# Postponed aging and desiccation resistance in *Drosophila melanogaster*

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## Abstract

Studies with the fruit fly, *Drosophila melanogaster*, have repeatedly shown that selection for postponed reproduction leads to increases in mean life span and increased stress resistance; including increased resistance to desiccation, starvation and ethanol vapors. We show that desiccation resistance declines with age in both short- and long-lived flies suggesting that desiccation resistance may serve as a useful biomarker for aging-related declines in physiological performance. We examined the physical basis of desiccation resistance in five replicate populations selected for postponed reproduction and five replicate control populations. The variables examined were water content, rates of water loss during desiccation, and water content at time of death due to desiccation. In the absence of desiccation stress, both the flies exhibiting postponed senescence and their controls maintained constant water content throughout their lifetimes. In the presence of desiccation stress, the short-lived flies showed significantly higher rates of water loss at all ages than did the long-lived flies. Flies from the two treatments did not differ in water content at death. Our results indicate that water loss rates are the major determinant of desiccation resistance. Water loss rates are under genetic control and covary with age in populations with genetically-determined postponed senescence. © 2000 Published by Elsevier Science Inc.

*Keywords*: *Drosophila*; Biomarker; Stress resistance; Desiccation; Water loss

## 1. Introduction

Laboratory selection for postponed reproduction in *Drosophila melanogaster* has been shown to produce populations which exhibit increased longevity and postponed senescence compared to control populations (Rose, 1984; Luckinbill et al., 1984).

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These populations have served as valuable systems for the study of demography, as well as the physiological and genetic bases of aging.

Populations of *D. melanogaster* exhibiting postponed senescence have frequently also shown increased resistance to a variety of environmental stresses including desiccation, starvation and ethanol vapors (Service et al., 1985). Although the correlation between genetically-determined postponed aging and stress resistance in *Drosophila* has been known for a number of years, the physiological basis of this resistance has only recently begun to be elucidated.

In a series of studies aimed at examining selection for stress resistance, Hoffman and Parsons (1989a,b, 1991) suggested that increased resistance to a variety of stresses might be functionally correlated with a reduced metabolic rate. They proposed that reduced metabolic rate, and concomitantly reduced respiration, might confer increased starvation resistance, reduced respiratory water loss, and decreased exposure via the respiratory system to aerial toxins. This hypothesis is also attractive as a possible explanation for increased longevity in populations exhibiting increased stress resistance, in view of theories of aging which attribute the adverse affects of aging to aerobic activity and oxidative damage (Sohal and Orr, 1994).

Djawdan et al. (1996) examined this hypothesis using five replicate long-lived populations (O populations) and five short-lived control populations (B populations) developed by Rose (1984). Contrary to the hypothesis of metabolic change, they found no significant difference in the age-specific metabolic rates of the populations.

Djawdan et al. (1998) conducted investigations to determine the physiological basis of increased starvation resistance not just in the B and O lines but in a variety of demographically and stress selected populations. They found that they could explain 99% of the variability in starvation resistance in these populations by quantifying the energy content of lipid and carbohydrate stores in these flies. Metabolic rate did not change with selection for enhanced starvation resistance.

Gibbs et al. (1997) using populations of *D. melanogaster* selected for desiccation resistance and their control populations, demonstrated that a variety of physiological traits had evolved in flies exhibiting increased desiccation resistance, particularly water content and cuticular permeability. Archer (1998) demonstrated that these two parameters were sufficient to explain 99% percent of the variability in desiccation resistance in a variety of lines. She also verified that these same traits differentiated in a second set of lines selected for enhanced desiccation resistance (Archer, 1998).

In the present paper, we are specifically interested in the physiological basis for differential stress resistance in populations that are genetically differentiated with regard to longevity. While several forms of increased stress resistance are correlated with increased longevity in populations of *D. melanogaster*, these do not necessarily show similar age-specific changes. Starvation resistance, for example, increases with age in adult female *D. melanogaster* as the females accumulate metabolic storage compounds. Desiccation resistance, by contrast, declines with adult age in both short-lived and long-lived populations of flies. Desiccation resistance may therefore serve as a physiological biomarker (similar to cardiac function or muscle strength in humans) which is correlated in an individual both temporally and physiologically with the progression of the aging process. Adult desiccation resistance is of particular interest in *D. melanogaster* because it: (1) has been shown to

be under genetic control; (2) varies between short- and long-lived populations; (3) has been shown to decline with age; and (4) is a functional character which is likely to be of significance for survival in wild habitats.

At the organismal level, desiccation resistance can be described as an issue of water balance, which involves three variables: (1) the initial water content of the flies; (2) the rate of water loss during desiccation; and (3) the threshold at which water loss becomes lethal. All the forms of physiological variability between the selected populations (e.g. cuticular, metabolic or hormonal) must affect one of these three variables if they are to influence desiccation resistance. In the present paper, we have undertaken an analysis of these three variables, and their relation to desiccation resistance during aging, in flies that are genetically differentiated with regard to longevity. We examine each of these variables as physiological characters that may decline with age.

## 2. Materials and methods

### 2.1. Stocks

The populations of *D. melanogaster* used for this study were derived from a South Amherst, Massachusetts population established by P.T. Ives in 1975. Ten lines were derived from this population by M.R. Rose in 1980. Five replicate lines (O lines) were selected for postponed age of reproduction (postponed senescence) over several generations, until they could be maintained on discrete, 10-week generations (Rose, 1984). Five other replicate lines (B lines) were maintained in the same fashion as the ancestral Ives population, on a 2-week cycle of discrete generations, to serve as controls for the O populations. For experiments, adults from both the B and O populations were maintained in cages at population densities of $10^3$–$10^4$ flies per cage. All populations were reared and maintained at 25°C on a 24-hour light cycle, on standard banana medium.

In order to eliminate parental and grandparental effects, and thereby ensure that all differences between the B and O lines were genetically based, the experimental populations were reared through two generations of common conditions. For the experimental generation, larvae were reared at densities of 60–80 per 10-ml vial. Fourteen days after oviposition, when emerging adults were ~4 days old, flies were dumped into cages. For most experiments, all five replicate lines for the B and O treatment groups were collected on the same day. Because of the limited capacity of our respirometer, egg collections for flies used for water-loss measurements were staggered over a 5-day period, so that pairs of B and O lines could be assayed at the same age. All assays were performed on female flies.

### 2.2. Desiccation resistance

Desiccation resistance was measured every 7 days, beginning 4 days after eclosion. For each population, ten female flies were placed individually in 30-ml vials. A polyethylene sponge restricted them to the lower half of the vial, and 5 g silica gel desiccant was added above the sponge. The vial was sealed with Parafilm, and flies were checked hourly. When a fly was deemed dead, as indicated by the inability to right itself or move its limbs when

agitated, time of death was noted and the fly was immediately removed for determination of water content (see below).

## 2.3. Water content

Water contents of unstressed flies were measured every 4 days, beginning with the day of eclosion. Flies were anesthetized with $CO_2$, placed in previously weighed micro-centrifuge tubes, and immediately weighed on a Cahn microbalance to a precision of 1 μg. After drying at 55°C for 1 h, the flies were reweighed to give the dry weight. Water contents were calculated as the difference between wet and dry weights. Five samples of ten flies per replicate line were used to determine a mean value for each population.

The water content at the time of death (dehydration tolerance) was measured in a similar manner to initial water content. As each fly died in the assay for desiccation resistance (above), it was immediately weighed on a tared piece of aluminum foil, dried at 55°C, and reweighed to estimate the water remaining when the fly died.

## 2.4. Water-loss rates

Rates of water loss were measured using a TR-3 flow-through respirometry system (Sable Systems, Henderson, Nevada) with a Li-Cor LI-6262 water-vapor detector (Gibbs et al., 1997). The entire system was contained in a 25°C room, and the air-flow rate was 25 ml/min. Water-loss rates were measured every 7 days, beginning with day 4 post-eclosion. Four groups of 20 female flies were assayed from each population, at each age. Because empty respirometry chambers exhibited small but measurable differences in water-vapor signal, baselines were recorded for each chamber and subtracted from water-loss rates measured with flies present. Placement of flies in the chambers was staggered so that all flies had been in the respirometry chambers for 3 h at the start of measurement. In order to minimize wash-out artefacts, dry air was flushed through the chambers not being recorded at 25 ml/min, the same flow rate as during recording.

We calibrated the respirometer by injecting known volumes (0.5–5 μl) of water into the air stream, while the system was running under identical conditions to our assays. Rates of water loss from groups of *Drosophila* were calculated from our calibration regression curve, and loss rates per fly were calculated by dividing by the number of flies in the chamber (20).

## 2.5. Cuticular lipids

Surface lipids of B and O flies were analysed in a separate series of experiments. Flies were reared for two generations of selection and otherwise handled in the same manner as for experiments described above. At 4-days post-eclosion, adult females were frozen at −70°C. Cuticular hydrocarbons were isolated from groups of ten flies by silica gel chromatography (Toolson, 1982). Lipids were analysed by two methods: gas chromatography (GC) and Fourier transform infrared spectroscopy (FTIR). For GC analyses, lipid amounts were quantified by comparison to a peak corresponding to 2.5 μg of *n*-docosane, which was added as an internal standard (Graves et al., 1992). The distribution of hydrocarbon

chain lengths was determined by comparison to known *n*-alkane standards. Melting temperatures of hydrocarbon mixtures were determined using FTIR, as described previously (Gibbs and Crowe, 1991; Gibbs et al., 1997).

### 2.6. Statistical analyses

In the present study, we compare two treatment groups, each containing five populations subjected to differing life-history selection. Because we are interested in differences resulting from the selection regime, statistical analyses were performed using the population means, for $n = 5$ populations per treatment, using Tool Pack software in Microsoft Excel and SYSTAT 5.0. Because the B flies lived a much shorter time than the Os, we could not sample both sets of populations at later ages. We continued to assay the O population as long as flies were available, but direct comparisons between the B and O populations (e.g. repeated measures ANOVAS) were only performed for ages in which all populations were alive.

## 3. Results

In the present study, we were seeking genetically-determined differences, at the population level, between flies which have undergone selection for short generation times (the B populations) and those which have undergone selection for postponed reproduction (the O populations). The sample size for each of the points shown on the graphs is therefore five populations in each treatment.

### 3.1. Effects of age on desiccation resistance

Patterns of changing desiccation resistance with age are very interesting in the B and O populations. The O flies are more resistant on day 4 of adult life, the first day we tested, and this statistically significant difference persists throughout life ($P < 0.001$) (Fig. 1). A second very important aspect of the desiccation resistance is that it declines with adult age in both the B and O populations.

### 3.2. Effects of age on water content

Neither the B nor O populations exhibited significant changes with age in initial water content, as indicated by least-squares regression slopes that were not significantly different from zero ($P > 0.15$ for both B and O populations) (Fig. 2). A repeated measures ANOVA indicated that the percent water content of the B populations was slightly but significantly higher than that of the O populations ($P < 0.001$). This difference is in the opposite direction, however, from that expected to provide greater desiccation resistance to the O flies.

### 3.3. Effects of age on water content at death from dehydration

Least-squares regressions of age versus water content at the time of death indicated no statistically significant changes with age in either the B or O populations in their tolerance
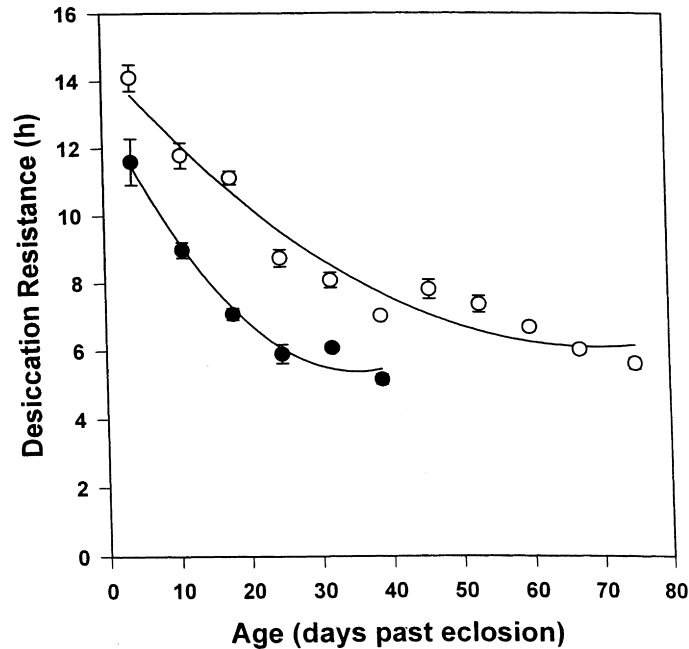
Fig. 1. The desiccation resistance in hours of flies as a function of adult age post-eclosion. The values presented are the means of the average values obtained from the five B populations (●) and the five O population (○) (mean ± S.E). The lines through the points were fit as a second-order regression.

to dehydration ($P > 0.1$ for both B and O populations) (Fig. 3). A repeated measures ANOVA revealed a slight but statistically significant difference between B and O flies ($P = 0.01$), with the O flies exhibiting greater tolerance.

### 3.4. Effects of age on rates of water loss

The mean rate of water loss of the B populations was significantly higher than that of the O populations as tested by repeated measures ANOVA ($P = 0.01$) (Fig. 4). Given that the populations were grown under identical conditions for two generations and tested under identical conditions, this demonstrates that the B and O populations are genetically differentiated with regard to the rates of water loss in dry air. Rates of water loss increased significantly with age for both the B ($P = 0.002$) and O ($P < 0.001$) populations. Comparison by $t$-test of the slopes of water loss versus age indicated that the O flies showed a slower rate of increase in water loss with age ($P < 0.001$). As a result, the difference in rates of water loss between B and O flies increased with age.

### 3.5. Cuticular lipids of B and O flies

Differences in either the amount or physical properties of surface lipids may affect rates of water loss (Gibbs, 1998; Rourke and Gibbs, 1999). In 4-day old flies, lipid amounts did not differ between B and O females (Table 1). No novel cuticular hydrocarbons were
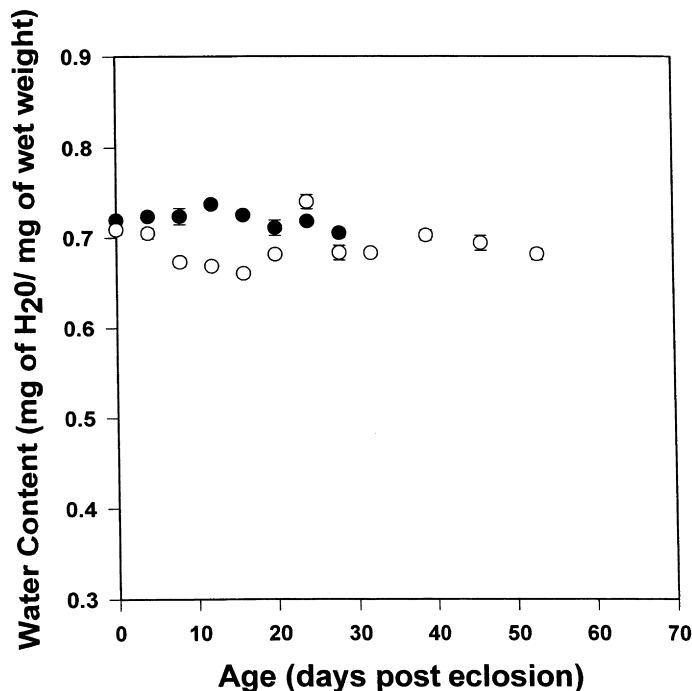
Fig. 2. Whole body water content of the B and O flies in the absence of desiccation stress, i.e. immediately after removal from the colony, as a function of adult age post-eclosion. The values presented are the means of the average values obtained from the five B populations (●) and the five O populations (○) (mean ± S.E).

detected in either set of populations, and the distribution of chain lengths was similar. Hydrocarbons ranged from 21 to 31 carbons in length, with 27-carbon species being the most abundant. Although hydrocarbons from O females were slightly longer on average than those of B females, lipid melting temperatures did not differ between selection treatments.

## 4. Discussion

*Drosophila* populations selected for postponed aging are useful models for the study of aging, because they exhibit genetically-determined differences in life span. A functional understanding of these genetic differences depends on the elucidation of age-specific changes in physiological characteristics of these populations. In particular, physiological systems that exhibit both genetic differentiation and age-specific decline in function are candidates for systems subject to late-life selection. Because reduced stress resistance may play a role in age-specific increases in mortality, a knowledge of which physiological systems decline with age may provide insight not only into the mechanisms of aging but mortality as well.

Our results in this study corroborate those obtained by Service et al. (1985), when the O
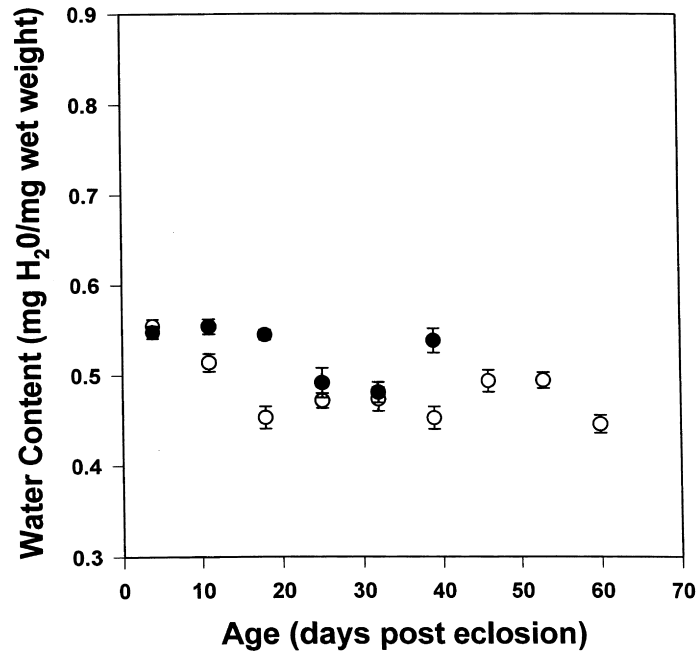
Fig. 3. Whole body water content of the B and O flies immediately following death by desiccation, as a function of adult age post-eclosion. The values presented are the means of the average values obtained from the five B populations (●) and the five O populations (○) (mean ± S.E).

populations had undergone approximately 24 generations of selection. After >100 generations of selection in the Os (and >450 generations of the B flies), the previously observed differences in desiccation resistance are still present, and the O flies remain more resistant at all ages (Fig. 1). What are the physiological mechanisms responsible for these differences? At the organismal level and under conditions where ingestion is precluded, differences in desiccation resistance at a given age can be explained by one or more of three variables: (1) the initial water content; (2) the water content at which death occurs; and (3) the rate of water loss. Age-related declines in desiccation resistance could be due to age-specific changes in any or all of these factors.

The B and O flies exhibited no age-specific changes in water content (Fig. 2). In contrast to expectations, the B flies actually had a higher percent water content than the Os. This may be due to increased lipid content in the O females, which would increase the dry mass relative to water content. We did not measure lipid contents in these experiments, but previous studies of these populations have documented higher lipid contents in O females (Service, 1987; Chippendale et al., 1998; Djawdan et al., 1998). Our results suggest that selection- and age-related differences in desiccation resistance are not caused by differences in initial water contents.

The ability of flies to tolerate loss of water, as indicated by water content at death, did not change with age (Fig. 3). This character cannot, therefore, explain age-dependent changes in desiccation resistance. The B and O flies did differ slightly in their water
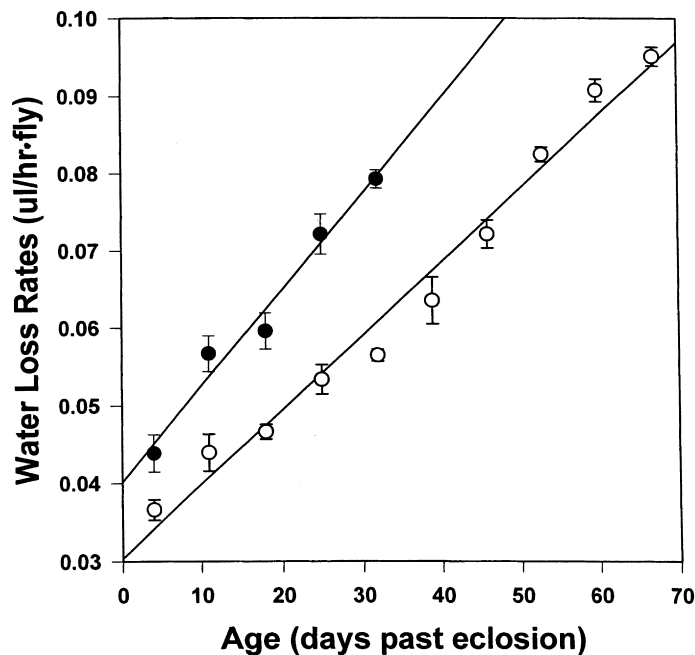
Fig. 4. Water loss rates measured by the release of water vapor into a dry stream of air, as a function of adult age post-eclosion. The values presented are the means of the average values obtained from the five B populations (●) and the five O populations (○) (mean ± S.E). The lines through the points were fit as a linear regression.

contents at death, but we conclude that these differences play only a minor role in explaining differences in desiccation resistance between the B and O populations (see further discussion of this issue below).

Because neither initial nor final water contents differ substantially as a function of age or selection treatment, we are left with water loss as the only remaining cause for changes in desiccation resistance. Beginning with our initial measurements on day 4 of adult life, the O flies exhibited consistently lower rates of water loss than the B flies (Fig. 4). In both selection treatments, water-loss rates increased with age, but the rate of increase was slower in the O populations. Water loss rates were the character that changed most with age, and that differed most between selection treatments (B versus O). The quantitative correlation between changes in the rate of water loss and changes in desiccation resistance

Table 1
Cuticular lipids of B and O females, at 4-days post-eclosion (data are means ($\pm$SE) for $n = 5$ populations per selection treatment. *P*-values are for unpaired *t*-tests ($n = 5$)

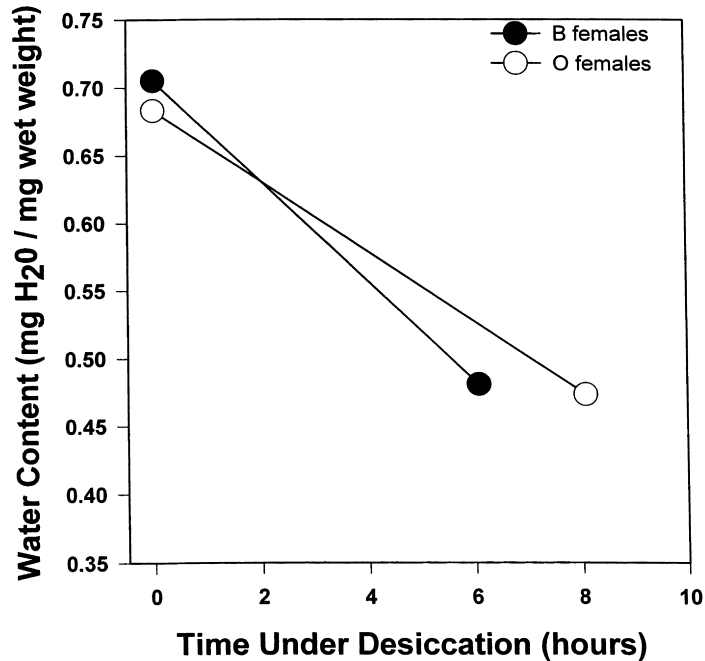| Lipid property | B flies | O flies | *P*-value |
| --- | --- | --- | --- |
| Amount ($\mu$g fly$^{-1}$) | 1.19 (0.12) | 1.06 (0.08) | 0.41 |
| Mean hydrocarbon chain length | 25.74 (0.30) | 25.98 (0.09) | 0.04 |
| Melting temperature (°C) | 34.45 (0.77) | 33.19 (0.69) | 0.26 |

Fig. 5. A visual depiction of water loss during a desiccation bout in adult flies 32-days post-eclosion. The points at time zero indicate the water content of the B and O flies at the initiation of desiccation. With time, the B flies lose water faster than the O flies. As a result, water content declines to a lethal level sooner in the B than in the O flies. Since both groups have the same tolerance for water loss (a percent water content around 48%) the O flies resist desiccation longer than the B flies solely due to a slower rate of water loss.

is demonstrated by the observation that the rate of water loss increased 1.87-fold from day 4 to day 32 in the B populations while desiccation resistance decreased 1.90-fold. Similarly, water loss rates in the O flies populations increased 1.61-fold over this same time period and desiccation resistance decreased 1.60-fold. The physiological character that quantitatively dominates in determining changes in desiccation resistance is therefore water loss rate.

This point is well illustrated in Fig. 5, which shows the changes in water content during desiccation in flies aged 32 days post-eclosion. It can be seen that the B and O populations begin with a very similar water content, although the B flies contain slightly more. As desiccation proceeds, the flies lose water. The line depicting water loss for the B flies has a more negative slope, illustrating a more rapid loss of body water. The B flies die following 6 h of desiccation, while the O flies can survive for 8 h. Since the water content at death is essentially identical in the B and O flies, the difference in desiccation tolerance is solely due to the difference in water loss rate.

We have thus identified a change in the rate of water loss as the physiological basis for both selection- and age-related changes in desiccation resistance. Clearly, it would be interesting to gain a more detailed understanding of the specific mechanisms responsible. Insects lose water through three distinct routes: cuticular, respiratory and excretory. Our

experiments were not designed to distinguish which of these pathways has been modified in the B and O flies. Pathways of water loss have been investigated in detail in *D. melanogaster*, however, particularly in populations selected for desiccation resistance and their controls (see reviews by Bradley et al., 1999; and Gibbs, 1999).

Excretory events in our flow-through respirometry system can be identified as peaks of water vapor appearing at the humidity sensor (Gibbs et al., 1997). Excretory water loss has previously been quantified by integration of these peaks, in two sets of populations descended from the O flies. These include the desiccation-selected D populations, and their control C populations. In these O-derived stocks, excretory water loss accounted for <5% of total water loss (Gibbs et al., 1997). We did not perform a detailed analysis of excretory water loss in our study, but inspection of water-loss recordings revealed no evidence that excretion accounted for a significant fraction of total water loss, at any age in any of the B or O populations. Although excretory water loss may differ with age, it was not sufficient to account for the two-fold increase in total losses as flies aged.

Insects can modulate respiratory loss from the tracheal system by changing the patterns with which the external spiracles are opened and closed (Lighton, 1994). Williams et al. (1997) examined B and O flies and found no differences in respiratory patterns, whereas the desiccation-selected D populations had differentiated patterns of spiracular control compared to their control (C) populations. Subsequent analyses of simultaneous $CO_2$ and $H_2O$ release patterns revealed that these changes actually had no effect on overall water loss (Williams and Bradley, 1998; Williams et al., 1998). Because the B and O flies exhibit no differences in respiratory pattern at day 4 (when overall water loss differed by 1.22-fold; Fig. 4), and highly differentiated patterns of respiratory control have no effect on water loss, it is unlikely that respiratory losses play an important role in the differences in water-loss rates between B and O flies.

Of the three routes of water loss from insects, only the cuticular pathway remains. Can we further narrow our focus to a particular aspect of cuticular structure? An obvious candidate is the epicuticular lipids, which provide the primary barrier to transpiration through the cuticle (Gibbs, 1998). Our analyses of surface lipids extracted from 4-day old flies revealed no significant differences in lipid amounts or physical properties. In accordance with previous work by Graves et al. (1992), gender was the primary factor affecting surface lipid composition (data not shown). A similar lack of differentiation was obtained in comparisons of the D and C populations, which exhibit even greater differences in rates of water loss (Gibbs et al., 1997). These results suggest that we can exclude cuticular lipids as the cause of differentiation between water-loss rates of young flies. Other components of the cuticle (e.g. chitin, cuticular proteins), or hormonal changes affecting cuticular permeability, may be involved.

A limitation of this analysis is that detailed studies of respiration, cuticular lipids, and excretion in these populations have been performed only on young flies. Further mechanistic studies of the effects of aging on pathways of water loss in *Drosophila* clearly are in order. The above studies indicate that the techniques for conducting such studies are available.

In summary, we have identified an important physiological process, water balance, that exhibits age-related deterioration and genetic differentiation between aging-selected and control populations of *D. melanogaster*. Our understanding of the life histories and

ecologies of these populations suggests that water stress is an important proximate cause of mortality in aging-selected populations. Several lines of evidence indicate that cuticular function is the primary site of selection on water balance. The cuticle therefore provides a concrete, tractable system for investigating mechanisms that influence age-related physiological performance, stress resistance and mortality.

## Acknowledgements

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