

Physiology of Aging

Selected Contribution: Long-lived *Drosophila melanogaster* lines exhibit normal metabolic rates

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Van Voorhies, Wayne A., Aziz A. Khazaeli, and James W. Curtsinger. Selected Contribution: Long-lived *Drosophila melanogaster* lines exhibit normal metabolic rates. *J Appl Physiol* 95: 2605–2613, 2003. First published August 29, 2003; 10.1152/japplphysiol.00448.2003.—The use of model organisms, such as *Drosophila melanogaster*, provides a powerful method for studying mechanisms of aging. Here we report on a large set of recombinant inbred (RI) *D. melanogaster* lines that exhibit approximately a fivefold range of average adult longevity. Understanding the factors responsible for the differences in longevity, particularly the characteristics of the longest-lived lines, can provide fundamental insights into the mechanistic correlates of aging. In ectothermic organisms, longevity is often inversely correlated with metabolic rate, suggesting the a priori hypothesis that long-lived lines will have low resting metabolic rates. We conducted ~6,000 measurements of CO₂ production in individual male flies aged 5, 16, 29, and 47 days postemergence and simultaneously measured the weight of individual flies and life spans in populations of each line. Even though there was a wide range of longevity, there was no evidence of an inverse relationship between the variables. The increased longevity of long-lived lines is not mediated through reduction of metabolic activity. In *Drosophila*, it is possible to both maintain a normal metabolic rate and achieve long life. These results are evaluated in the context of 100 years of research on the relationship between metabolic rate and life span.

aging; rate of living; longevity

AGING IS A UBIQUITOUS, BUT poorly understood process that results in a decline in physiological function over time. It is an inherently complex phenomenon that reflects the sum of an organism's genotype and the interaction of these genes with the environment. Although the mechanisms responsible for aging have recently become the subject of an intensive research effort (13, 17, 32), the multifactorial nature of aging makes it difficult to disentangle the factors responsible for senescence. The use of model organisms and genetics can provide a powerful method toward understanding the mechanisms of aging.

In our investigations of the mechanisms of aging, we employ a large set of recombinant inbred (RI) lines of *Drosophila melanogaster*, which offer several advantages. Each line is highly inbred, which makes it possible to obtain replicated measurements on the same genotypes, thereby increasing statistical power. The lines exhibit an unusually large range of life span variation, because they derive from populations artificially selected for long life, which live, on average, about twice as long as unselected controls (7). The main drawback of inbred lines is the potential for inbreeding depression, which is expected to arise from homozygosity of deleterious alleles in inbred lines. However, for these lines, we have shown that inbreds derived from selected and control populations retain the life span differences observed in the source populations (7) and also that our elite inbreds actually live longer in the laboratory environment than genetically heterogeneous laboratory-adapted populations and recently collected wild stocks (21). Understanding the factors responsible for differences in longevity, particularly the characteristics of the longest lived lines, can provide fundamental insights into the mechanistic correlates of aging.

Currently, the most widely proposed mechanistic explanation for the aging process links the production of free radicals and other oxidants produced during aerobic respiration to biomolecular damage that results in aging. The scientific origins of this explanation were first elucidated nearly 100 years ago when Rubner (44) compared metabolic rates in five different species of mammals and concluded that despite their large differences in chronological longevity, these mammals had approximately equal, mass-specific (metabolic output standardized to a given body mass), lifetime energy output. Pearl (40), expanded on Rubner's work and proposed the "rate of living hypothesis," which directly linked the metabolic output of an organism to its longevity. The causal mechanism linking these two was unclear at the time, but Pearl proposed that the duration of life was determined by the exhaustion of some vital cellular component. This component was consumed

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at a rate proportional to the metabolic rate, and death occurred on its depletion (reviewed in Ref. 5).

Harman (14) formulated a more modern interpretation of causal mechanisms linking metabolic rate and longevity when he proposed that free radicals produced during aerobic respiration were responsible for the link between metabolic rate and longevity. By this hypothesis, free radicals produced in aerobic respiration caused cumulative oxidative damage that eventually resulted in aging and death (5, 30, 49). Interpreted in its most simple form, the rate of living theory predicts that factors that decrease an organism's metabolic rate would increase longevity and factors that increase its metabolic rate will shorten longevity.

Support for an inverse relationship between the metabolic rate and longevity comes from the observation that environmental manipulations that decrease metabolic activity tend to increase longevity. This is best documented in the effects of ambient temperature on ectothermic organism longevity, and it has been known for many decades that ectotherms reared at lower temperatures live longer than warm-temperature counterparts (1, 28, 33, 35, 46, 55). This effect of temperature on longevity is thought to be mediated through the effects of temperature on metabolic rate, with metabolic rates typically changing two- to three-fold for every 10°C change in temperature (18, 47).

Changes in metabolic rate could be responsible for the wide range of longevities observed between the different RI fly lines. This explanation is consistent with the reported inverse correlation between the metabolic rate of wild strains of *D. melanogaster* and their longevity (34), and the initial observation that *D. melanogaster* lines selected for increased longevity had reduced metabolic rates relative to normal-lived control lines (45). This reduction in metabolic rate could also explain the generally increased ability of the long-lived lines to withstand environmental stresses as a hypometabolic state may in itself be sufficient to induce a great increase in the stress response (48). As such, it would not be necessary to invoke any other novel or unknown pathways of stress resistance to explain the increased stress tolerance of these lines.

If the increased longevity of the long-lived RI lines is due primarily to a reduction in metabolic rate, the relatively long-lived lines may have a chronologically extended life span, but their physiological life span (24, 51), the relative number of physiological events carried out, would be equivalent to that of normal animals. Alternatively, if the long-lived lines have normal metabolic rates, the increased longevity of these lines could be due to factors such as an increased ability to repair or prevent biological damage that is responsible for aging. Because of the potential importance of metabolic rate to longevity, it is critical to know the relationship between metabolic rate and longevity of the RI lines. To determine this relationship, we conducted nearly 6,000 metabolic measurements on ~3,000 individual *D. melanogaster* of various ages from 52 RI lines and their 2 parental lines inbred lines. To ensure that the metabolic rate measurements were representative of

the metabolic rates experienced by the flies in the longevity assays the metabolic measurements were done under conditions that closely matched those used to assay longevity.

MATERIALS AND METHODS

We measured the metabolic rate and longevity of virgin male flies from 51 RI lines and the two parental inbred stocks. One line used in the metabolic measurements was unavailable for the longevity assay (*line 56*).

Fly Stocks

RI lines of *D. melanogaster* derive from a genetically heterogeneous population artificially selected for long life and an unselected control population (27). Details of stock construction are presented elsewhere (7). Briefly, inbred lines were constructed by sib mating flies from selected and control populations for 28 generations. Life spans were characterized in 70 resulting inbred populations (7), and then one control-derived inbred line and one selected-derived inbred line were intercrossed. Progeny were sib mated for another 28 generations, producing a set of 58 RI lines (51 surviving for use here) plus 2 parental inbred genotypes. All stocks were maintained on standard yeast-cornmeal-agar medium at 24°C with constant illumination and 60–70% relative humidity.

Longevity Measurements

Life spans were measured in the University of Minnesota laboratory. Stocks were expanded in half-pint bottles under controlled larval density by using the method of Fukui et al. (10, 11). Adult flies of both sexes emerging within a 6-h period were collected under light CO₂ anesthesia, and sexed. Approximately 160 males from a single 6-h cohort were transferred into specially designed 4.6-liter population cages (41). The mouth of each cage was covered with a fine mosquito netting and inverted over an 11-cm-diameter disk of cooked medium. A total of 53 population cages were established, 1 per RI line. Flies were transferred without anesthesia to clean cages every 10 days, and food cookies were replaced every other day. Dead flies were removed by suction, and the number was recorded daily until the last death. Exact numbers of flies in each population were determined at the conclusion of the survival experiments by summing all deaths. Cages were assigned to randomized locations within a walk-in incubator.

Life spans were also measured on unmated males maintained on standard *Drosophila* food medium in 8-dram shell vials. Virgin male flies were collected under light CO₂ anesthesia and placed in vials at a density of 15 flies/vial, with 2 vials established for each line. Dead individuals were counted and removed from the vials daily until the last death. Flies were transferred to new vials every other day and maintained at 24°C with constant illumination and 60–70% relative humidity in the same incubator that housed the population cages used in the longevity assay. Longevity assays of all lines were done simultaneously to minimize environmental variations.

Metabolic Measurements

Resting metabolic rates were measured on flies at 4 ages. Flies ranged in age from young adults to old adult flies (flies measured at 5, 16, 29, and 47 days postemergence). The *D. melanogaster* were reared at constant temperature for two generations in an incubator at New Mexico State University before the collection of the animals used in this study. Flies

were reared on a standard cornmeal *Drosophila* food source, which was shipped overnight twice weekly from the University of Minnesota. Flies were maintained in 8-dram plastic vials covered with cotton plugs and were transferred to new food vials twice weekly. The flies were housed at 24°C, under continuous-light conditions.

Individuals used in the study were collected from eggs laid by approximately five female flies from each line. Females were allowed to lay eggs for 48 h, after which the adults were removed. On emergence, ~140 virgin male offspring were collected under light CO₂ anesthesia and placed in new food vials, at a density of ~20 flies per vial, with 7 vials established per line. Metabolic rates were measured on flies from these lines at different ages. For these lines, each fly was used for a single set of age-specific metabolic measurements.

For metabolic measurements the flies from a vial were first immobilized with a stream of humidified N₂ gas. An individual fly from this group was then placed in a 2.2-ml glass measurement chamber, either by gently transferring the fly via a glass pipet with mouth suction or by using a fine-tipped paint brush to move the fly into the chamber. The chamber was then sealed with a rubber stopper. This process took <1 min, and the flies quickly recovered and began moving within a few minutes of immobilization. In a series of control experiments, we determined that the fly's metabolic rate recovers to its preimmobilized value within 5 min of being immobilized with nitrogen. The chambers were then flushed for 15 s at a flow of 90 ml/min with CO₂-free, water-saturated (100% relative humidity) room air and left sealed for ~1 h. At the end of the sampling period, a 1-ml (STPD) gas sample was removed from the chamber with a Hamilton SampleLock syringe (Hamilton, Reno, NV) and injected into a Li-Cor 6251 CO₂ gas analyzer (Li-Cor, Lincoln, NE). The chamber was then refilled with CO₂-free air, and a second air sample was taken 1 h later. The amount of CO₂ produced by each fly was calculated by using DATACAN software (Sable Systems International, Henderson, NV).

The second metabolic measurement on each fly was usually less variable than the first reading, a result noted in other metabolic studies (15). For this reason, only the second metabolic measurements are used in the data analysis. A total of 16 flies from each line was typically measured for each sampling period, divided between 8 individuals measured in the morning and 8 in the afternoon. Each set of experiments also included six chambers without flies to control for background CO₂ production or the presence of CO₂ in the air used to flush the chambers. The CO₂ concentration in these blanks was typically <5% of that measured in a chamber with a fly. The CO₂ gas analysis system was zeroed daily against CO₂-free air and calibrated weekly against a 51 parts/million certified gas standard (Air Products, Long Beach, CA).

Currently, one of the most sensitive and accurate methods for assaying the metabolic rate of a single *Drosophila* is through the detection of CO₂, which is produced as a direct by-product of oxidative metabolism. Because the ratio of CO₂ produced to O₂ consumed varies with the metabolic substrate utilized, knowledge of the respiratory quotient (RQ) of the organism is necessary to accurately compare metabolic measurements based on CO₂ production to standard energy units (9, 56). To collect these data, we measured both the O₂ consumption and CO₂ production from groups of several dozen, 40-day-old flies from 22 of the RI lines and one parental line. These groups of flies were sealed in 50-ml glass metabolic chambers for ~1 h. At the end of this time, the air in the chamber was flushed into a paramagnetic O₂ analyzer (model PA-1, Sable Systems), linked in series with a Li-Cor

CO₂ analyzer, and both the O₂ consumption and CO₂ production were determined and a RQ calculated.

Fly Weight

To account for the possible effect of body mass differences on metabolic rate, we individually weighed each fly used in the metabolic study. After the second CO₂ measurement was recorded, the fly was left sealed in the metabolic chamber and frozen at -80°C until it was weighed. The mass of a frozen fly was found to be stable over at least a 1-mo period. Flies were thawed and weighed on microbalances either at the University of Minnesota or at New Mexico State University on a Mettler microbalance (model AT261) or a Sartorius M2P microbalance. Flies weighed at the University of Minnesota were sent frozen on dry ice and stored frozen until the time of weighing. To determine whether transporting flies from New Mexico to Minnesota affected the fly weight a subset of 50 flies was weighed in New Mexico and then sent to Minnesota for reweighing. The weight of these two groups of flies was within 4% of that expected from the original weighing in New Mexico. A total of 2,508 individual flies from the 53 different lines were weighed. A mean weight for each line was calculated from the weights of flies in a given line combined from the four time points at which the flies were weighed. An average of 46 flies were used to calculate the mean weight of each line.

Data Analysis

Metabolic output values were calculated from the mean metabolic rates of the lines as measured when 5, 16, and 29 days old. Mean output was calculated by summing the *day 5* and *day 16* line average metabolic rate, dividing by two, and correcting for the number of hours between these two sampling periods. This same calculation was then done on the *day 16* and *day 29* metabolic data.

RESULTS

Metabolic Rate Measurements

There was approximately a twofold range of metabolic rates between the various RI lines for any given measurement age (Fig. 1). Significant differences in inter-line metabolic rates existed at all the time points measurements (multivariate analysis of variance with weight included as a covariate; *day 5*: $F = 3.82$, $n = 807$; *day 16*, $F = 12.13$, $n = 860$; *day 29*, $F = 6.52$, $n = 837$; *day 47*: $F = 6.43$, $n = 308$; $P < 0.001$ for all analyses). There is a significant drop in resting metabolic rate between *day 5* the later ages (Fisher LSD, $P < 0.001$, $df = 180$), but there was no evidence of a uniform decline in metabolic rate with age. The slight apparent increase in metabolic rate at the highest ages compared with the middle ages could be due to demographic selection; i.e., only the most vigorous and healthy flies survived to be measured at the most advanced age.

Although there was a large range of metabolic rates observed for the different time points, the metabolic rate of a RI line at one age was significantly correlated with the metabolic rate of that line at other ages (Table 1). This indicates that the metabolic measurements provide a reasonably consistent measure of the metabolic rate of a particular RI line and that the different

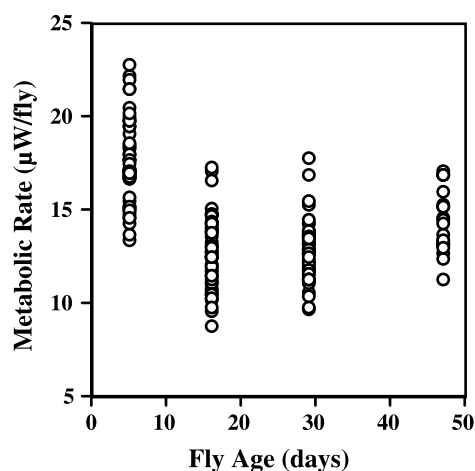


Fig. 1. Average metabolic rate of 52 recombinant inbred (RI) and 2 parental inbred lines derived from an artificial selection experiment for increased longevity. Within each sampling period, there are significant ($P < 0.001$) interline differences in the metabolic rates [multivariate analysis of variance with fly weight included as a covariate]. Day 5: $F = 3.8$, $df = 53,752$; day 16: $F = 12.1$, $df = 53,805$; day 29: $F = 6.5$, $df = 53,782$; day 47: $F = 6.4$, $df = 22,284$. The average metabolic rate of the 5-day-old flies is significantly higher than for all other ages measured [Fisher's least significant difference (LSD) test, $P < 0.001$, $df = 180$]. The average metabolic rates of the lines on day 47 are slightly higher than on day 16 (Fisher's LSD test, $P = 0.03$, $df = 84$) but are not significantly different from the day 29 metabolic rates (Fisher's LSD test, $P = 0.06$, $df = 84$). The metabolic rates at the other ages do not significantly differ from each other. Each symbol represents the mean metabolic rate for a single RI line. By day 47, 23 RI lines remained with a sufficient number of flies to conduct metabolic measurements. Metabolic rates on an average of 16 flies per line were measured at days 5, 16, and 29, and an average of 14 flies per line was measured on day 47. Metabolic rates were measured on a total of 2,866 individual flies.

RI lines have a characteristic metabolic rate. The factors responsible for this variation in metabolic rate are not known, but the range of metabolic rates cannot be explained by differences in body mass (54). Because of the lack of correlation between metabolic rate and body mass, it is inappropriate to express metabolic rate in mass-specific units, and therefore metabolic rates are expressed as per whole animal (16, 39). To account for the potential effects of body mass on metabolic rate body mass was included as a covariate in a multiple analysis of variance (39).

Longevity Data

There was a large range of interline longevity. Line means varied four- to fivefold depending on whether vial or cage longevity was used (vial mean longevity of the different RI lines ranged from 16 to 65.5 days, and cage longevity data ranged from 10.8 to 59 days). These differences in longevity are primarily due to differences in adult life spans and not developmental rates, and all the lines complete development from egg to adulthood within 2 days of each other. We assayed longevity data for populations in both vial and cage conditions to determine how robust the mean longevity of a line was under different environmental conditions. Flies housed in vials tended to live longer than those in cages, but

the two sets of longevity data were highly correlated ($r = 0.62$, $P < 0.01$). The conditions of the flies in vials were very similar to the conditions under which metabolic rates were measured because the flies could freely walk, but not fly, in the vials.

Correlation Between Metabolic Rate and Longevity

At none of the ages studied was there an inverse correlation between the average metabolic rates of the RI lines and their longevity. The only correlation seen was a slight, but often significant, positive correlation between metabolic rate and longevity, particularly for the older flies. (Fig. 2). This positive correlation is observed for both the cage estimates and vial estimates of longevity (Fig. 2). This same pattern also is observed between the metabolic rate and longevity of a subset of the RI lines that lived either approximately as long or longer than the long-lived parental stock (Fig. 3).

The correlation between metabolic rate and longevity was further examined by dividing the RI lines approximately into quartiles on the basis of their mean longevity and examining the correlation between metabolic rate and longevity in each group. This correlational analysis was done with metabolic rate data from 5-, 16-, and 29 day old flies against cage and vial longevity data that were divided into quartiles for each sample age. This analysis would account for the potential effect of flies in the short-lived lines dying from causes other than those expected to be associated with aging. Such an effect could potentially obscure the correlation between the mean metabolic rates of the RI lines and their longevity. In none of these 24 analyses was there a significant ($P < 0.05$) correlation between metabolic rate and longevity. There was a slight, but nonsignificant, trend for the shortest lived lines to show a negative correlation between metabolic rate and longevity and for the longest lived lines to show a positive correlation between these two variables. The two most significant correlations were a negative correlation between metabolic rate and longevity in the shortest-lived quartile of the 29-day-old flies ($r = -0.55$, $P = 0.052$) and a positive correlation between metabolic rate and longevity for the next longest lived quartile of the day 16 flies ($r = -0.54$, $P = 0.059$).

A comparison of the long-term metabolic output of the different RI lines and longevity revealed no inverse correlation between the average metabolic output of a line and its longevity. As shown in Fig. 4, there is a slight but positive correlation between the average

Table 1. Correlations between the metabolic rates determined on a RI line and subsequent metabolic rate measurements on the same line at a different age

Measurement Day	Measurement Day	Correlation Coefficient (r)	P Value
5	16	0.61	<0.01
5	29	0.54	<0.01
16	29	0.80	<0.01

RI, recombinant inbred.

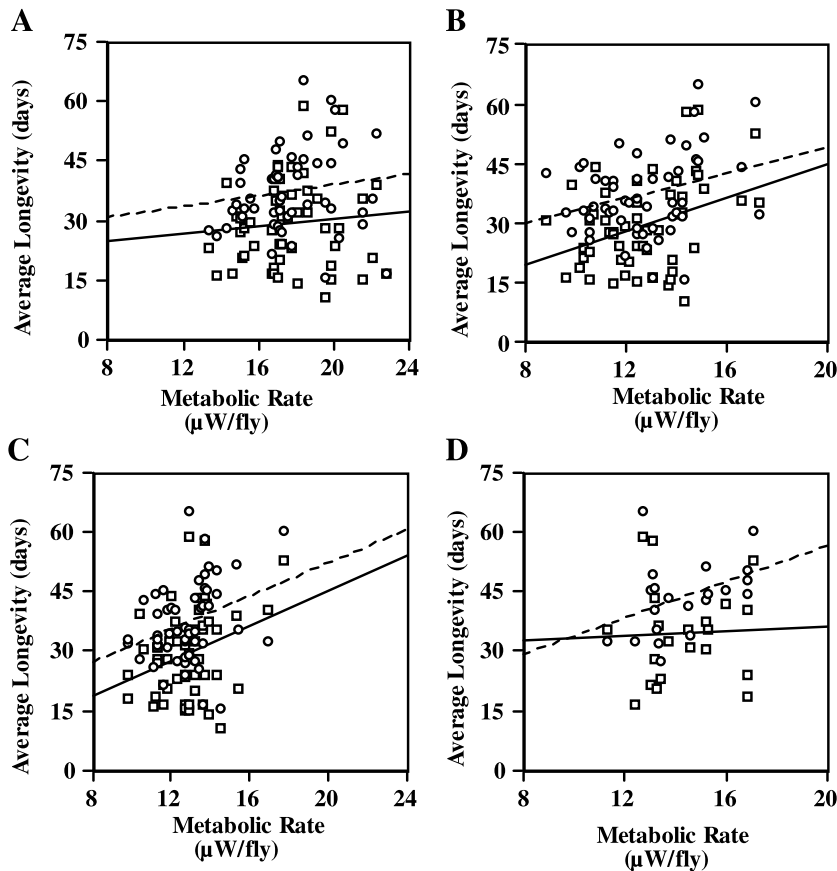


Fig. 2. Correlations between metabolic rate and longevity in 51 RI *Drosophila* lines and parental stocks measured at different ages. The average metabolic rate of each RI line is plotted against the average longevity of the line as measured either in vials (○) or cages (□). The plotted lines were fitted with a least squares linear line fitting equation. Dashed line, metabolic rate vs. vial longevity; solid line, metabolic rate vs. cage longevity. For 5-day-old flies (A), no significant correlation exists between either vial longevity or cage longevity and metabolic rate ($r = 0.16$, $P > 0.05$, $n = 53$; $r = 0.113$, $P > 0.05$, $n = 53$). For 16-day-old flies (B), there is a significant positive correlation between both vial longevity and cage longevity and metabolic rate ($r = 0.31$, $P < 0.05$, $n = 53$; $r = 0.38$, $P < 0.01$, $n = 53$). For 29-day-old flies (C), there is a significant positive correlation between both vial longevity and cage longevity and metabolic rate ($r = 0.31$, $P < 0.05$, $n = 53$; $r = 0.29$, $P < 0.05$, $n = 53$). For 47-day-old flies (D), there is no significant correlation between vial longevity or cage longevity and metabolic rate ($r = 0.41$, $P > 0.05$, $n = 22$; $r = 0.04$, $P > 0.05$, $n = 22$). The correlations between metabolic rates expressed in mass-specific units and longevity were also analyzed. The results of these analyses were essentially the same as those based on whole animal metabolic rates.

metabolic output of a line and its average longevity (Fig. 4).

Fly weight. The average body mass of the 54 lines was 0.63 mg and ranged from 0.72 to 0.53 mg. The correlation between the average mass of the lines and cage or vial longevity of the lines was not significant, indicating that differences in body mass do not appear responsible for differences in line longevity ($r^2 = 0.06$ for average line weight vs. vial longevity = 3.1, $df = 52,53$, and $r^2 = 0.04$ for average line weight vs. cage longevity, $F = 2.3$, $df = 52,53$; $P > 0.05$ for both correlations).

RQ

The RQ of the 23 lines assayed averaged 0.95 (SE = 0.01) a value consistent with the use of carbohydrates as a primary metabolic substrate. A RQ value of 0.95 was used to convert CO_2 measurements to International System of Units with an energy equivalent of 20.1 kJ/l O_2 .

DISCUSSION

The different RI lines assayed showed a wide range of average metabolic rates and longevity. There was, however, little correlation between these two variables, and the only significant relationship observed between metabolic rate and longevity was a slight positive correlation (Figs. 2–4). These positive correlations were not large, with r^2 values generally in the range of

0.05–0.10. The reasons for such a positive correlation between metabolic rate and longevity are not clear. One speculation is that the longer lived lines could have higher rates of biological repair than the shorter lived lines and that such repairs require an increased metabolic output.

There was also no apparent decline in metabolic rate from middle to old age. Such a decline might be predicted based on data obtained from *D. subobscura* (36), showing significant decreases in the levels of several key respiratory enzymes with age. One reason such a decline was not seen here may be because metabolic rates were measured on relatively inactive flies. The effects of mitochondrial decline may only be apparent at higher activity levels, such as flight. Another factor potentially responsible for the lack of decline in metabolic rate with age is the metabolic measurements only extended to 47-day-old flies, and many of the lines had average longevity much longer than this.

Clearly, the increased longevity of the long-lived RI lines does not appear to be mediated through a reduction in metabolic activity. This contrasts with other experimental results with *Drosophila*, houseflies (*Musca domestica*), and the nematode *Caenorhabditis elegans* that have found negative correlations between the metabolic rate and longevity (35, 55, 57). These differences may be due to the different mechanisms responsible for extending longevity in ectotherms. Among the factors that can increase longevity in ectotherms are either

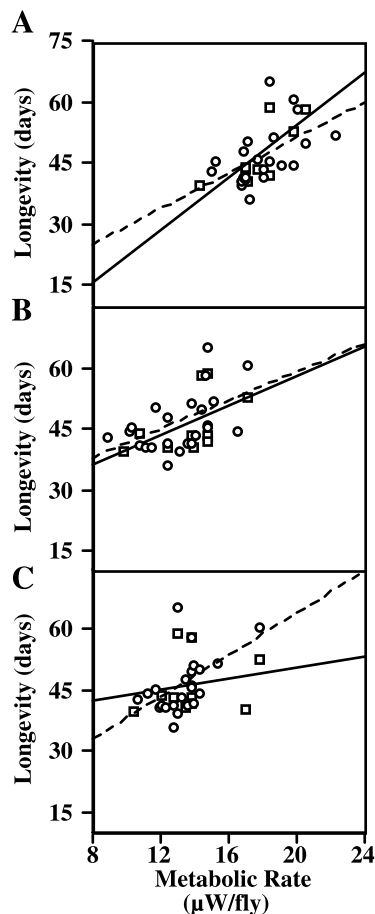


Fig. 3. Correlations between metabolic rate and longevity in RI *Drosophila* lines with an average longevity that was approximately the same or longer than the long-lived parental line used to construct the RI lines. The average metabolic rate of these long-lived RI lines is plotted against the average line longevity measured either in vials (\circ) or cages (\square). The plotted lines were fitted with a least squares linear line fitting equation. Dashed line, metabolic rate vs. vial longevity; solid line, metabolic rate vs. cage longevity. For 5-day-old flies (A), a significant correlation exists between both vial longevity and metabolic rate ($r = 0.52$, $P > 0.05$, $n = 23$; $r = 0.76$, $P > 0.01$, $n = 10$). For both 16-day-old flies (B) and 29-day-old flies (C), there is a significant positive correlation between vial longevity and metabolic rate but no significant correlation between cage longevity and metabolic rate (day 16: $r = 0.51$, $P < 0.05$, $n = 23$; $r = 0.52$, $P > 0.05$, $n = 10$; day 29: $r = 0.53$, $P < 0.01$, $n = 23$; $r = 0.20$, $P > 0.05$, $n = 10$).

mechanisms that reduce the metabolic rate of the organism or methods that augment the ability of the organism to withstand stresses associated with aging. Mutations or manipulations that induce the former condition would produce animals that live their lives at high temperature as if they are at a low temperature, producing so called "refrigerator mutants" (30). This class of long-lived animals is unlikely to provide basic insights into mechanisms that alter aging. Of far greater potential value toward understanding the mechanisms of aging are mutations or manipulations that allow the organism to both live longer and retain normal metabolic function such as exhibited in the long-lived RI lines.

We suggest that the critical factor allowing the long-lived RI lines to retain normal metabolic function is the method by which long-lived genotypes are generated. Most of the single-gene mutations that are known to extend life span in *C. elegans* and *Drosophila* were discovered in mutant screens (6, 20, 23, 43, 52). These discoveries have significantly advanced our understanding of the genetic control of life span, but the particular mutations uncovered have been sheltered, in the following sense: in order for a novel mutation to be recovered in a mutant screen, all that is required is that it have the desired effect on life span and that it be able to survive and reproduce in isolated culture. One could imagine, for instance, that a novel mutation that depresses resting metabolic rate, developmental rate, and mating ability could survive in isolated culture. In contrast to the "pampered" lifestyle of mutant screens, longevous genotypes produced by artificial selection in genetically heterogeneous populations, such as the experimental material used in our studies, have undergone multiple generations of fitness challenges; they are required to undergo larval development, eclosion, feeding, mating, and reproduction under competitive conditions in high-density genetically heterogeneous population cages. It seems very likely that mutations that reduce metabolic rate would be selectively disadvantaged in such a competitive environment. In short, we suggest that there are two classes of life span-extending genes: artificial selection experiments produce long-lived genotypes that are robust and competitive in all aspects of organismal fitness, whereas mutant screens produce longevous stocks that may be defective in one or more components of fitness, perhaps often including impaired metabolism. Our proposal is testable. We are presently engaged in measuring metabolic rates and life spans in many life span variants of *Drosophila*, including single-gene mutants and artificially selected stocks. By using standardized culturing and measurement techniques on a wide variety of

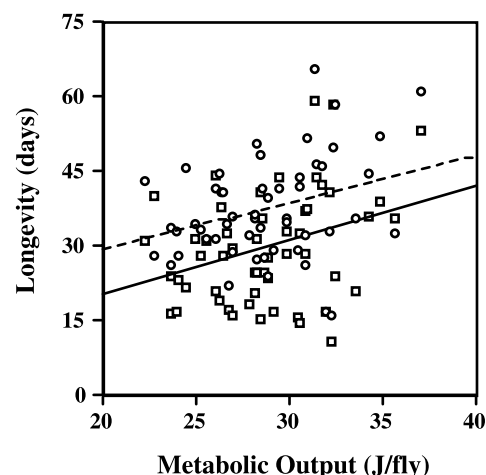


Fig. 4. Correlation between the estimated metabolic output of RI lines from day 5 to day 29. There is a significant positive correlation between both vial longevity and cage longevity and metabolic output ($r = 0.31$, $P < 0.05$, $n = 53$; $r = 0.33$, $P < 0.05$, $n = 53$).

genetic material, we hope to contribute to a general understanding of the interplay between life span, metabolic rate, and the method employed to produce long-lived genotypes. A recent report by Marden et al. (29) indicates it is possible for the long-lived, single-gene *Drosophila* mutants to retain normal metabolic rates.

Although our results indicate that long-lived *Drosophila* can maintain normal metabolic rates, they do not necessarily indicate that reactive oxygen species (ROS) generated during aerobic metabolism are unimportant in aging. Rather, the apparent uncoupling of metabolic rate and longevity in the RI lines may be due to factors such as an attenuation in the production of ROS during aerobic respiration or to increased ability to repair such damage. Organisms could potentially cope with ROS-induced damage by either reducing the levels of ROS produced during aerobic respiration or by increasing levels of antioxidant defenses or mechanisms that repair oxidative damage (12, 38). Long-lived species appear to use the former method, and there is little correlation between the level of antioxidant defenses possessed by a species and its longevity (4).

Researchers have studied the relationship between metabolic rate and longevity in *Drosophila melanogaster* for more than 85 yr (22). Since that time, numerous, often conflicting, results have been published on the relationship between these two variables (discussed in Ref. 25). For example, Service (45) compared the metabolic rates of *D. melanogaster* lines selected for increased longevity with the control lines from which they were derived and found that the long-lived lines showed age-specific decreases in metabolic rate compared with the control lines. However, a more recent comparison of the metabolic rate of these same lines by Djawden et al. (8) found no differences in metabolic rate between the long-lived and control lines. A possible explanation for these different results is the experimental conditions used to assay metabolic rates. In the original study comparing metabolic rates (45), the flies were confined to a small chamber, which greatly limited their ability for normal movement. In the second study comparing the metabolic rates between the lines, the metabolic rates were measured with the flies maintained in the same conditions as used to assay longevity. To meaningfully correlate the metabolic rate of an organism to its longevity, the metabolic rates of the organism must be measured under conditions that closely match the conditions used to assay longevity. For this reason, the metabolic rates of the RI lines were measured under conditions that were representative of the conditions under which the vial longevity were assayed. In both cases the flies were in an environment in which they could freely walk but not typically fly. Whereas the flies in the metabolic chambers were unfed and the flies in the longevity assay did have access to food, we found that the metabolic rate of unfed flies was quite stable over the time span that the flies were in the metabolic chambers (53). This was particularly true for the older

flies with the final metabolic reading typically within 5% of the initial metabolic reading.

The relationship between metabolic rate and longevity is complex within species other than *Drosophila*. Experimental manipulations with mammals have generally found that factors increasing metabolic rate do not cause a concomitant decrease in longevity (19). Similarly, there is an estimated threefold range in the lifetime energy output between different mouse strains (42). One reason why metabolic rate and longevity do not follow a simple inverse relationship is the fact that ROS production is not always directly proportional to metabolic rate (26). Additionally, metabolic rate and longevity may not vary as predicted because standard techniques for measuring metabolic rate do not differentiate between the different mitochondrial respiratory states, which is an important parameter affecting mitochondrial ROS production (37). Finally, variation in defenses against ROS can also be an important mechanism affecting the relationship between metabolic rate and aging.

The relationship between metabolic rate and longevity is also complex between species, and it is now well established, despite the early conclusions of Pearl (40) and others (44), that when compared at higher phylogenetic levels, metabolic rate and longevity are not linked in a simple, inverse relationship. For example, in mammals there is nearly a 30-fold variation between the actual longevity and that predicted by an inverse relationship between these two factors (2). Even larger differences occur when the estimated mass-specific, lifetime energy output is compared across a wider range of animals with the lifetime mass-specific energy output of long-lived pelagic birds, estimated to be around 750 times more than that of a soil nematode (54). These large differences in lifetime energy output can be explained by the vastly different life history characteristics of these animals, as well as differences in selective pressures to which they have been subjected (3, 50).

Although metabolic rate remains a potentially important determinate of the longevity of an organism, these results indicate that, at least in *Drosophila*, it is possible to both maintain a normal metabolic rate and have extended longevity. As such, these RI lines can provide valuable insights into the causes of aging and mechanisms by which it can be attenuated.

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DISCLOSURES

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