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Age-specific metabolic rates and mortality rates in the genus *Drosophila*

Daniel E. L. Promislow and Tamara S. Haselkorn

Department of Genetics, University of Georgia, Athens, GA 30602–7223, USA

Summary

Early theories of aging suggested that organisms with relatively high metabolic rates would live shorter lives. Despite widespread tests of this 'rate of living' theory of aging, there is little empirical evidence to support the idea. A more fine-grained approach that examined age-related changes in metabolic rate over the life span could provide valuable insight into the relationship between metabolic rate and aging. Here we compare age-related metabolic rate (measured as CO_2 production per hour) and age-related mortality rate among five species in the genus *Drosophila*. We find no evidence that longer-lived species have lower metabolic rates. In all five species, there is no clear evidence of an age-related metabolic decline. Metabolic rates are strikingly constant throughout the life course, with the exception of females of *D. hydei*, in which metabolic rates show an increase over the first third of the life span and then decline. We argue that some physiological traits may have been shaped by such strong selection over evolutionary time that they are relatively resistant to the decline in the force of selection that occurs within the life time of a single individual. We suggest that comparisons of specific traits that do not show signs of aging with those traits that do decline with age could provide insight into the aging process.

Key words: *D. affinis*; *D. hydei*; *D. melanogaster*; *D. simulans*; *D. virilis*; *Drosophila*; metabolic rate; mortality.

Introduction

An enormous number of theories have been proposed to explain why organisms age (over 300 by one account written over a decade ago – Medvedev, 1990). One of the oldest and most well studied of these theories is the 'rate of living' hypothesis. Almost a century ago, Rubner (1908) noted that large species have relatively long life spans but slow mass-specific metabolic rates. He concluded that among very different species, the total lifetime caloric expenditure was relatively constant. That is, animals living at a faster rate live shorter lives. Experimental tests of this theory came first from work by Loeb and Northrop in the early part of the 20th century (references cited in Rose, 1991). They noted that fruit flies kept at lower temperature had longer life spans. Raymond Pearl then popularized this idea in his 1928 book, *The Rate of Living* (Pearl, 1928).

Evidence to support the rate of living hypothesis is decidedly mixed. In addition to the finding that lower temperature extends life span and lowers metabolic rate (Rose, 1991; Sohal *et al*., 2000), studies of *Shaker* mutants in *Drosophila melanogaster* found that more metabolically active genotypes had shorter life span (Trout & Kaplan, 1970). Some proponents have argued that the relationship between enhanced longevity and stress resistance (e.g. Lithgow *et al*., 1995) is actually due to the fact that increased stress resistance is correlated with decreased metabolic rate (Hoffmann & Parsons, 1991), and that the decrease in metabolic rate, rather than stress *per se*, leads to extended longevity (Sohal *et al*., 2000). However, evidence for a link between stress and metabolism is equivocal (e.g. Harshman & Schmid, 1998).

Recent conceptions of Pearl's theory address the fact that metabolic processes generate free radicals. These free radicals can lead to cellular damage and may reduce life span. Thus, metabolism may be linked to survival rates, albeit indirectly. In support of this argument, comparative and experimental studies have shown that increased expression of free radical scavengers can extend life span (Orr & Sohal, 1994; Barja, 1998; Parkes *et al*., 1998; Sun & Tower, 1999; Melov *et al*., 2000).

Broad comparisons across species often contradict Rubner's initial claims. Birds, for example, live much longer than comparably sized mammals but have much higher metabolic rates. Among mammalian taxa, there is little relationship between metabolic rates and life history strategies (Harvey *et al*., 1991) and the patterns relating temperature to aging in insects turn out to be rather complex (Miquel *et al*., 1976). In this light, at least one author has argued that 'the rate-of-living theory is untenable' (p. 114, Rose, 1991).

Overall, differences within and among species in average metabolic rates may have little effect on variation in longevity, but a sole focus on variation in mean metabolic rate may overlook essential patterns expressed in terms of age-specific variation in metabolic rates within individuals. For instance, the rate of increase in age-specific mortality (the 'rate of aging', *sensu* Promislow, 1991) may be influenced by the rate of decline in metabolic capacity. In humans, there is a clear decline in metabolic rate among the elderly (e.g. Klausen *et al*., 1997). If this decline in metabolic rate mirrors the decline in other physiological parameters, and if it is related to the age-related

Correspondence
Daniel Promislow, Department of Zoology, University of British Columbia, 6270 University Blvd., Vancouver BC V6T 1Z4, Canada.
Tel.: +1 604 822 6973; fax: +1 604 822 2416; e-mail: promislow@arches.uga.edu

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decline in survival, then metabolic rate may be a useful 'biomarker' of aging (Miller *et al*., 1997; Miller, 2001). Furthermore, if rates of decline in age-specific metabolic rate are related to rates of aging, this would provide a powerful clue towards understanding the physiological basis of aging.

Although few studies have measured age-related change in metabolic rate, the few data that exist suggest substantial variation among groups. While species as diverse as humans (e.g. Klausen *et al*., 1997) and nematodes (Van Voorhies & Ward, 1999) show a decline in metabolic rate with age, others, including mussels (*Mytilus edulis*, Sukhotin *et al*., 2002) and common terns (*Sterna hirundo*, Galbraith *et al*., 1999), have age-constant metabolic rate.

In the following study, we measure age-specific metabolic rates (determined from levels of CO_2 production per hour) and age-specific mortality rates in five species of fruit flies. These data provide an opportunity not only to test whether survival rate and metabolic rate are correlated across species, but also to begin to determine whether age-related changes in metabolic rate might be useful as a biomarker of aging (Hershey & Wang, 1980; Shock, 1981).

Results

When comparing patterns across species, one should account for the fact that each data point (i.e. each species) in the statistical test is not independent of each other point (Felsenstein, 1985; Harvey & Pagel, 1991). This non-independence, which violates the assumptions of standard statistical models, occurs because species with shared phylogenetic histories are expected to be similar in physiology, morphology or behaviour as well. While numerous statistical packages now exist that allow us to correct for phylogenetic non-independence, the number of species analysed here is too small to carry out such corrections. Given the limited number of species in this study due to the effort required to collect both metabolic rate and mortality rate data simultaneously, one should interpret these results with due caution.

Mortality analysis

For all but one species (*D. affinis*), the Gompertz–Makeham model (Equation 2, Experimental procedures) provided an excellent fit to the natural log-transformed mortality data (Fig. 1, Table 1). Both males and females in *D. affinis* had very short life span. It appears as though either the food medium or the cage itself was not suitable for adult survival in *D. affinis*.

Longevity decomposition analysis was used to determine the relative contribution to differences in longevity produced by differences in the rate of demographic aging (slopes of the Gompertz function) and the baseline mortality (intercepts of the Gompertz function). Among males, interspecific variance in longevity was due to changes in both mortality parameters (Fig. 2) since the proportional contribution of each is relatively similar (Fig. 2). Females across species, however, differ in longevity primarily due to variation in Gompertz slopes (Figs 1 and 2). In females, the relatively short-lived *D. simulans* has both high intercept and high slope, *D. virilis* has low intercept and low slope, and *D. hydei* and *D. melanogaster* have intermediate values for both intercept and slope.

Body mass and metabolic rate

Within species, mass increased with age in males of *D. affinis* ($F_{1,5} = 6.85$, $P = 0.047$) and *D. hydei* ($F_{1,13} = 11.94$, $P = 0.0043$), and showed a slight but non-significant increase in *D. simulans* ($F_{1,5} = 5.71$, $P = 0.062$). No age-dependent change in mass was seen in other males or in any female as a function of age (Fig. 3a,b).

Some species appeared to gain mass with age but then declined later in life. Based on a second-order polynomial regression, *D. hydei* females, *D. melanogaster* males and *D. melanogaster* females showed a significant increase in early life mass ($P < 0.01$ in each case) followed by a significant decline in mass later in life ($P < 0.005$ in each case).

In this study, we defined metabolic rate (MR) as the volume of CO_2 produced per hour per fly, measured in groups of several flies (see Experimental procedures, below). For all species and both sexes, metabolic rate showed no significant change with age, with the exception of *D. melanogaster* males, in which MR increased with age ($F_{1,33} = 18.5$, $P < 0.00014$) (Fig. 4a,b). This effect was still significant after correction for bias due to multiple comparisons (Holm, 1979).

Among females, in four of the five species (all but *D. melanogaster*) metabolic rate declined from the first measurement at week 1 (which tended to be relatively high) to the next measurement 1 week later. In *D. simulans*, MR declined by 5%, *D. affinis* and *D. virilis* showed initial declines of 15–17% in MR, while *D. hydei* females showed a decline of 27%. Accordingly, we tested for age-related changes in MR after omitting the first age-class. This had little effect on the results reported above.

D. hydei females present an exceptional pattern. Metabolic rates increased during the first 30 days, after which they declined steadily over the next 2 months, from a peak of 1.5×10^{-3} mL CO_2 h^{-1} to a low of 6.5×10^{-4} mL CO_2 h^{-1} (Fig. 4).

As a broad empirical observation, metabolic rate typically scales with mass to the power of 0.75 ($MR = kM^{0.75}$) among species. In our data, MR scaled with mass to the power of 0.34 in females and 0.44 in males, though neither correlation was statistically significant (Fig. 5).

Metabolic rate and longevity

Across species, there was a positive but non-significant correlation between mean MR (averaged across all ages) and mean life span (Fig. 6, $F_{1,3} = 4.43$, $P = 0.13$) in males, and there was no correlation between mean MR and longevity in females (Fig. 6, $F_{1,3} = 0.07$, $P = 0.81$). Metabolic rate was

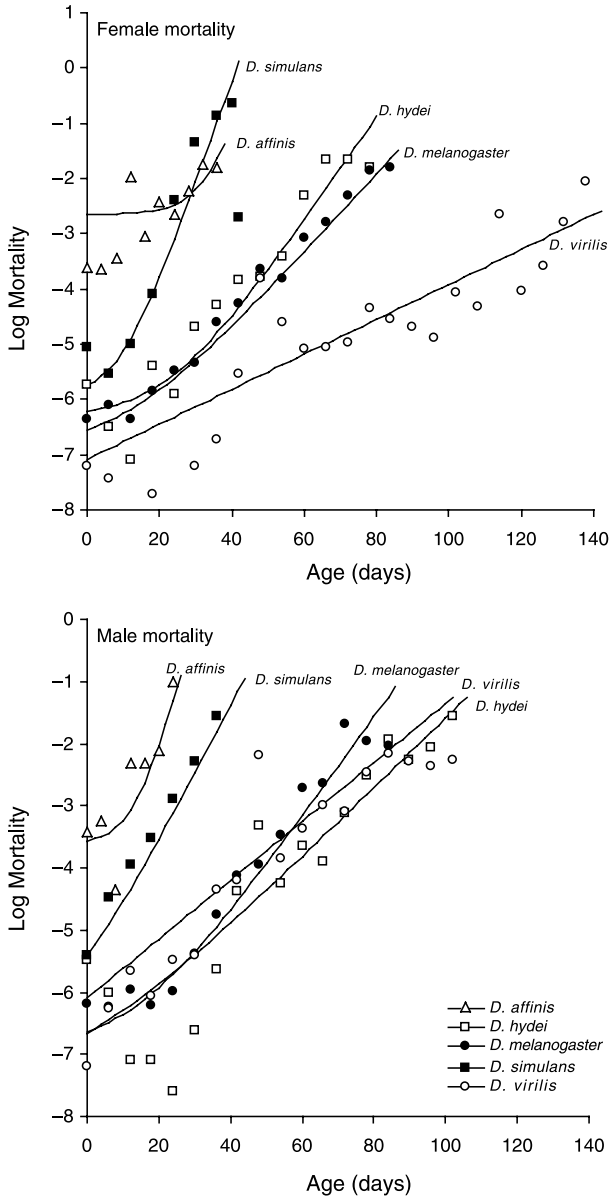

Fig. 1 Age-specific mortality rates for males and females, with fitted Gompertz–Makeham curves. Parameter values for the slope, intercept and Makeham term are given in Table 1. Note that mortality trends for females exhibit greater variation among species in slopes than mortality trends for males, where most of the variation is due to changes in intercept (see Fig. 2).

not significantly correlated with the intercept, slope or Makeham terms from the Gompertz–Makeham model. In a multiple regression model that included both dry mass and MR as predictors of life span, there was no relationship between mean MR and mean longevity in either males or females ($P > 0.25$ in each case). Finally, males and females both showed a negative relationship between mean mass-specific metabolic rate and life span, as predicted by theory, though in neither case was the regression coefficient statistically significant (males: $r = -0.42$, $F_{1,3} = 0.63$, $P = 0.49$; females: $r = -0.55$, $F_{1,3} = 1.32$, $P = 0.33$).

Discussion

Demography

Results from the demographic analysis were rather surprising. A series of experiments has now shown that substantial differences in longevity within species are often due to variation in the baseline mortality rate (the intercept of the Gompertz mortality curve – *a* in Eq. 2), rather than differences in the rate of aging (the slope *b* in the Gompertz equation) (Promislow *et al*., 1999; Pletcher *et al*., 2000). Here we found that males among species usually differed in both intercepts and slopes, whereas females differed primarily in the slope, or rate of aging. This contrast among sexes may reflect how costs of reproduction have evolved in females relative to males (Partridge *et al*., 1987). Future studies will be needed to address this question more directly.

The rate of living theory

The data presented here do not to support the idea that species with higher metabolic rates die at faster rates. In fact, among males, species with higher mean metabolic rates tended to be longer lived, although not significantly so. This positive association may have been due in part to the effects of body mass (e.g. Reeve *et al*., 2000; Norry & Loeschcke, 2002) since size, metabolic rate and longevity may be inextricably linked (West *et al*., 2001). Across species, longevity generally increases with body mass (reviewed in Calder, 1984), as does total metabolic rate (Schmidt-Nielsen, 1984). Thus longevity may be positively associated with absolute MR, but this trend disappears when mass is treated as a covariate.

Among females we observed no association of mean MR to longevity, with or without mass as a factor. The decline in age-specific MR from week 1 had no impact on this result and may reflect central metabolism used to initiate egg production.

Although metabolic rates decline with age in humans (e.g. Piers *et al*., 1998), a more common pattern may be the relative constancy of metabolic rate that we observed here. An enormous range of physiological processes decline with age, from lung capacity (Bode *et al*., 1976) to running speed (Moore, 1975) to oocyte chiasma frequency (Henderson & Edwards, 1968). We might expect that metabolic rates would decline with age in fruit flies for two reasons. First, normal physiological deterioration in flies might reduce the efficiency of central metabolism. In addition, metabolic rates might appear to decline due to the effects of cohort heterogeneity (Vaupel & Yashin, 1985). If individuals vary in metabolic rate, and if high metabolic rate is correlated with increased risk of mortality then, all else being equal, we would expect average metabolic rates to decrease with age as individuals with high metabolic rates die off early in life, leaving behind individuals with relatively low metabolic rates. Neither explanation seems to apply since metabolic rates were found to be relatively age-constant. Remarkably, age-constancy of MR appears in other recent cases (e.g. mussels (Sukhotin *et al*., 2002), common terns (Galbraith *et al*., 1999) and grasshoppers (M. Tatar, personal communication)).

Table 1 Parameter values for Gompertz–Makeham mortality model. *P*-values for Makeham term are without correction for multiple comparisons

Species	Sex	Mean life span (days)	*a*	*b*	*M*	Significance of Makeham term (*M*)
D. affinis	female	12.6	0.00014	0.189	0.07082	$P < 0.0001$
D. affinis	male	15.6	0.00150	0.212	0.02688	$P < 0.0001$
D. hydei	female	54.9	0.00022	0.094	0.00178	$P < 0.0001$
D. hydei	male	66.6	0.00074	0.056	0.00054	$P = 0.0601$
D. melanogaster	female	59.8	0.00049	0.071	0.00092	$P = 0.0097$
D. melanogaster	male	58.8	0.00034	0.080	0.00098	$P = 0.0001$
D. simulans	female	27.9	0.00052	0.183	0.00271	$P < 0.0001$
D. simulans	male	27.3	0.00308	0.110	0.00145	n.s.
D. virilis	female	96.7	0.00084	0.032	0	n.s.
D. virilis	male	56.1	0.00226	0.047	0	n.s.

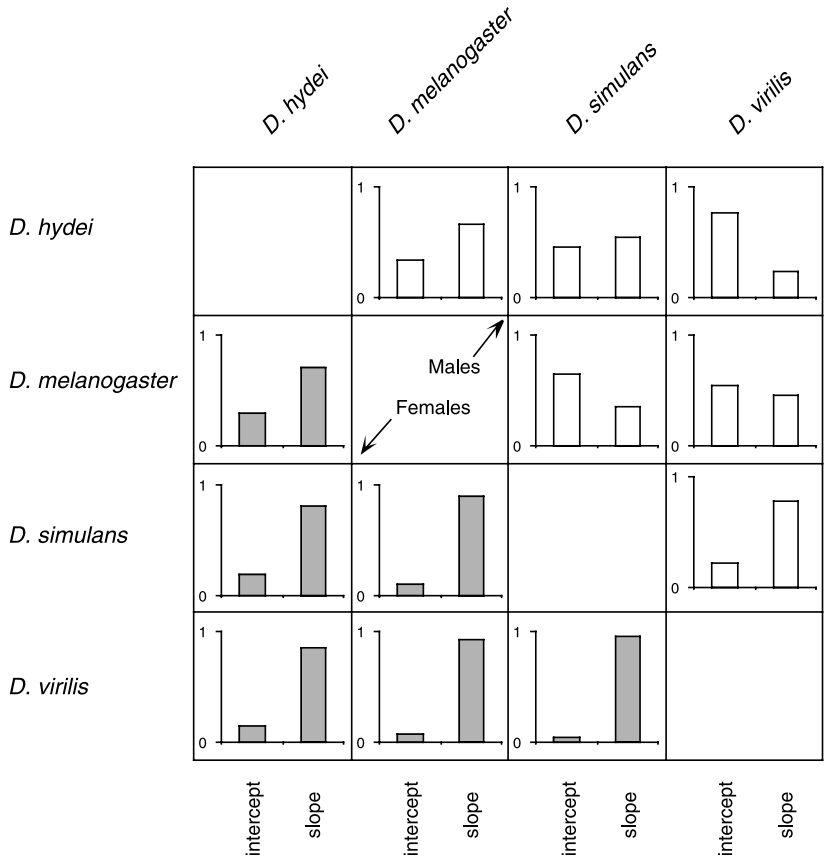

Fig. 2 Longevity decomposition comparing species pairs. This figure shows the proportional effect of slope vs. intercept on determining differences in longevity between species. Each square contains a comparison in life span between a pair of species. Male comparisons (above diagonal) have open bars, and female comparisons (below diagonal) have filled bars. Longevity differences between populations can be due to variation in either the slope (i.e. rate of aging) or the intercept (i.e. baseline mortality parameter) of the Gompertz model. For each comparison, the two bars sum to one. Note that for males of different species, variation in slope and intercept are roughly of equal importance. In contrast, for females, variation in slope tends to be much more important than variation in intercept.

We may have also expected metabolic rates to change with age for one trivial reason. We measured CO_2 production in a desiccating environment, and most *Drosophila* become more susceptible to the effects of desiccation as they age (Gibbs & Markow, 2001). The lack of change in metabolic rate suggests that desiccation was not a serious problem for flies in the respirometry chambers.

One explanation for constant metabolic rate with age is that traits associated with central metabolism are under extremely strong selection. Typically, traits are 'engineered' by natural selection to carry out the functions necessary for an organism to survive and reproduce and this force is strongest at ages where reproductive value is high. If these traits were designed by natural selection to work indefinitely, they would presumably exact a cost that would reduce fitness. This fundamental trade-off underlies standard evolutionary models of aging (Williams, 1957; Kirkwood, 1977). If central metabolic function were a dichotomous trait – either efficient or non-operable – any loss of function could be catastrophic at any age. Metabolic networks may have been shaped by selection to be relatively immune to deterioration (Albert *et al*., 2000; Kacser & Burns, 1973; Wagner, 2000). This resilience to deterioration does not mean that metabolic rates cannot evolve. Among the species we examined here, metabolic rate varied as much as three-fold. While much of the variation we observed here may have been due to interspecific

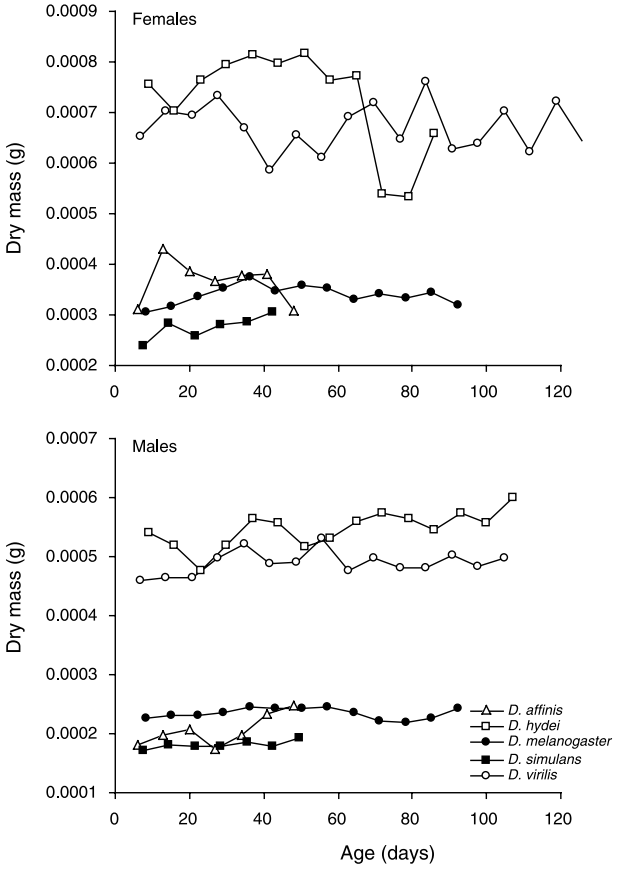

Fig. 3 Age-specific dry mass per fly sampled from aging cohorts. Females (top) and males (bottom) are shown for five *Drosophila* species.

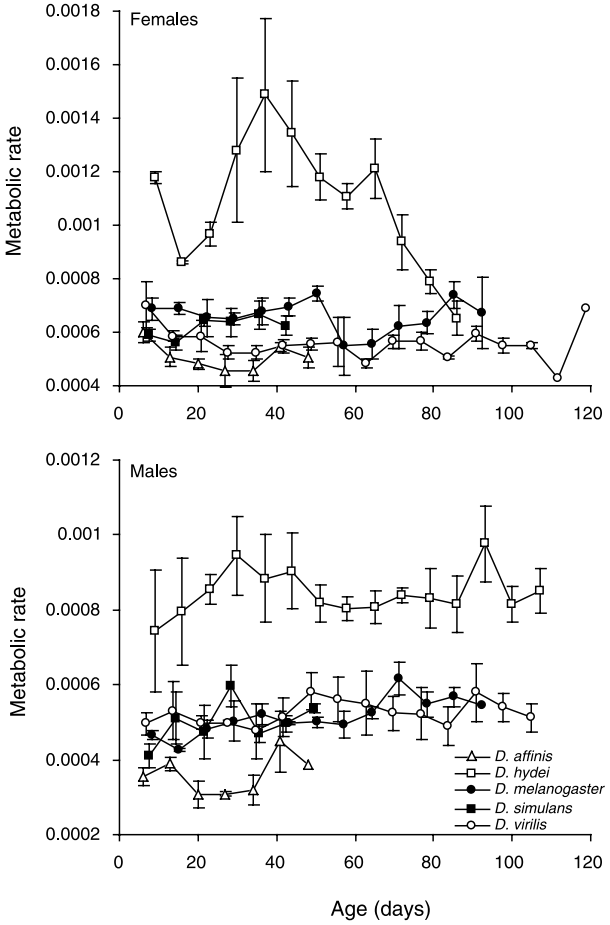

Fig. 4 Age-specific absolute metabolic rate per fly. Females (top) and males (bottom) are shown for five *Drosophila* species.

variation in mass, single gene mutants can also exhibit marked variation in metabolic rate (e.g. Van Voorhies & Ward, 1999).

The exceptional pattern of age-dependent MR by females in *D. hydei* may occur because females in this species have unusually high remating rates and males ejaculate a very small number of extremely large sperm (Markow, 1996). Future studies of other 'giant sperm' species may help to determine whether their unique reproductive physiology accounts for the observed unusual pattern of metabolic rate with age.

Biogerontology strategically aims to understand mechanisms for age-related decline in physiological, behavioural or demographic traits. Finch & Austad (2001) have argued, however, that we may be able to learn as much about aging from organisms that manage to avoid this degenerative process. At a mechanistic level, we may learn a great deal about aging by understanding how some, but not all, physiological traits avoid what we generally think of as the inevitable toll of aging. To this paradigm we may now add the observation that high metabolic rate does not necessarily promote elevated mortality rates or accelerated aging.

Experimental procedures

Five species of fruit flies were used in this study: *Drosophila affinis*, *D. hydei*, *D. melanogaster*, *D. simulans* and *D. virilis*, representing four species groups, including the obscura group (*D. affinis*), the repleta group (*D. hydei*), the virilis group (*D. virilis*) and the melanogaster group (*D. simulans* and *D. melanogaster*) (Fig. 7).

D. affinis, *D. melanogaster* and *D. simulans* were collected in the summer of 2000 in a peach orchard at the University of Georgia Horticultural Farm in Watkinsville, GA. *Drosophila hydei* and *D. virilis* were obtained from the *Drosophila* species stock centre.

All stocks were maintained in 1/3-pint plastic bottles (Applied Scientific) on 40 mL of standard molasses–agar–yeast–cornmeal medium at 24 °C on a 12/12 h L/D cycle. Flies for mortality and metabolic rate measurements were produced from stocks grown over two generations at controlled density, allowing flies to lay eggs for 2 days in 1/3-pint plastic bottles until a density of approximately 300 eggs per bottle was reached. Since each of the five species had different development times, the stock schedules were set to produce cohorts from each population with simultaneous emergence.

Emerging flies were collected as virgins over a 48-h period, separated by sex and placed in 2-pint plastic cages designed for measuring mortality (e.g. Promislow & Bugbee, 2000) at

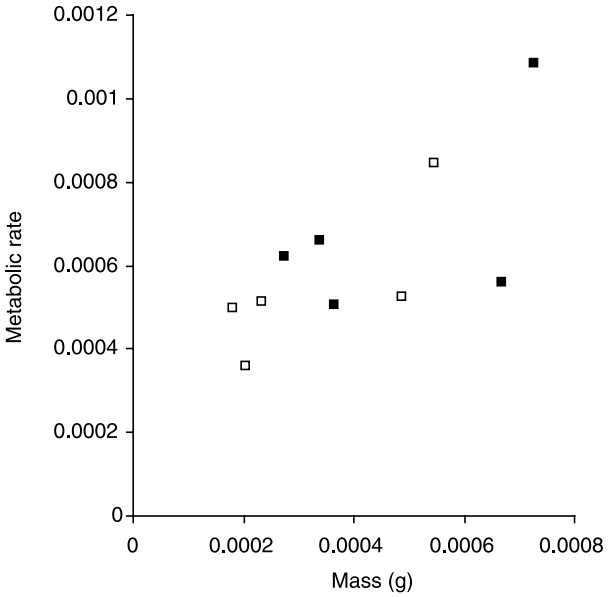

Fig. 5 Mean metabolic rate vs. mean dry mass. Females are shown by closed squares, and males by open squares. Lines represent least squares regression analysis, with a solid line for males and dotted line for females.

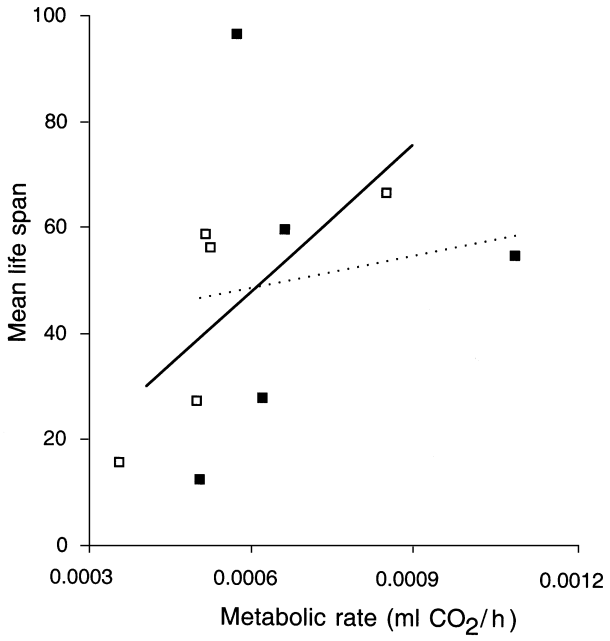

Fig. 6 Mean longevity vs. mean metabolic rate. Females are shown by closed squares, and males by open squares. Lines represent least squares regression analysis, with a solid line for males and dotted line for females.

a density of 0.4 g live weight per cage (between 135 and 600 flies per cage, depending on the species, Table 2). The number of cages for each sex and species was adjusted to ensure that we had approximately 800–1200 flies per sex per species. One extra cage per sex per species was set up to provide flies for metabolic rate measurements; flies from each cage were sampled without replacement at 1-week intervals. Each cage contained

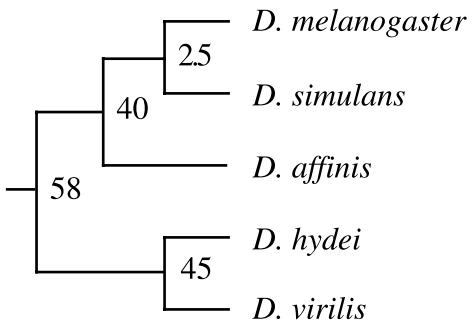

Fig. 7 Schematic phylogeny of species used in this study. Numbers at nodes refer to the time (millions of years) separating species.

Table 2 Number of cages and number of individuals per cage

Species	Sex	No. of flies tested	No. of flies per cage (± SE)	No. of cages
D. affinis	female	797	265.7 (21.7)	3
D. affinis	male	854	427.0 (10.0)	2
D. hydei	female	1037	148.1 (3.5)	7
D. hydei	male	1061	212.2 (6.8)	5
D. melanogaster	female	990	330.0 (25.9)	3
D. melanogaster	male	1226	613.0 (0.0)	2
D. simulans	female	1026	342.0 (21.2)	3
D. simulans	male	1140	570.0 (8.0)	2
D. virilis	female	1139	227.8 (4.7)	5
D. virilis	male	890	296.7 (9.3)	3

Table 3 Sample size and dry mass of flies in metabolic measurement chambers

	No. flies per chamber		Dry mass per chamber (g)	
Species	males	females	males	females
D. melanogaster	7	5	0.00164	0.00166
D. simulans	7	5	0.00127	0.00128
D. affinis	6	4	0.00121	0.00146
D. virilis	4	3	0.00194	0.00202
D. hydei	3	2	0.00162	0.00146

an 8-dram vial with 3 mL of standard fly medium, which was replaced every other day, at which time dead flies were removed and counted.

Mortality rates

To measure mortality rates, we counted and removed dead flies from each cage every other day. Deaths summed over all ages from each cohort estimate the initial cohort size N_0; the number alive N_x at age x was calculated from N_{x-1} less the deaths from the period $x - 1$ to x. Mortality rate was estimated as

$$\mu_x \cong -\ln\left\{\frac{N_{x+\Delta x}}{N_x}\right\}\frac{1}{\Delta x} \quad (1)$$

(Lee, 1992), where Δx is the interval over which deaths are observed. From the distribution of deaths per interval, maximum likelihood methods executed in *Winmodest* (see Pletcher, 1999) estimated mortality parameters of the Gompertz–Makeham model:

$$\mu_x = ae^{bx} + M \quad (2)$$

where a is the initial, baseline intrinsic mortality rate, b is the rate at which mortality rates accelerate with age, and M is the age-independent (Makeham) mortality rate. The exponent b can be interpreted as the 'demographic rate of aging' (MRDT, Finch *et al*., 1990). *WinModest* (Pletcher, 1999) was also used to estimate mean life span for each species and sex, and to evaluate hypotheses on homogeneity of the Gompertz mortality parameters among species. *WinModest* provides unbiased estimates and incorporates censored data.

Finally, *WinModest* was used to conduct 'longevity decomposition' to determine the proportional contribution of Gompertz slope and intercept to differences in mean longevity. Previous studies have found that variation in life span may be due primarily to differences among genotypes or species in the slope of the Gompertz curve (Service, 1998), or the intercept of the Gompertz curve (Promislow *et al*., 1999; Pletcher *et al*., 2000; Linnen *et al*., 2001). To determine the relative impact of slope and intercept on the mean longevity, *WinModest* solves the partial derivative of longevity with respect to the slope or intercept of the Gompertz curve. The magnitude of these derivatives estimates the relative impact of each mortality parameter on the longevity differential between groups.

Metabolic rate

A TR-2C constant-volume respirometry system (Sable Systems Inc., Las Vegas, NV, USA) was used to assess metabolic rates. CO_2 production was measured via a CA-1B CO_2 analyser (Sable Systems Inc.) after gas passed through a mass flow control valve and a multiplexer controlling eight 2×7.5-cm glass respirometry chambers. The chambers were housed in a closed (dark) wooden box with 1/2-inch styrofoam insulation to reduce thermal fluctuations. To initiate a reading, flies were lightly anaesthetized under moistened CO_2 and placed in chambers at densities of 2–7 like-sex individuals (Table 3); numbers were adjusted to maintain similar mass among different species per measurement episode. Following a 15-min recovery period, the chambers were flushed for 2 min with dry CO_2 free air (150 mL min^{-1} flow rate). Although a previous study has shown that desiccation can increase the variance of CO_2 respired on a minute-to-minute basis in flow-through measurement methods, the average amount of CO_2 respired over durations as long as 4 h was not affected by moisture (Williams *et al*., 1997).

After 90 min, the chambers were reflushed, pushing newly generated CO_2 to the gas analyser. Data were collected with Datacan V v5.4 software (Sable Systems), and the peaks were integrated using Convol v1.1 software (Sable Systems). Empty chambers were run simultaneously as controls. All work was performed at room temperature (22 ± 1 °C).

CO_2 production was assessed once per week, starting with 7-day-old flies and continued until the cage was extinct from sampling and death. Flies from each of the five species were measured in triplicate samples at each age. Males and females were measured on alternate days. Following the MR measurement, samples were weighed as a group to the nearest 0.01 mg, dried overnight at 65 °C and reweighed to obtain dry weights. Metabolic rate (MR) was calculated as the production of CO_2 per hour per fly (mL h^{-1} per fly). Mass-specific metabolic rate (MMR) was calculated as MR per single-fly dry mass (mL h^{-1} mg^{-1}).

Accurate measures of metabolic rate require estimates of both O_2 consumption and CO_2 production. CO_2 alone is a valid measure if we assume that the respiratory quotient is 1.0 in all tested species, as previously shown to be true for *D. melanogaster* (Berrigan & Partridge, 1997; Berrigan & Hoang, 1999).

To evaluate age-related changes in metabolic rate, we carried out least squares, second-order regression of absolute MR on age with each sample as independent replicates. Sex- and species-specific values of mean metabolic rate were calculated as the arithmetic average of metabolic rate across all ages. We estimated the relationship between body mass and age using least squares regression analysis of mean age-specific body mass on age. In several cases, there appeared to be an initial increase in mass, followed by a decline later in life. In this case, the significance of these trends was tested using a second-order least squares regression analysis. To determine whether metabolic rate was a significant predictor of mortality, we used multiple regression analysis to compare mean metabolic rate (averaged over the entire life span) with mean longevity, as well as with the intercept, slope and Makeham terms from the Gompertz model. This analysis was carried out both with and without mass as a covariate in the regression model, analysing males and females separately. In addition, we regressed longevity on mass-specific metabolic rate.

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