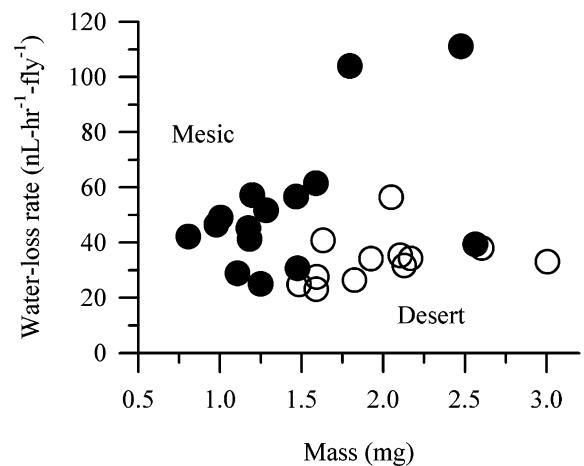


Fig. 3. Water-loss rates of female *Drosophila* from arid and mesic habitats. Mesic species lose water at significantly higher rates (ANCOVA;  $P < 0.002$ ). From Gibbs and Matzkin (2001).

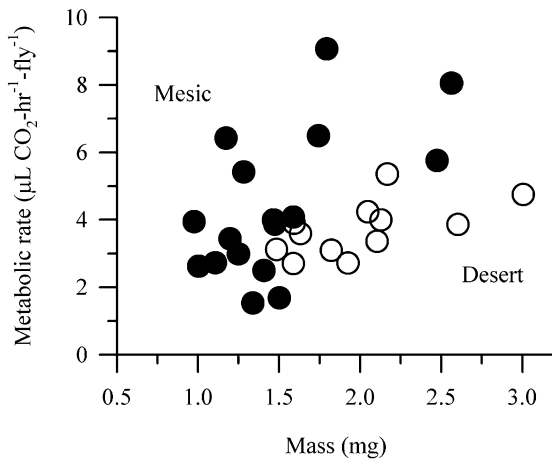


Fig. 4. Metabolic rates ( $\text{CO}_2$  production) of female *Drosophila* from arid and mesic habitats. Mesic species have significantly higher metabolic rates (ANCOVA;  $P < 0.005$ ).

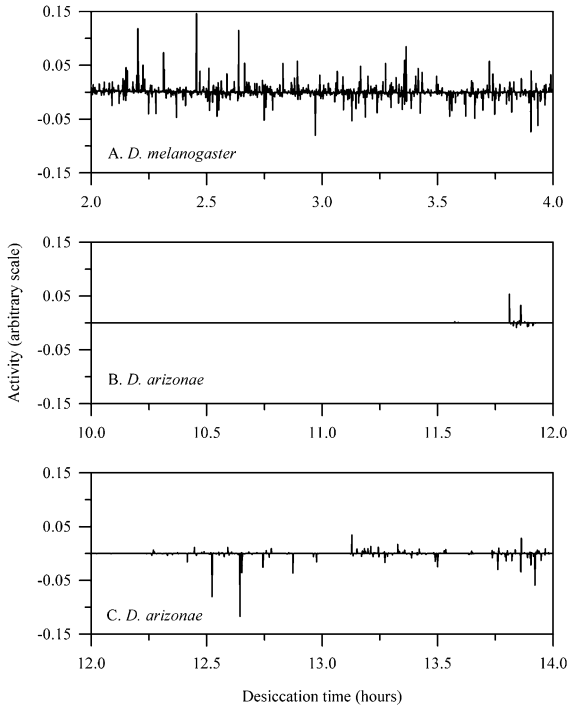


Fig. 5. Activity patterns of *Drosophila* species. Spikes (up or down) indicate movement by the insect. (a) *D. melanogaster* during the first 2 h of desiccation stress. (b) *D. arizonae*, a cactophilic species, after 10–12 h of desiccation. (c) Same *D. arizonae* between 12 and 14 h.

Very minor differences in cuticular lipids are seen in laboratory populations, and they appear insufficient to explain the 40% reduction in water-loss

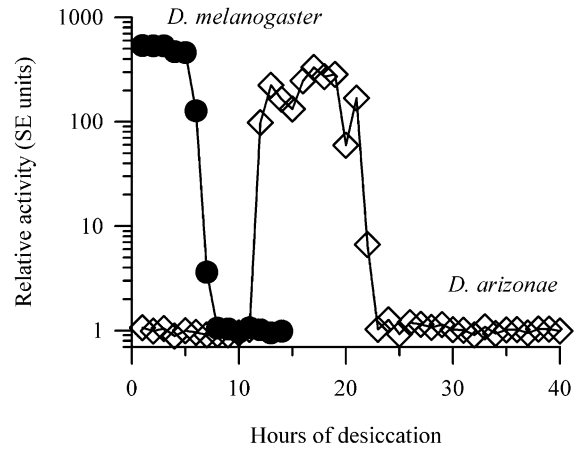


Fig. 6. Overall activity of the same individuals as in Fig. 5. For each 1-h period, the best-fit linear regression line through the activity data was calculated, and the standard error of the regression was used as a measure of average activity.

rates in desiccation-selected lines (Gibbs et al., 1997). Surface lipids differ greatly among *Drosophila* species (Bartelt et al., 1986; Jallon and David, 1987; Markow and Toolson, 1990; Tompkins et al., 1993), but this variation is not well correlated with rates of water loss (Gibbs, unpublished data). In summary, differences in surface lipids do not seem sufficient to account for reduced water loss in either laboratory or natural populations. If cuticular transpiration is reduced, it may be accomplished by changes in other portions of the cuticle, or in the distribution of surface lipids over the cuticle.

By elimination, reduced water loss must have been achieved by reductions in respiratory losses. One potential mechanism is to reduce the need for respiration, i.e. to reduce metabolic rate and the demand for oxygen. Our preliminary data suggest that cactophilic *Drosophila* have lower metabolic rates than mesic species (Fig. 4). Similar results have been obtained for desiccation-selected populations in the laboratory (Hoffmann and Parsons, 1989; Blows and Hoffmann, 1993; but see Djawdan et al., 1997; Harshman and Schmid, 1998).

Locomotory activity comprises a significant fraction of metabolism in many ectothermic organisms, and reduced activity has been identified as a behavioral response to desiccation selection (Hoffmann and Parsons, 1993; Williams, 1998). Reduced activity has also been implicated as a mechanism to reduce water stress in desert taxa (see other articles in this symposium). Fig. 5

depicts representative recordings of activity patterns of *D. melanogaster* and a cactophilic species, *D. arizonae*. Fig. 6 depicts overall activity patterns for the same flies, recorded continuously until the flies died from desiccation stress. In general, mesic species are active continuously and die within 15 h. Cactophilic species are much less active initially, but become active after 10–15 h of desiccation. Metabolic rates approximately double during activity (Gibbs, unpublished data); thus, reduced activity alone may be able to account for the lower metabolism of cactophilic species (Fig. 4).

It is important to note that reduced metabolic rates will only contribute to lower water-loss rates if the spiracles can then be kept closed more often. If *Drosophila* lack spiracular control, then water may be lost at the same rate no matter how low metabolism is. Unfortunately, ventilatory patterns in *Drosophila* have not been studied very well. Desiccation-selected populations of *D. melanogaster* exhibit discontinuous gas-exchange cycles (DGC; Lighton, 1996), whereas neither ancestral nor control populations do so (Williams et al., 1997). In some cases, simultaneous changes in water-loss rates can also be detected (Williams and Bradley, 1998), suggesting that ventilatory control does in fact reduce water loss significantly.

Some desert species of *Drosophila* also exhibit cyclic gas-exchange patterns, but these are not the same as classical DGCs. In *D. mojavensis*, a distinctive pattern of cyclic CO<sub>2</sub> release is associated with increased locomotory activity and higher metabolic rates (Fig. 7), whereas desiccation-selected *D. melanogaster* exhibit DGCs while inactive (A.E. Williams, personal communication). The function of cyclic ventilation in desert *Drosophila* remains uncertain, but it may help to conserve water during activity by matching spiracular conductance to metabolic demand (Lehmann, 2001).

## 6. Energetics and metabolism: energy storage

Several selection experiments have implicated energy storage in stress resistance. Fruit flies selected for postponed senescence store larger quantities of lipid and glycogen than short-lived control populations (Service, 1987; Graves et al., 1992), and starvation-selected flies store very large amounts of lipid (Chippindale et al., 1996, 1998; Djawdan et al., 1998). In the case of desiccation selection, selected populations store less energy

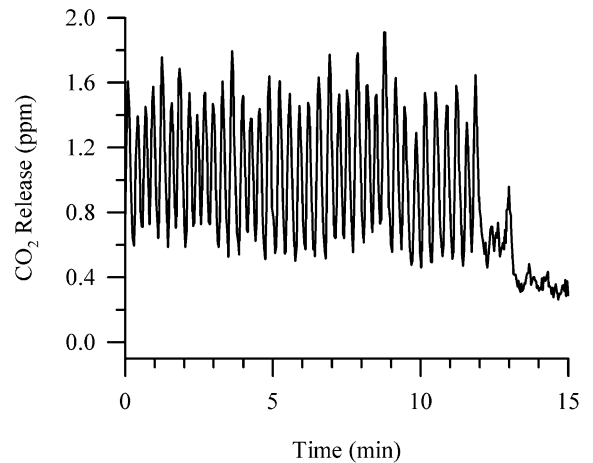


Fig. 7. Cyclic CO<sub>2</sub> release in *D. mojavensis*. The fly was active for the first 12 min of the recording, then became inactive for the last 3 min.

than their controls in the form of lipids, but more in the form of carbohydrates (Djawdan et al., 1998). Most of this is presumably glycogen, although trehalose levels may also increase (Folk et al., 2001).

Few comparative studies of the relationship between energy storage and stress resistance have been performed. Species containing more lipid survive starvation and desiccation stress longer (van Herrewege and David, 1997), but unfortunately that study included only one cactophilic species. In general, cactophilic *Drosophila* do not contain more lipids and carbohydrates than mesic ones (Marron et al., unpublished). Thus, laboratory and natural populations of desiccation-resistant flies exhibit different patterns of energy storage. Surprisingly, there has been little effort to determine which energetic substrates flies actually use under different types of stressful conditions. Fig. 8 depicts changes in lipid and carbohydrate contents of *D. melanogaster* exposed to desiccation stress. Whereas both lipids and carbohydrates are metabolized while flies are starved in the presence of water (not shown), glycogen provides most of the energy produced under desiccation.

It is unclear why flies switch to carbohydrate metabolism under desiccation stress. One possible benefit is increased metabolic water production, but even extremely desiccation-resistant flies with very large amounts of glycogen can obtain only a few extra hours of desiccation survival from this source (Gibbs et al., 1997). Bound water is prob-

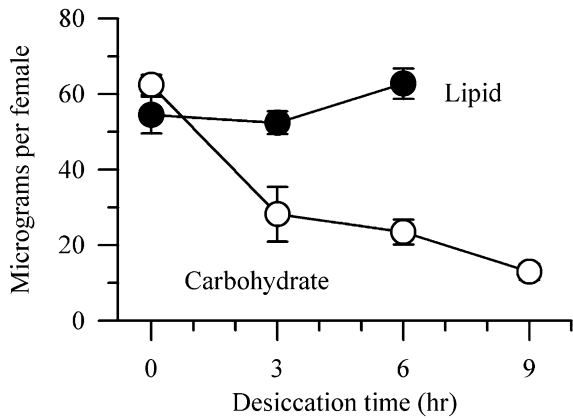


Fig. 8. Changes in carbohydrate and lipid levels in *D. melanogaster* exposed to desiccation stress. Each point represents the mean ( $\pm$ S.E.) of 12–15 individual females.

ably a more important water resource. Glycogen binds 3–5 times its mass in water, equivalent to 7 times the amount of metabolic water. This water will only be available, however, if the glycogen is metabolized, so that flies exposed to desiccation must metabolize carbohydrates to access this water source. In addition, certain tissues that require glycogen may increase their activity under desiccation stress.

### 7. Why do selection responses differ between nature and the laboratory?

The goal of this review has been to compare the evolutionary responses of *Drosophila* to water stress, under natural conditions and in artificial, laboratory deserts. Not surprisingly, many of the same responses are evident: flies from arid environments conserve water better and are less active. Other parameters have evolved differently in the lab and in nature, including water content, energy storage, and ventilatory patterns. What is different about these two superficially similar environments that could have caused divergent evolutionary responses? An obvious possibility is temperature; conditions in nature are much more variable, with temperatures in the Sonoran Desert ranging between sub-freezing and  $>40$  °C annually (and nearly as much in a single day; Gibbs et al., unpublished). It is not clear why temperature should exert these particular effects, however. For example, increased water storage can only help flies maintain water balance at high temperatures, but cactophilic *Drosophila* do not contain larger

amounts of water. One possibility is that genetic correlations between characters limit selection responses, so that there are trade-offs between the ability to tolerate temperature and desiccation stress.

Perhaps a more important environmental difference concerns the behavioral options available in these habitats. In nature, cactophilic flies are routinely exposed to desiccating conditions for short periods, but water is usually available from necrotic cactus tissues. These flies have no need to store large quantities of water, and indeed water storage may compromise flight performance and the ability to escape predators (Gu and Barker, 1995; Lehmann and Dickinson, 2001). Severe desiccation stress is most likely to occur if a cactus dries up completely, at which point the only option is to migrate to another host plant. The increased activity observed in dehydrated cactophiles (Fig. 6) therefore makes sense as a response to the loss of proper habitat. Desiccation-selected laboratory populations do not have the option of finding new habitats, and tend to be especially inactive when dehydrated (Williams, 1998). These flies have no place to go, but also do not have to avoid predation and can avoid this cost of water storage.

A final point is that not all expected physiological differences evolve, even under laboratory selection, where the environment is relatively well defined and understood. Thus, if expected differences between species or selection treatments are not present, this can not be taken as evidence that water stress, temperature, or any other environmental variable of interest is not important. Evolutionary pathways may be constrained by other factors, such as lack of genetic variation for a character, phylogenetic constraints, genetic correlations between characters, or functional constraints between different characters (e.g. flight performance vs. water storage). There is always more than one solution to a physiological problem. By using laboratory models and natural populations as complementary study systems, we can gain a better understanding of both.

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