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ORIGINAL PAPER

Age-Dependent Stability of Sensorimotor Functions in the Life-Extended *Drosophila* mutant *Methuselah*

Agavni Petrosyan · I-Hui Hsieh · Kourosh Saberi

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Abstract *Methuselah* is a *Drosophila* mutant with a 35% increased lifespan. We examined the robustness of *methuselah*'s sensorimotor abilities in tethered flight as a function of age in experiments designed to test visuomotor synchronization and phototaxis in simulated flight. A total of 282 flies from different age groups (4 hours to 70 days) and genotypes (*mth* and *w1118*) were individually tethered under an infrared laser-sensor system that digitally recorded wing-beat frequency (WBF). We found that *mth* has a higher average WBF throughout most of its lifespan compared to parental control flies (*w1118*) and develops flight ability at a younger age. Its WBF at late life, however, is not significantly different than that of its parental control line. We further found that *mth* entrains during flight to motion of a visual grating significantly better than its parental line. These findings suggest that the *mth* gene not only delays chronological aging but enhances sensorimotor abilities critical to survival during early and middle, but not late life.

Keywords *Drosophila* · *Methuselah* · Lifespan · Sensorimotor

Introduction

Drosophila melanogastar is a leading animal model in the study of aging, life extension, and functional senescence (Curtsinger et al. 1995; Osiewacz 1997; Lin et al. 1998). Popularity of this organism stems partly from its short lifespan, which provides a practical means of investigating life extension at a population level, and partly due to the high homology of its genes to other species including humans. Recent studies have shown, for example, that a substantial proportion of human disease genes are conserved in *Drosophila* (177 of 289 genes examined by Rubin et al. 2000; Fortini et al. 2000). Furthermore, *Drosophila* has a large repertoire of motor and sensory behaviors that facilitate investigation of sensorimotor decline as a function of aging.

The current study describes sensorimotor experiments on *methuselah*, a *Drosophila* chromosome 3 mutant with a 35% increased longevity (Lin et al. 1998; Wang et al. 2004). The *methuselah* gene (*mth*) is speculated to increase lifespan, at least in part, by regulation of *Drosophila*'s antioxidant defense system (Lin et al. 1998) which mitigates the cumulative damage to DNA and cell structure resulting from reactive oxygen species (Harman 1995, 2003; Barja 2004; Bokov et al. 2004; Landis and Tower 2005). While a number of studies have investigated the genetic, biochemical, and neural mechanisms affected by *mth* (West et al. 2001; Song et al. 2002; Aigaki et al. 2002; Wang et al. 2004; Cvejic et al. 2004) little is known about this gene's influence on sensory decline throughout *Drosophila*'s lifespan, particularly in late life. To date, the only study that has examined functional senescence in *mth* has found no age-dependent differences between *mth* and its parental line in noxious-odor and electric-shock avoidance, phototaxis, geotaxis, and exploratory behaviors (Cook-Wiens and Grotewiel 2002). This is surprising in that Lin et al. (1998) in the original paper on the discovery of the *mth* gene had shown that this gene increases resistance to stress-inducing stimuli such as heat and toxic supplements that cause oxidative stress.

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A. Petrosyan · I-HuiHsieh · K. Saberi (✉)
Department of Cognitive Sciences, University of California, Irvine, CA 92697-5100, USA
e-mail: saberi@uci.edu

Clearly, the degree to which functional senescence is delayed by life-extension genes is a critical question. Here we examine functional decline in *methuselah* and its parental control line for an entirely different set of natural behaviors than previously examined. These include wing-beat frequency (WBF) and coordinated visuomotor entrainment to motion during simulated flight. The goal is to determine whether the age-dependent decline of these functions is delayed in *methuselah* or coincides chronologically with that of the parental control group.

Methods

Verification of life extension in *methuselah*

The *mth* mutant and *w1118* parental control stock from which *mth* was derived were a generous gift from the laboratory of Dr. Seymour Benzer (California Institute of Technology, Pasadena, CA, USA). We verified delayed mortality in the *mth* line with small samples of ~100–150 virgin flies per genotype. The purpose was not a full replication of the previously published mortality curves but to simply verify life extension in *mth* flies. Flies were maintained in small groups of no more than 50 per vial containing fresh food media (Genesee Scientific Corp., San Diego, CA, USA; polystyrene, O.D. x H 25 × 95 mm; standard cornmeal-agar medium, IU Bloomington formula). Vials were kept in a low-temperature incubator at 25°C and 20% humidity (ambient) on a 12/12 h light/dark cycle (VWR Scientific, Model 2015). Surviving *Drosophila* were transferred to fresh food vials every 3–4 days and scored for survival.

Variation in WBF as a function of age and genotype

Flies were sex-segregated within 4 h of eclosion and maintained in the incubator at 25°C and 40% humidity on a 12/12 h dark/light cycle. They were transferred to fresh food vials every 3–4 days. WBF was measured in a tethered-flight paradigm at ~5 h after onset of subjective day (except for very young flies as described below). The tethering process involved several steps. First, an individual fly was lightly CO_2 anesthetized and transferred to a custom-made aluminum block in a Peltier cooler (Boekel Scientific, Model 260014) on which a small opening ($2 \times 1 \times 1$ mm^3) had been drilled to allow accurate positioning of an anesthetized fly. The fly remained under cold anesthesia at 4°C. Individual flies were gently handled either with a small brush or a jeweler's vacuum tweezers. The tip of a tungsten wire (130 μ in diameter) was dipped in glass glue (Loctite, New york, NY, USA) and, under a stereo microscope (Olympus SZ40) lowered using a micropositioner (Stoelting Co./Prior, England) onto the anesthetized fly's thorax. The glue was cured with a UV gun (Electro-Lite Corp., Model ELC-403) for 20 s and the fly was removed from the Peltier cooler using the micropositioner. Flies usually recovered from cold anesthesia and began flight within 3–4 min. Tethered flies were moved to the experimental chamber, fed with a small piece of filter paper dipped in sugar-water, and allowed to rest and become acclimated to the experimental environment for an additional 30–60 min prior to data collection.

The tethered fly was positioned under a solid-state infrared (IR) laser (808 nm; Lasermate Group Inc., Pomona, Ca, USA, Model PLC8082AE) with an adjustable focus that cast shadows of the wing beats onto fast-response IR photodiode sensors (Photonic Detectors Inc., Simi Valley, CA, USA, Part no. PDB-C615-2). The sensors were placed in a small plastic box covered with an IR filter (Edmund Industrial Optics, Barrington, NJ, USA, Part. no. NT32769). The IR laser and filter system were used to isolate changes in luminance associated with wing-beat shadows from extraneous light (especially from moving visual gratings used in a later experiment). The experiment was run in complete darkness in a steel chamber ($2 \times 2 \times 2$ m^3; IAC) with only a single green LED (555 nm) positioned directly in the fly's line of sight at a distance of 15 cm to provide a visual target for phototaxis (Hadler 1964; Miller et al. 1981).

The output of the photodiode sensors were sent to an amplifier and fed into an analog-to-digital converter (Sound Blaster Live, –120 dB noise floor) positioned outside the chamber, and recorded at a sampling rate of 10 kHz. The experimenter, who was in the chamber, listened to the fly's wing beats through a pair of headphones connected to the amplifier. This allowed an informal monitoring of the experimental run. WBF was measured during the first minute of flight after the rest period. Each fly's wing-beat data were saved in a digital file for later processing. An individual fly's WBF was determined as the average of five to ten 1-s samples of flight. This sampling scheme was sufficiently representative of the average WBF. For each 1-second sample, the wing-beat waveform was Fast-Fourier Transformed and the frequency corresponding to the peak of this function was determined as the WBF for that sample (i.e., 1-Hz frequency resolution; Fig. 1).

To verify that WBF measured using the laser system is accurate, wing-beats of one individual fly were recorded using the laser-system and, simultaneously, with a probe-tube acoustic microphone (Etymotic Research, Elk Grove Village, IL, USA ER-7C). The output of the two measurement systems were led to two separate microprocessors where they were digitally recorded. We found a near-perfect correlation between the acoustic measure

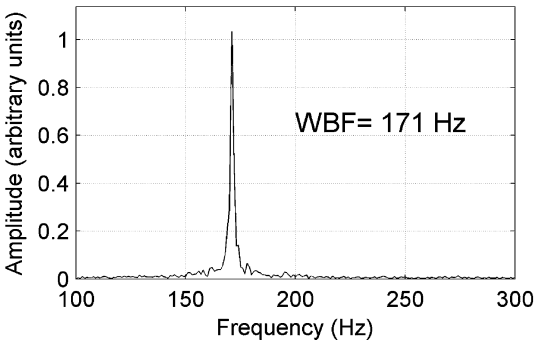

Fig. 1 Fast-Fourier Transform of a 1-second segment of an *mth* wing-beat waveform

of wing-beat waveform and laser measurements ($r = 0.994$). After each experimental run, flies were CO_2 anesthetized, removed from tether, and discarded in citrus oil.

Onset of first flight

Five *mth* and five parental-line flies were tethered and tested for flight ability at 4 h after eclosion. WBF could not be measured before 4 h of age because at younger ages their wings were not fully expanded. Parental-line flies (but not *mth* flies; see results) were kept on tether and fed throughout the day and retested at 10 h of age. All parental-line flies remained on tethered for 2 days, and were fed several times per day/night. Their WBF was measured at 4, 10 h, 1, and 2 days of age.

WBF measured at 5 days of age conditioned on lifespan

We measured WBF of a group of *mth* and parental flies at 5 days of age, and maintained the tested flies in individual vials to track their longevity with the goal of determining if particular frequencies are associated with a longer lifespan. The testing age of 5 days was selected because from the first experiment we determined that it is an ideal age for testing flight. Others have also reported that the age range from 3 to 5 days is optimum for testing *Drosophila* flight (Lehmann and Dickinson 1998, 2001).

All procedures were identical to those described earlier, with the following exceptions. After measuring WBFs at 5 days of age, flies were CO_2 anesthetized and removed from tether under a microscope by gently pressing on their thorax. Each fly was then placed in an individually numbered fresh-food vial and maintained in the incubator. Their behavior, after recovery from anesthesia was informally monitored and appeared to be normal. Sixteen male *mth* flies and 14 male parental-line flies were tested in this experiment.

Lifespan variation in WBF for individual *Drosophila*

To better understand age-dependent changes in WBF of individual *Drosophila*, compared to population-level variations, WBF was measured for six individual flies (three males *mth* and three males controls). All procedures were similar to those described previously, with the following exceptions. Flies were tethered at an early age, kept on tether, and fed several times per day/night for the first few days, and their WBFs measured several times during the first few days. They were then removed from tether and returned to the incubator with no noticeable change in behavior after recovery from anesthesia usually within 2 min.

The six individual flies were maintained in six separate fresh food vials and moved to new vials every 3–4 days. Flies were then re-tethered at approximate ages of 10, 20, 30, and in one case 42 days and their WBF measured. Immediately after measurement of WBF, the fly was anesthetized, removed from tether, and placed back in a fresh food vial. Their behaviors, after removal from tether, were again informally monitored and appeared to be normal.

Visuomotor synchronization to moving visual stimuli

Individual flies were tethered as described earlier. They were positioned in front of a high-speed LCD (Viewsonic VX 924, 4 ms rise-decay time) at a distance of 5 cm. An LCD was selected because unlike a CRT it does not flicker (i.e., a pixel remains on until changed) and since *Drosophila* have a high flicker-fusion rate, CRT monitors do not provide a temporally stable image. Vertical black and white square waves (bars) with a spatial frequency of 30° served as stimuli. In front of the LCD, a 30×30 cm^2 Schott Borofloat 3.3 mm thick high-energy hot mirror (heat shield; Navitar Coating Labs Lot no. 10-815-10) was positioned to eliminate near IR as well as LCD heat (passband between 390 and 700 nm). Temperature measurement at the position of the tethered fly (5 cm from the LCD) was equal to ambient temperature in the experimental chamber (25°C). Each trial of a 25-trial run consisted of 4 s of a stationary grating followed by 3 s of visual motion at a temporal frequency of 4 s^{-1}. These spatial and temporal frequencies were selected because in earlier pilot work we found that they induce a strong optomotor response in wildtype flies. Flies were tested from two age groups of 5- and 20-day olds. Most runs consisted of visual motion toward the right. However, for a subset of flies we also examined upward or downward motion of a horizontal grating. All aspects of the stimulus generation, presentation, and digital recordings were microprocessor controlled via software written in Matlab (Mathworks).

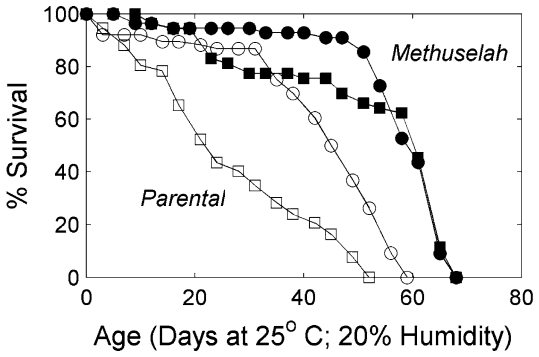

Fig. 2 Survival curves for small samples of virgin *mth* and parental line flies (*w1118*). *Filled squares*: male *mth* ($n = 53$), *filled circles*: female *mth* ($n = 55$), *open squares*: male *w1118* ($n = 92$), *open circles*: female *w1118* ($n = 76$)

Results

Verification of life extension in *methuselah*

Mortality curves are shown in Fig. 2 with filled symbols representing data from *mth* and open symbols from its parental line (circles = females; squares = males). The *mth* line clearly outlives its parental line. However, the mean lifespans of all genotypes are shorter than that previously reported, likely due to dry environmental conditions known to reduce survival rates (Al-Saffar et al. 1996); e.g., 20% vs. 50–65% humidity used in studies that have shown longer overall lifespans (Cook-Wiens and Grotewiel 2002; Lin et al. 1998). Stocks used in experiments, however, were maintained at 40% humidity at all times to increase survival rates. Kaplan–Meier survival analyses (log rank tests) showed that *mth* males significantly outlive parental-line males ($\chi^2 = 66.37$, $P < 0.001$) and *mth* females significantly outlive parental females ($\chi^2 = 48.59$, $P < 0.001$). Lin et al. (1998) have shown that *mth* males outlive controls by 35% ($n = 1{,}000$). Here we have observed a larger effect of genotype than previously reported, and no significant sex-specific effect for *mth*; these differences may possibly be attributed to a higher variance in our estimation of mortality functions associated with small-sample statistics.

Variation in WBF as a function of age and genotype

Figure 3 shows WBF as a function of age for *mth* and its parental line. Each circle is the average WBF from an individual fly. The *mth* data (top panel) is from 84 individually tethered flies, and the parental strain (*w1118*; middle panel) from 71 individuals. Solid lines represent mean WBF replotted for both genotypes in the bottom panel to facilitate visual comparison. Error bars are one standard error.

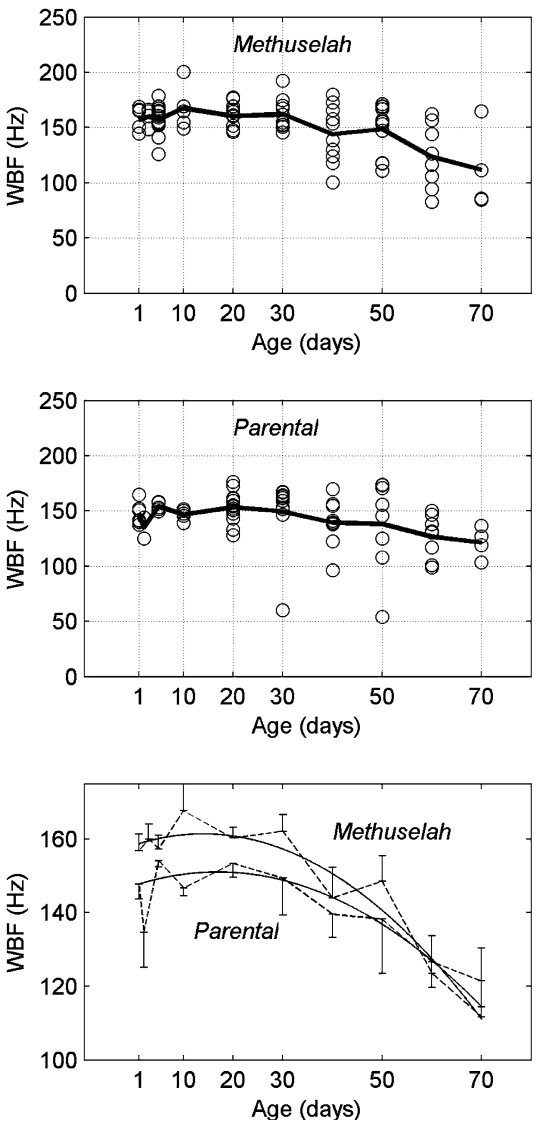

Fig. 3 Wing beat frequency (*WBF*) measured for 84 *mth* and 71 parental-line flies as a function of age. Each symbol represents average WBF from an individual fly. *Bottom panel* shows averaged WBFs with error bars representing one standard error. The curves in the bottom panel are fits from a multiple-regression model (see text)

A clear effect of age is observed. WBFs generally ranged from ~100 to 170 Hz. Rarely did WBF exceed 180 Hz and in only one case (a 10-day old *mth*) did WBF exceed 200 Hz. At every tested age from early life to 50 days of age, *methuselah* has a higher average WBF. However, for the oldest age groups (e.g., 70 days) the *mth* flies do not show a higher WBF than the parental group [$t(6) = 0.49$, n.s.] suggesting that *mth*-related delay in mortality is not coupled with delayed functional decline. This finding, however, should be tempered by the fact that the older age groups of both genotypes are sampled from progressively smaller populations, i.e., the population of survivors.

A multiple regression analysis was performed using SPSS on the data of Fig. 3 in which we included as predictor variables, linear terms for age and genotype, a quadratic term for age, and an age by genotype interaction term. Model fits are shown as the curves in the bottom panel of Fig. 3, with a coefficient of determination $R^2 = 0.255$, a significant effect of genotype ($t = 2.31$, $P < 0.05$), a significant effect of quadratic age ($t = -2.87$, $P < 0.01$), non-significant effects of the linear-age term and the genotype by age interaction. The statistical model yielded two main findings: (1) that *mth* flies show a significantly higher WBF throughout most of their lifespan compared to the parental-line flies, and (2) that age has a significant non-linear effect on WBF.

Onset of first flight

Data from five individual *mth* and five parental flies are shown in Fig. 4. Of the five parental flies tested, only one flew at 4 h of age. At ~24 h of age, still only 3 of 5 could fly, though these three now showed a higher WBF compared to when they were 10 h old. At age 48 h, all five could fly, and on average showed a higher WBF compared to that of earlier ages. As there were no visible injuries to any of the flies and since all were tethered using the same protocol, it appears that the *mth* gene not only has a life-extending effect, but also facilitates the initiation of flight at an earlier age, an ability that is likely important to survival. A one-way repeated measures analysis of variance on the parental-line data (all five flies at the four age categories) showed a significant effect of age on WBF ($F_{3,12} = 8.20$, $P < 0.01$). An ANOVA on the restricting data set from only the three flies that showed flight ability at ages 10 h, 1, and 2 days was also statistically significant ($F_{2,4} = 7.39$, $P < 0.05$). A comparison of the data from *mth* at 4 h of age to the three 10 h old parental flies that showed flight ability did not show a statistically significant difference in WBF [$t(6) = 0.55$, n.s.]. However, there was a significant difference between WBF for 4-h old *mth* and 1-day [$t(6) = 2.52$, $P < 0.05$) and 2-day old parental-line flies [$t(8) = 3.105$, $P < 0.05$).

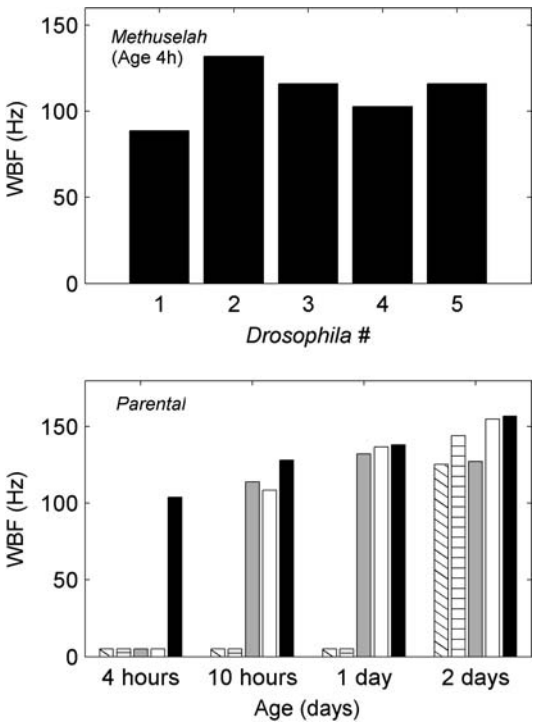

Fig. 4 Onset of first-flight development for five *mth* and five parental-line flies

WBF Measured at 5 days of age conditioned on lifespan

How does WBF measured at a given age vary as a function of the age at which an individual fly dies? In this experiment we measured WBF at 5 days of age conditioned on an individual fly's lifespan. This approach to determining the correlation between biological function at a particular age and longevity has previously been used to study functional aging in flies, for example, to determine the rate of egg laying at a particular age conditioned on lifespan (Carey et al. 2005), effects of the onset of dietary restrictions on subsequent longevity (Mair et al. 2003; Vaupel et al. 2003), and association between the intensity of a male fly's sexual signaling (e.g., calling behavior) and lifespan (Papadopoulos et al. 2004; Zhang et al. 2006).

Figure 5 shows results of this experiment. The abscissa represents the age at which each fly eventually dies, and the ordinate represents WBF. Error bars are one standard deviation measured from several estimates during the first minute of flight after rest. The most interesting finding is that the lowest wing-beat frequencies obtained from *mth* flies were from those that lived the longest. Of the 16 *mth* flies tested at age 5 days, only one did not fly. This single fly had the longest lifespan of 77 days. We did not however test this fly at a later stage of life to determine if it did begin flight. The two *mth* flies with the second and third longest lifespan had mean WBFs below 150 Hz, the lowest WBFs of the entire group. The highest WBF, exceeding 180 Hz, was produced by an *mth* that lived 52 days. No significant change in average WBF is observed for flies whose lifespan ranges from about 35 to 65 days.

Of the 14 parental-line flies tested, the shortest-lived one (32 days) did not fly. Excluding this fly, three out of the four parental-line flies that showed the lowest WBFs had the longest lifespan, and the fourth had the shortest lifespan. The highest WBF for the parental group exceeded

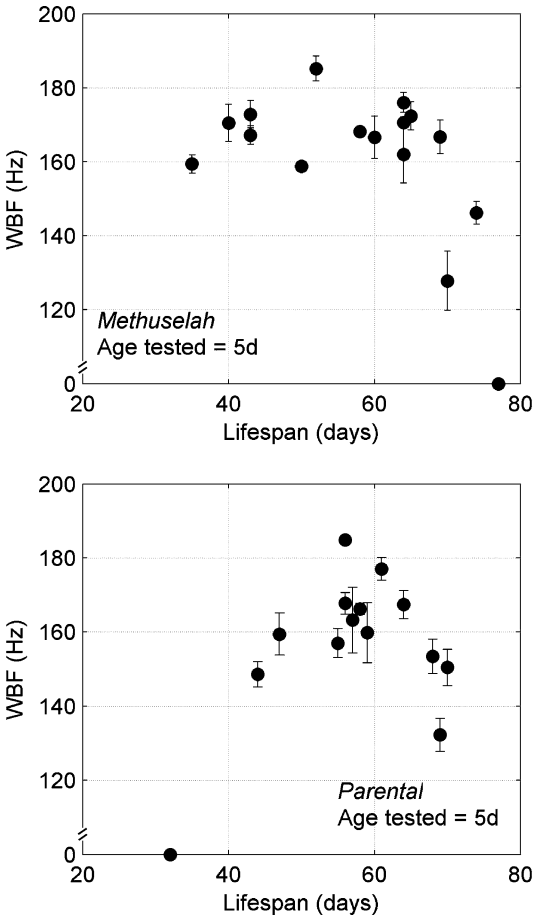

Fig. 5 WBF at 5-days of age conditioned on lifespan. Data are from 16 *mth* and 14 parental-line flies. Error bars are one standard deviation

180 Hz and was produced by a fly with a lifespan of 56 days (measured at age 5 days).

A multiple-regression analysis on the data of this experiment using lifespan, genotype, and a second-order term for lifespan (given the non-monotonic functions in Fig. 5) showed a statistically significant effects of lifespan (t = 47.31, $P < 0.001$), lifespan^2 (t = -45.30, $P < 0.001$), and genotype (t = -15.842, $p < 0.001$).

Lifespan variation in WBF for individual *Drosophila*

Top and bottom panels of Fig. 6 show data from three *mth* and three parental-line flies, respectively. Data were collected throughout each fly's life until an age at which the individual fly would no longer fly. Flies that could no longer fly were still maintained in fresh food vials and transferred to new vials every 3–4 days until they died. Within each panel the age at which each fly died is indicated. The form of the age-dependent WBF function is similar to that observed for the population (Fig. 3).

Of course, given the small sample size, we did not expect a significant difference between the *mth* and its parental line in this latter study. Nonetheless, even with this small sample, the longest-lived individual was a *methuselah* (60 days), and the shortest-lived individual was of the parental line (32 days). Further, the oldest age at which WBF could be measured before failure of flight was for an *mth* (42 days) and the youngest age after which flight failure was observed was for a parental fly (20 days; bottom right panel). In addition, in spite of the small sample, the age at which peak WBF is observed is higher for all three *mth* flies (9, 10, and 20 days) than that for the three parental flies (3, 4, and 5 days); a Mann–Whitney U-test showed that the two sets of peak values are statistically significantly different (U = 0, P = 0.05).

Visuomotor synchronization to moving visual stimuli

Sample WBF patterns for an *mth* and a parental-line fly are shown in Fig. 7. White and gray regions represent, respectively, times at which the visual grating was either moving or stationary. We quantified the strength of entrainment (i.e., phase-locking) to the stationary-motion period by subjecting each WBF function (e.g., waveforms shown in Fig. 7) to a Fast-Fourier Transform (FFT). The amplitude spectrum of each FFT was then re-plotted with the abscissa transformed to units of 1/frequency, i.e., transformed to waveform period in units of seconds. The purpose was to determine the dominant periodicity in the WBF function. If the dominant period was near or at 7 s, this was taken as evidence that the individual fly entrained to the stationary-motion period of 7 s (4 s stationary + 3 s motion). For each fly from each genotype we calculated this dominant period and plotted the results in the histograms shown in Fig. 8.

The top histogram is based on 68 runs from 48 *mth* flies, and the bottom histogram is based on 60 runs from 41 parental-line flies. For a subset of flies we examined entrainment to motion in different directions, hence, the additional runs. The *mth* population clearly shows a strong entrainment to a 7-s period, while the parental-line population does not, although a peak at 7 s and a larger peak at around 9 s are observed. A non-parametric χ^2 median test with Yates' continuity correction showed a significant difference between the *mth* and parental-line distributions of peaks periods (χ^2 = 4.49, $P < 0.05$). A Kolmogorov–Smirnov test on the differences in the *shapes* of the two distributions (derived from cumulative functions), however, did not yield statistical significance (Kolmogorov–Smirnov Z = 1.135, P = 0.152).

The data shown in the histograms of Fig. 8 are from the entire population tested, which included both 5-day and 20-day old flies. To determine if entrainment to visual motion

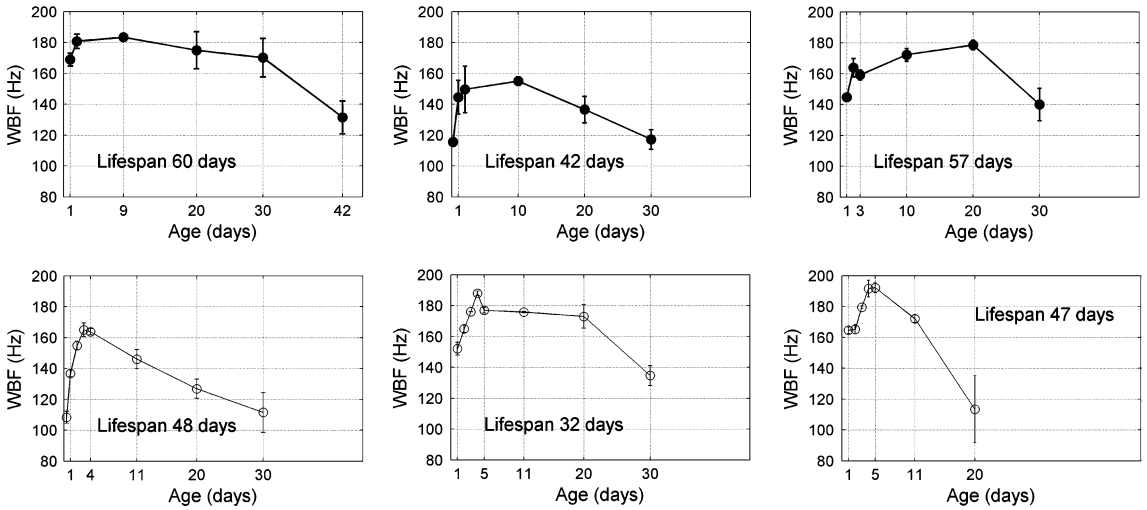

Fig. 6 Each panel shows lifespan variation in WBF for an individual *Drosophila*. The *top panels* show data from three male *methuselah* flies and *bottom panels* from three male controls

is age dependent, we re-plotted in Fig. 9 the data of Fig. 8 separately for the 5- and 20-day old flies. Note that a larger number of 5-day olds were tested. Also note that entrainment is not age specific, at least for the two tested age groups, since a main peak at 7 s is observed for both *mth* age groups. A χ^2 median test did not show a statistically significant difference between the 5 and 20 days old distributions for the *mth* (left panels, $\chi^2 = 2.42$, n.s.) or the 5 and 20-day old parental-line flies (right panels, $\chi^2 = 0.95$, n.s.). The distribution of periodicity peaks for the 5-day old *mth* and parental-line flies, however, were statistically significantly different (Fig. 9, top two panels, $\chi^2 = 5.90$, $P < 0.05$). However, the distributions for the 20-day old *mth* and parental line flies (Fig. 9, bottom two panels) were not significantly different ($\chi^2 = 0.096$, n.s.).

Figure 10 shows the data re-sorted by direction of visual-grid motion. Vertical-bar gratings were used to simulate motion toward the right, and horizontal gratings to simulate motion in either up or down directions. Strong entrainment of *mth* to motion is observed for right and up directions of motion with no significant difference between these two directions of motion ($\chi^2 = 0.627$, n.s.), but not for downward motion, which appeared to suppress flight.

Discussion

We described functional aging in the *Drosophila* life-extended mutant *mth* and found that it has a higher WBF throughout most of its lifespan, begins flight at an earlier age, and entrains to visual motion significantly better than its parental control line. In addition to genotype differences in WBF, a number of other findings are of interest. First, the association between WBF at a young age and longevity suggested that those individual flies that have the longest and shortest lifespan produce relatively low WBFs (Fig. 5). We suspect that the low WBFs for short-lived flies are possibly due to a fragile biological state, whereas the low WBFs for long-lived flies likely reflect a lower metabolic rate. Previous studies that have compared metabolic rate of life-extended *Drosophila* to controls have found no difference as measured by CO_2 output at a resting state (Lin et al. 1998; Tatar et al. 2001; Marden et al. 2003). Our results, measured in a highly active state of the fly are consistent with reports of a negative correlation between metabolic rate and lifespan extension (Van Voorhies and Ward 1999). It is however unknown whether the longest-lived flies whose data are shown in Fig. 5 developed a higher WBF in later life, i.e., were characterized by a more gradual increase in WBF as they aged.

Wing-beat frequency measured for individual flies at several points during their lifespan also yielded interesting results (Fig. 6). An observation from individual WBF curves, which could not be determined from a population curve, was the number of days that an individual survived after failure of flight. The oldest age at which WBF could be measured from the six flies was 42 days for an *mth* fly. This individual lived 60 days, suggesting that at least this type of motor failure does not signify a near-death state. Similarly, the *mth* fly whose data are shown in the top-right panel of Fig. 6 lived nearly a month after the oldest age at which WBF could be measured.

The superior motor abilities of *mth* flies observed in WBF measurements were also observed in more complex tasks requiring in-flight sensorimotor synchronization to a moving visual target. For flight navigation *Drosophila*

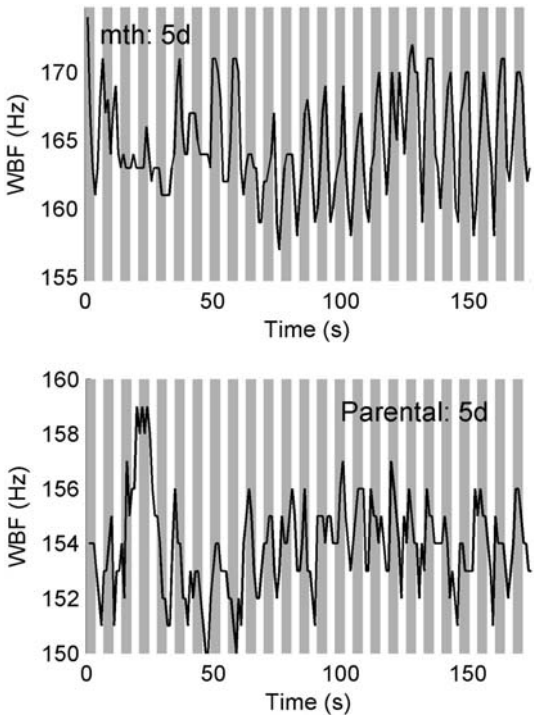

Fig. 7 WBF measured for a 5-day old *mth* and a 5-day old parental-line fly in response to moving visual gratings. Each of 25 trials consisted of 4 s of a stationary visual grating (*gray* regions) followed by 3 s of visual motion stimulation (*white* regions)

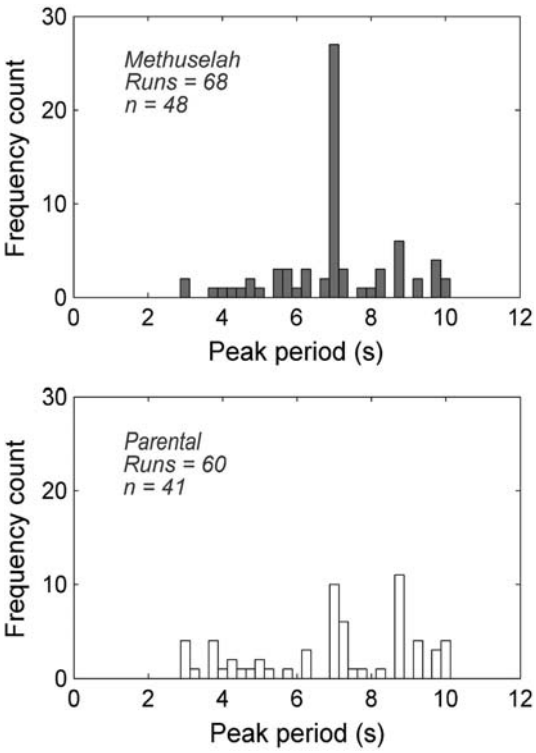

Fig. 8 Histograms of dominant periodicity derived from the Fourier spectrum of WBF functions (Fig. 7) from 48 *mth* and 41 parental-line flies

depends primarily on its vision, which has poor spatial acuity (Buchner 1976) but an extremely high temporal resolution, with flicker-fusion rates exceeding 200 Hz (Cosen and Spatz 1978; Sherman and Dickinson 2004; Autrum 1958; cf. 50 Hz in humans). When placed in a circular arena with a rotating wall of vertical stripes, flies tend to circle in the same direction as the rotating stimulus. A similar visuomotor response may be obtained from *Drosophila* in tethered flight, with rapid WBF adjustments in response to a moving grating (Lehmann and Dickinson 1998; Heisenberg and Wolf 1984). This optomotor response is presumed to indicate the fly's attempt to stabilize its visual world (Heisenberg and Wolf 1984) though one recent study has suggested that it may be related to avoidance of collision during flight (Tammero and Dickinson 2002).

In the current study we examined the strength of the optomotor response in tethered flight for *mth* and its parental line. A number of intriguing behavioral patterns emerged from this latter experiment. Figure 7 shows WBFs for two flies as a function of visual motion. Note that the peaks of the WBF functions occur in gray regions, i.e., during presentation of a stationary grating, and the dips in white regions. This, however, was not always the case and some flies produced a phase-reversed pattern. The reason for this phase difference is not clear and may simply reflect different response strategies to unwanted changes in the visual scene. A number of flies, from both genotypes, did not entrain to visual motion. Often, WBF patterns for these flies were random. However, occasionally, other systematic patterns emerged; for example, a non-periodic monotonically increasing function (not shown). Age groups older than 20 days were not tested in this latter study because they would not sustain flight long enough to allow a reliable estimation of entrainment. We should caution that the comparison of WBF entrainment to visual motion for the two age groups of 5 and 20 days should not be extrapolated to other age categories given the non-monotonic nature of the functions that relate age to sensorimotor ability (Fig. 3).

In investigating entrainment to visual motion we also found that a downward moving grating tended to suppress flight. We speculate that in nature, gusts of wind often thrust the fly in directions that are not its current heading. This may evoke rapid visuomotor corrective measures in heading. A downward thrust results in an up-moving visual scene, which may be interpreted as an undesired (or unplanned) downward fall, initiating a corrective WBF. A down-moving grating, however, may simulate an unplanned lift away from current heading, initiating a shutdown (or partial suppression) of flight to reduce elevation.

Finally, our findings may be contrasted to those of Cook-Wiens and Grotewiel (2002) who reported no significant

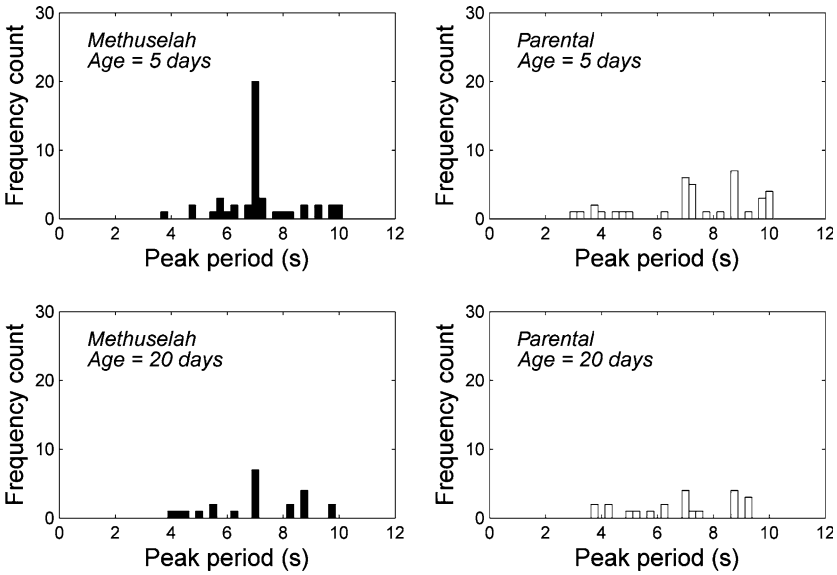

Fig. 9 Histograms of dominant periodicity in WBF functions for *mth* and its parental line as a function of age

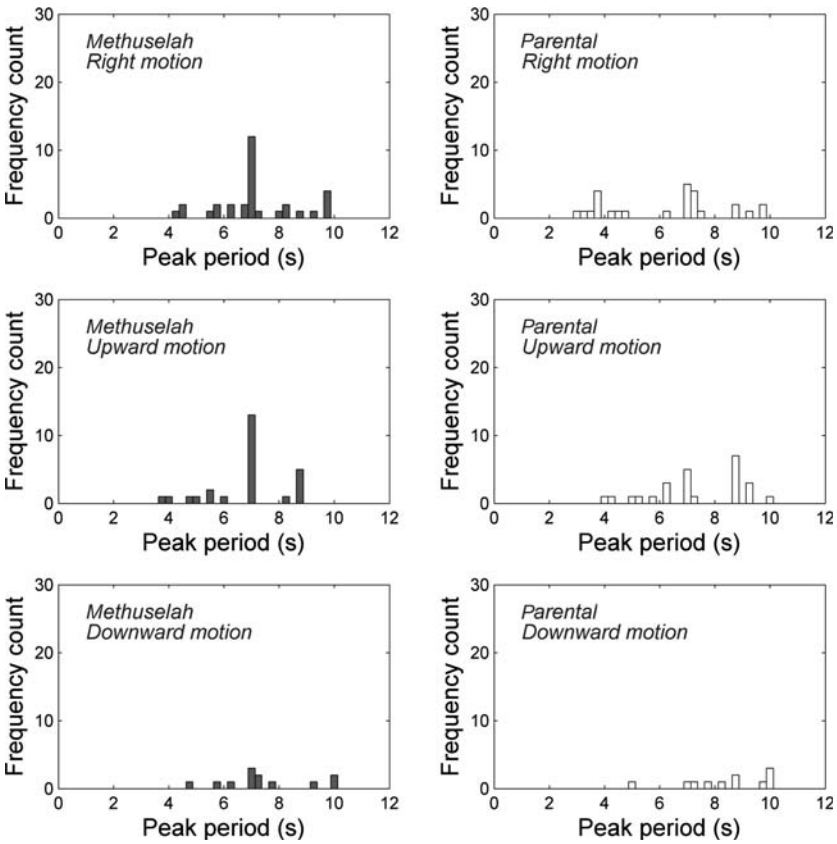

Fig. 10 Histograms of dominant periodicity in WBF functions for *mth* and its parental line as a function of the direction of visual motion

difference in functional aging between *mth* and control lines for a different set of functions, e.g., noxious-stimulus avoidance and exploratory behaviors. The *mth* gene, thus, appears to affect the robustness of some behaviors more significantly than others, though the reasons for these differences are unclear. We have, in a supplementary study, examined exploratory behaviors such as locomotor activity, quantified as a random-walk index for small groups of *mth* flies (~10) at different ages (50–77 days) in a gridline-marked arena. We found, consistent with Cook-Wiens and Grotewiel's results, no significant difference between the *mth* and parental groups at most age categories, although the parental line, surprisingly, appeared slightly more active at 50 days of age (data not shown).

In summary, our results demonstrate that the *mth* gene enhances some sensorimotor abilities, such as WBF or visuomotor reactions to moving stimuli, during much of the fly's life. However, although this gene does delay population mortality and enhances certain sensorimotor functions, the enhancements appear during early and middle life. For the oldest *mth* flies we did not find enhanced abilities, suggesting that their delayed chronological aging is not coupled with delayed functional decline.

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