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Genetic and environmental factors impact age-related impairment of negative geotaxis in *Drosophila* by altering age-dependent climbing speed

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Abstract

Age-related locomotor impairment in humans is important clinically because it is associated with several co-morbidities and increased risk of death. One of the hallmarks of age-related locomotor impairment in humans is a decrease in walking speed with age. Genetically tractable model organisms such as *Drosophila* are essential for delineating mechanisms underlying age-related locomotor impairment and age-related decreases in locomotor speed. Negative geotaxis, the ability of flies to move vertically when startled, is a common measure of locomotor behavior that declines with age in *Drosophila*. Toward further developing *Drosophila* as a model for age-related locomotor impairment, we investigated whether negative geotaxis reflects climbing or a combination of climbing and other behaviors such as flying and jumping. Additionally, we investigated whether locomotor speed in negative geotaxis assays declines with age in flies as found for walking speed in humans. We find that the vast majority of flies climb during negative geotaxis assays and that removal of hind legs, but not wings, impairs the behavior. We also find that climbing speed decreases with age in four wild type genetic backgrounds, in flies housed at different temperatures, and in control and long-lived flies harboring a mutation in *OR83b*. The decreases in climbing speed correlate with the age-related impairments in the distance climbed. These studies establish negative geotaxis in *Drosophila* as a climbing behavior that declines with age due to a decrease in climbing speed. Age-related decreases in locomotor speed are common attributes of locomotor senescence in flies and humans.

Keywords

locomotor; senescence; aging; behavior; speed

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Introduction

Aging is associated with a number of age-related functional deficits in animals (Arking 1998). In humans, age-related locomotor impairments are particularly important. Elderly individuals with impaired locomotor abilities are less independent and require more clinical care (Boyd et al. 2005), are at much greater risk for being institutionalized (von Bonsdorff et al. 2006), and are at greater risk for depression (Braam et al. 2005). Additionally, age-related locomotor impairment is associated with increased risk for falling and skeletal fractures as well as a variety of co-morbidities including coronary artery/cerebrovascular disease, obesity, diabetes, and hypertension (Boyd et al. 2005; Hillsdon et al. 2005; Onder et al. 2005; Volpato et al. 2005; Cesari et al. 2006a; Cesari et al. 2006b). Interestingly, one of the key features of locomotor senescence in the elderly population is reduced speed of walking (Oberg et al. 1993; Bohannon 1997; Kozakai et al. 2000; McGibbon and Krebs 2001; Samson et al. 2001; Laufer 2005). Geriatric individuals with reduced walking speed have the greatest locomotor impairment (Brach et al. 2007) and consequently are at greater risk for several co-morbidities and death (Woo et al. 1999; Hardy et al. 2007). Age-related locomotor impairments and reduced walking speed are therefore significant clinical challenges.

The *Drosophila* model is proving to be a powerful system for understanding the genetic basis of age-related locomotor impairment (Grotewiel et al. 2005). Negative geotaxis (startle-induced vertical movement) is a frequently used index of locomotor behavior in flies (Miquel et al. 1976; Arking and Wells 1990; Chavous et al. 2001; Mockett et al. 2001; Kang et al. 2002; Ruan et al. 2002; Goddeeris et al. 2003; Morrow et al. 2004; Gargano et al. 2005; Simon et al. 2006). Despite being commonly assessed, it was previously unclear whether negative geotaxis is principally a climbing behavior or a combination of climbing and other behaviors such as jumping and flying. Additionally, it was previously unclear whether age-related impairment in negative geotaxis is due to a decreased speed of vertical movement, an increased latency to initiate vertical movement, or other age-related behavioral changes.

We addressed these issues by using a number of different groups of surgically, environmentally and genetically manipulated flies. We find that the vertical distance moved in negative geotaxis assays predominantly reflects climbing. Jumping and flying, in contrast, have little if any impact on the vertical distance moved in negative geotaxis tests. Additionally, the speed of climbing is progressively reduced and the latency to initiate climbing is increased as flies age. Age-related reductions in climbing speed correlate with age-related decreases in the distance climbed. The changes in climbing latency, however, do not correlate with the changes in the distance climbed. Our studies suggest that senescence of negative geotaxis in *Drosophila* is primarily due to an age-dependent decrease in climbing speed. These results highlight age-related reductions in locomotor speed as a conserved feature of aging in humans and flies.

Materials and Methods

Fly stocks, husbandry and collection

Canton-S flies were supplied by Ron Davis (Baylor College of Medicine) and *OR83b* mutants and *w¹¹¹⁸* controls were from Scott Pletcher's laboratory (Baylor College of Medicine). The *OR83b* mutants were previously backcrossed to *w¹¹¹⁸* (Libert et al. 2007). Oregon-R, Lausanne-S and Samarkand flies were obtained from the Bloomington *Drosophila* Stock Center in Bloomington, IN.

All flies were reared at 25°C and 55% relative humidity under a 12-hour light/dark cycle in bottles with standard food medium (10% sucrose, 2% yeast, 3.3% cornmeal, 1% agar) seeded at a density of 12 male and female parents per bottle. Adult progeny (2–4 days-old) were collected under brief CO₂ anesthesia, separated according to sex and placed in standard food

vials with 25 flies per vial. Flies were allowed to recover at least 18 hours at 25°C/55% humidity before negative geotaxis assays were performed. For aging, flies were housed at 25°C/55% relative humidity under a 12-hour light/dark cycle and transferred to new food vials twice weekly.

Surgeries

For wingless studies, adult Canton-S and Samarkand males (2–4 days old) were collected under CO₂ and randomly assigned to one of two groups. The wings of the surgical ablation group of males were removed using surgical scissors by clipping off both wings from each animal. The other group (intact) was anesthetized under CO₂ in parallel for the same amount of time as the surgical ablation group. Both groups of flies were placed into food vials and allowed to recover at 25°C/55% relative humidity for 18 hours. The two groups of flies were tested using standard Video RING assay protocols (described below) at 1- and 4-weeks of age. For legless studies, adult Canton-S males (2–4 days-old) were collected under CO₂ anesthesia and randomly assigned to an intact or a surgically ablated group that had their 2 hind legs removed with scissors. The intact and the surgical groups were exposed to CO₂ in parallel and for the same amount of time. Surgically treated and intact flies were placed into food vials and housed at 25°C/55% relative humidity overnight to recover prior to behavioral testing. Negative geotaxis was assessed in flies with their hind legs removed at 1 week of age only. Greater than 95% of flies survived during overnight recovery from removal of their hind legs.

Video-taped negative geotaxis assays

Negative geotaxis was measured in RING (Rapid Iterative Negative Geotaxis) assays as described previously (Gargano et al. 2005) except that the behavior of the animals was recorded via a digital video camera (JVC Everio G). After RING testing, flies were transferred back to their original food vials and housed in 25°C/55% relative humidity. Flies were tested once per week for 6–9 weeks. The digital video of the flies' behavior was downloaded to a PC and Adobe Premiere Elements 2.0 was used to generate digital still images from the videos at 0.5 or 1 second intervals out to 4 seconds post test initiation. Data (distance climbed) were extracted from the images using Scion Image as described (Gargano et al. 2005). The distance climbed for living flies that did not perform the behavior was scored as zero. Dead flies were censored (not scored). Climbing speed (slope) and climbing latency (X-intercept) were determined by linear regression of the negative geotaxis time-courses for each vial of flies.

Prior to initiating the studies reported here, we found that static electricity on the vials used for testing negative geotaxis could at times produce an artifact by suppressing performance of the behavior and that treatment of the vials with Staticide (an anti-static spray) eliminated this problem (data not shown). We therefore treated all negative geotaxis vials used in these studies with Staticide prior to performing RING assays. Briefly, Staticide (diluted 1:99 in water) was sprayed on the inside and outside of each vial to be used for RING assays. Any excess Staticide solution was removed from each vial with a Kim wipe. The vials were stored open ended overnight to allow evaporation of any remaining Staticide. Staticide-treated vials were used the following day in RING assays.

Linear regression and parametric statistical analyses

Linear regression with Runs tests for non-linearity on negative geotaxis time-course data were performed using Prism (GraphPad, San Diego, CA, U.S.A.). The slopes (i.e. climbing speeds) and X-intercepts (i.e. response latencies) were determined for each vial of flies in all experiments. Two-way ANOVAs with Tukey HSD multiple comparison tests were performed with JMP 5.01 (SAS Institute, Cary, NC, U.S.A.). Pearson's correlations and t tests were performed with Prism. Statistical significance was determined as $p \leq 0.05$. Statistical comparisons with $p > 0.05$ were considered not significant (n.s.).

Decomposition analyses

Decomposition analysis was used to assess the contributions of and climbing speed to age-dependent changes in negative geotaxis. These methods were adapted for climbing speed from techniques developed for analysis of general demographic data (Pletcher et al. 2000; Horiuchi et al. 2008). In brief, the total distance climbed was decomposed into an additive function of latency and climbing speed. If $D = (4-l)s$, where D is the total distance traveled in 4 seconds, l is the response latency, and s is the climbing speed, then the difference in negative geotaxis measures between two ages or genotypes can be broken down into additive contributions from l and s as

$$\Delta D \approx \frac{\partial D}{\partial s} \Delta s + \frac{\partial D}{\partial l} \Delta l = (4-l) \Delta s - s \Delta l.$$

In practice, the contribution from each parameter to the total difference in negative geotaxis was calculated based on the aggregate from a series of sub intervals, which allows an arbitrary degree of accuracy to be obtained (Pletcher et al. 2000). Decomposition values are represented as the percentage contribution to the total difference in negative geotaxis. For example, the contribution of differences in climbing speed to the overall difference in distance is

$$s_c = \frac{\frac{\partial D}{\partial s} \Delta s}{\Delta D}.$$

Results

Negative geotaxis (startle-induced vertical locomotion) is frequently used to measure locomotor ability and to track age-related locomotor impairment in *Drosophila* (Grotewiel et al. 2005). Previous studies (Gibert et al. 2001; Gargano et al. 2005; Martinez et al. 2007) have not resolved, however, whether the vertical distance moved in negative geotaxis assays reflects climbing, jumping, flying, or some combination of these behaviors. Additionally, it was unclear from published reports whether the age-related impairment in negative geotaxis is due to decreased speed of vertical movement, increased latency to initiate vertical movement, or some combination of these two age-related changes.

Negative geotaxis is principally a climbing behavior

We video-taped wild type Canton-S and Samarkand adults in RING (Rapid Iterative Negative Geotaxis, (Gargano et al. 2005)) assays to address the contribution of climbing, jumping and flying to the distance moved in negative geotaxis experiments. In the videos we could readily identify flies that used climbing, but not other behaviors, to ascend the vial walls. We could not, in contrast, consistently distinguish between flies that jumped and flies that flew during the RING tests. Our inability to distinguish between fliers and jumpers was likely due to the intimate links between these two behaviors in *Drosophila* (Wymann et al. 1984; Trimarchi and Schneiderman 1993). We therefore treated jumping and flying as the same behavior in these analyses.

Negative geotaxis measured in RING assays is robust in 1-week-old control flies whereas this behavior is reduced significantly by week 4 of adulthood (Gargano et al. 2005). We assessed jumping and flying in 1-week-old and 4-week-old flies to determine whether the contribution of these behaviors to negative geotaxis performance varied with age. We found that the vast majority of young wild type Canton-S (Figure 1A) and Samarkand (Figure 1C) flies climbed during RING tests while only 5–10% of animals jumped or flew. The strong propensity of flies to climb and the limited jumping/flying behavior during RING assays was maintained in both wild type strains at 4 weeks of age (Figure 1A and C). These results suggest that the vertical distance moved during negative geotaxis assays largely reflects climbing in both young and older animals.

We compared the performance of climbers to all flies (climbers, jumpers and fliers) to further address the contribution of jumping, flying and climbing to the vertical distance moved in RING assays. With 1-week-old Canton-S (Figure 1B) and Samarkand (Figure 1D) animals, the vertical distance moved in flies that climbed was indistinguishable from that of all flies compiled (climbers, fliers and jumpers together). Four-week-old Canton-S and Samarkand flies performed substantially worse than their 1-week-old counterparts as expected. The vertical distance moved in 4-week-old climbers compared to that of all 4-week-old flies compiled, however, remained indistinguishable (Figure 1B and D). These data indicate that most young and older flies climb during RING tests and that the small fraction of animals that jump or fly does not contribute significantly to the vertical distance moved in young or older flies.

If the vertical distance moved principally reflects climbing behavior, then ablation of legs, but not wings, should depress performance in RING assays. We addressed this possibility by quantitating the vertical distance moved in flies with 2 hind legs or both wings removed. Canton-S wild type flies without wings performed indistinguishably from intact flies at 1- and 4-weeks of age (Figure 2A). In contrast, flies with 2 hind legs removed had marked reductions in the vertical distance moved (Figure 2B). Negative geotaxis in RING assays is principally a measure of legged climbing that is not influenced significantly by flying.

Age-related impairment in negative geotaxis is associated with reduced climbing speed and increased climbing latency

Previously published data from our laboratory suggest that flies might climb at a constant speed during RING tests (Gargano et al. 2005), although in these prior studies we did not thoroughly investigate this possibility. To formally assess whether flies climb at a linear rate, we evaluated negative geotaxis as time-courses of the distance climbed during RING assays. The distance climbed in Canton-S control males increased with the duration of RING tests in pilot studies (Figure Supplemental), consistent with our previously reported data (Gargano et al. 2005). The pilot time-course data were fit well by a linear equation, had a significant slope, and did not have significant nonlinearity (Figure Supplemental). These analyses indicate that the distance climbed increases linearly across the first 4 seconds of RING tests in young males.

To address whether the linearity of climbing speed is influenced by genetic background, sex and age, we evaluated the distance climbed in time-courses using males and females of 4 wild type stocks (Canton-S, Oregon-R, Lausanne-S and Samarkand) at one-week age intervals (Figure 3). All 87 time-courses evaluated were fit well by linear regression (Table S1), had significant positive slopes (Table S2), and were not significantly nonlinear (Table S3). These data demonstrate that the distance climbed increases linearly during the first 4 seconds of RING tests independently of genetic background, sex and age.

The distance climbed in RING assays decreases as flies age (Figure 3). We reasoned that the age-related reduction in the distance climbed could be due to reduced speed of climbing, increased latency to initiate climbing, or a combination of these two changes. A reduction in climbing speed (from 1 cm/s in young animals to 0.5 cm/s in aged animals) with no change in climbing latency would result in a substantial reduction in the distance climbed in aged flies (Figure 4, Age Δ Speed model). An increase in climbing latency (from 0.5 seconds in young animals to 2.5 seconds in aged animals) with no change in climbing speed would also result in a significant reduction in the distance climbed in aged animals (Figure 4, Age Δ Latency model). Age-related reduction of climbing speed with an increase in climbing latency would result in an even greater reduction in the distance climbed in aged animals since these two parameters interact (Figure 4, Age Δ Both model). The linearity of the negative geotaxis time-courses (Figure 3, Table S1–Table S3) allowed us to determine values for climbing speed (slope in cm/s) and climbing latency (X-intercept in seconds) via linear regression analyses. Our

experimental design also allowed us to assess the distance climbed 4 s after initiating RING tests, the standard static measure of negative geotaxis behavior (Gargano et al. 2005).

As shown in Figure 3, the distance climbed at 4 seconds decreased markedly with age in Canton-S, Oregon-R, Lausanne-S and Samarkand females and males housed at 25°C (4 second data summarized in Figure 5A and B). Samarkand had the most robust negative geotaxis scores across age, followed in rank order by Lausanne-S and then Oregon-R and Canton-S (Table 1). Climbing speed also decreased markedly with age in all groups of wild type animals tested (Figure 5C and D). The rank order of climbing speed (Samarkand > Lausanne > Oregon-R ≥ Canton-S) was the same as for the distance climbed at 4 seconds (Table 1). There was also a statistically significant increase in the latency to initiate climbing in all wild type strains (Figure 5E and F). Overall, Canton-S and Oregon-R had the greatest latencies (took the longest to initiate the behavior) while Lausanne-S and Samarkand had the shortest latencies (Table 1). These results raise the possibility that changes in climbing speed or climbing latency cause the age-related impairment in the distance climbed.

We postulated that climbing speed and latency might change in parallel with accelerated locomotor impairment. We therefore evaluated negative geotaxis across age in short-lived flies housed at elevated temperature. Age-related impairment in the distance climbed was accelerated in wild type Canton-S and Samarkand animals housed at 29°C versus 25°C (Figure 6A and B). Climbing speed decreased (Figure 6C and D) and climbing latency increased (Figure 6E and F) with age in all groups tested. The age-related changes in climbing speed and latency were accelerated in both wild type strains housed at 29°C (Figure 6C–F, Table 1), further suggesting that age-related impairment of negative geotaxis might be caused by altered climbing speed or latency.

In *Drosophila*, OR83b oligomerizes with other receptors to produce functional receptors for odorants (Elmore et al. 2003; Larsson et al. 2004; Neuhaus et al. 2005). *OR83b* mutant flies are anosmic and long-lived (Libert et al. 2007), suggesting that odorants influence *Drosophila* life span as found for *C. elegans* (Libert and Pletcher 2007). We analyzed time-courses of negative geotaxis behavior in control and long-lived *OR83b* mutants to determine (a) whether senescence of negative geotaxis was delayed in the long-lived flies and if so (b) whether age-related changes in climbing speed and latency were altered in conjunction with senescence of negative geotaxis. The distance climbed in negative geotaxis assays was more robust across age in *OR83b* mutants compared to *w¹¹¹⁸* control flies (Figure 7A). To address whether the robust negative geotaxis behavior in *OR83b* mutants reflected a change in behavioral, we assessed negative geotaxis behavior integrated across age (total negative geotaxis) and the time required for negative geotaxis behavior to undergo age-related decline (decline time). Total negative geotaxis is a measure of the behavior capacity across age and decline time reflects the rate of senescence (Martin et al. 2005; Martin and Grotewiel 2006). Total negative geotaxis behavior and the time required for negative geotaxis to senesce were significantly increased in *OR83b* mutants (Table 2). These characteristics strongly suggest that senescence of negative geotaxis is delayed in *OR83b* flies (Martin et al. 2005; Martin and Grotewiel 2006). Importantly, the age-related reduction in climbing speed (Figure 7B) and the age-related increase in latency (Figure 7C) were forestalled in *OR83b* mutants. This pattern of age-related changes is consistent with altered climbing speed or latency impacting senescence of negative geotaxis.

Age-related change in climbing speed, but not latency, is the major contributor to age-dependent reduction in the distance climbed

The data in Figure 5–Figure 7 demonstrate that age-related changes in climbing speed and latency accompany senescence of negative geotaxis. Cohorts with more rapid senescence of the distance climbed had accelerated reductions in climbing speed and increases in latency

(Table 1). As a first step, we evaluated correlations between changes in climbing speed and changes in the distance climbed in RING assays as well as correlations between changes in latency and changes in the distance climbed. We reasoned that age-related changes in climbing speed and latency should correlate with changes in the distance climbed if speed and latency are important contributors to senescence of negative geotaxis. In all 14 groups analyzed, there was a significant correlation between the age-related decreases in climbing speed and distance climbed (Supplemental table 4). The correlations between age-related changes in climbing speed and distance climbed strongly implicate climbing speed in senescence of negative geotaxis. In contrast, age-related changes in latency and distance climbed did not significantly correlate in any of the 14 groups studied (Supplemental table 4).

To more formally address the relative contribution of age-related changes in climbing speed and latency to impairment of the distance climbed, we attempted to use linear models in which we held the climbing latency constant while varying the climbing speed and vice versa. Unfortunately, this type of modeling was unreliable (data not shown). It consistently overestimated the contribution of changes in climbing speed, changes in latency, or both because climbing speed (slope) and latency (X-intercept) interact in linear equations. To circumvent these problems with estimating the contribution of changes in climbing speed and latency, we performed decomposition analyses. The age-dependent change in climbing speed accounts for at least 80% of the age-related reduction in the distance climbed in all groups (Figure 8). In 7 of the 14 groups analyzed, the change in climbing speed accounts for all of the age-dependent reduction in distance climbed (Figure 8). Our interpretation of the correlations in Supplemental Table 4 and the decomposition analyses in Figure 8 is that age-dependent change in climbing speed, but not latency, is the driving force behind senescence of negative geotaxis.

Discussion

Drosophila is emerging as an important model for investigating age-related locomotor impairment, a significant clinical issue for the elderly (Woo et al. 1999; Boyd et al. 2005; Braam et al. 2005; Hillsdon et al. 2005; Onder et al. 2005; Volpato et al. 2005; Cesari et al. 2006a; Cesari et al. 2006b; von Bonsdorff et al. 2006). In flies, negative geotaxis is a commonly used measure for locomotion and its senescence (Grotewiel et al. 2005). Two previous studies suggested that senescence of negative geotaxis in flies is due to age-related decreases in climbing speed (Gibert et al. 2001; Martinez et al. 2007). If true, the age-related decrease in speed would be an intriguing parallel between functional aging in humans and flies. The conclusions in Gibert et al. 2001 and Martinez et al. 2007, however, were based on the assumptions that (1) negative geotaxis is a climbing behavior, (2) that a cohort of flies at a given age climbs at a constant speed during negative geotaxis tests, and (3) that the latency to initiate negative geotaxis does not change with age. We addressed these issues in several groups of animals with genetic or environmentally altered senescence of negative geotaxis.

We find that the vast majority of flies climb during negative geotaxis assays and that partial amputation of legs impairs their ability to move vertically during these tests. Additionally, we find that jumping and flying do not contribute significantly to the vertical distance moved and that wingless flies perform indistinguishably from intact animals. These data establish negative geotaxis in RING assays as a climbing behavior that is largely independent of other forms of locomotion such as jumping and flying.

Since negative geotaxis is a climbing behavior, senescence of this behavior must be due to age-related changes in climbing speed, latency to initiate climbing, or some qualitative change in how the flies climb (i.e. punctuated versus constant climbing). We investigated these possibilities through careful video tape analysis of flies in RING assays. Our results show that

flies climb at linear rates during RING tests at all ages, indicating that there are not large qualitative changes (e.g. punctuated climbing) in the behavior with age. Additionally, our studies show that climbing speed is decreased and response latency is increased with age in flies. The age-related decrease in climbing speed correlates with the age-related decrease in the distance climbed, whereas the change in latency does not. Additionally, decomposition analyses indicate that a decrease in climbing speed, but not an increase in latency, accounts for the vast majority of the age-dependent change in distance climbed. Our interpretation is that the age-related decrease in climbing speed is the major contributor to senescence of negative geotaxis in *Drosophila*. Flies with slow or rapid senescence of negative geotaxis, therefore, have slow or rapid age-dependent decreases in locomotor speed.

Importantly, we used males and females from several different genetic backgrounds with known differences in senescence of negative geotaxis (Gargano et al. 2005; Martin and Grotewiel 2006), flies housed at normal and elevated temperature with normal and accelerated senescence of negative geotaxis (Grotewiel et al. 2005), and long-lived *OR83b* mutants and controls (Libert et al. 2007) in our studies. Age-related decreases in climbing speed account for most if not all of the age-dependent reductions in the distance climbed in all of these groups, strongly suggesting that the contribution of age-related decrease in climbing speed to senescence of negative geotaxis is a universal feature of aging in *Drosophila*.

One of the hallmarks of locomotor senescence in elderly individuals is a decrease in walking speed (Oberg et al. 1993; Bohannon 1997; Kozakai et al. 2000; McGibbon and Krebs 2001; Samson et al. 2001; Laufer 2005). Importantly, age-related reduction in walking speed in the elderly population is correlated with walking impairment (Brach et al. 2007) and is associated with increased risk of several co-morbidities and death (Hardy et al. 2007). Our studies demonstrate that flies share with humans the key feature of age-related decline in walking speed.

Flies harboring a null mutation in *OR83b*, an olfactory receptor, have extended life span (Libert et al. 2007) possibly because the absence of *OR83b* mimics a state of olfactory deprivation seen with dietary restriction (Libert and Pletcher 2007). *OR83b* mutants also have delayed senescence of negative geotaxis. Olfactory cues, therefore, appear to impact both quality and quantity of life in flies. Interestingly, although mutation of *OR83b* extends life span and delays senescence of negative geotaxis, dietary restriction extends life span but does not alter negative geotaxis senescence (Bhandari et al. 2007). It seems likely that *OR83b* participates in at least some processes unrelated to dietary restriction.

The studies reported here, in conjunction with previous work from our group (Goddeeris et al. 2003; Gargano et al. 2005) and others (Miquel et al. 1976; Arking and Wells 1990; Chavous et al. 2001; Mockett et al. 2001; Kang et al. 2002; Ruan et al. 2002; Morrow et al. 2004; Simon et al. 2006), indicate that locomotor senescence might be mechanistically connected to life span in *Drosophila*; i.e. long-lived flies tend to have better locomotor function across age. Thus, life span and health span could be served by common mechanisms in flies. The connection between life span and health span is not absolute, however, since at least some long-lived flies have unaltered locomotor senescence (Cook-Wiens and Grotewiel 2002; Bhandari et al. 2007).

Negative geotaxis is a reliable measure of locomotor function across age in flies (Grotewiel et al. 2005). The studies reported here define negative geotaxis in *Drosophila* as a climbing behavior that decreases with age due to reduced speed of locomotion. Since age-related reductions in walking speed are also observed in humans (Oberg et al. 1993; Bohannon 1997; Kozakai et al. 2000; McGibbon and Krebs 2001; Samson et al. 2001; Laufer 2005), our studies highlight the conserved effects of age on locomotor function in humans and flies. It

will be intriguing to further investigate genetic mechanisms that influence senescence of negative geotaxis in *Drosophila* and explore whether these mechanisms play a role in locomotor senescence in other species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Contribution of climbing and jumping/flying behavior to negative geotaxis performance Canton-S (CS), panels A and B; Samarkand (Sam), panels C and D. The percentage of flies that climbed or jumped/flew in RING assays was determined by careful manual inspection of video-taped Canton-S (A) and Samarkand (C) flies at 1- and 4-weeks of age. The percentage of flies that climbed was significantly greater than the percentage that jumped/flew (two-way ANOVA, $p < 0.0001$, $n = 5$). Age had no effect on the percentage of climbers or jumpers (n.s.). The distance climbed was decreased with age in Canton-S (B) and Samarkand (D) flies, but the behavior in flies that climbed (climbers) was indistinguishable from that of all flies combined (climbers, jumpers/fliers) (two-way ANOVA; effect of age, $p < 0.0001$; effect of climbing, n.s.; $n = 5$).

Figure 2. Negative geotaxis is impaired by removal of legs, but not wings

A. Negative geotaxis was lower in 4-week-old than in 1-week-old Canton-S flies, but was not affected by removal of the wings (two-way ANOVA; effect of age, $p < 0.0001$; effect of wing removal, n.s.; $n = 10-20$). B. The vertical distance climbed in one-week-old Canton-S flies with no hind legs was significantly less than in age-matched intact flies (unpaired two-tailed t test, $p < 0.0001$, $n = 5$).

Figure 3. Negative geotaxis time-courses across genetic background and age
 Canton-S (CS, A and B); Oregon-R (OR, C and D); Lausanne-S (LS, E and F); Samarkand (Sam, G and H). Negative geotaxis was assessed during 4-second RING assays in females (left panels) and males (right panels) at the ages (weeks) indicated by arrows. Lines are best-fit linear regression. Data are mean $\pm$ S.E.M. ($n=12$). The results from statistical tests are summarized in Table 1, Table S1, Table S2 and Table S3.

Figure 4. Linear models for negative geotaxis time-courses

Linear models of mock negative geotaxis time-courses in young flies and aged flies with decreased climbing speed only (Age Δ Speed), increased climbing latency only (Age Δ Latency), or both (Age Δ Both). Climbing speed and latency are 1 cm/s and 0.5 seconds, respectively, in the Young model. Climbing speed is changed to 0.5 cm/s while the climbing latency is maintained at 0.5 s in the Age Δ Speed model. Climbing latency is changed to 2.5 seconds while the climbing speed is maintained at 1 cm/s in the Age Δ Latency model. Climbing speed is changed to 0.5 cm/s and climbing latency is changed to 2.5 s in the Age Δ Both model.

Figure 5. Effect of age on the distance climbed, climbing speed and climbing latency—genetic background

Data in Figure 4 were analyzed to determine the distance climbed at 4-seconds (A and B), climbing speed (C and D), and response latency (E and F) in females (left panels) and males (right panels). Individual two-way ANOVAs revealed that the distance climbed at 4 s and climbing speed decreased with age while climbing latency increased with age in all experiments ($p < 0.0001$). Genetic background indications are the same as in Figure 4. Symbols indicating genetic background are the same in all panels.

Figure 6. Effect of age on the distance climbed, climbing speed and climbing latency—elevated housing temperature

The distance climbed at 4-seconds (A and B), climbing speed (C and D), and climbing latency (E and F) in Canton-S (left panels) and Samarkand (right panels) males. The distance climbed at 4-seconds and climbing speed decreased with age; the climbing latency increased with age (two-way ANOVAs, $p < 0.0001$, $n = 8-10$). Negative geotaxis at 4-seconds (A and B) and climbing speed (C and D) were lower in flies housed at 29°C than at 25°C ($p < 0.0001$). Climbing latency (E and F) was increased at the elevated temperature ($p \leq 0.0283$).

Figure 7. Effect of age on the distance climbed, climbing speed and climbing latency—long-lived *OR83b* mutants

The distance climbed at 4-seconds (A) and climbing speed (B) decreased with age while climbing latency (C) increased with age (two-way ANOVAs, $p < 0.0001$, $n = 20$). The distance climbed at 4-seconds (A) and climbing speed (B) were significantly higher overall in *OR83b* mutants compared to *w¹¹¹⁸* controls ($p < 0.0001$, $n = 20$). Climbing latency was significantly lower in *OR83b* mutants (C, $p < 0.0001$).

Figure 8. Decomposition of age-dependent changes in climbing speed and climbing latency

Data are the proportional contribution of the changes in climbing speed and latency on the change in distance climbed determined by decomposition analyses of the data from Figure 5–Figure 7. The age-dependent change in climbing speed is the major contributor to age-dependent reductions in the distance climbed in control females (A), control males (B), flies aged at 25°C (CS-25 and Sam-25) or 29°C (CS-29 or Sam-29), and in *Or83b* mutants and controls. CS, Canton-S; OR, Oregon-R; LS, Lausanne-S; Sam, Samarkand. Data are mean and S.E.M. for all vials of flies for each group.

Pair-Wise Comparisons of Distance Climbed, Climbing Speed and Climbing Latency
Rows not connected by the same letter are significantly different (Tukey HSD multiple comparisons, $p < 0.05$). Letter indicates rank order of measured value (A, largest, B, second largest, etc.)

Genotype	Group	Distance Climbed		Climbing Speed		Climbing Latency	
Canton-S	females						
Oregon-R	females	D				A	A
Lausanne-S	females						B
Samar kand	females		B		B		B
Canton-S	males			A		A	
Oregon-R	males	C					
Lausanne-S	males	C				A	
Samar kand	males		B		B		C
Canton-S	25°C			A			
Canton-S	29°C	A				B	B
Samar kand	25°C		B				A
Samar kand	29°C	A			B		A
<i>w¹¹¹⁸</i>	males	A			A	B	
<i>OR83b</i>	males		B		B		A

Table 2**Total negative geotaxis and decline-time metrics in OR83b mutants**

AUC (area under the curve, total negative geotaxis activity) and decline-times (DT25, DT50 and DT75) were calculated as described (Martin et al. 2005; Martin and Grotewiel 2006). AUC (unpaired two-tailed t-test), DT25, DT50 and DT75 (Bonferroni's multiple comparison tests) were significantly increased in *OR83b* mutants compared to *w¹¹¹⁸* controls ($p < 0.0001$; n shown in parentheses).

Metric	Genotype	Mean $\pm$ S.E.M.
AUC (cm $\times$ weeks)	<i>w¹¹¹⁸</i>	7.6 $\pm$ 0.4 (20)
	<i>OR83b</i>	14.6 $\pm$ 0.6 (20)
DT25 (weeks)	<i>w¹¹¹⁸</i>	2.7 $\pm$ 0.1 (16)
	<i>OR83b</i>	3.9 $\pm$ 0.2 (20)
DT50 (weeks)	<i>w¹¹¹⁸</i>	4.0 $\pm$ 0.1 (16)
	<i>OR83b</i>	6.0 $\pm$ 0.2 (20)
DT75 (weeks)	<i>w¹¹¹⁸</i>	5.4 $\pm$ 0.1 (15)
	<i>OR83b</i>	7.8 $\pm$ 0.1 (19)