

The effects of genetic background on exercise performance in *Drosophila*

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ABSTRACT

The use of the *Drosophila* model for studying the broad beneficial effects of exercise training has grown over the past decade. As work using *Drosophila* as an exercise model becomes more widespread, the influence of genetic background on performance should be examined in order to better understand its influence on assessments used to quantitatively measure and compare exercise phenotypes. In this article, we review the various methods of exercise training *Drosophila*, and the performance of different wild-type *Drosophila* strains on various physiological assessments of exercise response. We conclude by summarizing the performance trends of commonly used strains.

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Introduction

Exercise is a well-established therapy for improving health and wellness across species and within various disease states. Physical activity is known to improve indices of health in multiple systems within the body, including the cardiovascular system [1–4], the brain [5–12] and the gut [13]. Furthermore, exercise can slow the progression of multiple debilitating diseases, including Alzheimer's Disease [11], Parkinson's Disease [11,12], diabetes [14], and cardiovascular disease [15]. Despite growing knowledge in the fields of exercise science, there are still gaps in our understanding of the impact that genetics have on exercise capacity and the ability to adapt to training. The reasons why some individuals have large, positive systemic responses to exercise while others fail to gain any benefits are not fully understood. While several pathways have been identified through which exercise exerts positive effects, novel pathways are still being discovered, and the relative contribution of these pathways in genetically distinct individuals is still an open question. Understanding the genetics of individual variation in exercise adaptation is key to maximizing the potential of exercise training or exercise mimetics as therapeutic treatments.

Over the past decade, *Drosophila* has emerged as a new model system for the study of exercise. *Drosophila* are an ideal model for complicated multi-factorial responses such as exercise because of their tractable genetics, cost-efficient maintenance, and high homology to the human genome. In addition, there are many fully sequenced wild-type lines available to address the role that genetic variation plays in exercise ability and adaptation.

Multiple methods exist for exercise training in *Drosophila* [16,17]. Current research using these methods demonstrates that flies are able to adapt to exercise training with similar phenotypic responses to those seen in humans [18–20], including improved endurance, preservation of mobility with age, and lengthening of healthspan [16,21–23]. Therefore, results from *Drosophila* have a high probability of relevance to mammalian systems, including humans.

The suite of exercise adaptations observed in *Drosophila* occur robustly in a variety of genetic backgrounds. However, the influence that genetic background has on adaptive responses to exercise is not well understood. Here, we will examine the available methods for exercise training in flies, the known modifiers of exercise, and the role that genotype appears to have on exercise adaptation based on our own data and interpretations of other published

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works. Gaining a better understanding of the impact that genetics has on exercise ability will aid in answering remaining questions in the field.

Current methodology for exercise training in *Drosophila*

A few methods have been established for exercise training in *Drosophila*. Each of these methods takes advantage of the fly's natural escape behaviour which causes them to perform negative geotaxis in response to a stimulus. Each is a highly useful training platform for studying endurance exercise and adaptation. Here, we will review the common platforms used for endurance training, the broad beneficial effects of each, and some of the available assessments for exercise performance.

The swing boat

The Swing Boat is a relatively gentle form of exercise training that works through pendulum-shaped movements of a motor-powered metal swing holding vials of flies [24]. This pendulum movement constantly changes which end of the vial is 'up' causing the flies to continuously climb towards the 'top' end [24]. The metal swing is also equipped with a *Drosophila* activity monitoring system (DAM) which allows the fly's activity to be recorded prior, during, and after training [25]. Using this method, flies are exercised for 12 days, with exercise lasting 30 minutes per day [24]. The Swing boat has been used to investigate the effects of light exercise in an Alzheimer's disease (AD) fly model and found that their gentle training method increased the lifespan of trained AD flies but did not affect the lifespan of wild-type controls [24].

The treadmill

Another *Drosophila* exercise platform used to study exercise-induced changes is the Treadwheel. In order to induce exercise, the treadmill gently rotates sets of vials around a central axle [16]. This rotating motion stimulates the innate negative geotaxis response in the flies, causing them to walk with the turns of the vial as it rotates [16,23]. Exercising flies for 2 hours per day for 5 days resulted in significant improvements to

metabolic parameters such as decreased glycogen, triglycerides, and weight as well as improvements in physiological parameters such as climbing speed [16]. The Treadwheel is a gentle method of studying endurance since it slowly rotates to induce negative geotaxis in the fly. However, unlike the Swing Boat, it was not originally designed to track activity. For this reason, the original Treadwheel setup has been modified to generate another similar apparatus, called the Rotating Exercise Quantification System (REQS) [26]. Similar to the DAM system, the REQS uses lasers to record fly activity before, during, and after exercise [26]. The REQS was recently used to investigate the variation in activity levels between DGRP lines and identified possible candidate genes responsible for baseline activity variation [27].

The power tower

The Power Tower is set up to repeatedly raise and drop multiple vials of flies, stimulating their climbing instinct in response to the fall. Flies will continuously respond to the drops by climbing until they are physically fatigued [17]. The Power Tower is able to consistently provide broad beneficial effects to health in response to chronic exercise in the fly, and has been successful in the identification of single-molecule exercise mimetics [17,28–32]. Of the approaches to *Drosophila* endurance training outlined in this section, the Power Tower results in the most intense stimulation of negative geotaxis through forceful and repetitive dropping of vials. Exercise training is performed for 3 weeks, Monday–Friday, and the time on the machine is increased by a half an hour each week making the Power Tower a good method for chronic training [17]. Studies using the Power Tower have shown improvements in exercise parameters such as climbing speed, endurance, flight performance, cardiac resistance to stress, neurological health, mitochondrial function and healthspan in both wild-type and disease models, as well as across *Drosophila* species [17,21,22,28,32–34]. These results will be further dissected in subsequent sections of this review.

Each of the three approaches reviewed in this section differ in intensity and duration of exercise, which likely contribute to differences observed in

exercise-induced adaptation between methods. For instance, flies exercised on the Power Tower show no increase in basal activity levels during training [33], while flies exercised using the Treadwheel do appear to increase basal activity [27]. Such discrepancies between methodologies should be taken into account in determining which platform is most well suited for a particular experimental design.

Assessments of exercise performance

There are a multitude of available assessments for measuring exercise adaptation that can be performed before, during, or after completing a chronic training protocol in order to measure the various components of exercise adaptation. Additionally, we will highlight a few experiments that are exceptional for measuring the physiological adaptations that occur with chronic endurance training.

Climbing speed

In *Drosophila*, climbing speed decreases with age in multiple genetic backgrounds [28,33,35]. One way to measure both acute climbing speed and the rate of age-related decline in climbing speed is by using the rapid iterative negative geotaxis (RING) assessment [35]. The RING assessment tests multiple flies of a single cohort as opposed to other negative geotaxis assessments which only test single flies [35]. Briefly, flies are placed into vials and then tapped down to the bottom in order to induce negative geotaxis [17]. A picture is taken 2s after the induction of climbing to record how high the flies were able to climb [17]. The climbing height can then be analysed using a common imaging software such as Image J [17]. After training, normalized climbing speed of trained wild-type flies is significantly better than untrained siblings [17]. Depending on the purpose of the experiment, the data collected can be analysed and displayed in multiple ways and the analysis of acute and chronic climbing speed in response to exercise training is possible.

Endurance

Increased endurance is the defining adaptation that occurs with endurance training [36]. Measuring endurance in the fly can be done using the Power

Tower, since flies will continually run on the Power Tower until they reach complete physical exhaustion [17]. Endurance is measured by placing at least eight vials ($n = 20$) per experimental group on the Power Tower and letting the flies run until fatigued. A fatigued vial is defined as when 80% of the flies in a vial have stopped running $\frac{1}{2}$ cm upwards in the vial [17]. Once fatigued, the vial is removed, and the time is recorded. Endurance data can then be presented and analysed in multiple ways, but is often graphed as a survival curve and analysed using a log-rank analysis [17]. This assessment can be performed at any time-point throughout the training. Results have shown that training improves endurance of wild-type flies compared to age matched siblings [22].

Cardiac pacing

In *Drosophila*, as in humans [37], heart rate, rhythmicity, and the ability to increase heart rate in response to cardiac stresses decrease with age [38]. Stress response can be effectively measured in *Drosophila* through external electrical pacing of anesthetized flies. Cardiac pacing is performed by electrically stimulating the heart to beat twice its normal heart rate [39]. After 30 seconds of electrical stimulation, the fly's heart is visually scored for a normal heartbeat [17]. Young wild-type flies have a low failure rate (24%), meaning their hearts return to beating normally after removal of the stimulus, while older wild-type flies have a higher failure rate (72–76%) [39]. As exercised wild-type flies have a lower failure rate than unexercised age-matched siblings [21,33], pacing can serve as a rapid, non-specific indicator of the effects of exercise training on general cardiac health.

Flight

For winged organisms, flight is a physically demanding activity that requires high motor function and coordination. Acute flight ability can be measured by inverting and releasing vials of flies into a drop tube. At the end of the drop tube, the flies are ejected into a vertical cylinder lined with an adhesive substance. In response to being ejected from their vial, the flies open their wings and initiate flight in an outward direction, promptly becoming stuck to the adhesive

substance on the outer walls of the cylinder. The location in which a fly lands corresponds to how quickly the fly was able to initiate flight in response to ejection, such that flies stuck closer to the top of the cylinder have better flight performance than flies stuck closer to the bottom [17,40]. Exercised wild-type flies land higher in the tube than unexercised siblings [41]. Flight requires muscle groups that are not active during the climbing movement that takes place during training. Thus, improvements in the flight ability of trained flies support the claim that systemic adaptations have occurred as a result of exercise training.

Potential resistance exercise training paradigms

As of this review, no protocols for investigating resistance exercise training in *Drosophila* have been published. However, increased weight-bearing experiments reminiscent of resistance training have been done in the fly model with promising physiological benefits. Resistance exercise training stimulates adaptations that differ from endurance training. These adaptations include increased muscle size, muscle strength, and improved neuronal function [42]. One method to increase load-bearing in flies is by simulating a hypergravity environment through centrifugation [42]. It is estimated that spinning flies at 12 g causes the leg muscles to support an additional 17–25 times the flies' body weight [43]. Exposing flies to 12g for a 24-hour period resulted in increased jumping, negative geotaxis ability, and altered troponin T isoform splicing [43]. Additionally, when exposed to either 3g or 5g for a 2-week time period, male flies gained improved longevity [44] and, as they aged, performed similar to or better than flies exposed to only 1g on various behaviour assessments (spontaneous movement and climbing ability), indicating that their rate of ageing may have been slowed by hypergravity treatment [45]. Based on these results, hypergravity appears to have promising effects on mobility and longevity.

Most hypergravity studies to date involve long term, continuous treatment. This is in contrast to typical mammalian models of resistance exercise, which involves the shortening and lengthening of a muscle under load for a particular number of

sets and repetitions that are interspersed by short periods of rest. A *Drosophila* model of resistance exercise should emulate this in order to be a valid model. We have attempted to create such a model by subjecting flies to various degrees of hypergravity for 1.5–3 hours per day for 3 weeks. Preliminary data shows that different treatments have different effects on climbing speed and flight ability in wild-type flies (Figure 1). However, these protocols still need to be fine-tuned to replicate mammalian resistance exercise (i.e. duration, rest periods, validation of shortening and lengthening of muscle) and reliable assessments to measure classic resistance exercise adaptations need to be performed (e.g. muscle size and strength, muscle protein synthesis, anabolic protein activity). Nonetheless, hypergravity is a unique method to load muscle and has the potential to be exploited to replicate resistance exercise. Even if a model of resistance exercise remains elusive, hypergravity can still be a valuable tool to study the effects of muscle loading in numerous contexts such as mechanical transduction and metabolism.

Physiological factors that affect exercise

Over the past decade the devices previously discussed for exercise training in *Drosophila* have been used to determine factors that influence baseline performance and adaptations to chronic exercise training. In this section, we will discuss some of those factors as well as the known genetic determinants of endurance that have been discovered using *Drosophila* as an exercise model.

Age, diet, and sex are all factors that affect exercise performance

Similar to humans [46], *Drosophila* undergo an age-related decline in physical health that can be slowed with exercise, but not stopped [21]. Climbing speed, endurance, flight performance, cardiac performance, and mitochondrial health all decline with age [21,28,41,47–49]. In addition to slowing the rate of age-related decline in climbing ability, chronic exercise training using the Power Tower can preserve endurance and flight performance, improve resistance to cardiac stress that declines with age, and

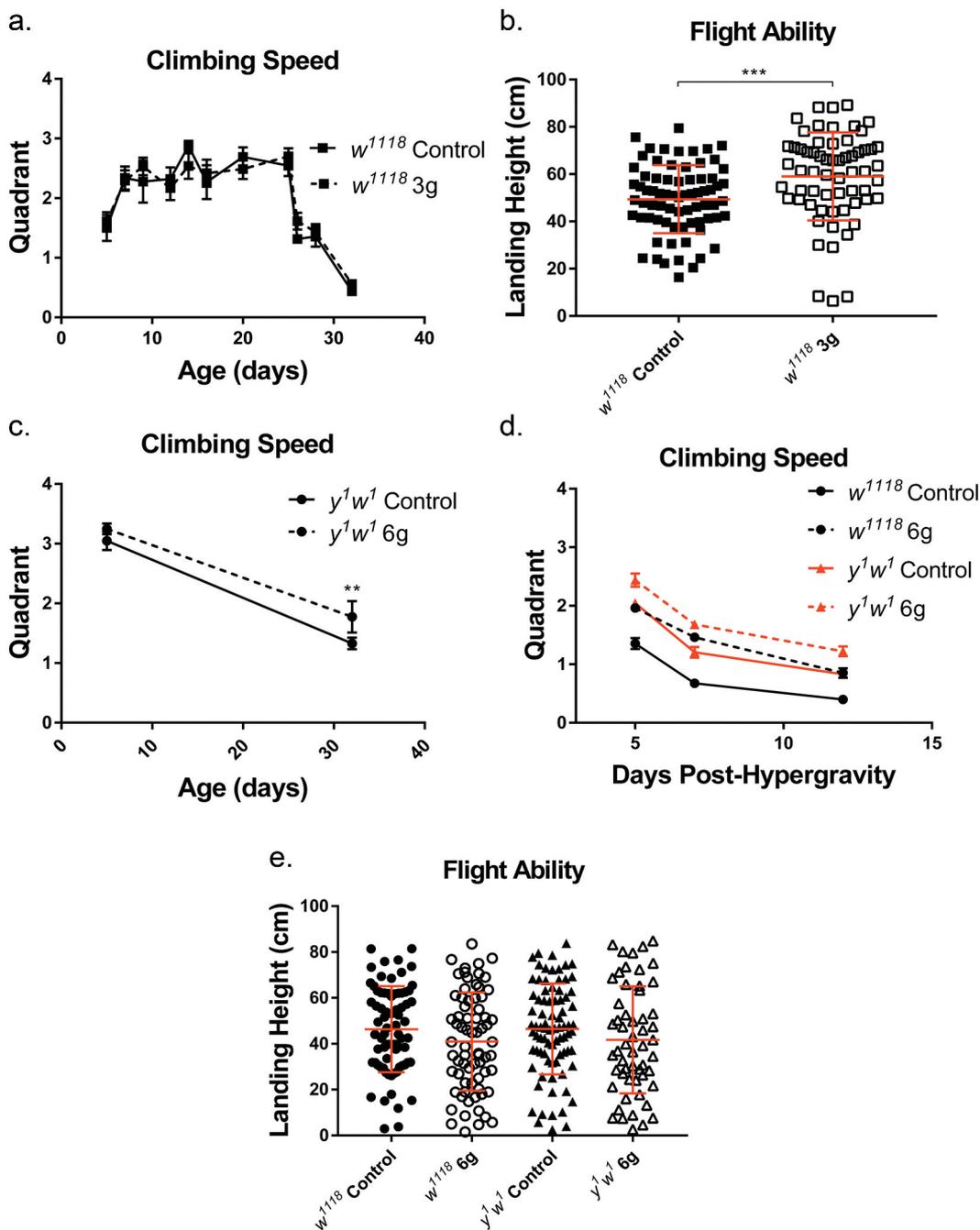


Figure 1. Hypergravity as an approximation of resistance exercise. Flies were subjected to varying degrees of hypergravity (by centrifugation) for different periods of time across three weeks to assess the effects of muscle loading on climbing speed and flight ability. (a,b) w^{1118} flies were exposed to 3 g every other day (Monday, Wednesday, Friday) for three weeks. Hypergravity treatment lasted 2 hours per session for the first week, 2.5 hours for the second week, and 3 hours for the third week. Climbing speed was not affected in this protocol but acute flight ability was significantly better ($p = 0.009$; student's t-test). (c-e) y^1w^1 and w^{1118} flies were subjected to 6 g for 1.5 hours for five days per week for three weeks (Monday-Friday). The climbing speed of hypergravity treated y^1w^1 flies was better than non-treated flies (2-way ANOVA with Tukey post-hoc comparison $**p < 0.01$). Additionally, this protocol improved the climbing speed of treated flies across ages after treatment (2-way ANOVA, $p < 0.0001$), but it did not affect acute flight ability (one-way ANOVA).

maintain mitochondrial health in trained flies [18,21,22,29,33,47,50], though the magnitude of the training response differs between wild-type strains [21,50].

While it is clear that exercise training offers protection against age-related decline in flies, optimal benefits have only been observed when training is started at a young age [21]. There is a clear reduction

in the beneficial effects of exercise when training is initiated in the second week of the fly's life, and even fewer benefits when started in the third week of the fly's life [21]. A possible explanation for this is that when training begins later in the life cycle, the endurance of the aged flies is already too low to allow them to complete the training protocol with the vigour required for adaptations to occur. Whatever the reason, it is apparent that ageing has a strong influence on both exercise performance and the ability to adapt to training.

Longevity and endurance work in part through similar mechanisms [41,51,52]. Flies bred for longevity have better endurance than their parental line [30]. Microarrays revealed that breeding for longevity activated similar genes as those activated by endurance training [41], implying overlapping mechanisms between increased longevity and endurance. To further support this claim, rats that were selectively bred to be high capacity runners lived longer than those bred to be low capacity runners [52]. Selective breeding in flies was mediated, at least in part, by changes to the mitochondrial genome, as the effects were reversed when mitochondria were exchanged between selected flies and progenitors [41]. No correlation between exercise-induced activity and lifespan was reported in multiple *Drosophila melanogaster* Genetic Reference Panel (DGRP) fly strains that were not bred for longevity [27]. Therefore, it appears that selective pressure for either longevity or endurance is required for a correlative response.

In the majority of studies, exercise does not extend maximum lifespan, rather, it extends healthspan [53–56]. Exercise training with the Power Tower [41] and exercise-induced activity with the Treadwheel do not extend maximum lifespan in the majority of lines tested [27,41]. In humans, exercise is used as both a preventive and a treatment for various diseases, and, as such, extends the healthspan of many individuals [46].

Diet influences acute endurance and adaptations to chronic exercise training, with dietary composition having a larger influence than caloric content [50]. After testing 10 diets that varied the amount of yeast, sugar, and calories, it was determined that caloric content was not a good predictor of endurance [50]. A balanced diet (equal parts sugar and yeast) was found to provide flies with the most benefits, including longer endurance,

preserved climbing ability across ages, and protection against cardiac pacing-induced stress [50]. Flies fed a balanced, low-calorie diet did run longer than those fed a balanced, high-calorie diet [50]. The effect that diet has on endurance is acute, with changes to endurance occurring within 48 hours [50]. The effects of diet on acute climbing speed were smaller in magnitude [50]. These results are understandable since activities requiring long-term energy expenditure, such as endurance running, are likely to be more susceptible to dietary changes and energy availability.

Diet alters the effect of chronic exercise training on age-related decline in climbing [21], specifically when protein content is changed. Exercised flies on a diet of 2.5% yeast climbed worse than unexercised flies, while flies on a 20% diet climbed so well that there was no apparent difference between exercised or unexercised flies [21]. However, independent of exercise training, all flies on a 20% yeast diet died by age day 15, indicating a strong negative trade-off [21]. The optimal amount of protein for the best climbing ability was 10% [21], which is why flies trained with the Power Tower are typically fed a 10% (w/v) yeast and sugar diet [17]. Although a large diet matrix examining diet, exercise training, and endurance or exercise-induced activity has not yet been performed, it is reasonable to hypothesize that diet likely influences the impact of training on endurance as well.

Sex has a strong impact on how flies respond to exercise training. When exercised using the TreadWheel, the activity levels of both male and female flies are similar, and correlate well with the amount of exercise being performed within a 2-h window [57]. However, when looking at the distribution of activity levels throughout that same 2-h bout of exercise, there is a strong correlation between sex and activity, with females undergoing early bursts of activity and males maintaining activity levels throughout training [58].

Differences in adaptation to chronic exercise training can also be observed using the Power Tower. Despite being equal in climbing and flight ability prior to any training, female *Drosophila* have reduced baseline endurance as measured by the Power Tower and do not gain benefits to performance following exercise training [33]. The

activity of neurons that produce the monoamine, octopamine, during training completely accounts for the sex difference in training response, and this difference has been used to examine the vital role in exercise adaptation that octopamine plays [33]. Octopamine is required for exercise adaptations to occur in flies [33] and is sufficient to induce exercise adaptations [33]. In fact, activation of octopaminergic neurons or feeding of octopamine to sedentary flies are sufficient to induce exercise adaptations without any exercise [33].

The sexual dimorphism in adaptation to chronic training is due to differences in the activation between male and female octopaminergic neurons during training [33]. Masculinization of female octopaminergic neurons through knock-down of the female-specific transcription factor, *transformer*, allows female flies to adapt to endurance training. Similarly, feminization of male octopaminergic neurons through *transformer* expression results in a loss of the ability to adapt to training in male flies [33].

Training adaptations occur through the activation of octopaminergic receptors, specifically the three β -adrenergic octopamine receptors (*Oct β 1 R*, *Oct β 2 R*, *Oct β 3 R*) and the α -adrenergic octopamine receptor (OAMB) [59]. A fifth octopamine receptor has not yet been tested [60]. Several tissue-specific receptor requirements for endurance training adaptations have been determined, with *Oct β 1 R* being required in all target tissues tested [59]. Though more work is needed to determine the downstream factors that are activated by each receptor in various tissues during exercise training, it is clear that octopamine and the activation of various octopamine receptors plays a vital role in exercise adaptations.

Other exercise devices, such as the Treadwheel, exert effects on both sexes, suggesting some differences in critical mechanisms induced by different training methods. When training takes place in both sexes, differences are revealed between male and female metabolic traits and climbing speed after exercise training. After Treadwheel training, triglycerides and glycogen levels of Oregon R, w^{1118} , y^1w^1 , and $y^1w^{67}c^{23}$ lines were decreased, though the magnitude of response varied between males and females and also by line [16]. Thus, both sex and genotype influence these metabolic traits in response to exercise training.

Spargel, *Sestrin*, *Su(z)2* and *Jarid2* are known genetic modifiers that improve exercise performance.

A large advantage of using the fly system to study exercise is the genetic tractability of the model. Virtually any gene of interest can be manipulated to determine its effect on exercise performance in a timely manner. In fact, in the past decade of research, four genes have been identified that positively influence exercise performance and, in some cases, are full mimetics of exercise. Both the Power Tower and the Treadwheel have added to the exercise field by identifying genes that increase endurance (*spargel*, *Sestrin*, *Su(z)2* and *Jarid2*).

Spargel (PGC-1 α homolog) was the first exercise gene to be studied in flies [29]. PGC-1 α is a conserved gene that increases aerobic capacity in mice [61] and when upregulated, increases mitochondrial biogenesis, transcription of mitochondrial genes, and fatty acid oxidation [62]. In flies, *spargel* is required for normal endurance, climbing and lifespan [29]. Upregulation of *spargel* in the muscle tissue is sufficient to increase endurance in young flies [28,29]. In older flies, muscle-specific overexpression of *spargel* increased endurance and flight ability to a further extent than exercise alone [28]. Additionally, exercised *spargel*-overexpressing flies gained little benefits to their endurance or flight ability. Together, these results support that *spargel* alone can mimic exercise training and is required for training adaptations [28,29].

Similar to *spargel*, *sestrin* overexpression is sufficient to provide all the benefits of endurance training, and overexpressing flies gain no further benefits from exercise training [28]. *Sestrin* acts in part through activation of *Spargel*, as knock-down of *spargel* in muscle prevents *sestrin* overexpression from improving endurance [28]. *Sestrin* is also required for baseline endurance and adaptation to training [28]. *Sestrins* are conserved proteins that are induced by stress and act as important signalling molecules [63]. *Sestrin* has several downstream effects, but in the context of exercise, *Sestrin* works by upregulating AKT (protein kinase B) through the activation of TORC2 (target of rapamycin complex 2), and by activation of AMPK (adenosine monophosphate activated protein kinase) and PGC-1 α [28].

Su(z)2, a member of the Polycomb Repressive complex 1 [64], and *Jarid2*, a Jumonji C domain-containing lysine demethylase associated with Polycomb Repressive Complex 2 [65], were identified as modifiers of activity level through a genome-wide association study [27]. Using the Treadwheel, *Su(z)2* and *Jarid2* were found to significantly increase basal activity as well as exercise induced activity in both males and females when expressed in neurons [27]. *Su(z)2* and *Jarid2* are chromatin modifying proteins, which suggests that these proteins enhance activity by altering the transcription of various genes [64]. *Mef2* is possibly one of those genes impacted by *Su(z)2* and *Jarid2*. However, *Su(z)2* and *Jarid2* had no prior direct link to animal activity and the precise mechanism through which they increase activity is still under study [64].

The discovery of these exercise-modifying genes is a good example of the power that the *Drosophila* exercise model has in making large advancements to the field. With continuing studies, more genetic modifiers of exercise are likely to appear, some of which could be possible therapeutic targets for multiple diseases. As research continues to grow in this area, understanding wild-type variation in exercise ability becomes even more pertinent in order to accurately identify pharmaceutical targets with the most likely chance of helping the general public.

Exercise phenotypes of various wild-type flies

Genetic background clearly influences endurance and training adaptations [16,18,27,50,66]. Therefore, background effects should be considered when analysing exercise experiments and the effectiveness of interventions. In this section, we will analyse the performance of commonly used genetic background strains in various physiological exercise assessments.

Baseline climbing speed and endurance varies among wild-type *Drosophila*

There are differences in the climbing ability of different genetic backgrounds both pre- and post-exercise [16,21,22,30,35]. The RING assessment can measure acute climbing speed across ages [16,35,17]. *w*¹¹¹⁸ flies are often slower climbers relative to other wild-type lines, while Berlin

K and Canton S flies are faster climbers [50]. To quantitate diversity in climbing ability among wild-types, we measured the climbing ability of four commonly used wild-type lines. At age day 5 and age day 25, climbing speed significantly varied between genotypes and, as reported previously, decreased with age (Figure 2a,b). Similar to previous findings, *w*¹¹¹⁸ flies performed the worst, while Berlin K and Canton S flies performed the best (Figure 2a,b) [50].

The rate of age-related decline in climbing speed also varies among wild-type lines (Figure 2c). The rate of age-related decline in climbing speed was measured by assessing the climbing speed of flies longitudinally over the course of 3 weeks [17] normalized to speed on the first day of climbing [17]. Similar to previously published data, we see significant differences in the rate of age-related decline in climbing speed between genotypes (Figure 2c), with *w*¹¹¹⁸ flies' rate dropping to less than half of its initial starting point (Figure 2c; Table 1) [30].

Knowing the general rank order of wild-type climbing speed is important, especially when testing interventions that can increase climbing speed and/or slow the rate of age-related decline. The magnitude of response to interventions tested in lines with poor climbing speed or a fast rate of age-related decline may be more robust since there is further room for improvement in those lines. If an intervention is only tested in lines with good climbing ability or a slow rate of age-related decline, it is possible that no benefits may be detected, leading to a false-negative result.

Endurance is influenced by genotype as well. We measured endurance by letting flies run to fatigue using the Power Tower. A vial of flies is scored as fatigued when 80% of the flies within the vial have stopped climbing ½ cm [17]. We see significant differences in *w*¹¹¹⁸, *y*¹*w*¹, Canton S, and Berlin K lines (Figure 2c,d). The rank order observed in Figure 2 is consistent with previous unpublished observations by the authors.

Canton S flies were previously reported to run significantly longer than Berlin K flies at age day 5 [50]. A possible explanation for this difference is the standard used for fatigue. In that study, a vial was fatigued when flies were climbing less than 2 inches [50]. The standard for fatigue used in the current study is less rigorous, requiring flies to climb at least

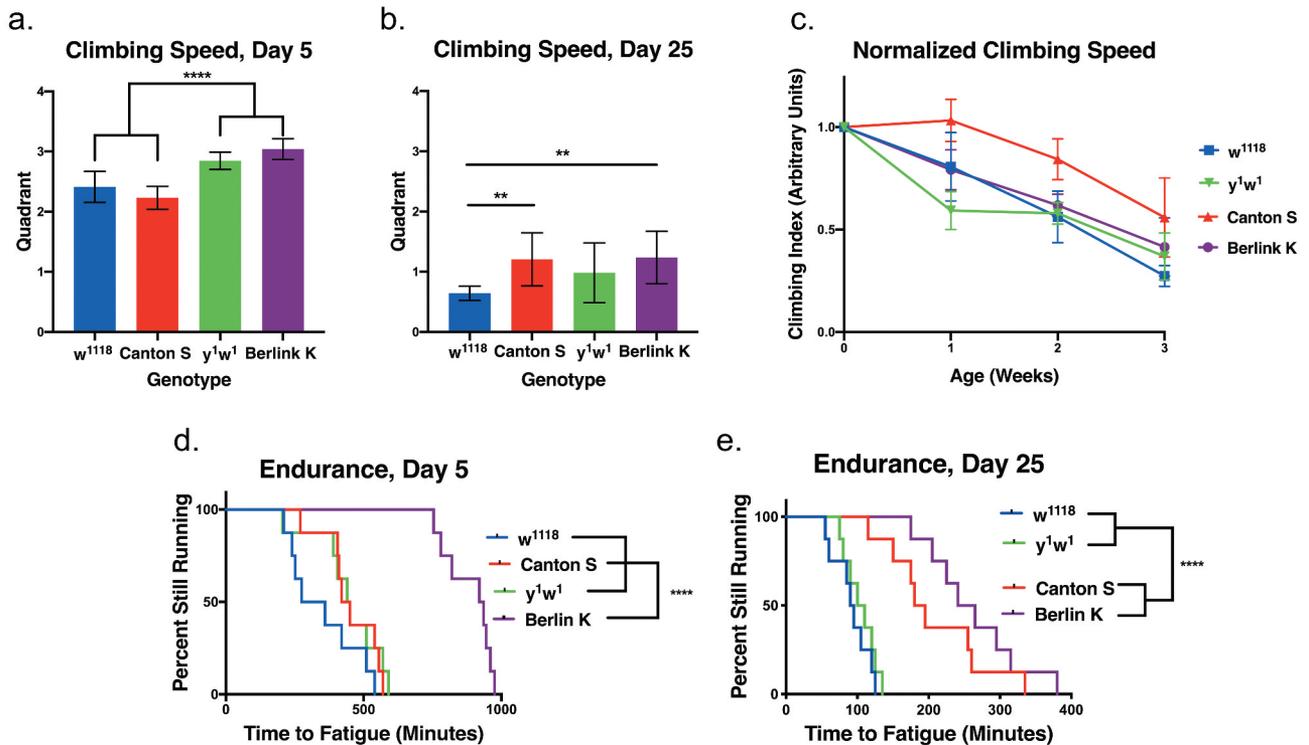


Figure 2. Endurance and climbing speed varies between common wild-types at age day 5 and day 25. (a,b) Climbing speed was measured by dividing a vial into quadrants and measuring the average quadrant height flies climbed to in two seconds (error bars indicate \pm SD, one-way ANOVA with Tukey post-hoc comparison * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). (c) Negative geotaxis climbing speed measured longitudinally across 5 weeks. Data is presented as a percentage of climbing height measured on day 5 by the same cohort. (d,e) Endurance graphs time-to-fatigue of several vials of flies. Each vial was considered fatigue when fewer than 20% of flies continued to respond to a climbing stimulus. Each data point represents one vial, while the curve represents the range of fatigue across the tested vials (log-rank test, $p < 0.0001$).

Table 1. Comparisons of the endurance and the climbing speed of standard genotypes. Arbitrary units (+) are displayed that correspond with performance level.

Age	Genotype	Endurance	Climbing Speed
Day 5			
w^{1118}	+	+	
y^1w^1	+	+++	
Berlin K	+++	+++	
Canton S	+++	++	
Day 25			
w^{1118}	+	+	
y^1w^1	+	++	
Berlin K	+++	+++	
Canton S	+++	++	

$\frac{1}{2}$ cm [17], which allows for a better measurement of maximum endurance [17].

Genetic background affects adaptation to chronic exercise

A survey of various isogenized wild-caught lines showed substantial variation in adaptation to exercise training among non-traditional

genotypes [16,27]. This holds true within commonly used laboratory backgrounds. After exercise training with the Power Tower, wild-type flies adapt with increases to endurance, climbing ability, flight ability, lysosomal activity, and resistance to electrical cardiac pacing [17]. With the Treadwheel, flies adapt with improved climbing ability, changes in metabolic traits, and increases in transcription of genes involved in mitochondrial function [16]. The majority of current studies use standard background control lines that match the genetic background of transgenic lines in the study. However, there are a few studies published that use multiple standard backgrounds in a single experiment [27]. In one study that compared the endurance of w^{1118} , Oregon R, and Ra lines, w^{1118} exercised flies performed similar to Ra exercised flies, with their endpoint endurance being similar [30]. However, Oregon R exercised flies ran longer than both the w^{1118} and the Ra exercised lines [30].

To further investigate variation in commonly used wild-type lines during exercise training, we examined endurance and climbing ability of w^{1118} and y^1w^1 after training (Figure 3). These lines were chosen because they are often used as backgrounds for transgenic fly lines. Exercised flies had increased endurance relative to age-matched siblings (Figure 3a), but no significant difference was found between the exercised flies of the different backgrounds (Figure 3a) [17]. At the end of week two and three, the climbing speed of y^1w^1 exercised flies was significantly faster than w^{1118} exercised flies (Figure 3b). Analysing differences between fully sequenced background genomes has already contributed to our knowledge of exercise mechanisms [27], and this is likely to continue to be a growing area in *Drosophila* exercise research.

Discussion

Exercise training is a low-cost intervention that can prevent the onset of numerous diseases, and maintain cardiovascular and muscle-skeletal health [46,66].

The baseline exercise ability and the magnitude of benefits gained from training varies on an individual basis [67]. A deeper understanding of the genetic mechanisms that contribute to differences in systemic response to exercise have great potential to point the way to therapies to provide benefits of exercise to patients with enforced sedentary lifestyles.

Over the past 10 years, *Drosophila* exercise models have been used to identify factors involved in promoting beneficial adaptations to exercise

[27–29]. The influence that ageing, diet, and sex have on performance and adaptation has been studied as well [16,33,41,50]. However, using *Drosophila* as an exercise model has some limitations, including having no established method for resistance exercise training and potential differences in endurance exercise adaptations between mammals and flies. Similar to mammals, flies contain both oxidative and glycolytic muscles [69], as characterized by the myosin heavy chain (MHC) isoform [69]. Oxidative muscles contain predominantly MHC type I and are often referred to as slow-twitch muscles, while glycolytic muscles contain mostly MHC type IIx and IIa, and are often referred to as fast-twitch muscles [69]. The flight muscles of flies, which are used for relatively long periods of time, are mostly oxidative muscles, while their leg muscles, which are used more periodically, are mostly glycolytic [68]. Skeletal muscle in mammals responds to chronic endurance training by switching fast-twitch muscle fibres to slow-twitch fibres [70], but fibre-type switching has yet to be examined in the fly.

Despite these limitations, the *Drosophila* system clearly executes exercise adaptations using pathways that are conserved with mammals, and has identified novel factors, such as Sestrin, that have been confirmed to be important in mammals.

Influence of genetic background

The collective data gathered using *Drosophila* exercise models clearly support an influence of

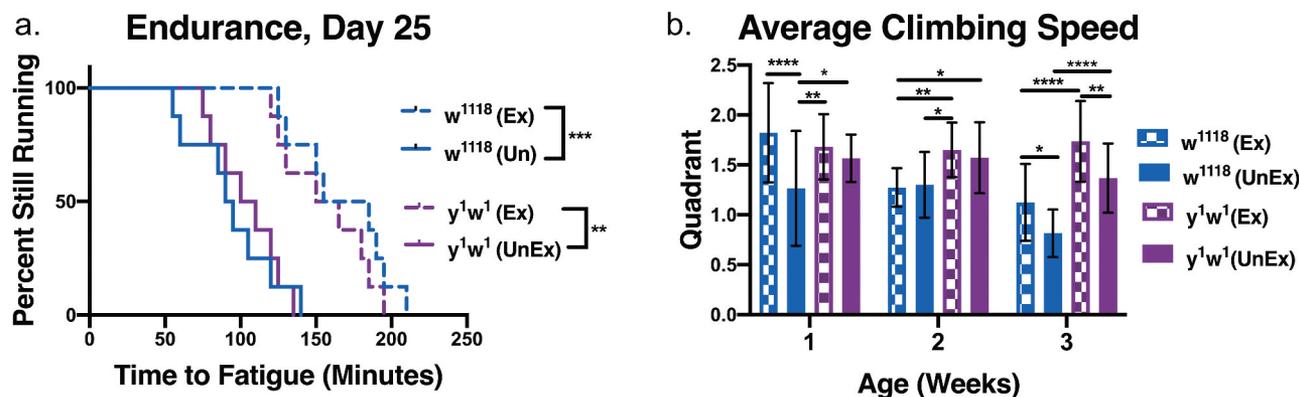


Figure 3. Endurance and climbing speed improve with exercise training in two common wild-type lines. (a) Endurance of 25 day old flies with or without exercise training (log-Rank test, ** $p < 0.01$, *** $p < 0.001$). (b) Average weekly climbing speed of trained y^1w^1 and w^{1118} flies (2-way ANOVA, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

genetic background on both endurance and climbing ability [16,30,50], but the sources of genotypic variation are multi-factorial and not completely understood. A possible source of variation in the magnitude of response to endurance training is the availability of octopamine to bind octopamine receptors on target tissues. Unlike male flies, young wild-type female flies have decreased baseline endurance and lack adaptive responses to chronic endurance training [33]. This difference is due to a lack of activation of octopaminergic neurons in female flies [33]. Furthermore, supplementing bioavailable octopamine through feeding is sufficient to provide females with the ability to adapt to training [33]. Since female flies have the same amount of octopamine in their heads as male flies regardless of exercise, the uptake of circulating octopamine is likely more directly involved in determining the degree of response to training than the concentration of octopamine in the body [34]. Taken together, these data support the hypothesis that in response to increased octopaminergic activation induced by exercise, there is an increase in bioavailable octopamine and therefore an increase in uptake by octopamine receptors for use in target systems, likely determining the adaptive response to training.

The amount of *sestrin* expressed in each wild-type line may be a key factor in exercise performance. *Sestrin* is required for normal endurance and overexpression of *sestrin* can mimic the benefits of exercise training [28]. Thus, it is feasible that the differences in both baseline exercise ability and adaptation observed in different wild-type lines is, at least in part, caused by differences in innate *sestrin* expression or activity of the Sestrin protein.

In addition to the phenotypes previously mentioned, cardiac remodelling is also different among wild-type lines. Briefly, cardiac performance declines with age in *Drosophila*, with age-related increases in arrhythmias, reduced resistance to stress, and cardiac remodelling [39,71,72]. Aged w^{1118} has more contractile dysfunction than yw or white-Canton S flies, due to decreased cortical stiffening [72]. Investigation into this genotypic variation identified the transcription of *Vinculin*, a cytoskeleton protein, to be increased in yw and white-Canton S, but not changed in w^{1118} flies [72]. Additionally, it was determined that vinculin

acts as a compensatory mechanism to maintain cortical stiffness, aiding in the preservation of cardiac function during ageing [72]. Determining the cause of genotype-dependent heart remodelling led to vinculin as a possible therapeutic target for reducing age-related heart failure.

This serves as a great example of what can be accomplished when investigating the mechanisms responsible for genotypic variation. With deeper understanding of the mechanism(s) responsible for genotypic variation in exercise ability, the underlying genetic pathways responsible for this highly conserved response across the animal kingdom will be further revealed, perhaps leading to generation of novel therapeutics with broad impact on human healthspan.

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Conflict statement

The authors declare no conflict of interest.

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