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Experimental and natural variation in hovering flight capacity in bees, Hymenoptera: Apidae

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EXPERIMENTAL AND NATURAL VARIATION IN HOVERING FLIGHT

CAPACITY IN BEES, HYMENOPTERA: APIDAE

By

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2000

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ABSTRACT

Experimental and Natural Variation in Hovering Flight Capacity in Bees, Hymenoptera: Apidae

By

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In honey bees, the capacity for flight underlies many behaviors which impact fitness and longevity, such as the ability to forage or evade predators. However, flight capacity is not fixed across bees' lifespan, which is punctuated by a suite of physiological changes that accompany age and the transition from in-hive to foraging behaviors; thus, flight capacity may vary during periods of development, senescence, or in response to morphological damage such as wing wear. This dissertation describes the biomechanics and aerodynamics which contribute to the scope of honey bee flight performance, and investigates how age, behavioral development, and wing-wear affects flight kinematics and maximal flight capacity. Three experiments were performed using high-speed (4347-6000 fps) digital videography and variable-density atmospheres, ranging from air (79%N₂, 21%O₂; 1.41 kg m⁻³) to heliox (79%He, 21%O₂; 0.41 kg m⁻³): 1) the detailed kinematics of honey bee foragers during hovering in air, performing simple enhanced-lift maneuvers, and hovering in heliox were compared; 2) I investigated the effects of age and behavioral development on the kinematics and flight capacity of honey bee

nurses and foragers; 3) I investigated the effects of symmetric and asymmetric experimental wing wear on the kinematics and flight capacity of honey bee foragers. Mature foragers vary aerodynamic force production almost exclusively by modulating wing stroke amplitude and holding wingbeat frequency constant, which yields greater wing angular velocities. Young (precocious) foragers and over-aged foragers increase stroke amplitude when challenged, however they are unable to maintain wingbeat frequency. Thus, their maximal flight capacity is impaired due to decreased wing angular velocity, relative to typical-aged foragers. Nurse bees demonstrate impaired kinematics similar to young foragers, but they are constrained by heavier body masses which further limits maximal flight capacity. Bees maintained robust flight in air in response to loss of wing area (wing wear), however maximum wingtip velocity and maximal flight capacity decreased in direct proportion to wing area. Bees with asymmetric wear produced lower maximum wingtip velocity than non-worn and symmetrically-worn groups, and despite less total wing area loss than the symmetric group, asymmetric wear caused a greater impairment in maximal flight capacity.

TABLE OF CONTENTS

ABSTRACT.....	iii
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
PREFACE.....	ix
CHAPTER 1 INTRODUCTION.....	1
Purpose.....	1
The Honey Bee Model System.....	3
Kinematic Mechanisms of Varying Aerodynamic Forces.....	4
The Effects of Age and Behavioral Development on Flight Performance.....	6
The Effects of Wing Wear on Flight Performance.....	7
CHAPTER 2 KINEMATIC MECHANISMS OF VARYING AERODYNAMIC FORCES.....	9
Abstract.....	9
Introduction.....	10
Materials and Methods.....	12
Results.....	14
Discussion.....	17
CHAPTER 3 THE EFFECTS OF AGE AND BEHAVIORAL DEVELOPMENT ON FLIGHT PERFORMANCE.....	27
Abstract.....	27
Introduction.....	28
Materials and Methods.....	31
Results.....	35
Discussion.....	43
CHAPTER 4 THE EFFECTS OF ARTIFICIAL WING WEAR ON FLIGHT PERFORMANCE.....	51
Abstract.....	51
Introduction.....	52
Materials and Methods.....	55
Results.....	58
Discussion.....	66

REFERENCES	72
VITA.....	81

LIST OF TABLES

Table 2.1	Kinematic maxima	16
Table 2.2	Results of one-way ANOVA for kinematics	16
Table 2.3	Results of two-way ANOVA for kinematics	17
Table 3.1	Results of MANCOVA for flight performance	36
Table 4.1	Honey Bee Morphology.....	58
Table 4.2	Results of MANCOVA for flight performance	59

LIST OF FIGURES

Figure 2.1	Wing stroke reversals and body angle.....	18
Figure 2.2	Mean kinematic patterns.....	19
Figure 2.3	Mean wingtip trajectory	20
Figure 2.4	Mean kinematic patterns.....	21
Figure 2.5	Aerodynamic forces.....	22
Figure 3.1	Maximal flight capacity vs. age.....	37
Figure 3.2	Maximal flight capacity vs. mass	38
Figure 3.3	Flight kinematics vs. mass.....	39
Figure 3.4	Flight kinematics vs. age	40
Figure 3.5	Flight kinematics for nurses	42
Figure 3.6	Maximal flight capacity vs. wing angular velocity	42
Figure 4.1	Maximal flight capacity vs. wing area.....	60
Figure 4.2	Wingbeat frequency vs. wing area.....	61
Figure 4.3	Wing stroke amplitude vs. wing area	62
Figure 4.4	Wing angular velocity vs. wing area	64
Figure 4.5	Wingtip velocity vs. wing area	65

PREFACE

“Tout d'abord pouss'e par ce qui fait en aviation, j'ai applique' aux insectes les lois de la resistance del'air, et je suis arrive' avec M. Sainte-Lague a cette conclusion que leur vol es impossible.” August Magnán (1934).

Translated: “First prompted by the fact of aviation, I have applied the laws of the resistance of air to insects, and I arrived, with Mr. Sainte-Lague, at the conclusion that their flight is impossible.”

“Aeronautics in its traditional form is usually presumed to have started as an engineering discipline somewhere in historical time between the mythological experiments of Daedalus and his ill-fated son, Icarus; and the dreams and schemes of Leonardo da Vinci during the Italian Renaissance, which eventually led to the Wright brothers’ success a century ago... “aeronautics” has a far richer and longer (though less disciplined) history extending over a period of about 300 million year beginning with the evolution of the ability of insects to fly. With the advent of the success of the early 20th Century pioneers, technologists quickly turned their attention from the inspirations and lessons provided by natural models of flying machines to a more practical quest for increasingly dramatic improvements in speed, range and altitude performance, far beyond the limits of what muscles and flapping wings could provide. Thus a field of further productive inquiry was left to a few amateur aeronauts, eccentrics and biologists.” John McMaster (2003).

I suppose my interest in ‘how stuff works’ has never been questioned, from my earliest experiments with bobby-pins and the electrical-outlet as a young child, to dismantling every motorized toy I was ever given, to dismantling almost every car I’ve owned (much to the chagrin of my wife and advisor). And probably because of these inclinations, the earlier years of my doctoral studies were challenged by my tendency to focus on intricate mechanisms of insect flight rather than the ‘big picture’. I would like to acknowledge those people who facilitated my interests and ultimately guided my professional development from that of an eccentric, simply fascinated by the mechanisms of flight, to a biologist. First, I thank John Mercer for offering me the opportunity to pursue independent research while I was an undergraduate student at the University of Oregon. You did so even after the department-office warned you about my abysmal GPA; for that rare opportunity I am truly grateful. I thank Douglas Altshuler, Will Dickson, and Michael Dickinson for our earlier collaboration which contributed greatly to my understanding of insect flight aerodynamics. Stephen Roberts, I’m grateful for the research tools, guidance and mentorship you’ve provided throughout these last six years. However, I’m most thankful that you never accepted “good enough”, or what I considered to be my “best” at that moment; you never backed down, so thank you for pushing me to meet those greater expectations. I thank my loving wife, Leanne, for her immeasurable patience, support, and sacrifice. Finally, for Emma and Anthony, when you find *that* question in your life’s journey, I pray you will exhaust all efforts in pursuit of its answer.

CHAPTER 1

INTRODUCTION

Purpose

A central challenge to all animal life is the optimization of those behaviors which impact longevity and fitness, such as the ability to forage and hunt, compete for a mate, and evade predators. The efficacy of these behaviors depends upon individual locomotor capacity, whether measured as metabolism/energetics, locomotor speed, endurance, maneuverability, or burst performance. However, such capacities are not fixed across an animal's lifespan. Locomotor capacity typically develops and improves early in life (Jayne and Bennett 1989; Vanberkum et al. 1989; Marden et al. 1998; Hale 1999; Irschick 2000; Domenici 2001; Roberts and Elekonich 2005a), plateaus during a middle-aged period (Schippers et al. 2006), and then senescens later in life (Weladji et al. 2002; Ridgel et al. 2003; Grotewiel et al. 2005; Ridgel and Ritzmann 2005). Furthermore, injury or damage to the locomotor apparatus can decrease performance, deviating from general, age-related trajectories (Bateman and Fleming, 2006; Cartar, 1992; Congdon et al., 1974; Fleming and Bateman, 2007; Fleming et al., 2007; Francis and Wood, 1989; Higginson and Barnard, 2004; Punzo, 1982; Robinson et al., 1970; Smith, 1992). The study of locomotion and the underlying biomechanics, particularly across an animal's life-history and in the context of ecologically-relevant constraints, can identify many mechanisms which explain how certain behaviors are governed. In

this dissertation, I investigated how the flight biomechanics and locomotor capacity of the European honey bee (*Apis mellifera*) is affected by development, senescence, and wing damage.

For the honey bee, flight capacity is a major contributor to the efficacy of foraging behavior. Their ability to fly long distances, evaluate the quality of nectar and pollen from several inflorescences during a foraging bout, and return these foraging loads to the colony contributes to the overall fitness of the colony. As such, foraging behavior generally appears to be regulated so that colony fitness is optimized. For example, foragers have the capacity to carry large nectar loads, which require a disproportionate amount of energy to carry, relative to the energy that quantity of nectar contributes to the colony. Thus, foragers carry small nectar loads and maximize foraging economy (energy gained from nectar relative to energy expended during the foraging bout); (Schmid-Hempel et al. 1985). Conversely, when pollen stores within the colony are depleted (or removed), foragers will increase the number of foraging bouts and load-size carried in order to replenish those stores (Fewell and Winston, 1992). The economy of this high foraging intensity is low, and imparts a high cost to the individual forager. By recovering these pollen stores, brood production and colony fitness can be maintained. Despite well-described individual- and colony-level dynamics, relatively little is known about the biomechanics and flight capacity that underlies foraging behavior. Even less is known about how factors such as development, senescence, or morphological damage (wing wear) affect flight capacity. These factors impose marked locomotor constraints throughout the life-histories of many organisms, including, I hypothesized, honey bee foragers. Thus, the purpose of my dissertation is to describe the kinematics of varying

aerodynamic force production in honey bee foragers, and investigate how maximal flight capacity is affected by age, behavioral development, and wing wear.

The Honey Bee Model System

The European honey bee is a holometabolous insect that spends its larval and pupal life within its cell in the honeycomb, and ecloses from its cell as an adult. Adult honey bees proceed through behaviorally-defined life-history stages as they age, a process of behavioral development called ‘temporal polyethism.’ Bees perform in-hive tasks, such as nursing and hive maintenance, during the first 2-3 weeks of adult life, after which they typically transition to tasks outside the hive such as foraging. This transition from in-hive to foraging behaviors generally occurs in an age-dependent fashion. However, if colony demographics change, the pace of behavioral development can be delayed or accelerated. For example, if a colony lacks a sufficient population of nurses to tend brood, young bees will continue to perform nursing behavior and delay their transition into foraging behavior (Robinson et al. 1989). Conversely, if there is a deficiency of foragers then young bees will begin to forage precociously, as early as 5-days of age (Robinson et al. 1989; Huang and Robinson 1992). This phenomenon contributes to the tractability of this model system, as colony demographics can be manipulated to separate the effects of age and behavioral development.

Honey bee foragers are the superstar athletes of the animal kingdom. Their mass-specific metabolic rates (up to 800 W kg^{-1}) are three orders of magnitude greater than the best human endurance athlete, and are the highest ever recorded in the animal kingdom (Roberts and Harrison, 1999). Bees can produce aerodynamic forces in excess

of 200% of their body weight, such as during undertaking where deceased bees are carried away from the colony. And in a typical day of foraging, bees may contract their flight muscle over 5 million times; this foraging routine may persist for 2 weeks or more (Winston, 1987; Harrison et al. 1996). Although these feats highlight bees' physical prowess and the demands necessary for successful foraging behavior, one might hypothesize that such a highly-tuned system would be sensitive to the timing of development and onset of senescence, or the wear and tear of the wings that accumulates with use during flight. However, before we can evaluate how honey bee flight performance is affected by these ecologically-relevant factors, we must first understand exactly how bees fly.

Kinematic Mechanisms of Varying

Aerodynamic Forces

In 1934, after applying simple, fixed-wing aerodynamic theory to the small wings of bees, August Magnan and his colleague Andre Sainte-Lague concluded that the flight of bees was impossible (Magnan, 1934). This claim illustrated the deficiencies in our understanding of aerodynamics at the small scales employed by insects, which continued throughout much of the 20th century. Progress was made by Weis-Fogh and Jensen (1956) through their 'quasi-steady' analysis, where they applied steady-state aerodynamic principles to the instantaneous velocity and geometry of the locust wing through its wingbeat. In 1984, Charles Ellington revised the 'quasi-steady' model to incorporate other 'unsteady' aerodynamic mechanisms, which included the unique vorticity produced by insect wing strokes (which differs from that of fixed wings).

From the early 1990's through today, Michael Dickinson and his colleagues (Altshuler, Birch, Fry, Gotz, Lehmann, Sane, Wang, etc.) have contributed greatly to the revision of 'quasi-steady' and 'unsteady' aerodynamic mechanisms, primarily through high-speed videography, detailed insect flight kinematics, analytical aerodynamic modeling, and the introduction of dynamically-scaled robot modeling. Dynamically-scaled modeling has become a valuable tool for the identification and verification of the aerodynamic mechanisms employed by hovering insects, especially by allowing the visualization of fluid flow and vorticity, and the measurement of the resulting aerodynamic forces on the wings of the robot model.

In 2005, preliminary data from the honey bee hovering kinematics I present in Chapter 2 were analyzed using the dynamically-scaled robot model in the Dickinson Lab at the California Institute of Technology (Altshuler, et al. 2005). We found that, similar to the well-studied fruit fly (*Drosophila melanogaster*), honey bees produce mid-stroke forces that are associated with the aerodynamic mechanism of 'delayed stall', a phenomenon that occurs when a vortex forms on the leading edge of the wing as the wing translates across its stroke. However, unlike *D. melanogaster*, honey bees' high frequency, low amplitude wing strokes produced significant forces towards the ends of each wing stroke via wing rotational mechanisms, and after the transitions of each stroke through wake capture where the wing encounters the vortices shed by the previous wing stroke. Increases in stroke amplitude, such as those associated with ascending flight or hovering in hypodense atmospheres, yields increased wing velocity and mid-stroke aerodynamic force production (Altshuler et al. 2005). This dynamically-scaled robot model yielded great insight into honey bee hovering aerodynamics, however it utilized

an artificially-generated stroke plane and wing angles of attack. Here, I address this gap in our understanding of honey bee flight kinematics and I describe the precise wing kinematics used by honey bees during hovering in air, and compare those kinematics used during simple, enhanced-lift maneuvers (ascending and descending) and hovering in hypodense heliox, a normoxic gas that is 1/3 the density of air. The purpose of this experiment was to identify the kinematic mechanisms bees use to vary aerodynamic force production.

The Effects of Age and Behavioral Development on Flight Performance

The development of locomotor capacity is strictly confined to the adult stage in honey bees, in contrast to other animals where development occurs through morphological growth during juvenile stages. However, even as adults, a broad suite of biochemical changes facilitate the attainment and development of flight ability. For example, 1-day-old bees (e.g. 1-day post-eclosion) are unable to fly, and 2-day old bees are limited to hovering flight (Roberts and Harrison, 1999). Increased thoracic glycogen levels, pyruvate kinase and citrate synthase activity facilitate a 200% increase in metabolic rate between 1 to 2 days of age (Fewell and Harrison, 2001; Harrison 1986; Harrison and Fewell, 2002; Moritz, 1988; Neukirch, 1982). Cytochrome concentrations increase by an order of magnitude from 1 to 20 days of age (Herold 1963), and the transition from in-hive to foraging behavior is accompanied by increased thoracic glycogen and citrate synthase levels, and increased Troponin T 10a expression (Harrison 1986; Fewell and Harrison 2001; Schippers et al. 2006). I predicted that flight capacity

should develop concomitant to physiological development. However, it was unknown whether flight capacity develops along a fixed, age-related trajectory, or if the plasticity of the honey bee behavioral transitions can accelerate this development.

The sustained flight which occurs during bouts of foraging is metabolically expensive. In house flies, *Musca domestica*, prevention of flight behavior greatly increases longevity and decelerates age-dependent oxidative stress, including the accrual of mitochondrial peroxide, carbonylation of select mitochondrial enzymes, and mitochondrial DNA damage (Sohal and Buchan 1981; Agarwal and Sohal 1994; Sohal and Dubey 1994; Yan et al. 1997; Yan and Sohal 1998, 2000). Thus, flight capacity cannot be maintained indefinitely and should progressively senesce due in large part to accumulation of oxidative stress and reduction in stress resistance (Sun and Tower 1999; Vieira et al. 2000; Amdam and Omholt 2002; Golden et al. 2002; Yoon et al. 2002; Martin and Grotewiel 2006; Seehuus et al. 2006; Yu and Chung 2006). To date only a small number of laboratory studies have experimentally linked expensive aerobic behaviors to longevity and its mechanistic underpinnings, but no research has experimentally linked the variation in the onset and duration of natural behaviors to development and functional senescence in a free-living organism. The purpose of this experiment was to investigate how age and behavioral development independently affects maximal flight capacity in honey bees.

The Effects of Wing Wear on Flight Performance

Mechanical damage or wear to the locomotor apparatus can result from encounters with predators, locomotion in physically heterogenous environments, and aging via

prolonged use and senescence. In flying insects any wing damage or functional impairment of these airfoils would be permanent. The loss of wing area, changes in mechanical properties such as stiffness, or symmetry of the wing pair can impair flight performance and ultimately reduce fitness. Foraging bees accumulate wing wear and damage with flight experience (Higginson and Barnard, 2004), and both natural and experimentally induced wing wear reduces longevity (Cartar, 1992). Wing wear also impacts foraging behavior through a reduction in the quantity, quality, and efficiency of nectar foraging (Higginson and Barnard, 2004). Despite increased wingbeat frequency, coefficient of lift, and induced aerodynamic power, wing wear does not affect metabolic cost (Hedenstrom et al. 2001). Instead, wing wear may impact maximal flight performance, which in turn may affect foraging behavior and the ability to evade predators (Cartar, 1992; Hedenstrom et al., 2001). To date, no research has identified a mechanism which unequivocally links wing wear to mortality. The purpose of this experiment was to investigate how variation in the magnitude and type (symmetric *vs.* asymmetric) of artificially-induced wing wear affects the kinematics and flight capacity of honey bees. Here, I present an aerodynamic power budget that describes how the reduction in maximal flight capacity impacts the aerodynamic power available for carrying foraging loads and the maneuverability necessary for evading predators.

CHAPTER 2

KINEMATIC MECHANISMS OF VARYING AERODYNAMIC FORCES

Abstract

During hovering flight, animals can increase the net aerodynamic force per stroke by increasing wing stroke velocity through modulation of wingbeat frequency, wing stroke amplitude, or both. However, aerodynamic forces will also vary with other kinematic features including angle of attack, timing of wing rotation, wing contact, and the pattern of deviation from the primary stroke plane. Most of the kinematic data available for flying animals are average values for wing stroke amplitude and wingbeat because these features are relatively easy to measure, but it is frequently suggested that the more subtle and difficult to measure features of wing kinematics can explain variation in force production for different flight behaviors. Here, we tested this hypothesis with high-speed (6,000 fps) recording and digitization of honey bee (*Apis mellifera*) wing kinematics for bees hovering in air, a hypodense gas (heliox: 21% O₂, 79% He), and during simple, enhanced-lift maneuvers in air. Bees employed low stroke amplitudes ($86.7 \pm 7.9^\circ$) and high wingbeat frequencies (226.8 ± 12.8 Hz) when hovering in air. When performing these maneuvers or hovering in heliox, bees increased stroke amplitude by 30-45%, which yielded a much higher wing angular velocity relative to that during simple hovering in air. Among the three flight conditions, there were no statistical differences

in wing stroke deviation, angle of wing rotation, wing rotation velocity, angle of attack, or even in wingbeat frequency. Thus, our data indicate that, at least for honey bees, modulation of wing angular velocity is sufficient, and that the overall time course of wing angles is highly preserved across a diverse set of flight behaviors.

Order of authors: Jason Vance, Douglas Altshuler, Will Dickson, Michael Dickinson, Stephen Roberts.

Introduction

Among flying animals only insects and hummingbirds are capable of sustained hovering. The reciprocation of their wings at high frequencies affords high maneuverability, rapid ascent, and carriage of loads greater than body mass, a feat that is routinely accomplished by many insects, such as during undertaking in honey bees, blood-feeding in mosquitos and prey carriage in cicada-hunting wasps. Thus, many hoverers possess substantial aerodynamic reserves beyond baseline requirements for stationary hovering. Studies of insects and hummingbirds filmed from single perspectives and at low sampling rates suggested that such reserves are realized at least in part by modulation of kinematic parameters such as wingbeat frequency (n) and wing stroke amplitude (Φ) (Altshuler and Dudley, 2003; Dudley, 1995; Lehmann, 2004; Roberts et al., 2004) that contribute to the angular and translational velocity of the wing and lift produced via delayed stall (Dickinson et al., 1999; Sane, 2003; Sane and Dickinson, 2002). However, several other possible strategies exist for varying hovering flight forces, such as changing angle of attack, wing rotation velocity/timing, and ‘clap and fling’ (Sane, 2003). This study tests for such mechanisms via three-dimensional,

high speed videography of honey bees hovering in hypodense vs. normodense atmospheres and performing simple maneuvers requiring elevated lift.

Several animals increase wing angular velocity (ω) and translational velocity (U_t) to enhance lift during hovering flight, which can be accomplished by modulating Φ and/or n . For example, hummingbirds (*Archilochus colubris*) and carpenter bees (*Xylocopa varipuncta*) increase both Φ and n to augment lift during load-lifting or flight in hypodense heliox (a gas mixture consisting of 79% helium and 21% oxygen that is roughly one-third the density of sea-level air), relative to values for individuals hovering in air (Chai, 1997; Chai and Dudley, 1996; Altshuler and Dudley, 2003; (Roberts et al., 2004)). Three species of orchid bees (Apidae: Euglossini) also increase Φ by 30-45% during hovering in heliox, however they hold n constant (Dudley 1995). When challenged to increase total flight force in a tethered flight simulator, *Drosophila melanogaster* increase both Φ and n up to the point where they reach their ceiling of power output, after which further increases in Φ are accomplished only with concomitant decreases in n (Lehmann & Dickinson, 1997). Even during this decrease in n , the increase in Φ is sufficient to increase ω and U_t . Thus, these different strategies all increase aerodynamic forces, which scale in proportion to U_t^2 . However, it is not known whether other kinematic mechanisms also contributed to the increased aerodynamic forces in these studies.

The range of kinematic mechanisms available to flying insects is especially observed during turning maneuvers. For example, *Drosophila* use transient, bilateral asymmetries in Φ and stroke plane angle to generate torque about the yaw axis during simple turning maneuvers (saccades), (Fry et al., 2003). Dragonflies can vary wing angle of attack to

perform more complex roll and yaw turns (Alexander 1986). Even for hovering with pollen loads up to 18% of body mass, honey bees (*Apis mellifera*) maintain constant Φ and n (Feuerbacher et al., 2003), which suggests that honey bees may be capable of modulating angle of attack or non-steady mechanisms, such as wing-rotational effects, to augment lift for carrying light to moderate pollen loads. These examples highlight basic kinematic strategies used by insects to vary aerodynamic forces, but it is unknown whether honey bees or other insects vary multiple kinematic parameters (such as stroke plane angle, angle of attack or wing rotation velocities) to control hovering lift forces. I addressed this issue in this study by using high-speed video analysis to describe the wing movements of honey bees during hovering flight in air and while accommodating the aerodynamic challenges of simple maneuvers in air and hovering in heliox.

Materials and Methods

Collection and Filming of Bees

European honey bees (*Apis mellifera*) were collected as they exited a hive at the University of Nevada Las Vegas campus apiary and were immediately transferred to an 8 liter transparent acrylic flight chamber in an adjacent laboratory. A sucrose solution and pollen grains were placed on a pedestal centrally located within the flight chamber, which provided the honey bees with sustenance and created a focal target for video recording. Three high-speed video cameras (Photron Ultima APX; San Diego, Ca, USA), oriented orthogonal to each other, recorded honey bee flight at 6000 frames per second with a resolution of 512 x 512 pixels. Two to three bees occupied the flight chamber during any given collection run, but only one bee was in flight during any

recorded trial. Bees were monitored until an acceptable sequence of hovering flight (e.g. a bee was recorded in focus in all three cameras) was recorded in one or two flight conditions, the bees demonstrated lethargy, or 30 minutes elapsed. Body mass and wing morphology for all honey bees were measured after each collection run.

Flight Conditions

Bees were filmed during flight in a normodense atmosphere (air; 21% O₂/79% N₂; 1.21 kg m⁻³) under three conditions: sustained hovering ($N = 5$), ascending flight ($N = 3$), and during the decelerations at the end of descending flight ($N = 1$). Ascent and deceleration from descent represent a class of simple, symmetric maneuvers during which we predicted the bees would require kinematic expenditures and flight forces above those required for simple hovering in air. In addition to bees hovering and performing enhanced-lift maneuvers in air, we filmed bees while hovering in a hypodense atmosphere (heliox; 21% O₂/79% He; 0.41 kg m⁻³; $N = 4$). Although heliox is approximately 1/3 the density of air, both atmospheres are normoxic. The gasses were mixed using calibrated bi-metal thermo-actuated valves (Tylan FC-460; San Diego, Ca, USA), metered by an electronic flow controller (Sable Systems MFC-4; Las Vegas, Nv, USA) at a total flow rate of 1 L min⁻¹ during the video acquisition period.

Video Processing and Kinematic Analysis

Digital video recordings were processed and analyzed using methods detailed by Altshuler et al (2005). Flight sequences were analyzed as individual bitmap images using custom software (Fry et al., 2003) written in Matlab (The Mathworks; Natick, Ma, USA). Prior to the analysis of each trial, the focal space was calibrated using anatomical landmarks on the bee that were visible from all three cameras. The kinematic analysis

of each trial utilized six landmarks that were digitized using at least two camera views: head, tip of abdomen, left and right wing hinges, and left and right wing tips. To determine the angle of wing rotation (α), I superimposed a wire-frame wing image over the bee wing in all three camera views and rotated the wire-frame about the long axis (wing hinge to wing tip) until optimal overlap was achieved. The mean stroke plane angle (β_r) was calculated as the average of the angle of the wing tip vectors across each wing stroke (β) relative to the body angle (χ). Wing position angle (ϕ) within the stroke plane and the deviation angle (θ) from the stroke plane were analyzed using a principle component analysis to calculate a rotational adjustment for each bee that would standardize the body coordinates; this rotational adjustment yielded ϕ , θ , and α relative to the body. Wing angular velocity (ω) was calculated from ϕ , θ , and n . The geometric wing angle of attack (α_r) was calculated as the wing rotation angle (α) relative to the angle of wing translation. The rate of change of wing rotation angle was calculated as wing rotational velocity ($\dot{\alpha}$). A cubic spline was used to smooth the data.

Results

Wing length (9.19 ± 0.11 mm; mean \pm S.D.; $N = 23$), area (52.7 ± 1.5 mm²) and aspect ratio (6.42 ± 0.17) were consistent across all bees collected for the study, however body mass varied greatly (117.5 ± 26.6 mg) and contributed to considerable variation in wing loading (21.9 ± 5.0 N m⁻²). I did not record individual body mass prior to each collection run and the bees were free to consume the sucrose solution and pollen grains that were provided in the flight chamber. Therefore, it is unknown how much mass each bee gained while inside the flight chamber. Bees ϕ and n during hovering in

air and heliox (Table 2.1) are consistent with those kinematics used by mature foragers under similar conditions (Vance et al. in press). Thus, I am confident that the kinematics represent the lighter individuals of the 2-3 bees which were present in the flight chamber during a given trial, and were likely of lesser body mass at the time of video recording than when collected and weighed.

The kinematics of honey bees hovering in air and heliox, and performing simple, enhanced-lift maneuvers are characterized by high frequency wingstrokes ($n = 228.6 \pm 17.8$ Hz) that were biased dorsally (Figs 2.1 and 2.2; ϕ). The downstroke was planar and nearly horizontal, and the upstroke was “U” shaped (θ , Figs 2.2 and 2.3). The combination of these patterns for ϕ and θ resulted in a wingtip trajectory shaped like ‘Cheshire cat-like grin’ (Fig 2.3) where ω (Fig 2.4) never reached zero. Honey bees hovered in air with relatively short Φ ($86.7 \pm 7.9^\circ$) and increased Φ by 30% during simple, enhanced-lift maneuvers, and by 47% during hovering in heliox. Despite no significant differences in n across the three conditions, the increase in Φ contributed to greater ω (Fig 2.4) during simple, enhanced-lift maneuvers and hovering in heliox as compared to air. During enhanced-lift maneuvers, ω was 18% greater during the downstroke ($\omega_{\text{down}} = 749.0 \pm 82.7$ rad sec⁻¹) and 25% greater during the upstroke ($\omega_{\text{up}} = 817.3 \pm 162.2$ rad sec⁻¹) than bees hovering in air. Likewise, during hovering in heliox ω was 46% greater during the downstroke ($\omega_{\text{down}} = 927.7 \pm 160.3$ rad s⁻¹) and 45% greater during the upstroke ($\omega_{\text{up}} = 949.6 \pm 153.4$ rad s⁻¹) than bees hovering in air. There were no significant differences in the maximum α , $\dot{\alpha}$, α_r , and α_r averaged across the wingstroke ($\alpha_{r, \text{avg}}$) across the three conditions; however, $\alpha_{r, \text{avg}}$ during the downstroke was greater than the during the upstroke for all bees analyzed. There were also no

Table 2.1: Kinematic maxima during hovering in air and heliox, and maneuvering in air.

	Air ($N=5$)	Heliox ($N=4$)	Maneuver ($N=4$)
n	226.8±12.8	238.6±11.4	221.1±26.6
Φ_{total}	86.7±7.9	127.5±25.6	112.4±17.9
ϕ_{dorsal}	72.8±5.7	90.3±12.1	84.4±10.0
ϕ_{ventral}	13.9±3.6	37.2±14.3	28.1±8.1
Θ_{down}	4.8±1.2	5.1±1.9	4.8±0.9
Θ_{up}	16.1±3.0	14.1±4.7	14.1±4.7
α_{down}	61.2±4.8	64.6±6.2	64.6±3.5
α_{up}	68.1±5.7	62.3±1.6	67.9±5.4
$\alpha_{\text{r, down}}$	26.1±3.2	27.1±2.0	24.1±1.8
$\alpha_{\text{r, up}}$	27.9±4.7	28.7±5.2	25.9±3.6
$\alpha_{\text{r, avg down}}$	45.6±3.6	42.8±3.6	42.6±1.0
$\alpha_{\text{r, avg up}}$	36.6±2.1	40.3±3.5	36.6±2.4
ω_{down}	635.5±58.5	927.7±160.3	749.0±82.7
ω_{up}	653.6±88.4	949.6±153.4	817.3±162.2
$\dot{\alpha}_{\text{dorsal}}$	2546±197	2831±298	2827±377
$\dot{\alpha}_{\text{ventral}}$	2564±128	2514±219	2483±340
χ	39.6±3.7	53.3±19.0	38.5±9.6
β_{r}	44.5±2.8	50.4±10.9	41.8±6.7
Re	1163±102	526±75	1419±180
V_{vert}	0.01±0.04	0.01±0.09	0.11±0.15

Units, mean±S.D.: n , Hz; Φ , Θ , α , χ , β_{r} , degrees; $\dot{\alpha}$, rad sec⁻¹; ω , rad sec⁻¹; Re (dimensionless); V_{vert} , m sec⁻¹.

Table 2.2: Results of one-way ANOVA for kinematic response to hovering in air and heliox, and maneuvering in air (condition).

	Condition	
	$F_{2,10}$	P
n	1.02	0.397
Φ_{total}	6.08	0.019*
ϕ_{dorsal}	4.13	0.049*
ϕ_{ventral}	7.26	0.011*
χ	1.77	0.220
β_{r}	1.49	0.271

Significant results in bold.

* denotes difference between bees hovering in air and heliox; Tukey's HSD.

Table 2.3: Results of two-way ANOVA for kinematic response to hovering in air and heliox, and maneuvering in air (condition); and for kinematic differences between the downstroke and upstroke (stroke). There were no significant *Condition x Stroke* interactions.

	Condition		Stroke	
	$F_{2,22}$	P	$F_{1,22}$	P
Θ	0.49	0.618	83.2	<0.001
α	0.60	0.555	2.19	0.154
α_r	1.44	0.259	1.56	0.225
$\alpha_{r, \text{avg}}$	0.57	0.573	16.9	<0.001
ω	14.3	<0.001*	0.58	0.453
$\dot{\alpha}$	0.52	0.604	3.46	0.076

Significant results in bold.

* denotes significant difference between all three groups.

significant differences in body angle (χ) or the stroke plane angle relative to the body (β_r) across the three conditions; however, 3 of the 4 bees hovering in heliox had 55% greater χ than bees hovering in air.

Discussion

Hovering flight requires mass-specific metabolic rates among the highest ever recorded in the animal kingdom (Coelho and Mitton, 1988; Harrison, 1986; Roberts et al., 2004; Suarez et al., 2005; Suarez et al., 1996; Withers, 1981). Even so, hovering animals must possess significant aerodynamic reserves to allow for load-carriage, accommodating atmospheric perturbations, and maneuvering. Although the kinematics of hovering flight are complex, it appears that many animals modulate aerodynamic output by manipulating a reduced set of parameters, primarily n and Φ , to control ω (Altshuler et al., 2005; Altshuler and Dudley, 2003; Altshuler and Dudley, 2004; Chai et al., 1997; Dudley, 1995; Lehmann, 2004; Lehmann and Dickinson, 1997). To meet the demands of enhanced-lift maneuvers and hovering in heliox, honey bees augment

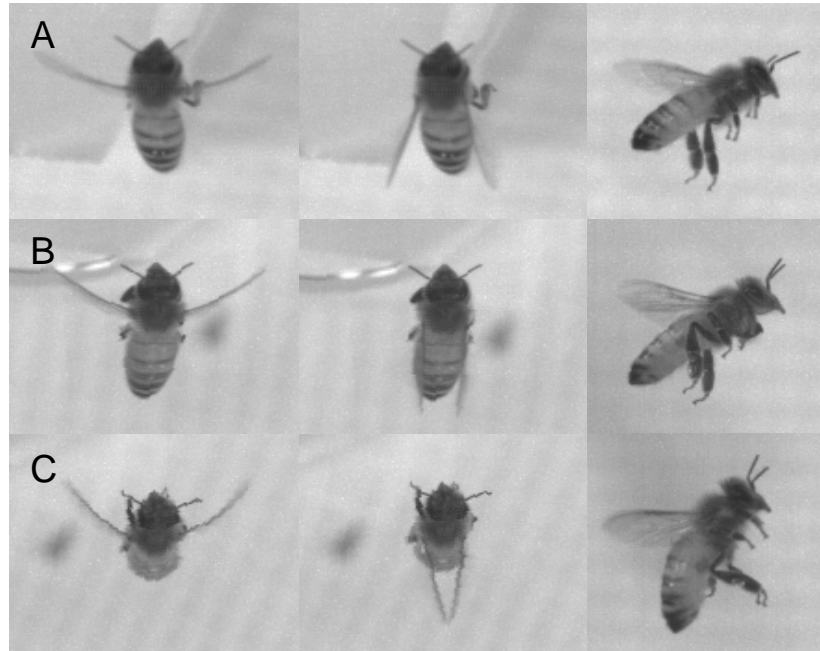


Figure 2.1: Wing stroke reversals and body angle in honey bees during hovering in air (A), simple, enhanced-lift maneuvers in air (B), and hovering in heliox (C). Columns (from left to right) are ventral stroke displacement (ϕ_{ventral}), dorsal stroke displacement (ϕ_{dorsal}), and body angle (χ).

aerodynamic force production almost exclusively by increasing Φ while maintaining constant n (Fig 2.4), a strategy used by other hymenopterans (Dillon and Dudley, 2004; Dudley, 1995; Roberts et al., 2004). As Φ and ω increased, the aerodynamic force profile is affected by large increases in mid-stroke lift and, to a lesser degree, by increases in lift associated with the stroke transitions (Fig 2.5; Altshuler et al., 2005). Honey bees are able to vary Φ by 47% (Figs. 2.1 and 2.2), and maximum Φ during hovering in heliox were similar to those of *X. varipuncta* (Roberts et al., 2004) and *Euglossine* spp. (Dudley, 1995), which suggests that these bee species may share common thorax and wing-hinge morphologies that limit ventral excursion and overall range of motion. The ability to vary Φ across such a large range contributes to a

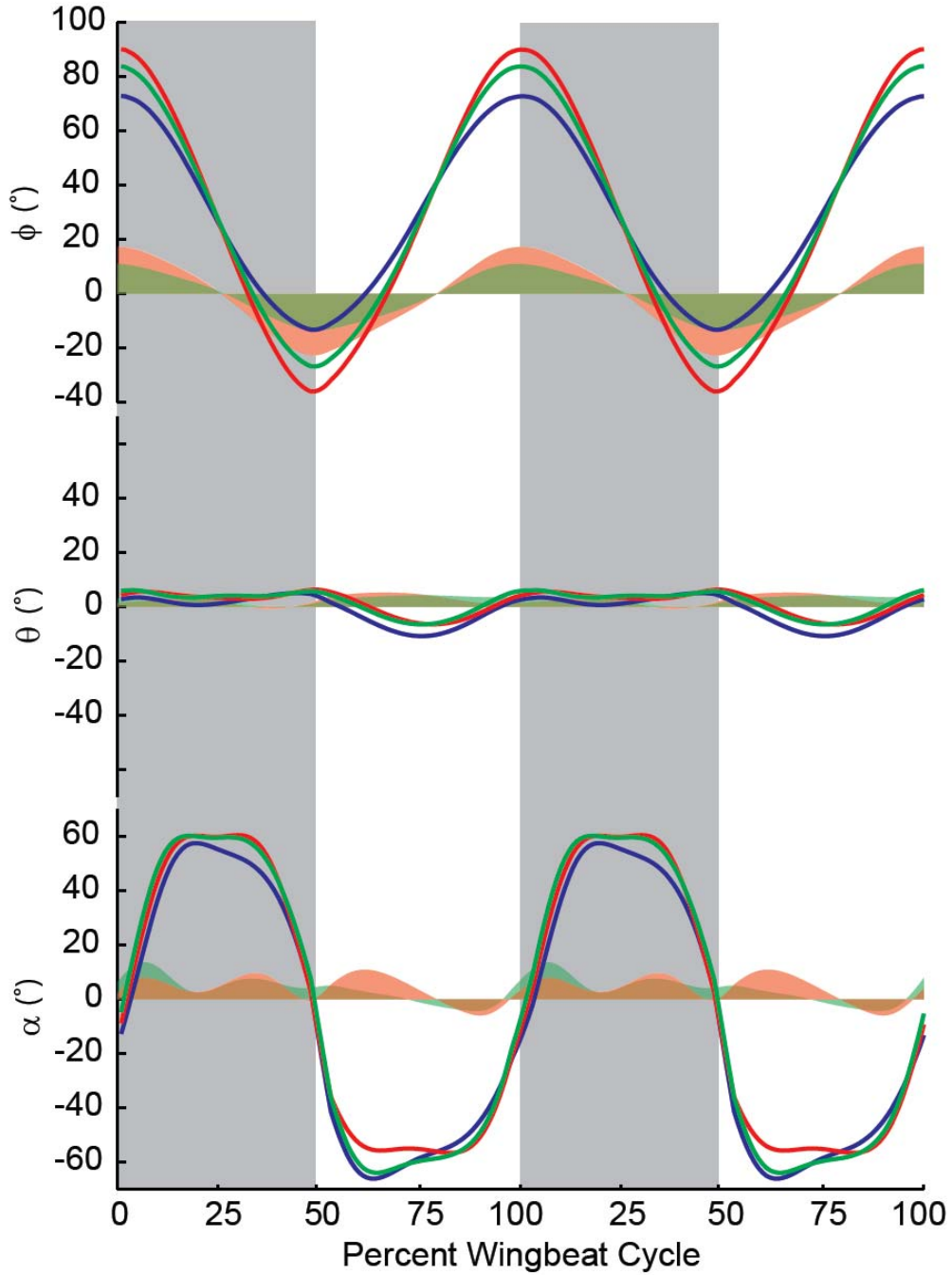


Figure 2.2: Mean kinematic patterns of wing stroke amplitude (ϕ), deviation from the mean stroke plane (θ), and wing rotation angle (α) during hovering in air (blue trace), simple maneuvers in air (green trace), and hovering in heliox (red trace). The kinematic patterns are normalized across 100% of the wingbeat cycle and averaged across all wingbeats per bee per group. The shaded plots represent the mean difference between hovering and maneuvering in air (green) and the mean difference between hovering in air and heliox (red). The grey and white columns indicate the downstrokes and upstrokes, respectively.

substantial aerodynamic reserve capacity that allows these foraging bee species to accommodate the perturbations of a dynamic environment even when laden with pollen, nectar or water, or when transporting dead bees away from the colony (undertaking).

Despite no significant differences in the minima, maxima, or average values for several kinematic parameters, there was discernable variation in the patterns of α , $\dot{\alpha}$, and α_r across the entire wingbeat cycle. During hovering in air, the timing of maximum $\dot{\alpha}$ had a tendency to be delayed relative to the dorsal stroke reversal, whereas, during hovering in heliox, the timing of maximum $\dot{\alpha}$ was advanced relative to the ventral stroke

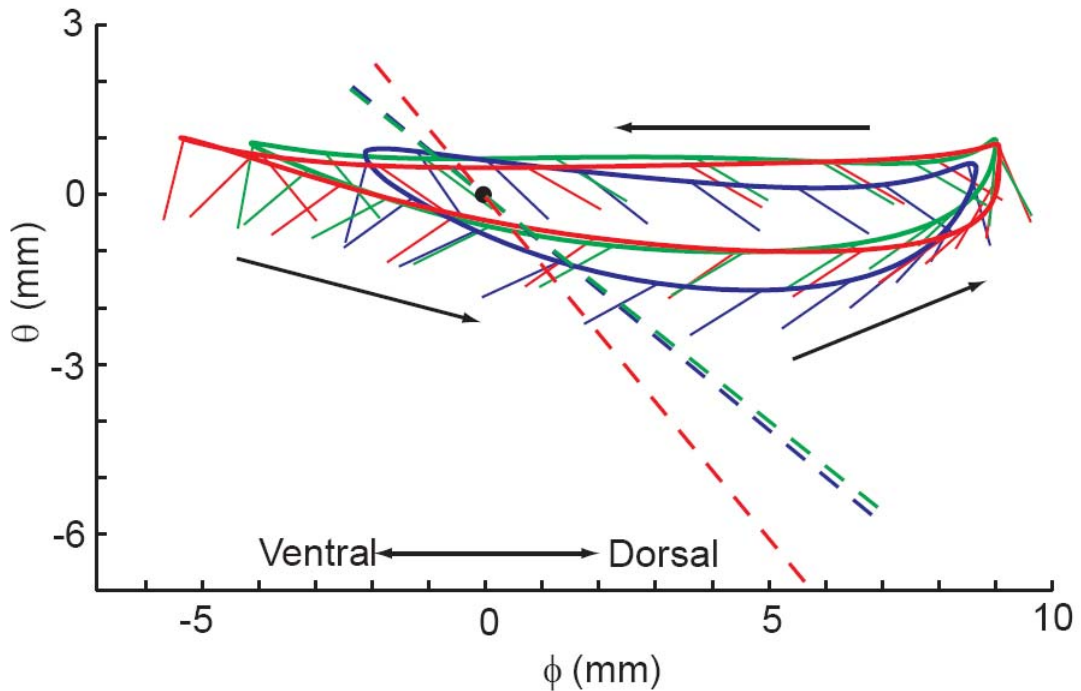


Figure 2.3: Mean pattern of the wingtip trajectory (in two dimensions) during hovering in air (blue), simple maneuvers in air (green) and hovering in heliox (red). The line segments trailing each of the three respective traces indicate the wing rotation angles (α) across the wing beat cycle. The broken lines represent the mean body angle for the bees in each of the three conditions.

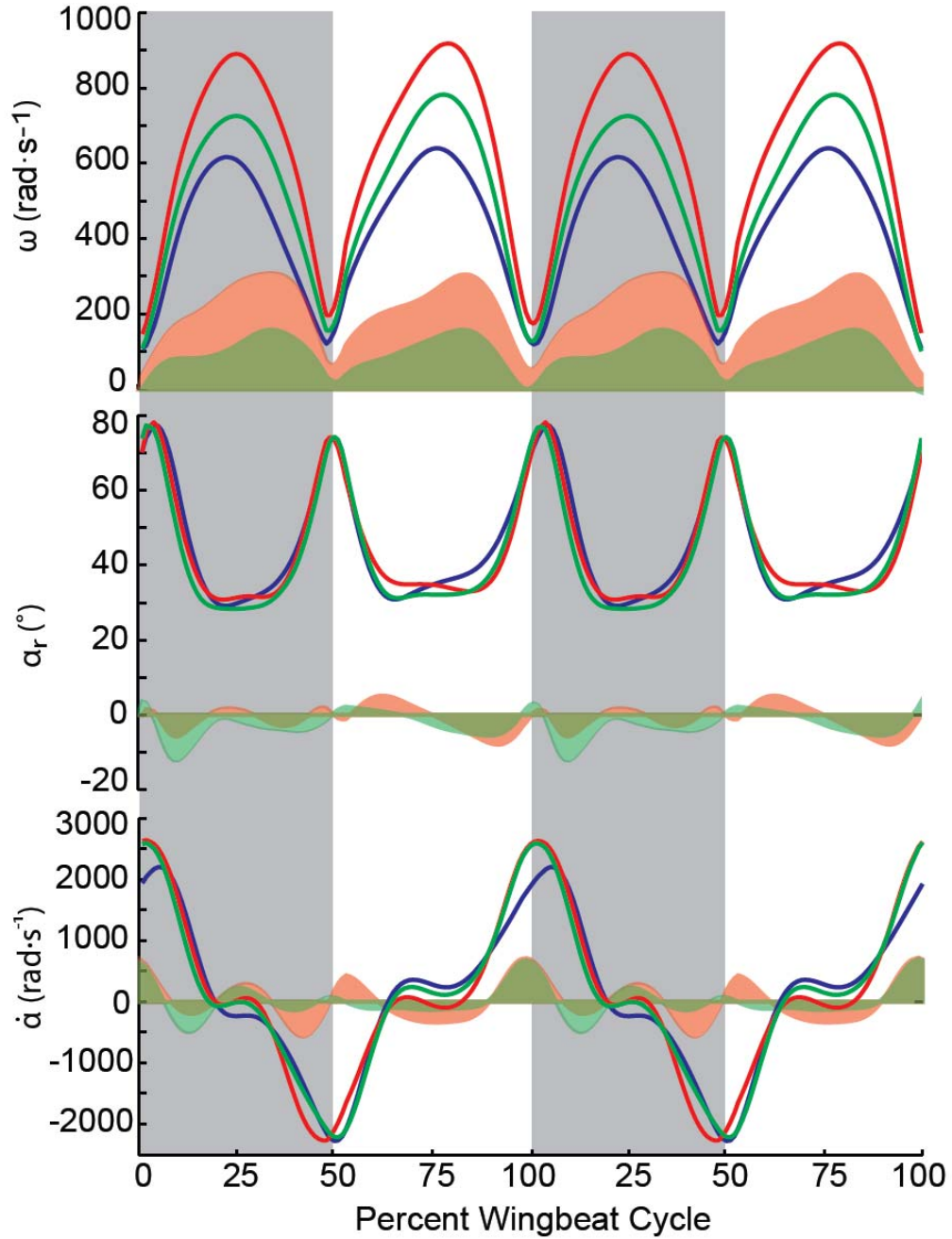


Figure 2.4: Mean kinematic patterns of wing angular velocity (ω), wing angle of attack (α_r), and wing rotation velocity ($\dot{\alpha}$) during hovering in air (blue trace), simple maneuvers in air (green trace), and hovering in heliox (red trace). The kinematic patterns are normalized across 100% of the wingbeat cycle and averaged across all wingbeats per bee per group. The shaded plots represent the mean difference between hovering and maneuvering in air (green) and the mean difference between hovering in air and heliox (red). The grey and white columns indicate the downstrokes and upstrokes, respectively.

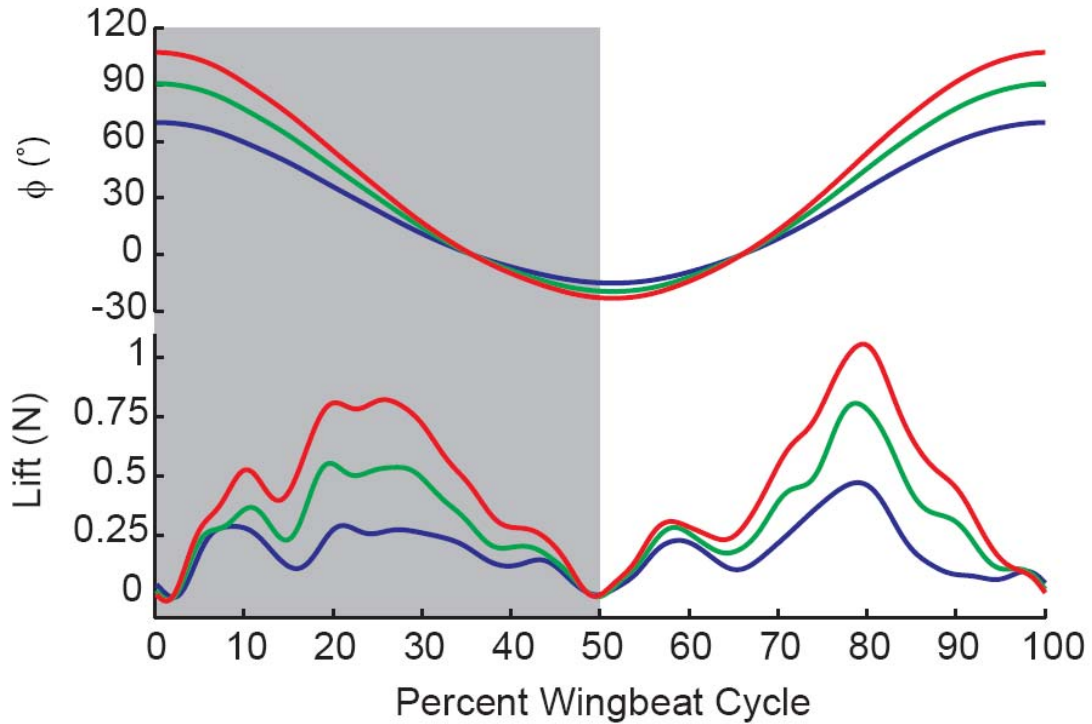


Figure 2.5: Aerodynamic forces (lift) resulting from kinematics representative of hovering in air (blue), simple maneuvers in air (green), and hovering in heliox (red). Forces were measured using a dynamically-scaled robot model as described in (Altshuler et al., 2005). The grey and white columns indicate the downstrokes and upstrokes, respectively.

reversal (Fig 2.4). Although subtle, the timing of maximum α could affect the production of lift through wing rotational mechanisms. Less subtle, however, is the pattern of α_r , which appears markedly different across the three conditions (Fig 2.2, α_r).

To understand the potential for this variation in α_r to affect translational-lift production, I estimated the aerodynamic forces (quasi-steady method; Sane and Dickinson, 2002) for each bee's wingstroke kinematics using all patterns of α_r observed in this experiment. For bees hovering in air, average lift forces increased by 3.7% when α_r was replaced by those patterns of α_r used in the maneuver and heliox conditions.

Even though variation in α_r contributes only a small percentage to the estimated aerodynamic forces, future work (including dynamically-scaled modeling) may provide insight as to whether these kinematic variations contribute to flight control and stability.

As stroke amplitude increased in response to the aerodynamic challenges of hovering in heliox, body angle also increased. It is unknown whether this trend resulted from a lack of pitch control, either due to the physical properties of the heliox atmosphere or from wing kinematics approaching functional limitations. However, another explanation may be that the increased body angle moved the abdomen away from the path of the wing stroke-induced air flow and vorticity, especially at stroke amplitudes where dorsal excursion exceeded 90 degrees (Fig 2.1C). If the body angle did not increase under such wing kinematics, the latter portion of the wing stroke and dorsal stroke transition would occur directly above the abdomen. Although increasing body angle cannot be viewed as a lift-enhancing mechanism, it may simply minimize losses in lift that would otherwise be attributed to interference with the abdomen. In either case, increasing body angle will transfer the bees' center of mass further below the height of the wing hinges. Passive stability should improve simply by this increase in inertia about the roll axis, which could be beneficial during near-maximal effort flight where diminished kinematic and muscle reserve capacities may not provide active compensation to perturbations.

During hovering in heliox, some bees exhibited extreme dorsal stroke excursions that caused contact between the distal region of the wings at the dorsal stroke transition (Fig 1). While this was not investigated with a dynamic model, these kinematics are similar to those that produce aerodynamic force via 'clap-and-fling.' In application, the

‘clap’ forces air out from between the area where the two wings are in contact, and the ‘fling’ causes air to rush over the leading edges as the wings peel apart, speeding up the development of vorticity (Ellington, 1999; Lehmann et al., 2005). This aerodynamic mechanism is utilized by a variety of insects, ranging from small parasitic wasps (Miller and Peskin, 2005) to damselflies (Wakeling and Ellington, 1997a; Wakeling and Ellington, 1997b), and has been successfully exploited at much larger scales in micro aerial vehicle (MAV) development, such as the *Mentor* MAV (Zdunich, 2007). However, the degree to which a clap-and-fling pattern increases lift is crucially dependent on the precise kinematics of the wing (Lehmann and Pick, 2007; Lehmann et al., 2005), and its mere presence does not necessarily indicate a substantial augmentation in force. Currently, I do not know what forces result from the bees’ wing-contact at the dorsal stroke transition. However, bees’ dorsally-biased wing strokes suggest that their biomechanics and/or thorax morphology are conducive to eliciting clap-and-fling when magnitudes of Φ are great enough. Further investigation is necessary to determine whether this contributes to bees’ flight ability, and to what extent increased dorsal stroke excursion and wing-contact affect pitch control and body angle during flight in heliox.

Several hymenopteran species operate at n that are very high relative to their body mass, compared to other hovering insects. Many endothermic bees vary n inversely with air temperature in an apparent thermoregulatory manner (Borrell and Medeiros, 2004; Harrison et al., 1996; Roberts and Harrison, 1998; Spangler and Buchmann, 1991; Unwin and Corbet, 1984), whereas other bee species have demonstrated at least some capacity to increase n as a mechanism to augment lift. For example, the carpenter bee, *Xylocopa varipuncta*, modestly increases wingbeat frequency in response to hypodense

gas; this effect is most pronounced in lighter bees, but diminishes with mass as heavier bees must operate near or at their maximal wingbeat frequency during hovering in air (Roberts et al., 2004). Likewise, the solitary desert bee, *Centris pallida*, increases wingbeat frequency by 12% during transient “bursts” when defending territory during mate competition (Roberts, 2005). However, neither Euglossines (Dudley, 1995) nor the honey bees in this study varied wingbeat frequency in response to hovering aerodynamic challenges. This might indicate that the flight muscle of these species may simply have a narrow operating range, respective of the resonant properties of the surrounding structures. In contrast, dipteran muscle, which relative to body size operates at a much lower frequency, has a broader operating range. In this study, if bees hovering in heliox had instead held Φ constant, n exceeding 320 Hz would be required to produce the same ω . This strategy could allow for significant contribution of lift from wing-wake interactions and wing-rotation mechanisms, but it would come at the cost of metabolic work to overcome the increased inertial power and drag associated with small wing strokes (Altshuler et al., 2005). It is possible that honey bees also are capable of increasing n during short bursts of maximal aerodynamic output, such as what might be required to evade predators. Thus, hypodense atmospheres may not reveal transient bouts of true, maximal flight, and instead may reveal only sustained near-maximal efforts. Nonetheless, honey bees maintained n across normal, sub-maximal and near-maximal flight efforts, which suggests that this operating frequency is tuned to maximize economy across modes of flight that bees experience during extended bouts of foraging.

Honey bees use high-frequency, low-amplitude wing strokes during hovering in air, in contrast with insects that use low-frequency, high amplitude strokes such as *Drosophila*. Bees perform sustained, long-distance flight in dynamic environments and are capable of accommodating gusts of wind, maneuvers (including ascending and descending flight), and pollen/nectar loading. Moreover, these aerodynamic challenges are compounded for bee populations and species from high-altitude habitats with low atmospheric densities. A substantial reserve capacity is necessary for successful flight under such conditions, yet despite complex wing kinematics, bees accommodate the aerodynamic challenges of simple, enhanced-lift maneuvers and flight in hypodense atmospheres by simply increasing Φ . The kinematics of these simple maneuvers lie within the continuum of kinematics between hovering in air and heliox, which suggests that the response to heliox is not novel, but a routine strategy that bees employ when conditions require near-maximal aerodynamic output. These results suggest that the output degrees of freedom with which insects can modulate aerodynamic performance are limited, perhaps by constraints of their musculoskeletal system. The reduction of the control of aerodynamic output to simply manipulating stroke amplitude in bees provides validation and support for the kinematics yielded by studies utilizing single camera views and/or low temporal resolution (Dudley, 1995; Roberts et al., 2004). However, further research is necessary to characterize the aerodynamic consequences of the more subtle kinematic patterns I observed. Specifically, future work should address how variation in the patterns of α_r and $\dot{\alpha}$, and dorsal wing contact contribute to the production of aerodynamic force, flight control and stability.

CHAPTER 3

THE EFFECTS OF AGE AND BEHAVIORAL DEVELOPMENT ON FLIGHT PERFORMANCE

Abstract

A critical but seldom studied component of life history theory is how behavior and age affect whole-organism performance. To address this issue I compared the flight performance of honey bees (whose behavioral development and age can be assessed independently via simple manipulations of colony demographics) between distinct behavioral castes (in-hive nurse bees *vs.* out-of-hive foragers) and across lifespan. Variable-density gases and high-speed video were used to determine the maximum hovering flight capacity and wing kinematics of age-matched nurse bees and foragers sampled from a single-cohort colony over a period of 34 days. The transition from hive-work to foraging was accompanied by a 42% decrease in body mass and a proportional increase in flight capacity (defined as the minimum gas density allowing hovering flight). The lower flight capacity of hive-bees was primarily due to the fact that in air they were functioning at a near maximal wing angular velocity due to their high body masses. Foragers were lighter and when hovering in air required a much lower wing angular velocity, which they were able to increase by 32% during maximal flight performance. Flight performance of hive-bees was independent of age, but in foragers the maximal wingbeat frequency and maximal wing angular velocity were lowest in

precocious (7 to 14-day-old) foragers, highest in normal-aged (15 to 28-day-old) foragers and intermediate in foragers older than 29 days. This pattern coincides with previously described age-dependent biochemical and metabolic properties of honey bee flight muscle.

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Introduction

A critical issue in life history theory is how behavior and age affect the lifetime patterns of whole-organism performance (Roff, 2007; Rose et al., 2007). Studies of this issue should ideally separate the effects of age and behavior without ambiguity, focus on performance traits that are ecologically relevant, and utilize free-living animals, whose behavior and physiology may be quite different from those of laboratory-reared counterparts (Ricklefs and Wikelski, 2002). These challenges can be met by comparing the flight performance of honey bees (*Apis mellifera*, whose behavioral development and age can be assessed independently via simple manipulations of colony demographics) among distinct behavioral castes and across lifespan. Flight is a principal trait (along with eusociality, memory, communication and navigation) contributing to honey bee fitness and success via colony-level resource acquisition. Flight is unique among these traits in that its capacity is subject a suite of physiological changes during development, yet chronic performance of this behavior entails exposure to stressors (e.g. high temperature, reactive oxygen species, mechanical wear) that may hinder these same beneficial physiological traits and cause senescence (Roberts and Elekonich, 2005).

Adult honey bees proceed through behaviorally-defined life-history stages as they age, a process of behavioral development called temporal polyethism. These insects increasingly rely on flight ability during this process, which normally involves in-hive tasks such as brood care (nursing) and hive maintenance during the first 2-3 weeks of adult life followed by a transition to tasks outside the hive, predominantly foraging, which typically last for 2-3 weeks prior to death (Dukas, 2008). Among the many physiological and biochemical changes occurring between eclosion and the onset of foraging are a 10-fold increase in cytochrome concentrations (Herold, 1963), a doubling of thoracic glycogen levels (Fewell and Harrison, 2001; Harrison, 1986), and increased citrate synthase levels and Troponin T (TnT) 10A expression (Schippers et al., 2006) that combine to yield an 8-fold increase in flight metabolic rate (up to 800W kg^{-1}) during this period (Harrison and Fewell, 2002; Roberts and Harrison, 1999).

For many metabolically expensive behaviors such as insect flight, peak capacity is transient and progressively senesces (Carey et al., 2006; Grotewiel et al., 2005; Leffelaar and Grigliatti, 1984; Miller et al., 2008), presumably due in large part to oxidative stress and the impairment of mechanisms resisting it (Amdam and Omholt, 2002; Golden et al., 2002; Martin and Grotewiel, 2006; Seehuus et al., 2006; Sun and Tower, 1999; Vieira et al., 2000; Yoon et al., 2002; Yu and Chung, 2006). In *Drosophila melanogaster*, the frequency and duration of flight bouts as well as wing kinematic performance decreases with age beginning 1-2 weeks after eclosion (Carey et al., 2006; Leffelaar and Grigliatti, 1984; Miller et al., 2008). In house flies (*Musca domestica*), flight behavior accelerates age-dependent oxidative damage including the accrual of mitochondrial peroxide, carbonylation of select mitochondrial enzymes, and

mitochondrial DNA damage, while preventing flight prevents such damage and increases longevity (Agarwal and Sohal, 1994; Sohal and Buchan, 1981; Sohal and Dubey, 1994; Yan et al., 1997; Yan and Sohal, 1998; Yan and Sohal, 2000).

Oxidative stress produced by the intense aerobic demands upon honey bee foragers is likely mitigated by upregulation of flight muscle Hsp70, catalase and CuZn superoxide dismutase (Williams et al., 2008; Wolschin and Amdam, 2007). However, the diurnal upregulation of Hsp70 and catalase (along with total antioxidant capacity) in the flight muscles of foragers subsides with age (Williams et al., 2008), and honey bee mortality sharply increases following 12-14 days of foraging experience (Dukas, 2008). Thus, oxidative stress that accrues with age, especially following the transition to foraging behavior, may accelerate senescence of flight capacity in honey bees.

The present study investigated how age and behavioral development independently affect honey bee flight capacity. I hypothesized that changes in flight capacity reflect physiological and biochemical changes in flight muscle that are known to occur during behavioral development and with age as described above. I predicted that, independent of age, bees collecting pollen and nectar (foragers) will have greater flight capacity than bees performing brood-care (nurses). I also predicted that the flight capacity of foragers will initially improve with age, reach some maximum level in intermediate-aged individuals, and senesce in older individuals. To separate the effects of age and behavioral development on normal vs. maximal hovering flight capacity, I created a single-cohort colony (SCC) comprised only of 1- to 2-day old honey bees. About 10% of bees in a SCC will transition to foraging precociously (i.e. about 1 week after eclosion) while others remain normal-aged nurses. In the following 1-2 weeks more bees

transition into foraging behavior at a typical age while others remain in the hive as over-aged nurses. Thus, a SCC allows for comparisons of flight performance between age-matched groups of nurses and foragers, to assess the effects of behavior independently of age, and within behavioral castes, to assess the effects of age independently of behavior. I assayed maximal flight capacity by permitting bees to hover in a series of normoxic, variable-density gasses to determine the minimal gas density (*MGD*) that allowed for hovering flight (Roberts et al., 2004). A high-speed (4348 fps) digital video camera was used to record hovering sequences, from which the following kinematics were derived: wingbeat frequency (*n*), wing stroke amplitude (Φ), and wing angular velocity ($\bar{\omega}$). I found that honey bee flight capacity is limited and age-independent in nurses but greatly improves at the transition to foraging behaviors. Moreover, flight capacity further improves with age if the transition to foraging is premature, and then senesces in very old foragers.

Materials and Methods

Single Cohort Colony; Sampling and Weighing

A SCC containing 2240 European honey bee workers was created from 6 frames of immature bees from 3 different source colonies (each derived from multiply-mated queens) at the University of Nevada, Las Vegas apiary during late June, 2007. The frames were placed in an incubator (32 °C, 75% RH, 24-hour dark cycle) and newly-eclosed adult bees were removed every 24 hours. The SCC was founded from adult bees that eclosed on two consecutive days. On the first of these two days, 1000 bees were fitted with small, unique, color- and number-coded tags (Opallitplätchen, Graze, KG,

Endersbach, Germany) glued to the dorsal thorax for the purpose of individual identification. Of these bees, 400 were individually weighed immediately following tagging. The SCC was provided with an unrelated queen bee, one frame each of honey and pollen, and three empty frames for egg-laying/brood development. The SCC was kept closed in an environmental chamber (30 °C and 30% RH) for the following five days post-eclosion to allow the queen to lay eggs and maturation of the workers before being moved to the outdoor apiary to permit normal colony activity. Only tagged nurses and foragers were collected for assessment of flight capacity.

Maximal Flight Capacity and Wing Kinematics

Forager and nurse bees were assessed for maximal flight capacity. Foragers generally exit the hive at a relatively high velocity (relative to bees performing guarding behavior, or in-hive bees performing orientation or defecation flights) and in a straight-line trajectory towards the perimeter of the apiary. I intercepted individual out-going foragers ($N = 57$, ranging in age from 8 to 40 days old) as they flew into a 1-quart, clear plastic bag held approximately 30 cm from the entrance of the hive. Nurses ($N = 40$, ranging in age from 8 to 27 days old) were collected from the comb using light forceps after they performed the caste specific behavior of repeatedly sticking their heads into cells that contained larvae. I was unable to collect nurses older than 27 days of age from the original cohort of tagged bees because these individuals were gradually replaced by younger bees from brood laid by the resident queen. Bees were transported to an environmental chamber maintained at a temperature of 30 °C where maximal flight capacity was determined. Bees were weighed to the nearest 0.0001 g following assessment of maximal flight capacity.

The methods used to assess individual flight capacity were similar to those used by Roberts et al. (2004). Forager and nurse bees were immediately transferred to a flight chamber which consisted of a 5-L Erlenmeyer flask fitted with an inlet port at the base for gas perfusion and a lucite cover to prevent the bees from escaping. Bees were exposed to variable density, normoxic gas mixtures which consisted of oxygen and nitrogen and/or helium, and ranged from normodense air (21% O₂, 79% N₂; 1.21 kg m⁻³) to hypodense heliox (21% O₂, 79% He; 0.41 kg m⁻³) in 0.16 kg m⁻³ increments. The gasses were mixed using calibrated bi-metal thermo-actuated valves (low flow: Tylan F C-260; San Diego, CA, USA) and solenoid-actuated valves (high flow: Tylan FC-2910), and mixtures and flow rates were metered by an electronic flow controller (Sable Systems MFC-4; Las Vegas, NV, USA). When assessing maximal flight capacity and filming hovering flight, total gas flow rate was maintained at 1 L min⁻¹. Each trial began with air and the 5 hypodense gas mixtures were then administered in random order. In between gas mixtures, the flight chamber was flushed with the new gas mixture at a flow rate of 25 L min⁻¹ for one minute to ensure complete washout. Bees were flown in each gas mixture until either: 1) sustained hovering flight was observed and recorded; 2) hovering flight was attempted but failed (typically distinguished by the bee skimming across the floor of the chamber, unable to generate enough lift to hover); or, 3) three minutes elapsed, in which case the inactive bee was excluded from analysis. Bees that landed on the floor or sides of the chamber were persuaded to fly by agitating them with a small magnetic stir-bar, directed by a magnetic wand outside of the chamber. Maximal flight capacity was determined as *MGD*, the minimal gas density that allowed hovering flight.

Honey bees hover in air and heliox using a horizontal stroke plane (Altshuler et al., 2005; Ellington, 1984); therefore, hovering flight kinematics were determined from the wing trajectories in the horizontal plane recorded by a single, high-speed (4348 fps) digital video camera (Vision Research, Phantom v5.1; Wayne, NJ, USA). The camera was oriented directly above the flask and focused such that the focal plane was at the center of the flask. Hence, hovering bees in focus and viewed directly through the mouth of the flask were away from the narrow-circumference(s) near the top of the flask and centered in the chamber at least five wing-lengths (i.e. 50 mm) away from the chamber floor and walls. The central positioning within the chamber minimized the possibility of kinematic variation due to the boundary effect – when vortices become ‘trapped’ between the flyer and nearby surfaces (Raynar and Thomas, 1991). Ascending, descending or maneuvering flight was ignored. The digital video sequences were analyzed using custom software (Matlab, The Mathworks; Natick, MA USA) to determine the following kinematic variables for individual bees during hovering in air (subscript: “norm”) and hovering in the *MGD* (subscript: “max”): n (in Hz) was calculated from the duration to complete 10 successive wingbeats; Φ (in degrees) was calculated as the average of the downstroke and upstroke angular displacement for each of the 10 wingbeats; and $\bar{\omega}$ (in radians sec^{-1}), the average wing angular velocity, was calculated from the duration to complete the total angular displacement of one downstroke and one upstroke for each of the 10 wingbeats.

Statistical Analysis

Analysis of variance (ANOVA) was used to evaluate how body mass (M_b) differed between foragers, nurses and one-day old bees (eclosion mass). Multivariate analysis of

covariance (MANCOVA; $\alpha = 0.05$) was used to determine the effect of behavioral caste, with M_b and age as covariates, on flight performance and kinematic variables. Our *post hoc* analyses consisted of evaluating specific relationships using linear or polynomial regression. Model I (least squares) linear regression was used to analyze relationships that included age or maximal flight capacity (*MGD*). Other relationships where both continuous variables were subject to measurement error were analyzed with Model II linear (reduced major axis) regression. Because our *a priori* prediction was that flight capacity and kinematics in foragers would improve and then decline with age, I also used a 2nd-order polynomial regression to test the effects of age on these variables.

Results

Behavioral Development and Body Mass

The M_b of a random sample of adult honey bees (exclusive of those used in flight assays) within 24 hrs of eclosion was 93.9 ± 13.3 mg (mean \pm S. D.; $N = 40$). The youngest age at which bees began to forage was 8 days post-eclosion. Collection of nurses and foragers for flight analyses began at this time and concluded at 27 days of age for nurses and at 40 days of age for foragers. Body mass was significantly different between bees at eclosion, nurses and foragers (ANOVA: $F_{1,94} = 376.9$; $P < 0.001$), with foragers (76.0 ± 7.4 mg, $N = 57$) being 42.9% lighter than nurses (133 ± 19.1 mg, $N = 40$). However, age did not significantly affect M_b for either nurses (Model I linear regression: $P = 0.154$) or foragers (Model I linear regression: $P = 0.345$).

Flight Performance and Kinematics

There was a significant effect of behavioral caste, mass and age on flight performance (MANCOVA: $P < 0.001$; $P < 0.001$; $P = 0.006$ respectively, see Table 3.1). Behavioral caste had a significant effect on MGD (MANCOVA: $P < 0.001$), with foragers being able to fly in gas densities 34% lower than nurses, after correcting for variation in mass and age (Table 3.1). Approximately 20% of foragers could hover in pure heliox, while the same fraction of nurses were capable of hovering only in normal air or could not fly at all. Age had a significant effect on MGD (MANCOVA: $P < 0.001$). Because my hypothesis predicted that maximal flight capacity would improve with age in young foragers and senesce in older foragers, I fitted a 2nd order polynomial curve to the MGD vs. forager age data (Fig. 3.1); this polynomial regression was significant ($r^2 = 0.26$, $P < 0.001$).

Body mass also had a significant effect on MGD (MANCOVA: $P = 0.005$). Because M_b varied greatly between the two behavioral castes, I further evaluated the relationship between M_b and MGD using linear regression (Fig. 3.2). MGD was independent of M_b in

Table 3.1: Multivariate Analysis of Covariance (MANCOVA) for the effects of caste, mass and age on flight performance.

	Parameter Estimates ^a (mean \pm S.E.)		Caste ^b		Mass ^c		Age ^d	
	Nurse	Forager	$F_{1,93}$	P	$F_{1,93}$	P	$F_{1,93}$	P
n_{norm} (Hz)	233.7 \pm 3.7	229.1 \pm 2.7	0.63	0.429	3.36	0.070	1.37	0.244
Φ_{norm} (deg)	121.1 \pm 2.9	108.7 \pm 2.1	7.21	0.009	15.3	< 0.001	0.45	0.503
$\bar{\omega}_{\text{norm}}$ (rad s ⁻¹)	985.1 \pm 24.4	866.5 \pm 18.1	9.28	0.003	6.33	0.014	0.01	0.925
n_{max} (Hz)	220.6 \pm 4.3	219.9 \pm 3.2	0.01	0.923	0.12	0.733	3.07	0.083
Φ_{max} (deg)	139.0 \pm 2.8	143.6 \pm 2.1	1.03	0.314	0.98	0.326	5.86	0.017
$\bar{\omega}_{\text{max}}$ (rad s ⁻¹)	1065.1 \pm 22.6	1102.6 \pm 16.8	1.08	0.302	0.59	0.446	15.83	< 0.001
MGD (kg m ⁻³)	0.99 \pm 0.04	0.65 \pm 0.03	19.23	< 0.001	8.40	0.005	18.5	< 0.001

^a Least squares means evaluated at mass = 100.3 mg, and age = 19.9 days

^b MANCOVA: Pillai's Trace, $F_{7,87} = 5.04$; $P < 0.001$

^c MANCOVA: Pillai's Trace, $F_{7,87} = 5.18$; $P < 0.001$

^d MANCOVA: Pillai's Trace, $F_{7,87} = 3.09$; $P = 0.006$

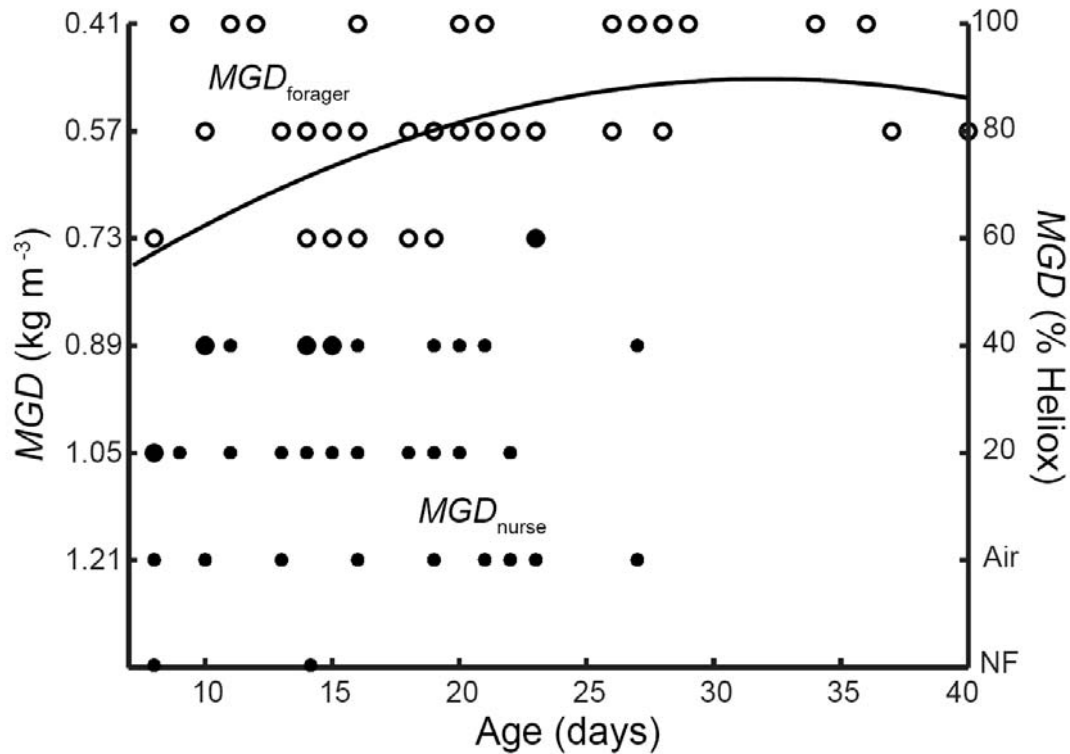


Figure 3.1: Maximal flight capacity (minimal gas density: MGD) vs. age for foragers (MGD_{forager} ; open symbols) and nurses (MGD_{nurse} ; filled symbols). Large filled circles indicate overlapping forager and nurse data. Values of MGD (kg m^{-3}) are inverted to reflect the increasing aerodynamic demand of flying in lesser-density gas mixtures. Bees that were unable to fly in air (no flight: NF; secondary y-axis) were plotted for descriptive purposes and were not included in the calculated MANCOVA or regressions. 2nd order polynomial regression for foragers: $MGD = 0.954 + 0.029\text{age} - 0.0005\text{age}^2$, $r^2 = 0.26$, $P < 0.001$ (solid line).

foragers (Model II regression: $MGD_{\text{forager}} = 0.619 - 0.001M_b$, $r^2 = 0.002$, $P = 0.772$), but significantly increased with M_b in nurses (Model II regression: $MGD_{\text{nurse}} = 0.613 + 0.003M_b$, $r^2 = 0.177$, $P = 0.006$). This effect was subtle, with variation in M_b explaining just 18% of variation in MGD in nurses. However, each bee in our experiment is an independent observation, and when behavioral castes were pooled, MGD significantly increased with M_b (i.e. lighter bees - primarily foragers - were better able to fly in hypodense gases), with variation in M_b explaining 66% of variation in MGD for all bees

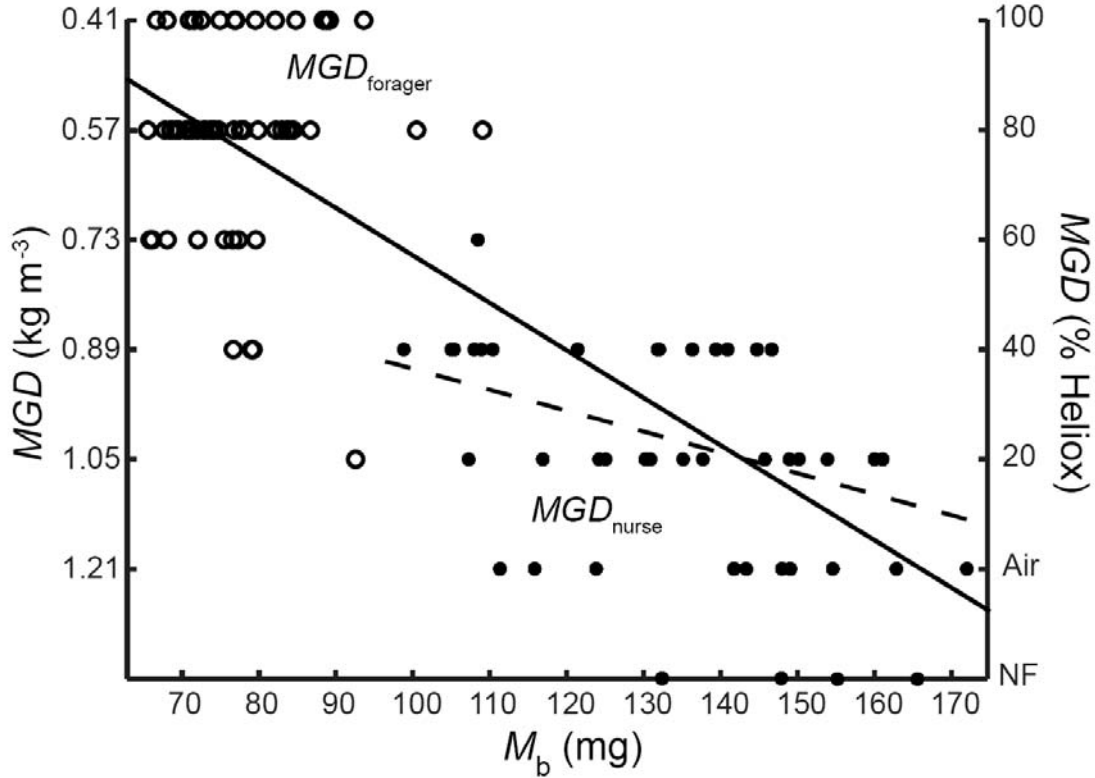


Figure 3.2: Maximal flight capacity (minimal gas density: MGD) vs. body mass (M_b) for foragers (MGD_{forager} ; open symbols) and nurses (MGD_{nurse} ; filled symbols). Values of MGD (kg m^{-3}) are inverted to reflect the increasing aerodynamic demand of flying in lesser-density gas mixtures. Bees that were unable to fly in air (no flight: NF; secondary y-axis) were plotted for descriptive purposes and were not included in the calculated MANCOVA or regressions. Model II regression: $MGD_{\text{forager}} = 0.619 - 0.001M_b$, $r^2 < 0.01$, $P = 0.772$; $MGD_{\text{nurse}} = 0.613 + 0.003M_b$, $r^2 = 0.18$, $P = 0.006$ (broken line). $MGD_{\text{total}} = 0.061 + 0.007M_b$, $r^2 = 0.66$, $P < 0.001$ (solid line).

combined (Model II regression: $MGD_{\text{total}} = 0.061 + 0.007M_b$, $r^2 = 0.660$, $P < 0.001$).

For bees hovering in air, n_{norm} tended to decrease across M_b , but this trend was not significant (MANCOVA: $P = 0.070$). However, M_b significantly affected Φ_{norm} and $\bar{\omega}_{\text{norm}}$ (MANCOVA: $P < 0.001$, $P = 0.014$, respectively). During hovering in air, Φ_{norm} significantly increased with M_b (model II regression: $P < 0.001$), with variation in M_b explaining 67% of the variation in Φ_{norm} (Fig. 3.3). The heaviest bees had Φ_{norm} values approximately 45% higher than the lightest bees. Likewise, $\bar{\omega}_{\text{norm}}$ significantly

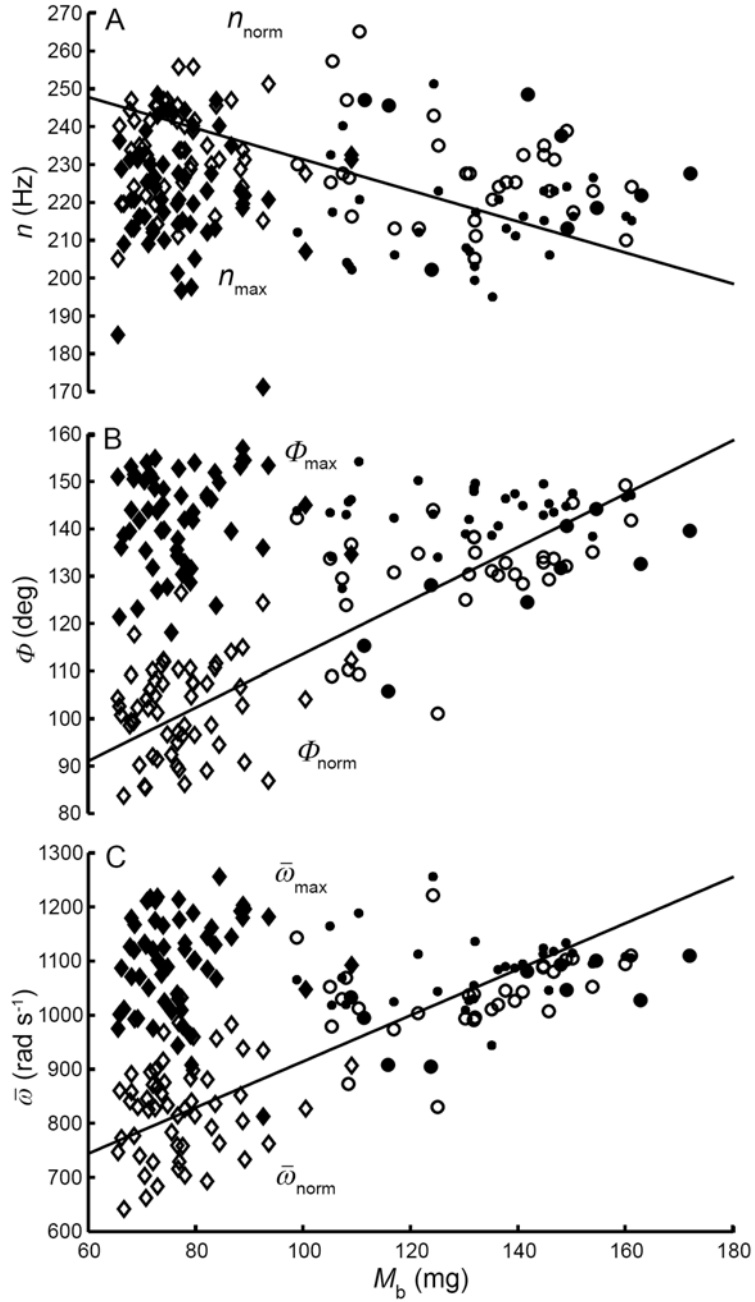


Figure 3.3: Wingbeat frequency (n ; panel A), wing stroke amplitude (Φ ; panel B), and wing angular velocity ($\bar{\omega}$; panel C) vs. body mass (M_b) for foragers (diamonds) and nurses (circles) during flight in air (norm; open symbols) and maximal flight in the *MGD* (max; closed symbols). Model II regression for n (panel A): $n_{\text{norm}} = 272.18 - 0.411M_b$, $r^2 = 0.07$, $P = 0.008$ (solid line); $n_{\text{max}} = 267.71 - 0.474M_b$, $r^2 = 0.01$, $P = 0.401$. Model II regression for Φ (panel B): $\Phi_{\text{norm}} = 56.85 + 0.568M_b$, $r^2 = 0.67$, $P < 0.001$ (solid line); $\Phi_{\text{max}} = 173.54 - 0.318M_b$, $r^2 < 0.01$, $P = 0.823$. Model II regression for $\bar{\omega}$ (panel C): $\bar{\omega}_{\text{norm}} = 488.49 + 4.256M_b$, $r^2 = 0.58$, $P < 0.001$ (solid line); $\bar{\omega}_{\text{max}} = 1356.80 - 2.689M_b$, $r^2 = 0.01$, $P = 0.347$.

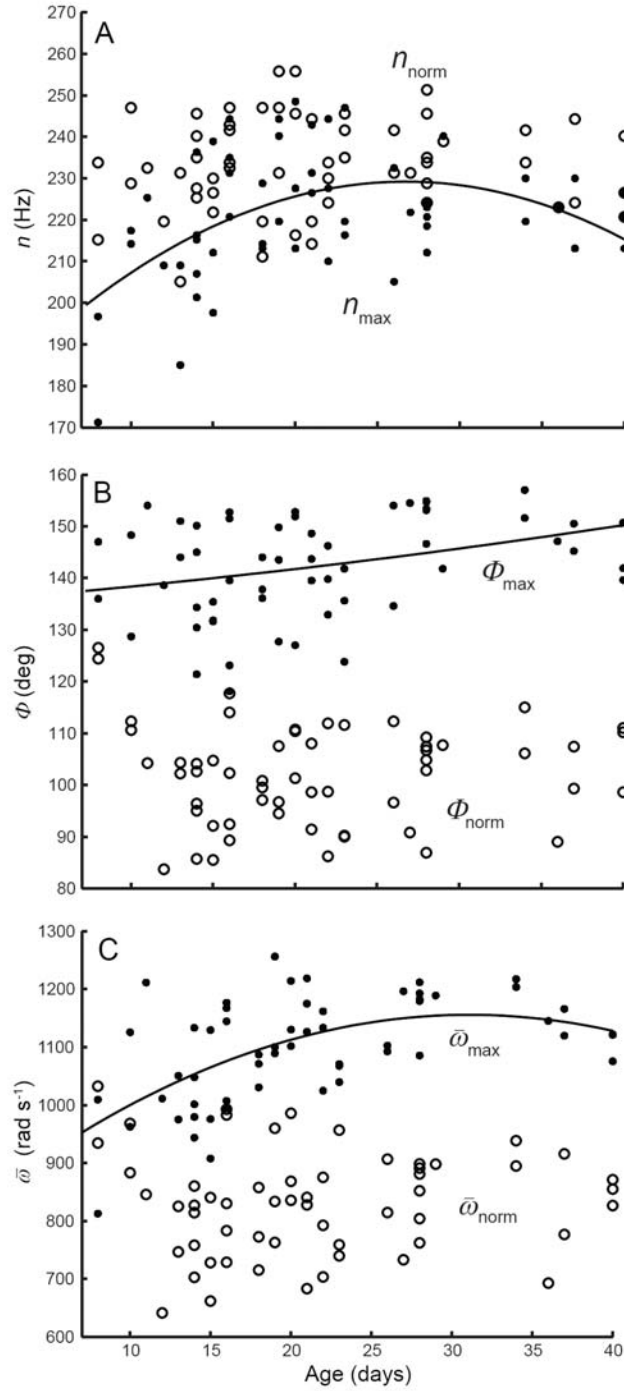


Figure 3.4: Wingbeat frequency (n ; panel A), wing stroke amplitude (Φ ; panel B), and wing angular velocity ($\bar{\omega}$; panel C) vs. age for foragers. 2nd order polynomial regression for n_{\max} : $n_{\max} = 173.27 + 4.19\text{age} - 0.079\text{age}^2$, $r^2 = 0.24$, $P < 0.001$. 2nd order polynomial regression for Φ_{\max} : $\Phi_{\max} = 135.57 + 0.252\text{age} - 0.003\text{age}^2$, $r^2 = 0.11$, $P < 0.001$. 2nd order polynomial regression for $\bar{\omega}_{\max}$: $\bar{\omega}_{\max} = 813.50 + 22.14\text{age} - 0.355\text{age}^2$, $r^2 = 0.34$, $P < 0.001$ (solid line).

increased with M_b during hovering in air (model II regression: $P < 0.001$), with variation in the latter explaining 58% of the variation in the former. During hovering in the *MGD*, M_b did not affect n_{\max} , Φ_{\max} , or $\bar{\omega}_{\max}$ (MANCOVA: $P = 0.733$, $P = 0.326$, $P = 0.446$, respectively). Behavioral caste had a significant effect on *MGD*, Φ_{norm} , and $\bar{\omega}_{\text{norm}}$ (MANCOVA: $P < 0.001$, $P = 0.009$, $P = 0.003$, respectively; Fig. 3.3). The effects of behavioral caste are similar to those of M_b (Table 3.1), in large part due to the significant difference in M_b between the nursing and foraging castes.

Age did not affect n_{norm} , Φ_{norm} , and $\bar{\omega}_{\text{norm}}$ during hovering in air (Table 3.1). Although age did not affect n_{\max} during hovering in the *MGD*, age had a significant effect on Φ_{\max} and $\bar{\omega}_{\max}$ (MANCOVA: $P = 0.017$, $P < 0.001$, respectively). However, the MANCOVA is a linear model and thus cannot reveal the predicted parabolic relationships between kinematic capacity and age. To test whether maximal kinematic capacities peaked in middle-aged foragers, I fitted a 2nd order polynomial curve to the forager data. The polynomial regression for n_{\max} vs. age was significant ($r^2 = 0.24$, $P < 0.001$) for foragers hovering in the *MGD* (Fig. 3.4A). For Φ_{\max} vs. age, the 2nd order polynomial regression curve fit for foragers hovering in *MGD* was significant but explained only a small percentage of the variation in Φ_{\max} across age ($r^2 = 0.11$, $P = 0.040$; Fig. 3.4B). The 2nd order polynomial curve fit to $\bar{\omega}_{\max}$ vs. age was significant ($r^2 = 0.34$, $P < 0.001$) for foragers hovering in *MGD* (Fig. 3.4C). Hence, n_{\max} and $\bar{\omega}_{\max}$ increased with age in precocious foragers, reached a plateau in middle-aged foragers, and senesced to a small degree in older foragers. In foragers, n_{\max} was less than n_{norm} , while Φ_{\max} and $\bar{\omega}_{\max}$ were greater than Φ_{norm} and $\bar{\omega}_{\text{norm}}$, respectively (Paired T-Test:

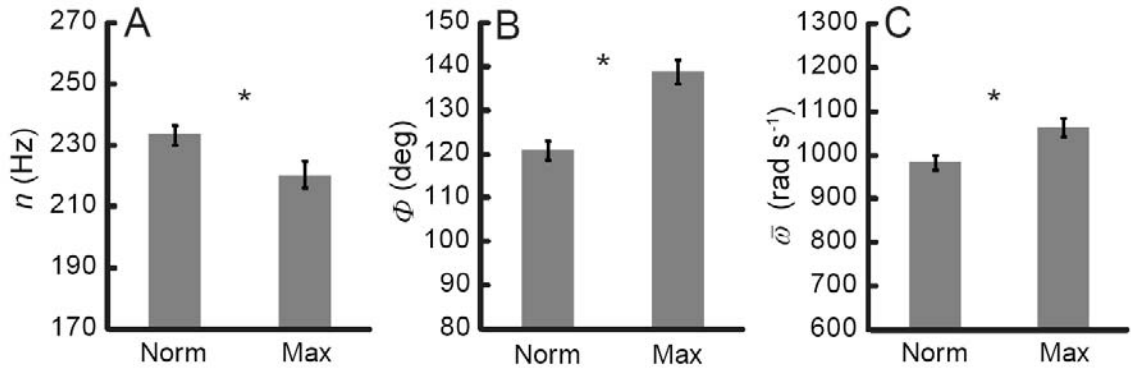


Figure 3.5: Wingbeat frequency (n ; panel A), wing stroke amplitude (Φ ; panel B), and wing angular velocity ($\bar{\omega}$; panel C) for nurses during hovering in air (norm) and hovering in the *MGD* (max). Asterisks indicate significant differences between normal and maximal hovering for n , Φ , and $\bar{\omega}$ (Paired T-Test: $P < 0.001$ in each case).

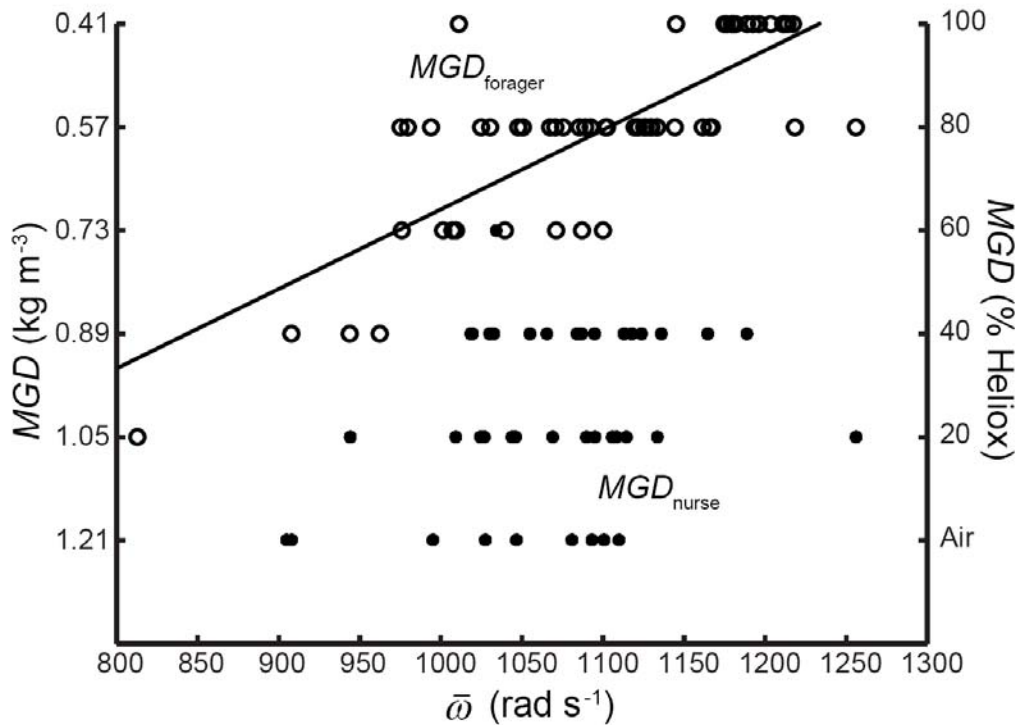


Figure 3.6: Maximal flight capacity (minimal gas density: MGD) vs. maximal wing angular velocity ($\bar{\omega}_{\max}$) for foragers (MGD_{forager} ; open symbols) and nurses (MGD_{nurse} ; closed symbols). Values of MGD (kg m^{-3}) are inverted to reflect the increasing aerodynamic demand of flying in lesser-density gas mixtures. Model I regression: $MGD_{\text{forager}} = 1.930 - 0.0012\bar{\omega}_{\max}$, $r^2 = 0.62$, $P < 0.001$ (solid line); $MGD_{\text{nurse}} = 1.594 - 0.0006\bar{\omega}_{\max}$, $r^2 = 0.08$, $P = 0.083$.

$P < 0.001$ in each comparison). There were no significant regressions of n , Φ , or $\bar{\omega}$ across age for nurses hovering in air or *MGD*. In nurses, n_{\max} was slightly, but significantly, less than while Φ_{\max} and $\bar{\omega}_{\max}$ were significantly greater than Φ_{norm} and $\bar{\omega}_{\text{norm}}$ (Fig. 3.5; Paired T-Test: $P < 0.001$ in each comparison). In order to better understand how kinematic performance might affect the caste-specific flight performance, I performed an ANCOVA to investigate the effects of caste on *MGD* with $\bar{\omega}_{\max}$ as a covariate (Fig. 3.6). There was a significant interaction between caste and $\bar{\omega}_{\max}$ on *MGD* (ANCOVA: $F_{1,93} = 5.38$, $P = 0.023$). In foragers, *MGD* significantly increased with $\bar{\omega}_{\max}$, with variation in $\bar{\omega}_{\max}$ explaining 62% of the variation in *MGD*. However, there was no relationship between $\bar{\omega}_{\max}$ and *MGD* in nurses.

Discussion

Using SCCs and variable-density gas mixtures, I was able to show that both age and behavioral development affect the flight performance of honey bees. To my knowledge this is the first study to experimentally segregate these factors and test their effects on the locomotor capacity of a free-living organism over a lifetime. The ability to fly in hypodense atmospheres greatly improves at the transition from nursing to foraging behaviors, and this improvement is facilitated predominantly by a large decrease in body mass that accompanies this transition. Although precocious (8-14 day old) foragers had greater flight capacity than age-matched nurses, flight capacity generally improved with age in young (15-21 days old) and typical-aged (22-28 day old) foragers. Peak kinematic performance was lowest in precocious (7 to 14-day-old) foragers, highest in normal-aged (15 to 28-day-old) foragers and intermediate in foragers older than 29 days.

Kinematic performance and flight ability strongly increased following the transition to foraging (although this improvement was not complete if the behavioral transition occurred too early), and also showed modest, but perhaps ecologically important, signs of senescence in the oldest foragers in the study.

Body Mass and Flight Performance

The primary basis for improved flight ability in foragers was the large (~ 43%) decrease in M_b that occurred prior to the transition to foraging behavior, regardless of age. The reduction in M_b prior to the behavioral transition is restricted to tissues of the abdomen and is primarily due to gut emptying; hence, thoracic mass remains constant (but relative thorax mass increases) across the behavioral transition (Harrison, 1986). The strong effect of M_b on flight capacity was not apparent by comparing the two variables within each behavioral caste, as flight capacity was unaffected by M_b in foragers and only weakly correlated with M_b in nurses. However, when the two behavioral castes were pooled, yielding a much broader range of independent observations of mass and flight capacity in honey bees as a general group, a strong inverse relationship between M_b and MGD was revealed (Fig. 3.2).

Nurse bees had a very limited reserve capacity for kinematic and aerodynamic performance due predominantly to their heavy bodies, but also to their immature flight muscles. While hovering in air, the Φ_{norm} and $\bar{\omega}_{\text{norm}}$ of heavier, younger bees (nurses) were at or just below maximal attainable levels. Moreover, nurses were unable to sustain normal n when challenged to hover in hypodense gases – to the extent that $\bar{\omega}_{\text{max}}$ exceeded $\bar{\omega}_{\text{norm}}$ air by only 8% (after adjusting for the effects of age and mass). Precocious foragers and very old foragers were similarly unable to maintain n when

challenged with hypodense gases, but their ability to strongly increase Φ still offered greatly elevated $\bar{\omega}$ during maximal hovering performance. When challenged with hypodense gases, only middle aged foragers were able to increase Φ and maintain n . Hummingbirds (Altshuler and Dudley, 2003; Chai et al., 1997), euglossine bees (Dudley, 1995) and carpenter bees (Roberts et al., 2004) similarly increase Φ and maintain (or even slightly increase) n during maximal hovering flight. Although there is no information on the age-dependence of flight performance in these taxa, it seems plausible that kinematic performance might be similarly affected in very young or old individuals.

Across closely-related hovering insects, n decreases with M_b during hovering flight (Dillon and Dudley, 2004; Dudley, 2000), but this negative relationship does not always hold true for the few available datasets allowing intraspecific comparisons of n and M_b . In honey bees, there is a slight negative relationship between n_{norm} and M_b , although this is unlikely due to resonance issues and an increase in the induced power required to move a larger wing (factors typically associated with the negative relationship between n and M_b across similar species) because neither wing size nor thorax dimensions differ between foragers and nurses (personal observation). Instead, the heaviest honey bees (nurses) require elevated Φ just to fly in air, but their immature flight muscles do not allow them reach n values attainable by many (particularly middle aged) foragers, which are much lighter than nurses. For carpenter bees (*Xylocopa varipuncta*) hovering in air, heavier individuals have higher Φ (as do honey bees; Fig. 3.3B) and n during due to disproportionately heavier abdomens and high wing loading (Roberts et al., 2004), although peak kinematic performance and M_b are independent of each other in both of

these species. This is not the case during flight in heliox and maximal load lifting across several species of euglossine bees whose M_b span over an order of magnitude, in which case Φ_{\max} is highly conserved near 140° , but n_{\max} decreases with M_b (Dudley 1995; Dillon and Dudley, 2004).

Variation in M_b was smallest in foragers, and M_b had no effect on MGD in this group. This is not so for *X. varipuncta*, in which body mass varies by 3-fold, with lighter individuals capable of hovering in lower gas densities than heavier individuals due to lower wing loading, relatively larger flight muscles and smaller abdomens (Roberts et al., 2004). The ability of honey bee foragers to fly in hypodense gases was positively correlated with $\bar{\omega}_{\max}$ (Fig 3.6). In several species of *Drosophila*, aerodynamic forces scale to the square of wing translational velocity (Lehmann and Dickinson, 1998), which is determined by $\bar{\omega}$, and hence it is not surprising that the honey bees capable of generating the highest values of $\bar{\omega}_{\max}$ were also the ones capable of hovering in the lowest gas densities. To our knowledge this is the only study to date linking individual variation in kinematic capacity (in this case largely due to age plus random effects) to peak flight performance.

The Development and Senescence of Flight Performance

The improvement of flight muscle performance at the transition to foraging and during foraging (if the transition is premature) is likely due to a suite of biochemical and structural changes in the flight muscle that occur during honey bee maturation and behavioral development. For example, young honey bees (~3 days old) that have acquired the ability to fly express an isoform of TnT similar to the 46 kDa TnT isolated

only to the mature flight muscle of adult *Drosophila* (Domingo et al., 1998). This TnT isoform is absent in juvenile stages in *Drosophila* as well as 1-2 day old bees that are unable to fly, suggesting that the muscle function necessary for flight is dependent upon the expression of specific TnT isoforms. Furthermore, honey bee foragers express more TnT 10A (> two-fold increase) in their flight muscles than younger hive bees (Schippers et al., 2006). The effects of the differential TnT isoform expression on honey bee flight are unknown, but in the dragonfly *Libellula pulchella* the differential expression of TnT isoforms affects flight muscle calcium sensitivity and is correlated with an increase in wingbeat frequency and amplitude as the dragonflies progress from the teneral stage to sexual maturity (Fitzhugh and Marden, 1997; Fitzhugh et al., 1999; Marden et al., 2001; Marden et al., 1998; Marden et al., 1999). Elevated TnT 10A expression may contribute to the age and behavior-dependent increase in maximal wingbeat frequency in honey bees, and attempts to determine if the expression of TnT isoforms (and other flight-motor proteins) are similarly affected by age and behavioral development are ongoing in our laboratories.

The reduction in maximal kinematic and flight capacity in the older foragers likely reflects senescence via oxidative stress within the flight muscles. The intense aerobic metabolism of forager flight muscle (over 2000 W kg⁻¹ muscle) yields high levels of reactive oxygen species (ROS), the effects of which are mitigated by the upregulation of stress and antioxidant proteins such as Hsp 70, catalase and CuZn superoxide dismutase (Schippers et al., 2006; Williams et al., 2008; Wolschin and Amdam, 2007). However, resistance to oxidative stress declines with age, as old (30-32 days) honey bee foragers express less catalase and have lower total antioxidative capacity than precocious

foragers (Williams et al., 2008). Cytochrome *c* oxidase activity also decreases in aged honey bee flight muscle (Schippers 2006), but other cellular pathologies of honey bee flight muscle senescence are unknown. In *Drosophila* and other dipterans, such pathologies include depressed actin transcription, decreased sarcomere length, enlarged/degraded mitochondria, depressed mitochondrial respiration and depressed aconitase activity (Ferguson et al., 2005; Labuhn and Brack, 1997; Miller et al., 2008; Yarian and Sohal, 2005).

The mechanical wear of wings has also been implicated as an important factor contributing to the senescence of flight performance and mortality in eusocial bees (Cartar, 1992; Dukas, 2008; Hedenstrom et al., 2001; Higginson and Barnard, 2004). These authors hypothesize that degraded wings in older bees limits flight performance with consequences for foraging ability and predator evasion. Wing wear was not a factor contributing to senescence of flight performance in our study because in our experiments I only assayed bees that possessed intact, unworn wings. However, our finding of impaired n_{\max} in very old foragers may compound the problems of worn wings. For example, bumblebees increase n in response to wing clipping (Hedenstrom et al., 2001), and such compensation may be unavailable to older honey bee foragers. I have no information about the foraging history of the bees in our study (i.e. the absolute age of the foragers is known, but not how long they had been foraging), but I believe that the declines in n_{\max} , $\bar{\omega}_{\max}$, and maximal flight capacity in the older foragers probably mark the *onset* of senescence in the flight muscle. The *pace* of senescence of overall flight ability is still unknown but should be a function of both flight muscle and wing degradation.

The Ecological Significance of Honey Bee

Flight Performance

A honey bee colony can shift worker demographics in response to a deficiency of workers in a particular caste (Huang and Robinson, 1992; Robinson et al., 1989) or worker effort in response to a shortage of pollen stores (Fewell and Winston, 1992). Such shifts might involve precocious or very old foragers, both of which have reduced maximal flight capacity, and negatively affect foraging loads and rate of foraging intake (Higginson and Barnard, 2004; Schippers et al., 2006), with potential consequences for colony-scale economy and energy flux (Schmid-Hempel et al., 1985). Likewise, colony-level intake should be higher when, all else being equal, the foraging caste is represented by middle-aged individuals. Indeed, the amount of food collected per trip increases by over 300% throughout a bee's first week of foraging behavior (Schippers et al., 2006). Finally, precocious and aged foragers may be subject to higher predation risk due to their limited burst flight capacities (Cartar, 1992; Dukas, 2008). There are no data to confirm this linkage in honey bees, although wing damage resulting from male-male combat in the burrowing bee *Amegilla dawsoni* increases the risk of predation by birds and shortens longevity (Alcock, 1996).

Certain honey bee genotypes are predisposed to early or late initiation of foraging (Calderone and Page, 1988; Giray and Robinson, 1994), and it is possible that the trajectory of the age-dependent development of maximal flight capacity varies genetically as well. For colonies genetically predisposed to begin foraging at an earlier age, any potential colony-level costs of precocious foraging may be mitigated by a faster rate of development and shorter periods of sub-optimal maximal flight capacity.

Conversely, in colonies predisposed to a later onset of foraging, the costs of precocious foraging may be prolonged by a slower rate of development, or foraging onset may be temporally coordinated with slower development of flight capacity. Experiments addressing the temporal kinetics of foraging initiation and flight capacity among such genotypes would be valuable to test these possibilities.

Conclusion

The development of the flight capacity necessary for effective foraging in honey bees depends upon the sharp reduction in body mass at the transition from nursing to foraging behavior. Following this transition, the age-dependent development and senescence of maximal flight capacity in foragers reflects the ability to, when aerodynamically challenged, increase Φ while simultaneously maintaining n . Importantly, our experiment does not allow us to determine if the timing of the initiation of foraging affects the onset and pace of senescence (which would require lifetime ethography of individual bees), although precocious foraging does shorten lifespan (Rueppell et al., 2007). Even so, our results suggest that variation in honey bee flight capacity across age is an important factor explaining known life-history patterns of foraging behavior and mortality rates. However, future research is needed to directly link the ontogeny of flight capacity to foraging efficacy, predation risk and mortality.

CHAPTER 4

THE EFFECTS OF ARTIFICIAL WING WEAR ON FLIGHT PERFORMANCE

Abstract

The wings of bees and other insects accumulate permanent wear, which increases the rate of mortality and impacts foraging behavior, presumably due to effects on flight performance. In this study, I investigated how experimental wing wear affects flight performance in honey bees. Variable-density gases and high-speed video were used to determine the maximum hovering flight capacity and wing kinematics of bees from three treatment groups: no wing wear, symmetric and asymmetric wing wear. Wing wear was simulated by clipping the distal-trailing edge of one or both of the wings. In all bees, increases in wingbeat frequency were inversely proportional to wing area. During hovering in air, the kinematic response to accommodate symmetric and asymmetric wing wear produced wingtip velocities similar to those bees with no wing wear. However, maximum wingtip velocity and maximal flight capacity decreased in direct proportion to wing area. Bees with asymmetric wear produced lower maximum wingtip velocity than the control and symmetric groups, and despite less total wing area loss than the symmetric group, asymmetric wear caused a greater impairment in maximal flight capacity. These results demonstrate that the magnitude and type of wing wear affects maximal aerodynamic power production and, potentially, the control of

hovering flight. Wing wear reduces aerodynamic reserve capacity and, subsequently, the capacity for flight behaviors such as load carriage, maneuverability, and evading predators.

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Introduction

Damage or wear to the locomotor apparatus can result from encounters with predators and prey, locomotion in physically heterogeneous environments, and aging via prolonged use and senescence. In some cases even self-amputation of an appendage (autotomy) is a strategy to avoid predation (Congdon et al., 1974; Fleming et al., 2007; Robinson et al., 1970), although at great cost to locomotor capacity (Fleming and Bateman, 2007; Punzo, 1982). Most animals possess healing or restorative processes that improve or preserve functionality in locomotor appendages so damaged. However, such processes are conspicuously absent for the wings of insects so that any damage and functional impairment of these airfoils are permanent. The loss of wing area, changes in mechanical properties such as stiffness, or symmetry of the wing pair can impair flight performance and may ultimately reduce fitness. In this study I examined the consequences of artificially induced wing wear on the kinematics and flight capacity of honey bees (*Apis mellifera*).

Research attempting to unequivocally link wing damage, flight performance and longevity in insects is rare, although one exception is the case of honey bees and bumblebees (*Bombus* spp), both of which are eusocial. Foraging bees accumulate wing wear and damage with flight experience (Higginson and Barnard, 2004), and both

natural and experimentally induced wing wear reduces longevity (Cartar, 1992). In honey bees, longevity is inversely related to foraging effort (Neukirch, 1982; Schmid-Hempel and Wolf, 1988), and if wing wear increases flight metabolism via kinematic effort to offset loss of wing area, then this effect may reduce longevity in the context of a fixed lifetime energy budget. However, for *Bombus terrestris*, wing wear has no effect on flight metabolism, despite elevated kinematic and aerodynamic costs through increased wingbeat frequency (n), coefficient of lift, and induced power (Hedenstrom et al., 2001). Wing wear may also cause behavioral impairments with colony-level consequences. For example, as foraging honey bees accumulate wing wear, they accept inflorescences that are smaller or aged, suggesting that the efficiency of nectar foraging is reduced via acceptance of lower nectar quality/quantity by wing-worn bees (Higginson and Barnard, 2004).

The absence of a clear link between wing wear and metabolic cost indicates that wing wear impacts longevity and foraging behavior through a deficit in a different currency related to flight performance. Cartar (1992) and Hedenstrom et al. (2001) hypothesized that elevated mortality due to wing wear could be due to higher rates of predation resulting from impaired flight maneuverability. Lepidopterans, which rely on erratic zigzags to deter predators from a potentially energetically costly and unsuccessful hunt, are capable of flying with their hindwings completely removed, although such marked reductions in wing area in *Limantria dispar* and *Pieris rapae* decreases linear and turning acceleration, resulting in flight behavior that may predispose individuals to predation (Jantzen and Eisner, 2008). To a point, the magnitude and asymmetry of artificial wing wear in foraging *B. flavifrons* has little effect on flight distance and

velocity, and only when magnitude of wear and asymmetry is extreme do they begin to deviate from linear flight paths and require longer flight distances with higher velocities between flowers (Haas and Cartar, 2008). Although hymenopterans and lepidopterans are robust in their ability to mitigate substantial wing wear, these studies suggest that the costs of wing wear are reductions in kinematic and aerodynamic reserves available for elevated flight effort.

The kinematic and aerodynamic reserve capacities of flying insects are necessary for behaviors such as load carriage, gust mitigation, and predator evasion. The few studies have been able to elucidate these capacities during free flight have focused on bees, which are capable of vertical force production in excess of 2-times their body mass (Dillon and Dudley, 2004) and flight in atmospheres 1/3 the density of sea-level air (Altshuler et al. 2005; Dudley 1995; Roberts et al. 2004). These aerodynamic demands are met by increasing stroke amplitude (Φ) up to 45%, which in turn increases the angular and translational velocities ($\bar{\omega}$ and U_t , respectively) of the wings. Studying bees during challenging flight should reveal how wing wear affects maximal flight capacity and underlying functional reserves. Wing-worn *B. terrestris* increase n during normal hovering flight (Hedenstrom et al., 2001), but it is not known how wing-worn bees modulate Φ and n during maximal flight performance. If wing wear, and the subsequent reduction in wing area (S) and wing length (R), reduces maximal flight capacity, then the efficacy of flight behaviors dependant on those aerodynamic reserves should decrease. The present study investigates how the reduction in S due to symmetric and asymmetric experimental wing wear affects maximal hovering flight capacity in the honey bee (*Apis mellifera*), with the predictions that maximal flight

capacity will inversely vary with wing wear and that asymmetric wear will have the greatest affect on flight capacity due to likely impairment of both lift and control. I simulated symmetric and asymmetric wing wear across an ecologically-relevant range by clipping the distal-trailing edge of the wings (Hedenstrom et al., 2001; Higginson and Barnard, 2004). Maximal flight capacity was evaluated by allowing bees to hover in a series of normoxic, variable-density gasses to determine the minimal gas density (*MGD*) that permitted hovering flight (Roberts et al., 2004; Vance et al., Accepted). A high-speed (4348 fps) digital video camera recorded hovering sequences, from which I derived n , Φ , average $\bar{\omega}$ and average U_t . Our results show that honey bee flight capacity is progressively limited by the reduction in wing area, and that this effect is compounded by asymmetric wear.

Materials and Methods

Collection and Experimental Treatment

Honey bees of the foraging caste were collected as they exited a hive at the University of Nevada Las Vegas campus apiary and were randomly assigned to one of three experimental wing wear treatment groups: no wing wear (control; $N = 17$), symmetric ($N = 16$) and asymmetric ($N = 15$) wing wear. Only bees that possessed complete, unworn wings were chosen for the study (i.e. bees with pre-existing natural wing wear were not used). To create the experimental wing-wear, bees were held by the thorax using soft forceps, and the distal trailing edge of the forewing was trimmed using micro-shears. The symmetric group had both left and right forewings trimmed equally, and the asymmetric group had only one forewing trimmed and the opposite forewing left

intact. The magnitude of wing wear varied across an ecologically-relevant range, from 3 to 37% (Higginson and Barnard, 2004). For the control group, the bees were held by the thorax for 10 seconds, and the distal trailing edges of the forewings were touched with the shears, but no *S* was removed, similar to the sham-treatment used by Hedenstrom et al. (2001). Following the flight assay, bees were weighed, sacrificed, and the wings were removed and photographed with a digital camera. Wing images were digitized using custom software (MatLab; The Mathworks; Natick, Ma, USA) to calculate wing length and area.

Maximal Flight Capacity and Flight Kinematics

The methods used to assess individual flight capacity were similar to those used by Roberts et al. (2004) and Vance et al. (accepted). Following the experimental wing wear treatment, bees were immediately transferred to a flight chamber, which consisted of a 5-L Erlenmeyer flask with a lucite cover. Bees were exposed to six variable density, normoxic gas mixtures composed from oxygen and nitrogen and/or helium, and ranged from normodense air (21% O₂, 79% N₂; 1.21 kg m⁻³) to hypodense heliox (21% O₂, 79% He; 0.41 kg m⁻³) in 0.16 kg m⁻³ increments. The gases were mixed using calibrated bi-metal thermo-actuated valves (low flow: Tylan FC-260, San Diego, Ca, USA) and solenoid-actuated valves (high flow: Tylan FC-2910, San Diego, Ca, USA), and mixtures and flow rates were metered by an electronic flow controller (Sable Systems MFC-4, Las Vegas, Nv, USA). When assessing maximal flight capacity, total gas flow rate was maintained at 1 L min⁻¹. In between trials, the flight chamber was flushed with a new gas mixture at a flow rate of 25 L min⁻¹ for 1 minute. The initial trial began in air and the remaining gas mixtures were administered in a non-repeating, random order.

Bees that landed on the floor or sides of the chamber were persuaded to fly by agitating them with a small magnetic stir-bar, directed by a magnetic wand outside of the chamber. Maximal flight capacity was determined as the minimal gas density (*MGD*) of these gas mixtures that allowed hovering flight. Thus, lower *MGD* corresponds to greater maximal flight capacity, and the highest *MGD* corresponds to flight that was limited to air. Bees that failed to fly in air (e.g. the wing wear was too severe, the bee lacked motivation, etc.) were omitted from the analysis.

Hovering flight kinematics were calculated from the wing trajectories in the horizontal plane recorded by a single, high-speed (4348 fps) digital video camera (Vision Research, Phantom v5.1, Wayne, Nj, USA) which was oriented above the flask and focused such that the focal plane was at the center of the flask and away from the narrow-circumference(s) near the top of the flask to minimize boundary effects (Raynar and Thomas, 1991). The digital video sequences were analyzed using custom software (Matlab, The Mathworks; Natick, MA USA) to determine the following kinematic variables for individual bees during hovering in air (subscript: “norm”) and hovering in the *MGD* (subscript: “max”): n (in Hz) was calculated from the duration to complete 10 successive wingbeats; Φ (in degrees) was calculated as the average of the downstroke and upstroke angular displacement for each of the 10 wingbeats; and $\bar{\omega}$ (in radians sec⁻¹), the average wing angular velocity, was calculated from the duration to complete the total angular displacement of one downstroke and one upstroke for each of the 10 wingbeats.

Results

The morphology of honey bees analyzed in this study is summarized in Table 4.1. Body mass (M_b) was consistent across all three wing treatment groups. There was low variation in total S and wing asymmetry for bees in the control group. The symmetric wing wear treatment reduced wing area by approximately 15% on average, and ranged from 3% to approximately 40%. Wing asymmetry in the symmetric treatment group was comparable to the control group. The asymmetric wing wear treatment reduced the wing area of the treated wing by an average of 15%, the area of the untreated wings in this group was similar to wings of bees in the control group. Asymmetry in wing area between the treated and untreated wings averaged 14.6 ± 6.6 (mean \pm S.D.) %, and ranged from 6 to 26%. The reduction in total S for the asymmetric treatment was approximately one-half that of the symmetric group, although the wing area loss per treated wing was similar between the symmetric and asymmetric treatments. For all bees, S significantly correlated to R ($P < 0.001$; $r = 0.92$); because S reflects both spanwise (R) and chordwise wing dimensions, S was chosen, along with M_b , as covariates for the following multivariate analyses of covariance (MANCOVA) for flight performance.

Table 4.1: Honey bee morphology.

Treatment	N	M_b (mg)	S (mm ²)	R (mm)	<i>Asymmetry</i> (%)	<i>MGD</i> (kg m ⁻³)
Control	17	79.7 ± 8.7	52.4 ± 3.6	9.2 ± 0.4	2.4 ± 2.7	0.50 ± 0.10
Symmetric	16	77.3 ± 4.5	45.0 ± 4.7	8.3 ± 0.7	4.0 ± 2.6	0.77 ± 0.23
Asymmetric	15	78.7 ± 6.7	47.8 ± 3.2	8.5 ± 0.4	14.7 ± 6.6	0.90 ± 0.25

Units are mean \pm S.D.: Body mass, M_b ; Total wing area, S ; Average wing length, R ; Asymmetry in wing area between right and left wings, *Asymmetry*; Minimal gas density allowing for hovering flight, *MGD*.

Wing area and wing treatment group had a significant effect on the overall model of flight performance (MANCOVA: wing area $F_{9,35} = 18.4$, $P < 0.001$; wing treatment group $F_{18,70} = 2.70$; $P = 0.002$). Although the effect of M_b on the entire model was significant (MANCOVA: $F_{9,35} = 3.00$; $P = 0.009$), M_b had no effects on any individual variables of flight performance (Table 4.2). There was a significant effect of S (MANCOVA: $F_{1,43} = 8.26$; $P = 0.006$) and wing treatment (MANCOVA: $F_{2,43} = 9.39$; $P < 0.001$) on maximal flight performance (minimal gas density: MGD ; Fig 4.1A). Pairwise comparison of least squares means (evaluated at $S = 48.5 \text{ mm}^2$, $M_b = 78.6 \text{ mg}$) revealed that bees from both the control ($P < 0.001$) and symmetric ($P = 0.009$) treatments had greater maximal flight performance than the asymmetric treatment (Fig 1B). Wingbeat frequency during hovering in air (n_{norm} ; Figure 4.2A) increased as S decreased (MANCOVA: $F_{1,43} = 18.1$; $P < 0.001$), but there was no effect of wing treatment (MANCOVA: $F_{2,43} = 1.16$; $P = 0.323$). Similarly, wingbeat frequency during

Table 4.2: Multivariate Analysis of Covariance (MANCOVA) for the effects of area, mass and wing treatment on flight performance.

	Parameter Estimates ^a (mean \pm S.E.)			S^b		M_b^c		Treatment ^d	
	Control	Symmetric	Asymmetric	$F_{1,43}$	P	$F_{1,43}$	P	$F_{2,43}$	P
n_{norm} (Hz)	240.7 \pm 5.3	231.6 \pm 5.3	241.9 \pm 4.8	18.1	<0.001	0.41	0.528	1.16	0.323
Φ_{norm} (deg)	107.2 \pm 3.2	116.3 \pm 3.1	111.7 \pm 2.9	3.98	0.052	0.34	0.565	1.70	0.195
$\bar{\omega}_{\text{norm}}$ (rad s ⁻¹)	909.3 \pm 32.8	938.0 \pm 32.6	944.0 \pm 29.8	21.1	<0.001	0.63	0.433	0.30	0.745
U_{tnorm} (m s ⁻¹)	7.75 \pm 0.26	8.16 \pm 0.26	8.05 \pm 0.24	1.42	0.240	1.07	0.308	0.53	0.590
n_{max} (Hz)	238.1 \pm 4.2	236.0 \pm 4.2	242.9 \pm 3.8	25.7	<0.001	0.03	0.862	0.89	0.419
Φ_{max} (deg)	146.2 \pm 3.5	140.0 \pm 3.5	129.5 \pm 3.2	0.001	0.976	2.66	0.110	6.71	0.003
$\bar{\omega}_{\text{max}}$ (rad s ⁻¹)	1211.5 \pm 38.7	1157.1 \pm 38.5	1097.8 \pm 35.1	7.10	0.011	1.82	0.184	2.37	0.105
U_{tmax} (m s ⁻¹)	10.46 \pm 0.32	10.07 \pm 0.32	9.41 \pm 0.29	0.62	0.434	1.61	0.212	3.11	0.055
MGD (kg m ⁻³)	0.59 \pm 0.05	0.69 \pm 0.05	0.89 \pm 0.05	8.27	0.006	0.04	0.846	9.39	<0.001

Body mass, M_b ; Total wing area, S ; Wing treatment group, Treatment; wingbeat frequency, n ; stroke amplitude, Φ ; wing angular velocity, $\bar{\omega}$; wingtip velocity, U_i ; Minimal gas density allowing for hovering flight, MGD ; kinematics during flight in air, n_{norm} ; kinematics during flight in the MGD , n_{max} .

^a Least squares means evaluated at $S = 48.49 \text{ mm}^2$, and $M_b = 78.57 \text{ g}$

^b MANCOVA: $F_{9,35} = 18.4$; $P < 0.001$

^c MANCOVA: $F_{9,35} = 3.00$; $P = 0.009$

^d MANCOVA: $F_{18,70} = 2.70$; $P = 0.002$

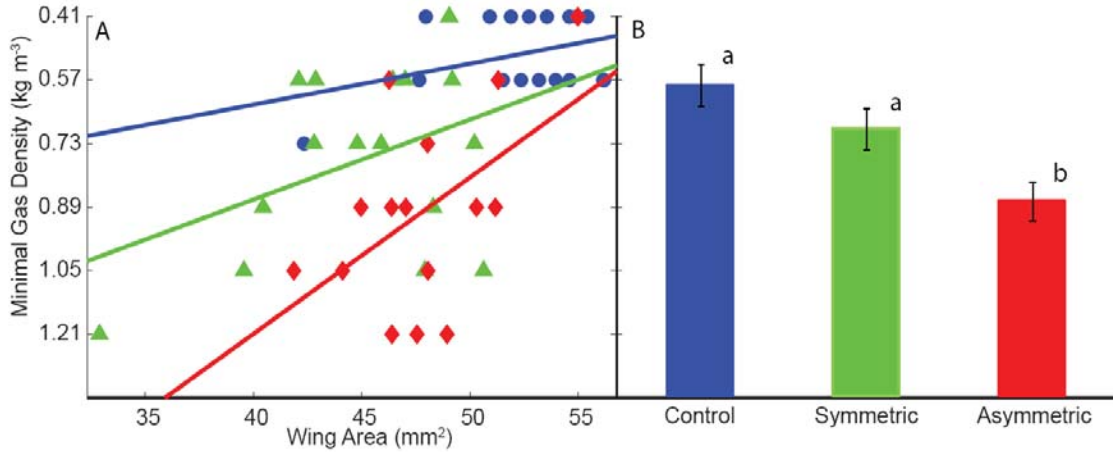


Figure 4.1: A) Maximal flight capacity (minimal gas density: MGD) vs. wing area (S) for control (blue ●), symmetric (green ▲), and asymmetric (red ◆) wing wear. Values of MGD (kg m^{-3}) are inverted to reflect the increasing aerodynamic demand of flying in lesser-density gas mixtures. There was a significant effect of treatment ($P < 0.001$) and S ($P = 0.006$) on MGD (MANCOVA): $MGD_{\text{sham}} = 1.04 - 0.0103S$ (blue line); $MGD_{\text{sym}} = 1.68 - 0.0264S$ (green line); $MGD_{\text{asym}} = 2.78 - 0.0394S$ (red line). B) Least squares means evaluated at $S = 48.49 \text{ mm}^2$ and $M_b = 78.57 \text{ g}$. Pairwise comparisons revealed that bees with the control and symmetric wing treatments had greater maximal flight capacity than bees with asymmetric wings after accounting for variation in S and M_b .

hovering in the MGD (n_{max} ; Fig 4.2A) increased as S decreased (MANCOVA: $F_{1,43} = 25.7$; $P < 0.001$), but there was no effect of wing treatment (MANCOVA: $F_{2,43} = 0.89$; $P = 0.419$). After adjusting for variation in wing area (evaluated at $S = 48.5 \text{ mm}^2$, $M_b = 78.6 \text{ mg}$), there were no differences in the least squares means for n_{norm} or n_{max} between the three treatment groups (Fig 4.2B).

Wing stroke amplitude during hovering in air (Φ_{norm} ; Fig 4.3A) was not affected by the wing treatment (MANCOVA: $F_{2,43} = 1.70$; $P = 0.195$). Although there was a general linear trend between S and Φ_{norm} for all bees (model I regression: $F_{1,46} = 15.8$; $P < 0.001$), the MANCOVA did not reveal a significant relationship between S and Φ_{norm} ($F_{1,43} = 3.98$; $P = 0.052$). During hovering in the MGD , wing stroke amplitude (Φ_{max})

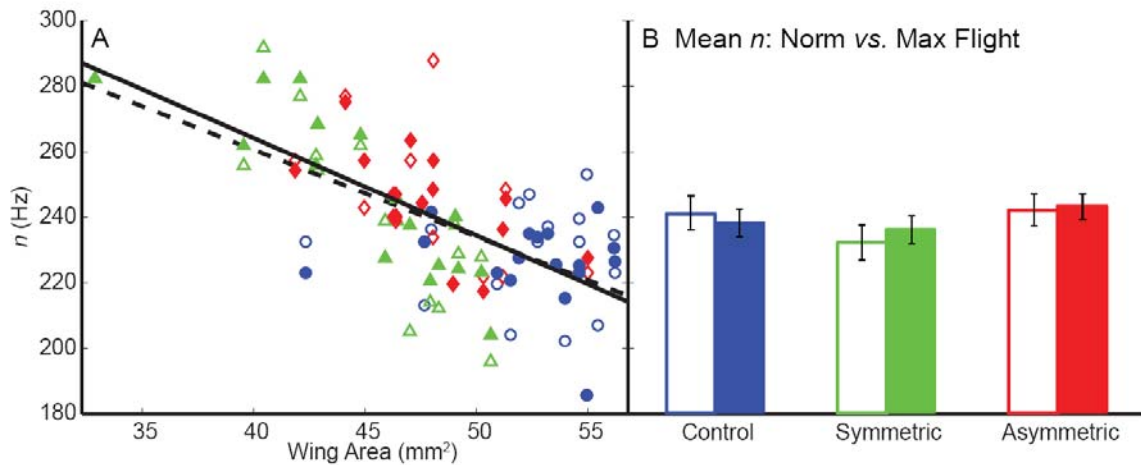


Figure 4.2: A) Wingbeat frequency (n) vs. wing area (S) for control (blue ●), symmetric (green ▲), and asymmetric (red ◆) wing wear. Data during flight in air (n_{norm}) are represented by open symbols and flight in MGD (n_{max}) are represented by filled symbols. There was a significant effect of S ($P < 0.001$), but no effect of treatment ($P = 0.323$), on n_{norm} (MANCOVA): All bees, $n_{\text{norm}} = 367 - 2.66S$ (broken line). There was also a significant effect of S ($P < 0.001$), but no effect of treatment ($P = 0.419$), on n_{max} (MANCOVA): All bees, $n_{\text{max}} = 383 - 2.98S$ (solid line). B) Least squares means for n_{norm} (open) and n_{max} (filled) evaluated at $S = 48.49 \text{ mm}^2$, and $M_b = 78.57 \text{ g}$. There were no differences in n_{norm} or n_{max} between the three groups after accounting for variation in S .

was not affected by S (MANCOVA: $F_{1,43} = 0.001$; $P = 0.976$), but was affected by wing treatment (MANCOVA: $F_{2,43} = 6.71$; $P = 0.003$). Pairwise comparisons of least squares means (evaluated at $S = 48.5 \text{ mm}^2$, $M_b = 78.6 \text{ mg}$) showed that bees from both the control ($P = 0.001$) and symmetric ($P = 0.027$) treatments were capable of greater Φ_{max} than bees from the asymmetric treatment (Fig 4.3B). To evaluate the potential effects of natural and experimental asymmetry in S on Φ , I compared Φ between the smaller and larger wing in each bee. During hovering in air (Fig. 4.3C), there were no differences in Φ_{norm} between the smallest and largest wings for the control and symmetric treatment groups (Paired T-Test: $P = 0.055$, $P = 0.205$, respectively). However, for the asymmetric treatment group, the smaller (treated) wing had significantly greater Φ_{norm}

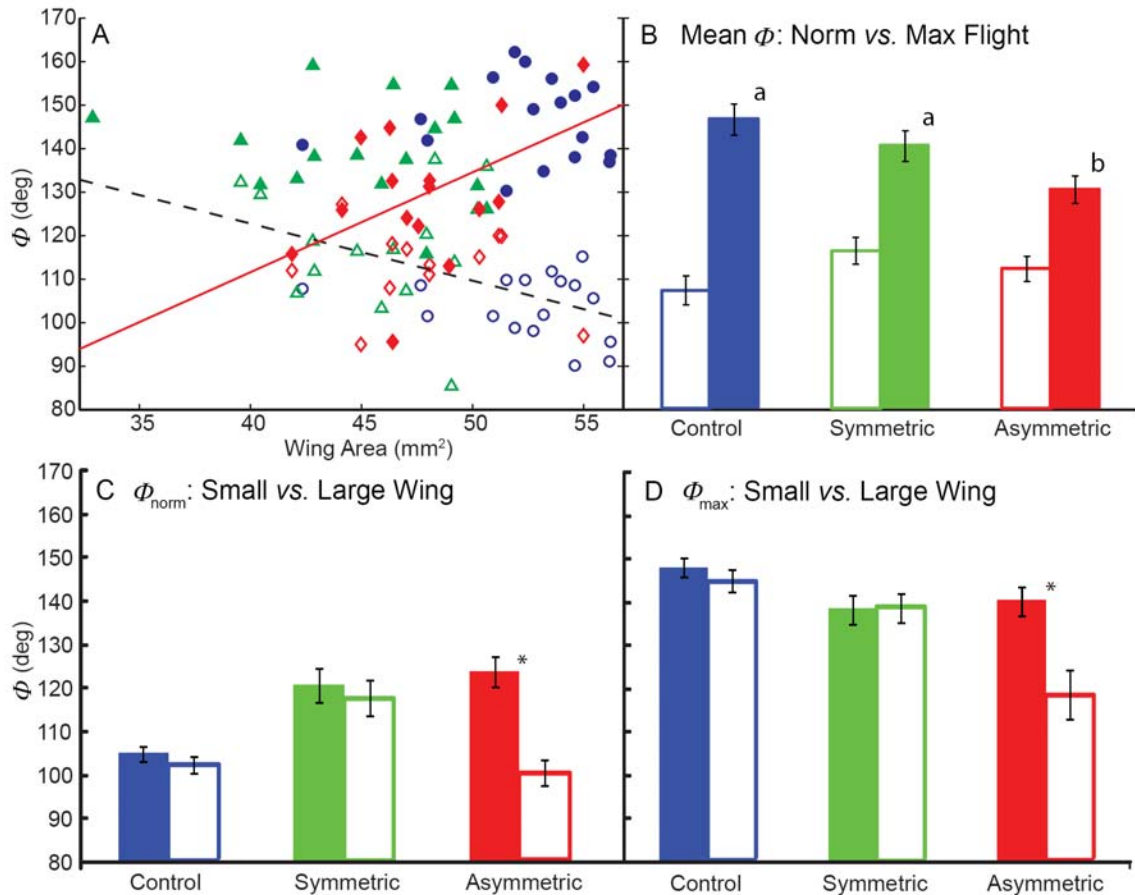


Figure 4.3: A) Wing stroke amplitude (Φ) vs. wing area (S) for control (blue ●), symmetric (green ▲), and asymmetric (red ◆) wing wear. Φ is the mean amplitude of the left and right wings. Data during flight in air (Φ_{norm}) are represented by open symbols and flight in MGD (Φ_{max}) are represented by filled symbols. Although there was an inverse trend between S and Φ_{norm} (broken line: $\Phi_{\text{norm}} = 175 - 1.31S$), there was no effect of S ($P = 0.052$) or treatment ($P = 0.195$), on Φ_{norm} (MANCOVA). There was no effect of S ($P = 0.967$), but there was a significant effect of treatment ($P = 0.003$) on Φ_{max} (MANCOVA): Sham, $\Phi_{\text{max}} = 142 + 0.084S$; Symmetric: $\Phi_{\text{max}} = 161 - 0.472S$; Asymmetric: $\Phi_{\text{max}} = 198 + 2.30S$ (red line). B) Least squares means for Φ_{norm} (open) and Φ_{max} (filled) evaluated at $S = 48.49 \text{ mm}^2$, and $M_b = 78.57 \text{ g}$. Pairwise comparisons revealed that there were no differences between the three groups for Φ_{norm} , but bees with the control and symmetric wing treatments had greater Φ_{max} than bees with asymmetric wings after accounting for variation in S and M_b . C) Independent Φ_{norm} for the smallest (filled) and largest (open) wings. For the asymmetric wing treatment group, the smallest wing had significantly greater Φ_{norm} than the largest wing (Paired T-Test: $P < 0.001$). D) Independent Φ_{max} for the smallest (filled) and largest (open) wings. For the asymmetric wing treatment group, the smallest wing had significantly greater Φ_{max} than the largest wing (Paired T-Test: $P < 0.001$).

than the larger (untreated) wing (Paired T-Test: $P < 0.001$). During hovering in the *MGD* (Fig. 4.3D), there were also no differences in Φ_{\max} between the smaller and larger wings for the control and symmetric treatment groups (Paired T-Test: $P = 0.096$, $P = 0.877$, respectively). As during hovering in air, the asymmetric treatment group exhibited greater Φ_{\max} in the smaller (treated) wing than the larger (untreated) wing during hovering in the *MGD* (Paired T-Test: $P < 0.001$).

Wing angular velocity during hovering in air ($\bar{\omega}_{\text{norm}}$; Fig 4.4A) had an inverse relationship to S (MANCOVA: $F_{1,43} = 21.1$, $P < 0.001$), but was not affected by wing treatment (MANCOVA: $F_{2,43} = 0.30$; $P = 0.745$). Likewise, during hovering in the *MGD*, $\bar{\omega}_{\max}$ had an inverse relationship to S (MANCOVA: $F_{1,43} = 7.10$, $P = 0.011$), but was not affected by wing treatment (MANCOVA: $F_{2,43} = 2.37$; $P = 0.105$). Pairwise comparisons of least squares means showed no significant differences between the three treatment groups for $\bar{\omega}_{\text{norm}}$. The control treatment group had greater $\bar{\omega}_{\max}$ than the asymmetric group ($P = 0.040$), after adjusting for variation in S (evaluated at $S = 48.48 \text{ mm}^2$, $M_b = 78.6 \text{ mg}$). During hovering in air (Fig. 4.4C), there were no differences in $\bar{\omega}_{\text{norm}}$ between the smaller and larger wings for the control and symmetric treatment groups (Paired T-Test: $P = 0.051$, $P = 0.180$, respectively). However, for the asymmetric treatment group, the smaller (treated) wing had significantly greater $\bar{\omega}_{\text{norm}}$ than the larger (untreated) wing (Paired T-Test: $P < 0.001$). During hovering in the *MGD* (Fig. 4.4D), there was also difference in $\bar{\omega}_{\max}$ between the smaller and larger wings for the sham (Paired T-Test: $P = 0.109$). Both the symmetric and asymmetric treatment groups exhibited greater $\bar{\omega}_{\max}$ in the smaller (treated) wing than the larger (untreated) wing during hovering in the *MGD* (Paired T-Test: $P = 0.010$, $P < 0.001$).

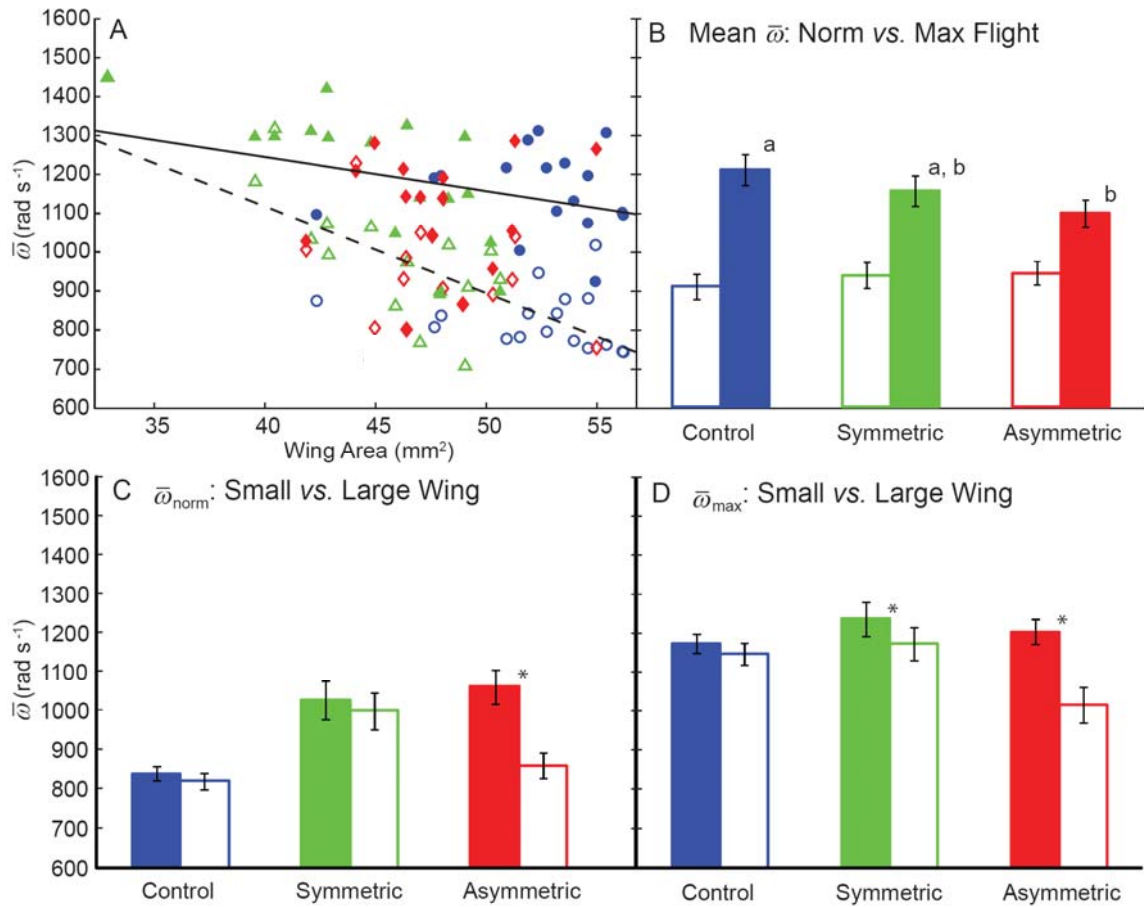


Figure 4.4: A) Wing angular velocity ($\bar{\omega}$) vs. wing area (S) for control (blue ●), symmetric (green ▲), and asymmetric (red ◆) wing wear. Data during flight in air ($\bar{\omega}_{\text{norm}}$) are represented by open symbols and flight in *MGD* ($\bar{\omega}_{\text{max}}$) are represented by filled symbols. There was a significant effect of S ($P < 0.001$), but no effect of treatment ($P = 0.745$) on $\bar{\omega}_{\text{norm}}$ (MANCOVA): all bees, $\bar{\omega}_{\text{norm}} = 2011 - 22.3S$ (broken line). There was an effect of S ($P = 0.011$), but no effect of treatment ($P = 0.105$) on $\bar{\omega}_{\text{max}}$ (MANCOVA): all bees, $\bar{\omega}_{\text{max}} = 1597 - 9.05S$ (solid line). B) Least squares means for $\bar{\omega}_{\text{norm}}$ (open) and $\bar{\omega}_{\text{max}}$ (filled) evaluated at $S = 48.49 \text{ mm}^2$, and $M_b = 78.57 \text{ g}^2$. There were no differences in $\bar{\omega}_{\text{norm}}$ between the three groups, but the control group had greater $\bar{\omega}_{\text{max}}$ than the asymmetric group after accounting for variation in S and M_b . C) Independent $\bar{\omega}_{\text{norm}}$ for the smallest (filled) and largest (open) wings. In the asymmetric treatment group, $\bar{\omega}_{\text{norm}}$ for the smallest wing was significantly greater than in the largest wing (Paired T-Test: $P < 0.001$). D) Independent $\bar{\omega}_{\text{max}}$ for the smallest (filled) and largest (open) wings. For the symmetric and asymmetric treatment groups, $\bar{\omega}_{\text{max}}$ for the smallest wing was significantly greater than in the largest wing (Paired T-Test: $P = 0.010$ and $P < 0.001$, respectively).

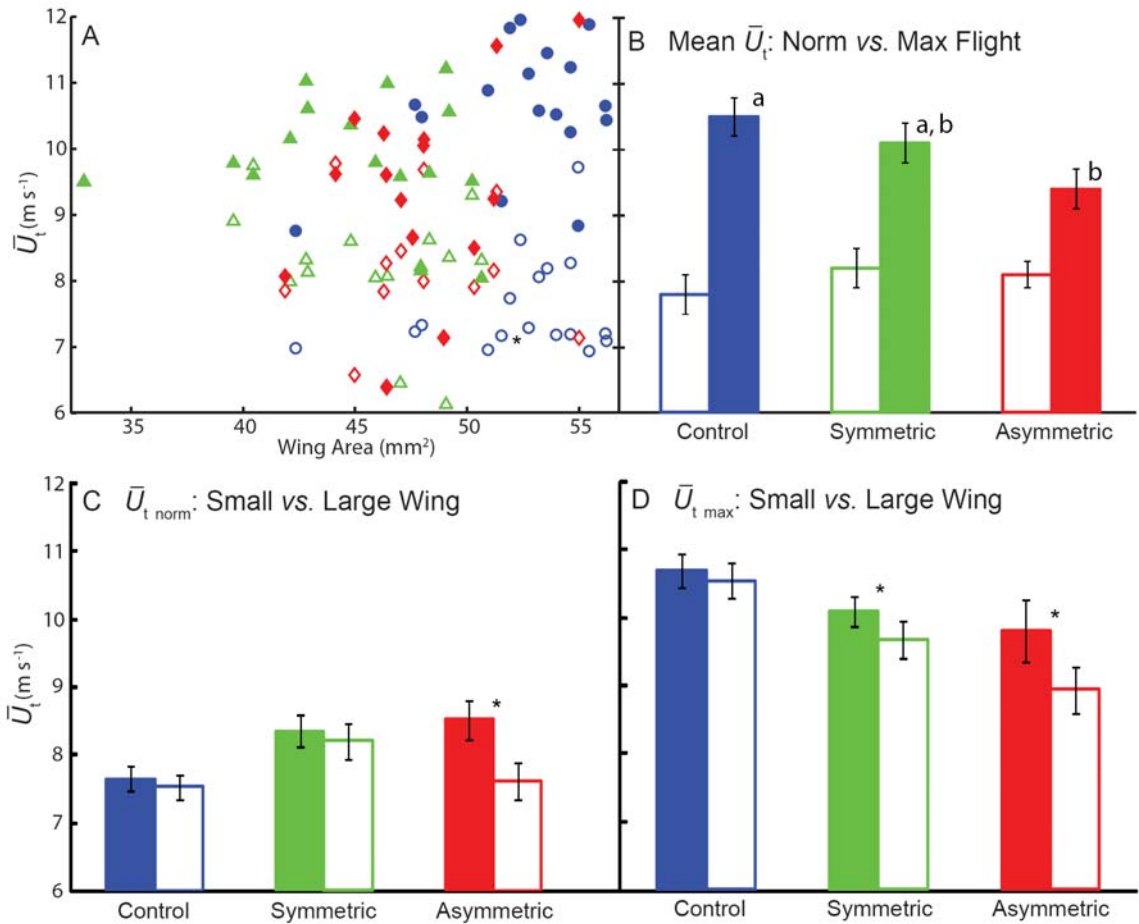


Figure 4.5: A) Wingtip velocity (U_t) vs. wing area (S) for control (blue ●), symmetric (green ▲), and asymmetric (red ◆) wing wear. Data during flight in air ($U_{t \text{ norm}}$) are represented by open symbols and flight in *MGD* ($U_{t \text{ max}}$) are represented by filled symbols. There was no effect of S ($P = 0.240$) or treatment ($P = 0.590$), on $U_{t \text{ norm}}$ (MANCOVA). There was no effect of S ($P = 0.434$) or treatment ($P = 0.055$) on $U_{t \text{ max}}$ (MANCOVA). B) Least squares means for $U_{t \text{ norm}}$ (open) and $U_{t \text{ max}}$ (filled) evaluated at $S = 48.49 \text{ mm}^2$, and $M_b = 78.57 \text{ g}$. Pairwise comparisons revealed that there were no differences between the three groups for $U_{t \text{ norm}}$, but the control group had greater $U_{t \text{ max}}$ than the asymmetric treatment group after accounting for variation in S and M_b . C) Independent $U_{t \text{ norm}}$ for the smallest (filled) and largest (open) wings. In the asymmetric group, $U_{t \text{ norm}}$ for the smallest wing was significantly greater than the largest wings (Paired T-Test: $P = 0.009$). D) Independent $U_{t \text{ max}}$ for the smallest (filled) and largest (open) wings. For the symmetric and asymmetric groups, $U_{t \text{ max}}$ for the smallest wing was significantly greater than the largest wing (Paired T-Test: $P = 0.040$, $P = 0.003$, respectively).

Wingtip velocity during hovering in air ($U_{t \text{ norm}}$; Figure 4.5A) was not affected by S (MANCOVA: $F_{1,43} = 1.42$; $P = 0.240$) or wing treatment (MANCOVA: $F_{2,43} = 0.53$; $P = 0.590$). Likewise, $U_{t \text{ max}}$ was not affected by S (MANCOVA: $F_{1,43} = 62$; $P = 0.434$) or wing treatment (MANCOVA: $F_{2,43} = 3.11$; $P = 0.055$). However, pairwise comparisons of least square means (evaluated at $S = 48.5 \text{ mm}^2$, $M_b = 78.6 \text{ mg}$) did reveal that bees from the control treatment were able to maintain greater $U_{t \text{ max}}$ than bees from the asymmetric treatment ($P = 0.024$; Figure 4.5B). During hovering in air (Fig. 4.5C), there were no differences in $U_{t \text{ norm}}$ between the smaller and larger wings for either the control or symmetric groups (Paired T-Test: $P = 0.223$, $P = 0.265$, respectively). However, bees with asymmetric wing wear had greater $U_{t \text{ norm}}$ in the smaller wing, as compared to the larger wing (Paired T-Test: $P < 0.001$). There was no difference in $U_{t \text{ max}}$ between the smaller and larger wings during hovering in the *MGD* (Fig. 4.5D) for the control group (Paired T-Test: $P = 0.316$). However, the symmetric and asymmetric groups had greater $U_{t \text{ max}}$ in the smallest wing than the largest wing (Paired T-Test: $P = 0.040$, $P < 0.001$, respectively).

Discussion

As bees accrue wing wear with foraging experience, foraging behavior is altered and mortality increases (Higginson and Barnard, 2004; Cartar, 1992). Hedenstrom et al. (2001) found no metabolic cost associated with wing wear, and Haas and Cartar (2008) observed little variation in free flight performance across varying degrees of wing wear magnitude and asymmetry. Our results confirm that, during non-challenging flight conditions, bees are resilient to wing wear, but also that wing wear, especially that

which is asymmetric, profoundly impairs maximal flight capacity. Such reductions in maximal flight capacity limit the aerodynamic reserves available for maneuvers and burst performance associated with evading predators and flight under adverse conditions such as high wind. Reduced flight capacity likely compromises the performance of other flight-dependent behaviors such as foraging. Thus, I proposed that limited maximal flight capacity, and the consequences for flight behaviors that require those aerodynamic reserves, are the mechanisms that link wing wear to mortality and foraging behavior.

In normal and maximal hovering, the reduction in wing area due to wing wear is mitigated by enhanced kinematics to maintain aerodynamic force production. During hovering in air, there was a tendency for Φ_{norm} to vary inverse to S (Figure 4.3A; broken line), but the predominant strategy to compensate for decreased S was to increase n (Figure 4.2A; broken line), which is similar to how *B. terrestris* responds to wing wear (Hedenstrom et al., 2001). This variation in n across S may simply result from passive resonance properties, as n is inversely related to wing moment of inertia (Sotavalta, 1952). However, it is not known whether any active mechanisms also contribute to the modulation in n across S . For *Drosophila melanogaster*, the asynchronous flight muscle, and control and accessory muscles are sensitive to the spike rate of neural inputs, and contribute to the active modulation of n (Dickinson et al., 1998). Some bee species, such as *Xylocopa varipuncta* and *Centris pallida*, exhibit the capacity to increase n when augmenting aerodynamic output (Roberts et al., 2004; Roberts et al., 1998). Whereas, other bee species, including Euglossines and honey bees, do not vary n under similar conditions (Altshuler et al., 2004; Dudley, 1995), although they exhibit n

that is sensitive to ambient temperature (Roberts and Harrison, 1999), development and age (Vance et al., accepted). The general absence of modulation of n in honey bees (under normal, non-impaired conditions) may reflect the selection of muscle strain rates that maximize muscle efficiency (Dickinson et al., 1998), which would be favorable for foraging bouts of long duration. Considering that other hymenopterans do not incur greater metabolic costs associated with the increase in n due to S loss (Hedenstrom et al., 2001), it is plausible that n is modulated across S loss to optimize muscle strain rates and efficiency.

The modulation of $\bar{\omega}$ and U_t (by varying n and/or Φ) is a general strategy used by hoverers to control aerodynamic output (Altshuler et al. 2005; Chai and Dudley, 1996; Chai and Dudley, 1997; Dudley, 1995; Roberts et al. 2004; Vance et al. in press), as aerodynamic forces generally scale to U_t^2 (Lehmann and Dickinson, 1998). I expected $U_{t \text{ norm}}$ to increase as a mechanism to mitigate the loss of S and R in the symmetric and asymmetric groups. Although $\bar{\omega}_{\text{norm}}$ varied inversely to S , $U_{t \text{ norm}}$ was maintained across the 3 groups. In order to produce normal, hovering flight forces at a given $U_{t \text{ norm}}$ with smaller wings, the coefficient of lift may be augmented by exploiting wake capture or rotational mechanisms common to the high-frequency, short wingstrokes of honey bees during flight in air (Altshuler et al., 2005). However, as Φ increases during flight in hypodense atmospheres, midstroke force production related to $U_{t \text{ max}}$ would dominate aerodynamic force production. Bees with wing wear did not sufficiently increase $\bar{\omega}_{\text{max}}$ to offset the loss of S and R , and $U_{t \text{ max}}$ decreased in response to the magnitude and type of wing area loss, which ultimately impaired maximal flight performance. In the asymmetric treatment group, the smaller (treated) wing exhibited greater Φ , $\bar{\omega}$, and U_t

than the larger (untreated) wing during normal and maximal hovering. The putative aerodynamic and inertial imbalances imposed by asymmetric U_t may have disrupted stability during hovering near or at *MGD*. Indeed, bees with high asymmetry were prone to spiraling flight as they approached the point of aerodynamic failure and spiraling plummets as they achieved aerodynamic failure. Bees were capable of mitigating asymmetric wear during normal flight but appeared unable to overcome the effects of the wing and stroke asymmetries during challenging flight, and thus maximal flight capacity in these bees was below that of bees with symmetric wear.

The mechanism relating wing wear to longevity and mortality rates has long been elusive. In the absence of a metabolic link between wing wear and mortality, it has been hypothesized that wing wear may reduce bees' ability to evade predators (Alcock, 1996; Cartar, 1992; Hedenstrom et al. 2001). Aerodynamic power reserves (P_{res}) in excess of the power required to sustain normal flight (P_{norm}) facilitate the maneuverability and burst performance of evasive flight. However, the P_{res} available to the insect is determined by its maximal aerodynamic capacity (P_{max}),

$$P_{res} = P_{max} - P_{norm}$$

Thus, as maximal capacity (P_{max}) is impacted by the magnitude and type of wing wear, the subsequent reduction in P_{res} may limit bees' maneuverability and burst performance, and increase risk of predation and mortality (Cartar, 1992; Dukas, 2008). In addition to evading predators, P_{res} is required to augment aerodynamic output beyond the requirements of steady flight for many transient tasks, such as rapid ascent, maneuvering, and burst performance to defend territory or compete for a mate. It is also

important to consider other ecologically-relevant factors that reduce aerodynamic reserves,

$$P_{\text{res}} = P_{\text{max}} - P_{\text{norm}} - P_{\text{load}} - P_{\text{wind}}$$

such as the aerodynamic power required to carry additional load (P_{load}) during foraging or mating (Pettersson, 1995), and the aerodynamic power necessary to overcome wind (P_{wind}). In the context of a given flight behavior (where P_{norm} may be constant), a reduction in P_{max} , or an increase in P_{load} or P_{wind} , will reduce P_{res} . It is plausible that bees govern flight behavior, such as limiting load carriage, in order to maintain P_{res} as a margin of safety, especially when foraging within dynamic environments that demand variable P_{wind} . For example, despite the capacity to carry large nectar loads, *A. mellifera* only partially fill their crop during foraging bouts (Schmid-Hempel et al., 1985). Although this regulation of nectar loading has been attributed to colony-level energetics and foraging efficiency, a further increase in the foraging load and P_{load} would reduce P_{res} and bees' ability to ascend, maneuver, and evade predators. Likewise, as P_{max} and P_{res} are reduced by wing wear, foraging bees may limit P_{load} (Higginson and Barnard, 2004) as a mechanism to maintain adequate P_{res} . P_{max} may be constrained by other factors, such as parasitic tracheal mites which limit flight metabolic rates (Harrison et al., 2001), or developmental trajectory (Vance et al., accepted); under such conditions, reduced P_{res} may be insufficient for optimal flight performance without otherwise compromising task-specific flight behaviors.

When imposed with significant symmetric and asymmetric wear, honey bees maintained normal flight performance by increasing n_{norm} and $\bar{\omega}_{\text{norm}}$, which produced $U_{t, \text{norm}}$ similar to unaffected bees. However, bees could not increase $U_{t, \text{max}}$ sufficient to

offset loss in S , which impacted maximal flight capacity. Asymmetry further compounds the effects of wing wear and impaired maximal flight capacity, possibly due to inertial and aerodynamic imbalances which limit flight control and stability. Thus, wing wear reduces the aerodynamic reserve capacity that is available for flight behaviors which require enhanced aerodynamic output, such as predator evasion, and I believe this is the mechanism that links wing wear to mortality in foraging bees. Flying insects must balance the aerodynamic power expended on task-specific flight behaviors with their remaining aerodynamic reserves so that they can accommodate environmental perturbations, maneuver, or evade predators if necessary. Besides wing wear, a broad suite of other physiological, developmental and environmental factors may reduce maximal flight capacity and hinder flight performance. Thus, future research that links these sources of variation in maximal flight capacity to the performance of ecologically-relevant flight behaviors will be critical in understanding how such behaviors are governed.

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