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Different age-dependent performance in *Drosophila* wild-type Canton-S and the *white* mutant w1118 flies

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ABSTRACT

Aging has significant effects on the locomotor performance of insects including *Drosophila*. Using a protocol for the high-throughput analysis of fly locomotion in a circular arena, we examined age-dependent behavioral characteristics in adult flies. There are widely used wild-type and genetically engineered background lines including the Canton-S strain and the w1118 strain, which has a null mutation of the *white* gene. Under standard rearing conditions, we found similar survival and median lifespans in Canton-S (50 days) and w1118 (54 days) strains, however, w1118 flies maintained stable body mass for up to 43 days, whereas Canton-S flies gained body mass at young age, followed by a gradual decline. We also tested the behavioral performance of young and old flies. Compared with young w1118 flies (5–10 days), old w1118 flies (40–45 days) had an increased boundary preference during locomotion in small circular arenas, and increased speed of locomotor recovery from anoxia. Old Canton-S files, however, exhibited unchanged boundary preference and reduced recovery speed from anoxia relative to young flies. In addition, old w1118 flies showed decreased path length per minute and reduced 0.2 s path increment compared with young flies, whereas old Canton-S flies displayed the same path length per minute and the same 0.2 s path increment compared with young flies. We conclude that age-dependent behavioral and physiological changes differ between Canton-S and w1118 flies. These results illustrate that phenotypic differences between strains can change qualitatively, as well as quantitatively, as the animals age.

1. Introduction

The fruit fly, *Drosophila melanogaster*, is extensively used as a model organism in modern biological sciences because of its remarkable adaptability in almost all habitats (Ayrinhac et al., 2004; Stratman and Markow, 1998) with minor geographical genetic variation and microevolution (David and Capy, 1988). More importantly, with its powerful accessibility to genetic analysis, *Drosophila* is one of the most attractive model organisms to study neural mechanisms and brain function. So far, various wild-type and genetically engineered background fly strains have been described, among which Canton-S is one of the most used wild-type fly lines while w1118 is often used as the appropriate control because it carries isogenic background for convenient genetic transformations. Behavioral differences have been found between Canton-S and w1118 strains at a young age, however, whether the differences persist into their old age is still unclear.

The Canton-S strain was first established by Bridges from wild flies collected from Canton, Ohio in the 1920s (Stern, 1943; Stern and Schaeffer, 1943). Later, it was studied by Seymour Benzer due to its rapid phototaxis, and subsequently was used as a control strain in neurogenetics research (Benzer, 1967). The w1118 strain originates from the wild-collected Oregon-R strain rather than the Canton-S strain. The w1118 strain contains a spontaneous partial deletion in the *white* gene, resulting in white eyes (Bingham, 1980; Hazelrigg et al., 1984). The *white* gene, present on the X chromosome of *Drosophila* (Lefevre and Wilkins, 1966) and discovered more than a hundred years ago (Morgan, 1910), encodes a member of the ATP-binding cassette (ABC) transporter superfamily (Allikmets et al., 1998) and is responsible for the transportation of eye pigment precursors, guanine and tryptophan, into pigment cells for pigment synthesis during pupation (Ewart et al., 1994; Sullivan et al., 1980; Summers et al., 1982). The chromosomes in Canton-S flies are polygenic. In contrast, the first chromosome except for the *white* locus, second and third chromosomes are isogenic in w1118 flies (Cingolani et al., 2012; Platts et al., 2009), and the cytoplasmic background is different between Canton-S and w1118 flies (Greenspan and Hafen, 1997).

The w1118 strain is commonly used as the genetic background to generate P-element insertion lines including the widely-used GAL4/UAS system (Duffy, 2002; Kain et al., 2012). However, mutations at the *white* locus in w1118 result in decreased capacity for the deposition of pigment in compound eyes, ocelli pigment cells, testes sheathes and larval Malpighian tubules (Hazelrigg, 1987; Pirrotta and Brockl, 1984).

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In addition, neurobiological roles of the *white* gene have been discovered, such as the control of male-male courtship (Zhang and Odenwald, 1995) and anesthetic sensitivity (Campbell and Nash, 2001). Besides being associated with retinal degeneration, the w1118 strain also exhibits neurodegenerative phenotypes such as poor place memory and abnormal climbing ability (Campbell and Nash, 2001; Colley, 2012; Krstic et al., 2013; Pérez et al., 2014; Schilman et al., 2011). The fly strains, including w1118, used in those studies were within the age range of 0–10 days and underwent multiple experiments such as distribution assay, courtship assay and memory test.

Age-related behavioral decline is common in many organisms. Invertebrates such as *C. elegans*, have reduced movement towards bacterial food as they age (Glenn et al., 2004; Hosono, 1978). This decline of movement is related to muscle deterioration (Fisher, 2004) and could be delayed by the loss-of-function mutations in genes such as *daf-2* and *age-1* (Glenn et al., 2004; Huang et al., 2004; Murakami et al., 2005). The spider *Zygiella x-notata* builds orb-webs with decreased length of capture spiral and reduced parallelism of silk thread with age (Anotaux et al., 2012). *Blaberus discoidalis* cockroaches have a decreased spontaneous locomotion with increasing age (Ridgel et al., 2003). The 60-week-old adult cockroaches reduce walking speed to half of the level of 1-week-old ones (Ridgel et al., 2003). Age-related reduction of activity is observed in vertebrates as well, such as mice (Ingram et al., 1981; Sprott and Eleftheriou, 1974), rats (Barrett and Ray, 1970; Wallace et al., 1980) and humans (Caspersen et al., 2000; Sallis, 2000). With regard to flies, young ones move away from a release point more often than old ones, and walking and flying frequencies decrease with increasing age (Carey et al., 2006; Le Bourg, 1983). This is likely due to the increased sensitivity to oxidative stress as flies age, and the inability to resist oxidative stress in old age (Amdam and Omholt, 2002; Golden et al., 2002). In houseflies *Musca domestica*, flight behavior could shorten lifespan by accelerating age-related oxidative damage (Yan and Sohal, 2000). Old flies also have reduced negative geotaxis and mating success (Gargano et al., 2005; Miquel et al., 1976).

Different *Drosophila* strains behave differently (Colomb and Brembs, 2014; Faville et al., 2015; Ruebenbauer et al., 2008; Walcourt and Nash, 2000) and behavioral aging is also strain-specific. *Drosophila* wild-type Oregon-R has a longer lifespan and later onset of locomotion deterioration than wild-type Canton-S (Ganetzky and Flanagan, 1978). Therefore, it is possible that age-related changes in behavioral performance differ between fly strains, including Canton-S and w1118 flies. We have previously described behavioral and neural differences between Canton-S and w1118 strains at a young age. At 4–9 days old, compared with w1118 flies, Canton-S flies show a higher boundary preference, shorter travel distance per minute and lower 0.2 s path increments in small circular arenas (Xiao and Robertson, 2015). Locomotion of Canton-S flies recovers faster from a transient anoxia than that of w1118 (Xiao and Robertson, 2016). The current study was designed to determine whether such differences persist into old age, and whether behavioral aging was different in these two strains. If behavioral aging is strain-dependent as hypothesized, it suggests that the choice of fly strains for aging studies needs careful consideration.

2. Materials and methods

2.1. Flies

Wildtype Canton-S (Bloomington stock center) and mutant w1118 strains (L. Seroude laboratory, Queen's University) were raised with standard medium (0.01% molasses, 8.2% cornmeal, 3.4% killed yeast, 0.94% agar, 0.18% benzoic acid, 0.66% propionic acid) at room temperature 21–23 °C and 60–70% humidity. A 12 h/12 h light/dark cycle was provided by three light bulbs (Philips 13 W compact fluorescent energy saver) with lights on at 7 a.m. and off at 7 p.m. Male flies were collected within 2 days after eclosion for these experiments: (1) lifespan, (2) body weight measurement, (3) locomotor assay. Male flies were selected in this study to avoid complications due to egg production in female flies. Flies for the locomotor assay and the recovery from anoxia experiments were raised for at least 3 days free of nitrogen paralysis before experiments. All experiments were performed between 10 am and 4 pm during the photophase. Flies were transferred into fresh food vials every 4 days.

Male Canton-S and w1118 flies were collected and raised in vials (20–30 flies per vial). Flies were continuously transferred to fresh food vials once every 3–4 days. The survival dynamics of Canton-S and w1118 male flies were investigated starting on Day 1. The number of dead flies were recorded every 1–2 days. Flies were considered to be dead when neither voluntary movement nor responses to external stimulation could be observed. The lifespan measurement was replicated four times. Each lifespan cohort contained 60–120 flies for each genotype.

Flies were raised in 3 vials for each strain. We weighed wet mass (fresh mass) of all the live flies together from each vial and calculated the average mass per fly for each vial. Therefore, three independent measures were taken at each time point for each strain. Body mass during aging was measured once every four days starting at Day 3 post-ecdysis using a Denver Instrument SI-234 balance (accuracy 0.1 mg).

Flies for body weight measurement and flies for the lifespan study were collected at the same time and raised under the same conditions.

2.2. Locomotor assay

Flies were tested either on Days 5–10 (d5–10, defined as "young") or Days 40–45 (d40–45, defined as "old") as indicated in the results section. The locomotor assay was conducted using a previously described protocol (Qiu et al., 2016; Xiao and Robertson, 2015). Individual fly was restrained in a circular arena (1.27 cm in diameter and 0.3 cm in depth). Locomotion was video-captured and analyzed with scripts written using Open Computer Vision 2.0 (OpenCV2.0). A 30 s anoxia treatment (delivery of pure nitrogen gas) at the desired time was applied in some experiments. After a 5 min adaptation period in the arena, locomotor parameters including percent time on perimeter (% TOP) over a period of 60 s, 0.2 s path increments, and travel distance within first 60 s were examined between different groups of flies. % TOP per min has been shown to be maintained at steady levels without decline for five consecutive minutes (Xiao and Robertson, 2015), indicating that the selection of the first 60 s for evaluating % TOP, 0.2 s path increments and travel distance could all be considered to represent the behavior of a period within at least 5 min. The sample sizes for Canton-S and w1118 flies at young or old ages are shown in the results section.

2.3. Statistics

Survivorship and lifespan data were processed based on the number of deaths and analyzed using Log-rank (Mantel-Cox) Test in Prism version 5.0 set for survival curve algorithm. Two-way ANOVA with Bonferroni post test (2wAN) was conducted for analyzing body mass data over age in two strains. For the locomotor assay, statistical analysis was performed using Prism version 5.0 (GraphPad Software, San Diego, CA). D'Agostino & Pearson omnibus normality test was carried out to examine data distribution. % TOP, 60 s path length and median 0.2 s path increment of two strains were box-plotted and examined with Mann-Whitney test (MW). A sigmoidal function with variable slope was applied for curve-fitting of path length per minute during recovery from anoxia. The slope and time to half recovery were derived from sigmoidal curve-fitting. Percent recovery was calculated by the equation: % recovery = 100% × (maximal path length per minute during 60 min recovery) / (average path length per minute before anoxia). A $P < 0.05$ is considered statistically significant.

3. Results

3.1. Lifespan in Canton-S and w1118 flies

The longevity of Canton-S and w1118 flies was examined under standard rearing conditions. Both Canton-S and w1118 strains demonstrated a lag period before entering an exponential death phase with median lifespans of 50 (n = 64) and 54 days (n = 81), respectively, indicating similar lifespans of Canton-S and w1118 strains (Log-rank Test, $P > 0.05$) (Fig. 1A). Repeated experiments showed consistently that there was no difference between the two strains under these conditions. This indicates that Days 5–10 or Days 40–45 selected in the locomotor assay would be the same physiological age in Canton-S and w1118 strains.

3.2. Body mass with age in Canton-S and w1118 flies

In order to understand the basic physiological changes with age, the body mass was investigated between these two strains throughout the lifespan (Fig. 1B). The fresh mass per fly at Day 3 was the same in male Canton-S and male w1118 ($P > 0.05$, 2wAN). At Day 19, fresh mass per fly in Canton-S was higher than that of w1118 males ($P < 0.01$, 2wAN). Fresh mass per fly of Canton-S males was lower than w1118 males at Day 35 and Day 43 ($P < 0.01$ or 0.001, respectively, 2wAN). Clearly, Canton-S flies displayed a gradual gain of body mass before 20 days, and a decline after. In contrast, fresh mass per fly in w1118 males was relatively stable throughout 3–44 days. Therefore, the dynamic changes of body mass were different between Canton-S and w1118 males.

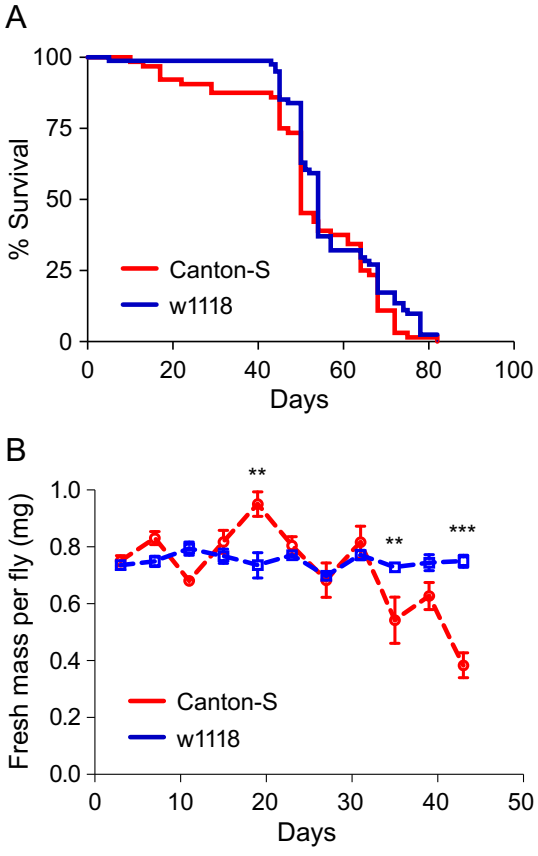

Fig. 1. Longevity and body weight of Canton-S and w1118 flies. (A) Survival dynamics of Canton-S (red) and w1118 (blue) strains. (B) Mean body weight ± SEM over time of CS (red circle) and w1118 flies (blue square). Asterisks ** or ***$P < 0.01$ or 0.001, respectively, two-way ANOVA with Bonferroni post-hoc test.

3.3. Age-related increase of boundary preference during locomotion in w1118 flies

To examine locomotor performance with age, we analyzed the boundary preference of young and old flies in the circular arenas. Young Canton-S flies walked in the perimeter of the arena for most of the time. The boundary preference during locomotion was highly consistent from fly to fly (Fig. 2A). The perimeter preference and the consistency between individuals were largely retained in old Canton-S flies. Young w1118 flies, however, walked and turned actively in the arenas. Each fly showed a preference for staying on the perimeter and also a high probability of crossing the central region of the arena (Fig. 2A). In contrast, old w1118 flies increased their preference for the perimeter during locomotion, and reduced the probability of moving in the central area. The age-related change appeared to be common in w1118 males.

Within 60 s, % TOP in young Canton-S flies (n = 13) was similar to old Canton-S flies (n = 7) ($P > 0.05$, MW) (Fig. 2B). However, % TOP in old w1118 flies (n = 11) was significantly higher than that in

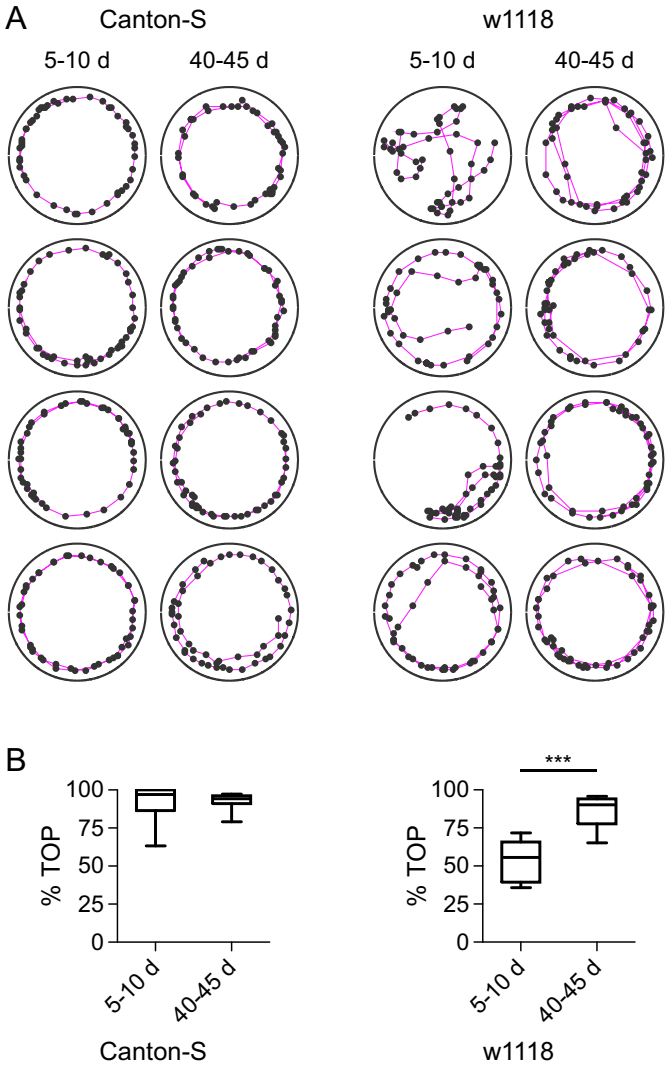

Fig. 2. Age-dependent changes of % TOP in Canton-S and w1118 flies. (A) Locomotor trajectories in the circular arenas for four representative flies at young (5–10 days) and old (40–45 days) age. Each circle represents an arena (1.27 cm diameter). Dots indicate fly positions (calculated centers of mass) and the connecting lines show the trajectories during 20 s of locomotion for Canton-S and w1118 flies at young and old ages. Positions are calculated once per 0.2 s. (B) % TOP in Canton-S and w1118 flies at young and old ages. ***$P < 0.001$, Mann-Whitney test.

young w1118 flies (n = 8) ($P < 0.001$, MW). Therefore, the boundary preference remained stable between young and old Canton-S flies, whereas it increased in w1118 old flies compared with young flies.

3.4. Age-related locomotor recovery from anoxia in Canton-S and w1118

With an anoxia treatment, the time to locomotor recovery is different between Canton-S and w1118 males at young age (Xiao and Robertson, 2016). We asked whether there was an age-related change of locomotor recovery from anoxia in each strain, and whether the change was common in two different strains.

During anoxia, flies were quickly knocked down. The recovery of locomotion after anoxia displayed a dynamics that could be nicely fitted by a sigmoidal function. Old Canton-S flies (n = 12) displayed slower recovery speed, longer time to half recovery but higher % recovery of locomotion compared with young Canton-S flies (n = 16) (Fig. 3A). In contrast, old w1118 flies (n = 13) displayed faster recovery speed, shorter time to half recovery, and higher % recovery of locomotion compared with young w1118 flies (n = 14) (Fig. 3A).

Locomotion before anoxia was also analyzed. Old w1118 flies (n = 13) traveled markedly shorter distances than young ones (n = 14) ($P < 0.0001$, MW) (Fig. 3B). The travel distance was the same in Canton-S young and old flies (n = 16 and 12, respectively) ($P > 0.05$, MW). Thus, w1118 but not Canton-S displayed an age-related reduced travel distances without anoxia.

3.5. Age-related changes of 0.2 s path increments in Canton-S and w118 flies

We examined locomotor performance by comparing 0.2 s path increments in young and old flies of both strains. This parameter is equivalent to average step distance of a single leg at a body speed around 40–50 cm/min (Mendes et al., 2013; Xiao and Robertson, 2015). Canton-S young flies walked in a relatively steady 0.2 s path increment during the 300 s locomotion. Canton-S old flies walked with similar 0.2 s path increment but had an increase of pausing or stopping compared with young flies (Fig. 4A). There was no statistical difference of median 0.2 s path increment between young and old Canton-S males ($P > 0.05$, MW) (Fig. 4B).

Unlike the Canton-S flies with steady 0.2 s path increment, both w1118 young and old individuals showed large variance (Fig. 4A). Old w1118 males also showed greatly reduced median 0.2 s path increment compared with young files. These data provided detailed age-related changes of median 0.2 s path increment to support the earlier observation of reduced 60 s path length in w1118 old flies (see Fig. 3B). Clearly, median 0.2 s path increment was lower in old w1118 males than young males ($P < 0.01$, MW) (Fig. 4B).

4. Discussion

Our general conclusion is that aging differed markedly in Canton-S and w1118 flies, not only for body mass, but also at the level of

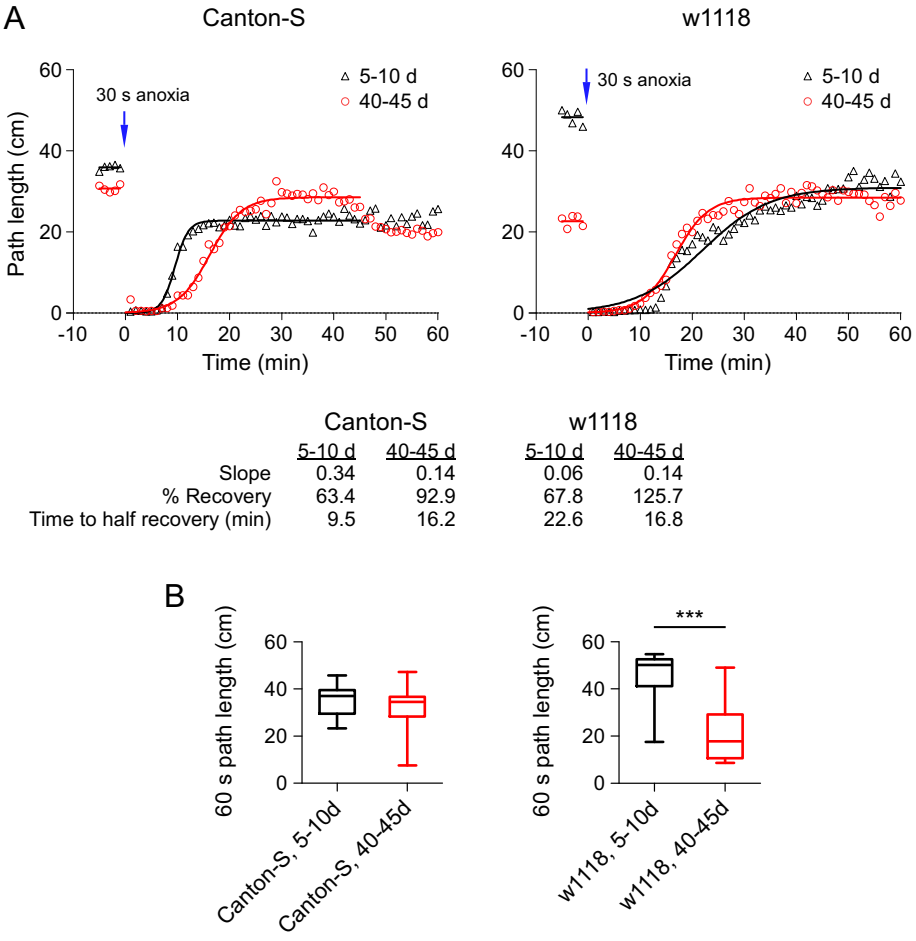

Fig. 3. Age-related locomotor recovery from anoxia in Canton-S and w1118. (A) Locomotor activities with a 30 s anoxia (start indicated with blue arrows) in Canton-S and w1118 flies. Both young (5–10 days) and old (40–45 days) flies were tested. Path length during recovery was fitted with a sigmoidal curve. Horizontal lines represent mean path length per minute before anoxia. (B) 60 s path length in Canton-S and w1118 at young and old ages. ***$P < 0.001$, Mann-Whitney test.

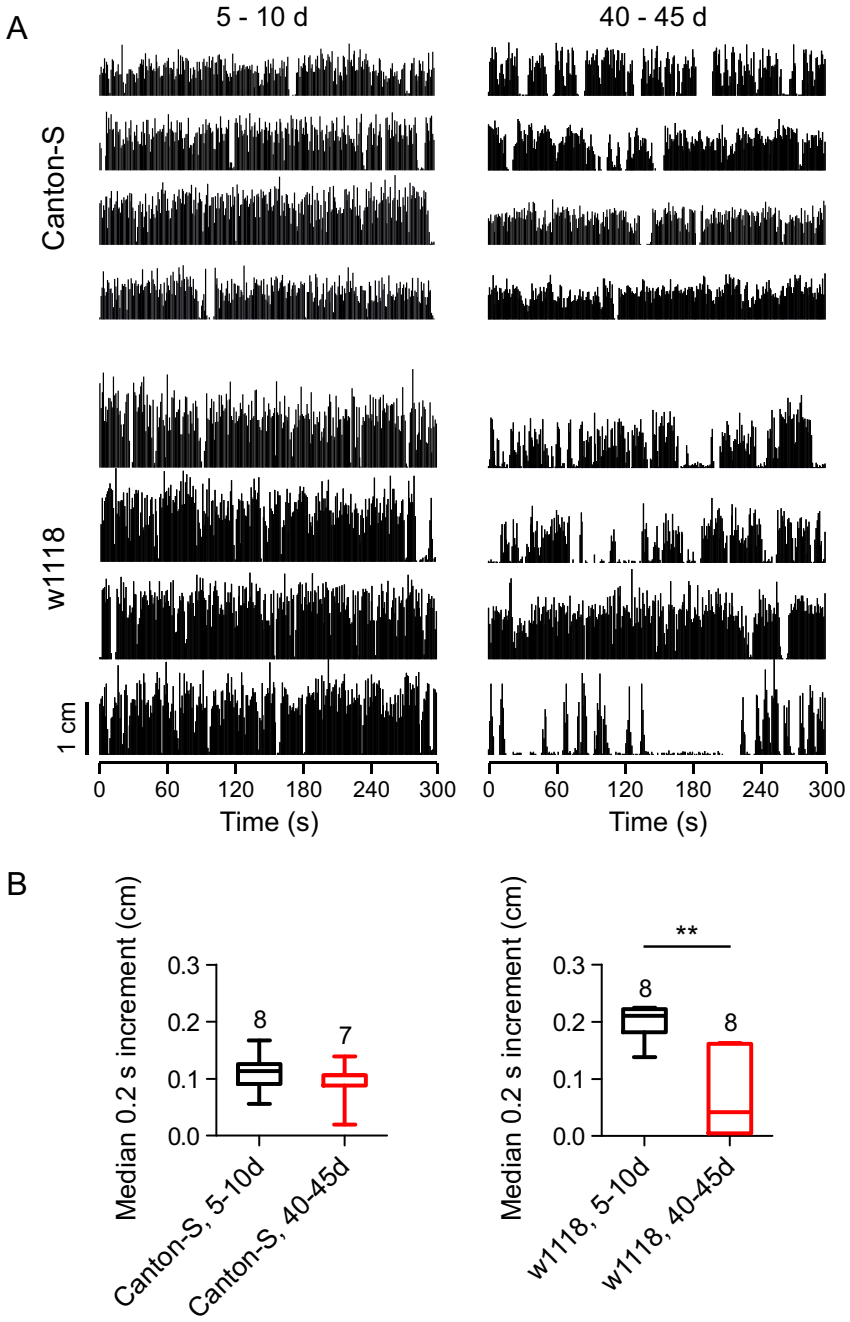

Fig. 4. 0.2 s path increments in Canton-S and w1118 flies. (A) Distances traveled every 0.2 s are plotted during 60 s of locomotion for four representative flies at young (5–10 days) and old (40–45 days) ages. (B) 0.2 s path increments in Canton-S and w1118 flies at young and old ages. $^{**}P < 0.01$, Mann-Whitney test.

behavioral performance. w1118 males maintained consistent body mass for up to 43 days, however Canton-S flies gained body mass at young age, followed by a gradual decline. In the locomotor assay, old w1118 flies had increased boundary preference, faster recovery speed from anoxia as well as decreased travel distance per minute and median 0.2 s path increment compared with young w1118 flies. However, old Canton-S flies displayed unchanged boundary preference, reduced recovery speed from anoxia, similar path length per minute and similar 0.2 s path increment relative to young ones. Thus, these two fly strains exhibit different behavioral aging.

The alfalfa leaf-cutting bee, *Megachile rotundata*, reduces allocation to developmental processes and reproduction as their body mass decreases (Abdelrahman et al., 2014), resulting in poor performance (Scheiner, 2012). We hypothesize that the difference of the body mass over time might explain why the behavioral changes differed in Canton-S and w1118 flies across the age. In addition, Oregon-R flies also gain body mass during the first five weeks with spontaneous feeding (Gill et al., 2015), which is consistent with the body mass trend of Canton-S flies in the current study. Resistance to desiccation decreases with age in flies, suggesting an age-related decline in water balance (Gibbs and Markow, 2001). The body mass measured in the current study was wet mass rather than dry mass. w1118 flies might have a better ability to maintain water homeostasis over age compared with Canton-S flies, which indicates a stable metabolic rate in w1118 flies as reported previously (Hoffmann and Parsons, 1989a, 1989b). The body mass was only

measured until Day 43, because some flies had already died on Day 43.

Wild-type flies exhibit a boundary preference in square or circular arenas (Colomb et al., 2012; Ewing, 1963; Liu et al., 2007; Martin, 2004; Soibam et al., 2012; Valente et al., 2007; Xiao and Robertson, 2015) and travel slower with less variation in speed (Xiao and Robertson, 2015). Compared with young w1118 flies, old w1118 flies displayed improved boundary preference and were likely more focused on the exploratory task by spending more time on perimeter. In the open field test, Tenascin-R-deficient mice, which have a severe motor coordination deficit, spend shorter time on the edge than in the central area, indicating that increased boundary preference requires a high degree of motor coordination (Montag-Sallaz and Montag, 2003). Therefore, the improved boundary preference in old w1118 flies suggests an enhanced motor coordination compared with young w1118 flies.

Young w1118 flies tend to move faster than young Canton-S flies to cross the circular arena. This faster travel speed may be due to the reduced amount of serotonin and dopamine in the heads of w1118 flies, which are neurotransmitters associated with locomotor performance (Borycz et al., 2008; Chen et al., 2013; Lebestky et al., 2009; Riemensperger et al., 2013; Sitaraman et al., 2008). Serotonin and dopamine decrease with age (Luine et al., 1990). It suggests that old flies display an increased travel distance and step size compared with young ones. However, old w1118 flies show a decreased step size and travel distance relative to young w1118 ones. It indicates that the reduced travel distance in old w1118 flies is attributable to other mechanisms which outweigh the changes of serotonin and dopamine levels. However, to the best of our knowledge, such mechanisms are still unclear.

Our investigation sheds light on how anoxia tolerance could be affected by fly age and fly strain. w1118 flies showed faster recovery speed from anoxia with aging, however, Canton-S old flies showed the opposite trend. It is possible that the faster recovery speed in old w1118 flies is due to a reduced metabolic rate, which could result in a reduced metabolic disturbance resulting from the coma as suggested (Schilman et al., 2011). It is worth noting that one previous study showed that w1118 old flies have a delayed recovery after submersion (wet anoxia) at 23 °C compared with young ones (Benasayag-Meszaros et al., 2015), which is inconsistent with our findings shown here. There are critical differences in the experimental settings. One important difference is that flies were exposed to pure N_2 in the current experiment whereas wet anoxia was used in their study without sufficiently exhausting oxygen levels in the air sac, reducing fly surface tension and removing oxygen from water. Therefore, there is a possibility that oxygen residual in the trachea, around the body and in the water leads to a hypoxic environment rather than an anoxic environment, and this might contribute to the different recovery trends across age in w1118 flies between two studies.

Differences in behavioral performance and neural function between Canton-S and w1118 strains are obvious at a young age. The current study expands the age range and demonstrates different age-related changes in body mass, behavioral performance and neural function. However, whether the age-related differences between Canton-S and w1118 strains are related to the *white* gene or the different genetic backgrounds in Canton-S and w1118 flies is still unclear, although it is established that the *white* gene does influence locomotor recovery from anoxia (Xiao and Robertson, 2016).

This investigation is limited by focusing on only two fly strains. Nevertheless, it is clear that differences in behavioral performance and neural function between Canton-S and w1118 flies persist into their old age. Moreover, it is interesting that the quality of the difference, rather than just the magnitude, is dependent on the age at which the flies are tested. The general implication is that phenotypic differences between strains cannot be considered to be constant and can vary considerably as the animals age. Thus, due to the marked difference between Canton-S and w1118 strains during aging, whether they could both be considered as ideal controls for studies of fly aging is unclear at present and the issue deserves further attention.

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