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RESEARCH ARTICLE

The effects of age and lifetime flight behavior on flight capacity in *Drosophila melanogaster*

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ABSTRACT

The effects of flight behavior on physiology and senescence may be profound in insects because of the extremely high metabolic costs of flight. Flight capacity in insects decreases with age; in contrast, limiting flight behavior extends lifespan and slows the age-related loss of antioxidant capacity and accumulation of oxidative damage in flight muscles. In this study, we tested the effects of age and lifetime flight behavior on flight capacity by measuring wingbeat frequency, the ability to fly in a hypo-dense gas mixture, and metabolic rate in *Drosophila melanogaster*. Specifically, 5-day-old adult flies were separated into three life-long treatments: (1) those not allowed to fly (no flight), (2) those allowed – but not forced – to fly (voluntary flight) and (3) those mechanically stimulated to fly (induced flight). Flight capacity senesced earliest in flies from the no-flight treatment, followed by the induced-flight group and then the voluntary flight group. Wingbeat frequency senesced with age in all treatment groups, but was most apparent in the voluntary- and induced-flight groups. Metabolic rate during agitated flight senesced earliest and most rapidly in the induced flight group, and was low and uniform throughout age in the no-flight group. Early senescence in the induced-flight group was likely due to the acceleration of deleterious aging phenomena such as the rapid accumulation of damage at the cellular level, while the early loss of flight capacity and low metabolic rates in the no-flight group demonstrate that disuse effects can also significantly alter senescence patterns of whole-insect performance.

KEY WORDS: Flight, Insect, Senescence

INTRODUCTION

A fundamental issue in life history theory is how an organism's past behavior affects its present and future physiological performance and survival. For example, vigorous exercise training in humans and other mammals has been shown to increase cognitive capacity (Suominen-Troyer et al., 1986), locomotor performance (Skalicky et al., 1996), motor coordination (Dorner et al., 1997), antioxidant capacity (Gündüz et al., 2004; Kayani et al., 2008), resistance to cellular oxidative damage (Radák et al., 1999), immune function (Utsuyama et al., 1996) and lifespan (Paffenbarger et al., 1993; Lee and Paffenbarger, 2000). Alternatively, prolonged sedentarism in otherwise active mammal species can decrease running capacity (Swallow et al., 1998), lower the maximum rate of O_2 uptake (Overton et al., 1986; MacNeil and Hoffman-Goetz, 1993), increase body mass (Mlekusch et al., 1996; Swallow et al., 1998) and decrease lifespan (Goodrick, 1980; Holloszy, 1988; Franco et al., 2005; Bronikowski et al., 2006).

While such effects are now well-established for humans and other vertebrates, far less is known about the effects of lifetime behavior patterns on physiological performance in invertebrates, the most speciose and abundant of which – the flying insects – lead much more aerobically variable and active lives than nearly all vertebrates because of their small size, poikilothermy and extremely high energy costs associated with flight. Research to date suggests that flight activity (and suppression of it) in insects has few long-term parallels with cardiovascular/musculoskeletal exercise and sedentarism in vertebrates. For example, experimental, non-invasive suppression of flight behavior in certain dipterans extends lifespan (Sohal and Buchan, 1981; Sohal et al., 1993; Agarwal and Sohal, 1994; Yan and Sohal, 2000; Magwere et al., 2006), elevates levels of the antioxidant enzymes aconitase and adenine nucleotide translocase (Yan and Sohal, 2000), and slows the age-based accumulation of protein carbonylation, lipid peroxidation and oxidative stressors (e.g. 8-hydroxydeoxyguanosine, glutathione and hydrogen peroxide) (Sohal et al., 1984; Sohal et al., 1993; Agarwal and Sohal, 1994; Yan and Sohal, 2000; Magwere et al., 2006).

Similar flight-related patterns of longevity and cellular stress phenomena are seen among behavioral castes of the honey bee *Apis mellifera*. In this species, workers perform few behaviors requiring flight in the early (or nursing) stages of their adult lives, and later transition to behaviors that increasingly involve flight, culminating in numerous daily, long-distance foraging flights. Early onset (i.e. precocious) foraging or extended daily flight duration while foraging decrease lifespan in honey bee workers (Neukirch, 1982), and certain antioxidant proteins are upregulated in foragers compared with nurse bees (Schippers et al., 2006; Wolschin and Amdam, 2007). Likewise, levels of flight muscle phosphofructokinase and cytochrome *c* oxidase decrease in older foragers (Schippers et al., 2006; Schippers et al., 2010), as does flight kinematics (Vance et al., 2009). Young (8–10 days old) foragers diurnally upregulate antioxidant and heat-shock proteins in flight muscle in greater amounts than age-matched nurse bees, and this response largely disappears in old (30–32 days old) bees of either group (Williams et al., 2008). In bumblebees, the suppression of foraging activity slightly increases body mass and the immune response later in life (Doums and Schmid-Hempel, 2000; Skandalis and Darveau, 2012), suggesting trade-offs between flight and other energy-demanding traits over the lifetime of a forager.

While flight behaviors decrease longevity and hasten senescence at the cellular and system levels in flying insects, very little is known about the effects of locomotory sedentarism in insects. As described above, suppression of flight behavior extends lifespan and slows the accumulation of oxidative damage within flight muscles. However, there are few reported examples of muscle or neurological disuse

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List of abbreviations

FMR	flight muscle ratio
IF	induced flight
NF	no flight
VF	voluntary flight
WBF	wingbeat frequency

pathologies in insects. Experimental wing removal in crickets accelerates weight and protein loss within the dorso-longitudinal flight muscle (Tanaka, 1991; Gomi et al., 1995), while wing removal in newly eclosed tsetse flies causes an increase in the sarcoplasmic fraction and a decrease in the mitochondrial and myofibrillar fractions of the dorso-longitudinal flight muscle as the flies age (Anderson and Finlayson, 1976). When *Drosophila melanogaster* Meigen 1830 are prevented from flying during the first 5 days post-eclosion, steering control during flight is less precise, but maximal flight performance is not significantly different from flies permitted to fly beginning at eclosion, suggesting that the steering impairment is not due to a muscle disuse phenomenon, but instead a lack of neurological 'practice' of fine control of aerial performance (Hesselberg and Lehmann, 2009).

In this study, we investigated how lifetime patterns of flight behavior affect the age-based trajectory of flight performance in *D. melanogaster*. As described above, there are significant effects of varying flight behavior on flight muscle composition, control and muscle oxidative damage, and we hypothesized that these effects, resulting from either disuse or overuse of the flight muscles, would alter the flight performance of flies as they age. Specifically, we predicted that both the experimental suppression and elevation of flight behavior would, relative to control flies, lead to an early-onset reduction of relative flight muscle mass, flight ability and metabolism. To test these predictions, we separated 5-day-old female fruit flies into three lifetime flight behavior groups: (1) those not allowed to fly (no flight; NF), (2) those allowed – but not forced – to fly (voluntary flight or control treatment; VF) and (3) those mechanically stimulated to fly (induced flight; IF). For each group we measured flight capacity in a hypodense gas mixture (see Roberts et al., 2004), fresh body mass, relative thorax mass [as a proxy for relative flight muscle mass, or flight muscle ratio (FMR)], wingbeat frequency (WBF) and flight metabolism (CO_2 emission) at 15, 35 and 65 days of age.

RESULTS

Flight capacity

Flight capacity was affected by age and lifetime flight behavior, and there was an interaction between them (Table 1). Among 15-day-old flies, nearly all flies from the VF and IF groups could fly, but 30% of the NF flies were flight-impaired (Fig. 1). At 35 days of age, only 10% of the VF flies were flight impaired, compared with 50% and 30% for the NF and IF groups, respectively. By 65 days of age, nearly all flies in the NF and IF groups were flight-impaired, although 30% of the VF flies were still capable of flight.

Table 1. Ordinal logistic regression analysis evaluating the effects of age and treatment and their interaction on flight capacity in *Drosophila melanogaster*

Variable (d.f.)	Coefficient	s.e.m.	*P*	Odds ratio
Age (2)	−3.5036	0.5498	0.0000	0.0301
Treatment (2)	−1.9023	0.4772	0.0001	0.1492
Age × Treatment (4)	1.0886	0.3379	0.0013	2.9702

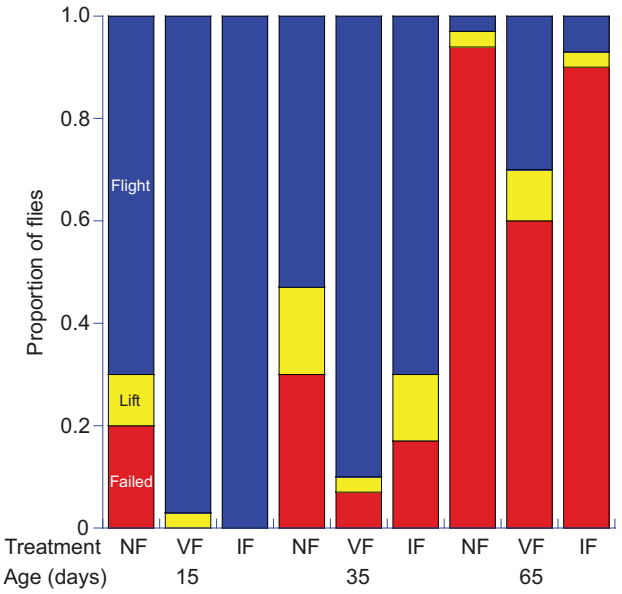

Fig. 1. Flight capacity as a function of age and lifetime flight behavior in *Drosophila melanogaster*. Blue indicates the proportion of flies that could fly normally, yellow indicates generation of lift (limited flight), and red indicates failed or no flight (see Materials and methods for details). NF, no flight; VF, voluntary flight or control treatment; IF, induced flight. *N*=27–30 flies for each age × treatment group combination.

Morphology, WBF and metabolic rate

There was an effect of age on body mass, as well as an interaction between age and lifetime flight behavior (Table 2). At 15 days of age, NF and VF flies were slightly (10%) heavier than IF flies, but body mass was not different among lifetime behavior groups at 35 and 65 days of age. Except for the slightly heavier flies in the 15-day-old NF and VF flies, body mass was generally constant at 1.05–1.12 mg across all ages (Fig. 2). Estimated fresh thorax mass was not significantly affected by age, but was significantly affected by treatment, with a significant interaction between age and treatment (Table 2, Fig. 2). FMR was affected by age and lifetime flight behavior, and there was an interaction between the main effects (Table 2). At 15 days of age, IF flies had a slightly higher FMR than NF and VF flies (Fig. 3). The FMR of IF flies remained constant with age at approximately 41%, while the FMR of NF flies was approximately 37% at all ages. The FMR of the VF flies increased from 37% to 40% over the duration of the experiment. Variation in FMR was not due to any consistent age or treatment-based variation in either total mass or thorax mass.

There was an effect of age on WBF, as well as an interaction between age and lifetime flight behavior (Table 2). In the NF group, WBF was highest at 35 days of age and not different from the highest WBFs of the VF and IF groups, which occurred at 15 days of age (Fig. 4). In the VF group, WBF decreased between 15 and 35 days of age, but did not further decrease at 65 days of age. In the IF group, WBF decreased between 15 and 35 days of age and between 35 and 65 days of age, but only the decrease in the latter period was statistically significant.

Metabolic rate was affected by age and lifetime flight behavior, and there was an interaction between the main effects (Table 2). In the NF group, metabolic rate was low and unaffected by age (Fig. 5). In the VF and IF groups, metabolic rates were highest at 15 days of age and dropped significantly at older ages. Metabolic rates of flies in the VF group fell significantly by 35 days of age and again by

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Table 2. ANOVA results for factors affecting body mass, estimated thorax mass, flight muscle ratio, wingbeat frequency and metabolic rate in *D. melanogaster*

	Variable (d.f.)	*F*	*P*
Body mass (mg)	Age (2)	10.64	<0.001
	Treatment (2)	2.47	0.087
	Age × Treatment (4)	5.62	<0.001
Estimated thorax mass (mg)	Age (2)	1.71	0.182
	Treatment (2)	6.44	0.002
	Age × Treatment (4)	4.55	0.001
Flight muscle ratio (% body mass)	Age (2)	5.63	0.004
	Treatment (2)	27.23	<0.001
	Age × Treatment (4)	2.58	0.038
Wingbeat frequency (Hz)	Age (2)	49.55	<0.001
	Treatment (2)	2.9	0.06
	Age × Treatment (4)	10.58	<0.001
Metabolic rate (ml g^{-1} h^{-1})	Age (2)	86.95	<0.001
	Treatment (2)	13.86	<0.001
	Age × Treatment (4)	13.36	<0.001

65 days of age. In the IF group, metabolic rate dropped dramatically (57%) between 15 and 35 days of age, but did not further drop between 35 and 65 days of age.

DISCUSSION

The senescence of flight ability in aged insects is well established (Le Bourg and Minois, 1999; Magwere et al., 2006; Simon et al., 2006; Miller et al., 2008), and the results of this study expand the understanding of this process by demonstrating that variation in lifetime flight behavior, in addition to age, affects the onset and pace of flight senescence. Elevated flight frequency in the IF group moderately hastened the onset of flight senescence compared with the VF group, and the pace of flight senescence in the VF and IF flies between 35 and 65 days of age was the most rapid over any time period in the study. The accelerated pace of flight senescence in older VF and IF flies may have been due to a more rapid accrual of molecular, cellular and/or internal morphological breakdown in the flight apparatus. Suppression of flight behavior slows the accumulation of cellular oxidative damage in flight muscles and increases lifespan (Agarwal and Sohal, 1994; Yan and Sohal, 2000; Magwere et al., 2006), suggesting that flight ability in the NF group should have persisted later into life compared with the VF and IF groups. However, the NF flies lost their flight ability earlier than the VF and IF groups, indicating the presence of disuse phenomena in NF flies that negatively affected flight performance to the extent that presumed beneficial effects of mitigated oxidative/ultrastructural damage on flight performance were masked.

Aging and senescence of flight performance

Flight performance, WBF and metabolic rate decreased in older flies, and such trends are well documented for *Drosophila* and other insects (Le Bourg and Minois, 1999; Tofilski, 2000; Simon et al., 2006; Miller at al., 2008; Williams et al., 2008; Vance et al., 2009).

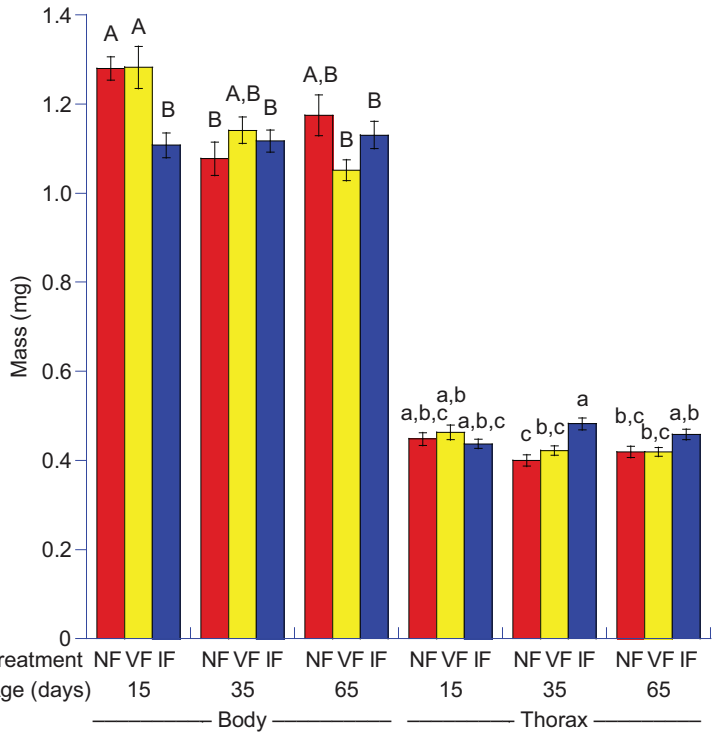

Fig. 2. Body mass and thorax mass as a function of age and lifetime flight behavior in *D. melanogaster*. Means that do not share a letter (uppercase for body mass and lowercase for thorax mass) are significantly different. Error bars represent s.e.m. *N*=27–30 flies for each age × treatment group combination.

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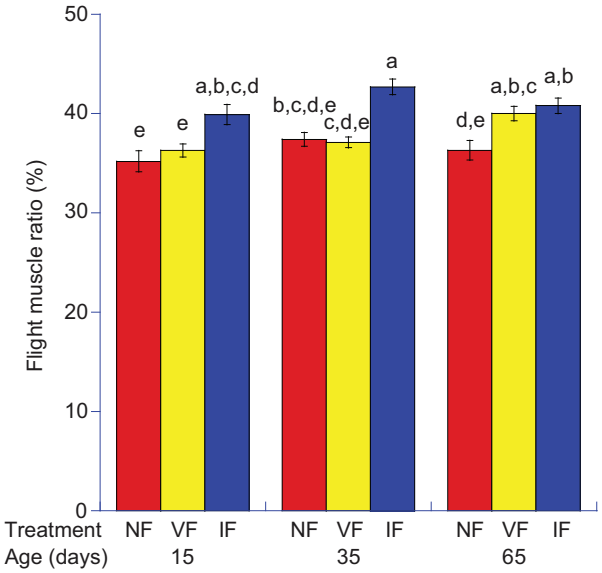

Fig. 3. Flight muscle ratio as a function of age and lifetime flight behavior in *D. melanogaster*. Means that do not share a letter are significantly different. Error bars represent s.e.m. *N*=27–30 flies for each age × treatment group combination.

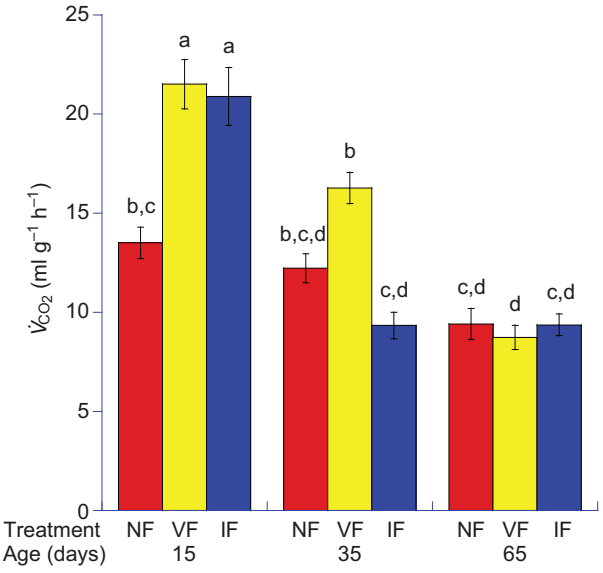

Fig. 5. Metabolic rate ($\dot{V}_{CO_2}$) during 'agitated' flight as a function of age and lifetime flight behavior in *D. melanogaster*. Means that do not share a letter are significantly different. Error bars represent s.e.m. *N*=6 for each age × treatment group combination.

In the Oregon R strain of *D. melanogaster*, flight performance and WBF decrease after 28 and 42 days of age, respectively (Miller et al., 2008). Exploratory activity (distance travelled away from a central location per unit time), the proportion of time spent flying and negative geotaxis also decrease with age in *D. melanogaster* (Le Bourg and Minois, 1999; Gargano et al., 2005; Magwere et al., 2006; Simon et al., 2006). Wing kinematics and the frequency of foraging trips decrease in old honey bee foragers, although age has no effect on the flight performance of nurse bees (Tofilski, 2000; Vance et al., 2009). Flight behavior and flight muscle mass decrease soon after the imaginal moult in crickets and other orthopterans, although this represents an adaptive reallocation of mass and energy towards reproductive capacity as opposed to classical senescence (Shiga et al., 1991; Zera et al., 1997).

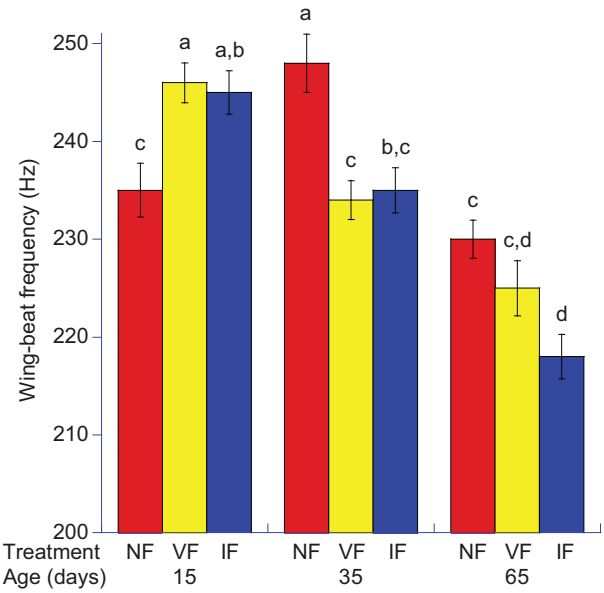

Fig. 4. Wingbeat frequency as a function of age and lifetime flight behavior in *D. melanogaster*. Means that do not share a letter are significantly different. Error bars represent s.e.m. *N*=12 flies for each age × treatment group combination.

The senescence of flight performance in insects can result from numerous molecular, cellular and morphological impairments associated with age. The flight muscles lose antioxidant capacity and accrue oxidative damage to lipids, proteins and DNA (Yan and Sohal, 2000; Magwere et al., 2006; Seehuus et al., 2006; Williams et al., 2008), and such damage possibly underlies the age-based impairment of glycolytic and electron transport chain enzymes (Schippers et al., 2006; Schippers et al., 2010) and the ultrastructural degeneration of flight muscle mitochondria and sarcomeres (Sacktor and Shimada, 1972; Fernandez-Winckler and da Cruz-Landim, 2008; Miller et al., 2008), which in turn might explain the lower metabolic rates of aged flies in the VF and IF groups. Flight muscle mass decreases steadily with age in certain insects (Ready and Josephson, 1982; Stjernholm and Karlsson, 2008), although this effect did not occur in our study (Fig. 3). Wing wear is another aging phenomenon common to flying insects, especially large species (see Foster and Cartar, 2011). *Drosophila* have an extremely well-characterized wing morphology, which includes a continuous vein along the entire anterior margin of the wing blade and a smooth posterior margin. Hence, any wear to the wing due to age or use would be very evident, and we did not notice any fractures or loss of wing area during the experiment.

Flight activity and senescence

Elevated levels of flight activity accelerated the onset of flight senescence. At 15 days of age, flight performance, WBF and metabolism did not differ between the VF and IF groups and were at peak levels. However, by 35 days of age, flight performance and metabolism had dropped approximately twice as much in the IF group than in the VF group, while WBF in both groups had dropped equivalently (~11 Hz, or 4%). By 65 days of age, approximately 30% of VF flies could still fly (compared with 10% in the IF group), and the average WBF in both groups dropped another 10–15 Hz. We did not observe any loss of wing area in any of the treatment groups at any age (which by itself would have the effect of increasing

WBF). The lower WBF in aged VF and IF flies could be due to sarcomeric lesions, perhaps affecting elasticity and storage of wing inertial energy (Miller et al., 2008). At 65 days of age, the metabolic rates of VF flies had decreased to the same levels as the IF flies (whose metabolic rates remained similarly low between 35 and 65 days of age). Compared with the young VF and IF flies, the lower metabolic rates in all other age × treatment groups could have been due to the inability to maintain continuous/near-continuous flight performance during continuous agitation, reduced metabolic rates of flight itself (a possibility somewhat supported by the WBF data), or both. Variation in fresh body mass and FMR played little if any role in the differences in flight senescence patterns between the IF and VF flies. Fresh body mass [more of which limits flight performance (see Roberts et al., 2004)] was slightly, but significantly, higher in VF flies than in IF flies only at 15 days of age, and was the same in the two groups at older ages. Furthermore, FMR (which should aid flight performance) was slightly higher in IF flies than in VF flies at 15 and 35 days of age, but was equivalent between these groups at 65 days of age.

Suppression of flight behavior also led to a premature decrease in flight ability, as well as a failure to attain the peak metabolic rates exhibited by 15-day-old VF and IF flies. The NF flies did not attain the peak WBF of 15-day-old VF and IF flies (~247 Hz) until 35 days of age, at which time they were even more flight impaired than 15-day-old IF flies and 35-day-old VF and IF flies. This fact, along with the small variation in fresh body mass and FMR among ages and behavior groups, indicates that the premature loss of flight ability of NF flies was not due to major morphological or gross kinematic disadvantages (although a more complete aerodynamic analysis is necessary to confirm the latter). Instead, disuse phenomena limiting flight control and metabolic capacity could be the major factors underlying the impairment of flight ability in the NF group. Indeed, when the flight of *D. melanogaster* is suppressed during the first 5 days after eclosion, flight properties associated with steering control (e.g. turning rate, control of stroke asymmetry, etc.) in 5-day-old flies are impaired, although there is no effect on WBF and peak horizontal speed (Hesselberg and Lehmann, 2009). Using three treatment groups very similar to ours, Anderson and Finlayson (Anderson and Finlayson, 1976) showed that post-eclosion suppression of flight behaviors in tsetse flies causes an increase in the sarcoplasmic fraction and a decrease in the mitochondrial and myofibrillar fractions of flight muscle, while long-term increases in flight frequency (via daily tapping on the cage) induces opposite long-term effects. We are currently conducting experiments to determine whether similar muscle composition phenomena, which could help to explain the observed patterns in metabolism, are occurring in our NF, VF and IF groups of *D. melanogaster*.

The results of other studies that have manipulated insect flight activity strongly suggest that flight behavior accelerates senescence and decreases longevity because of the production of reactive oxygen species and the accumulation of oxidative damage within cells. The strongest evidence for this argument is that the developmental or experimental suppression of flight behavior in flies and bees slows the senescence of antioxidant defenses and the accumulation of oxidative damage (Sohal and Buchan, 1981; Sohal et al., 1984; Agarwal and Sohal, 1994; Yan and Sohal, 2000; Magwere et al., 2006; Williams et al., 2008). However, no studies to date have tested whether experimental increases in flight behavior yield supra-normal accumulation of reactive oxidative species and oxidative damage to cellular macromolecules. Our preliminary comparisons of longevity in the NF, VF and IF groups suggests this may be the case, as the longevity of VF flies is greater than IF flies but lower than NF flies (S.R., personal observation). In an interesting parallel to human exercise, experimental increases in the frequency of walking behavior in *D. melanogaster* slows age-based decreases in walking mobility and cardiac function (Piazza et al., 2009), indicating that the type of locomotor behavior, perhaps related to the contraction frequency and mass of muscles involved, can have strong effects on health and senescence.

Conclusions

This is the first study to assess how age and the experimental suppression and enhancement of lifetime flight activity affect flight performance in a volant species. Elevated levels of flight activity hasten the senescence of flight performance and metabolism in a pattern similar to the more rapid senescence of antioxidant defenses and accumulation of oxidative damage in flight-active versus flight-inactive insects. The loss of flight ability is likewise hastened by the lifetime suppression of flight behavior. This effect is likely due to disuse phenomena that are not yet completely understood rather than oxidative damage, but may include lack of neuromuscular exercise and changes in flight muscle composition. Research is ongoing in our laboratory to further determine how variation in lifetime flight behavior, including alternating periods of activity and sedentarism, affects longevity and age-based patterns of flight performance, flight muscle ultrastructure, fuel substrate usage and energetic allocation strategies.

MATERIALS AND METHODS

Fly culture and age cohorts

Flies used in all experiments were derived from a colony established from several hundred females collected in 2008 from a winery near East Lansing, MI, USA, and maintained as a large, outbred population. The fly stock was maintained on standard *Drosophila* medium (Standard Medium, Indiana University Stock Center, Bloomington, IN, USA) in a climate-controlled incubator at 25°C under a 14 h:10 h light:dark photoperiod. In order to obtain age-matched cohorts of flies, females from the stock colony were permitted to lay eggs on a grape-agar laying medium, from which first instar larvae were collected 24 h later and placed into standard food vials at a density of 100 larvae per vial. Adult flies were collected from these rearing vials at 24 h intervals and used to populate the experimental treatment groups with age-matched (to within 24 h) flies.

Lifetime behavior treatment groups

Three different treatment groups were created to generate a broad range of daily flight activity. In all of the treatment groups, young (5-day-old post-eclosion) female flies were placed into 0.24 l bottles at a density of 200 flies per bottle and maintained thereafter at 22±1°C. The lid of each bottle consisted of an inverted standard medium food dish that was changed daily. In the NF group, nylon mesh was placed in the bottles to create a baffling that prevented the flies from flying but still allow walking and access to food. Flies in the VF group were kept in bottles without mesh. Flies in the IF group were also kept in bottles without mesh, but the bottles were adhered via Velcro to a horizontal cardboard deck attached to the head of a vortex-type shaker. Using an SLC programmable timer (Allen–Bradley, Milwaukee, WI, USA), the shaker was programmed to gently shake, during the 14 h light photoperiod, for 0.3 s at random intervals between 5 and 10 min. The brief, abrupt movement of the bottle caused the flies to fly en masse ~120 times per day over the 14 h light period. Preliminary control experiments were conducted with flies housed in bottles containing mesh and similarly agitated to assess the effect of brief shaking independent of increased flight frequency. The flight capacity and egg-laying rate of these flies did not differ from the NF group, indicating that the intermittent shaking alone did not affect flies. Female flies were sampled from the treatment groups at ages 15, 35 and 65 days for measurements of flight performance, metabolic rate and body mass.

Flight performance assay

Individual flies were sampled from treatment groups and screened for their ability to fly in a low-density, normoxic gas mixture of 21% O_2, 39.5% N_2 and 39.5% He (density 0.81 g l^{-1}) at 22±1°C. Because of the greater power requirements of flying in a low-density gas, this approach effectively reveals variation in insect flight performance (Roberts et al., 2004; Vance et al., 2009). Specifically, individual flies were placed into a 1.9 l covered plastic flight chamber that was perfused with the hypodense gas at a rate of 500 ml min^{-1}. The gas was mixed and regulated using a Sable Systems MFC-4 (Sable Systems Inc., Las Vegas, NV, USA). Flight performance was scored according to Frazier et al. (Frazier et al., 2008). Flies that showed normal, upward take-off flight behavior and could fly the full width of the chamber (12 cm) were categorized as performing a 'flight'; those that flew >5 cm but <12 cm, conventionally a take-off followed by an arching loop ending on the chamber bottom or a controlled decent off the chamber wall, were categorized as performing 'lift' (these flies were unable to maintain flight, but were considered different from 'no flight' because they could travel further than the maximum jumping distance observed); and flies that traveled <5 cm and displayed an uncontrolled descent behavior were classified as performing 'no flight'. We tested a fly until it performed a flight or 3 min had passed. Flight performance was determined for 27–30 flies for each age × treatment group combination. An optical tachometer was used to measure WBF of 12 flies for each age × treatment group in the flight performance assay (Frazier et al., 2008). Additional flies were screened with the optical tachometer in the 65-day-old NF and IF groups (which had severely impaired flight ability) so as to obtain WBF measurements of 12 flies in these groups. The tachometer recordings were then digitized and visualized using the RAVEN sound analysis program (Cornell University, Ithaca, NY, USA). Each recorded sequence contained five to 15 continuous wing beats. For each fly, WBF was determined to the nearest 0.2 Hz by dividing the number of clearly distinguishable, uninterrupted wing beats in the sequence by the duration of the sequence (measured to the nearest 0.001 s) (Frazier et al., 2008).

Body mass and metabolic rate

Immediately after the flight assay, the live fly was weighed to the nearest 0.01 mg on a Sartorius BP211D balance (Sartorius Corporation, Edgewood, NY, USA). The fly was then frozen and its wings were removed. The head, thorax and abdomen were separated and dried for 24 h at 55°C, then immediately weighed using an Orion Cahn C-35 microbalance (±1 µg; Thermo Electron Corp., Beverly, MA, USA). The thorax primarily houses flight muscle and thus provides an index of flight muscle mass (Frazier et al., 2008). We estimated FMR as the ratio of dry thorax mass to dry body mass, and estimated fresh thorax mass by multiplying FMR by the fresh body mass.

CO_2 emission was measured using flow-through respirometry, in which a single trial consisted of 10 flies from a given age × treatment group combination placed together in a 30 ml glass chamber maintained at 22±1°C. Six trials using different flies were run for each age × treatment group combination. Dry CO_2-free air was passed through the chamber at 50 ml min^{-1}, controlled by a Tylan FC-260 mass-flow valve and an MFC-4 controller (Sable Systems International). Water vapor produced in the chamber was scrubbed from the air stream with a magnesium perchlorate drying column, and CO_2 was quantified in the excurrent air with a Sable Systems CA-10 analyzer. Included in each run was a 2 min measurement of the chamber without any flies to establish baseline. Flies were placed in the chamber and, after an 8 min resting period, the chamber was tapped at 2 Hz for approximately 5 min to stimulate them to fly as continuously as possible. Data were acquired by a UI-2 interface and analyzed with Expedata software (Sable Systems International). Immediately after each metabolic trial, flies from that trial were weighed to the nearest 0.01 mg. The highest average age × treatment group metabolic rates obtained using this method were in the 15-day-old VF and IF groups, and were similar to metabolic rates measured by others for individual, continuously flying *D. melanogaster* (Wigglesworth, 1949; Lehmann et al., 2000; Lehmann and Schützner, 2010).

Statistical analysis

To assess the effects of age and lifetime flight behavior on flight performance, an ordinal logistic regression was conducted because the flight performance data were an ordered categorical response variable (see Frazier et al., 2008). We used a two-way ANOVA to analyze WBF, body mass, FMR and metabolic rate in response to treatment and age. The normality of the data was tested and confirmed with an Anderson–Darling test. *Post hoc* analyses were conducted using Tukey's test to determine which means were significantly different from one another. Type I error was set at 0.05.

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Competing interests

The authors declare no competing financial interests.

Author contributions

M.M.E. and S.P.R. contributed to the conception of the project. All authors contributed to the design of the project, interpretation of the data, and the drafting and revision of the article. S.J.L. and S.P.R. contributed to the execution of the experiments.

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Maresh, Samantha Simmons and Birgitte McDonald travelled to the Año Nuevo State Park just north of Monterey Bay in California, where elephant seals go to breed. Selecting 12 animals over a period of months, the scientists injected two different types of modified water into the animals (to measure their energy expenditure) and strapped a wooden block to their backs to increase their exertion by increasing the amount of water that they must push aside while swimming. In addition, they also attached GPS trackers, time-depth recorders, accelerometers and magnetometers to the animals' backs to monitor their behaviour, before driving the animals 50 km down the coast to the Hopkins Marine Station where they were released. 'Elephant seals have a tendency to return to their rookery', says Maresh, who was confident that she would be able to retrieve the priceless data when the animals returned home. The team also repeated the exercise with the same animals, but this time they removed the drag block, to find out how the animals behave and how much energy they consume under normal circumstances.

After successfully retrieving the data loggers, the team was impressed to see that the unhampered animal's movements are incredibly efficient. 'Elephant seals had low energy requirements (106.5 kJ kg^{-1} day^{-1}), approaching or even falling below predictions of basal requirements', says Maresh. However, when the animals had to drag the blocks through the water, their metabolic rates rocketed by 65% to 175.2 kJ kg^{-1} day^{-1}. Yet, when the team scrutinised the seals' behaviour, they were surprised that the animals had barely altered their swimming style: they did not beat their flippers wider or faster to compensate and they continued diving to the same depths and for as long as the unhindered seals. However, the animals spent 46% longer recovering at the surface after returning from a dive with the drag block and they took longer to ascend and descend. Instead of altering their swimming style to maintain efficiency, the seals stuck to their usual technique, which became increasingly inefficient.

Explaining that increasing time at the surface puts the seals at increased risk of predation by sharks and orcas, Maresh adds, 'Elephant seals are considered to be a relatively hardy species … that they were so easily affected by a disruption to their routine swimming behaviours was thus somewhat surprising', and she warns that other more sensitive species are likely to be more seriously affected by human disturbance of the sea.

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Kathryn Knight

Inactivity exacerbates flies' senescence

The fruit fly *Drosophila melanogaster* on necrotic fruit. Photo credit: Stephen Roberts.

When a honey bee embarks on its foraging career, its days are numbered. The relentless schedule takes its toll and most of the insects die within 3 weeks. 'Foraging has really profound effects on longevity, flight ability and physiological performance', says Stephen Roberts from Central Michigan University, USA.

Intrigued by the ageing process – known as senescence – in insects, Roberts and his colleague Michelle Elekonich are keen to understand how flight, the most energetically expensive behaviour known, affects senescence. However, when it comes to answering complex questions about the mechanisms that govern ageing, *Drosophila* are better insects than bees for experimentally disentangling the effects of age and flight on senescence. Yet, little was known about the effects of a lifetime of activity on these ageing flies, so Roberts decided to get to grips with how different flight histories impact on geriatric fruit flies (p. 1437).

Teaming up with master's student Steven Lane, Roberts monitored how three populations of flies that had experienced different amounts of flight activity through their lives fared as they aged. The team allowed the first population to fly whenever they wished, but imposed a complete flight ban on the second group by loosely stuffing the insects' jar home with light wedding-veil gauze, only allowing the insects to walk around the interior. The third population was also free to fly, but their jar was strapped to a vortex shaker that was programmed to give the insects a brief nudge at random times over the day, frequently forcing them to take flight. Then, Lane analysed the insects' flight performance, weight and metabolism as they aged – starting with 15 day old youngsters, moving up to 35 day old middle-aged insects and concluding with 65 day old geriatrics – to find out how they had senesced.

Comparing the three populations, the team could see that insects that had been allowed to fly whenever they wished fared reasonably well. Testing the insects' ability to take to the air in low density air (where half of the nitrogen had been replaced with helium), they found the majority flew well in middle age and 30% were still able to take to the thin air as geriatrics. However, when the team tested the flight performance of flies that had been forced to fly throughout their lives, they had essentially burned out. By 35 days their metabolic rate had plummeted by 57% and by 65 days none of them were able to get off the ground in the helium-supplemented air. Roberts explains that this dramatic decline was predictable because of wear and tear and the insects' increased exposure to toxic oxygen by-products produced by their hectic lifestyles.

However, the big surprise came when the team investigated the insects that had been prevented from flying. Instead of benefiting from their leisure, the couch potatoes paid a high metabolic price for their sloth. 'Their flight ability was compromised the earliest and the most out of all of the treatment groups', says Roberts. By 65 days, none of the flies could get off the ground in the test atmosphere. However, unlike immobile humans, they had not gained weight.

'We didn't know what we were going to see with the no-flight group', says Roberts, who explains that it was possible that inactivity might have slowed the ageing process, but evidently it had not. In fact, it exacerbated the insect's decline. He says, 'Behaviour can have profound effects on an organism's future; what it has done in the past has profound effects on how good its life may be in the future.'

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