Fly far, lift more? What patterns exist within interindividual capacity of flight performance traits in *Bombus impatiens*?

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Thesis submitted to the Faculty of Graduate and Postdoctoral Studies
University of Ottawa
In partial fulfillment of the requirements for the
M.Sc. Degree in the Ottawa Carleton Institute for Biology

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ACKNOWLEDGEMENTS

The completion of my thesis would not have been possible without the generous help of several individuals. I would like to thank my thesis supervisor, Dr. Charles Darveau for all of his guidance and enthusiasm on this project. I would also like to thank all of the students in the Darveau lab for their help and for all that I have learned from them. I would like to thank Dr. DeSilva for kindly taking the time to assist me by setting up an excel file to process the RFID data. I would like to thank Mike Murphey for technical help and building of equipment. I would like to thank Dr. Bertram and Dr. Standen for being my committee members and for all their helpful input on this project. Finally, I would like to thank my family, my mother, father, stepmom and brother as well as my extended family for all their support during my degree.

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AIC - Akaike information criterion

AR – Aspect ratio

PMR – Peak metabolic rate

SA – Stroke amplitude

VF – Vertical Force Production

WA – Total wing area

WBF – Wingbeat frequency

WL – Wing loading

ABSTRACT

Locomotion is central to the survival of many animal species; however large variation in performance, for example in speed or endurance, exists between individuals within a species. Using the bumblebee species, *Bombus impatiens*, I studied the extent of the variation in several flight performance traits and how they are associated. I first addressed how bumblebee workers vary in foraging effort and observed that only around half of the monitored individuals underwent foraging activity. Additionally, significant variation in metabolic rate between foragers and non-foragers was uncovered. I further investigated if such variation could be associated with flight performance capacity, such as an individual's ability to carry a load, their flight speed and distance traveled, their wing morphology and kinematics, and their flight metabolic rate. These traits are commonly measured to characterize flight capacity in insects, however the links between them have yet to be investigated. Links between morphology, wing kinematics and peak metabolic rate previously uncovered in the literature were observed in my analysis, although variation in their scaling with body mass was detected. Vertical force scaled isometrically with body mass but was not related to it when expressed in on a mass specific basis (VF $m^{-1}g^{-1}$, where m is gravitation acceleration). In regard to forward flight speed, body mass does have an affect, however it alone does not have a great degree of explanatory power and other factors such as morphology and wing kinematics are likely to play a greater part in its determination. Finally, maximum flight speed had a significant relationship with total flight time. Together, these results demonstrate that some links do exist between flight performance traits, however links are not present between all traits and certain flight performance traits should be treated as independent of each other.

1. Introduction

1.1 Interindividual variation

Between species the capacity to perform energy demanding activity such as moving quickly, dispersing long distances or lifting heavy loads varies widely, however these traits also vary widely between individuals within a species. For example, within a species certain, individuals will be faster or stronger than others. Intraspecific variation of repeatable and heritable traits is the basis of natural selection and evolution, and associations among such traits are indicative of genetic or functional links between them (Garland and Carter, 1994).

In insects, such as bumblebees, certain flight performance characteristics like wing beat frequency and flight metabolic rate have been shown to be repeatable (Darveau et al, 2014), meaning, for example, that the same individual will have the same wingbeat frequency or metabolic rate if tested multiple times. Additionally, certain traits related to locomotion are known to be heritable. For example, flight metabolic rate has been linked to certain genotypes in honeybees (Coelho and Milton, 1988) and butterflies (Niitepold et al., 2011). Among butterflies, dispersal capacity is heritable and linked to polymorphism in the phosphoglucose isomerase gene (Niitepold et al., 2011). These interindividual differences are of biological importance and examination of interindividual variation can be useful for a wide variety of purposes including determining functional correlations between morphology and behaviour or physiology, searching for factors which affect a given physiological or behavioural trait, determining if a trait is variant and heritable at the species level and determining if individual traits provide adaptive advantages which may be acted upon by natural selection (Bennett, 1987). In fish, the examination of interindividual variation rather than group means has led to the observation of relationships between morphology, physiology and swimming performance that might have been masked by using group means instead (Kolok 1999). Additionally, in a study by Cortés et al. (2008), individual repeatability of traits in a marsupial mammal *Dromiciops gliroides,* including resting metabolic rate and temperature control, were tested to determine if those traits could be acted upon by natural selection. Repeatability sets the lower bounds of heritability and traits which are not repeatable will not be acted upon by natural selection. Low repeatability of physiological variables led the authors to conclude that those traits could not exhibit evolutionary potential. In another study, Lebeau et al. (2016) looked at interindividual variation in butterflies based on environment to assess the effects of fragmented landscape on the butterflies' flight performance and metabolic rate. They found that butterflies from low nectar environments had superior flight performance on a flight mill to those from nectar rich environments and had different reactions to low nectar treatments than those from nectar rich environments. The low nectar environment butterflies had no change in flight performance on the low nectar treatment while the nectar rich environment butterflies' flight performance decreased. Garland (1984) looked at interindividual variation of burst speed in lizards and measured body mass, along with several other traits. Through the use of multiple regression analyses, the study found that nearly 90% of variation in burst speed could be accounted for by maximum oxygen consumption, skeletal muscle mass, heart muscle mass and hepatic aerobic enzyme activity, when controlling for body mass. Clearly the study of interindivdual variation is a useful tool for the study of animal physiology and of how physiology relates to animal locomotion.

1.2 Locomotion and survival

A main goal of comparative physiology is to understand the ways in which animals meet their functional requirements (Garland and Carter, 1994). Locomotion is central to animal biology allowing crucial activities such reproducing, escaping predators, foraging and avoiding challenging environments. Many forms of locomotion exist in animals, the main forms being walking, running, swimming and flying, and an animal’s locomotory performance, for example how fast or how far it can walk, will affect its ability to meet these needs. The study of animal locomotion has therefore been a focal point in comparative physiology given that it provides a clear illustration of the links between physiology, ecology and evolution (see Garland and Losos, 1994). For example, semi-aquatic animals such as the platypus must compromise between their locomotion on water and on land. Their semi-aquatic lifestyle has led to the evolution of morphology with limbs shaped and positioned such that they are not as efficient at walking as their walking specialized counterparts and with a body shape not designed to reduce drag as well as their diving specialized counterparts, making diving more energetically costly for them (Bethge et al., 2001). In another example, the relationship between peak metabolic rate and dispersal capacity is different in male and female Glanville fritillary butterflies and this difference is believed to be linked to mating and reproductive behaviour in which dispersal is beneficial for females whereas it is more beneficial for males to undergo territorial behaviour, remaining in one place (Niitepold et al., 2011). In another study by Watanabe (2016) the migration distances of various bird species were linked to morphological traits of the species, wing aspect ratio and wing loading. It is clear that animals’ locomotory performance is reflective of several factors related to their environment, physiology and morphology which together produce variation in interspecific and interindividual performance.

In insects, such as bumblebees, flight is a dominant form of locomotion. Bees fly to find food, find mates, protect their colony, and to find adequate locations to start new colonies. As social insects, worker caste bees undergo foraging to supply food for their colony and they are reliant on their ability to fly to perform this crucial activity (Tenczar et al., 2014; O’Donnell et al., 2000) In honeybees, foraging is allocated to a specific age group of bees which make up the foraging caste of the colony and foraging effort among bees is not consistent between individual members of a given colony, within this specialized caste (Tenczar et al., 2014). In a study done by Tenczar et al. (2014), wide interindividual variation within the foraging cast of honey bees was observed. The study used RFID tagging to monitor the foraging behaviour of individual honey bees. These tags are light weight and capable of being encoded with a unique ID number. They allow the monitoring of many individuals for extended periods which is not possible with observational monitoring. With the use of this system, every foraging trip of each individual can be recorded. Using this tagging system, Tenczar et al. (2014) monitored the lifetime foraging of hundreds of foraging caste honeybees across 5 colonies and discovered that 50% of the foraging for the colony was done by only 20% of the workers in this cast. This is known as elitism, the phenomenon in which a relatively small proportion of a group of social insects performs tasks at a higher activity level than the others (Weller 2015). Additionally, manipulation of the foraging workforce (through the removal of foragers) increased activity in previously less active or inactive bees, suggesting that the wide variation in foraging effort may allow for flexibility of response to unexpected needs of or damages to the colony. Similar studies have also been done on bumblebees. O’Donnell et al. (2000) observed that certain individuals tended to specialize as pollen or nectar foragers and this specialization led to variation in foraging effort of individual bees with specialized bees contributing more to the colony food stores than unspecialized bees.

In addition, certain traits have been observed to be related to foraging specialization. Feuerbacher et al. (2003) examined the differences between pollen and nectar specialized honey bees and found that pollen specialists had a 10% higher metabolic rate than nectar specialists. This difference in metabolism was maintained even in unloaded bees and it was suggested that it may be due to genetic variation in muscle, wing morphology or neuroendocrine properties which influence hovering metabolism and/or wing kinematics. Additionally, in previous studies, there have been differences in metabolic rate observed between bees with different phenotypes of the metabolic enzyme malate dehydrogenase (Harrison and Fewell 2002) as well as observed effects of genetics on foraging behaviour (Calderone and Page, 1988; Robinson and Page, 1989; Fewell and Page, 1993) so it is possible that pollen and nectar foragers vary from each other genetically. Additionally, Carter (1992) looked at the effect of food source manipulation on colony foraging effort and found that bees which only started foraging when food stores were lowered were less efficient foragers than the bees which had been foraging already, indicating that there is a difference in the performance between bees which regularly foraged and bees which did not. More efficient foragers were defined as bees which had a shorter trip length, carried more per load and underwent more foraging trips. Foraging effort has also been linked to individual survival, with higher rates of foraging relating to shorter individual lifespan in honey bees (Neukirch, 1982), indicating that this behaviour, though essential, is costly for the individual which might explain why such inter-individual variation is observed. Neukirch (1982) found that bees died quickly after a total flight distance of about 800 km and proposed that the shorter lifespan of bees with higher rates of foraging is possibly due to exhaustion of enzymatic mechanisms of carbohydrate metabolism after a certain degree of flight performance.

The results of Carter (1992) indicate that this variation in effort might be reflective of interindividual variation in the animals’ flight performance. However, flight is composed of many different performance characteristics, including speed, dispersal capacity, and in-flight load lifting capacity, and not all bees are equal in their performance of these characteristics. Even though all worker bees can fly, one bee might be able to fly faster than another bee. The ability of a bee or any other flying animal to fly at a certain speed, or to lift a certain load is its flight performance.

1.3 Flight Performance

Flight performance is a broad term used to describe a variety of flight related traits including flight speed, dispersal capacity, ability to carry a load, and the ability to fly in adverse conditions. Flight performance trait and flight performance characteristic, are terms used to describe the individual traits. A wide variety of traits are defined as flight performance in the literature including flight duration and distance (Abdelrahman et al, 2014; Ávalos et al, 2014; Castro et al., 2014; David et al, 2014; Ferrer et al, 2014; Hargrove 1975; Henry and Harrison, 2014), load lifting capacity (Johnson and Carter, 2014; Dillon and Dudley, 2004; Lehmann, 1999; Vance and Roberts, 2014), flight speed (Ávalos et al, 2014; Berwearts et al, 2002; Castro et al., 2014; David et al, 2014), as well as flight kinematic characteristics such as wingbeat frequency and stroke angle (Dudley 1994; Esch, 1976; Hargrove 1975; Henderstrom et al, 2011; Vance and Roberts, 2014).

Due to the importance of these traits to flying animals, flight performance traits are examined often in the literature for a wide variety of reasons. For example, because these traits are vital to the survival of these animals, they are often tested to assess the quality of individuals when they are exposed to certain conditions. Abdelrahman et al, 2014 measured flight performance as flight metabolic rate, ability to fly and flight duration in order to examine the effects of hypoxia during rearing of leaf cutting bees and found no difference in flight performance between those bees raised in hypoxic conditions and those not. They did however find a significant decrease in longevity in hypoxia reared bees. Ferrer et al. (2014) Esterhuizen et al. (2014) and Frazier et al. (2008) all looked at the effects of extreme temperature during development measuring ability to create lift or fly and flight distance and duration respectively. Each study found that insects reared at lower temperatures outperformed insects reared at higher temperatures in cold ambient temperatures and performed about the same at higher temperatures, allowing for further understanding of dispersal and reproduction of these animals. However, they also found that females reared at lower temperature were smaller and had reduced egg production relative to body size suggesting that, in terms of the effect of rearing temperature, there is a trade off between flight performance and fecundity.

Effects of extreme or varying ambient temperatures have also been examined using flight performance. Crespo et al. (2014) compared the effects low and high ambient temperature during takeoff time on the airspeeds of male moths attracted by female pheromones. The study concluded that males at lower temperatures warm their flight muscles less, compromising their flight performance in order to take off sooner, while moths at higher ambient temperatures take off more quickly and with warmer flight muscles indicating a possible effect of temperature on mating success. Esch (1976) examined the effects of thoracic temperature on flight performance and found that increased thoracic temperature as ambient temperature increased led to better lift and flight speed in honeybees. Marden (1995) compared the effects of temperature on the vertical force production of newly emerged and fully mature dragonflies, and found age related changes in vertical force capacity in response to temperature. Mature adults reached peak performance at higher temperatures than newly emerged dragonflies, which reached peak performance at lower temperatures and maintained their performance into higher temperatures. Alaux et al. (2014), looked at the effects of immune challenge and parasitism on honeybees to assess the effects of these conditions on foraging behaviour. They found, when compared to control bees, parasitized bees spent more time foraging while immune challenged bees spent less. Another study by Dillon and Dudley (2014) tested male alpine bumblebees at increasing lower barometric pressures in order to assess their hovering flight ability in high altitude conditions and uncovered that bees were able to fly in conditions mimicking elevations of over 9000 m, surpassing Mt. Everest.

Dispersal capabilities of insects may be studied for their potential effects on human health as well as their commercial applications. Castro et al. (2014) looked at the effect of infection on the dispersal capacity of the insect vector *Rhodnius pallescens*. The study found that beetles infected with the causative agent of Chaga's disease, *Trypanosoma cruzi,* did not significantly differ in their dispersal capacity from non-infected beetles, results important for developing management strategies to prevent the spread of the disease. Maes et al. (2014) compared flight performance and the effect of diet on that performance on invasive and non-invasive ladybirds', two species used as biocontrol agents. They found invasive ladybirds outperformed non-invasive ones in terms of flight speed, distance and duration and ladybirds fed on a commercial diet had better flight performance than those fed on a natural diet. They concluded that comparative flight studies are useful in risk assessment of potential biocontrol agents and that rearing conditions should be considered when assessing them.

Due to its importance, individual traits have been examined for their relationships with flight performance. Hargrove (1975) looked at flight performance across age groups of tsetse flies and found increases in wingbeat frequency, aerodynamic lift and flight duration with increasing age. These changes were linked to increase in thoracic muscle, cuticle and proline levels as the insects matures. The variation in flight performance between morphotypes of Asian citrus psyllids was examined by Martini et al., (2014) to further understand the dispersal patterns of the species. They found that a significantly greater proportion of blue green morphotype performed long duration flights than the gray-brown morphotype. Another study by Vance and Roberts (2014) looked at the effects of wing wear on honeybees and found a significant decrease in load lifting capacity and maneuverability with increased wing wear.

Flight performance characteristics are associated with a range of ecological features of insect species. Saastamoinen et al., 2012 stated that dispersal capability of flying insects is an important aspect to look at in order to understand a population's persistence and the ability of an individual to respond to environmental change. In butterflies, mating and reproductive success and behaviour are believed to be linked to dispersion and flight metabolic rate (Niitepold et al., 2011) and Niitepold and Hansky (2013) found a positive correlation between flight metabolic rate and lifespan in butterflies. In the broader picture, flight performance plays a central role in the pollination by bees (Bennett et al, 2013) and dispersal capacity is important to understand in order to assess the potential damage caused by insect pests (Maes et al., 2014; David et al, 2014; Ávalos et al., 2014). Individual flight performance traits provide important information about the condition, survival capacity and broader ecological and commercial impacts of flying animals such as bumblebees. However, there is limited understanding of how these traits relate to each other. Are faster bees also able to lift more or fly further? An increased comprehension of how these traits relate to one another will be useful for future research involving insect locomotion.

1.4 Body Mass

Body mass is an important determinant of interindividual variation of several traits including metabolic rate and wing morphology (Skandalis and Darveau, 2012), foraging behaviour (Spaethe and Wiedenmuller, 2002) as well as several flight performance traits such as vertical force production (Dillon and Dudley 2004, Buschwald and Dudley 2010), wingbeat frequency (Skandalis and Darveau, 2012; Darveau, 2005) and forward flight speed (Ellington 1991; Berwearts et al., 2002). Traits such as wingbeat frequency are known to scale with body mass (Darveau 2005), however not all traits scale proportionally. Traits such as peak metabolic rate scale allometrically with body mass, larger bees have a lower CO_2 output per gram than smaller bees do even as larger bees have a greater overall CO_2 output (Darveau 2005). Other traits such as vertical force production scale isometrically with body mass. For example, larger bees can produce proportionally more vertical force than smaller bees (Dillon and Dudley 2004). An understanding of the scaling of traits is important in order to understand how they will be affected by size variation among individuals. For this reason, body mass is measured and analysed for its relationship with the traits measured in this study.

1.5 Peak metabolic rate

Metabolism is directly linked to locomotion as it is the source of the energy required for movement (Schippers et al. 2006). Peak metabolic rate is heritable among insect species. In honeybees, variations in the slow, fast and medium in alleles for the gene encoding malate dehydrogenase were found to affect flight metabolic rate (Harrison and Fewell 2002) and in butterflies, peak metabolic rate is related to variation in the gene for the enzyme phosphoglucose isomerase. Darveau et al. (2014) looked at peak metabolic rate in individual bumblebee workers and drones and found that among workers it was highly repeatable. This repeatability was associated with functional links to body mass, thorax mass, wing size and wingbeat frequency and these functional links were predictive for peak metabolic rate differences between sexually dimorphic workers and drones, even though drones did not display the same repeatability of peak flight metabolic rate as workers. Peak metabolic rate had a positive relationship with both body mass and thorax mass and a mass independent positive relationship with wingbeat frequency. It was also indirectly related to wing size, which was related to body size and directly related to wingbeat frequency. Similarly, Skandalis and Darveau (2012) observed links between peak flight metabolic rate and body mass, wing morphology, wingbeat frequency and potential links between peak flight metabolic rate and flight muscle metabolic enzyme activities in bumblebees. Vogt et al. (2000) also found a significant positive relationship between metabolic rate and wingbeat frequency in fire ant species *Solenopsis invicta*. Similarly, a relationship between wing form, wing kinematics, body size and peak metabolic rate has been found across species of orchid bees (Darveau et al., 2005; Casey and May., 1985), indicating that the functional links between these traits exists both inter and intraspecifically.

Flight metabolic rate has also been correlated with flight performance traits. Niitepold et al. (2009) found that flight metabolic rate had a significant positive effect on the dispersion of individual butterflies and Niitepold et al. (2011) observed that variation in phosphoglucose isomerase genotypes explained variation in both metabolic rate and mobility. However, other studies have found no relationship between certain traits and metabolic rate. Ellington et al. (1990) did not find any significant changes in metabolic rate (O_2 uptake) with increasing forward flight speed in insects and Dillon and Dudley, 2004 found that flight metabolic rate had no influence on vertical force production in bees. Henderstrom et al. (2011) did not observe any significant metabolic costs of *compensatory* increased wingbeat frequency in *Bombus terrestris* after reduction of wing area in bees, stating that it was likely because there was no observed effect on mechanical power output as a result of the experiment and concluded that wing area reduction does not lead to an increase in flight cost. However, conversely, Skandalis and Darveau (2012) found a significant relationship between reduction in wing area and wing symmetry and metabolism as well as wing stroke frequency and thoracic temperature in *Bombus impatiens.* With no clear consensus, the existence of relationships between these traits and metabolism remains an important area to explore. In this study, I will measure the peak metabolic rate of bumblebees in order to compare it to various flight performance traits including vertical force production, forward flight speed and dispersal capacity with the goal of furthering the understanding of the relationships between metabolism and flight performance.

1.6 Load Lifting

Load lifting capacity is vital to many insect species. Cohelo and Ladage (1999) found a strong correlation between maximum load capacity and actual prey mass in female *Sphex ichneumoneus* (wasps) meaning that the more a female wasp could carry back to its nest, the more it would carry. The same group also found a positive effect of body mass, thorax mass and wing length on maximum prey size. They concluded that larger prey size would result in greater reproductive output for these wasps which use their prey to feed their offspring. Load lifting is also important for bees which forage for both pollen and nectar (Feuerbacher et al., 2003, Schippers and Dukas, 2010) which the colony relies on to grow and continue. Animals which lift food loads in flight benefit from being able to carry larger loads. However, not all animals have equal load lifting capacity. Dillon and Dudley (2004) found that among orchid bee species, load lifting capacity varied by body mass with larger bees having a greater load lifting capacity. Additionally, Spaethe and Wiedenmuller (2002) observed that in foraging bumblebees, larger bees carried more nectar back to the colony per trip, implying a potential relationship between load lifting capacity and body mass in bees that is reflected in their foraging behaviour. Intraspecifically, Johnson and Carter (2014) found that wing wear in bumblebees was related to a decrease in load lifting capacity. Marden (1985) looked at dragon flies and showed an effect of age on vertical force in response to temperature with younger individuals reaching peak performance at lower temperatures than older individuals. This study will look at the interindividual variation of load lifting capacity in bumblebees and how it relates to traits such as body mass and wing morphology, as well as other flight performance traits.

1.7 Tethered flight mills

Flight mills are often used in the literature to study the speed and endurance of insects such as beetles, honey bees, and moths (Castro et al., 2014; Blanken et al., 2015; Ferrer et al, 2014) and are used in this study to measure speed, distance and flight duration. They have been used in a wide variety of studies. Castro et al. (2014) used flight mills to determine there was no difference in the dispersal capacity of infected and uninfected insect vectors *Trypanosoma cruzi*. A study by Ferrer et al. (2014) used flight mills to determine the beneficial effects of acclimation at cold rearing temperatures on the dispersal capacity of invasive moths, which had important implications for monitoring their spread. Maes et al. (2014) used a tethered flight mill on invasive and non-invasive ladybird species and determined that the invasive species had a greater dispersal capacity and that a commercial diet led to animals having a greater dispersal capacity than those fed on a natural diet.

Ávalos et al, (2014) used a computer monitored flight mill to measure flight distance, duration, and maximum and average speed in the red palm weevil, an invasive species threating the palm tree population and found that there was no effect of age, body mass or sex on flight capacity, information useful in the future design of control measures against this insect pest. Although they have their limitations, for example in limiting or eliminating the insect's need to generate lift, and may overestimate or underestimate an animal's performance (Castro et al, 2014; David et al, 2014), flight mills are useful for comparison of individual ability and appropriate for this study for which the primary goal is to compare flight performance between individuals.

1.8 Established links between flight performance traits

Links between certain flight performance traits have been established in the literature. Interspecifically, in birds, Watanabe (2016) tracked migratory patterns of different species and found that species with higher aspect ratio and lower wing loading underwent longer migrations. The same study also found a link between body mass and migration distance with smaller bird species having smaller migration distances. In insects, Betts and Wooton (1988) found a link between wing shape and flight performance among butterfly species and among orchid bee species links have been found between peak hovering flight metabolic rate, wingbeat frequency and wing loading (Darveau et al., 2005). Links between flight performance traits have also been observed between individuals of the same species. Niitepold et al. (2009) found a positive link between flight metabolic rate and dispersion capability in free flying Glanville fritillary butterflies. In bumble bees, Johnson and Carter, 2014 determined that reduction in wing area through wing wear linearly decreased load lifting capacity. Also among bumblebees, Skandalis and Darveau (2012) observed links between peak hovering flight metabolic rate and wing surface area and wingbeat frequency.

Studies have been done primarily on morphological and kinematic flight performance traits in comparison to traits such as speed and dispersal capacity and several links have been established, but flight performance is a term widely used in the literature to describe a much wider variety of flight related characteristics ranging from load lifting capacity, to maximum flight speed to dispersal capacity. Additionally, despite the interchangeable use of the term, the ways in which these traits relate to each other have yet to be thoroughly investigated and it remains questionable whether or not these traits are interchangeable with each other. Does measuring one flight performance trait of an individual, such as flight speed, also provide information about the animal’s other flight performance traits such as its dispersal capacity and load lifting capacity? Does a bee with “better flight performance” of one trait also excel at another? Or are these traits functionally distinct from each other among individuals? Further investigation of the links between flight performance traits, such as flight distance and load lifting capacity, will provide useful information for future research.

1.9 Predicted Outcomes

It is predicted that bumblebee foragers will have unequal division of labour similar to that observed in honeybees by Tenczar et al. (2014). Carter (1992) found similarly uneven recruitment in bumblebee foragers in response to a reduction in food sources, indicating that it is likely that bumblebees follow a similar patter of unequal division of foraging effort to honeybees to allow for flexibility of their foraging response. Carter (1992) also observed among bumblebees that earlier recruits, bees which are foragers under initial conditions when there is no increase in foraging demand on the colony, were more efficient foragers than later recruits. More efficient foraging was defined as bees which had shorter trip times, underwent more foraging trips and carried more per trip. Spaethe and Wiedenmuller (2002) found that nectar load per foraging trip was predicted by body size with larger bees carrying more than smaller bees. Therefore, it is predicted that, on average, foragers will have a greater body mass than non-foragers because larger bees will be able to carry more nectar per trip. For similar reasons, it is also predicted that larger bees will have a greater load lifting capacity, which has been observed in previous studies on bumblebees (Spaethe and Wiedenmuller, 2002; Buchwald and Dudley, 2010) and in orchid bees (Dillon and Dudley, 2004).

It is also predicted that vertical force production and forward flight speed will be related as both are influenced by power output during flight (Dillon and Dudley 2004, Ellington 1991). However, they also represent two different directions, vertical and horizontal, and loads lifted vertically are not necessarily congruent with whole-body acceleration in other directions (Dillon and Dudley 2004).

Forward flight speed might also have a negative relationship with total flight time as work requiring high power output, such as higher flight speeds, leads to shorter endurance time (Morton and Hodgson, 1996). Faster flying animals will tire faster and will not fly as long. In vertebrates, the trade-off between speed and endurance is believed to stem from different proportions of slow and fast twitch muscles in individuals (Bennett et al., 1984; Garland, 1988). However, although arthropods such as insects similarly have fast and slow motoneurons, several differences between the muscles of vertebrates and arthropods exist (Pfluger and Duch, 2011) and even in vertebrates several other studies (Garland, 1988; Tsuji et al., 1989; Sorci et al., 1995) have reported no trade-off between speed and endurance on the whole animal level. It is therefore also possible that there will be no relationship between forward flight speed and total flight time in bumblebees. Overall flight performance traits may be related to each other as the animals' performances will be related to their physiology and morphology, however each trait is also a distinct characteristic which may be differently affected by morphological and physiological characteristics of the animal such as body mass or peak metabolic rate.

2. Study Objectives

Although flight performance is of great biological importance in flying insects such as bumblebees, the relationships between many flight performance traits remains unknown. This study will consist of two parts examining locomotion in bumblebees. A first part will examine if individual bumblebees vary in their foraging effort and how it relates to the flight performance traits, peak (hovering) flight metabolic rate and hovering flight wingbeat frequency. A second part will examine flight performance traits such as flight speed, flight distance and peak flight metabolic rate in bumblebees and ascertain if there are any connections between the bees' performances of these traits. This study will allow to us address if, for example, faster bees are also able to fly for longer, or if there are correlations between a bee's average flight speed and how much it can lift. Ultimately, I am interested in linking these traits to animal energetics. Since metabolism is the source of energy needed for flight, is there a correlation between these flight performance traits and peak metabolic rate? The goal of this project is to find out and create a broader understanding of interindividual variation in flight performance.

4. Materials and Methods

4.1 Animals and Holding Conditions

Bumble bees used in this study are commercially bred *Bombus impatiens* donated by Biobest Canada Ltd (Leamington, ON, Canada). A total of 108 worker bees was used in this study, with masses ranging from 91 to 298 mg. Colonies were maintained in their housing boxes in a room with the temperature controlled at 25°C. All individuals tested were capable of flight. Bees incapable of hovering flight were not used in this study.

4.2 Experiment 1: Foraging Effort

4.2.1 Colony Setup

A first experiment aimed at quantifying differences in foraging effort and association with flight performance. In order to examine foraging effort, an RFID tagging and reading system was used. A total of 49 bees were tagged with both RFID tags and coloured paper tags. Each bee's foraging effort was recorded as the number of foraging bouts the bee took and the amount of time it took for each bout using RFID readers. Due to our limited ability to follow many individuals simultaneously, multiple cohorts of bees from three different colonies were used. 22 individuals were tagged and monitored in a first colony (Colony A) which foraged in July, August and September of 2015. 14 individuals were tagged and monitored in Colony B, which foraged in February and March of 2016. 13 individuals were tagged and monitored in Colony C which foraged in May and June of 2016. The foraging colonies were kept in a housing box in a room with free access to sugar water in a flight cage (170 x 180 x 170 cm) and pollen balls placed directly in the colony box. The bees had continuous access to the flight cage through a clear plastic tube leading into it from the colony in order to allow the bee's access to the sugar water placed on a table inside. Individuals were marked at emergence, their foraging effort was measured from day 7 to 14, and they were captured from their colonies at day 14 of age in order to measure their peak flight metabolic rate and their wingbeat frequency.

4.2.2 RFID Tagging and Foraging Effort

Bees were tagged with 4 mg micro Sensys (city, country) ID tags on the dorsal side of their thorax using a small amount of cyanoacrylate glue. Bees were additionally tagged with paper tags weighing approximately 2.1-2.3 mg on their thorax which had a colour and a number to facilitate recapture. An RFID tag of this size is expected not to affect the overall flight performance of the bees as it is small in comparison to their recorded nectar and pollen loads of 70 mg (Decourtye et al., 2011, Molet et al., 2008, Streit et al., 2003) and the additional weight of the paper tags is low in comparison to the bee's average weight and natural lifting capacity (Decourtye et al., 2011). The tube leading out of the hive was placed through a tunnel in two micro Sensys RFID tag readers located 10 cm and 20 cm from the hive entrance following Gill et al., (2012). The tagged individual's entrances and exits from the hive were recorded in the ID controller and stored for transfer to a laptop computer and conversion to an excel file. In this way, the total number of nectar foraging bouts for each tagged bee could be calculated as well as the amount of time spent on each bout. To avoid recording non-foraging hive exits, recordings were considered a foraging bout only if the bee took longer than 4 minutes to return. This decision was made following a few days of visual observation of foraging behaviour in the flight cage. Bees which underwent a foraging bout at least once were considered "foragers" and bees which did not forage even once were considered "non-foragers". The information gathered through the readers was used to determine the total foraging time of each bee (mins), the number of days the bee foraged, the average time spent foraging per day of the bee (time foraging per day) and average number of foraging bouts per day (foraging bouts per day expressed as bouts/day) of the bee.

4.2.3 Respirometry and Wingbeat Frequency

All bees in Experiment 1 were tested for peak metabolic rate. Additionally, wingbeat frequency measurements were introduced at the acquisition of colony B (after the Colony A testing period) and 27 individuals, those individuals from colonies B and C, were tested for wingbeat frequency. To acquire the animal's peak (hovering flight) metabolic rate, the bees were flown in a 1 L chamber connected through tubing (PharmMed BPT tubing) to a FoxBox flow-through respirometry system (Sable Systems International, Las Vegas, NV, USA). Air dried using Drierite^{TM} was pushed into the chamber at a rate of 500 ml/min using the same tubing. The respirometry system was connected to a laptop and the data was collected using Expedata (Sable Systems International) and exported as Excel documents. Since bees only metabolize carbohydrates (Suarez et al., 2005), only CO_2 output was considered in order to estimate metabolic rate. The CO_2 detector was calibrated each day using both pure nitrogen (0 ppm CO2) and a 430 ppm CO_2 span gas. Following Darveau et al. (2014), a flight duration of 3-5 minutes was used to obtain a period of good quality flight. Hovering flights which showed at least a 30 second period of stable CO_2 output were accepted as good quality flight. Wingbeat measurements were performed using an optical detector and the program T-Rex. Bees were captured directly from their colonies and flown within five minutes of capture.

Metabolic rate was calculated into ml CO_2 output per hour per gram of bee by multiplying the metabolic rate (CO_2 output x flow rate in ml/minute) by 60 minutes. Metabolic rate was analysed using both whole-animal (ml CO_2 hr^{-1}) and mass-specific (ml CO_2 hr^{-1} g^{-1}) values.

4.3 Experiment 2: Flight Performances

4.3.1 Experimental setup

A second experiment investigated the association between various flight performance measurements. Data for experiment 2 consisted of 59 individuals with 43 individuals flying long enough, above the threshold time of 5 minutes of flight, to be counted for flight distance and flight time. In observations of flight mill flight done prior to the study, bees which failed to fly for over five minutes would not maintain steady flight for the duration of the trial necessary for flight time and distance to be measured. Individuals were assessed for flight time, distance and speed using a circular flight mill, load lifting capacity, wing stroke amplitude, peak metabolic rate and wingbeat frequency. The methods for assessing peak metabolic rate and wingbeat frequency were identical to those used in Experiment 1. Additionally, wing measurements were taken to calculate aspect ratio, wing loading, and wing surface area.

The bees were kept in housing boxes with free access to pollen and sugar water inside the box. They were randomly selected from their colony at the start of the experiment and marked for recapture throughout the experiment. Certain colonies used later in experiment 2 were prone to individuals escaping and needed to be kept in small mesh flight cages to prevent escaped bees from having free run of the room. This setup made marking and recapture difficult and, additionally, bees which escaped from the colony into the flight cage had no access to their food source, which meant that any tagged bees which escaped risked undergoing starvation or death before the completion of the experiment. So, bees from these colonies which were used in the experiment 2 flight performance trials, were kept in a recycled colony box separate from their main colony, along with at least one other bee and free access to pollen and sugar water for the duration of the testing period. The bees went through 3 days of trial, with a day of rest between each day, for a total of five days. On the first day, the bees were flown on the flight mill, on the third day they underwent the load lifting trial and on the fifth day their metabolic rate and wing beat frequency were measured. Bees were frozen and killed immediately after the last trial and both their forewings and hindwings were cut off and stored for wing measurements. All bees were placed in the refrigerator at 4°C for 40-60 minutes in order to immobilize them prior to flight performance trials, with the exception of respirometry measurements in which they were flown within 5 minutes of capture and not placed in the refrigerator.

4.3.2 Flight Mill trials

A flight mill was used to determine flight speed, duration and distance flown. These flight trials took place on a flight mill which was connected to a laptop computer. Data was analyzed using Expedata (Sable Systems International). The flight mill design was based on Dr. Jones' design (http://entomology.tfrec.wsu.edu/VPJ_Lab/Flight-Mill.html) with a circumference of 32 cm. Bees were placed in the refrigerator at 4°C for 40-60 minutes in order to immobilize them prior to gluing. The bees were then attached to the pins using Solarez thick, hard formula UV drying glue. Once glued they were immediately placed on the flight mill, waking up within 1-5 minutes. The pin with the bee was inserted on one side and a weight made from poster putty was attached to the other side in order to counterbalance the weight of the bee. Bees that did not initially begin flight were encouraged to do so through stimulation of the tarsal reflex. Individuals that flew uninterruptedly for over 30 seconds were counted for speed and bees that flew uninterruptedly for over 5 minutes were counted for speed, distance and flight time. Distance was calculated by multiplying the number of rotations by the circumference of the flight path and speed was calculated by continuously dividing distance flown every ten seconds over that time.

4.3.3 Load Lifting Experiments

In order to assess vertical force production and load lifting capability, bees were subjected to load lifting experiments using a beaded string. Based on the design of Dillon and Dudley (2003), thread tied around the bee's petiole, which represents their centre of gravity, was tied to plastic monofilament string which had beads tied at regular intervals from the bee. The first segment of beads weighed 24 mg (3 x 8 mg beads) and each segment after that weighed 16 mg (2 x 8 mg beads). The first bead segment was 3.5 cm from the bee with consecutive segments 2 cm apart. Bees were flown in a 40 x 31 x 25.5 cm Plexiglas container, with a piece of lined paper taped to the back to better visually view their lifting capacity. The flight was filmed with an EXILIM camera (Casio) to facilitate the counting of the number of bead groups lifted and the total load lifted was used to calculate vertical force production. Maximum load was calculated by multiplying the number of centimetres of string lifted by the weight of each cm of string and adding that to the number of bead groups lifted after the first bead group, multiplied by the weight of each group (16 mg) and added to the weight of the first bead group (if it was lifted) (24 mg). From that, vertical force (VF) was calculated as the product of gravitational acceleration (g) and the bee's body mass (kg) combined with maximum load lifted (kg). Vertical force was also expressed on a body mass specific basis in VF $m^{-1}g^{-1}$ where m is the body mass of the bee in kg and g is gravitational acceleration, as done in Dillon and Dudley (2004).

4.3.4 Stroke Amplitude

Bees were filmed immediately prior to load lifting for stroke amplitude. Bees were filmed from above using an EXILIM camera (Casio) at 600 frames per second. The obtained video was then cut using Windows Movie Maker and analyzed using Tracker (http://physlets.org/tracker/).

The stroke amplitude measured as the angle between upstroke and down stroke of the bee was measured using the protractor function in Tracker and averaged over 30 frames.

4.3.5 Wing Measurements

Wing measurements were done using photographs taken by a powershot A640 camera (Canon) attached to a SteREO Zeiss compound microscope (Discovery V8). The wing area of both the forewing and the hindwing as well as the length of both the forewing and the hindwing were measured using AxioVision (Zeiss). Forewing and hindwing surface area were added together and then multiplied by 2 to get total wing surface area. The left wings were used unless they were damaged after the death of the bee, in which case the right wings were used. Bees with damaged wings during the flight trials were removed from the experiment.

4.4.6 Measurements of mass

The body mass of each bee was taken after completion of the first trial. Bees were placed in the refrigerator at 4°C for 40-60 minutes in order to immobilize them and then measured using a Mettler Toledo (Excellence) laboratory balance. Measurements of thorax mass were taken at the end of the experiment.

4.4 Statistical Analyses

The data was analysed using R and graphs were created using R and Sigma Plot.

4.4.1 Experiment 1

An ANOVA was used to test for variation in mean body mass (g) among the three colonies, followed by pairwise comparisons using t-tests with pooled standard deviations to determine differences between colonies. This test was used in place of standard t-tests to account for multiple comparisons, which would raise the probability of a type 1 error.

ANCOVAs with body mass as a covariate were used to determine if there was a significant difference in wingbeat frequency, mean peak metabolic rate (ml CO2 hr-1) and foraging effort between colonies.

ANCOVAs were also used to determine if the relationships between these traits and body mass differed significantly between the three colonies. No significant differences between colonies were detected for peak metabolic rate, foraging time or time spent foraging per day. Because no significant differences existed between colonies, the colonies were pooled for further analyses of these traits. The results of the ANCOVAs indicated that the relationships between body mass and total days foraged, as well as body mass and foraging bouts per day did have significantly different slopes between colonies A, B and C and wingbeat frequency of colony B and C varied significantly in intercept. For these reasons, analyses of these traits were done separately for each colony.

Colony data was then pooled for further analyses because only mean body mass, which did not affect foraging effort, varied significantly between colonies.

Flight performance and foraging traits were tested for their relationships with peak metabolic rate using multiple linear regression with body mass a covariate and peak metabolic rate as the independent variable. Peak metabolic rate was compared to average number of foraging bouts per day, total days foraged, time spent foraging per day and total time spent foraging.

T-tests were performed to determine if significant differences in body mass existed between foraging and non-foraging bees. Because Colony B varied significantly from Colonies A and C in body mass, ANCOVAs with body mass as a covariate were also performed to determine if significant differences between foraging and non-foraging bees existed in wingbeat frequency or peak metabolic rate. Log values were used for all variables to eliminate heteroscedasticity detected by the Bruesch-Pagan test, except for total days foraged and average number of bouts per day which did not display heteroscedasticity.

4.4.2 Experiment 2

Due to the documented effects of body mass on certain traits such as wingbeat frequency and peak metabolic rate in bumblebees (Skandalis and Darveau, 2012), all flight performance traits were analysed for their relationships with body mass. Linear regression was used to determine if relationships existed between body mass and thorax mass on flight performance traits including peak metabolic rate (ml CO_2 h^{-1}), wingbeat frequency (Hz), vertical force production (N), mass dependent vertical force (VF $m^{-1}g^{-1}$ where g is gravitational acceleration), distance flown (m), average flight speed (m/s), maximum flight speed (m/s), flight time (mins), stroke amplitude (φ), aspect ratio, wing area (mm^2) and wing loading (g cm^{-2}). For analyses of these traits' relationships with body mass, log values were used and the slopes of the log regressions were taken to determine the scaling exponent of the power function of each relationship. These scaling exponents were used to determine if the relationships were allometric or isometric.

Additionally, multiple regressions with body mass as a covariate were used to determine if there was significant relationship between peak metabolic rate and vertical force production, distance flown (m), average flight speed (m/s), maximum flight speed residuals (m/s), stroke amplitude (φ), aspect ratio and flight time (mins).

Linear regression was also used to determine if a significant relationship of peak metabolic rate with wing loading (g cm^{-2}) and wingbeat frequency (Hz) using the residuals from the relationship with body mass for all three factors.

Other flight performance characteristics were analysed using a correlation matrix. Traits which related to each other were further investigated using using multiple linear regression with body mass as a covariate.

To analyse the effects of multiple independent flight performance traits on a single dependent variable flight performance trait, multiple regression analyses were performed. The Akaike Information Criterion (AIC) was used to help find the AIC best model (the model with the lowest AIC) for the data. Body mass, metabolic rate, wingbeat frequency, stroke amplitude, aspect ratio, and wing loading were assessed for their effects on the flight performance traits vertical force, flight distance, average flight speed, maximum flight speed and flight time (tables 5.5, 5.6 and 5.8) using AIC. In addition, correlation analyses were done for relationships which did not have obvious dependent and independent variables.

Tests of assumptions were performed on the data for regression, correlation and ANOVA analyses. In order to test for heteroscedasticity, I used the Breucsch-Pagan test. The Durbin-Watson test was used to ensure no autocorrelation between variables. Additionally, the RESET test was used to test for the assumption that the data was linear in regressions and multiple regressions. Finally, Shapiro-Wilk normality test was used to determine if the data met the assumption having a normal distribution. Diagnostic graphs were generated including residuals vs fitted, normal Q-Q, scale-location and residuals vs leverage in order to visually assess whether the data met the statistical assumptions of linearity, normality and homoscedasticity and to detect outliers respectively.

5. RESULTS

5.1 Experiment 1: Foraging

5.1.1 Variation among colonies and effects of body mass on foraging and flight performance traits

Because many traits, such as wingbeat frequency, have an established relationship with body mass, the colonies were assessed for variation in body mass. The three colonies did differ significantly by body mass ($P<0.001$) (See Table 5.1). The average body mass of colony B tagged individuals was 0.215 g (SE), significantly higher than that of colony A and C tagged individuals which had average body masses of 0.137 g and 0.162 g respectively (See Table 5.1). Additionally, ANCOVAs were performed with mass as a covariate to determine if wingbeat frequency, peak metabolic rate or foraging related traits varied significantly between colonies. No significant variation was found (see Table 5.2).

Due to the variation in body mass among the colonies, ANCOVAs were performed on foraging and flight related traits with body mass as a covariate to determine if significant variation in slope and intercept existed between the two colonies. No significant differences between colonies were detected for peak metabolic rate, foraging time or time spent foraging per day. Because no significant differences existed between colonies, the colonies were pooled for further analyses of these traits. Across the colonies, peak metabolic rate was significantly related to body mass ($r^2 = 0.628$, $P<0.001$) with larger bees having a higher metabolic rate. However, no significant relationship between body mass and foraging time or time spent foraging per day were detected.

Table 5.1 Summary of the body mass, peak metabolic rate (PMR) (ml CO_2 hr^{-1}), wingbeat frequency (WBF) (Hz), foraging time (mins), foraging time/day (min/day), total days foraged and foraging bouts per day of three colonies of *Bombus impatiens* workers. Foragers were bees which foraged at least one time, non-foragers were bees which never foraged. In total 23 bees foraged and 26 did not. Body mass was used as a covariate for PMR and WBF comparisons. (N=26 for wingbeat frequency, N = 23 for foraging measurements and N = 49 for all other factors).

Colony (N)	**Foragers (%)**	**Mean body mass (g) (SE)**	**Mean PMR (ml CO_2 hr^{-1}) (SE)**	**Mean WBF (Hz) (SE)**	**Mean foraging time (mins) (SE)**	**Mean time foraging/day (min/day) (SE)**	**Mean total days foraged (SE)**	**Mean foraging bouts per day (SE)**
Colony A (22)	41	0.137 (0.005)	9.26 (0.399)	-	907.6 (15.4)	143.5 (42.8)	4.3 (0.943)	9.99 (3.07)
Colony B (14)	57	0.215 (0.014) [1]	13.31 (0.933)	181.52 (2.70)	256.5 (26.4)	39.29 (15.4)	4.9 (0.854)	5.04 (1.97)
Colony C (13)	46	0.162 (0.012)	11.15 (0.812)	192.69 (7.02)	401.6 (42.8)	58.23 (26.4)	4.5 (1.02)	2.83 (0.946)

1 Colony B body mass varied significantly from colonies A and C $p<0.001$. All other variables did not vary significantly between colonies when accounting for body mass variation

Table 5.2 Summary of the ANCOVA results of the effect of colony on peak metabolic rate (PMR) (ml CO_2 hr^{-1}), wingbeat frequency (WBF) (Hz), foraging time (mins), time spent foraging/day (mins/day), total days foraged and number of foraging bouts per day with body mass (g) as a covariate in *Bombus impatiens* workers. (N=26 for foraging measurements, N=49 for all other factors). Wingbeat frequency is compared between colonies B and C, all other factors are between colonies A, B and C.

		F	**df**	**P**
WBF (Hz)	Mass	0.238	1, 24	0.630
	Colony	1.20	1, 24	0.305
	Intercept	108.0	1, 24	<0.001
Total days foraged	Mass	0.047	1, 17	0.831
	Colony	5.99	2, 17	<0.005
	Interaction	5.74	2, 17	<0.005
	Intercept	0.499	1, 17	0.490
Foraging bouts per day	Mass	0.146	1, 17	0.707
	Colony	3.976	2, 17	<0.005
	Interaction	5.897	2, 17	<0.005
	Intercept	0.022	1, 17	0.883

The results of the ANCOVAs indicated that the relationships between both body mass and total days foraged, as well as body mass and foraging bouts per day did have significantly different slopes between colonies A, B and C. Wingbeat frequency of colony B and C varied significantly in intercept. For this reasons, analyses were done separately for each colony. Foraging bouts per day had a significant positive relationship with body mass for Colony A bees with larger bees tending to have more foraging bouts per day than smaller ones ($P<0.05$), but there was no significant relationship with body mass for the other two colonies. Additionally, this relationship was based on a relatively small sample size and the pooled date for the three colonies shows no relationship between body mass and number of foraging bouts per day. Neither number of days foraged nor wingbeat frequency were related to body mass for any of the three colonies so it is unlikely that variation in slopes or intercepts between colonies is meaningful.

5.1.2 Effects of peak metabolic rate on foraging and flight performance traits

Peak metabolic rate had no significant relationship with the wingbeat frequency of the tested bees. It also had no significant relationship with any of the foraging traits of the bees including foraging bouts per day, total days foraged, average foraging time per day and total time spent foraging.

5.1.3 Variation between foragers and non-foragers

Table 5.1 displays the proportion of foraging to non-foraging tagged bees of each colony as a percentage. For all colonies, a total of 23 individuals were recorded foraging and 26 individuals did not forage. Colony B had the largest proportion of foragers with 57% of tagged individuals foraging at least once. Colony A had the lowest proportion of foragers at only 41% and in Colony C, 46% of tagged individuals foraged at least once.

The results of the comparisons between foragers and non-foragers are displayed in Table 5.3. Foragers and non-foragers did not differ significantly in their wingbeat frequencies or body mass. However, peak metabolic rate did differ significantly between the two groups when controlling for body mass ($P<0.05$) with foraging bees having a 22.0% higher peak metabolic rate than non-foraging bees (Figure. 5.1).

Table 5.3 Summary of the t-test results of comparisons of body mass between foragers and non-foragers as well as the ANCOVA results for comparisons, peak metabolic rate (PMR) (ml CO_2 hr^{-1}) and wingbeat frequency (WBF) (Hz) in RFID tagged *Bombus impatiens* workers. Body mass was used as a covariate for PMR and WBF. (N= 26 for WBF and N=49 for all other factors.)

		F-stat	**P**	**df**	**Forager average (SE)**	**Non-Forager average (SE)**
Body Mass (g)			0.124	47	0.178 (0.010)	0.155 (0.010)
PMR (ml CO2 hr^{-1})	Mass	72.61	<0.001	1, 46	12.07 (0.666) [a]	9.90 (0.546) [b]
	Foraged	4.22	0.046	1, 46		
	Intercept	7.76	<0.01	1, 46		
WBF (Hz)	Mass	1.33	0.260	1, 24	186.45 (2.67)	187.38 (4.97)
	Foraged	0.008	0.930	1, 24		
	Intercept	203.1	<0.001	1, 24		

a, b – letters used to indicate significant difference between groups

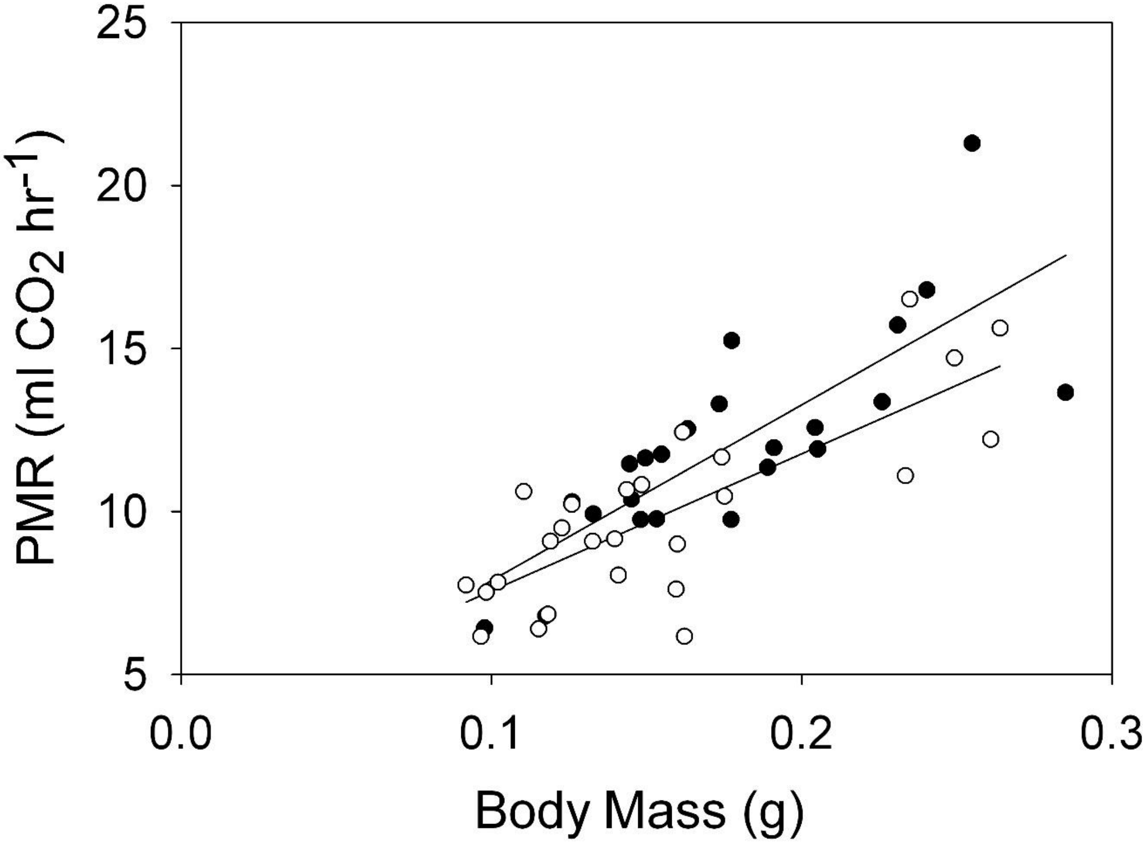

Figure 5.1 Linear regression of peak metabolic rate (PMR) (ml CO_2 hr^{-1}) and body mass (g) for foragers (filled circles) and non-foragers (open circles) in *Bombus impatiens* workers (n =49). Peak metabolic rate is significantly different between foragers and non-foragers when controlling for body mass ($P<0.005$)

5.2 Experiment 2 Flight Performance

5.2.1 The effects of mass on flight performance traits

Figure 5.2 shows the relationships between flight performance traits and body mass in log transformed values among the tested bees. Certain flight performance traits were shown to be significantly related to body mass with larger bees tending to have higher wing loading, a greater maximum speed, greater vertical force production, lower wingbeat frequencies and a higher peak metabolic rate than smaller bees. 62% of individual variation in vertical force production is explained by body mass. However, contrastingly, mass specific vertical force, expressed as VF $m^{-1}g^{-1}$, had no significant relationship with mass (P=0.644). This makes sense as vertical force scales isometrically with body mass, having a scaling exponent of 1.07 (See Table 5.4). Other traits scaled allometrically with body mass (See Table 5.4) including wing loading, wingbeat frequency, peak metabolic rate and maximum flight speed. Body mass explained 27.8% of variation in wing loading with a scaling exponent of 0.51, 48.0% of variation in wingbeat frequency with a scaling exponent of -0.23, 22.5% of variation in peak metabolic rate with a scaling exponent of 0.49, 7.3% of variation in maximum speed with a scaling exponent of 0.36 and 31.6% of variation in total wing area with a scaling exponent of 0.49. Flight performance traits showed similar relationships to thorax mass and thorax mass variation does not contribute further to variation among flight performance traits measured.

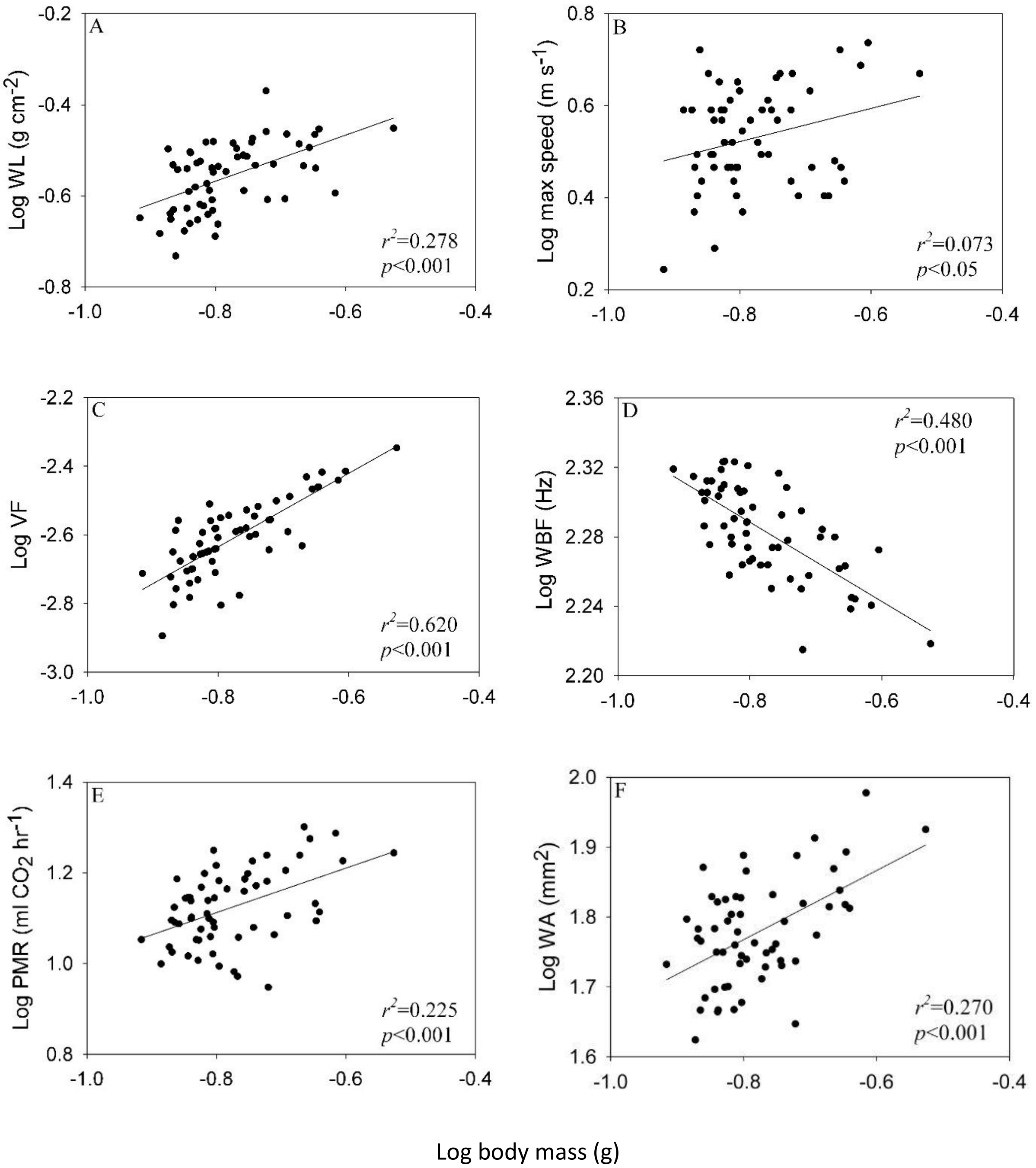

Figure 5.2 Linear regressions of the log of wing loading (WL) (g cm^{-2}), maximum (max) speed (m s^{-1}), vertical force (VF) (N), wingbeat frequency (WBF) (Hz), peak metabolic rate (PMR) (ml CO$_2$ hr^{-1}) and total wing area (WA) (mm^2) with the log of body mass (g) in *Bombus impatiens* workers (n=59)

Table 5.4 Results of the linear regressions of the log of wing loading (WL) (g cm^{-2}), maximum (max) speed (m s^{-1}), vertical force (VF) (N), wingbeat frequency (WBF) (Hz), total wing area (WA) (mm^2) peak metabolic rate (PMR) (ml CO_2 hr^{-1}) with the log of body mass (M_b) (g) showing equations, standard error of the scaling exponent and correlation coefficients in *Bombus impatiens* workers (N=59).

Trait	**Equation**	**SE of Exponent**	**P**	**r^2**
WL (g cm^{-2})	$0.686M_b^{0.51}$	0.109	<0.001	0.278
Maximum Speed (m s^{-1})	$6.47M_b^{0.36}$	0.171	<0.05	0.073
VF (N)	$0.017M_b^{1.07}$	0.111	<0.001	0.620
WBF (Hz)	$128.8M_b^{-0.23}$	0.031	<0.001	0.480
WA (mm^2)	$145.7M_b^{0.49}$	0.109	<0.001	0.270
PMR (ml CO_2 hr^{-1})	$32.0M_b^{0.49}$	0.120	<0.001	0.225

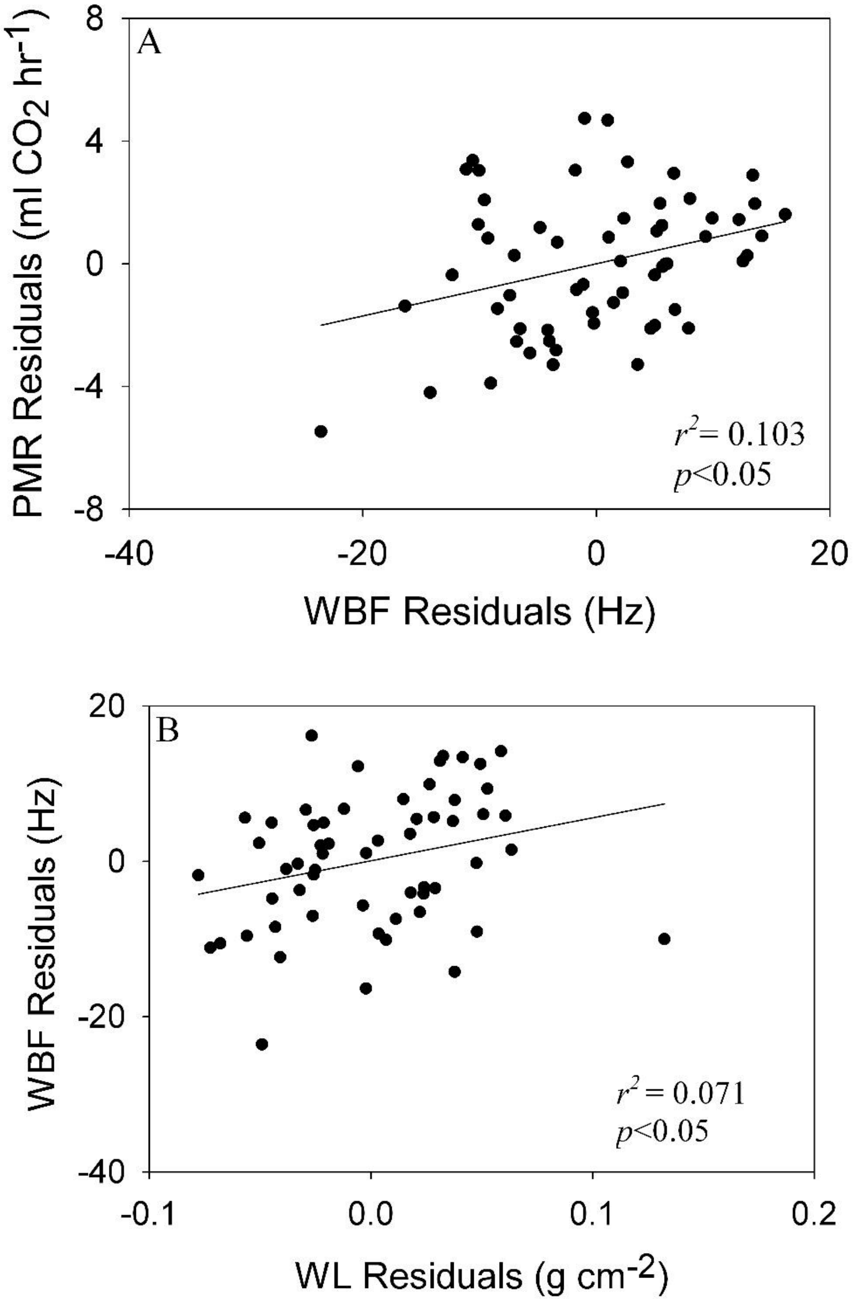

Figure 5.3 Linear regressions of body mass specific peak metabolic rate residuals (PMR) (ml CO_2 hr^{-1}) with body mass specific wingbeat frequency residuals (WBF) (Hz) and body mass specific WBF with body mass specific wing loading (WL) (g cm^{-2}) in *Bombus impatiens* workers (n=59).

5.2.2 The effects of peak metabolic rate on flight performance traits

Of all the flight performance traits analysed, only wingbeat frequency showed a significant relationship with peak metabolic rate ($P<0.05$) (See Figure 5.3). This relationship was positive, with peak metabolic rate explaining 10.3% of the variation in wingbeat frequency independent of body mass. No other flight performance traits were found to have a relationship with peak metabolic rate.

5.2.3 Relationships between other flight performance traits

Several relationships were uncovered between flight performance traits. Among the bees tested, wing loading and wingbeat frequency had a positive relationship when residuals were used to control for mass (Figure 5.3) ($P<0.05$), with wing loading explaining 7.14% of variation in wingbeat frequency. Additionally, wingbeat frequency and stroke amplitude were related with wingbeat frequency explaining 8.07% of variation in stroke amplitude ($P<0.05$). However, this relationship does not remain significant when mass is used as a covariate.

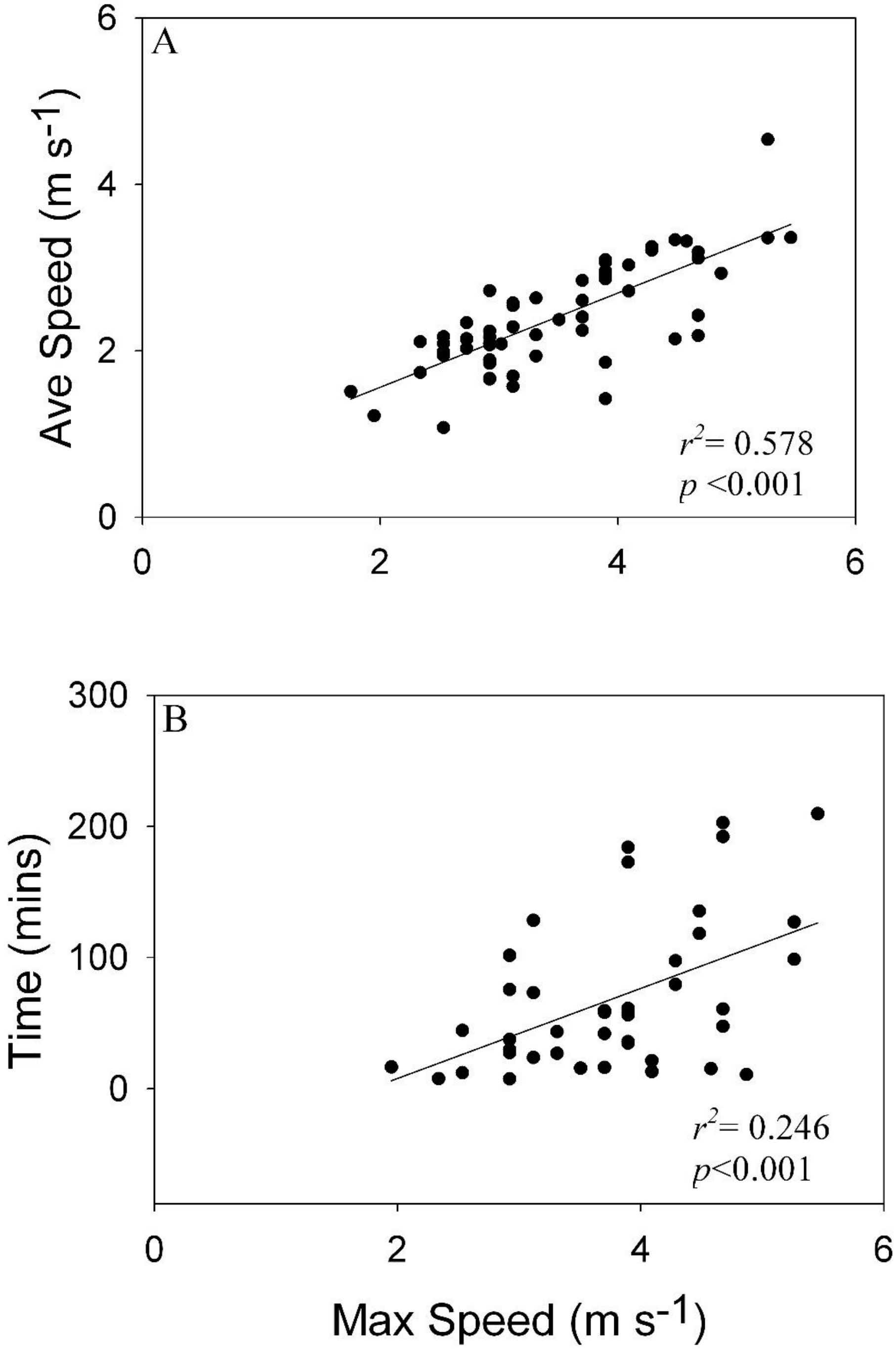

Figure 5.4 Linear regression of average (ave) speed (m s^{-1}) and time (mins) with maximum (max) speed (m s^{-1}) in *Bombus impatiens* workers (n=59 for A and n=43 for B).

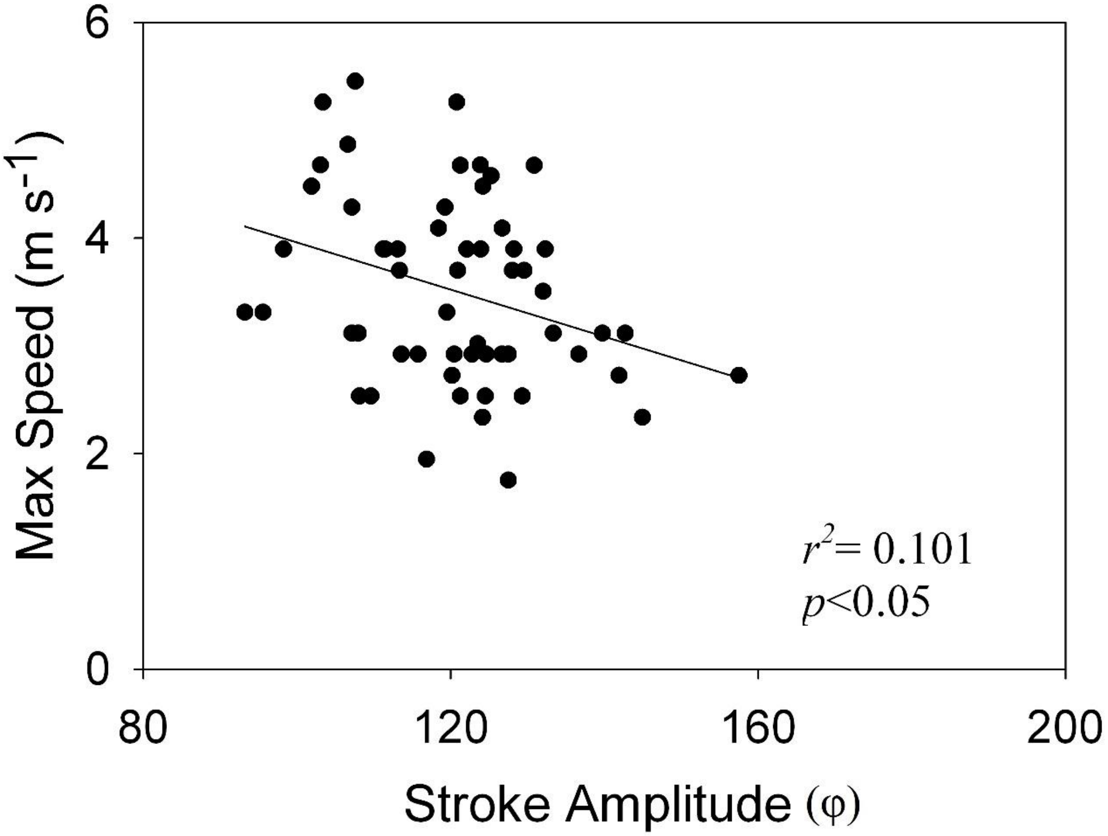

Figure 5.5 Linear regression of maximum (max) speed (m s^{-1}) and stroke amplitude (φ) in *Bombus impatiens* workers. (n=58)

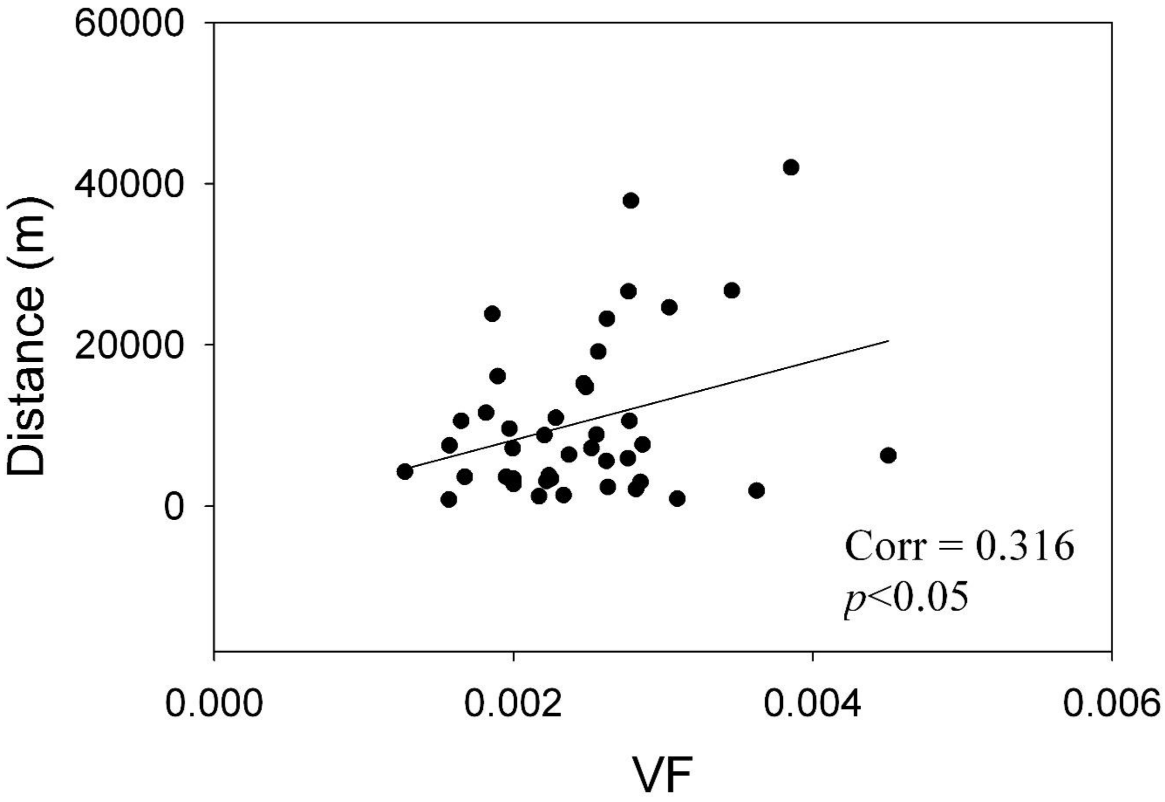

Figure 5.6 Correlation between flight distance (m) and vertical force production (VF) (N) in *Bombus impatiens* workers. (n=43)

Average flight speed and total flight time were both significantly related to maximum flight speed (see Figure 5.4). Bees with a higher maximum flight speed also tended to fly faster on average, and bees with a greater maximum flight speed tended to fly longer. Maximum flight speed explained 57.8% of the variation in average flight speed and it explained 24.6% of the variation in flight time. The relationship between maximum speed and average speed was significant even when controlling for body mass ($P<0.001$), as was the relationship between maximum speed and flight time ($P<0.001$). Additionally, shown in Figure 5.5, maximum speed was related to stroke amplitude with 10.1% of variation in maximum speed explained by stroke amplitude. However, this relationship does not remain significant when controlling for wingbeat frequency. Vertical force production and flight distance were significantly correlated, with bees that had a higher vertical force production tending to fly further than bees with a lower vertical force production (Figure 5.6). However, this relationship is not significant when body mass is used as a covariate.

The best AIC model for average speed only included wingbeat frequency (See Table 5.5). Bees which flew faster on average had a lower wingbeat frequency (Figure 5.7) however wingbeat frequency only explained 11.5% of variation in average flight speed. This relationship was still significant when mass was used as a covariate ($P<0.05$). Similarly, maximum flight speed was significantly related to wingbeat frequency, with individuals with lower wingbeat frequencies tending to have a higher maximum flight speed (Figure 5.7) and this relationship remained significant when mass was used as a covariate ($P<0.05$). The use of AIC yielded an AIC best multiple regression model for maximum flight speed with wingbeat frequency, stroke amplitude and aspect ratio as the independent variables (adjusted $r^2 = 0.177$, $P < 0.01$). AIC results can be seen in Table 5.6. All relationships were negative. However, only wingbeat frequency had a significant effect on maximum speed ($P < 0.01$) and when removing stroke amplitude and aspect ratio, wingbeat frequency alone still had a significant negative relationship with maximum speed (adjusted r^2=0.140, $P < 0.01$). A comparison of the two models is shown in Table 5.7. Due to missing data for certain traits, the full data set could not be used for AIC (n=57 instead of 59) and a so a regression analysis was done with the full data set and results yielded an r^2 of 0.157 ($P < 0.01$).

Wingbeat frequency was also significantly related to distance flown, with bees that flew further tending to have a lower wingbeat frequency (Figure 5.7). This relationship remained significant when using mass as a covariate ($P < 0.05$) However it was no longer significant when maximum flight speed and average flight speed were used as covariates ($P = 0.298$). In a multiple regression analysis done on flight distance, in the AIC best model, flight distance was related to peak metabolic rate, aspect ratio, wing loading, stroke amplitude and wingbeat frequency (adjusted r^2= 0.288, $P < 0.01$) (See Table 5.8). All variables were negatively related to distance, except peak metabolic rate which was positively related. However, once again, wingbeat frequency was the only factor significantly related to distance and was significantly related in a linear regression model independent of the other two factors (adjusted r^2=0.183, $P < 0.01$). The comparison between these two models can be seen in Table 5.9. Due to missing data for certain traits, the full data set could not be used for AIC (n=42 instead of 43) and a so a regression analysis was done with the full data set and results yielded an r^2 of 0.177 ($P < 0.01$).

Model selection is not an indicator of significance, but only an indicator of the best fit model for the data (Burnham and Anderson 1988), therefore only terms significantly affecting the flight performance trait ($P < 0.05$) were taken into consideration for further analysis.

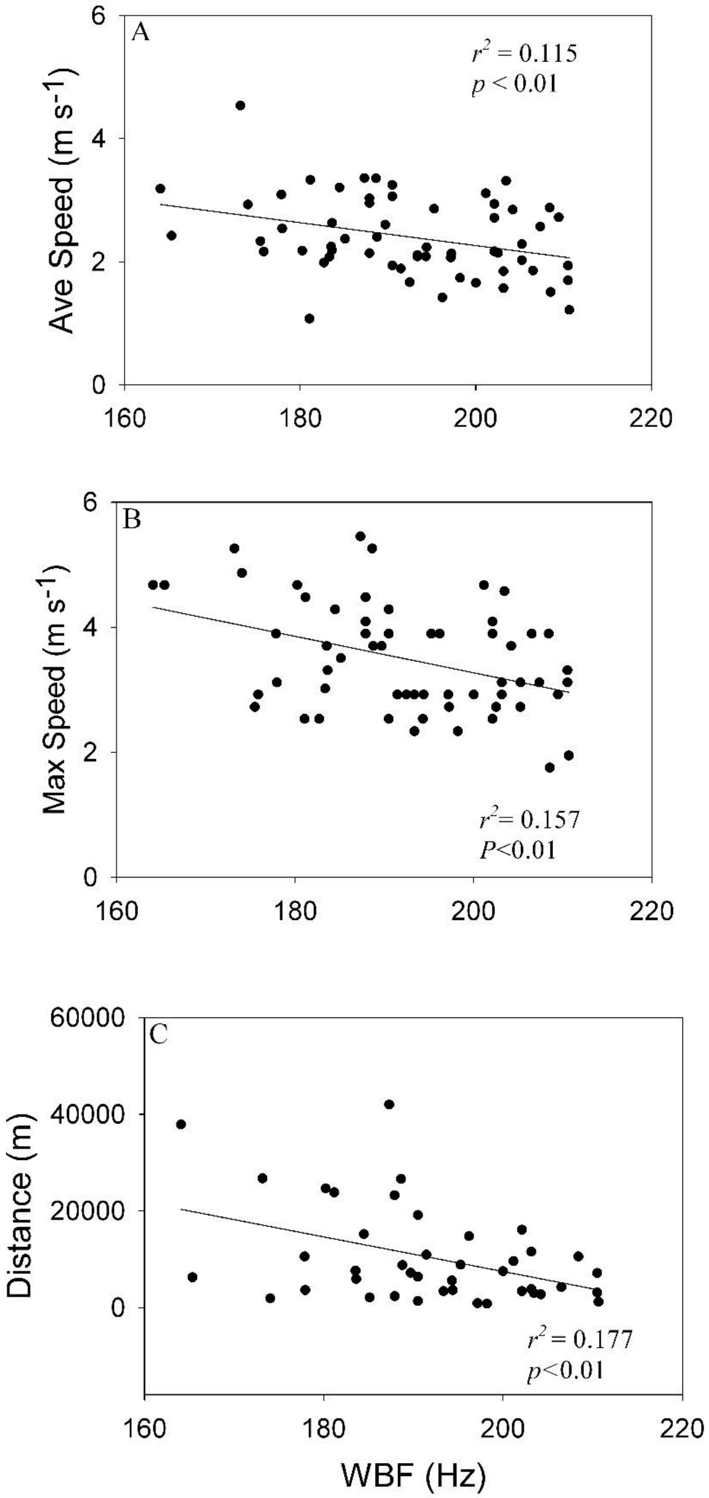

Figure 5.7 Linear regressions of average (ave) speed (m s^{-1}), maximum (max) speed (m s^{-1}) and distance (m) with wingbeat frequency (WBF) (Hz) in *Bombus impatiens* workers (n=59 for A and B and n=43 for C).

Table 5.5 A summary of the multiple regression models for average flight speed as the dependent variable and body mass, peak metabolic rate (PMR), wingbeat frequency (WBF), stroke amplitude (SA), aspect ratio (AR) and wing loading (WL) as the independent variables from a single AIC set in *Bombus impatiens* workers. (n=57)

	Model 1		**Model 2**		**Model 3**		**Model 4**		**Model 5**		**Model 6**	
Variable	Beta	Beta SE	Beta	Beta SE	Beta	Beta SE	Beta	Beta SE	Beta	Beta SE	Beta	Beta SE
Intercept	10.0**	2.87	9.67**	2.80	8.51***	2.36	8.16***	2.34	7.48***	2.30	5.845***	1.321
Body Mass (g)	-7.43	5.10	-8.32	4.87	-8.78	4.81	-5.971	4.05	-2.97	3.42	-	-
PMR (ml CO_2 hr^{-1})	0.0499	0.0399	0.0551	0.0388	0.06013	0.0381	0.0500	0.0370	-	-	-	-
WBF (Hz)	-0.0296*	0.0114	-0.032**	0.0106	-0.0316**	0.0106	-0.0282**	0.0101	-0.0238*	0.00966	-0.0179*	0.00685
SA (φ)	-0.00456	0.00733	-	-	-	-	-	-	-	-	-	-
AR	-0.204	0.226	-0.168	0.217	-	-	-	-	-	-	-	-
WL (g cm^{-2})	1.63	2.20	2.03	2.09	2.23	2.06	-	-	-	-	-	-
Adjusted r^2	0.0891		0.1		0.107		0.104		0.0905		0.0945	
P	0.0970		0.0638		0.0418		0.0317		0.0290		0.0115	
df	50		51		52		53		54		55	
Delta i	-4.9		-3.34		-2.01		-1.28		-1.21		0	

* p-value < 0.05 ** p-value < 0.01 *** p-value < 0.001

Table 5.6 A summary of the multiple regression models for maximum flight speed as the dependent variable with body mass, peak metabolic rate (PMR), wingbeat frequency (WBF), stroke amplitude (SA), aspect ratio (AR) and wing loading (WL) as independent variables from a single AIC set in *Bombus impatiens* workers. (N=57)

	Model 1		**Model 2**		**Model 3**		**Model 4**	
Variable	Beta	Beta SE	Beta	Beta SE	Beta	Beta SE	Beta	Beta SE
Intercept	13.8***	3.58	13.0***	3.152	13.8***	2.99	12.6***	2.69
Body Mass (g)	-3.1	6.37	-	-	-	-	-	-
PMR (ml CO_2 hr^{-1})	0.0481	0.0498	0.0332	0.0392	-	-	-	-
WBF (Hz)	-0.0312*	0.0142	-0.0259**	0.00916	-0.0268**	0.00906**	-0.0253*	0.00890
SA (φ)	-0.0152	0.00915	-0.0165	0.00871	-0.0168	0.00867	-0.0154	0.00854
AR	-0.357	0.282	-0.383	0.275	-0.412	0.272	-0.384	0.270
WL (g cm^{-2})	-1.42	2.74	-2.21	2.21	-2.06	2.19	-	-
Adjusted r^2	0.158		0.171		0.175		0.177	
P	0.0216		0.0116		0.00688		0.00390	
df	50		51		52		53	
Delta i	-4		-1.65		-1.04		0	

* p-value < 0.05 ** p-value < 0.01 *** p-value < 0.001

Table 5.7 A summary of the comparison of AIC best and the model with only significant variables for maximum flight speed as the dependent variable and wingbeat frequency (WBF), stroke amplitude (SA) and aspect ratio (AR) as the independent variables in *Bombus impatiens* workers. (n=57)

	AIC best model			**Significant variable only model**		
Variable	Beta	Beta SE	p-value	Beta	Beta SE	P
Intercept	12.6***	2.69	0.000	8.79***	1.67	0.000
WBF (Hz)	-0.0253**	0.00890	0.00635	-0.0276**	0.00867	0.00238
SA (φ)	-0.0154	0.00854	0.07596	-	-	-
AR	-0.384	0.270	0.16102	-	-	-
Adjusted r^2	0.177			0.140		
P	0.00390			0.00238		
df	53			55		

* p-value < 0.05 ** p-value < 0.01 *** p-value < 0.001

Table 5.8 A summary of the multiple regression models for distance as the dependent variable and body mass, peak metabolic rate (PMR), wingbeat frequency (WBF), stroke amplitude (SA), aspect ratio (AR) and wing loading (WL) as the independent variables from a single AIC set in *Bombus impatiens* workers. (N=41)

	Model 1		**Model 2**	
Variable	Beta	Beta SE	Beta	Beta SE
Intercept	166446.8***	41031.7	164795.7***	38356.6
Body Mass (g)	-9583.9	75642.0	-	-
PMR (ml CO_2 hr^{-1})	-756.7	587.5	-803.3	451.6
WBF (Hz)	-397.7*	158.0	-383.2**	108.0
SA (φ)	-213.7	122.9	-218.2	115.9
AR	-5367.9	3438.2	-5517.4	3183.4
WL (g cm^{-2})	-36521.7	30430.0	-38776.7	24333.0
Adjusted r^2	0.267		0.288	
P	0.00933		0.00410	
df	34		35	
Delta i	1.70		0	

* p-value < 0.05 ** p-value < 0.01 *** p-value < 0.001

Table 5.9 A summary of the comparison of AIC best and the model with only significant variables for distance as the dependent variable and peak metabolic rate (PMR), wingbeat frequency (WBF), stroke amplitude (SA), aspect ratio (AR) and wing loading (WL) as the independent variables in *Bombus impatiens* workers. (n=57)

	AIC Best Model			**Significant variable only Model**		
Variable	Beta	Beta SE	p-value	Beta	Beta SE	P
Intercept	164795.7***	38356.6	0.000131	73496.2***	20296.6	0.000834
PMR (ml CO_2 hr^{-1})	-803.3	451.6	0.0840	-	-	-
WBF (Hz)	-383.2**	108.0	0.00112	-332.6**	105.5	0.00311
SA (φ)	-218.2	115.9	0.0681	-	-	-
AR	-5517.4	3183.4	0.0919	-	-	-
WL (g cm^{-2})	-38776.7	24333.0	0.120	-	-	-
Adjusted r^2		0.288			0.183	
P		0.00410			0.00311	
df		35			39	

* p-value < 0.05 ** p-value < 0.01 *** p-value < 0.001

6. DISCUSSION

This study aimed to examine the extent of variation in flight performance traits and determine how these traits are related to each other. This included examining variation in foraging effort and examining variation in peak metabolic rate, flight morphological and kinematic traits, load lifting capacity and flight distance, time and duration among individual *Bombus impatiens*. Foraging effort among individuals was unequal, with only 41-57% of bees undergoing active foraging at least once. Peak metabolic rate was significantly higher in foraging than in non-foraging bees. Links between morphology, wing kinematics and metabolism were uncovered and vertical force production was found to scale isometrically with body mass, but not when expressed in mass dependent units. Body mass was also significantly linked to several other flight performance traits including forward flight speed. Additionally, maximum flight speed had a significant relationship with total flight time. Overall, body mass is a significant factor affecting many flight performance traits and links between certain flight performance traits exist, however not all traits were related.

6.1 Experiment 1: Foraging

6.1.1 Variation among colonies

The colonies varied by average body mass, with colony B having a significantly higher average body mass than colonies A and C (See Table 5.1). Adult bumblebee size is not determined genetically but by how much the immature bees (callows) eat before they emerge from their pollen balls and differences in the average body masses of colonies have been known to occur in bumblebees (Couvillon et al., 2015). Furthermore, Couvillon et al., 2015 observed that small workers were not the result of an insufficient foraging force and Spaethe and Wiedenmuller (2002) did not observe an effect of size on foraging behaviour other than the volume of nectar carried by the bee per trip, which was not measured in this study. This is consistent with the results of this study which only found a relationship between body mass and number of foraging bouts per day for one colony and only with a small sample size. Overall, these results indicate a minimal or absent effect of body mass on foraging effort. Therefore, it is not likely that the size variation among colonies affected the measured foraging effort of the bees and, additionally, controlling for mass while undergoing statistical analyses should have eliminated any possible effects.

6.1.2 Unequal distribution of labor

Throughout the three colonies, a total of 23 individuals were recorded foraging and 26 individuals did not forage. Colony foraging rates varied, with between 41-57% of tagged, flight capable bees undergoing active foraging for nectar at least once (See Table 5.1). This is comparable to the study done on honeybees by Tenczar et al. (2014) in which 51% of tagged bees underwent nectar foraging, indicating that the bumblebees I tested had a similar proportion of foragers to non-foragers as the honeybee foraging caste tested in their study. Reasons for variation in foraging effort require further investigation, however, a study by Fewell and Bertram (1999) observed that among honeybees, foraging caste workers adjust task allocation in response to external stimuli in accordance with genetic variation among colony members. Certain individuals were genetically predisposed to be more sensitive to recruitment in response to external conditions and so certain individuals were recruited earlier than others. This allows the genetic diversity of a colony to lend it foraging flexibility. A similar genetic variation in recruitment sensitivity, if present in bumblebees, may be partially responsible for the variation in foraging effort among the bumblebees in my study, which were held in stable laboratory conditions. If so, the foragers in this study would be synonymous with the “early recruits” observed by Fewell and Bertram (1999). Additionally, bees which undergo foraging may have better foraging performance than non-foragers. Carter (1992) found that among bumblebees, workers which were recruited from in-nest tasks to foraging tasks after a reduction in food source were less efficient foragers than those which were foragers before the reduction, indicating that, in addition to variation in foraging effort providing foraging flexibility to the colony, performance differences may exist between bees which forage initially and bees which do not forage unless pressured by environmental conditions such as low nectar stores. However, conversely, other studies have found changes in honeybees with increasing foraging experience including an increase in efficiency of the rate of nectar and pollen acquisition (Dukas and Visscher 1994) as well as biochemical changes in protein composition and enzyme activity (Schippers et al. 2006) by individuals increasing with foraging experience. It is therefore possible that the differences in experience of early vs late recruits are responsible for the difference in their foraging activity observed by Carter (1992).

Foraging bees has a significantly higher metabolic rate than non-foraging bees. This difference may be due to interindividual variation; however, it may also be due to foraging experience. Schippers and Dukas (2010) observed flexibility in peak metabolic rate among honeybees, with foraging activity increasing metabolic rate in foraging caste bees which had 4-5 days of foraging experience. Metabolic rate in these bees gradually increased with foraging experience, peaking at 4-5 days. Additionally, Schippers et al. (2006) observed plasticity in flight muscle structural and metabolic components related to increased foraging experience. Since the bees in my study were assessed for peak metabolic rate after 7 days of foraging activity, it is possible that the differences in metabolic rate which I observed between foragers and non-foragers are due to the effects of being active foragers on the metabolic activity of the bees, rather than innate differences between foraging and non-foraging bees. However, peak flight metabolic rate is also a stable and repeatable trait in worker bumblebees (Darveau et al., 2014; Skandalis and Darveau 2012) and Skandalis and Darveau (2012) did not observe any significant changes in peak metabolic rate, wingbeat frequency or enzyme activity with increased flight experience. They did note, however, that the amount of flight recorded in their study was different than that expected in the field, which the previous studies were examining. It is possible that the discrepancy between their results and the results of the previous studies may be due to difference in testing. Conditions in which my bees were kept is closer to the conditions in Skandalis and Darveau (2012) study so it is more likely that the effects of flight experience on flight performance in my bees is more similar to theirs. Additionally, several studies have shown a degree of genetic control over peak metabolic rate such as the association with allelic variation in the gene for malate dehydrogenase in honeybees (Coelho and Mitton, 1988; Harrison et al., 1996; Nielsen et al., 1994) including between workers within a colony (Coelho and Mitton, 1988). It is therefore also possible that the differences observed in my study between foragers and non-foragers are inherent to the individuals rather than a product of foraging experience. Future research in this area would benefit from measuring metabolic rate prior to onset of foraging to determine if differences between foraging and non-foraging individuals exist independent of foraging experience.

6.2 Experiment 2: Flight Performance Traits

6.2.1 Morphology, flight kinematics and metabolism

Wingbeat frequency, wing loading and peak flight metabolic rate were all related to body mass (See Figure 5.2) with wingbeat frequency having a negative relationship with body mass and wing loading and peak flight metabolic rate having a positive relationship. Peak metabolic rate and wingbeat frequency were also related to each other independent of body mass as were wingbeat frequency and wing loading (See Figure 5.3). It has been established in previous studies that these traits all correlate with each other (Darveau et al., 2005; Skandalis and Darveau, 2012). Size affects wing form which affects wing kinematics, which affects the scaling of metabolic rate with body mass (Darveau et al., 2005). Additionally, Skandalis and Darveau (2012) found that body mass alone did not fully explain variation in metabolic rate among *Bombus impatiens* and peak metabolic rate was further explained by variation in wing morphology as well as variation in flight muscle metabolic enzyme activity. Mass independent wing loading and wingbeat frequency had a weak positive relationship in my study which was contrary to the negative relationship found in Darveau et al. (2005), however Byrne et al. (1988) found that among smaller size ranges, insect wing loading had a positive relationship with wingbeat frequency. Therefore, it is likely that the relationship in my study, which observed a relatively small size range (within a single species) is following the pattern observed by Byrne et al. (1988). In addition, I found that stroke amplitude was positively related to wingbeat frequency. However, this relationship was no longer significant when body mass was used as a covariate and, although no relationship between body mass and stroke amplitude was found in my study, previous studies have observed both wingbeat frequency and stroke amplitude to scale with body mass (Roberts et al., 2004). It is therefore likely that it is acting as a third factor producing the observed relationship between the two variables. Overall, the relationships observed in my study are in congruence with previous studies which indicate that peak metabolic rate is strongly influenced by wing morphology and wing kinematics which scale with body mass and the interindividual variation in all of these factors is connected (Darveau et al., 2005; Skandalis and Darveau, 2012; Byrne et al., 1988).

The scaling exponents for the traits tested in my study are shown in Table 5.4. In geometrically similar animals, maximum wingbeat frequency should scale with body mass with an exponent of -0.33 and a minimum of 0.167 Norberg (2006). Among bird species, Pennycuick (2010) modeled a scaling exponent similar to their minimum at 0.167. My value was between these at -0.23 with a standard error of ± 0.031, indicating in my study, wingbeat frequency scaled allometrically with body mass. This is contrary to Darveau et al. (2005) who found that wingbeat frequency scaled with body mass close to Norberg (2006) maximum predicted values, with a scaling exponent of -0.31 among orchid bee species and Skandalis and Darveau (2012) who reported wingbeat frequency scaling with body mass with an exponent of -0.33 in *Bombus impatiens*. Buschwald and Dudley (2010), however, reported a similar value to mine with an exponent of -0.20. Wing loading should have a scaling exponent of 0.33 with mass in geometrically similar animals (Norberg 2006), which is similar to what Buschwald and Dudley (2010) found among bumblebees, reporting a scaling exponent of 0.32. However, previous studies done interspecifically among orchid bee species have reported this scaling exponent to be much lower at only 0.14 (Dareveau et al., 2005). In my study, the scaling exponent for the relationship between wing loading and body mass was larger than in the other two studies, with wing loading scaling with body mass with an exponent of 0.51 and a standard error of ± 0.109 among *Bombus impatiens*. Wing area should scale with body mass with an exponent of 0.67 in geometrically similar animals (Norberg 2006). Among bumblebees in my study, it scaled with an exponent of 0.49 with as standard error of ± 0.109, however in other studies on bumblebees it was higher at 0.73 in Skandalis and Darveau (2012) and 0.68 in Buschwald and Dudley (2010), which is consistent with the predicted value. The scaling of peak flight metabolic rate in my study, with an exponent of 0.49, was closer to that found among orchid species by Darveau et al.

(2005) which was 0.68 than to what Skandalis and Darveau (2012) observed among *Bombus impatiens* which was 0.98. The Skandalis and Darveau (2012) results in bumblebees were much closer to isometry than both my results in bumblebees and the results of Darveau et al. (2005) in orchid bees. The differences between my observations and those by Skandalis and Darveau (2012) may be due to a smaller sample size (59 compared to 230). The differences between my study and theirs may also be due to differences in morphology between my bees and theirs, as wingbeat frequency and wing area also scaled differently with mass between their study and mine.

6.2.2 Vertical Force Production

Among the tested bees, VF was positively related to body mass, as the body mass of the bee increased the vertical force production of the bee also increased (See Figure 5.2). This relationship was isometric with a scaling exponent of 1.07 ± 0.111 standard error (See Table 5.4). Dillon and Dudley (2004) similarly concluded that vertical force scaled positively and isometrically with body mass among orchid bee species of varying sizes, with a scaling exponent of 0.97, as did Buchwald and Dudley (2010) among bumblebees with a scaling exponent of 1.09. However, contrastingly, when Dillon and Dudley (2004) expressed vertical force in body mass dependent units ($VF\ m^{-1}g^{-1}$, where m represents the mass of the bee and g represents gravitational acceleration) they found that it decreased linearly with body mass. My study found no significant relationship between body mass dependent vertical force and body mass. Dillon and Dudley (2004) and Buchwald and Dudley (2010) reasoned that the decrease in the maximum vertical force production with increasing size of their bees was due to the kinematic properties of the bee's wing movements with limitations on both stroke amplitude and wingbeat frequency at maximum load among orchid bee species and interindividually among bumble bees. Both studies found that wingbeat frequency and stroke amplitude at maximum load were limited and independent of size and for this reason, larger bees experience a decrease in mass dependent vertical force production capacity. One possible explanation for the discrepancy between these studies and mine is that the previous studies looked at larger bees than I did. Even in Buchwald and Dudley (2010), which looked only at *Bombus impatiens,* the sizes ranged from 109–372 mg, whereas my bees ranged from 121-298 mg despite having similar sample sizes (62 bees in their study and 59 bees in mine). It's possible that the smaller size range limited the restricting effects of maximum wingbeat frequency and stroke amplitude at peak load lifting capacity, resulting in no detectable relationship between mass-specific vertical force production and body mass in my study. Additionally, wing loading among the bees in my study scaled more steeply with body mass than it did for the bumblebees in Buchwald and Dudley (2010). Since previous studies have observed a high positive correlation between wing loading and maximum vertical force production (Dillon and Dudley 2004) it is possible that our diverging results are due to different variation in wing loading among the individuals studied. Overall, my study confirms the allometric relationship between vertical force production and body mass, but did not observe the predicted relationship between mass independent vertical force production and body mass among *Bombus impatiens.* This relationship between body mass and vertical force production is also similar to what was observed in foraging bumblebees by Spaethe and Wiedenmuller (2002) where larger bees were able to carry back more nectar per trip than smaller bees. The same study found that bees did not vary by body mass in any other foraging related traits including trip time or trip number and concluded that larger foragers contributed disproportionately more nectar to the colony. Taken together with the results of my study, and the previous study by Buchwald and Dudley (2010), it appears that increase in load lifting capacity with increasing body mass occurs among bumblebees and this may be affecting the foraging behaviour of the bees, causing larger bees to be more efficient nectar foragers.

6.2.3 Flight Speed

Maximum speed was positively related to average speed (see Figure 5.4), which was expected and has been observed in previous studies on insects (Dudley and Srygley, 1994). Maximum flight speed was also positively related to body mass (see figure 5.2). This is congruent with previous literature, finding a link between forward flight speed and body mass in flying animals (Alestram et al., 2007; Dudley and Srygley, 1994; Riskin et al., 2010; Ellington, 1991). In my study, a relationship between body mass and flight speed only occurred when the bee underwent its maximum forward acceleration (maximum flight speed) and no significant relationship was observed between mass and the average flight speed of the bees. A possible reasoning for this is that the predicted relationship between body mass and forward flight speed is derived from the relationship between body mass and power output (Ellington 1991), however flying animals such as birds (Tobalske et al., 2002) and honeybees (Nachtigall et al., 1995) display a u-shaped relationship between power and forward flight speed, with lower and higher flight speeds requiring more power output. It is possible that the average flight speeds recorded in this study reflect lower power requirements in the bees while the maximum flight speeds require the animal to output power closer to its maximum capacity and are therefore more reflective of its mass specific power output capacity.

It is predicted that for “fundamental aerodynamic reasons” flight speed will vary in a positive relationship with mass with an expected scaling exponent of 1/6 (0.167) in equivalent animals of different sizes (Alestram et al., 2007; Dudley and Srygley, 1994; Riskin et al., 2010; Ellington, 1991). With an exponent of 0.36 ± 0.171 standard error, the scaling of maximum speed and body mass in my study diverged from this predicted relationship. Other studies have also found deviation in scaling of flight speed with body mass from the predicted value. Alestram et al. (2007) measured free flight speeds in migrating birds using tracking radar and found a positive relationship between body mass and flight speed among bird species with a scaling exponent of 0.12. They noted that evolutionary restrictions and geometric differences between species such as variation in wing loading, maybe be responsible for the compression of flight speed ranges among species of varying mass. Another study by Dudley and Srygley, (1994) looked at scaling of flight speed among neotropical butterfly species and found that speed scaled with body mass with an exponent of 0.56. All three studies show different scaling of flight speed with body mass from that predicted as well as from each other.

Additionally, both my study and Alestram et al., (2007) found relatively low explanatory power of body mass on flight speed with variation in body mass only explaining 7.3% of variation in flight speed in my study and only 12% of variation in theirs. Various factors other than body mass have a significant effect on flight speed including taxonomy (Alestram et al, 2007; Dudley and Syrgley,1994), wing loading (Alestram et al, 2007; Dudley and Syrgley,1994; Norberg, 2006) and wingspan (Norberg, 2006) and Alestram et al. (2007) concluded that both taxonomy and wing loading had a much greater effect on average speed than body mass when measured interspecifically among birds. This explains why the scaling exponents can deviate so much from the predicted values, and may be why there is such variation in the values between the two studies and my own. However, no relationship between wing loading and maximum or average speed was observed among the bees in my study at the species level. Other factors may be involved which explain the low explanatory power of body mass on forward flight speed both interspecifically and intraspecifically.

Taken together, it appears that body mass does affect forward flight speed, however it alone does not have a great degree of explanatory power. Alestram et al. (2007) predicted that additional mechanisms besides body mass, such as differences in musculature or flight kinematics, were required to explain the variation in flight speed which observed. Indeed, in my study, both maximum and average flight speed were related to kinematic properties. Both were negatively related to wingbeat frequency, which explained 11.5% and 15.7% of variation in average and maximum flight speed respectively (see Figure 5.7). Maximum flight speed was also related to stroke amplitude, which explained 10.1% of variation (see Figure 5.5). However, since this relationship is no longer significant when wingbeat frequency is added as a covariate, it is likely due to the effect of wingbeat frequency being related to both maximum flight speed and stroke amplitude.

It appears that a variety of factors influence flight speed in animals with variation in morphology, ecology, size and wing movement all acting together. However, the negative relationship which I observed between wingbeat frequency and both maximum and average speed is opposite of what is expected (Ellington, 1991) as an increase in wingbeat frequency should be accompanied by an increase in power output for forward flight. Wingbeat frequency and maximum speed were both related to body mass, however since the relationship between these traits exists even when body mass is used as a covariate, it is not being influenced by body mass. Another possible explanation for the unexpected relationship is that wingbeat frequency was taken during hovering flight not during the tethered flight bout in which speed was recorded, however since wingbeat frequency is heavily influenced by morphology, it is reasonable to assume that individuals will fly with a characteristic wingbeat frequency related to their hovering flight wingbeat frequency (Skandalis and Darveau, 2012). Additionally, one study by Snelling et al. (2012) observed tethered flight wingbeat frequency in locust at similar frequencies to free flight values. However further investigation may be needed to determine if there is a relationship between tethered flight wingbeat frequency and hovering flight wingbeat frequency in insects such as bumblebees. Reasons for the contrary relationship between forward flight speed and wingbeat frequency may also be related to its close relationship and scaling with morphology and metabolism (Darveau et al., 2005; Skandalis et al., 2012) however none of the morphological characteristics other than body mass were related to flight speed in my study.

6.2.4 Flight Distance and Time

Flight distance was found to have a positive relationship with vertical force in a correlation analysis (see Figure 5.6). One possibility for the occurrence of these two traits together is that they are both good traits for foraging bees. If bees which forage do have superior flight performance of certain traits to bees which do not forage, having both a greater load lifting capacity and a greater dispersal capacity would be beneficial. Foragers would be able to travel further in search of resources and carry more per trip than non-foragers. The relationship, however, was not present when body mass was used as a covariate. Therefore, it is possible that, although there is no direct relationship between body mass and distance flown, body mass is influencing the relationship between these two flight performance traits. Additionally, Flight distance had a negative relationship with wingbeat frequency ($r^2 = 0.177$). However, because the relationship disappears when average speed and maximum speed are used as covariates, it is likely this relationship is a bi-product of the relationships between flight speed and wingbeat frequency.

Total flight time and maximum speed have a positive relationship (see Figure 5.4). This relationship is not a product of how maximum speed was calculated because total flight time was not used to calculate maximum speed. Maximum flight speed explained 24.6% of variation in flight time, even when mass was used as a covariate. One possible explanation for my observed relationship between flight time and speed could be on board fuel. Bees in my study were given free access to a sucrose solution in their colony boxes, however it was unknown how recently each bee had fed before beginning the trial. Bees with more on-board fuel may have been able to fly faster and further. It is also possible that individuals which fly for longer have characteristics which also allow them to fly faster. Maes et al. (2014) observed long-term diet affected both flight speed and flight time in lady bugs, implying that the condition of the animal might have an effect on both flight speed and flight time. However, in contrast, Blanken et al. (2015) found that exposure to pesticides affected only flight distance and time but not maximum flight speed in honeybees. This relationship was unexpected as previous studies in vertebrates have determined a trade-off between speed and endurance in muscles (Robbie et al., 2002) However the same study, and several other studies (Garland, 1988; Tsuji et al., 1989; Sorci et al., 1995) reported no trade-off between speed and endurance on the whole animal level. Additionally, Sorci et al. (1995) observed no negative genetic correlation between speed and endurance in lizards and concluded that since both were free to evolved independently of each other, natural selection could act to increase both. It is also possible that other factors such as morphology are affecting both flight speed and endurance. Fore example, low wing loading and pointed wings act to improve energy efficiency in migratory birds (Weber, 2006). Although no relationships were detected between wing loading or aspect ratio and maximum flight speed or total flight time, it is possible that other morphological aspects of the bees are affecting their flight efficiency, reflecting in a higher speed and longer flight time in certain body plans.

7. CONCLUSION

The aim of this experiment was to examine interindividual variation of flight in bumble bees by 1) determining if the variation in foraging effort among individuals observed in honeybees (Tenczar et al., 2014) existed among bumblebees and 2) to develop a greater understanding of how flight performance traits examined in the literature relate to each other in *Bombus impatiens.*

RFID tagged bees in the first experiment displayed unequal distribution of foraging effort with a similar proportion of foragers to that observed by Tenczar et al. (2014) which may possibly be explained by genetic variation among members of a colony creating graded responses to foraging pressure among individuals (Fewell and Bertram 1999). Additionally, foragers and non-foragers differed significantly only in their peak metabolic rate, which is possibly due to the effect of active foraging on the animals' physiology (Schippers and Dukas, 2010) but may also be due to the bees having repeatable (Skandalis et al., 2012) and heritable (Coelho and Mitton, 1988; Harrison et al., 1996; Nielsen et al., 1994) metabolic phenotypes.

In the second experiment, the relationships between flight performance traits were examined. Links between morphology, wing kinematics and peak metabolic rate previously uncovered in the literature (Darveau et al., 2005; Skandalis and Darveau, 2012) were observed in my analysis, although variation in scaling with body mass was detected. Additionally, maximum speed and vertical force production displayed positive relationships with body mass, as predicted by previous literature (Dillon and Dudley 2004; Alestram et al., 2007; Dudley and Srygley, 1994; Riskin et al., 2010; Ellington, 1991). Vertical force scaled isometrically with body mass but was not related to it when expressed as body mass dependent. Regarding forward flight speed, body mass does affect forward flight speed, however it alone does not have a great degree of explanatory power and other factors such as morphology and wing kinematics are likely to play a greater part in its determination. Maximum flight speed also had a significant relationship with flight time which may be due to individuals of greater quality being superior at both endurance and speed (Maes et al., 2014) however this is not observed in all cases. Blanken et al., (2015) for example, observed that exposure to insecticides reduced flight distance in bumblebees but had no affect on flight speed. Additionally, low wing loading and pointed wings act to improve energy efficiency in migratory birds (Weber, 2006) and although no relationships were detected between wing loading and maximum flight speed or total flight time in this study, it is possible that other morphological aspects of the bees such as wing shape are affecting their flight efficiency, reflecting in a higher speed and longer flight time in certain body plans.

This study provides useful information on how flight performance characteristics extensively examined in the literature are, and are not related in *Bombus impatiens.* The information will hopefully assist in research on or involving flight in flying animals by highlighting the simultaneously independent and interconnected nature of flight related characteristics which have been categorized as flight performance traits.

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