Journal of Insect Physiology 99 (2017) 8–14

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Starvation but not locomotion enhances heart robustness in *Drosophila*

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ARTICLE INFO

Keywords:
Drosophila
Physiological relationships
Heart robustness
Stress resistance
Locomotion

ABSTRACT

Insects and vertebrates have multiple major physiological systems, each species having a circulatory system, a metabolic system, and a respiratory system that enable locomotion and survival in stressful environments, among other functions. Broadening our understanding of the physiology of *Drosophila melanogaster* requires the parsing of interrelationships among such major component physiological systems. By combining electrical pacing and flight exhaustion assays with manipulative conditioning, we have started to unpack the interrelationships between cardiac function, locomotor performance, and other functional characters such as starvation and desiccation resistance. Manipulative sequences incorporating these four physiological characters were applied to five *D. melanogaster* lab populations that share a common origin from the wild and a common history of experimental evolution. While exposure to starvation or desiccation significantly reduced flight duration, exhaustion due to flight only affected subsequent desiccation resistance. A strong association was found between flight duration and desiccation resistance, providing additional support for the hypothesis that these traits depend on glycogen and water content. However, there was negligible impact on rate of cardiac arrests from exhaustion by flight or exposure to desiccant. Brief periods of starvation significantly lowered the rate of cardiac arrest. These results provide suggestive support for the adverse impact of lipids on *Drosophila* heart robustness, a parallel result to those of many comparable studies in human cardiology. Overall, this study underscores clear distinctions among the connections between specific physiological responses to stress and specific types of physiological performance.

1. Introduction

Organisms encounter and endure stress on a daily basis, with most stressors having an adverse impact. However, in animal experiments and patient clinical evaluations, such as cardiac stress tests, stress levels may be increased in order to reveal the physiological foundations of physical health and fitness. Stress resistance, for example, is often an important indicator of components of fitness in lab evolutionary studies that use *Drosophila* (Djawdan et al., 1998; Rose et al., 1992). Stress resistance and energy allocation have been strongly associated with life history and other physiological traits in many *Drosophila* studies of this kind over the years (e.g. Djawdan et al., 1998; Graves and Rose, 1990; Graves et al., 1988; Service et al., 1985; Service et al., 1988). Marked differences in the physiological machinery underlying such *Drosophila* characters have been revealed. For example, adult starvation resistance in *Drosophila* depends largely on total stored calories (e.g. Djawdan et al., 1998), while desiccation resistance depends predominantly on water content and rate of water loss (e.g. Gibbs et al., 1997). When Archer et al. (2007) applied sustained strong selection for desiccation resistance in *Drosophila*, water content and water loss rates were shown to evolve separately from each other.

Understanding responses to stress and stress resistance has been a major theme of invertebrate studies. As previously stated, applying stress can also help define the mechanisms and relationships among different physiological systems. The effects of thermal stress and hypoxia on respiratory and cardiac function have been studied in crustaceans, such as dungeness crab, spiny lobster, or crayfish (Airriess and McMahon, 1994; De Wachter and Wilkens 1996; Fitzgibbon et al., 2015; McMahon et al., 1974; McMahon, 2001a,b). Such stressors have also been studied for their effects on metabolite levels in Madagascar cockroaches and in blow flies (Chowanski et al., 2015; Muntzer et al., 2015). For adult mosquitoes, altering nutrition can have effects on heart contractility, longevity, flight performance, and fecundity (Ellison et al., 2015; Gary and Foster, 2001; Kaufmann et al., 2013).

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http://dx.doi.org/10.1016/j.jinsphys.2017.03.004
Received 25 January 2017; Received in revised form 27 February 2017; Accepted 6 March 2017
Available online 08 March 2017

Over the past 100 years, inducing flight has revealed clear relationships between insect locomotor function and metabolic reserves. Dipteran and Hymenopteran species use glycogen, not lipid, as the primary fuel for flight (Clements, 1955; Suarez et al., 2005; Vogt et al. 2000; Wigglesworth 1949). Others studies have shown that lipid is the primary flight fuel in Lepidoptera, Hemiptera, and Orthoptera (Beenakkers et al., 1984; Ziegler and Schultz, 1986; Zera et al., 1999; Canavoso et al., 2003; Arrese and Soulages, 2010; Amat et al., 2012).

Drosophila cardiac function and its relationship to other physiological processes has been of great scientific interest recently. There are important differences between *Drosophila* and mammalian cardiovascular structure and function. *Drosophila* have an open circulatory system and a reversible direction of hemolymph flow. There is also no relationship between the circulatory system and respiratory system in *Drosophila*. However, there are similarities in early heart development, age-dependent decline in heart function, and the genes associated with heart development, function, and diseases (Bodmer and Venkatesh, 1998; Cripps and Olson, 2002; Zaffran and Frasch, 2002; Bier and Bodmer, 2004; Ocorr et al., 2007; Birse and Bodmer, 2011; Nishimura et al., 2011; Diop and Bodmer, 2012). In 2004, Bier and Bodmer examined age-dependent decline in cardiac function in *Drosophila* by showing that adult responses to external electrical pacing decline with age. The fruit fly has become a powerful tool for understanding cardiomyopathies, metabolic homeostasis, and other obesity-related disorders (Birse and Bodmer, 2011; Diop and Bodmer, 2015; Smith et al., 2014; Trinh and Boulianne, 2013). *Drosophila* populations exposed to either a high-fat diet or a high-sugar diet have led to flies with cardiac and metabolic dysfunction (i.e. hyperglycemia, insulin resistance, lipid accumulation, reduced cardiac contractility, and cardiac arrhythmias: Birse et al., 2010; Hoffmann et al., 2013; Na et al., 2013; Trinh and Boulianne, 2013).

Here we explore the robustness of physiological interrelationships between flight performance, stress resistance, and cardiac function using experimental manipulation. We conducted electrical pacing assays with manipulative conditioning to determine how cardiac arrest frequency is impacted by flight exhaustion and exposure to desiccation or starvation stresses. Our findings underscore previous findings in some respects, while pointing to the value of combining direct experimental manipulation with other experimental strategies, such as mutation and experimental evolution, in the study of insect physiology.

2. Methods and materials

2.1. Experimental overview

The experimental populations, B_{1-5}, were derived from a single ancestor population, the IV population (Rose, 1984; Rose et al., 2004). The IV population originated in 1975 as a sample of *D. melanogaster* caught in Amherst, Massachusetts. After four and a half years of laboratory culture, the B_{1-5} populations were derived from the single IV population in 1980. The IV and five B populations share a discrete, non-overlapping, two-week generation cycle. These large, outbred populations are maintained on a banana-molasses medium with a 24L:0D light cycle. Seven different manipulative sequences were applied to these B_{1-5} populations: (i) flight then starvation, (ii) flight then desiccation, (iii) starvation then flight, (iv) desiccation then flight, (v) starvation then pacing, (vi) desiccation then pacing, and (vii) flight then pacing.

2.2. Assay methods

2.2.1. Rearing protocols

Two run-in generations of 14-day life-cycles were used to remove any parental or grand-parental epigenetic effects. The populations were cultured in banana-molasses medium from egg to adult, on a 24L:0D light schedule. Eggs were collected at a density of 60–80 eggs per vial after adults were allowed 24 h to lay eggs. At the end of each run-in generation (day 14 from egg), the populations were transferred to an acrylic cage. Replicate populations of the same number were handled in parallel at all stages. On day 14 of the second run-in generation, the adults were assigned at random to one of the four physiological assays.

2.2.2. Desiccation resistance assay

Individual female flies from each population were placed in their own desiccant straw. A piece of cheesecloth separated the fly from the pipet tip at the end of the straw that contained 0.75 grams of desiccant (anhydrous calcium sulfate). The pipette tip containing desiccant was sealed with a layer of Parafilm. Mortality was checked hourly, using lack of movement under provocation as a sign of death. Note that this was a materially different procedure than the one we have employed previously in our studies of desiccation resistance (e.g. Service et al., 1985; Graves et al., 1992; Djawdan et al., 1998), which used vials.

2.2.3. Starvation resistance assay

Individual female flies from each population were placed in their own starvation straw with agar. The agar plug provides adequate humidity, but no nutrients. Mortality was checked every four hours, using lack of movement under provocation as a sign of death.

2.2.4. Flight exhaustion assay

Female flies from each replicate per stock were first selected at random. The flies were briefly anesthetized using cold-shock by partially submerging a plastic vial with female flies into ice for one to two minutes, and then tethered singly to a monofilament string using Duco cement glue applied to the mesonotum region of the thorax. The flight response was stimulated by gently tapping the tethered string, with total flight duration being recorded. Flight was terminated if the fly could no longer be made to resume flight by tapping within a continuous three-minute interval, or at least seven brief flights were attempted but not sustained consecutively during that period. The protocol for this assay is described further in Graves et al. (1988).

2.2.5. Cardiac pacing assay

Female flies from each replicate per stock were first chosen at random. The flies were anesthetized for three minutes using triethylamine, also known as FlyNap, and then placed on a microscope slide prepared with foil and two electrodes. FlyNap was chosen as the anesthetic because of its minimal effect on heart function and heart physiology when administered for more than one minute (Chen and Hillyer, 2013). The cold-shock method was not used as an anesthetic for the cardiac pacing assay, because the flies need to be fully anesthetized throughout the procedure. If the flies regain consciousness, the added stress and abdominal contractions while trying to escape would alter heart rate and function more than FlyNap does. Paternostro et al. (2001) found that FlyNap has the least cardiac disruption compared to the two other substances commonly used for *Drosophila* anesthesia, carbon dioxide and ether. Two electrodes were attached to a square-wave stimulator in order to produce electric pacing of heart contraction. Anesthetized flies were attached to the slide between the foil gaps using a conductive electrode jelly touching the two ends of the fly body, specifically the head and the posterior abdomen tip. The shocking settings for this assay were 40 volts, six Hertz, and 10 ms pulse duration. Each shock lasted for 30 s. An initial check of the status of the heart was made after completion of the shock, followed by a check after a two-minute “recovery” period. Heart status was scored as either contracting or in cardiac arrest. The protocol for this assay is outlined in Wessells and Bodmer (2004).

2.3. Manipulative sequences

2.3.1. Flight then starvation

Thirty female flies from each of the five experimental populations underwent the flight exhaustion procedure. An additional 30 flies were tethered, but prevented from flying. If one of these non-flown flies began flying, simply touching the bottom of the legs ended the flight response. These flies were paired with the flown flies. When a flown fly reached exhaustion, the flown fly and the paired non-flown fly were removed from their tethering strings and placed into a straw with agar. The agar plug provides adequate humidity, but no nutrients. To calculate starvation resistance, flies were checked every four hours and time of death was noted if no movement was observed.

2.3.2. Flight then desiccation

Thirty female flies from each population underwent the flight exhaustion procedure. An additional 30 flies were tethered, but prevented from flying. If one of these designated non-flown flies began flying, simply touching the bottom of the legs ended the flight response. These flies were paired with the flown flies. When a flown fly reached exhaustion, the flown fly and the paired non-flown fly were removed from the string and placed into a straw with 0.75 grams of desiccant. The desiccant removes moisture, and this environment does not contain any nutrients or water. To record desiccation resistance, flies were checked every four hours and time of death was noted if no movement was observed.

2.3.3. Starvation then flight

Two hundred female flies from each population were placed into straws with agar. An additional 200 flies were placed into straws with banana-medium. Flies were starved until a 25% mortality threshold was reached (at ~20 h), and then 30 flies were chosen at random to undergo flight exhaustion. Thirty normally-fed flies were also flown to exhaustion.

2.3.4. Desiccation then flight

Two hundred female flies from each population were placed into straws with desiccant. An additional 200 flies from each population were placed into straws with banana-medium. Flies were desiccated for 6 h, which produced approximately 25% mortality, and then 30 flies were chosen at random to undergo flight exhaustion. Thirty normally-fed flies were also flown to exhaustion.

2.3.5. Starvation then cardiac pacing

Two hundred female flies from each population were placed into straws with agar. An additional 200 female flies from each population were placed into straws with banana-medium. Flies were starved until a 25% mortality threshold was reached (~20 h), and then 39–40 flies per population were chosen at random to undergo cardiac pacing. Forty-41 fed flies per population were also electrically paced.

2.3.6. Desiccation then cardiac pacing

Two hundred female flies from each population were placed into straws with desiccant. An additional 200 female flies from each population were placed into straws with banana-medium. Flies were desiccated for 6 h, which produced approximately 25% mortality, and then 40–45 flies per population were chosen at random to undergo cardiac pacing. Forty-41 fed flies per population were also electrically paced.

2.3.7. Flight then cardiac pacing

Fifty female flies from each population underwent the flight exhaustion procedure. An additional 50 flies were tethered, but prevented from flying. These flies were paired with the flown flies. When a flown fly reached exhaustion, the flown fly and the paired non-flown fly were removed from the string and placed into their own respective straw. Within five minutes of reaching exhaustion, both the flown fly and its paired non-flown fly were anesthetized using FlyNap and then electrically paced. Thirty-four to 44 flies per population were electrically paced.

2.4. Statistical methods

Linear mixed-effects (LME) models were used to analyze the effect of starvation on flight endurance, the effect of desiccation on flight endurance, the effect of flight exhaustion on starvation resistance, and the effect of flight exhaustion on desiccation resistance. The model for the effect on starvation on flight duration is described here. The models for the remaining three sequences follow the same format. Let *yijk* be the flight time for treatment – i (i = 1 (control), 2 (starved)), population – j (j = 1,...,5) and individual – k (k = 1,...,n_j). We predict flight time with the linear mixed effect model

$$yijk = \alpha + \delta_i\beta + b_j + \varepsilon_{ijk}$$

where δ_i = 1, if i = 2, otherwise, and b_j and ε_{ijk} are population and individual variation that are assumed to be normally distributed with a zero mean and variance σ_b^2,σ_e^2.

Cochran-Mantel-Haenszel (CMH) tests were used to analyze the rates of cardiac arrests between the "stressed" experimental cohort and the control cohort of flies. The CMH test is used when there are repeated tests of independence, or multiple 2 × 2 tables of independence. This is the equation for the CMH test statistic, with the continuity correction included, that we used for our statistical analyses:

$$X^2_{\mathrm{MH}} = \frac{\left\{\left|\Sigma\left[a_i - \frac{(a_i+b_i)(a_i+c_i)}{n_i}\right]\right| - 0.5\right\}^2}{\Sigma(a_i+b_i)(a_i+c_i)(b_i+d_i)(c_i+d_i)/(n_i^3+n_i^2)}$$

We designated "a" and "b" as the number of cardiac arrests in the stressed and control cohorts of population *i*. We designated "c" and "d" as the number of contracting hearts in the stressed and control cohorts of population *i*. The n_i represents the sum of a_i, b_i, c_i, and d_i. The subscript i (i = 1,...,5), representing one of the five replicate populations within the B stock.

3. Results

3.1. Effect of stress on flight duration

Experiencing either desiccation or starvation prior to flight negatively impacted the flies' flight endurance (see Figs. 1 and 2). Flies desiccated for a period of six hours flew on average 16.48 min, whereas the fed-control flies flew on average 59.69 min. The average difference

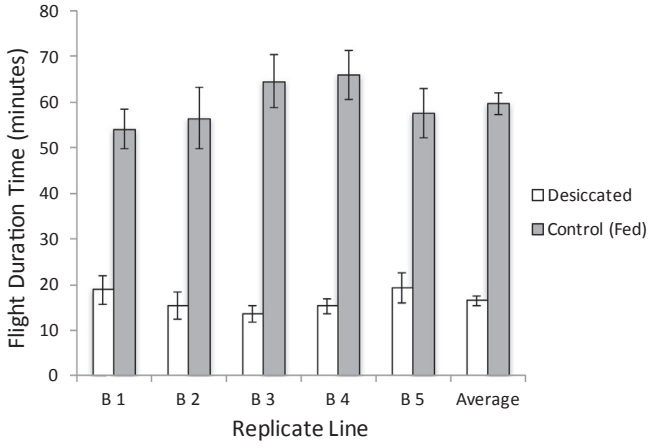

Fig. 1. The average flight duration of flies desiccated for a brief period of time compared to the flies fed the standard banana-medium (mean ± 1 SEM). Desiccated flies had an average flight duration significantly lower than the control fed flies (p-value < 0.005).

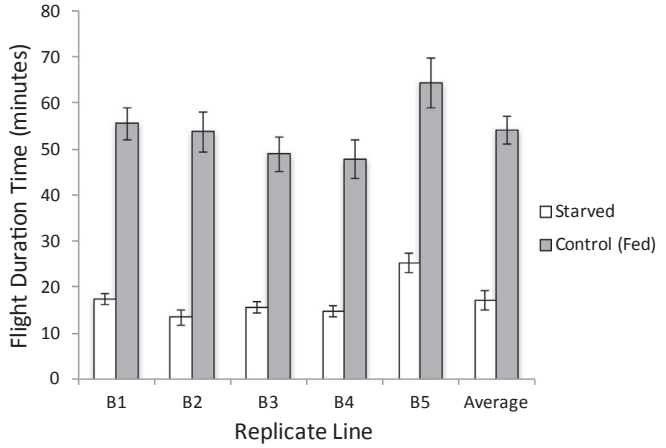

Fig. 2. The average flight duration of starved fruit flies compared to the average flight duration of fruit flies fed the standard banana-medium (mean ± 1 SEM). Starved flies had an average flight duration significantly lower than the control flies (p-value < 0.005).

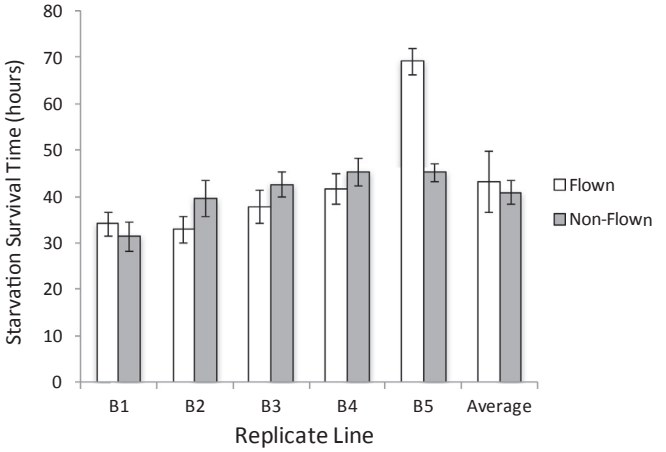

Fig. 4. The average survival time of fruit flies in a starvation environment after being flown to exhaustion (mean ± 1 SEM). The non-flown cohort was tethered, but prevented from flying. Exhausted flies had an average starvation survival time that was not significantly different than the non-exhausted flies (p-value < 0.2928).

of 43.21 min was significantly different when evaluated using the linear mixed effects model (p-value < 0.005). Flies starved for a period of 20 h flew on average 17.23 min, whereas the fed control flies flew on average 54.05 min. The average difference of 36.82 min was also significantly different when evaluated using the linear mixed effects model (p-value < 0.005). The significant impacts of both starvation and desiccation on flight duration support the notion that surviving these physiological stresses depends on the metabolites that also underlie flight.

3.2. Effect of flight on stress resistance

Prior flight to exhaustion significantly reduces the survival time of flies in a desiccating environment (Fig. 3). However, flight to exhaustion has a negligible effect on a fly starvation resistance (Fig. 4). The desiccation survival time of flown cohort of flies was on average one hour less than that of the non-flown tethered cohort of flies. This one-hour difference in survival time was statistically significant when tested using the linear mixed effects model listed above (p-value < 0.01). When starved, flown flies had a slightly longer average survival time. Flown flies survived starvation an average of 2 h and 18 min longer than non-flown flies However, this slight increase in survival time was not statistically significant when tested using the linear mixed effects model given above (p-value = 0.2928). Despite both starvation and desiccation prior to flight resulting in a lower average flight duration, flight prior to stress resistance only affected desiccation resistance to a degree that reached statistical significance.

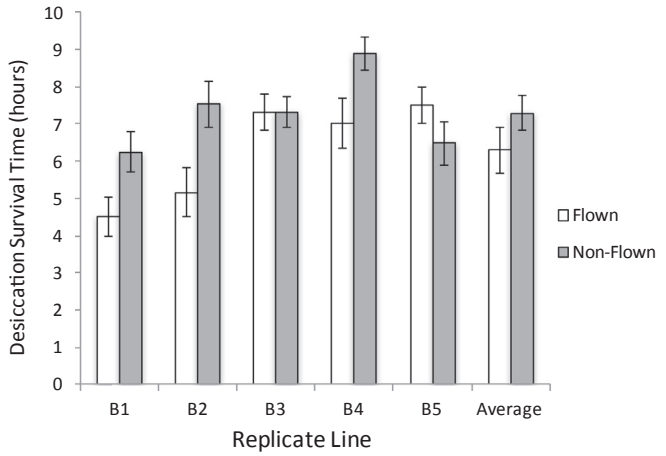

Fig. 3. The average survival time of fruit flies in a desiccated environment after being flown to exhaustion (mean ± 1 SEM). The non-flown cohort was tethered, but prevented from flying. Exhausted flies had an average desiccation survival time significantly lower than the non-exhausted flies (p-value < 0.0055).

3.3. Effect of stress on heart function

When observing the effect of stressors on heart robustness, only one of the three stressors had a statistically significant impact. Flying to exhaustion, or experiencing a brief period of desiccation, does not significantly alter the average cardiac arrest rates of our fruit flies after being electrically paced (Figs. 5 and 6, respectively). The average cardiac arrest rate among the five replicates of flown flies was 27.38%, whereas the average cardiac arrest rate among the five replicates of tethered but not flown flies was 26.26%. The slight difference in cardiac arrests was not statistically significant when evaluated using a Cochran-Mantel-Haenszel test (p-value = 0.933). The average cardiac arrest rate among the five desiccated replicates was 35.44%, whereas the average cardiac arrest rate among the five control replicates was 35.63%. The small increase of cardiac arrest rate in the control flies was not statistically significant when evaluated using a Cochran-Mantel-Haenszel test (p-value = 0.951).

Unlike the neutral effects of flight or desiccation on cardiac arrest rates, there was a decrease in the average cardiac arrest rate in flies

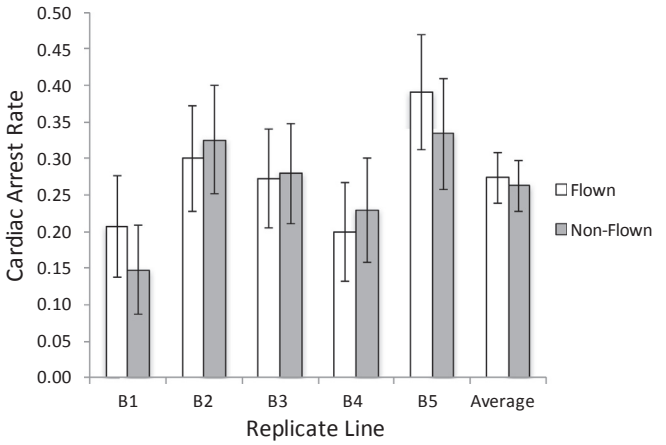

Fig. 5. The average rate of cardiac arrest of fruit flies after being flown to exhaustion, compared to flies that were tethered, but prevented from flying (mean ± 1 SEM). Exhausted flies had an average rate of cardiac arrests that was not significantly different than the non-exhausted flies (p-value = 0.933).

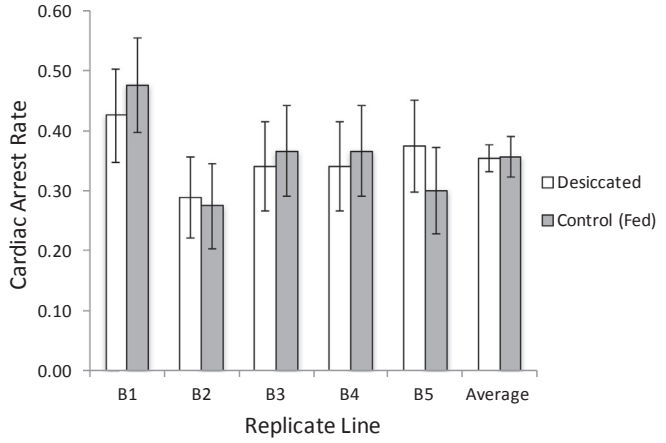

Fig. 6. The average rate of cardiac arrests in desiccated fruit flies compared to flies that were fed the standard banana-medium (mean ± 1 SEM). Desiccated flies had an average rate of cardiac arrests that was not significantly different than the control fed flies (p-value = 0.951).

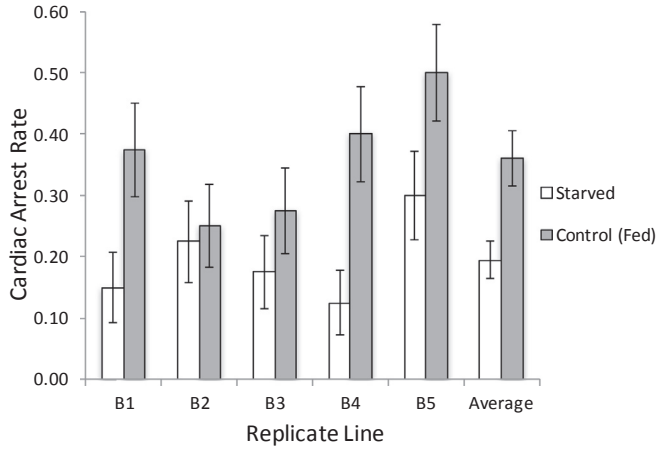

Fig. 7. The average rate of cardiac arrests in starved fruit flies compared to flies that were fed the standard banana-medium (mean ± 1 SEM). Starved flies had an average rate of cardiac arrests that was significantly lower than the control fed flies (p-value < 0.001).

exposed to starvation prior to electrical pacing (Fig. 7). The average cardiac arrest rate among the five replicates of starved flies was 19.5%, whereas the average cardiac arrest rate among the five replicates of control fed flies was 36%. The difference in cardiac arrests rates between the five starved and five control groups was statistically significant when evaluated using the Cochran-Mantel-Haenzsel test (p-value < 0.001). Starving a cohort of flies for a period of 20 h appears to lower the average cardiac arrest rate. The same cannot be said for the six-hour exposure to a desiccated environment, or being flown to exhaustion. Thus we find an association only between rates of cardiac arrest and prior starvation.

4. Discussion

4.1. Physiological interrelationships of stress resistance and flight performance

Exposure to starvation or desiccation significantly reduced flight duration. The results from these two manipulative sequences strengthened the hypothesis that stress of any kind for a prolonged period of time will weaken the body and thereby undermine at least some functions. These results supported what we initially expected, a diminished flight duration after imposition of either of these two environmental stressors. However, the only definitive conclusion we can make is that these two stressors affect flight duration. These two manipulative procedures only indicated one direction of causation, and accordingly we sought to characterize the converse pattern(s) of causation by testing for the effect of flight prior to stress resistance.

The experiments testing the effect of flight on stress resistance found that flight exhaustion significantly affected survival time under desiccation, but did not affect survival time under starvation. The lack of an adverse effect of flight on starvation resistance supports the hypothesis that *Drosophila* flight performance is not dependent on lipid content, a long-established conclusion about flight in dipteran species (Clements 1955; Wigglesworth, 1949; Williams et al., 1943). Our manipulative results indirectly corroborate what previous evolutionary physiology research has shown, specifically that (1) glycogen and trehalose are the major sources of energy for insect flight (Graves et al., 1992), and (2) there is a significant relationship between desiccation and glycogen, trehalose, and bulk water content (Archer et al., 2007; Gibbs et al., 1997). Trehalose and glycogen, which serve as the primary fuel reserves for flight, are more readily used by fly flight musculature than lipids (Graves et al., 1992; Nation, 2008). Despite the impact of starvation on flight endurance, our finding of minimal impact of flight on starvation resistance supports the common conclusion that there is little relationship between flight performance and lipid content in Diptera, as the most important determinant of starvation resistance in *Drosophila* is lipid content (vid. Djawdan et al., 1998). That is, these results are readily incorporated into previous accounts of the distinct roles of glycogen and lipid as substrates for specific physiological functions.

4.2. The response of cardiac robustness to Various stressors

We found that only one of three prior stressors significantly altered cardiac arrest rates, and in a direction we did not initially expect. Flight exhaustion or exposure to desiccation did not alter the rate of cardiac arrests in fruit flies. But starving a population of fruit flies for a period of 20 h significantly lowered the rate of cardiac arrests due to electrical pacing. The negligible effects of flight exhaustion and desiccant exposure prior to electrical pacing suggests that glycogen content and water loss do not affect the rate of cardiac arrest. That fruit flies subjected to starvation stress can have a *reduced* rate of cardiac arrests when electrically paced (Fig. 7) suggests that there is an important adverse effect of body fat on heart function in fruit flies, a parallel result to those of many comparable studies in human cardiology (Crewe et al., 2013; Heinrichsen and Haddad, 2012; Manrique et al., 2013).

4.3. A divide among the physiological interrelationships

From the effects of these seven manipulative sequences, we can infer an overall view of interrelationships among several major physiological systems in *D. melanogaster* (see Fig. 8). A significant impact was seen in four of the seven manipulative sequences. In certain manipulative sequences, one of the replicated populations may display a neutral response to the stressor, or a response opposite to the overall observed trend. Even though the observed overall trend may not be universal among all five replicated populations in each manipulative sequence, we can still find an overall trend moving in one direction or another when using all five replicated populations. As proposed in our earlier work (e.g. Graves et al., 1992), we continue to find a clear divide not only between certain physiological processes, but also between the metabolic reserves used in physiological responses to particular stressors and specific types of physiological performance. On one side, there is evidence of strong relationships among desiccation resistance, flight exhaustion, and carbohydrate content, once again corroborating the findings of past studies (e.g. Archer et al., 2007; Gibbs et al., 1997; Graves et al., 1992; Wigglesworth, 1949). And on the other side, we see a strong relationship among starvation resistance, cardiac robustness, and lipid content (Fig. 8). These results suggest that lipid content, not carbohydrate content, is a key determinant of cardiac robustness and a fly's ability to resist cardiac arrest. However, our interpretations of the

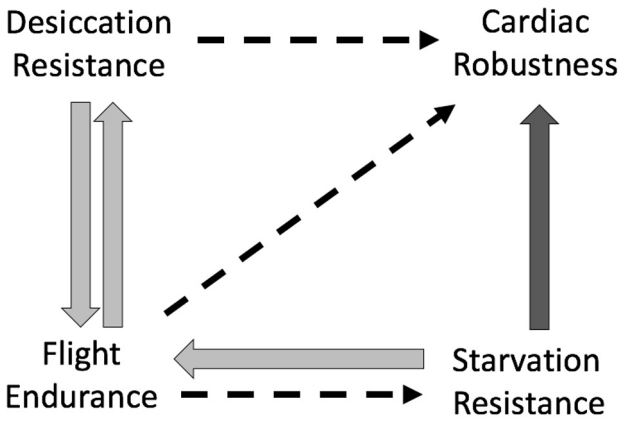

Fig. 8. The physiological interrelationships between *Drosophila* flight performance, stress resistance, and cardiac robustness. This is a visual summary of the seven manipulative sequences applied to the $B_{1\text{-}5}$ populations. A black, dotted arrow indicates a neutral effect of a pre-lethal stress on a physiological process (i.e. exposure to desiccation on cardiac arrest rates). A light grey, solid arrow indicates a negative effect of a pre-lethal stress on a physiological process (i.e. flying to exhaustion on desiccation survival time). A dark grey, solid arrow indicates a positive effect of a pre-lethal stress on a physiological process (i.e. exposure to starvation on cardiac arrest rates).

biochemical underpinnings of the effects we have found cannot be considered definitive. Instead, our results are no more than suggestive of the biochemical hypotheses we have offered.

With a high metabolic rate being typical in *Drosophila*, we hypothesize that flies undergoing starvation consume a significant amount of free-floating lipids in their hemolymph. The improved cardiac robustness after a fasting period is similar to the results found by Hardy et al. (2015). Fasting their starvation-selected, laboratory-evolved populations for seven days rescued heart function (Hardy et al., 2015). One possible mechanism behind the disruptive effect of fat on heart function is the mechanical effect of enlarged fat body structures applying pressure on the heart anatomically (Hardy et al., 2015). Another potential effect of a moderate period of starvation could be reduced hemolymph viscosity, which may then make fly heart contraction less demanding physiologically during cardiac pacing. To determine whether the hemolymph is in fact less viscous after starving for 20 h, future experiments should examine the lipid content in the hemolymph and fat bodies both before and after experiencing periods of starvation, among other measures of heart mechanics with and without starvation.

4.4. Understanding invertebrate and vertebrate physiology and disease

Experimentally probing these physiological interrelationships in *D. melanogaster* has improved our understanding of this complex metazoan as an experimental model for the study of health and disease. *Drosophila* has been a key model organism for many fields, including experimental evolution and physiology (e.g. Burke and Rose, 2009). *Drosophila* have short generation times, are easily maintained at large population sizes, and possess vast public genomic resources (e.g. FlyBase). Unlike most microbial models, the physiology of *Drosophila* is both complex and broadly analogous to some features of vertebrate physiology. *D. melanogaster* also have orthologous genetic mechanisms with those that are thought to determine lifespan among vertebrates, including such genetic systems as TOR and insulin/insulin-like signaling (e.g. Partridge and Gems, 2007).

With cardiovascular disease and heart-related defects being leading causes of death among Western patients, conducting useful heart experiments at great scale and intensity with model organisms should be of significant value (Olson, 2004). As mentioned earlier, fruit flies experience a decline in heart function and robustness with age, similar to what has been found in aging human adults (Bier and Bodmer, 2004; Nishimura et al., 2011; Ocorr et al., 2007; Paternostro et al., 2001; Wessells and Bodmer, 2007). Here we found that flies with higher lipid content have decreased heart robustness. Likewise, it is believed that higher circulating levels of triglycerides in humans are a major factor affecting cardiac disease. The *Drosophila* fly shares some of the genes that underlie its cardiac performance with those of human cardiac genetics, such as *tinman* (Bodmer, 1993; Bodmer and Venkatesh, 1998; Bodmer, 2006) and *opa1* (Shahrestani et al. 2009). In another study of *Drosophila* cardiac function, Birse et al. (2010) have shown that altering nutrient-sensing signaling pathways (e.g. insulin-TOR signaling) can combat the adverse cardiac effects of a high-fat diet. Additional genes and signaling pathways conserved between *Drosophila* and mammals might be used to develop therapies that could counteract the lipotoxicity and cardiac dysfunction of obesity (e.g. PGC-1/spargel: Diop et al., 2015). The combination of the present study's results with the results of other studies, such as Birse et al. (2010), Diop and Bodmer (2012), Diop et al. (2015) or Hardy et al. (2015), underscores the value of using laboratory-evolved *Drosophila* populations to parse the genetic and physiological mechanisms of heart function.

Acknowledgments

We thank James W. Hicks for helpful discussions and comments on the experiments. We are also grateful to the many undergraduate research students who contributed to the stock maintenance and experimental assays, especially Grigor Azatian, Jose Buenrostro, Annie Khong, Eric Leung, Jennifer Majdick, Adil Rahman, and Gabriel Reyes.

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