

Effects of Age on Water Balance in *Drosophila* Species

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ABSTRACT

Age-related declines in physiological performance have been documented in a wide variety of organisms. However, it is unknown whether related species age in a similar manner or whether physiological differences associated with aging differ widely among species. Previous work has shown that the desiccation resistance of *Drosophila melanogaster* decreases rapidly with age. Other *Drosophila* species may have delayed reproductive maturity or may inhabit arid habitats, so that age-related changes in water balance may be a significant physiological constraint on their ecological success. We may, therefore, predict that physiological systems involved in water balance will deteriorate with age at a reduced rate in these species. We quantified several components of water budgets for 14 species of *Drosophila*, including both desert and mesic representatives differing in their age of maturity. Desiccation resistance decreased with age in all but one species studied, primarily because of increased rates of water loss. However, there was no significant relationship between the rate of aging, as indicated by the rate of increase in water-loss rates as flies aged, and either habitat or age of maturity.

Introduction

Aging can be defined as an increase in the mortality rate of a cohort of organisms over time (Finch 1990). The evolutionary causes of aging are well understood. The intensity of natural selection declines after organisms attain reproductive maturity and have passed their genes on to the next generation (Rose 1991). However, knowing why organisms age does not allow us to predict how they will age. What is the physiological basis for increased mortality rates? Do all physiological systems de-

cline in function, or are some systems more vulnerable than others? Are the mechanisms of aging general across species, or do they differ even among closely related species?

Numerous studies have examined age-related changes in physiological performance, particularly in humans and model organisms such as mice, fruit flies, and nematodes. A common finding is that older individuals exhibit decreased resistance to stresses such as high or low temperature, exercise, starvation, desiccation, oxidative stress, and so forth. Most research on aging and stress resistance in insects has used the cosmopolitan human commensal, *Drosophila melanogaster*. Fruit flies become less resistant to desiccation stress as they age (Lamb 1984; Service et al. 1985; Drapeau et al. 2000), and selection for postponed senescence has resulted in populations (O populations) that are more desiccation resistant than unselected controls (B populations; Service et al. 1985; Chippindale et al. 1998). Both B and O populations undergo an age-related decline in desiccation resistance, which can be attributed to increased rates of water loss (Nghiem et al. 2000). However, the O flies exhibit a significantly slower increase in water-loss rates (WLR), indicating that their water conservation mechanisms age more slowly than those in the B flies.

Several factors suggest that patterns of aging in *D. melanogaster* may not be representative of other members of the genus. This species is relatively short lived, exhibits low desiccation resistance, and loses water rapidly for its size (van Herreweghe and David 1997; Hoffmann and Harshman 1999). Many other *Drosophila* species are cactophilic and inhabit desert environments where their host plants are found. Species from arid habitats lose water less rapidly when young (Gibbs and Matzkin 2001) but will still be at greater risk of desiccation stress at later ages. Thus, one would predict that desert flies will maintain their ability to conserve water at all ages.

In addition to ecological considerations, most *Drosophila* species attain reproductive maturity at a later age than *D. melanogaster*. For example, males of *Drosophila packera*, a species endemic to the hot Sonoran Desert of North America, require 2 wk to mature (Pitnick et al. 1995), compared with <1 d for *D. melanogaster*. Clearly, a fly must survive long enough to reproduce to have nonzero fitness. Thus, we can also predict that slowly maturing *Drosophila* will exhibit slower rates of physiological aging than will rapidly developing congeners. For example, rates of water loss might remain constant until flies have matured and then begin to increase, or they might increase throughout adulthood but do so at a slower rate than in *D. melanogaster*.

In this study, we compared the effects of age on desiccation resistance and water balance in 14 species of *Drosophila*. These

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species included representatives from desert, arid, and mesic habitats, which reach reproductive maturity at ages ranging from 1 to 14 d. Our first objective was to determine whether *Drosophila* species in general exhibit similar age-related changes in water balance. We also tested two hypotheses: first, that *Drosophila* species that mature more slowly will exhibit slower age-related changes in desiccation resistance and WLR; and second, that species inhabiting more arid environments will exhibit slower changes as they age.

Material and Methods

Species Used

Table 1 lists the species used in this study as well as collection data, reproductive ages, and habitat information. As adults, all flies were reared and maintained at 24°C on cornmeal medium with live yeast. Flies were held at densities of 10–20/vial and transferred to fresh medium twice each week. All flies used in experiments were virgins collected within 24 h of eclosion, or less if necessary. A single cohort of flies emerging from the same culture bottles was used for all experiments of a given type (e.g., all measurements of WLR for a given species).

Desiccation Resistance Assays

Groups of flies were transferred to empty 35-mL shell vials, and a foam rubber plug was used to keep flies in the bottom half of the vial. The remainder of the vial was filled with indicating silica gel desiccant. The vial was sealed with Parafilm, and the number of dead flies was counted hourly. Males and

females were assayed separately. Two assays were performed for each species, using a single cohort of flies collected from the same culture bottles. The first assay was performed at an age of 7–10 d, and the second was performed, using the remaining flies, approximately 3 wk later. Ten *Drosophila* species were assayed for desiccation resistance at both ages. Typically, six to eight groups of five flies each were assayed for each experiment. Data for percent survival were logit transformed, and we calculated median survival times by linear regression of the transformed data.

Rates of Water Loss

WLRs were measured essentially as described previously, except that a flow rate of 100 mL/min was used (Gibbs et al. 1997, 1998; Nghiem et al. 2000). Flies were placed in 5-mL glass–aluminum respiration chambers, which were placed in a flow-through respirometry system (Sable Systems, Henderson, Nev.). Average rates of water loss and metabolic rates (measured as release of carbon dioxide) were calculated from two 15-min measurements on each group of flies. Placement of flies in the chambers was staggered so that measurements were performed when the flies had been in the chambers for 3 h and again for 5 h. Typically, three groups of four to 10 flies of each gender were assayed at each age. Rates of water loss per individual were not affected by the number of flies in the chamber. Assays were begun at 4–10 d of age and were repeated weekly until all flies had died.

Table 1: Collection data for species of *Drosophila* used in this study

Species Name	Date of Original Collection	Collection Location	Age at Maturity (d)		Habitat Type
			Female	Male	
<i>Drosophila acanthoptera</i>	August 1988	Chiapas, Mexico	6	6	Arid
<i>D. anceps</i>	January 1998	Infiernillo, Michoacan, Mexico	5	3	Arid
<i>D. busckii</i>	October 1999	Holland	2	0	Mesic
<i>D. hydei</i>	July 1999	Madera Canyon, Arizona	3	9	Mesic
<i>D. melanogaster</i>	January 2000	Alamos, Sonora, Mexico	4	2	Mesic
<i>D. mettleri</i>	November 1999	Guaymas, Sonora, Mexico	3	2	Desert
<i>D. micromettleri</i>	May 1983	Jamaica	3	1	Arid
<i>D. mojavensis</i>	January 2000	San Carlos, Sonora, Mexico	4	5	Desert
<i>D. nannoptera</i>	July 1998	Tehuacan Valley, Puebla, Mexico	4	8	Arid
<i>D. navojoa</i>	March 1985	Navojoa, Sonora, Mexico	4	6	Arid
<i>D. nigrospiracula</i>	July 1999	Tucson	3	5	Desert
<i>D. pachea</i>	November 1999	Guaymas, Sonora, Mexico	3	14	Desert
<i>D. paulistorum</i>	March 1999	Panama	Mesic
<i>D. pseudoobscura</i>	July 1999	Madera Canyon, Arizona	3	1	Mesic

Note. Habitat designations are as follows: desert, cactophilic species endemic to Sonoran Desert; arid, cactophilic species not endemic to deserts; mesic, noncactophilic species.

Body Size

Flies were frozen at -80°C immediately after measurement of WLR. The flies were then weighed within 2 h, dried overnight at 55°C , and weighed again. Water content was calculated as the difference between total and dry mass. We corrected for water lost during respirometry, using the mean WLR measured for each group of flies multiplied by the length of time spent in the respirometer. A similar correction was made for dry mass used during metabolism. This was calculated from the mean carbon dioxide production under the assumption that carbohydrate was the sole metabolic fuel (Djawdan et al. 1996; M. T. Marron and A. G. Gibbs, unpublished data). Preliminary experiments indicated that this procedure provided estimates of mass and water content that were indistinguishable from those of untreated flies.

Data Analysis

Figure 1 shows a phylogeny for the species studied, which was based on several recent sources (Pitnick et al. 1995; Durando et al. 2000) and was used for data analysis. We analyzed our data using independent contrasts (Felsenstein 1985), using the Phylogenetic Diversity Analysis Package (Garland et al. 1993) or Comparative Analysis by Independent Contrasts (Purvis and Rambaut 1995). When necessary, data were transformed to meet the statistical assumptions of this approach. Standard statistical tests were performed using Systat version 9 (Systat, Ev-

anston, Ill.). Because female flies were larger than males in all species studied and differed from males in age of maturity and desiccation resistance, we analyzed data for males and females separately.

For comparisons based on habitat, species were divided into three categories based on their natural histories and species ranges (Table 1). In order of decreasing aridity, these were desert (Sonoran Desert endemics), arid (cactophilic species that may occur in, but are not restricted to, deserts), and mesic species.

Results

Water Balance in Young *Drosophila*

In young flies (7–10 d old), resistance to desiccation varied widely among *Drosophila* species (Table 2). Desert endemics survived >24 h in dry air, whereas mesic congeners died within 16 h. Rates of water loss varied threefold among species (Fig. 2). This variation was related to body size and habitat. For their size, desert endemic *Drosophila* tended to lose water less rapidly than species from less arid and mesic environments, although the differences were not statistically significant (ANCOVA; $0.05 < P < 0.1$ for both sexes).

Age-Related Changes in Water Balance within *Drosophila* Species

Desiccation resistance decreased with age for all 10 species of *Drosophila* assayed except *Drosophila micrometleri* (Table 2),

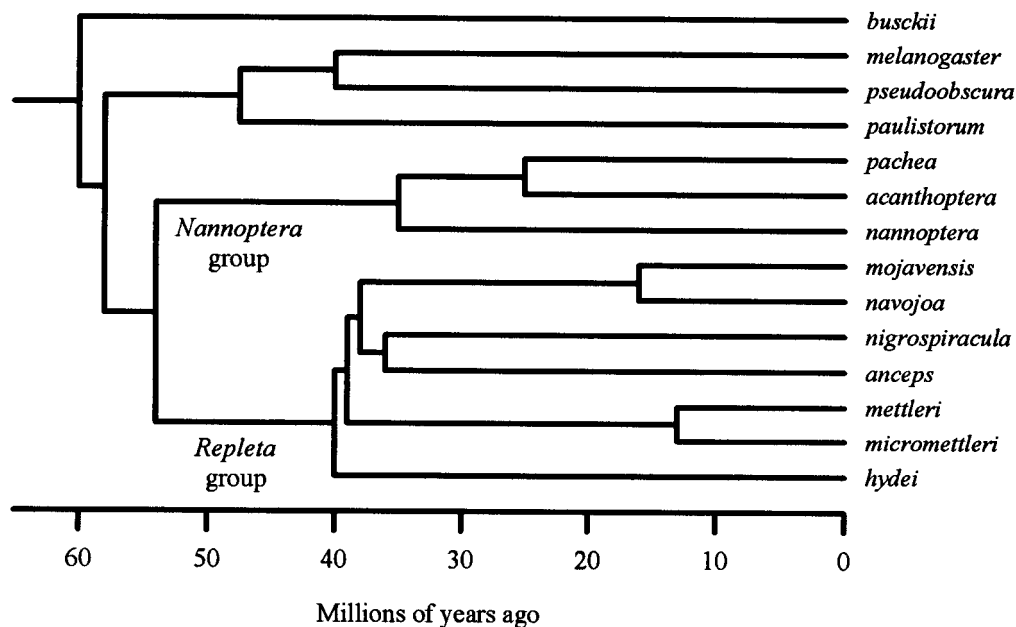


Figure 1. Phylogeny of *Drosophila* species used in this study, based on information in Pitnick et al. (1995) and Durando et al. (2000)

Table 2: Effects of age on desiccation resistance in *Drosophila* species

Species	Males		Females	
	Young Flies	Old Flies	Young Flies	Old Flies
<i>Drosophila acanthoptera</i>	12.18 (.33)	9.23 (.21)	13.13 (.12)	8.37 (.13)
<i>D. anceps</i>	16.87 (.83)	9.90 (.35)	29.72 (.38)	18.15 (.18)
<i>D. hydei</i>	15.27 (.67)	9.87 (.17)	12.83 (.06)	11.45 (.23)
<i>D. melanogaster</i>	7.50 (.09)	5.17 (.10)	10.39 (.12)	5.95 (.11)
<i>D. mettleri</i>	25.21 (.17)	22.03 (.18)	29.66 (.47)	21.61 (.25)
<i>D. micromettleri</i>	8.85 (.12)	8.73 (.26)	nd	nd
<i>D. mojavensis</i>	45.70 (.38)	28.69 (.33)	36.89 (.32)	28.58 (.31)
<i>D. nigrospiracula</i>	25.85 (.34)	22.87 (.19)	33.84 (.11)	27.11 (.28)
<i>D. paulistorum</i>	5.02 (.11)	3.67 (.24)	7.95 (.09)	6.22 (.07)
<i>D. pseudoobscura</i>	11.79 (.17)	10.87 (.20)	15.63 (.60)	14.11 (.22)

Note. Depending on the species, flies were assayed at 7–11 d (young flies) and 28–31 d (old flies). Desiccation resistance values are in hours. Data are medians (SE) calculated from regressions of logit-transformed data; nd = no data.

with the magnitude of the decline ranging between 8% and 43%. The average percentage decrease in desiccation resistance was 22.9% ($n = 10$ species; 95% CI = 13.7%–33.2%) for males and 25.5% ($n = 9$ species; 95% CI = 16.6%–35.0%) for females. An eleventh species, *Drosophila busckii*, was not assayed for desiccation resistance, but we observed significant and increasing mortality during water-loss measurements as these flies aged. (These data were excluded from analyses.) These observations are consistent with reduced desiccation resistance in older *D. busckii* as well.

Figure 3 depicts the effects of age on rates of water loss in adult *Drosophila*. In males, WLR increased significantly with age in 11 of 14 species studied. The exceptions were *Drosophila mojavensis*, *Drosophila navojoa*, and *Drosophila anceps*, which exhibited positive but insignificant increases in WLR. We found no evidence that WLR remained constant until reproductive maturity then began to increase.

Females followed a pattern to similar that of males. Calculated linear regression slopes (WLR slopes) were positive for all species except *D. navojoa*. Seven species exhibited a significant increase in WLR, and two exhibited a marginally significant increase ($0.05 < P < 0.10$). Female *Drosophila mettleri* and *Drosophila nanoptera*, in addition to the three species listed above for males, exhibited no significant age-related changes in WLR.

Within a given species, increased rates of water loss in older flies could be related to changes in body size. Older females might become larger because of egg storage, or flies of either sex might store more lipid or glycogen as they age (Fairbanks and Burch 1970; Service 1987; Djawdan et al. 1996). The consequent increase in surface area might then result in increased cuticular water loss. The effects of age on total mass, dry mass, and water content are shown in Figure 4 for four representative *Drosophila* species. No consistent age-related patterns were ob-

served. Both positive and negative correlations between age and size were obtained. In females, water contents tended to increase slightly, whereas dry weights decreased, leading to no net gain or loss in total mass. In males of several species, both water content and dry mass decreased, so that total mass also decreased. Water content, when expressed as the percentage of total mass, increased with age in both genders of all species studied.

Higher rates of water loss might also be caused by increased respiratory losses associated with higher activities or metabolic rates. However, metabolic rates did not consistently increase or decrease with age (Fig. 5). We therefore concluded that changes in body size and metabolic rates were not responsible for the increased WLR of older flies.

Comparison of Age-Related Changes among *Drosophila* Species

Variation in body size among *Drosophila* species had to be accounted for in our interspecific analyses. In general, one would expect larger species and mesic species to lose water more rapidly. One would also expect a positive correlation between absolute rates of water loss and age-related increases in WLR. For example, WLR might double over the first month of life for two different species, but the absolute rate of increase would be greater for the species that initially lost water more rapidly.

To account for interspecific variation in body size, we first investigated correlations between WLR, measured at 7–10 d of age, and mass (Fig. 2). No significant correlations were detected, either in simple linear regressions of raw data or in analyses using independent contrasts to control for phylogenetic relationships.

We then looked for a correlation across species between absolute WLR and WLR slopes, using independent contrasts. If

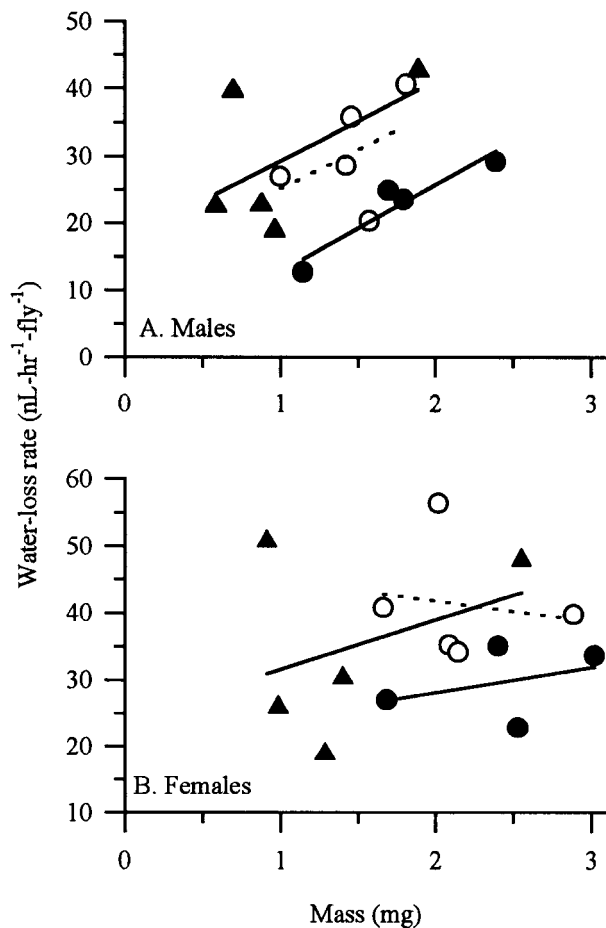


Figure 2. Relationship between mass and water-loss rates in *Drosophila* species from different habitats (see Table 1). Rates of water loss were measured in 7–10-d-old flies. Filled circles, desert endemic species; open circles, species from arid habitats; triangles, mesic species.

larger slopes were caused by higher absolute rates of water loss, then one would expect a significant positive correlation. In females, no significant relationship was detected. In males, however, WLR slopes were positively correlated with WLR. Inspection of plots suggested that this relationship was primarily driven by one species, *Drosophila acanthoptera*, which lost water faster than any other species and whose WLR slope was over twice as high as that of any other species (Fig. 6). The Studentized residual for *D. acanthoptera* was 3.72, indicating that this species was a significant outlier. When *D. acanthoptera* was omitted from the analysis, no correlation between WLR and WLR slope was detected.

We concluded from these analyses that differences in WLR slopes could not be attributed simply to differences in body size or absolute rates of water loss. We then used independent contrasts to examine the relationship between age of repro-

duction and WLR slope. We had predicted that species in which flies, especially males, matured more slowly would exhibit smaller WLR slopes (i.e., a negative correlation). However, the correlation was not significant in either males or females (Fig. 7).

Our second prediction was that *Drosophila* species from arid environments would exhibit a slower increase in WLR with age than mesic species because of the greater need for water conservation at all ages. In this analysis, habitat (desert endemic, arid, mesic) was used as a categorical variable to predict WLR slope (Purvis and Rambaut 1995). An important issue is assigning character states to ancestral nodes. On the basis of geological information about the history of the Sonoran Desert and southwestern North America (Scarborough 2000), we assumed that the ancestral state was always the more mesic choice. The statistical power of this analysis was low because there were only six habitat transitions available (see Table 1; Fig. 1). As shown in Figure 8, no significant relationship between habitat and WLR slope was obtained.

As an alternative to the explicit evolutionary assumptions of independent contrasts, we also compared species from different habitats using a standard ANOVA. The assumption here is that species have adapted to their current habitats recently (within the time since their most recent common ancestors) and, therefore, can be treated as being statistically independent of each other. No significant relationship between habitat and WLR slope was found for either sex ($P > 0.1$ for both cases).

As described earlier (see "Age-Related Changes in Water Balance within *Drosophila* Species"), WLR slopes were positive for 27 out of 28 total species–gender combinations, and 18 of these were significantly greater than 0. We noted that nine of the 10 nonsignificant slopes were in *repleta* group species, with one other in the related *nannoptera* group. This pattern suggested a possible phylogenetic component to variation in WLR slope (i.e., *repleta* group species having smaller values), so we compared *repleta* group species with all others by *t*-test. No correlation was found in females ($P > 0.9$), but in males the relationship was nearly statistically significant whether or not *D. acanthoptera* (an outlier, see Fig. 6) was included in the analysis ($0.05 < P < 0.1$).

Discussion

We found that an age-related decrease in desiccation resistance is characteristic of the genus *Drosophila*, with nine out of 10 species assayed exhibiting a significant decrease between 7–10 d and 1 mo in age (Table 2). In terms of organismal water balance, there are three possible causes for these changes: older flies could contain less water, they could lose water faster, or they could be less tolerant of low water content. In *Drosophila melanogaster*, several studies have shown that reduced desiccation resistance is primarily caused by increased rates of water loss rather than reductions in water content or dehydration

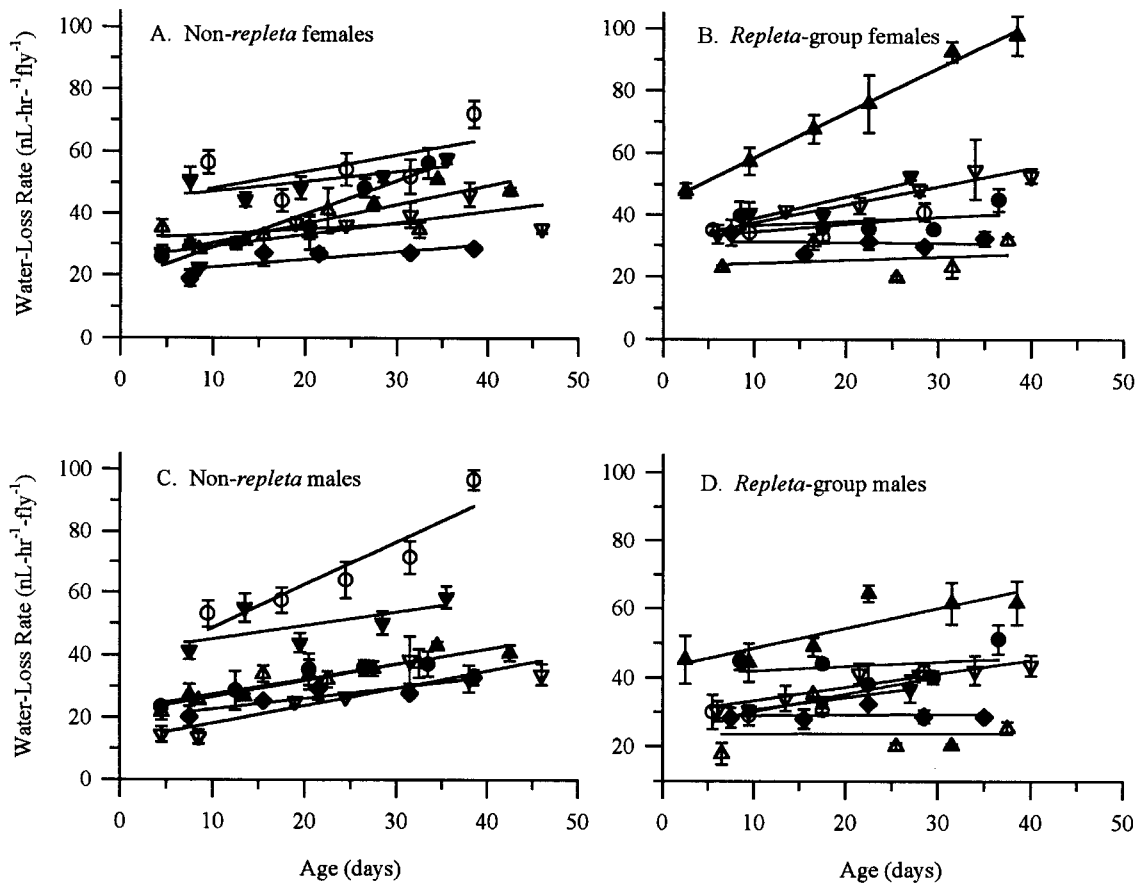


Figure 3. Effects of age on rates of water loss from *Drosophila* species. For clarity, the data have been separated into *repleta* group species and others.

tolerance (Fairbanks and Burch 1970; Lamb 1975, 1984; Service 1987; Graves et al. 1992; Nghiem et al. 2000). We therefore focused our attention on the contribution of changes in WLR to changes in desiccation resistance. Our study reveals that other *Drosophila* species exhibit similar patterns and further demonstrates that reduced desiccation resistance in older flies is not caused by changes in metabolic rate.

A critical factor to be considered in these experiments is body size, which can affect desiccation resistance in many ways. Larger flies will have greater surface areas, which may increase rates of water loss. However, larger flies may also have greater water stores available for resisting desiccation stress. We found no consistent age-related changes in body size or absolute water content (Fig. 4). The only pattern observed was an increase in the fraction of body mass consisting of water because of the fact that water content often tended to increase slightly, whereas dry mass decreased slightly. Thus, at the same body size, older flies tended to contain more water, which would serve to increase, not decrease, their resistance to desiccation.

We did not quantify the ability to tolerate dehydration stress (measured as water content at the time of death; Gibbs et al. 1997). Studies using *D. melanogaster* indicate that this parameter is not affected by age (Nghiem et al. 2000). Thus, neither water content nor dehydration tolerance appear to account for reduced desiccation resistance in older flies. This leaves increased rates of water loss the primary explanation. In agreement with this conclusion, in 27 out of 28 cases, plots of WLR versus age had positive slopes, and 18 slopes were significantly greater than zero (Fig. 3).

Our experiments were not designed to determine the mechanistic basis for increased rates of water loss, but our data do allow us to discount some potential causes. Water is lost from insects via three major routes: excretion from the anus or mouthparts, respiratory losses through open spiracles, and transpiration through the cuticle. Excretory losses could be detected as large bursts of water flux because of the evaporation of water from feces or saliva. We quantified the amount of water lost during these events in several dozen recordings from

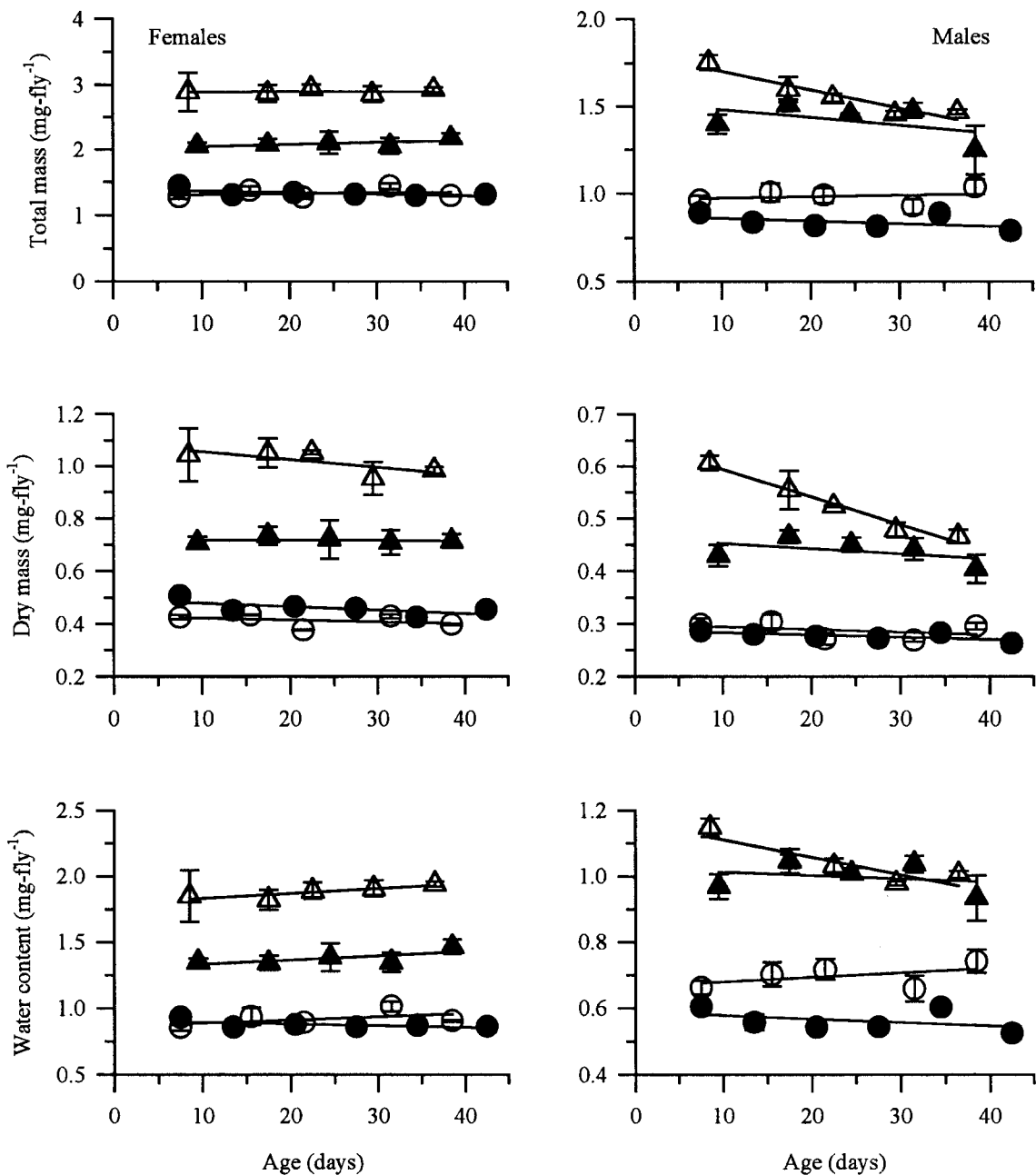


Figure 4. Effects of age on body size in four representative species of *Drosophila*. Data for females are on the left; data for males are on the right. Filled circles, *Drosophila melanogaster*; open circles, *Drosophila pseudoobscura*; filled triangles, *Drosophila acanthoptera*; open triangles, *Drosophila anceps*.

different species at different ages (data not shown). Excretory losses contributed <10% of total water loss and, therefore, could not be responsible for the twofold or greater increase in water loss observed in some species.

We could not rigorously distinguish respiratory and cuticular

water loss from each other, as these measurements require the use of individual insects (Lighton 1994; Williams and Bradley 1998). Two factors could cause an increase in respiratory water loss: higher metabolic rates or the loss of spiracular control. Metabolic rates did not generally increase with age and, indeed,

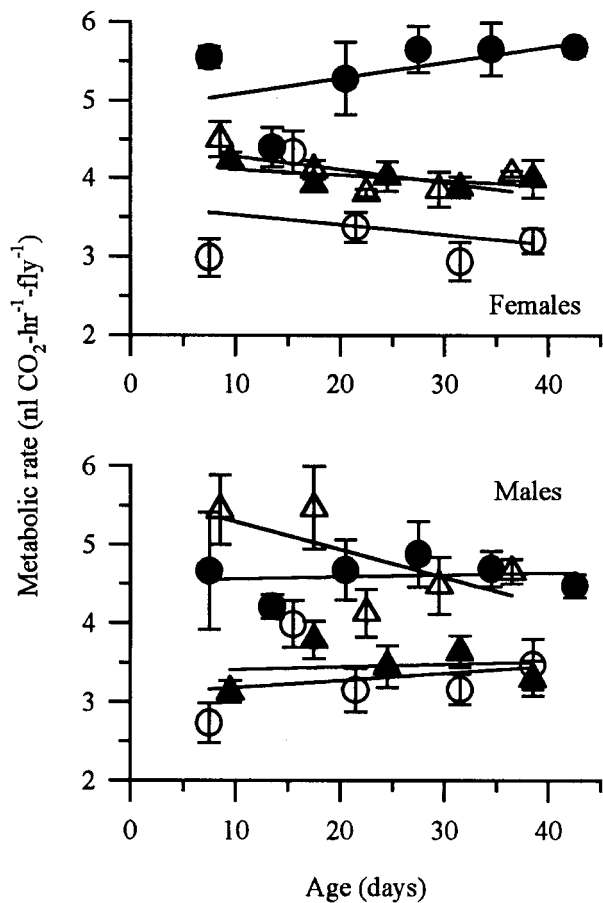


Figure 5. Effects of age on metabolic rates in four representative species of *Drosophila*. Species and symbols are the same as in Figure 4.

often decreased (Fig. 5). Similar findings have been reported for *D. melanogaster* (e.g., Service 1987; Arking et al. 1988; Djawdan et al. 1996). It should be noted, however, that we measured metabolic rates as the release of CO_2 rather than as oxygen consumption. Age-related changes in metabolic fuel used, from carbohydrates to lipids, could mask an increase in oxygen consumption and ATP production of up to one-third (Schmidt-Nielsen 1990). Increased oxygen consumption could require that the spiracles remain open longer and expose the insects to increased water loss. The ability of flies to regulate spiracular opening may also deteriorate as they age (Fairbanks and Burch 1970). If respiratory differences are not responsible for higher WLR as flies age, changes in cuticular permeability remain as the primary cause of higher WLR in older *Drosophila*, a conclusion reached in the case of aging-selected populations of *D. melanogaster* (Nghiem et al. 2000).

Whatever the mechanistic basis, our data clearly indicate that *Drosophila* lose water more rapidly as they age. Life history

theory and ecological considerations suggest that the pattern of increase in WLR should differ among species. One possibility is that rates of water loss could be low and constant until reproductive age in each species and only increase in post-reproductive flies. No evidence for this pattern was obtained either in our study or in previous work on populations of *D. melanogaster* that do not reproduce until they are 60 d old (Nghiem et al. 2000). Instead, we observed a consistent increase in WLR as flies aged.

An important factor in interspecific comparisons is body size, yet we found no correlation between mass and WLR. This was because desert species tended to be larger than *Drosophila* from more mesic habitats and lost water relatively slowly for their size (Fig. 2). Because size was not correlated with WLR, which were not correlated with WLR slopes, size turned out not to be a contributing factor to variation in slopes among species.

Our first hypothesis, that species that mature more slowly would exhibit slower increases in rates of water loss, was not supported. When the data were corrected for phylogenetic relatedness, neither males nor females exhibited the expected correlation (Fig. 7). Indeed, the only pattern we were able to detect for increased WLR was phylogenetic. Males of species from the *repleta* group exhibited a slower increase in WLR than other *Drosophila* species ($P = 0.055$; see Fig. 3). A phylogenetic component to mechanisms of aging is not entirely unexpected. Aging is the result of the decreased strength of natural selection in older individuals, which can allow the accumulation of mutations having deleterious late-life effects (Arking 1998). Thus, one would expect little selective pressure to modify the basic physiological changes associated with aging, so that a species'

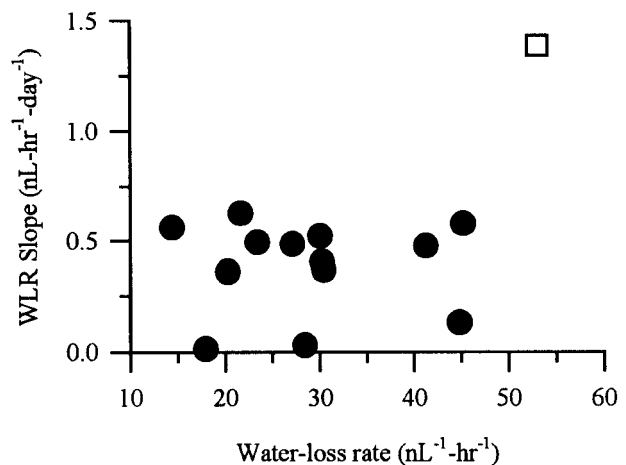


Figure 6. Relationship between water-loss rates (WLR; measured between 7 and 10 d in age) and WLR slopes in male *Drosophila*. The open square indicates data for *Drosophila acanthoptera*, which should be considered an outlier.

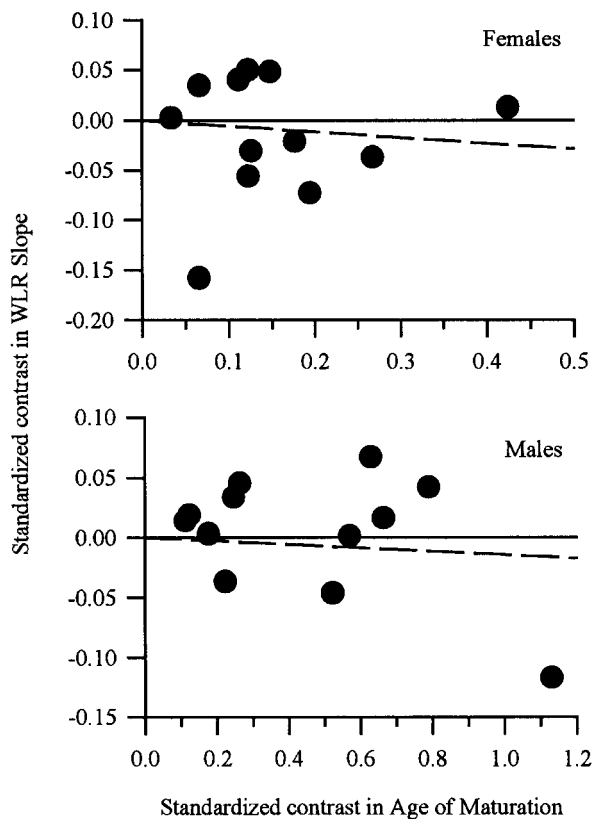


Figure 7. Independent contrasts analysis of the relationship between age of maturity and water-loss rates slope. Dashed regression lines are not statistically significant.

descendants are likely to retain similar features of the aging process. It is surprising, however, that *Drosophila* species exhibiting delayed reproduction did not differ from their rapidly developing relatives. Delayed maturity has been correlated with longer life span in *Drosophila*, and direct selection for delayed reproduction in *D. melanogaster* significantly reduces WLR slopes (Nghiem et al. 2000).

Our second hypothesis also was not supported. *Drosophila* from more arid habitats did not exhibit a significant difference in the age-dependence of WLR. Two potential limitations of this analysis need to be noted, however. First, because of the concentration of cactophilic species in the subgenus *Drosophila* (which includes the *repleta* and *nannoptera* groups), the number of transitions between habitat states (which are necessary for analysis using independent contrasts) was low. Second, we distinguished between Sonoran Desert endemics and other, nonendemic cactophiles, a distinction that may not be appropriate. Geological evidence suggests that the Sonoran Desert is ~12,000 yr old (Scarborough 2000), whereas the species in this study diverged from each other >15 million yr ago (Fig. 1).

Thus, the current habitat of these species is much younger than their respective lineages. Because a fossil record is lacking, we cannot know whether their ancestors were restricted to a pre-Sonoran desert habitat or whether endemism is a newly derived trait. On the basis of these considerations, one could also assume that the species are effectively evolutionarily independent and use standard statistics without concern for phylogeny. Doing so, however, did not change the conclusions of the analysis. No relationship between habitat and WLR slope was evident.

The lack of a correlation between habitat and WLR slopes is surprising, although several factors may compensate for the relatively poor ability of older flies to conserve water. Desert *Drosophila* have reduced rates of water loss at all ages, not just when they are young (Fig. 3). Thus, in comparison to their mesic relatives, desert *Drosophila* remain better at water conservation as they age. An important ecological factor is likely to be the availability of water in nature. Even desert *Drosophila* have access to water in the necrotic cacti they inhabit, so flies may compensate for increased water loss by increasing consumption of fluids.

Despite these considerations, necrotic cacti inhabited by desert *Drosophila* still may dry out at any time. Flies must then find a new host plant and are unlikely to have water available during the trip. Our results suggest that younger flies will disperse more successfully. Because males of some desert species (e.g., *Drosophila pachea*) mature so slowly, flies may benefit by dispersing before maturation, when their probability of survival is greatest. Thus, water balance may be an important factor affecting dispersal behavior and life history strategies of desert *Drosophila*.

A critical factor to consider in nature is temperature. Insects exposed to higher temperatures have shorter life spans (Rose

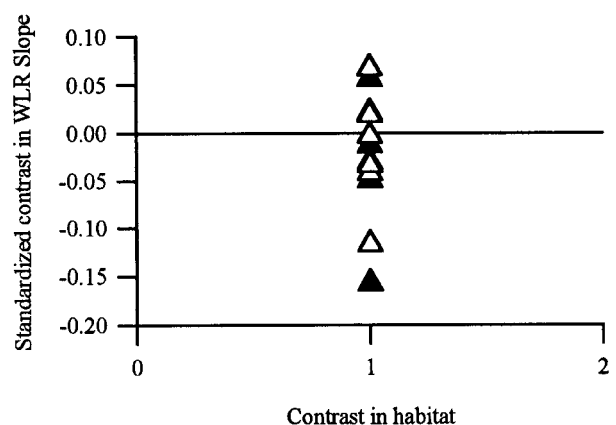


Figure 8. Independent contrasts analysis of the relationship between habitat and water-loss rates slope. Habitats were ranked in order of aridity: desert > arid > mesic. Ancestral character states were assumed to be the more mesic condition.

1991; Arking 1998) and appear to age more rapidly. Desert *Drosophila* are exposed to much higher temperatures than *D. melanogaster* (Junge-Berberovic 1996; Feder et al. 2000; Gibbs et al. 2001). This will increase the rate at which they mature but will also increase the rate at which physiological systems age. Thus, WLR slopes are likely to be even higher in desert species under natural conditions.

Finally, we do not know how long any *Drosophila* actually live in nature. In mark-release-recapture experiments, very few individuals are collected more than a few days after release (Powell 1997). However, the Sonoran endemic, *Drosophila nigrospiracula*, has been recaptured as many as 24 d after release (Johnston and Heed 1976). Clearly, we will not be able to understand the evolutionary significance of age-related physiological changes in *Drosophila* until we know more about the environmental conditions and life spans of flies in nature.

Acknowledgments

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