

## EVOLUTION OF WATER BALANCE IN THE GENUS *DROSOPHILA*

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### Summary

Fruit flies of the genus *Drosophila* have independently invaded deserts around the world on numerous occasions. To understand the physiological mechanisms allowing these small organisms to survive and thrive in arid environments, we performed a phylogenetic analysis of water balance in *Drosophila* species from different habitats. Desert (cactophilic) species were more resistant to desiccation than mesic ones. This resistance could be accomplished in three ways: by increasing the amount of water in the body, by reducing rates of water loss or by tolerating the loss of a greater percentage of body water (dehydration tolerance). Cactophilic *Drosophila* lost water less rapidly and appeared to be more tolerant of low water

content, although males actually contained less water than their mesic congeners. However, when the phylogenetic relationships between the species were taken into account, greater dehydration tolerance was not correlated with increased desiccation resistance. Therefore, only one of the three expected adaptive mechanisms, lower rates of water loss, has actually evolved in desert *Drosophila*, and the other apparently adaptive difference between arid and mesic species (increased dehydration tolerance) instead reflects phylogenetic history.

Key words: desert, desiccation, *Drosophila* spp., phylogeny, water loss, evolution.

### Introduction

The subject of water balance has been central to insect physiology for over a century. The high surface area:volume ratio of insects and other terrestrial arthropods results in a relatively large area from which to lose a relatively small amount of water, leaving them vulnerable to water loss and dehydration stress. In spite of this inherent limitation, arthropods are the dominant animal taxon in deserts worldwide.

Mechanisms of surviving water stress have been investigated in scores of insect species (Hadley, 1994). These studies have revealed some expected physiological differences, such as a reduction in rates of water loss, between desert and mesic arthropods. However, while highly informative, these studies have generally had a significant limitation. Species from deserts or other arid environments (e.g. grain pests) have either been studied in isolation or compared directly with only one or a few other species, sometimes from a different genus or even a different order. In addition, the experimental methods used by different researchers have varied, making comparisons between studies difficult.

The lack of phylogenetic rigor may explain some of the conflicting results obtained in comparative studies of insect water balance. For example, one might expect desert arthropods to be highly tolerant of water loss, relative to mesic species, yet the insect with the greatest reported tolerance for

water loss is a semi-aquatic beetle, *Peltodytes muticus* (Arlian and Staiger, 1979; see table 2.1 in Hadley, 1994). The adaptive value, if any, of this is not apparent and may simply reflect the ancestry of this species. Perhaps *P. muticus* is descended from beetles that were highly tolerant of dehydration, and this character has been retained despite its lack of current utility. Without both physiological and phylogenetic information on closely related species, it may be impossible to assign adaptive value to this or any other physiological trait (Garland and Adolph, 1994).

Recent years have seen an explosion in the development and use of comparative methods to control for the phylogenetic relatedness of study species (e.g. Felsenstein, 1985; Harvey and Pagel, 1991; Garland et al., 1992; Garland et al., 1993; Garland et al., 1999; Garland and Carter, 1994). We used these techniques to test whether interspecific differences in components of water balance are, in fact, adaptations that evolved to increase desiccation resistance. Our study system is the genus *Drosophila*, whose members include both xeric and mesic species. An important advantage of this genus is that the evolutionary relationships of *Drosophila* have been studied in detail, so that we can interpret putative adaptive differences in a phylogenetic context. In principle, desiccation-resistant *Drosophila* species can differ from their mesic congeners in one or more of three ways: they can lose water less rapidly,

they can store more water in their bodies or they can better tolerate low water content. We demonstrate here that desert *Drosophila* species have adapted to arid conditions primarily by reducing rates of water loss rather than by the other two potential mechanisms.

## Materials and methods

### Species used

Twenty-nine species of *Drosophila* were studied, including representatives of both major subgenera (*Sophophora* and *Drosophila*) and species from mesic and xeric environments. Because no single source of phylogenetic information was available for these species, we assembled the phylogeny shown in Fig. 1 from several sources (Russo et al., 1995; Pitnick et al., 1995; Pitnick et al., 1999; Spicer and Jaenike, 1996; Powell, 1997; Durando et al., 2000). The topologies of the published phylogenies were consistent across studies, but divergence times were not always available. In these few cases, the timing of divergence was estimated as the mean of adjacent nodes. Collection dates and locations and ecological information are provided in Table 1. All species were maintained as large cultures (several hundred individuals per generation) at 24 °C. With a few exceptions, flies were assayed within 2 years of their original collection date.

### Assays

Our experimental procedures were essentially identical to those described previously (Gibbs et al., 1997; Gibbs et al., 1998; Nghiem et al., 2000; Gibbs and Markow, 2001). All flies were 6–10 days post-eclosion at the start of the assays. They were sexed within 24 h of eclosion, or sooner if necessary, and maintained as virgins on cornmeal medium with live yeast. Desiccation resistance was measured by placing 15–20 flies in individual 35 ml shell vials. The flies were restricted to the bottom half of the vial with a foam rubber stopper, and indicating Drierite desiccant was added above the stopper. The vials were sealed with Parafilm, and the flies were checked hourly for survivorship. Survival data were logit-transformed, and median desiccation resistance was calculated from a linear regression of the transformed data.

Initial water content was measured as described previously (Gibbs et al., 1997; Gibbs et al., 1998). Five or six groups of 3–5 flies were frozen briefly at –80 °C, thawed and weighed (to 0.1 µg) on a Cahn microbalance. The flies were dried overnight at 55 °C, then re-weighed to obtain dry mass. Water content was calculated as the difference between wet and dry mass.

The ability to tolerate dehydration was determined using the flies from the desiccation resistance assays. Immediately after death, each fly was weighed, dried overnight at 55 °C and re-weighed. Water content at death was calculated as the difference between initial and final mass. Because water content can vary greatly as a consequence of age, physiological condition or other factors (Hadley, 1994), initial water content for a subset of flies was measured during these assays for direct comparison with final water content.

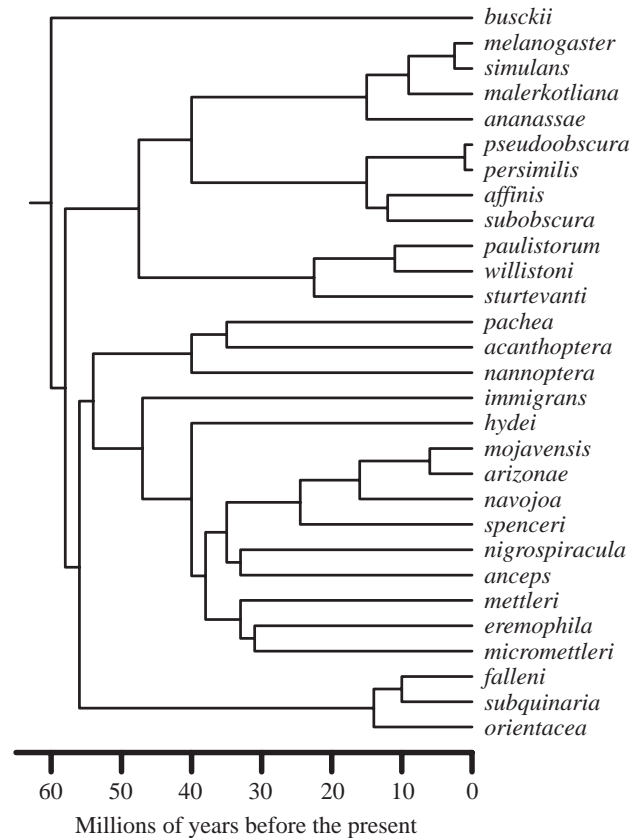


Fig. 1. Phylogeny of the *Drosophila* species used in this study. Collection data and further information are provided in Table 1.

Rates of water loss were measured using flow-through respirometry in a Sable Systems (Henderson, NV, USA) TR-2 respirometer. Five to twenty flies were placed in 5 ml glass/aluminum chambers, and dry CO<sub>2</sub>-free air was pumped through the chambers at a flow rate of 100 ml min<sup>-1</sup>. Three to eight groups of each sex were assayed for each species. The humidity sensor was calibrated by injection of small drops of water (0.5–3.0 nl) into the air stream.

### Data analysis

Because males and females differed substantially in body size (females averaged 53% larger in mass across all species), data from males and females were analyzed separately. Species were grouped according to dietary preference (i.e. cactophilic or non-cactophilic) because this distinction closely matched overall habitat differences (W. B. Heed, personal communication). Four of the 12 cactophilic species are endemic to the Sonoran Desert of southwestern North America (Table 1), whereas the others live in arid locations but are not restricted to deserts.

We used the Phenotypic Diversity Analysis Package (PDAP; Garland et al., 1993) to implement Felsenstein's (Felsenstein, 1985) method of phylogenetically independent contrasts. To account for potential effects of body size, we regressed our measures of water loss or water content against

Table 1. Collection and habitat information for the *Drosophila* species used in this study

Species name	Collection date	Collection location	Habitat or feeding preference
<i>busckii</i>	October 1999	Holland	Mesic, cosmopolitan
<i>melanogaster</i>	January 2000	Alamos, Sonora, Mexico	Mesic, cosmopolitan
<i>simulans</i>	November 1998	Tempe, Arizona, USA	Mesic
<i>malerkotliana</i>	March 1999	Panama	Mesic, tropical
<i>ananassae</i>	March 1999	Panama	Mesic
<i>pseudoobscura</i>	July 1999	Madera Canyon, Arizona, USA	Mesic
<i>persimilis</i>	1998	Mather, California, USA	Mesic
<i>affinis</i>	October 1999	Baton Rouge, Louisiana, USA	Mesic
<i>subobscura</i>	1996	Seattle, USA	Mesic
<i>paulistorum</i>	March 1999	Panama	Mesic, tropical
<i>willistoni</i>	1995	Brazil	Mesic, tropical
<i>sturtevanti</i>	March 1999	Panama	Mesic
<i>pachea</i>	November 1999	Guaymas, Sonora, Mexico	Cactophilic, Sonoran endemic
<i>acanthoptera</i>	August 1988	Chiapas, Mexico	Cactophilic
<i>nannoptera</i>	July 1998	Tehuacan Valley, Puebla, Mexico	Cactophilic
<i>immigrans</i>	September 1999	Vancouver, Canada	Mesic, cosmopolitan
<i>hydei</i>	July 1999	Madera Canyon, Arizona, USA	Mesic, cosmopolitan
<i>mojavensis</i>	January 2000	San Carlos, Sonora, Mexico	Cactophilic, Sonoran endemic
<i>arizonae</i>	April 1997	Lost Dutchman Mine, Arizona, USA	Cactophilic
<i>navojoa</i>	March 1985	Navojoa, Sonora, Mexico	Cactophilic
<i>spenceri</i>	1998	San Juan de Cabo, Baja California, Mexico	Cactophilic
<i>nigrospiracula</i>	July 1999	Tucson, Arizona, USA	Cactophilic, Sonoran endemic
<i>anceps</i>	January 1998	Infernillo, Michoacan, Mexico	Cactophilic
<i>mettleri</i>	November 1999	Guaymas, Sonora, Mexico	Cactophilic, Sonoran endemic
<i>eremophila</i>	March 1999	San Juan de Cabo, Baja California, Mexico	Cactophilic
<i>micromettleri</i>	May 1983	Jamaica	Cactophilic
<i>falleni</i>	1992	Rochester, New York, USA	Mesic, mycophagous
<i>subquinaria</i>	September 1999	Vancouver, British Columbia, Canada	Mesic, mycophagous
<i>orientacea</i>	1991	Japan	Mesic, mycophagous

For ease of comparison with Fig. 1, species are listed in the same order as they appear in the figure.

In descending order, *D. busckii* is in the subgenus *Dorsilopha*, *D. melanogaster* to *D. sturtevanti* are members of the *Sophophora* and the remainder are in the subgenus *Drosophila*.

body size (mass), then calculated independent contrasts using the residuals of these regressions (Garland et al., 1992). Other statistical analyses were performed using Systat 9 or Microsoft Excel software.

## Results

### Effects of size on desiccation resistance and water balance

An important factor in our study was body size, which varied fivefold among species. Cactophilic flies weighed approximately 40% more than mesic species, a difference that was statistically significant for females ( $t$ -test;  $P < 0.05$ ). Fig. 2 depicts the relationship between mass and desiccation resistance for 20 species of *Drosophila*. For both sexes, larger species tended to be more desiccation-resistant ( $P = 0.020$  for females;  $P = 0.058$  for males). The most resistant species, all of which survived over 24 h on average, included *D. arizonae*, *D. navojoa* and the four Sonoran Desert endemics, *D. mettleri*, *D. mojavensis*, *D. nigrospiracula* and *D. pachea*. Cactophilic species were significantly more desiccation-resistant than mesic ones ( $t$ -tests;  $P < 0.001$  for both sexes).

Rates of water loss varied sixfold among species, but were

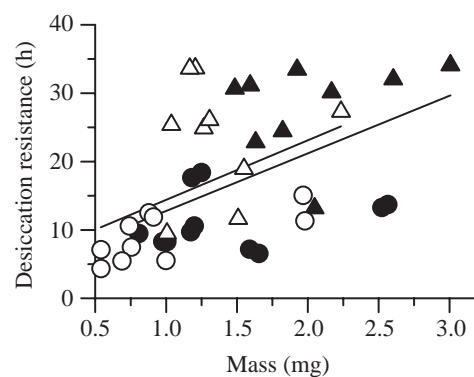


Fig. 2. Relationship between body mass and desiccation resistance (survival time) in 20 *Drosophila* species. Open symbols, males; filled symbols, females. Circles, mesic species; triangles, desert species. Regression equations were: males,  $T = 5.70 + 8.71M$ ;  $r^2 = 0.20$ ,  $P = 0.058$ ; females,  $T = 4.34 + 8.43M$ ;  $r^2 = 0.27$ ,  $P = 0.020$ , where  $T$  is survival time and  $M$  is body mass.

not significantly correlated with body size overall (Fig. 3). The data appeared to separate into two groups: cactophilic species, with rates of water loss below  $35\text{--}40\text{ nl h}^{-1}$ , and other species

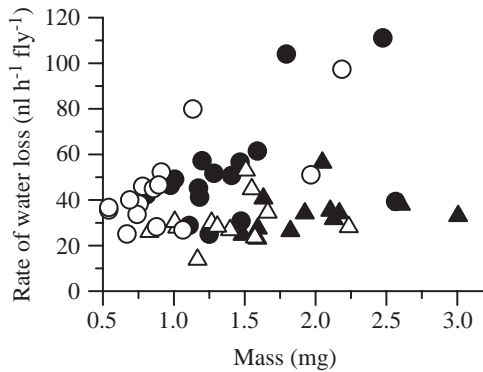


Fig. 3. Relationship between body mass and rates of water loss in 29 *Drosophila* species. Open symbols, males; filled symbols, females. Circles, mesic species; triangles, desert species.

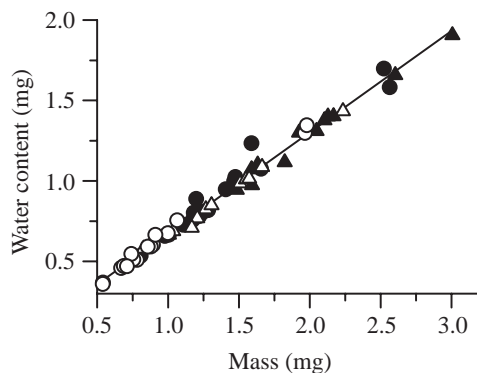


Fig. 4. Relationship between body mass and water content in 29 *Drosophila* species. Open symbols, males; filled symbols, females. Circles, mesic species; triangles, desert species. The regression line for the combined data from both sexes is  $W=0.0418+0.6305M$ ;  $r^2=0.986$ ,  $P=4.74\times 10^{-53}$ , where  $W$  is water content and  $M$  is body mass.

with generally higher rates of water loss. An analysis of covariance (ANCOVA) revealed that cactophilic species differed significantly from the other species studied ( $P<0.002$  for each sex). Within each ecological group, rates of water loss were significantly, positively correlated with mass (ANCOVA;  $P<0.05$  for each sex).

Fig. 4 depicts the relationship between mass and initial water content for 29 *Drosophila* species. Water content averaged 67% across species, ranging from 61 to 77%. Surprisingly, the lowest water contents were measured in the Sonoran Desert species (all contained less than 65% water), whereas the desiccation-sensitive mycophilic species had the highest water contents (>70%). No significant differences were found between cactophilic and mesic females, but cactophilic males had significantly lower water contents than males from mesic species (ANCOVA;  $P<0.002$ ).

The ability of flies to tolerate dehydration stress varied greatly among species. On average, flies could tolerate the loss of approximately 45% of their body water before death, but this value ranged from 30 to nearly 60% (Fig. 5). Dehydration

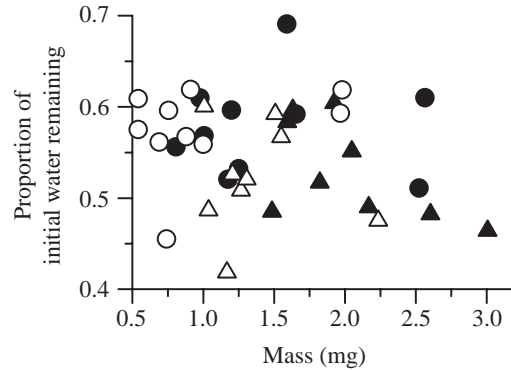


Fig. 5. Relationship between body mass and dehydration tolerance (proportion of initial water remaining) in 20 *Drosophila* species. Open symbols, males; filled symbols, females. Circles, mesic species; triangles, desert species.

tolerance was not correlated with body size ( $P>0.10$  for each sex), although cactophilic species tended to be more dehydration-tolerant than mesic species. On average, cactophilic males tolerated the loss of  $47.8\pm 2.0\%$  (mean  $\pm$  S.E.M.,  $N=9$ ) of their water before dying, whereas mesic species could tolerate the loss of only  $42.5\pm 1.5\%$  ( $N=11$ ) ( $t$ -test;  $P=0.042$ ). For females, cactophiles died after loss of  $46.9\pm 1.8\%$  ( $N=9$ ), whereas mesic females succumbed after losing  $42.1\pm 1.7\%$  ( $N=11$ ) of their water ( $t$ -test;  $P=0.068$ ).

#### *Correlations between desiccation resistance and components of water balance*

To determine the mechanistic basis for differences in desiccation resistance, we plotted survival times in dry air against each of the three components of organismal water balance. To correct for the effects of body size on rates of water loss and water content, we used the residuals of regressions against mass as the independent variables (Fig. 6). In males, desiccation resistance was significantly negatively correlated with all three components of water balance ( $P<0.001$ ,  $r^2=0.53$  for rates of water loss;  $P<0.01$ ,  $r^2=0.41$  for water content; and  $P<0.001$ ,  $r^2=0.52$  for dehydration tolerance). In females, only rates of water loss were correlated ( $P<0.001$ ,  $r^2=0.54$ ). No significant correlations were found for either water content ( $P>0.1$ ) or dehydration tolerance ( $P=0.098$ ).

In summary, increased desiccation resistance in cactophilic species of *Drosophila* appeared to result from a reduction in rates of water loss in both sexes. In addition, an increased ability to tolerate low water content was correlated with desiccation resistance in males. Water content was also correlated with desiccation resistance in males, but the direction of the relationship was the opposite of that expected on mechanistic grounds.

#### *Phylogenetic analyses*

The results presented above suggest that increased desiccation resistance in cactophilic *Drosophila* species results from a reduction in rates of water loss (in males and females)

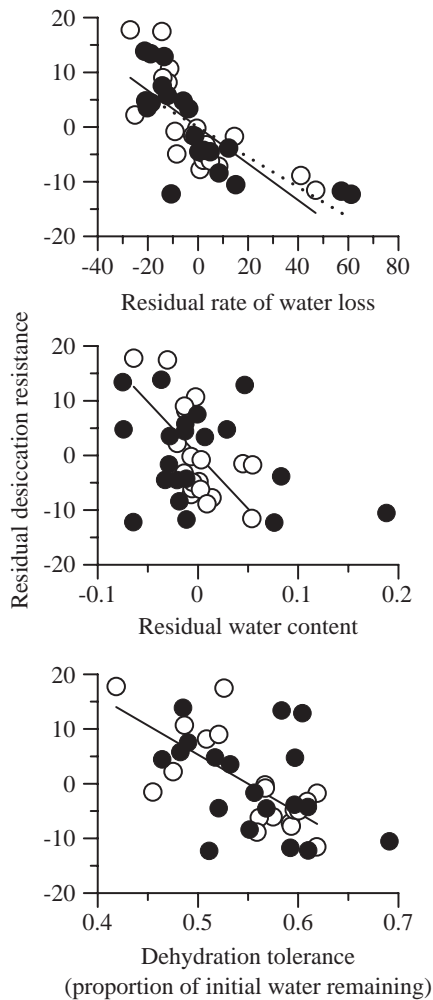


Fig. 6. Correlations between desiccation resistance and components of water balance in *Drosophila* species. To correct for the effects of body size, residuals from linear regressions are used for desiccation resistance, rate of water loss and water content. Open symbols, males; filled symbols, females. Solid lines, males; dashed line, females. See text for further details.

and greater tolerance of dehydration (in males) but not with increased initial water content. However, the cactophilic species are all members of the subgenus *Drosophila*, whereas most mesic species in this study were of the subgenus *Sophophora*. In addition, cactophiles and other drosophilans are generally larger flies, and desiccation resistance, water content and rates of water loss were significantly correlated with mass (Fig. 2, Fig. 3, Fig. 4). Thus, it is difficult to distinguish whether cactophilic species are more desiccation-resistant simply because of their size, which is associated with their phylogenetic history, or whether they indeed exhibit adaptive differences related to their habitat.

We therefore analyzed our data using Felsenstein's (Felsenstein, 1985) method of phylogenetically independent contrasts. To control for the effects of body size, we performed these analyses using residuals of regressions between mass and components of water balance. Branch lengths were obtained

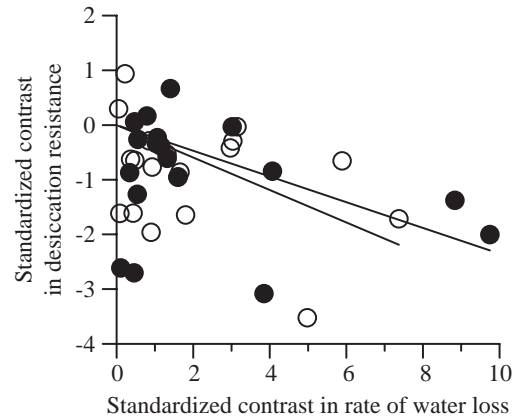


Fig. 7. Relationship between desiccation resistance and rate of water loss in 20 *Drosophila* species analyzed using the method of phylogenetically independent contrasts. Open symbols, males; filled symbols, females. Separate regression lines are drawn for males and females. See text for further details.

from the phylogeny depicted in Fig. 1. To assess the potential effects of inaccuracies in estimating branch lengths, we performed a separate analysis using constant branch lengths (Garland et al., 1992) and obtained similar results to those presented below (data not shown).

We had complete data sets available for 20 species, including nine cactophilic and 11 mesic representatives. Seven mesic species were from the subgenus *Sophophora*, two were mycophilic members of *Drosophila*, and the other two were *D. hydei* and *D. immigrans*. Two cactophiles (*D. pachea* and *D. acanthoptera*) were from the *nannopectera* group and seven were from the larger *repleta* group (which includes *D. hydei* to *D. micrometleri* in Fig. 1). This distribution minimized phylogenetic bias between ecological groups. Standardized contrasts were calculated and 'positivized' for these species, as described by Garland et al. (Garland et al., 1992), and regressions through the origin were calculated. Despite the correlations described above, we hypothesized that a phylogenetic analysis would find that desiccation resistance was positively correlated with water content and negatively correlated with rates of water loss and dehydration tolerance.

Fig. 7 depicts the results of an independent contrasts analysis of the relationship between rates of water loss and desiccation resistance. As predicted, these were negatively correlated ( $P < 0.01$  for both sexes;  $r^2 = 0.43$  for males and  $r^2 = 0.35$  for females). Thus, high rates of water loss were associated with reduced desiccation resistance.

Fig. 8 depicts the relationship between water content and desiccation resistance after correction for phylogeny. *A priori*, one would expect a positive correlation, but our initial analysis (described above) had suggested the opposite trend in males. In accordance with these analyses, females exhibited no significant correlation, but the water content of males was negatively correlated with desiccation resistance ( $P < 0.001$ ;  $r^2 = 0.59$ ).

Our initial analysis also suggested that greater desiccation

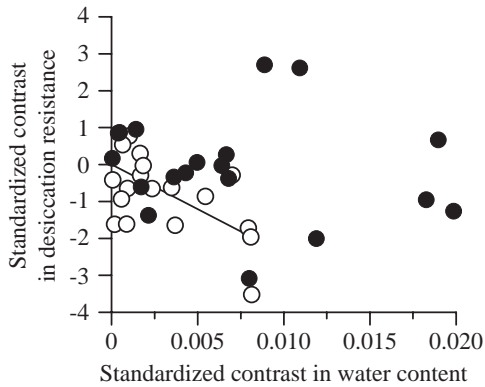


Fig. 8. Relationship between desiccation resistance and water content in 20 *Drosophila* species analyzed using the method of phylogenetically independent contrasts. Open symbols, males; filled symbols, females. Regression line is drawn for males. See text for further details.

resistance was correlated with the ability to tolerate dehydration stress, but this relationship was not supported after phylogenetic correction (Fig. 9). No significant correlation between dehydration tolerance and desiccation resistance was observed in either sex ( $P > 0.3$  for both sexes).

### Discussion

Comparative studies of desiccation resistance and water balance in *Drosophila* species have generally concluded that species from arid environments are more resistant to desiccation stress (Eckstrand and Richardson, 1981a; Eckstrand and Richardson, 1981b; Hoffmann, 1991; van Herrewege and David, 1997; Karan et al., 1998; Hercus and Hoffmann, 1999; see Hoffmann and Harshman, 1999). These studies, however, have often not included desert species of *Drosophila*, and the absence of microclimate data has made it difficult to be certain that more resistant species do in fact experience greater water stress in nature. A more important concern is that these studies have not taken into consideration the phylogenetic relationships of the study species (Garland and Adolph, 1994).

Our study illustrates the importance of considering phylogeny when performing multispecies comparisons of physiological characters. The obvious prediction one would make on the basis of habitat is supported by our work; cactophilic species, including desert endemics, survive desiccating conditions significantly longer than mesic species (Fig. 2). A simple correlative analysis suggests that cactophilic *Drosophila* exhibit two of the three expected differences in relation to their mesic congeners: reduced rates of water loss and an increased ability to tolerate dehydration (Fig. 3, Fig. 5). However, incorporation of phylogenetic relationships into the analysis reveals that the latter correlation is spurious (Fig. 9). Instead, evolved differences in desiccation resistance are due solely to changes in rates of water loss.

A potential complicating factor in comparative analyses is

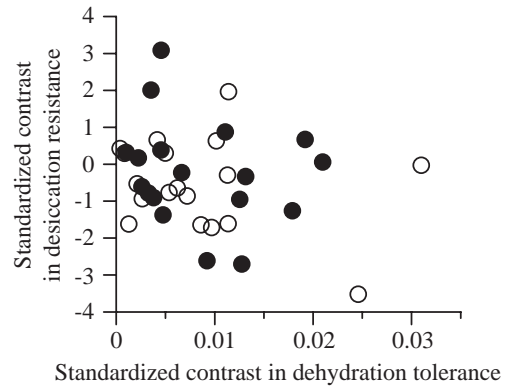


Fig. 9. Relationship between desiccation resistance and dehydration tolerance in 20 *Drosophila* species analyzed using the method of phylogenetically independent contrasts. Open symbols, males; filled symbols, females. See text for further details.

that different portions of a phylogeny may exhibit different evolutionary correlations (Garland et al., 1992). In our case, the concentration of cactophilic species within the subgenus *Drosophila* (Fig. 1; Table 1) may affect our conclusions, particularly if the relationship between desiccation resistance and components of water balance is different in these species. Within groups, however, similar trends to those obtained between cactophiles and mesic species were observed. For example, pair-wise comparisons of the Sonoran Desert endemics with each of their closest relatives revealed that Sonoran Desert endemics had a greater desiccation resistance, lower rates of water loss, lower water contents and no consistent differences in dehydration tolerance (data not shown). Within mesic *Drosophila*, the clade of mycophilic species, which occur in cool moist forests, lost water rapidly, had high water contents and were very susceptible to desiccation. Because we observed similar patterns within and between our two major ecological groups, we feel that our overall conclusions are robust and accurate.

Another factor that may affect inter-specific comparisons is intra-specific variation. Geographic variation in desiccation resistance has been documented for several *Drosophila* species (Coyne et al., 1983; Da Lage et al., 1990; Davidson, 1990; Hoffmann, 1991; Karan et al., 1998). If anything, however, our choice of populations should have minimized potential bias towards species differences. We studied three mesic species (*D. melanogaster*, *D. simulans* and *D. pseudoobscura*) that exhibit significant geographic variation in desiccation resistance (Hoffmann and Harshman, 1999). These were collected from Arizona or Sonora (Table 1), relatively arid regions of their ranges. Thus, these populations may have been adapted to local dry conditions, which would tend to minimize differences between them and cactophilic species.

Adaptation of *Drosophila* stocks to laboratory culture should also be considered. We used recently collected strains whenever they were available, but several had been in culture for over a decade (Table 1). We note, however, that the three species in culture the longest were cactophilic and that these

were similar in desiccation resistance, rates of water loss, etc. to more recently collected cactophiles. Because long-term culture had no apparent major effects on water balance in our study, nor does it affect thermal tolerance in *D. melanogaster* (Krebs et al., 2001), we believe that it did not significantly affect our conclusions.

Previous comparative studies of *Drosophila* species have generally not considered the mechanistic basis for differences in desiccation resistance, but our results are generally consistent with published work. For example, a non-phylogenetic study (Van Herrewege and David, 1997) found that desiccation resistance in 22 *Drosophila* species correlated positively with mass but not with water content. Other authors have reported that more desiccation-resistant species lose water less rapidly (Eckstrand and Richardson, 1981a), although Eckstrand and Richardson (Eckstrand and Richardson, 1981b) reported relatively high rates of water loss in the Sonoran endemic *D. nigrospiracula*. Variation in dehydration tolerance has received little attention from *Drosophila* researchers, and no consistent correlations with habitat have been detected (Eckstrand and Richardson, 1981b).

An important issue, which has received surprisingly little attention from researchers on *Drosophila*, is the actual environmental regime faced by flies in nature. Previous comparative and geographic studies in *Drosophila* have emphasized the importance of latitude as an environmental variable (David and Capy, 1988; van Herrewege and David, 1997; Karan et al., 1998). Deserts occur primarily at mid-latitudes, and their severity is strongly affected by local topography (Louw and Seely, 1982), so this approach risks conflating species from very different habitats within a single category. Because we were concerned solely with water balance, whereas previous studies were often also concerned with starvation resistance, we felt that a comparison based on habitat aridity was more appropriate for this work. Feeding habit (cactophily) is not a perfect correlate for habitat, but species feeding on necrotic cacti should be exposed to more desiccating conditions. This assumption is supported by the relatively few microclimate studies concerning *Drosophila* species that have been performed (e.g. Junge-Berberovic, 1996; Feder et al., 1997; Feder et al., 2000; A. G. Gibbs, M. C. Perkins and T. A. Markow, in preparation).

Our results provide an interesting contrast to those obtained using laboratory populations of *D. melanogaster* selected for increased desiccation resistance (Hoffmann and Parsons, 1993; Gibbs et al., 1997). Selected flies lose water less rapidly and may contain much more bulk water than unselected control populations (Gibbs et al., 1997). Clearly, water content can evolve under desiccation selection, but has not done so in nature. One potential explanation is that desert flies have water freely available from their food source, necrotic cacti, and therefore have not been subject to selection for water storage. Alternatively, water storage may come at a cost, such as decreased flight performance (Lehmann and Dickinson, 2001). Trade-offs may exist between survival of desiccation stress and

the locomotory ability required to find new necroses or to avoid predation.

Even if natural selection has not favored higher water content and increased dehydration tolerance, one would still expect these variables to be correlated with desiccation resistance. All else being equal, flies containing more water or better able to tolerate low water content should survive desiccation stress longer. The negative relationship between water content and desiccation resistance in males was particularly surprising. One possible explanation is that desiccation-resistant flies need larger stores of glycogen or lipid to survive, since the desiccation resistance assay involved removal of both food and water. This need for energy storage would result in a greater relative amount of dry mass, but one would then expect a similar pattern in females. Alternatively, the low water:mass ratios of cactophilic males may be a function of their large testes, which are needed for the production of their large ejaculates (up to 2% of their mass; Markow et al., 1990). Thus, the apparently low water contents of cactophilic species may result from differences in reproductive physiology.

In summary, our studies demonstrate that *Drosophila* species from the Sonoran Desert and other arid environments are more resistant to desiccation stress than other species. By incorporating phylogenetic information into our analysis, we have demonstrated that these differences have been achieved solely by reductions in rates of water loss. Although cactophilic species are more tolerant of dehydration stress than mesic congeners, this appears to be an ancestral trait in cactophiles. Their greater dehydration tolerance may have contributed to the ability of these flies to survive and diversify in arid environments, but cannot be considered an adaptation specifically related to evolution in these habitats. Our results indicate that future evolutionary studies should focus on the physiological mechanisms by which cactophilic *Drosophila* species conserve water rather than other components of organismal water balance.

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