

5-1-2013

## Kinematic analysis of axial rotations and the effects of stress selection on takeoff flight performance

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KINEMATIC ANALYSIS OF AXIAL ROTATIONS AND THE EFFECTS OF  
STRESS SELECTION ON TAKEOFF FLIGHT PERFORMANCE

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A Dissertation submitted in partial fulfillment  
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**Doctor of Philosophy in Biological Sciences**

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May 2013**

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## THE GRADUATE COLLEGE

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entitled

Kinematic Analysis of Axial Rotations and the Effects of Stress Selection on Takeoff Flight Performance

be accepted in partial fulfillment of the requirements for the degree of

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**May 2013**

## Abstract

The importance of flying insects cannot be understated; without them as pollinators, the great diversity of flowering plants could not have occurred. Flight requires a suite of highly derived morphological and physiological characteristics that may limit the evolutionary responses of other life history traits.

To investigate the complexities of maneuvering flight, I used high-speed videography to analyze wing and body motions in the Hoverfly *Syrirta pipiens* (Order: Diptera) during horizontal turns called saccades. I characterized the saccades by calculating the instantaneous rotational velocities throughout the saccade, maximum rotational velocity, and the mean rotational velocity. I then compared the shape of the rotational velocity curve to previous research on the saccades of *Drosophila melanogaster* to determine if, like *Drosophila*, saccades of *S. pipiens* are stereotypical behaviors triggered by an avoidance response and are consistent in the magnitude of the rotation and in the time to complete the turn. I also found that, unlike *Drosophila* that accelerate to the maximal rotation velocity more quickly than they decelerate, in *S. pipiens* saccades acceleration/deceleration times could be equal, or flies could take a longer time to accelerate than decelerate, or a shorter time to accelerate than decelerate. This suggests that *S. pipiens* saccades are more variable, and under greater control by *S. pipiens* than has previously been found in *D. melanogaster*. In *D. melanogaster*, body torque is generated by asymmetry in wing stroke amplitude between the inner and outer wings. I measured stroke amplitude during S.

*pipiens* saccades and found that no asymmetry in wing stroke amplitude could be found that could be associated with the generation of body torque.

Wing loading, the ratio of wing area to mass, has been shown to be a predictor of flight performance in flying animals. Increases in wing loading have been found to increase the energetic cost of flight and result in lower overall flight performance. I studied the effect of increased wing loading in flies that had undergone laboratory selection for resistance to desiccation and starvation.

Desiccation selected flies had wing loadings significantly higher than their controls but did not differ in flight velocity (total, horizontal, vertical) or flight angle during take-off. Starvation resistance flies, with higher wing loadings than their controls, had significantly lower vertical flight velocity and flight angle during take-off flights. However, starvation resistance flies did not have significantly different wing loadings than the desiccation resistant flies, suggesting that other mechanisms might be responsible for lower vertical flight velocities and take-off angles.

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## CHAPTER 1

### INTRODUCTION

The evolutionary paths of the plants and animals in existence today have been marked by significant adaptations that have given them a selective advantage. The transition from single cell to multicellular organisms that led to the differentiation of cells to perform specific functions, the development of sensory organs that allowed organisms to receive cues coming from their environment, and the ability of animals to actively adjust their position within the environment, have all been landmark adaptations leading to the complex forms we see today. It is the ability of animals to move in contra to environmental conditions (fly against the wind, swim upstream) that has facilitated the expansion of animals into every habitat on earth.

The morphological and physiological requirements for locomotion can vary significantly within and among taxonomic groups depending on the specific requirements of a species. For fish species, there are predictable fin morphologies for ambush hunters, long distance travelers, and high-speed swimmers. In addition, differing physiological adaptations are necessary to accommodate these differing swimming styles (Sfakiotakis et al., 1999; Blake, 2004; Palstra and Planas, 2012).

Gliding, a controlled decent, has evolved multiple times within the major taxonomic groups (McGhee, 2011). Due to the highly derived characteristics necessary for powered flight, the ability to remain airborne for extended periods of time, flight has only evolved in three extant groups, most abundantly in insects

and birds, as well as in one group of mammals (McGhee, 2011). Extant volant forms range in size from  $< 0.025$  mg with a wing span of  $\approx 0.5$  mm for fairyflies (Family: Mymaridae) to the Andean Condor (*Vultur gryphus*) with a wing span of over 3 m that can weigh as much as 15 kg. With a difference in wingspan of  $\approx 4$  orders of magnitude and  $\approx 9$  orders of magnitude in mass, adaptations for flight can vary almost as much as the species that employ them.

The abundance of flying insects, in both biomass and diversity of species, makes them an ideal subject for research into flight. Dipterans, in particular, are of great interest because they include some of the most highly derived insects for flight. Possessing asynchronous flight muscles, where a single impulse results in multiple muscle contractions, dipteran wing beat frequencies can be substantially higher than those of many other species. For example, midges from the genus *Forcipomyia* (Diptera: Ceratopogonidae) have the fastest wing beat frequency ever recorded at over 1000 Hz (Sotavalta, 1947).

A general evolutionary trend for insects that rely more heavily on flight, such as hymenopterans, has been the gradual reduction in rear wing size and the addition of a coupling mechanism to allow the front and rear wings to act as a single lifting surface (Grodzinsky, 1999). Dipterans have evolved even further with a reduction of the rear wings to the point where they no longer possess a lift-producing surface (Pringle, 1948; Dudley, 2000). With only one pair of wings, Dipterans have evolved to be some of the most acrobatic of flyers. This is illustrated by them being the only group of flyers that that can perform lateral

flight maneuvers without the incorporation of body roll (Dudley, 2000; Sudo et al., 2010).

Halteres (modified rear wings) are highly sensitive to deviations in rotations about the three body axes and are particularly sensitive to yaw rotations, a rotation about the vertical axis (Pringle, 1948; Chan et al., 1998; Sherman and Dickinson, 2003). In addition, they have evolved into such a highly acute sensory organ that if removed or damaged, flies are unable to maintain stability in flight (Sudo et al., 2010).

Many Dipteran groups also contain high levels of visual acuity that allow them to accurately detect relatively small objects in their field of view (Land, 1997; Sherman and Dickinson, 2003). Combined, their wing number, high visual acuity, and mechanosensory organs tuned to flight forces make Dipterans some of the most proficient flyers in the animal kingdom.

To understand how flight has become so prominent in insects, many levels of research are required to elucidate the characteristics necessary for flight, from the cellular level up to the whole organismal level. My research first focused on how syrphid flies modulate wing kinematics to produce torquing forces that are then used to elicit body rotations that, in turn, alter flight trajectories. These turns are of particular interest in that they play an important role in navigating cluttered habitats, defending territories from rival males, and in the 'capturing' of females to breed. Although the horizontal rotations (saccades) of syrphid flies have been described in general, to date no research has been conducted on the wing kinematics used to modulate these turns. In addition, a

description of the angular velocities or the magnitude of their rotations has yet to be quantified. My goal of this research was to: 1) provide an in-depth description of syrphid saccades that quantified rotational velocities during the acceleration and deceleration phases of the rotation, and the maximum velocities achieved; 2) quantify the specific kinematic patterns used by syrphids to initiate, terminate, and control rotational velocities during saccades; 3) compare axial rotations of syrphids to those described, in detail, in *Drosophila melanogaster*, and 4) test the hypothesis that saccades of *Syrphid pipiens* (Diptera: Syrphidae) will differ in wing kinematic patterns and rotational velocities from those described in *D. melanogaster*, due to the extremely high level of maneuverability they exhibit during territorial defense, male-male competitions, and breeding behaviors.

My final two experiments focused on organismal level adaptive responses to environmental stresses that resulted in morphological and physiological changes, by quantifying their effects on takeoff flight performance. One set of populations of *Drosophila melanogaster* that had undergone laboratory selection for resistance to desiccation and a second set selected for resistance to starvation was filmed using high-speed videography during takeoff flights. I tested the hypothesis that takeoff flight performance would be compromised due to energy storage adaptations for desiccation and starvation resistance and that this would result in significant reductions in: 1) total mean and maximum flight speeds; 2) mean and maximum horizontal flight velocities; 3) mean and maximum vertical flight velocities; 4) and finally, that adapted flies would have a significantly lower flight angle.

This research attempts to establish procedures that can be used to quantify changes in locomotion (flight) that result from the introduction of environment stressors. By understanding how specific stresses introduced in the lab can impact flight, hypotheses can be tested as to how wild populations of flying insects may respond to multiple stresses occurring in a changing environment.

## CHAPTER 2

### WING KINEMATICS AND ROTATIONAL VELOCITIES OF AXIAL ROTATIONS (SACCADES) OF THE HOVERFLY, *SYRITTA PIFIENS*

#### Introduction

Locomotion is a critical activity for most animals, even if only for a small part of their life cycle. For many pterygote insects, flight is a daily requirement during feeding, foraging, territorial defense, and reproduction, as well as other critical activities. Understanding the energetic cost of flight required for such critical behaviors is a significant factor in understanding the overall ecology of flying insects. Direct measurements of metabolic rates have shown that hovering flight is the most energetically costly form of locomotion yet measured (Snelling et al., 2012). While this area of research has uncovered many important factors regarding insect ecology, there are still many questions yet to be examined. The study of maximal flight performance, in order to better understand how insects interact with their environment, is one area of research currently under investigation. Understanding the kinematic mechanisms implemented by insects to achieve near instantaneous rotations and translations along the roll, pitch, and yaw axes can help elucidate what limits may exist during flight for insects (Fig. 2.1).

Figure 2.1

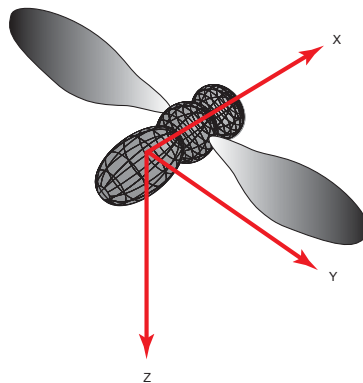


Figure 2.1. Flight requires the ability for animals to simultaneously translate along and rotate around the X, Y, and Z-axes. Maneuverable species are able to simultaneously make radial adjustments in roll (X), pitch (Y), and yaw (Z). In addition, high levels of maneuverability are the result of simultaneous translations along multiple axes.

For volant forms, flight maneuvers require continual adjustments in translational and rotational forces, as well as the coupling and decoupling along the three axes of orientation: roll, pitch, and yaw (Fig. 2.1) (Dudley, 2000).

Dipterans, being the only group able to perform lateral flight without the use of body roll, are considered to be some of the most acrobatic of the flying insects (Dudley, 2002). This makes them a desirable model for research into flight performance. Early research on the hoverfly *Syrirta pipiens* (Family: Syrphidae) showed that these insects are capable of extremely acrobatic flight. During many flight sequences analyzed, they often exhibited saccades, a rotation about the yaw (Z) axis only. Yaw rotations were found to occur during vertical, forward, rearward, and sideways flight (Collett and Land, 1975; Collett, 1980). Most importantly, they were also found to occur during hovering flight. Saccades that occur during hovering flight exclude axial translations and are limited to rotation about the yaw axis only (Collett and Land, 1975). This type of flight behavior should exhibit a limited number of variables in wing and body kinematics and is,



therefore, a desirable place to start research into the maneuverability of flying insects.

Research into the wing kinematics of saccades was conducted on *Drosophila melanogaster* placed within a visual arena that could be used to trigger a turning response from the fly (Dickinson, 2005). An infrared light source projected a shadow of the wings that allowed for the measurement of wing amplitude before, during, and immediately after the saccade. Additional research showed asymmetry between the outer and inner wing stroke amplitudes, with the fly initiating the saccade by increasing the stroke amplitude of the outer wing over that of the inner wing (Dickinson, 2005). Termination was achieved by increasing inner wing stroke amplitude over that of the outer wing, however, to a smaller degree due to the frictional dampening by the body slowing the rotation (Dickinson, 2005; Hesselberg and Lehmann, 2007; Cheng et al., 2010).

Further research focused on the rotational components of the wing stroke in conjunction with amplitude. Wing rotations, particularly the transition between the down and up strokes (ventral flip), were found to contribute significantly to the generation of torque, and any bilateral asymmetry in wing rotation timing plays a significant role in generating body torque (Dickinson et al., 1993; Sane and Dickinson, 2002; Altshuler et al., 2005a; Ramamurti and Sandberg, 2007).

My research tested the hypothesis that, like *Drosophila*, asymmetrical wing stroke amplitude between the inner and outer wings is the kinematic pattern employed by the hoverfly *Syrirta pipiens* to modulate saccades under free flight conditions. I also tested the hypothesis that saccades of more acrobatic fliers,

unlike *Drosophila*, are not stereotypical behaviors and will differ within and among individuals. Therefore, if *S. pipiens* actively control yaw turns, then saccades from the same individual can have different rotational velocity profiles for each turn. In addition, velocity profiles will differ among individuals.

### Methods

The syrphid fly *Syrirta pipiens* was chosen for this research due to its local abundance and the high levels of maneuverability described in other studies (Collett and Land, 1975; Collett, 1980; Dickinson, 2005; Sudo et al., 2010). Unlike many flying insects, this species will continue to fly voluntarily for extended periods of time, allowing the collection of multiple flight sequences from a single individual without interference from the researcher. This helps to eliminate possible differences between flights that occur after an external stimulus is introduced to maintain flight (escape flight) and flights that occur under strictly voluntary conditions.

Filming occurred during the months of April and May (2007), when hoverflies are abundant on the campus of the University of Nevada, Las Vegas. Flies were netted, transferred to a glass vial, and immediately taken to the lab and filmed. Time from capture to filming was typically no greater than 15 min.

Flies were allowed to acclimate for 5 - 10 min in the flight chamber with only the room lights on. Due to the heat generated by the halogen lights required for filming, these lights were only turned on when capturing maneuvers and were limited to 30 seconds before being turned off. This kept the flight chamber, and the fly within, from overheating and introducing a heat stress to the flies that

could affect their flight performance. When the flies came into the focal range of the cameras, the three cameras were triggered and the on-board memory saved. It took several minutes for the sequences to be reviewed; during this time, all lights in the laboratory were turned off, forcing the fly to land and remain relatively still while sequences were reviewed and saved.

### *Filming*

Filming of flight sequences was done using three orthogonally placed Phantom (v5.1) high-speed digital cameras (Fig. 2.2) under free flight conditions at a spatial resolution of 512 X 512 pixels at 8 bits per pixel and at a frame rate of 4,350 Hz. Initial filming showed this to be a high enough frame rate for insects that exhibit wing beat frequencies between 200 and 300 Hz. The focal volume of the cameras consisted of a 5 cm x 5 cm x 5 cm volume in the center of a 20 cm acrylic cube (Fig. 2.2).

One *S. pipiens* was collected at a time and immediately transferred into the flight arena. After the fly performed a maneuver within the focal volume the lights were extinguished, forcing the fly to land, and the flight sequence was saved from all three camera views. This conserved the fly for multiple flights from which the most suitable sequences would be digitized. A total of 5 saccades from 3 individuals were digitized.

Figure 2.2

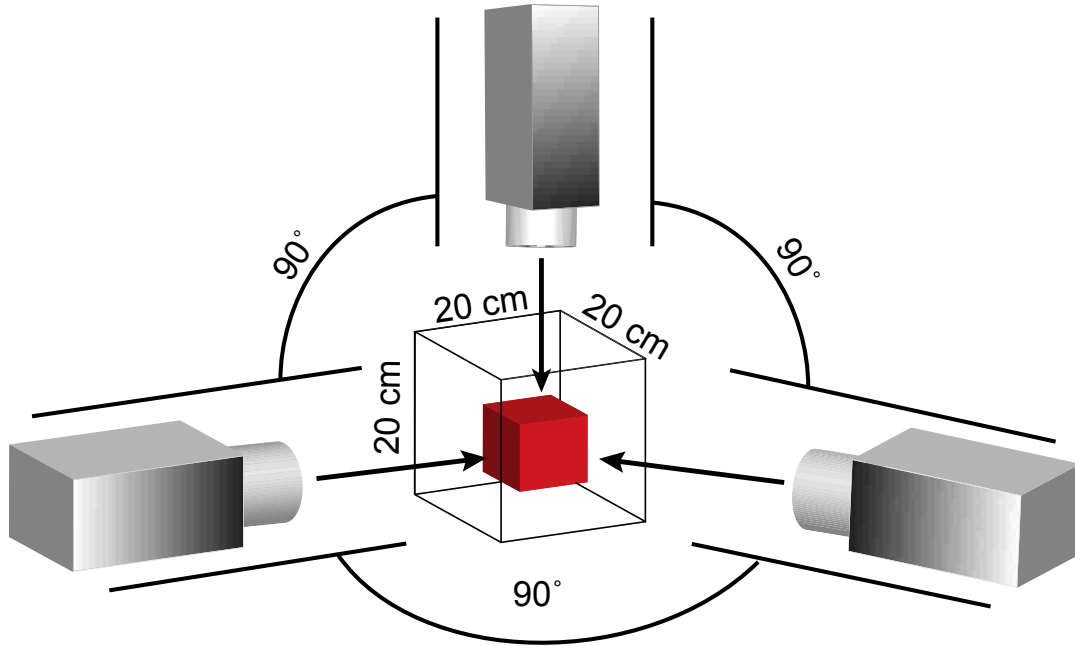


Figure 2.2. Digital videography was conducted by using 3 orthogonally placed high-speed digital cameras. Flies were allowed to freely fly within a 20 cm acrylic cube. The focal volume, represented by the solid inner box, of the cameras was a 5 cm cube near the center of the larger flight chamber.

### *Digitizing*

Saved sequences were visually inspected, and flights showing the least amount of movement other than the yaw rotation were digitized using DLTdataviewer2 (Hedrick, 2008) written for Matlab. The head, tail, wing tips, and wing bases were marked on each frame (Fig. 2.3). From these data points, stroke amplitude, stroke plane, stroke plane deviation, body rotations (pitch, roll, and yaw), were calculated. Calculations were performed using a custom program (Robert C. Reiner, Michael L. Brewer) written for Matlab.

Figure 2.3

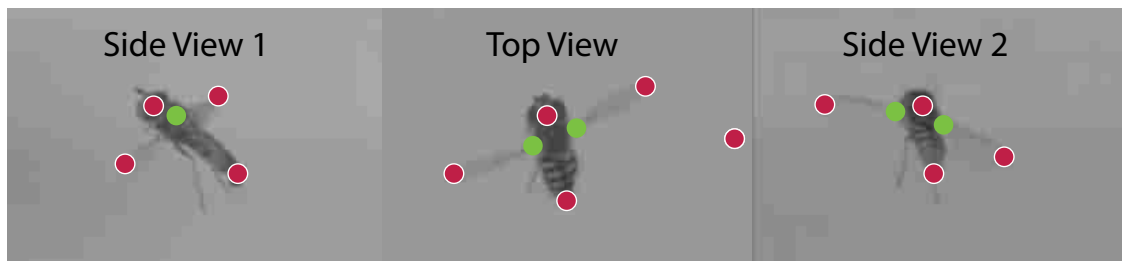


Figure 2.3. Screen shots from all three-camera views, illustrating the points digitized to calculate the kinematics of syrphid flight. The head and tail were used to calculate flight direction, body angle, and yaw rotation. The wing tips were used to calculate wing stroke amplitude, stroke plane, and stroke plane deviation. The wing hinges (green dots) were used to calculate body roll.

### *Saccades*

Rotational velocities were calculated as the change in angular position (yaw) divided by the change in time (1 frame = 0.23 ms). Excessive noise is a common issue when calculating velocities and accelerations from data sampled at high frequencies (Walker, 1998). To analyze the velocity data, several methods of filtering data were investigated in an attempt to reduce or eliminate excess noise. Filters, including a Butterworth low pass filter, generalized cross-validatory quintic spline, and a Savitzky-Golayf filter (moving polynomial regression) were applied to the raw data sets that were extracted from the 6 body locations of the flies (Walker, 1998)(Fig. 2.3). As expected, applying filters to the raw positional data did not reduce the noise found in the velocity data, and when stronger filtering methods were applied (i.e. larger window size), the result was to compromise the kinematic data. The same array of filtering methods was then applied to the rotational velocities calculated from the yaw data. Of the filtering methods, the moving cubic polynomial regression removed the greatest amount of noise without appearing to alter the overall results; however, it did not reduce

the noise sufficiently to calculate the initiation, termination, or maximal rotation point of the saccades.

The saccade interval was determined by fitting a cubic spline to the raw instantaneous rotational velocities (Fig. 2.4) and locating the two points (initiation point (IP) and the termination point (TP)) that most closely approximated 0 °/ms (Fig 2.5).

Figure 2.4

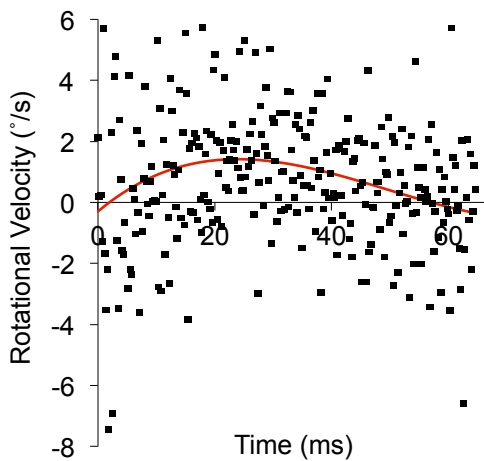
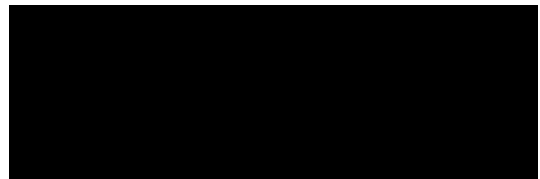
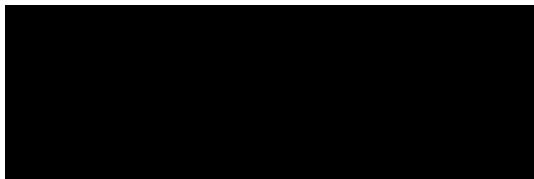
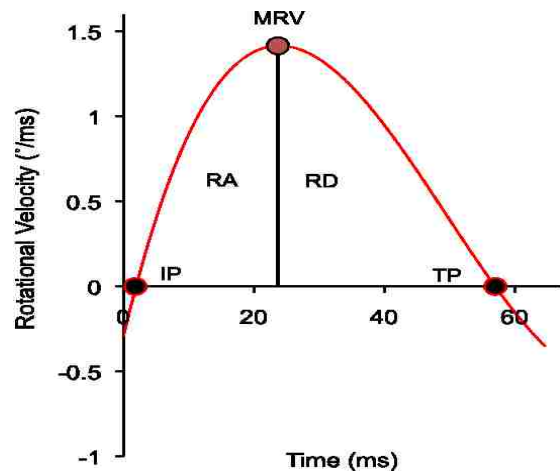


Figure 2.5



The goal of my research was to describe the general shape of saccades performed by *S. pippins*. To accomplish this, I tested whether a cubic spline fit applied to the raw data showed a trend that actually existed or simply forced a curve onto the data where one did not exist. I tested this by comparing a cubic spline fitted to the raw data to that of one fitted to data that was filtered using a moving polynomial regression (Table. 2.1 a, b). All 5 saccades were compared

using this method, and the saccades that showed the greatest dissimilarities (Fig. 2.6 a) and similarities (Fig. 2.6 b) are presented here.

Saccade 1.2 showed the greatest amount of dissimilarity between the curve fitted to the raw data and that fitted to the filtered data (Table. 2.1 a, Fig 2.6 a). The saccade based on the fitted data took 1.38 ms longer to complete, the initiation point occurred 1.61 ms earlier, the maximum rotational velocity occurred 1.61 ms later, and the termination point occurred 0.23 ms (1 frame) earlier. Overall, this resulted in the acceleration curve for the fitted data requiring 62% of the time, a 2% increase over the raw data (Table 2.1 a). Saccade 3.1 showed no difference between the raw and filtered data in any of the parameters measured (Table 2.1 b, Fig. 2.6, b.).

The minimal differences between curves fitted to raw and filtered data, suggest that the patterns elucidated by the cubic spline fitted to the raw data show an actual trend in rotational velocities and sufficiently describe the overall shape of saccades. All results described below were determined by a cubic spline fit to the raw data.

Table 2.1

a)

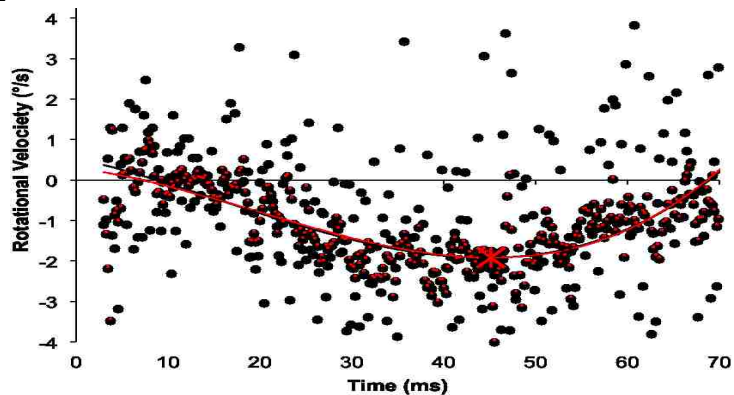
Saccade 1.2	Time (ms)	Mean RV (°/ms)	Max RV (°/ms)	% AC
Raw Data	60.26	0.915	-1.9178	60
Filtered Data	61.64	0.916	-1.9235	62

b)

Saccade 3.1	Time (ms)	Mean RV (°/ms)	Max RV (°/ms)	% AC
Raw Data	54.51	1.26	2.083	63
Filtered Data	54.51	1.26	2.082	63

Table 2.1 Comparisons of the rotational velocities and percent time of the acceleration curves (% AC) calculated from curves fitted to the raw and filtered data. The largest differences were found in saccade 1.2 (a) while saccade 3.1 (b) showed only a small difference in maximum rotational velocity of 0.001 °/ms.

a) Saccade 1.2



b) Saccade 3.1

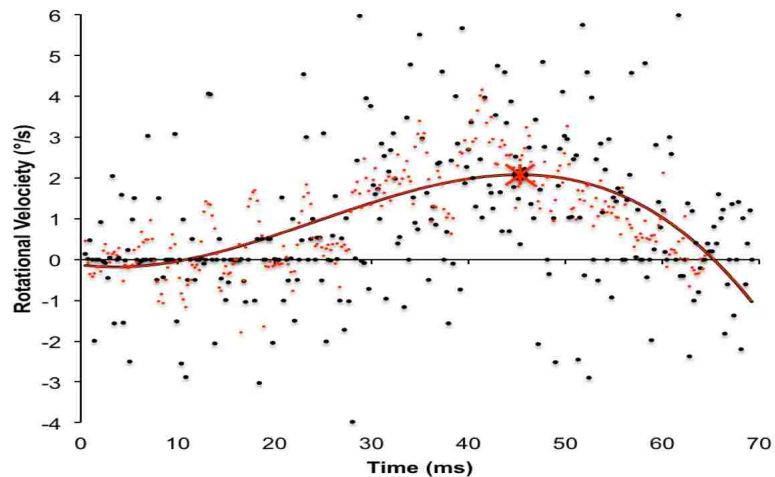


Figure 2.6. Plots showing the raw (black point) and the filtered (red dots) data of the saccades with the greatest (a) and the least (b) amount of variation of the fitted curve (lines). Maximum rotational velocities are shown as a black dot (raw data) and a red star (filtered data).

I calculated the following parameters from cubic spline fits (Fig. 2.5):

- Total Rotation (TR): difference in yaw position between termination point and initiation point ( $^{\circ}$ )
- Total Time (TT): time between IP and TP (ms)
- Average Rotational Velocity (ARV): average rotational velocity between IP and TP ( $^{\circ}/\text{ms}$ )
- Velocity Maximum (VM): point of maximal rotational velocity ( $^{\circ}/\text{ms}$ )
- Rotational Acceleration (RA): curve between IP and VM



- Mean velocity ( $^{\circ}/\text{ms}$ ) and percent of total curve
- Rotational Deceleration (RD): curve between VM and TP
  - Mean velocity ( $^{\circ}/\text{ms}$ ) and percent of total curve
- Percent Velocity Curve: time between IP/TP and VM divided by TT

## Results

### *Saccades*

I analyzed a total of 5 saccades performed by 3 *S. pippins*, and show clockwise rotations as a positive yaw values and counterclockwise turns as negative yaw values. Individual 1, sequence 1 (saccade 1.1) was a  $47.0^{\circ}$  (clockwise) yaw rotation that took 0.056 s to complete (Table 2.2). Rotational acceleration took up 40.8% of the total time required to complete the saccade. Maximal rotational velocity (MRV) occurred  $1.8^{\circ}$  after the rotational midpoint (RM, Table 1).

Saccade 1.2 was a  $-72.0^{\circ}$  (counterclockwise) yaw rotation that took 0.06 s to complete (Table 2.2). Rotational acceleration took up 60.1% of the total time required to complete the rotation, and maximum rotational velocity occurred  $7.8^{\circ}$  prior to the RM.

Saccade 2.1 was a  $-31.4^{\circ}$  yaw rotation that took 0.051 s to complete (Table 2.2). Rotational Acceleration took up 54.5% of the total time required to complete the rotation, and maximum rotational velocity occurred  $3.72^{\circ}$  prior to the rotational midpoint.

Saccade 2.2 was a  $-38.8^{\circ}$  yaw rotation that took 0.052 s to complete (Table 2.2). Rotational acceleration took up 52.2% of the total time required to

complete the rotation, and maximum rotational velocity occurred 5.33° prior to the rotational midpoint.

Saccade 3.1 was a 65.3° yaw rotation that took 0.055 s to complete (Table 2.2). Rotational acceleration took up 63.9% of the total time required to complete the rotation and MRV occurred 9.07° prior to the rotational midpoint.

Table 2.2

Saccade	Total Yaw (°)	Time (ms)	Mean RV (°/ms)	Max RV (°/ms)	RA Mean RV (°/ms)	RD Mean RV (°/ms)	RV Max (°)	Mid Rot (°)	% RA
1.1	47.9	56	0.89	1.4	0.95	0.85	24.0	22.2	41
1.2	-72.0	60	0.92	1.9	1.16	1.32	-36.0	-43.8	60
2.1	-31.4	51	0.62	0.9	0.61	0.64	-15.7	-19.4	54
2.2	-38.8	52	0.80	1.2	0.80	8.95	-19.4	-24.7	52
3.1	65.3	55	1.26	2.1	1.17	1.43	32.7	41.7	63

Table 2.2. The rotational components of the each saccade listed in order the sequences were filmed. Sequences 1.1 and 1.2 represents two saccades filmed from the same individual.

### *Wing Kinematics*

If hoverflies follow the same kinematic patterns as *Drosophila*, then we would expect to see greater stroke amplitude in the outer wing at the initiation of the saccade, followed by greater stroke amplitude of the inner wing (to a lesser degree) at the termination point. However, the sequences analyzed did not exhibit any consistent trends in any of the kinematic parameters measured, including wing amplitude asymmetry between the inner and outer wings, which could be associated to the initiation or termination of saccades.

Saccade 1.1 showed asymmetry in total wing amplitude (dotted lines) throughout the rotational phase of the sequence, with the outside wing (red) maintaining greater amplitude throughout the saccade (Fig. 2.7 a). The outer

wing did show a positive phase shift (black solid line) at the beginning of the saccade, however, at the end of the saccade there was no clear phase that correlated with the termination of the saccade (Fig. 2.7 a). In contrast, saccade 1.2, from the same individual, exhibited no clear deviations in amplitude or phase shift that would suggest a mechanism of yaw control (Fig. 2.7 b).

Saccades 2.1 and 2.2 were rotations of similar magnitude ( $-21.4^\circ$  and  $-38.8^\circ$  respectively) performed by the same individual, yet there were no similarities in any of the kinematic parameters measured that are thought to regulate yaw rotations (Fig. 2.8 a, b).

Saccade 3.1 also showed no patterns in wing kinematics that would suggest that wing amplitude, phase shift, roll, body angle, stroke plane, or stroke plane deviation was being used to regulate yaw rotation (Fig. 2.9 e).

Figure 2.7

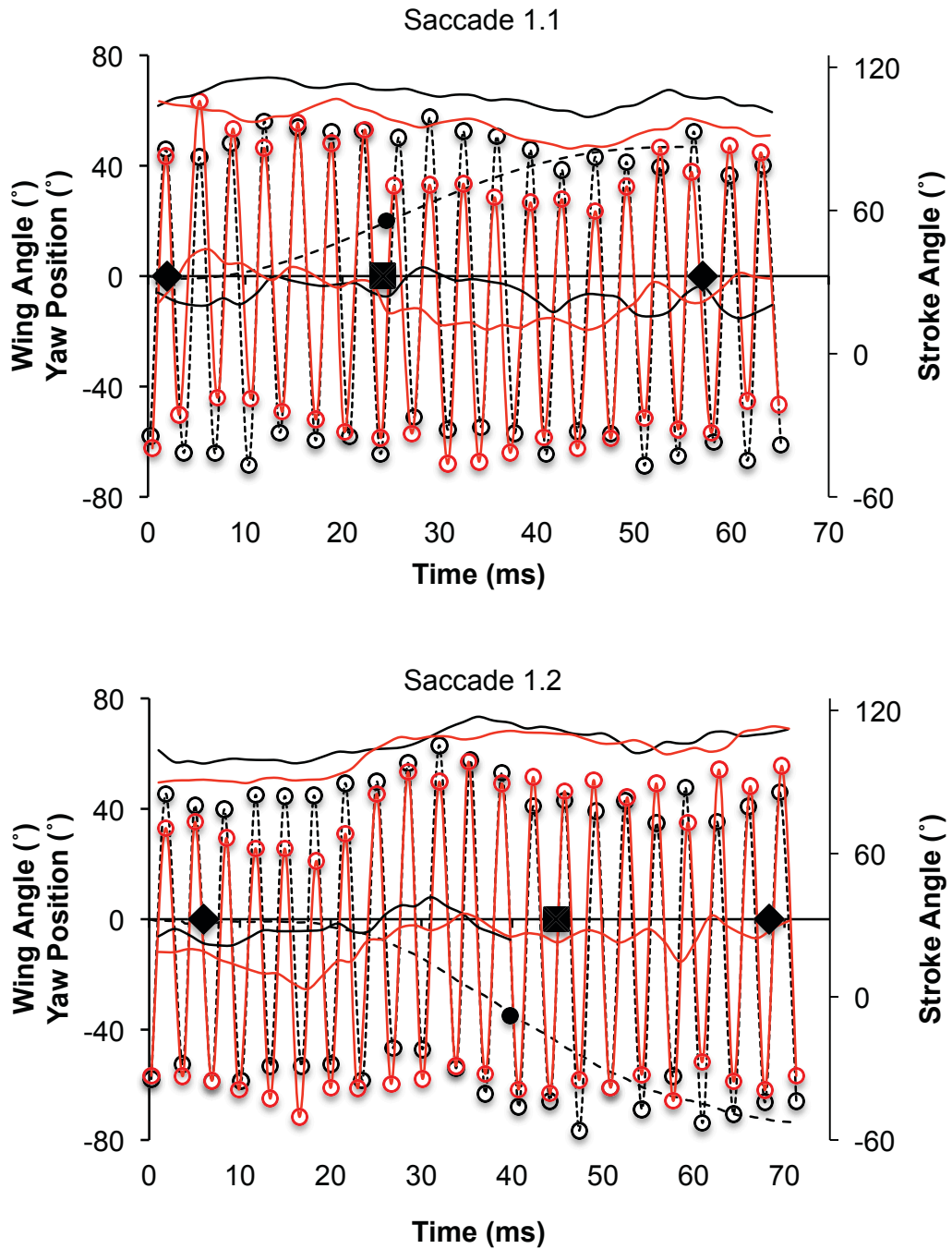


Figure 2.7. Plots of the major kinematic perimeters measured for the 2 saccades from syrphid fly 1, with the red lines representing the inner wing. Yaw rotation (black dashed line), mean stroke (lower solid lines), and wing oscillations are on the primary vertical axis (left), and total amplitude (upper solid lines) is on the secondary vertical axis. Black diamonds represent the initiation and termination points of the saccade, Black Square correlates to the maximum rotational velocity, and the black circle represents the point of mid rotation.

Figure 2.8

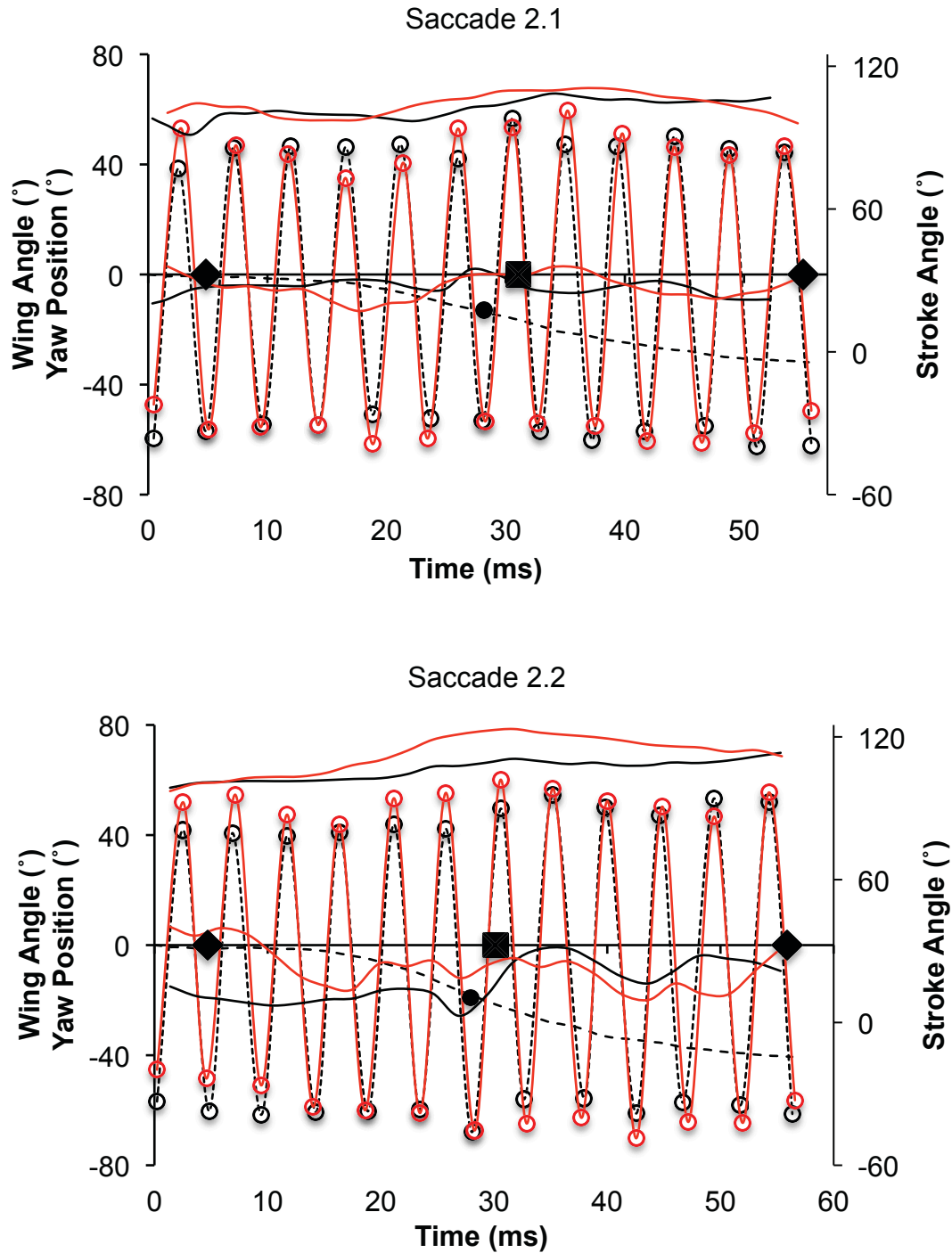


Figure 2.8. Plots of the major kinematic perimeters measured for the 2 saccades from syrphid fly 2, with the red lines representing the inner wing. Yaw rotation (black dashed line), mean stroke (lower solid lines), and wing oscillations are on the primary vertical axis (left), and total amplitude (upper solid lines) is on the secondary vertical axis. Black diamonds represent the initiation and termination points of the saccade, Black Square correlates to the maximum rotational velocity, and the black circle represents the point of mid rotation.

Figure 2.9

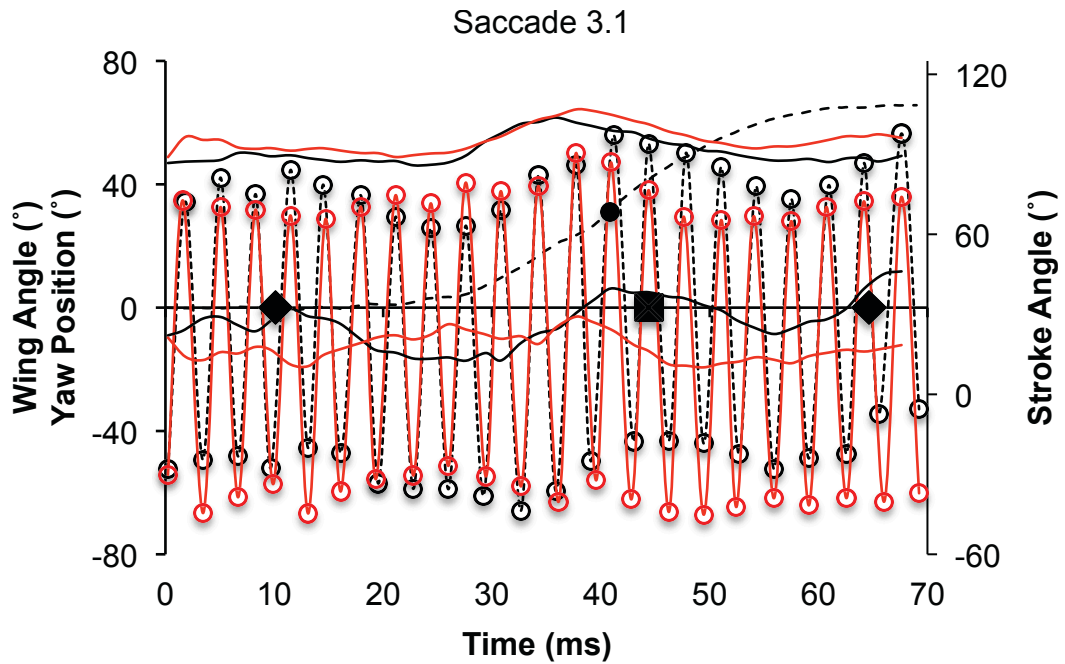


Figure 2.9. Plots of the major kinematic perimeters measured for the single saccade from syrphid fly 3 with the red lines representing the inner wing. Yaw rotation (black dashed line), mean stroke (lower solid lines), and wing oscillations are on the primary vertical axis (left), and total amplitude (upper solid lines) is on the secondary vertical axis. Black diamonds represent the initiation and termination points of the saccade, Black Square correlates to the maximum rotational velocity, and the black circle represents the point of mid rotation.

## Discussion

Saccades have been described as rapid, stereotypical maneuvers that are performed through the asymmetry of wing stroke amplitude between the outer and inner wings (Dickinson, 2005). This suggests that saccades, at least for *Drosophila*, are achieved by the use of consistent wing kinematics in the initiation, termination, and control of acceleration and deceleration during the turn. If variation exists in the shape of the rotational velocity curves, the timing of the point of maximum rotation and the point of mid rotation, and the shape of

acceleration and deceleration curves, it would suggest that *S. pippins* saccades are actively controlled and not the result of a stereotypical response.

Saccade 1.1 showed a rightward skew that resulted in the deceleration curve requiring 60% of the time to complete and an average rotational velocity that was  $\approx 10\%$  ( $857.6^\circ/\text{s}$ ) lower than acceleration curve ( $942.1^\circ/\text{s}$ , Fig. 2.10 a). This rotational velocity curve closely resembles the velocity curves documented in *Drosophila* saccades, where the flies accelerate more rapidly than they decelerate (Dickinson, 2005; Bender and Dickinson, 2006). In contrast, saccades 1.2 and 3 showed the flies decelerating more rapidly than accelerating, the opposite of what was found in *Drosophila*, with the acceleration curve requiring 62.4% and 62.9% of the saccade, respectively (Table 1, Fig. 2.10 b, e). In addition, Saccades 2.1 and 2.2 (from the same individual) showed nearly equal rates of acceleration and deceleration (Table 1, Fig. 2.10 c, d), resulting in 54% and 52%, of the saccade, respectively, occurring during acceleration phase.

Figure 2.10

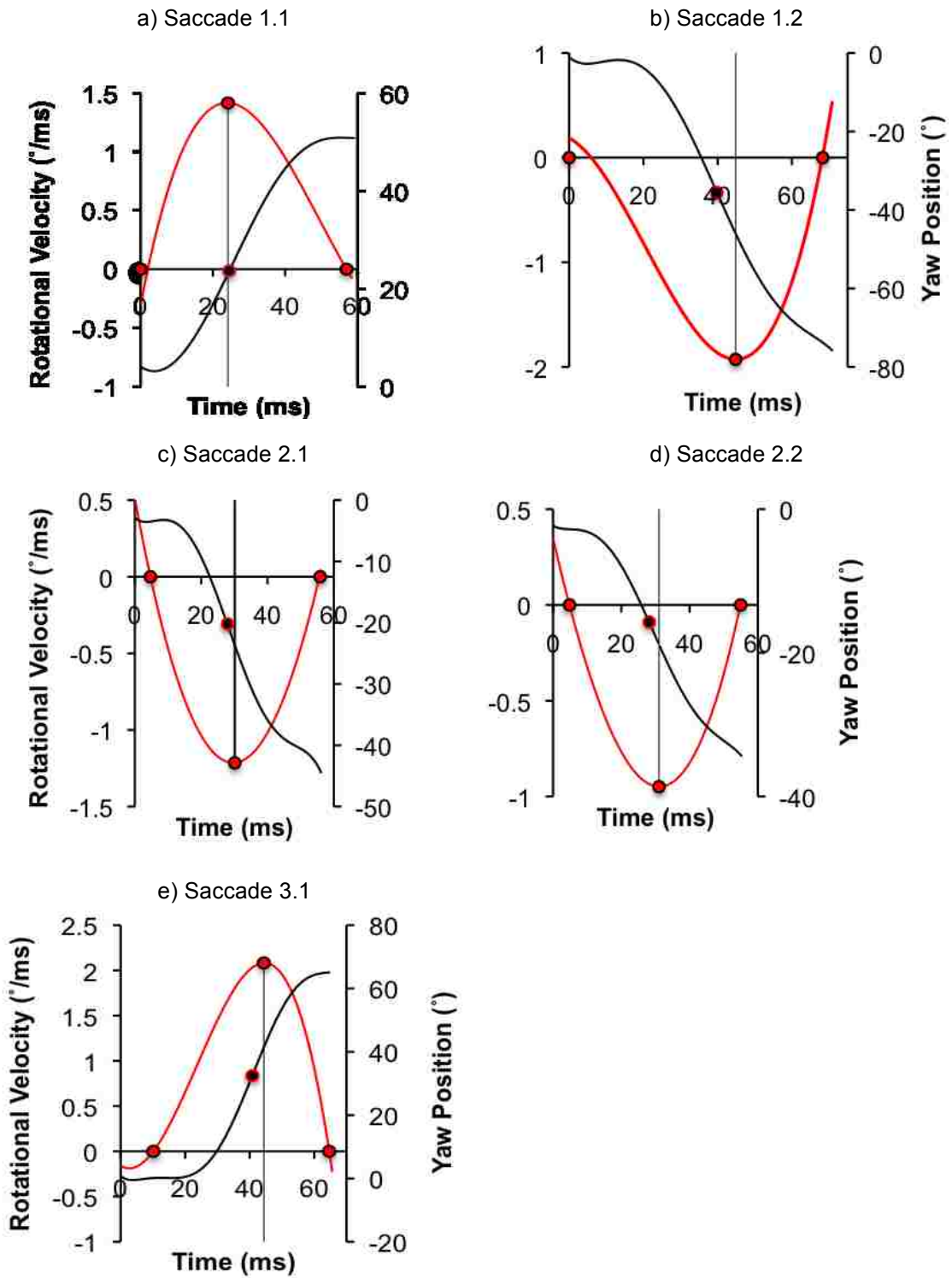


Figure 2.10. Plots showing the rotational velocities (red line) and yaw rotations (black line) calculated from fitted data. Red dots represent the initiations and terminations of the saccades (on the 0 axis) and the maximum rotational velocities. Black dot represents the point of mid rotation (secondary vertical axis).



My data show heterogeneity in kinematic parameters in voluntary turns of *S. pipiens*, and are in contrast to the stereotypical saccades described in *Drosophila*. Most of the research into *Drosophila* maneuverability has triggered a turning response by using an LED array to create a visual expansion in the fly's field of view (Dickinson, 2005; Fry et al., 2005; Bender and Dickinson, 2006). It is possible that *Drosophila* saccades are the result of a collision avoidance response and, therefore, may show a different profile than saccades that occur under voluntary conditions. Saccades of *S. pipiens* were collected under voluntary conditions within a focal volume that was much smaller than the overall flight arena. Because of this, saccades described in previous research could be fundamentally different from saccades occurring under voluntary conditions. Recent research into *Drosophila* saccades has shown that 90% of all saccades were the result of a visual stimulus (Censi et al., 2013), supporting the hypothesis that saccades are a stereotypical response with a limited amount of variation (Censi et al., 2013).

Typically we associate deviations in flight path to be caused by external stimuli such as wind speed and direction, obstacle avoidance, prey capture, escape from predation, and responses to olfactory cues from food or potential mates. Animals may also alter their flight path due to internally fixed foraging patterns based on an optimal foraging strategy. The differing shapes of the rotational velocity curves show that *S. pipiens* saccades are not simply the result of a fixed response to stimuli, but are likely the result of deliberate action by the fly to determine the magnitude of the turn, the rate of acceleration, and rate of

deceleration. This may also allow *S. pipiens* to alter the saccade after the rotation has started, further increasing the maneuverability of this species. This correlates well with the flight required for the visual tracking of rival males and breeding females previously described in this species (Collett and Land, 1975; Collett, 1980).

Short-amplitude, high-frequency flight, like that used by honeybees and *S. pipiens*, is a less efficient form of flight than the large-amplitude, low-frequency flight of *Drosophila* (Sane and Dickinson, 2001). The use of a less efficient, more energetically costly form of flight must, therefore, have a selective advantage to those groups of animals that use it. By having a large supply of high-energy floral nectar, honeybees are able to use short-amplitude, high frequency flight. The advantage for honeybees is, that during unladen flight, they can use a relatively short wing stroke amplitude. As they collect nectar and pollen, they increase their wing stroke amplitude (holding frequency constant), increasing wing tip velocity and total flight force production (Altshuler et al., 2005b). By using short-amplitude, high-frequency flight, honeybees are able to perform flight while maintaining a high level of power in reserve to perform ecologically important flight behaviors (Altshuler et al., 2005a).

During my research, *Syrirta pipiens* used high-frequency, low amplitude flight with stroke amplitudes ranging between 96° and 108° and frequencies of ≈ 220 Hz. This suggests that there is an abundance of high-energy food sources in their environments. It is probable that selection for flight performance is more dependent on environmental factors such as sexual selection, male-male

competition, and territorial defense than the conservation of energetic resources. Hoverflies have been shown to use complex visual tracking to maintain precise distances from other individuals of the same species. When tracking females, males will establish the correct distance and angle from the female before accelerating rapidly to the female to breed. The distance and head on angle is accomplished by the simultaneous modulation of axial rotations and multidirectional translational flight (Collett, 1980; Dudley, 2002).

Wing rotation has been found to contribute significantly to overall flight force production, and in particular, torquing forces that contribute to body rotations (Dickinson et al., 1993; Sane and Dickinson, 2002; Altshuler et al., 2005a; Ramamurti and Sandberg, 2007) The necessity for high levels of maneuverability, agility, acceleration, and deceleration performed by *S. pipiens* suggests that short-amplitude, high-frequency flight allows aerodynamic forces generated during the rotational phases to be redirected on a much smaller temporal scale, because wing rotations occur more frequently than when using long-amplitude, low-frequency flight (Altshuler et al., 2005b). It might also suggest that, as has been found in honeybees, a greater reserve in force production might be available when using short-amplitude, high-frequency flight by increasing wing stroke amplitude while holding frequency constant. Force generation during stroke reversal should increase due to the greater acceleration acting on the wing and the earlier timing of the stroke reversal. In addition, larger stroke amplitude increases the amount of force being generated during the translational phase of the wing stroke (Dickinson, 1994; Altshuler et al., 2005a).

The subsequent increase in force production coupled with more opportunities to redirect them, could account for the extremely high flight performance observed in syrphid flies.

The absence of stroke amplitude-mediated yaw rotations in *S. pipiens*, in contrast to *Drosophila*, illustrates that insects are capable of achieving complex flight maneuvers by utilizing differing suites of kinematic parameters that do not appear to be fixed among groups. Although *Drosophila* appears to use asymmetry in stroke amplitude to perform saccades, they also employ rotational forces generated during the transition between the down and up strokes (ventral-flip). In general, greater stroke amplitude results in increased force production during the translational phase of stroke due to increases in wing tip velocities. Increasing amplitude also changes the timing of the ventral-flip; the wings begin to rotate earlier in the stroke cycle as amplitude increases, also generating greater flight forces. Although increases in amplitude are closely tied to changes in the timing of the ventral-flip, *Drosophila* have shown a limited ability to decouple these two phases of the stroke cycle (Dickinson et al., 1993).

For insects using large wing stroke amplitude ( $>145^\circ$ ), maximum flight forces are generated during the midpoint of the translational phase, when wing tip velocities are highest (Altshuler et al., 2005a). For groups that use short wing stroke amplitude ( $<130^\circ$ ), accompanied with rapid stroke reversal and high wing beat frequencies, force peaks occur during the midstroke and at both transitional phases (Altshuler et al., 2005a). This supports the idea that high frequency-, short amplitude flight, like that used by *S. pipiens*, relies more heavily on the

rotational phase to produce lifting and torquing forces. If a more complete decoupling of stroke amplitude and the timing of the rotational phases was occurring in syrphid flight, this could explain the lack of consistent kinematic patterns observed during the translational phase. If forces generated during translation produced primarily lifting forces, and transitional phases produced primarily torque, this could explain how *S. pipiens* yaw rotations could occur in the absence of deviation in the wing kinematics that I investigated.

In summary, *S. pipiens* performed saccades that (1) took equal amounts of time to accelerate and decelerate through the turn, (2) took longer to accelerate than decelerate, and like *Drosophila*, (3) accelerated more quickly than they decelerated. In addition, none of the translational wing kinematic parameters measured showed any pattern that could be correlated to the initiation and termination of saccades. One possible explanation for the lack of kinematic patterns found in my research may be that *S. pipiens* have decoupled the translational and rotational phases of the wing stroke cycle and generate body torque primarily during the rotational (transitional) phases. To test this hypothesis, flies could be flown in a smaller flight arena, limiting translational movement, and saccades filmed. Analysis of wing kinematics would include measuring the timing of the initiation of wing rotation and compare the initiating of saccades with the timing of wing rotation. If asymmetry in the timing of wing rotation were found, then this would strongly support the hypothesis that body torque in *S. pipiens* is generated by asymmetry in timing of the transitional phases of the wing stroke cycle.

## CHAPTER 3

### THE EFFECTS OF SELECTION FOR DESICCATION RESISTANCE ON TAKEOFF FLIGHT PERFORMANCE IN *DROSOPHILA MELANOGASTER*

#### Introduction

The morphology and physiology of organisms is the result of a compromise of adapting to many environmental factors, such as locomotion, prey acquisition, predator avoidance, and reproduction, to name a few. For some organisms, certain environmental pressures can play a greater role in shaping the form of a population by favoring some characteristics while limiting the direction and magnitude of others. For most of the estimated 1 - 10 million insect species in existence today, flight is a critical component in at least some part of their life history.

Flight requires a specialized suite of morphological and physiological characteristics that can vary depending on flight requirements. For insects that operate at low Reynolds numbers ( $Re$ ), these characteristics may be further specialized by the utilization of unsteady aerodynamic force production, such as delayed stall, clap and fling, etc. (Dickinson and Gotz, 1993). For example, long distance, hovering, and high maneuverability fliers may exhibit very different characteristics based on their flight requirements (Brewer and Hertel, 2007). For species where life history traits such as predator escape or reproductive strategies (as well as other factors) require high levels of flight performance, flight may favor some evolutionary responses while placing limits on others.

Some of the most maneuverable fliers can be found in the order Diptera

(Collett and Land, 1975). With one pair of true wings and a pair of halteres, highly derived sensory organs tuned to the rotational forces created during flight, these advanced flyers have radiated into virtually every terrestrial habitat (Ashe et al., 1987). It is the ability of this group to adapt to many different environments, coupled with their relatively short life spans, that make them a preferred subject to study the effects of evolutionary pressures on flight.

In habitats where the threat of desiccation is prevalent, survival becomes a dominant pressure shaping the evolutionary process. In *Drosophila melanogaster*, physiological responses to desiccation selection include a reduction in water loss, increased glycogen content, and an overall increase in body mass (Gibbs et al., 1997; Gefen et al., 2006). *Drosophila melanogaster* could compensate for greater body mass by altering wing kinematics. By increasing wing stroke amplitude, increasing wing beat frequency, or by altering the timing of the wing rotation, they could increase flight force production. These compensatory mechanisms could offset increased body mass, and no reduction in takeoff flight performance would be evident. Flies could also change their flight behavior, only flying when winds are absent, at minimal velocities, or by flying in conjunction with wind direction. Desiccation adapted flies could also offset a greater flight load with a concurrent increase in wing size.

The increase in body mass, without concurrent evolutionary responses for greater flight performance, should result in an increased cost of flight and a reduction in takeoff flight performance. This chapter examines the effect of increased body mass, due to selection for desiccation resistance, on takeoff flight

performance of *D. melanogaster*.

## Methods

### *Selection*

The origins and development of the desiccation selected lines used in this research have been previously described in detail (Gefen et al., 2006). An overview of the selection process is as follows (Fig 1); (1) the founding population, from Terhune, New Jersey, was reared in the lab for 30 generations under no selection pressure before being randomly divided into 3 separate treatments (desiccation selected, starved control, and control lines) and further divided into three populations within each treatment; (2) the fed control treatment was reared with food and water being present during the entire adult life cycle; (3) starved control lines were reared with water but no food; (4) for 30 generations, desiccation selected flies were housed with a desiccating agent until  $\approx$  80 - 85% mortality was achieved, and the remaining flies were used as the founding population for the subsequent generation, (5) for  $\sim$ 100 generations the desiccation-selected flies were housed with the desiccating agent for 24h, and the remaining flies were used as the founding population for the subsequent generation. Flies from all three treatments were collected concurrently based on the status of the desiccation treatment flies.

Female flies were collected and sexed 6 - 8 hours post eclosion and housed in food vials until flight filming took place. Filming of flight sequences occurred between 4 and 6 days of age.



### *Flight*

Individual flies were transferred from food vials into a 200  $\mu$ l pipet tip modified to require the fly to climb out before taking off. This allowed time to close the flight arena and set the camera trigger before takeoff occurred. Flies were released into a 20 cm acrylic cube and given 10 – 15 s to take off before a visual expansion in the field of view of the fly (hand tap on the top of the flight arena) was introduced to elicit flight. If after 2 - 3 taps the fly did not take off, the trial was terminated and the fly replaced with a new randomly selected individual. Flight sequences were filmed using 2 orthogonally placed Phantom v5.1 (Vision Research) high-speed digital cameras at a spatial resolution of 768 x 768 pixels and a frame rate of 2100 fps.

The flight trajectory was digitized using DLTdataviewer4 (Hedrick, 2008), and a Savitzky-Golay filter was applied to the raw data (Walker, 1998). Processed data were analyzed using custom software I wrote for Matlab. Parameters calculated were: angle calculated between the point of take off to the end of flight; angle between the point of takeoff and the point of maximum elevation; maximum and mean velocity; maximum and mean horizontal velocity; maximum and mean vertical velocity. Wing beat frequency was also calculated during the initial takeoff, starting at the first down stroke and ending when the transition to forward flight was completed (body angle held constant).

### *Morphology*

Immediately following filming, flies were captured and anesthetized using CO<sub>2</sub> to confirm sex and then weighed using a Cahn microbalance. Flies were

then transferred to vials containing 70% ethanol and stored.

Wings were removed and placed on a micrometer slide and photos taken of a randomly selected wing. Wing circularity, the ratio of the length and width of an ellipse fitted to the wing perimeter, and wing area were measured using ImageJ 1.39 (Mac). Each image was calibrated using the scale bar on the slide.

### *Statistical Comparisons*

A one-way nested ANOVA was used to compare means, with selection treatments treated as fixed effects and replicate lines nested within selection as a random effect. Tukey's HSD tests were used for post-hoc comparisons of means.

## Results

### *Morphology*

**Circularity:** The TS line was found to be significantly lower ( $p < 0.01$ ) than either the TD or the TF lines, while the TD and TF lines did not differ (Fig. 3.2 a).

**Area:** The TF line was found to be significantly higher than the TD line, which in turn was significantly higher than the TS line (Fig. 3.2 b).

**Mass:** The TD line was significantly greater than either the TS or TF lines, which did not differ from each other (Fig. 3.2 c).

**Wing Loading:** The TD line was significantly greater than the TS line, which in turn was significantly greater than the TF line (Fig. 3.2 d).

### *Flight*

There was no significant difference found in mean or maximal velocity (Fig. 3.3 a, b), horizontal velocity (Fig. 3.3 c, d), or vertical velocity (Fig. 3.3 e, f).

In addition, end and maximum flight angles did not differ among groups (Fig 3.3 g, h).

### Discussion

Evolutionary responses to environmental pressures can often be measured as differences in morphological or physiological characteristics; however, the overall effects of these differences may not be easily understood, particularly in the case of complex behaviors such as flight.

Measurements of the wing (circularity and area) morphology and body mass did not reveal a clear pattern (Fig. 3.2 a-e). Although it is important to evaluate body mass and wing area separately to assess how they may be changing (or not changing) in conjunction with each other, wing loading (body mass/wing area) was used to predict changes in flight performance due to selection for desiccation resistance. The desiccation selected lines had a significantly higher wing loading that correlates with increased glycogen content (Gefen et al., 2006), the fuel used during insect flight. One might expect a reduction in flight performance in flies with an increased wing loading (Suarez et al., 2005; Gefen et al., 2006; Arrese and Soulages, 2010) but this was not found in the desiccation selected flies. To explain why a reduction in flight performance was not found, we must turn to the unsteady aerodynamic mechanisms used by insects operating at low Reynolds numbers.

Flight forces are the direct result of relative air speed over a wing (Hoerner, 1965; Hoerner and Borst, 1975), and deviation in flight performance is primarily the result of the combined effects between wing beat frequencies and

wing stroke amplitude (Walker, 1998; Dudley, 2000; Card and Dickinson, 2008; Fontaine et al., 2009). Deviations in either of these parameters, and to a lesser degree other kinematic patterns, result in changes in the direction and magnitude of flight forces being produced. Increases in either factor result in an increase in total flight force production (Sane and Dickinson, 2001; Floreano, 2009).

*Drosophila melanogaster* have been shown to increase force production by increasing wing stroke amplitude while holding frequency constant (Dickinson, 1994), increasing wing tip velocity during the translation phase and rotational velocities during the transition phases of the stroke cycle (Altshuler et al., 2005b), but this greater force output results in an increase in the energetic cost of flight.

In this study, the significantly higher wing loading measured in the desiccation resistant flies did not result in a measurable reduction in takeoff flight performance. The probable explanation for this is that an increase in force production, in the form of increased stroke amplitude, was used to maintain flight performance. The spatial scale over which these takeoff sequences occurred could also play an important role in the nonsignificant differences in flight performance. Ranging from 0.34 – 0.73 s, these sequences occurred over a relatively short period of time. With greater reserves of glycogen present, desiccation selected flies may have increased flight metabolism to maintain takeoff performance by operating at near maximal levels. This increase in the cost of flight, while masking the negative effect of higher wing loading over the distances traveled in the lab, should affect flight performance at greater distances and over longer flight times. For example, capture and release experiments

conducted using *Bactrocera tryoni*, found that these flies could be detected at distances of 400 m from the release site, with greater recapture rates in areas in line with the principal wind direction (Dominiak et al., 2003). It may be that desiccation selected flies in nature could be limited to substantially smaller ranges when searching for food, or be limited in flight direction by only traveling in conjunction with the prevailing winds.

In summary, the desiccation selected flies did not show any differences in any of the flight performance parameters measured, that would be predicted by an increase in wing loading. The lack of differences in take-off flight performance could not be associated with any differences in morphology, but may be the result of the desiccation selected flies operating at higher flight force output. To test this hypothesis, the three treatment lines could be flown in hypodense / normoxic environments (helox gas). If desiccation selected flies were operating at higher flight force output (greater wing stroke amplitude), then they should show a reduction in flight performance in air densities lower than normal but higher than would be necessary to reduce performance in the control lines.

Figure 3.1

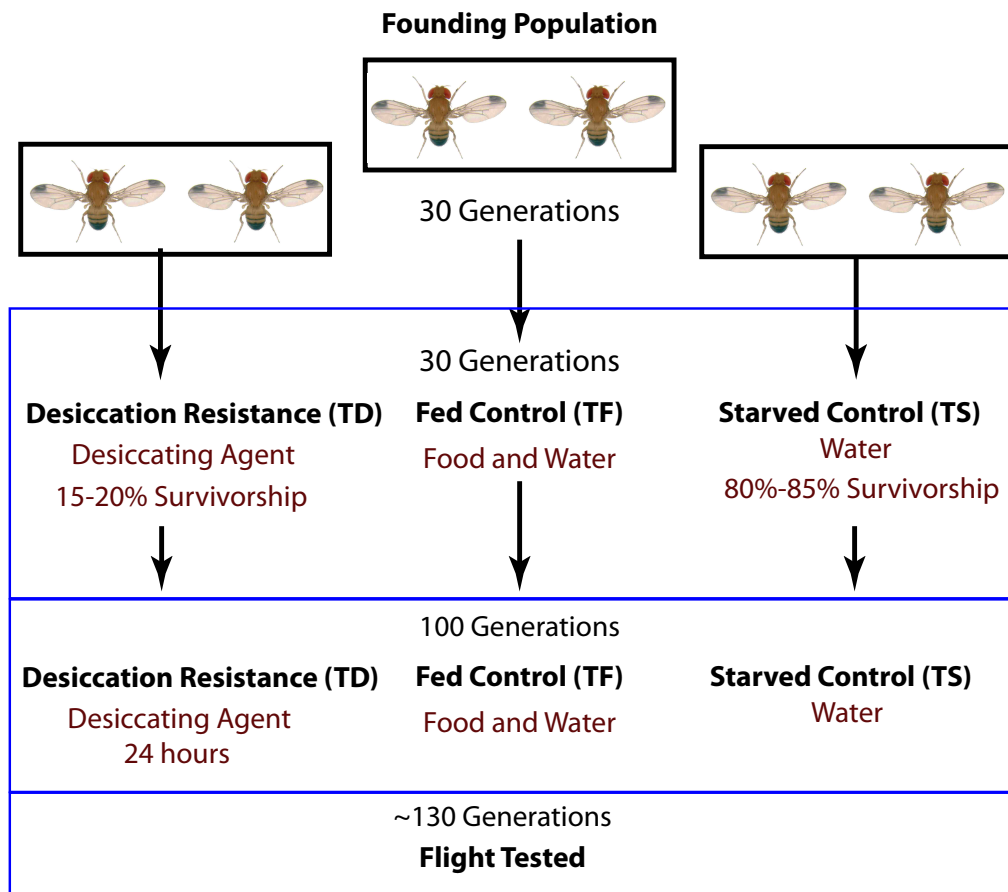


Figure 3.1. Illustration of the selection process used to establish the desiccation selected, fed- and starved-control lines.

Figure 3.2

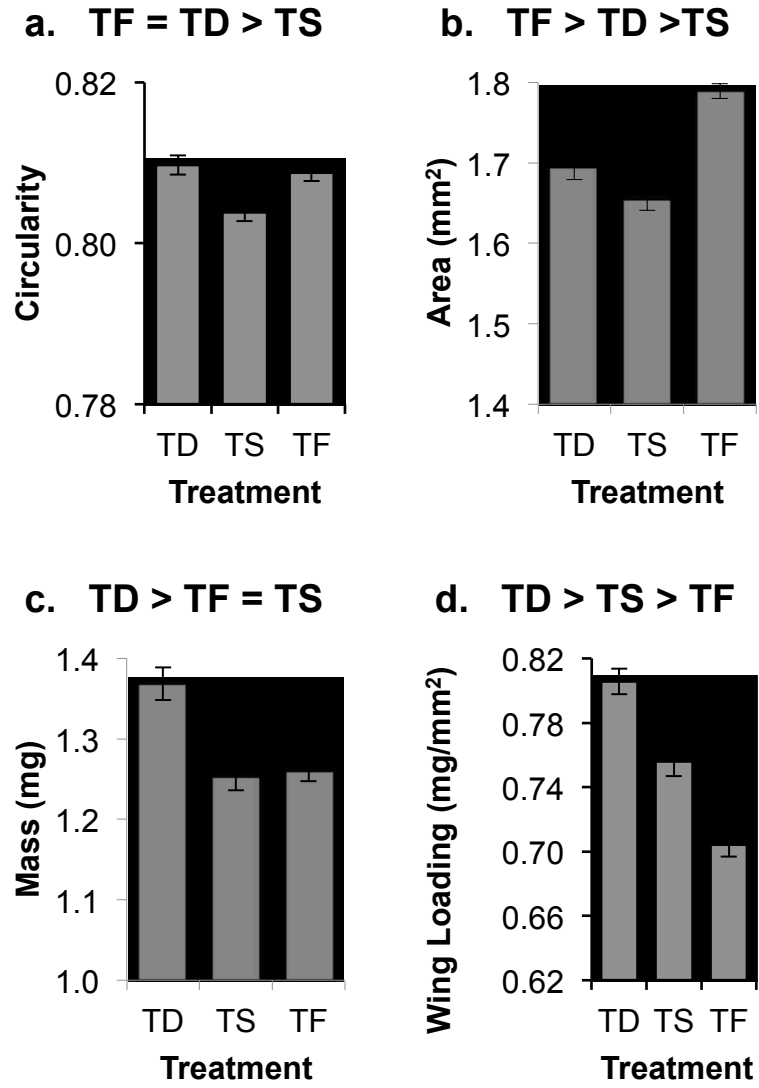


Figure 3.2. Morphological measurement of desiccation-selected flies and their controls. Significant differences between groups are denoted by '>' while non significant outcomes are denoted by the '=' symbol.

Figure 3.3

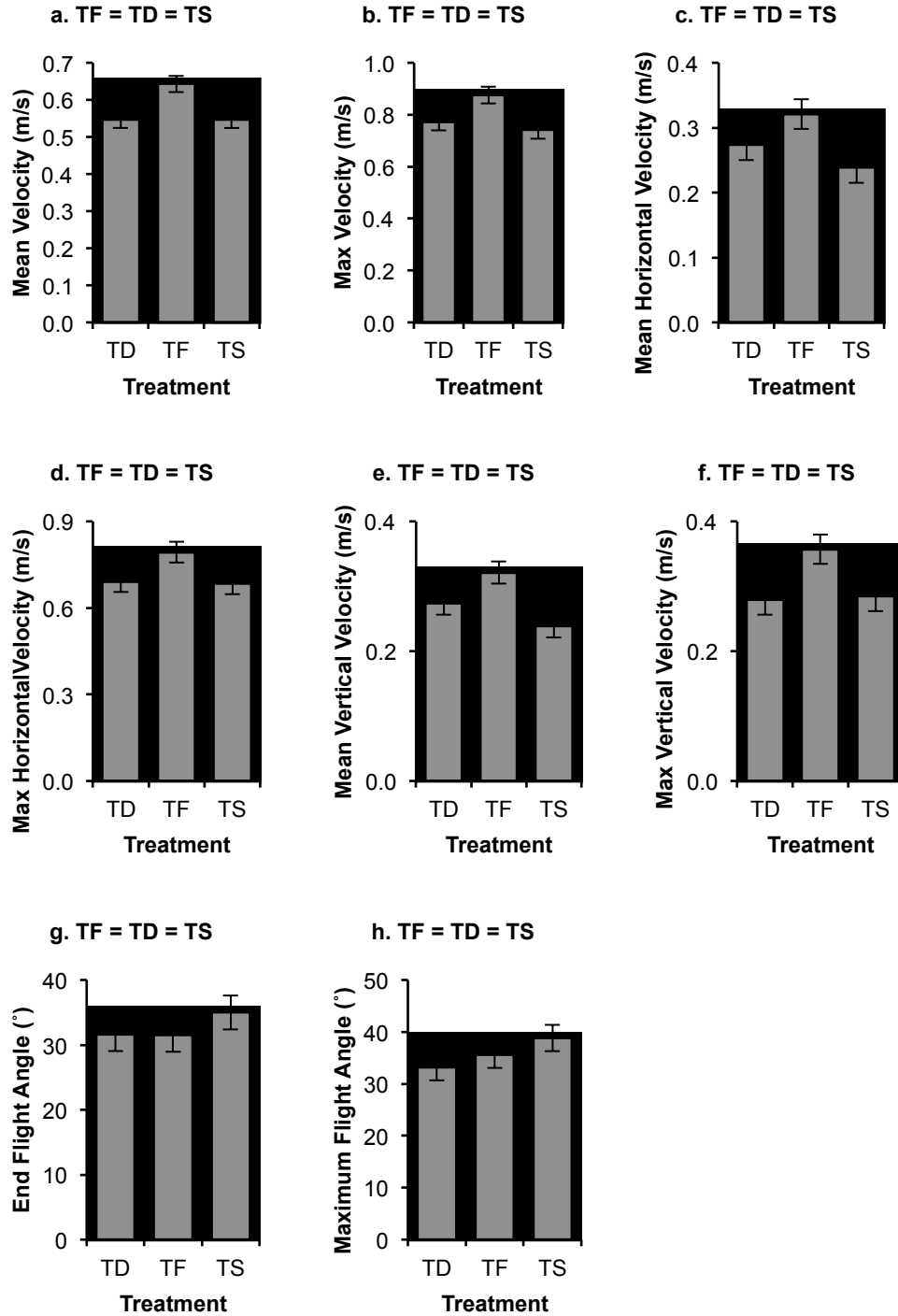


Figure 3.3. Flight perimeters measured in desiccation-selected flies and their controls. Perimeters measured were; mean and maximum velocity (a, b), mean and maximum horizontal velocity (c, d), mean and maximum vertical velocity (e, f), end flight angle (g), and maximum flight angle (h). Significant differences among groups are denoted by '>' while non significant outcomes are denoted by the '=' symbol.



## CHAPTER 4

### THE EFFECTS OF SELECTION FOR STARVATION RESISTANCE ON TAKEOFF FLIGHT PERFORMANCE IN *DROSOPHILA MELANOGASTER*

#### Introduction

It can be argued that the primary function of any species is reproduction. The strategies for reproductive success can be highly variable, even within similar taxonomic groups. For insects, in which adult life spans can be as short as 10 min, as in the mayfly *Dolania americana*, the opportunities for reproductive success can be limited (Sweeney and Vannote, 1982). In addition, the smaller size of insects can put them at a greater risk to acute changes in frequency, quality, and quantity of resources. For flying insects, whose energetic cost of locomotion can be much greater than larger animals, additional stress is placed on them to find enough resources to be reproductively successful (Schmidt-Nielsen, 1972).

Dipterans represent a group of insects that are highly evolved for flight (Chan et al., 1998). The high flicker rates of their compound eyes and the transition of rear wings into halteres, highly derived sensory organs sensitive to the rotational forces produced during flight, have allowed them to radiate into virtually every terrestrial (and some aquatic) habitats (Ashe et al., 1987; Convey and Block, 1996; Yeates and Wiegmann, 2005). It is the ability of Dipterans to expand into some of the most extreme habitats, coupled with relatively short life spans that make them of great interest for research into the interaction between

the reactive response to environmental stress and the subsequent effect on life history traits.

In habitats where the threat of starvation is prevalent, survival becomes a dominant pressure shaping the evolutionary process. In *Drosophila melanogaster*, the primary response to starvation is an overall increase in lipid content (Marron et al., 2003), increasing the load the flies must carry during flight. Flight requires the balance between the mass of animal and any additional payload (pollen, nectar, etc), flight metabolism to produce the muscle contractions that result in wing motion, and the wings that produce lift and thrust (Hoerner and Borst, 1975; Lehmann and Dickinson, 1998; Dudley, 2000). As mass increases, a corresponding increase in flight forces must be produced to offset the greater payload. Flight forces increase proportional to wingtip velocity, and greater force production could be accomplished by increasing the rate of muscle contraction (wing beat frequency), the length of muscle contraction (wing stroke amplitude), or by increasing wing size (Lehmann and Dickinson, 1998; Altshuler et al., 2005b). *Drosophila* increase flight force production by increasing wing stroke amplitude, resulting in a corresponding decrease in wing beat frequency, but with an overall increase in wing tip velocity (Lehmann and Dickinson, 1998). If the increased lipid content found in starvation selected flies reaches a level where flight metabolism and wing amplitude can no longer be modulated to produce additional flight forces, flies selected for resistance to starvation should show decreased flight velocities and lower flight angles during

takeoff. This chapter examines the effect of selection for starvation resistance on takeoff flight performance of *D. melanogaster*.

## Methods

### *Selection*

The starvation selected and fed control lines were derived from the starved control and fed control lines described in the previous chapter. After 70 generations of light selection (starvation time equal to that of desiccation stress for its paired desiccation-selected population; 10-15% mortality each generation), the starvation selected flies were transferred to a strong selection regime. For 30 generations, the starvation selected flies were subjected to ~80 - 85% mortality before collecting the founding population for the next generation. During the selection process, founding populations for the fed control lines were collected at the same time as the starvation selected flies.

Female flies were collected and sexed 6 - 8 hours post eclosion and housed in food vials until flight filming took place. Filming of flight sequences occurred between 4 - 6 days of age.

### *Flight and Morphology*

Flight and morphology techniques are exactly the same as described in chapter 3.

## Results

### *Morphology*

No significant differences were found in circularity (wing shape) or wing area between the starvation selected and control lines (Fig. 4.2 a, b). The

starvation selected flies, however, were found to be significantly heavier. This resulted in a significantly higher wing loading (Fig. 4.2 c, d).

### *Flight*

The overall mean and maximum flight velocities were calculated for each fly and the velocities compared between the starvation selected and control lines. No significant differences were found in total or horizontal flight velocities (mean and maximum) between the starvation selected and control lines (Fig. 4.3. a-d). When vertical velocities were compared, it was found that the starvation selected flies had significantly lower mean and maximum velocities.

The maximum flight angle, measured from the point of takeoff to the point of maximum elevation, was also found to be significantly lower for the starvation lines (Fig 4.3 g). The end flight angle, measured from the takeoff point to the end of the flight sequence, was found to be significantly smaller in the starvation lines (Fig 4.3 h).

Flight paths for the starvation selected lines also showed that the end flight angle was smaller than the maximum flight angle ( $14.8^\circ$ ,  $21.6^\circ$  respectively, Fig 4.4). The control lines showed maximum flight angles and end flight angles that were nearly identical ( $32.2^\circ$ ,  $31^\circ$  respectively, Fig 4.4). The starvation selected lines achieved maximum elevation earlier in the flight, at  $\approx 74\%$  of the total flight time, while the control lines achieved maximum elevation at  $\approx 94\%$  of the total flight time (Fig 4.4).

## Discussion

Starvation selected flies showed a significant reduction in flight performance that was restricted primarily to the vertical plane and presented as lower vertical flight velocities and trajectories skewed toward the horizontal plane. Lower flight angles and vertical flight velocities are consistent with the hypothesis that higher wing loading results in a higher energetic cost of flight and a reduction in flight performance (Norberg, 1990). However, results from the desiccation selected flies (Chapter 3) show that increased wing loading does not necessarily result in a change in flight performance. When comparing wing loadings between the starvation and desiccation selected flies, no difference in wing loading was found. Because of this, other factors must be considered to explain the reduced flight performance of the starvation selected flies. These factors may be found by considering the unsteady aerodynamic mechanisms that insects use during flight and by considering current research into the physiological changes that occur due to selection for starvation resistance (Hardy and Gibbs, personal communication).

Higher wing loading typically results in an overall increase in the cost of flight (Norberg, 1990; Dudley, 2000; Hedenström, 2002). Flight forces are the result of the combined effects of wing stroke frequency, amplitude, wing rotation, and to a lesser degree other kinematic parameters such as stroke plane and stroke plane deviation. For honeybees using high-frequency / low wing stroke amplitude wing kinematics, increases in flight forces are generated by holding wing beat frequency constant while stroke amplitude is increased, resulting in

greater wing tip velocities during translation and increased rotational velocities during the transition phases. During hovering flight, the starting wing stroke amplitude of honeybees is  $\approx 90^\circ$ ; as nectar and pollen are collected and wing loading increases, they produce additional flight forces by increasing stroke amplitude (while holding frequency constant), resulting in an increased wing tip velocity during the translation phase and greater rotational velocities during the rotational phases of the stroke cycle. It has been shown that a limit in amplitude is reached at  $\approx 132^\circ$ , after which no additional flight forces can be produced and any additional mass results in the bee's inability to maintain flight (Altshuler et al., 2005a). This maximum in flight force production has also been shown in hummingbirds and suggests that strict limits in maximal force production can restrict flight ability. For example, hummingbirds living at high elevation must produce greater flight forces, due to the lower air density, to perform the same maneuvers that they would at lower elevations (Altshuler and Dudley, 2003; Altshuler et al., 2004). This results in a greater cost of flight by operating at higher levels of force production and translates to a smaller reserve in force production to perform important flight behaviors such as hovering, maneuvering, and accelerating (Altshuler and Dudley, 2003; Altshuler et al., 2004). For starvation selected flies, with a significantly greater wing loading, they may not have the reserve output necessary to takeoff at relatively steep angles exhibited by control lines; limiting them to a more horizontal takeoff.

Lift is the direct result of airspeed over an airfoil; higher relative wing speed increases lift production (Hoerner and Borst, 1975). During hovering,

airflow over the wings is limited to the rate at which the wings can oscillate, resulting in a much more costly form of flight. During forward flight, wind velocity over the wings is the combination of relative ground speed, wind speed (which can increase or decrease flow depending on wind direction), and wing beat frequency (Hoerner, 1965; Hoerner and Borst, 1975; Dickson and Dickinson, 2004; Lehmann et al., 2005; Lehmann and Pick, 2007). By limiting the vertical flight component, starvation selected flies may be able to maintain similar airflow over the wings, resulting in overall and horizontal flight speeds comparable to those measured in the control lines. It's probable that they are operating closer to their maximal flight force output and have less capacity to generate additional flight forces, resulting in a smaller flight angle and lower vertical flight velocities.

*Drosophila* have been shown to perform two distinct types of takeoff flights, a voluntary takeoff and an escape flight that is a stereotypical behavior elicited by a visual stimulus (Card and Dickinson, 2008; Fontaine et al., 2009). Voluntary takeoffs occur with a high level of control over the flight path and occur with few deviations in the flight path. Escape flights typically have greater rates of translations and rotations about all three body axes that, on a small scale, result in a highly variable trajectory (Card and Dickinson, 2008). Approximately 90% of the flights from the starvation selected and the fed controls were escape flights, requiring a visual stimulus before the flies would take off.

Under natural conditions, predation rates might not be compromised in the starvation selected flies because successful predator avoidance may rely more on overall flight velocity, time from threat recognition to response, and the flight

pattern of the escape flight rather than on the takeoff angle. By reducing the takeoff flight angle, starvation selected flies may maintain overall flight velocities while maintaining a reserve in flight forces for small-scale deviations in translations and rotations that result in the unpredictable escape flight and could lead to a predation rate similar to non-selected flies.

Starvation selected flies have high levels of body fat (Hardy and Gibbs, personal communication), which may limit their ability to disperse to habitats with greater resources. *Drosophila's* small size already limits their ability to disperse, and starvation-selected flies may be further hampered by their reduction in flight performance. This may restrict their ability to fly against prevailing winds and, in the absence of wind, to fly for any great distance when compared to non-selected flies. Flight trajectories for flies dispersing from resource-poor environments may be limited to paralleling prevailing wind direction. In doing so, starvation resistant flies may be able to travel similar distances as non-adapted flies, but deviations from wind direction may still be severely compromised.

In summary, starvation selected flies showed a reduction in mean and maximal vertical flight velocities and in the end and maximal flight angles. This could be explained by the increase in wing loading that resulted from the increase in body mass, but the increase in wing loading was nearly identical to that found in the desiccation selected flies, which did not result in a reduction in take-off flight performance (Chapter 3). One possible explanation for this result could be the additional mass of the starvation selected flies was in the form of increased fat deposits in the thorax. This increased fat deposition may impede



cardiac output and could explain the reduction in flight performance found in the starvation selected and not the desiccation selected flies.

Initial research (Hardy and Gibbs, personal communication) has shown that cardiac function returns after thorax fat deposits are exhausted. To test the hypothesis that reduced flight performance is the result of reduced cardiac function, two sets of starvation selected flies could be flown 4-6 days post eclosion. After the initial flight trial, one group of flies would be housed on food and the other group starved until fat deposits were exhausted. If flight performance increases in the starved flies, it would support the hypothesis that reduced flight performance in the starvation selected flies is the result of impeded cardiac function and not simply due to the increase in wing loading.

Figure 4.1

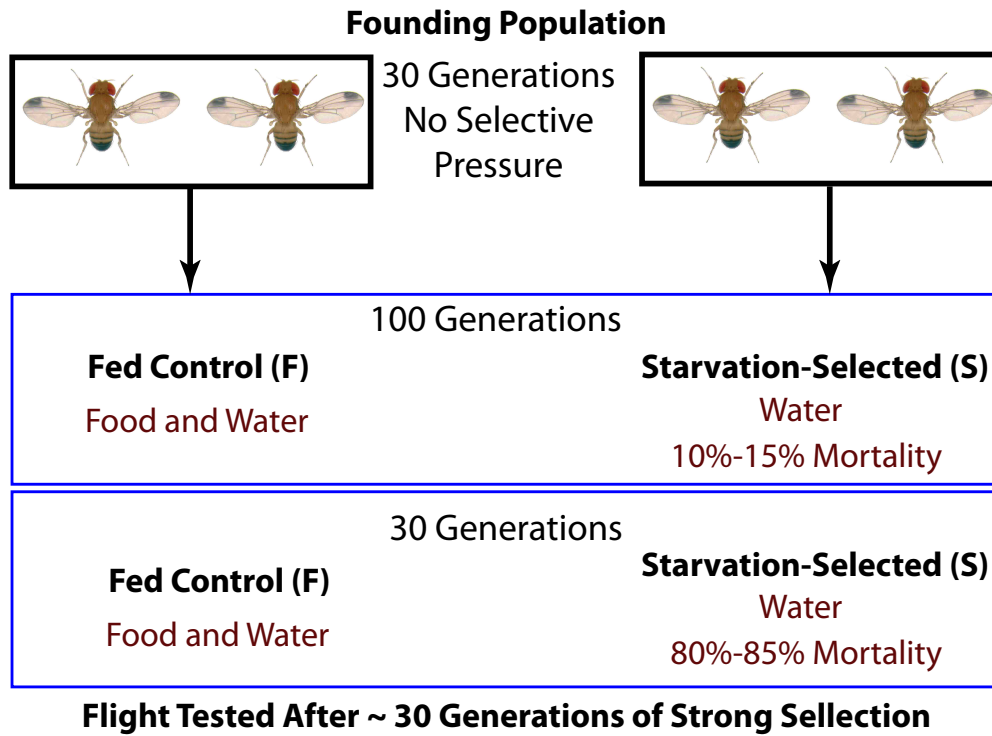


Figure 4.1. Illustration of the selection process used to establish the starvation selected, fed control lines.

Figure 4.2

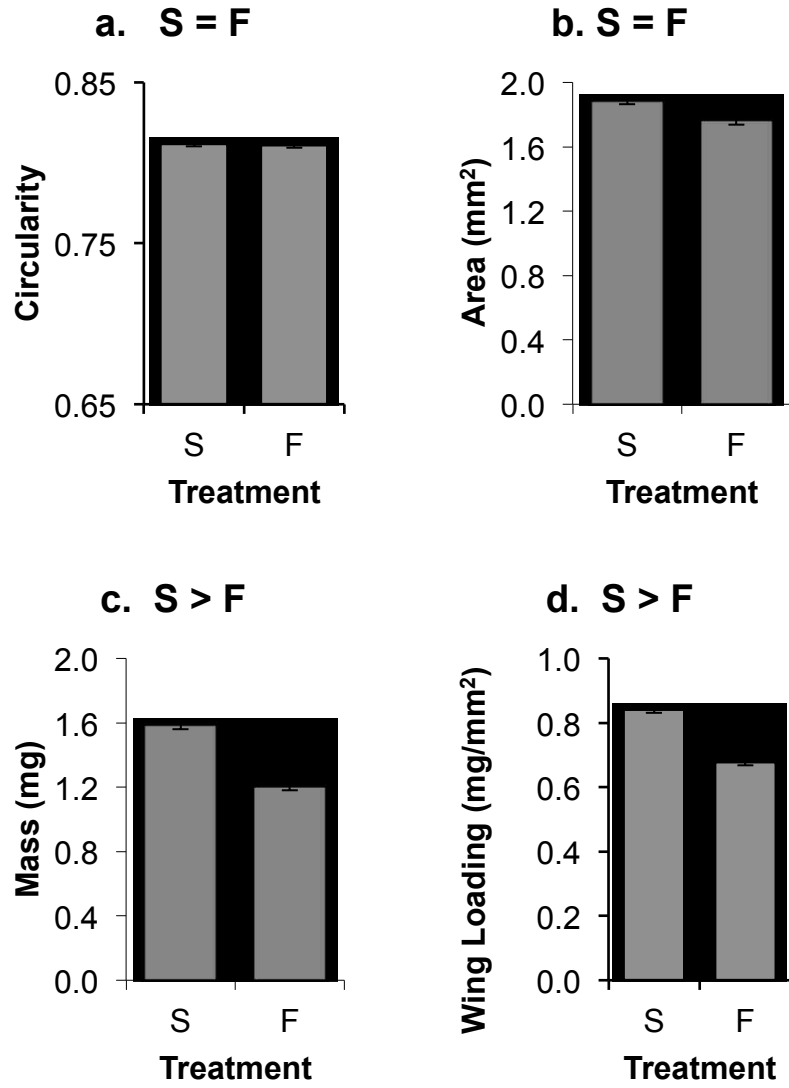


Figure 4.2. Morphological measurements of starvation selected flies and their controls . Significant differences between groups are denoted by '>' while non significant outcomes are denoted by the '=' symbol.

Figure 4.3

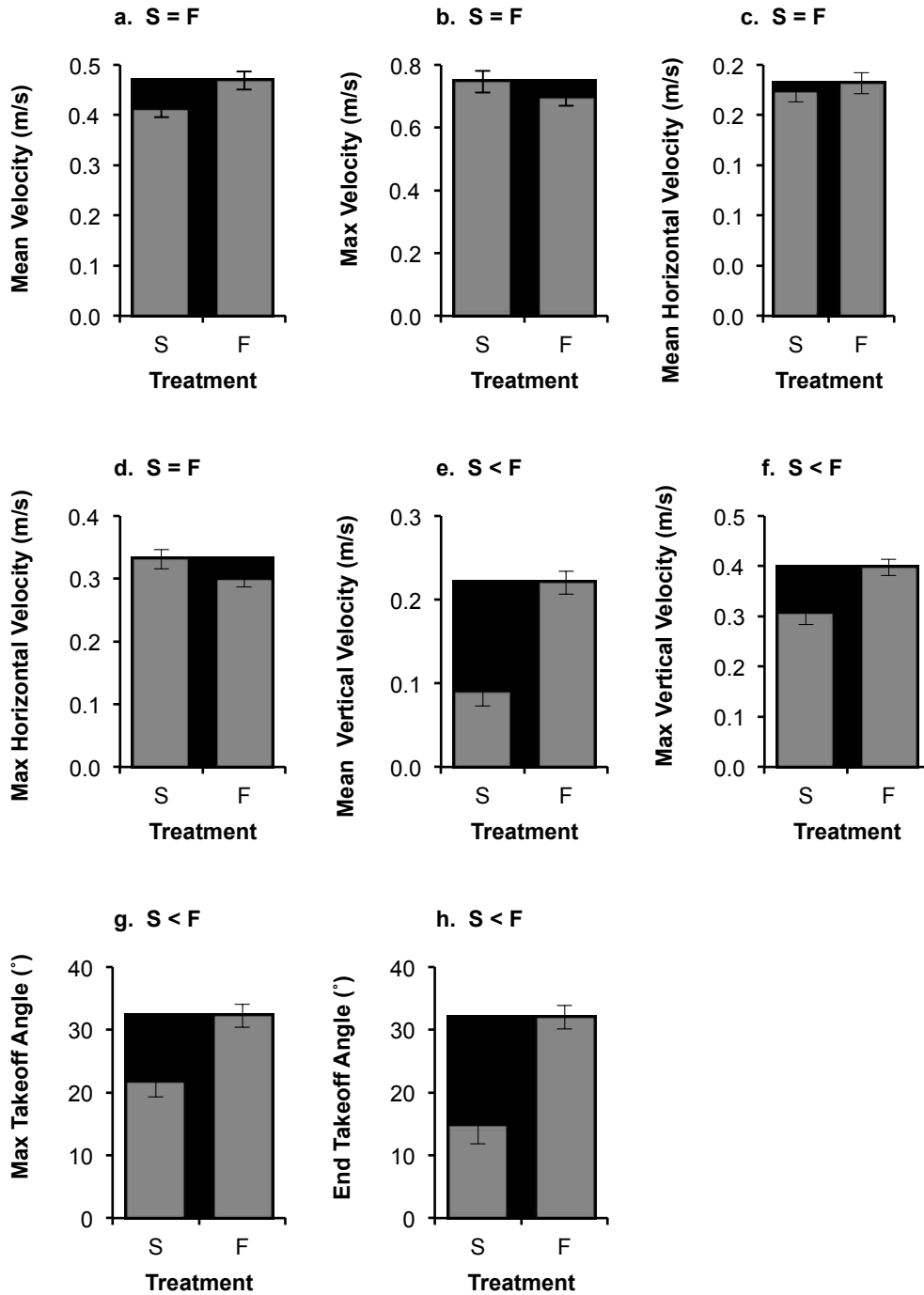


Figure 4.3. Flight perimeters measured from starvation selected flies and their controls. Perimeters measured were; mean and maximum velocity (a, b), mean and maximum horizontal velocity (c, d), mean and maximum vertical velocity (e, f), end flight angle (g), and maximum flight angle (h). Significant differences among groups are denoted by '<' while non significant outcomes are denoted by the '=' symbol.

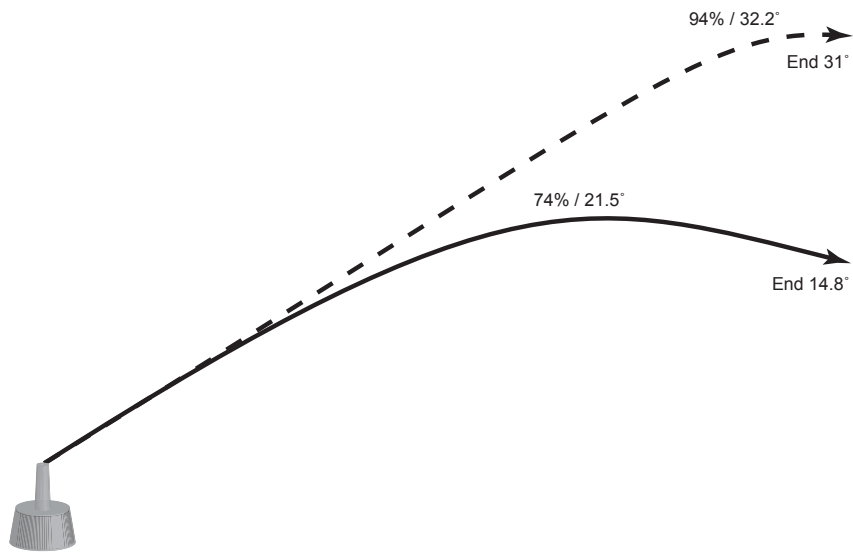


Figure 4.4. Approximate flight paths of the starvation-selected (solid line) and fed-control populations (dashed line), showing the end angle and the point of maximum flight angle (angle and percent of flight)

## CONCLUSION

In 1934, using calculations based on fixed wing aerodynamic theory, August Magnan determined that bumble bees are unable to generate the minimum forces necessary for flight (Magnan, 1934). Since that time, we have discovered that one of the most important mechanisms used by insects to generate lift is the formation of leading edge vortices (Dickinson and Gotz, 1993; Ellington et al., 1996; Dickinson et al., 1999). The oscillating stroke cycle that insects use during flight requires the wings to rapidly rotate at the end of each half-beat, and results in the leading edge vortices staying attached to the wing at much higher angles of attack. This in turn delays the point of stall (vortex separation from the wing), significantly increasing lift production (Ellington et al., 1996). The use of unsteady aerodynamic mechanisms, like delayed stall, illustrates the importance of the rotational phase of the wing stroke cycle in insect flight. It also helps to illustrate that wing kinematics can differ depending on the flight requirements of the species (Ellington, 1984).

In my research I attempted to show that the hoverfly, *Syrirta pippins*, used the same kinematic mechanism (asymmetrical stroke amplitude) that has been previously discovered in *Drosophila*. However, the lack of pattern in translational wing kinematics did not fit what had been found in other flying insects and suggests that *S. pippins* may rely more heavily on the rotational phase of the stroke cycle than has been previously found for other insects. The complete decoupling of the translational and rotational phases of the stroke cycle could

allow *S. pippins* to modulate body torque production separately from lift and thrust production.

Research has shown that vertical component of the flight path (ascending flight) in prey birds plays a significant role in predator avoidance (Hedenstrom and Rosen, 2001). Additionally, net flight speed is inversely correlated to predation rates in neotropical butterflies (Chai and Srygley, 1990). Combined, this suggests that flight path and flight speed are both important factors in avoiding predation. The small size of insects, such as *Drosophila*, allows them to take-off and ascend at steeper angles than could be achieved by their typically larger predators, and thus such take-offs have a selective advantage during predator avoidance (Hedenstrom and Rosen, 2001). Higher ratios of flight muscle to relative body mass typically results in increased flight performance and increased predator avoidance, however, the small size of insects make them highly susceptible to increases in body mass that lower this ratio (Srygley and Dudley, 1993; Almbro and Kullberg, 2008).

Desiccation and starvation resistant flies have increased body mass with the addition of glycogen and fat respectively, increasing their wing loadings and possibly lowering their flight muscle ratios. The predicted effect of increased body mass should be a reduction in flight performance and result in lower flight speeds and trajectories during take-off flights. The starvation resistant flies did show reductions in vertical flight velocities and lower take-off trajectories,

whereas the desiccation-selected flies did not show a difference in flight performance from their control groups.

The difference in take-off flight performance between the desiccation resistant flies, starvation resistant flies, and their respective control groups is most likely due to the location and the composition of added mass. Some of the additional fat accumulated by the starvation resistance flies is located in the thorax and may inhibit cardiac function, resulting in lower flight metabolism and reduced energetic output (Hardy and Gibbs, personal communication). The desiccation selected flies have increased glycogen content and possibly lowered flight muscle ratio, but can operate at near maximal flight metabolic rates due to the abundance of readily available fuel. This process of offsetting increasing fuel load with the addition of readily metabolizable substrates can also be found in migrating birds (Klaassen, 1996; Guglielmo, 2010). For non-soaring birds, pre-migration body mass is increased by addition of relatively large amounts of fat. Unlike mammals, they are able to utilize this energy-dense substrate during flight because of their high capacity for fatty acid transport. This allows birds to migrate for extended distance and over long periods of time even with a significant increase in the cost of flight (Klaassen, 1996; Guglielmo, 2010).

The ability of insects to use different flight kinematic parameters to modulate flight force production, coupled with their relatively short life spans that allow evolutionally responses to environmental stresses to occur relatively quickly, has likely played a significant role in the success of flying insects. The



goal of my research was to first look at the wing kinematics that allow for some insects to fly at extremely high levels of performance, by investigating mechanisms used by hoverflies to redirect flight force production, allowing them to change direction over extremely small temporal spans. I also investigated how environmental stress, in the absence of any selection for flight, impacted take-off flight performance.

*Syritta pipkins* showed no patterns in the wing kinematics that could be associated with the production of body torque, distinguishing them from the other species that have already been studied. Future research into the flight of this species should include a comprehensive analysis of both the translational and rotational phases of the wing stroke cycle to provide a complete picture of how wing rotation produces the yaw torque necessary for the initiation and termination of saccades.

Flies that underwent selection for resistance to desiccation showed no difference in take-off flight performance from their control groups. This could be the result of them operating at near maximal flight force output or that, in addition to glycogen, desiccation resistant flies also increased the amount of flight muscle. Additional research should include an analysis of body composition that includes the amount of flight muscle. In addition, flights should be filmed over longer distances to evaluate if reduction in flight performance for desiccation resistant flies occurs only during longer flights.

Starvation resistant flies did show reductions in take-off flight performance that were likely due to the addition of fat in the thorax that not only increased body mass but also may have reduced cardiac output. Current research into the effects of fat accumulation on cardiac output of starvation selected flies may confirm that flight performance is limited by the quantity and location of stored fat (Hardy and Gibbs, personal communication).

Future research projects will help to further explain the results of my research presented here. By examining the rotational phases of the *S. pipiens* saddaces in conjunction with the translational phase of the wing stroke cycle, an explanation may be found as to how this group of Dipterans generates body torque. By researching takeoff flight in a hypodense / normoxic environment, a greater understanding of how desiccation selected flies maintain flight performance in the face of increased wing loading. And finally, by testing starvation selected flies post eclosion and after the reduction of thoracic fat deposits, a better understanding of what mechanism (higher wing loading, cardiac dysfunction) is responsible for the reduction in flight performance found in the starvation selected flies.

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Dissertation Title: Kinematic Analysis Of Axial Rotations And The Effects Of Stress Selection On Takeoff Flight Performance

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