

FLIGHT PERFORMANCE OF BUMBLE BEES WITH WING WEAR

CLAUDIA A. HAAS

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Dedication

To the noble bumble bee.

Burly, dozing bumble-bee,
Where thou art is clime for me,
I will follow thee alone,
Thou animated torrid zone,
Zig-zag steerer, desert-cheerer,
Yellow-breeched philosopher,
Seeing only what is fair.
--Emerson

Abstract

This two-part study addressed the foraging flight performance of bumble bees (*Bombus* spp.) burdened with artificially induced wing wear between fireweed flowers (*Chamerion angustifolium*). The first part of the study examined the effects of wing wear and inter-flower distance on travel time. The second part of the study addressed the effect of mean wing clipping and wing asymmetry on flight biomechanics (flight distance, velocity, acceleration, and deceleration) and flight path (displacement from a bee-line). Bees with wing wear flew faster between flowers spaced more sparsely, possibly compromising accuracy in choosing rewarding flowers. Flight biomechanics were relatively unchanged by wing wear. Bees with low wing loss and little asymmetry increased slightly in acceleration and deceleration. Bees with high mean wing loss and high asymmetry flew less directly between flowers. Asymmetry had the largest effect on flight path when in conjunction with high mean wing loss. Bees with high wing loss and high asymmetry flew further and higher between flowers compared to control bees. When the high mean wing loss was symmetrical, bees flew as far and as high as control bees. The results of these studies suggest the relative resilience of bumble bees to induced wing wear, with little change in flight performance.

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DEDICATION.....	III
ABSTRACT	IV
ACKNOWLEDGMENTS	V
CHAPTER 1: GENERAL INTRODUCTION.....	9
WHAT IS WING WEAR?.....	9
PHYSIOLOGICAL AND BEHAVIOURAL CONSEQUENCES OF WING WEAR.....	10
ASYMMETRY IN WING WEAR	11
HOW INSECTS FLY AND HOW WING WEAR MIGHT MATTER	12
BASIC AERODYNAMIC PRINCIPLES	14
THIS THESIS	17
LITERATURE CITED	19
CHAPTER 2: TRAVEL TIMES OF BUMBLE BEES WITH ARTIFICIALLY INDUCED WING WEAR: THE INFLUENCE OF INTER-FLOWER DISTANCE.....	23
ABSTRACT.....	23
INTRODUCTION	25
METHODS.....	27
RESULTS.....	31
DISCUSSION.....	34
LITERATURE CITED	38
CHAPTER 3: FLIGHT PERFORMANCE OF BUMBLE BEES WITH ARTIFICIALLY INDUCED WING WEAR: THE INFLUENCE OF ASYMMETRY AND MEAN WING LOSS.....	41
ABSTRACT.....	41
INTRODUCTION	42
METHODS.....	44
RESULTS.....	48
DISCUSSION.....	56
LITERATURE CITED	59
CHAPTER 4: GENERAL DISCUSSION.....	61
LITERATURE CITED	64

TABLE 1: Mixed-model ANCOVA explaining travel times (ln-transformed) of experimental bumble bees (*Bombus* spp.) by wing treatment and distance between flowers. See methods for model details. The model error DF=5296.....**32**

TABLE 2: Mean (\pm SE) of the biomechanical and flight path variables calculated for worker bees (*Bombus flavifrons*) flying between fireweed (*Chamerion angustifolium*) flowers. To control for the large effect of flower distance, values include only the flights between 30 cm flower distances. Values in each row are based on 3 bees. “Maximum displacement” is a measure from the bee-line between flowers.**49**

TABLE 3: MANCOVA explaining flight distance, mean velocity, variability of velocity, maximum acceleration, maximum deceleration (all ln-transformed) and proportion of time spent in acceleration for 5 treatments (combinations of 2 levels of MWL and asymmetry and control) of bees (*Bombus flavifrons*). Overall model Pillai’s Trace $F_{126, 2304}=2.49$, $p<0.001$. See Methods for model details. The F-values for Treatment, Treatment*Time and Bee [Treatment] are Pillai’s Trace.....**50**

FIGURE 1: Power curve of bumble bee flight over various flight speeds from Cooper (1993). Symbols represent different individual bees.....16

FIGURE 2: Effect of wing clipping on the travel time of experimental bumble bees (*Bombus* spp.) at three flower distances. Standard errors are present but too small to be visible. Letters show results of contrasts using sequential Bonferroni within each flower distance.....33

FIGURE 3: Multivariate differences among means of the 5 wing treatment groups, represented in the first two canonical variables (CVs) of the MANCOVA (Table 3). Ellipses represent 95% confidence intervals for each mean. Letters represent the results of post-hoc contrasts of means in 6-dimensional space (for the six biomechanical variables). Contrasts between control and wing treatments use a p value of 0.05. A sequential Bonferroni correction was applied to 6 unplanned contrasts (between wing treatments). The first two canonical variates explain 60% of the correlation among wing treatment groups. The table on the right shows correlation coefficients of each CV with each biomechanical variable. Important correlations are bolded.....51

FIGURE 4: Total displacement (absolute value) along flight paths of bumble bees (*Bombus flavifrons*) flying 30 cm between fireweed flowers (*Chamerion angustifolium*) with varying amounts of MWL and wing asymmetry. Lines show 6th degree polynomial regression fits with 95% confidence intervals based on n=3 bees. Letters above peaks represent the result of a post-hoc analysis. See Table 2 for peak values.....55

Chapter 1: General Introduction

What is Wing Wear?

All flying animals can suffer from loss in wing area. It can be from general wear and tear of the wings or feathers, or from a purposeful loss in wing area during an annual moult (moult in birds reviewed in Jenni and Winkler 1994). Insects, unlike birds, do not have an opportunity to renew their flight apparatus and suffer from wing wear until their eventual death. An insect's wing can wear down with time, either by mechanical damage or photochemical reactions from prolonged sun exposure (as reviewed in Dudley 2000). Mechanical damage can result from severe weather, such as wind, that can cause an insect to fly off course and into solid objects. Failed predation attempts can also result in wing wear (as reviewed in Vermeij 1982 and 1987); for a pictorial example of failed predator attempts of birds on butterflies see Carpenter (1937). Other causes of wing wear include mating attempts (Ragland and Sohal 1973) and territorial disputes between males (Batra 1994).

Since wing damage is irreversible, older insects tend to suffer from higher accumulated wing wear (in flies - Ragland and Sohal 1973, Allsopp 1985, Burkhard et al. 2002; in bees - Mueller and Wolf-Mueller 1993, Visscher and Dukas 1997, Eltz et al. 1999). Older bees also have an increased mortality rate, linking it with senescence (Rodd et. al. 1980). Wing wear has been linked to senescence, with older bees having higher rates of accumulation of wear (Alcock 1996, Tofilski 2000, Higginson and Barnard 2004). The reason for the increased rate of mortality could be wing wear itself, possibly by increasing an individual's susceptibility to predator attacks or making them more clumsy fliers resulting in more collisions with obstacles. A decrease in wing area has been directly linked to decreased

survivorship of female flycatchers (*Ficedula hypoleuca*) with their wings clipped (Slagsvold and Dale 1996) and to an increased mortality in bumble bees (Cartar 1992).

Physiological and Behavioural Consequences of Wing Wear

The overall result of wing wear is a decreased wing area, a jagged forewing margin and increased load on the wings. A decrease in wing area results in less air moved per wing stroke, which decreases the lift generation capacity of the wings (Hargrove 1975). The capacity of a wing to create lift could be more related to wing length rather than to specific wing area (Chai 1997), but both area and length are expected to decrease with wing wear. Along with a decrease in lift, the less streamlined wings are the higher the drag force acting on the wing. A decrease in wing area will also increase the wing loading, these now smaller wings have to carry the same weight of the insect as before wear. Together these factors could make flying more costly, either by increasing energy expenditure for flight or decreasing manoeuvrability.

Insects have ways of compensating for aerodynamic burdens caused by non-optimal wings, for example, by changing their wing kinematics, including wing stroke angle, wingbeat frequency and amplitude. In one study, the wings of Western white butterflies (*Pontia occidentalis*) were clipped by 15-20% and individuals compensated by increasing wingbeat frequency by about 40% (Kingsolver 1999). A same increase in wingbeat frequency was observed when wings were clipped for other organisms as well (in tsetse flies - Hargrove 1975; in stink bugs - Gopalakrishna et al. 1983; in bumble bees - Hedenström *et al.* 2001; in zebra finches - Hambly et al. 2004). However, changes to wingbeat amplitude seem to be less desirable; bumble bees with their wings clipped did not increase their wingbeat

amplitude (Hedenström *et al.* 2001). In a similar wing loading experiment using changes in body mass instead of wing area, no significant increase in wingbeat amplitude was found (Cooper 1993). However, changing wingbeat kinematics could be costly. Increasing wingbeat frequency is expensive because energy metabolism during flight is closely related to wingbeat frequency (Casey and Ellington 1989).

Another strategy for insects coping with wing wear is to change flight behaviour.

According to Tofilski (2000), older honey bees took a longer time foraging, decreased the number of flowers visited per foraging bout, and took longer to fly between the flower patch and their colony than younger honey bees. Honey bees with experimentally induced wing wear accepted different inflorescences than their unclipped counterparts (Higginson and Barnard 2004). Baltra (1994) observed that territorial male solitary bees flew less often, were less aggressive, and flew slower as they accumulated wing wear. Reductions in wing area can also decrease flight speed (moulting birds - Chai and Dudley 1999, Swaddle and Witter 1998; locusts - Fischer and Kutsch 2000). However, flight speed is correlated with accuracy in choosing rewarding flowers in bumble bees (Chittka *et al.* 2003) since bees spend time assessing floral rewards by hovering in front of a flower without landing (Marden 1984). These compensatory behaviours could help a wing-worn insect adjust to their wings wear, however each at their own costs.

Asymmetry in Wing Wear

In addition to the factors mentioned above, it is important to note that wear on individual wings may differ. Processes that cause wing wear, such as failed predator attacks or collisions with solid objects, can affect one wing more than the other resulting in

asymmetric wing areas (see e.g. Carpenter 1937). Environmental stresses, such as temperature and organo-pesticides, may increase the level of wing asymmetry (Mpho et al. 2001). Mueller and Wolf-Mueller (1993) found that in nearly 5% of solitary Wool-Carder bees (*Anthidium manicatum*) wing wear was asymmetrical. For butterflies, natural levels of asymmetry were approximately 1% of various wing morphologies including wing area (Windig and Nylin 1999).

Asymmetry between wings will create more lift by one wing than the other, resulting in a rolling motion that must be compensated for with each wing stroke (Thomas 1993). Midges (*Chironomus plumosus* L.) with experimentally induced wing asymmetry flew less, decreased wingbeat frequency, and increased wingbeat amplitude (McLauchlan 1997). Birds with asymmetric wings were more clumsy fliers (Swaddle et al. 1996, Swaddle and Witter 1998) and had a higher rate of mortality (Brown and Brown 1988, Brommer et al. 2003). House flies (*Musca domestica*) with asymmetric wings had a decreased ability to avoid predators, a higher rate of infection by a deadly fungus, and a decreased mating success (Møller 1996). However, small amounts of asymmetry may be beneficial. Male butterflies were more manoeuvrable being able to turn faster while defending their territory (Windig and Nylin 1999) and finches with symmetrical wing cuts had higher flight costs than finches with equivalent asymmetrical cuts (Hambly et al. 2004).

How Insects Fly and How Wing Wear Might Matter

Bumble bees and other small insects create lift in an interesting way that is not yet fully understood. Unlike most birds and large insects such as butterflies and moths who keep

aloft by flapping their wings up and down, the wingtips of bumble bees follows an oval path that is at a sharp angle from their body. Much of the lift is generated by a rotational motion of the wing in that the wing is right side up during the downstroke, but flips over during the upstroke. This results in opposing flows of air around the wing which creates much of the uplift on the wing. Also important in flight in insects is a process known as delayed-stall. Due to the sharp angle of the wing air moves faster under the wing than over the wing resulting in vortices being created above and below the outer margin. These vortices create large amounts of lift, but are short lived and soon move off the wing. However before the bee would loose lift from a departure of the vortices, due to a high wingbeat frequency, new vortices have already been created in the following wingstroke.

However, delayed stall and the rotational motion of the wing do not explain all the lift an insect creates, leaving scientists trying to determine what other processes are working during bumble bee flight. A large robotic fly wing created to “fly” in a viscous liquid allowed for scientists to create an equivalent environment experienced by minute flies and other insects. This research has lead to the theory that vortices created on subsequent wingstrokes work together to create even more lift. This phenomenon is known as wake-capture. For a review of current studies on the aerodynamics of insect flight refer to Dickinson (2001) and Dudley (2000).

Wing wear is expected to influence the natural creation of vortices, but how has not been tested empirically. It is expected that smaller wings will move less air and create smaller vortices, thus creating less lift per wingstroke. Also, a jagged wing margin could influence the creation of the vortices, perhaps disrupting them along the wing. However, a detailed look at how within-individual changes in wing area changes flight mechanics has not been

systematically investigated, and would be of interest to fully understand how flying insects deal with the deterioration of their wings as they age.

A review of wing kinematics and morphological parameters, lift and power generation of forward flight of bumble bees with pristine wings is provided by Dudley and Ellington (1990a and b).

Basic Aerodynamic Principles

A variable traditionally used to compare flight ability across species is wing loading (Nm^{-2}), a measurement that describes the effect of the ratio of body weight to wing area (Norberg 1990). For bird species, Pennycuik (1989) described the calculation of wing area to include the area of the two wings and the area of the body between the two wings. This has not been the case for insects, where only the actual forewing and hindwing area are measured (e.g. Windig and Nylin 1999).

Two other important variables in explaining flight are lift (L) and drag (D) that the wings and body create during flight. L and D are related to individual lift and drag coefficients (C_L and C_D respectively):

$$L=1/2\rho SV^2C_L$$

and

$$D=1/2\rho SV^2C_D$$

where ρ is the density of air, V is speed and S is the total wing area (insects: reviewed in Dudley 2000; birds: reviewed in Rayner and Swaddle 2000). The lift and drag coefficients are related to the wing's angle of attack, the angle of the wing relative to horizontal (Norberg 1990). Using these equations it becomes evident that a decrease in wing area will decrease the lift and drag on an individual. However, suboptimal wing shape can also influence drag. Lift and drag are important components in the calculation of the power created by the wings that enables the individual to fly. A power curve is conventionally used to explain how a flying organism's power requirements change over various flight speeds. The following figure shows an example of a experimentally derived power curve found for bumble bees in Cooper 1993.

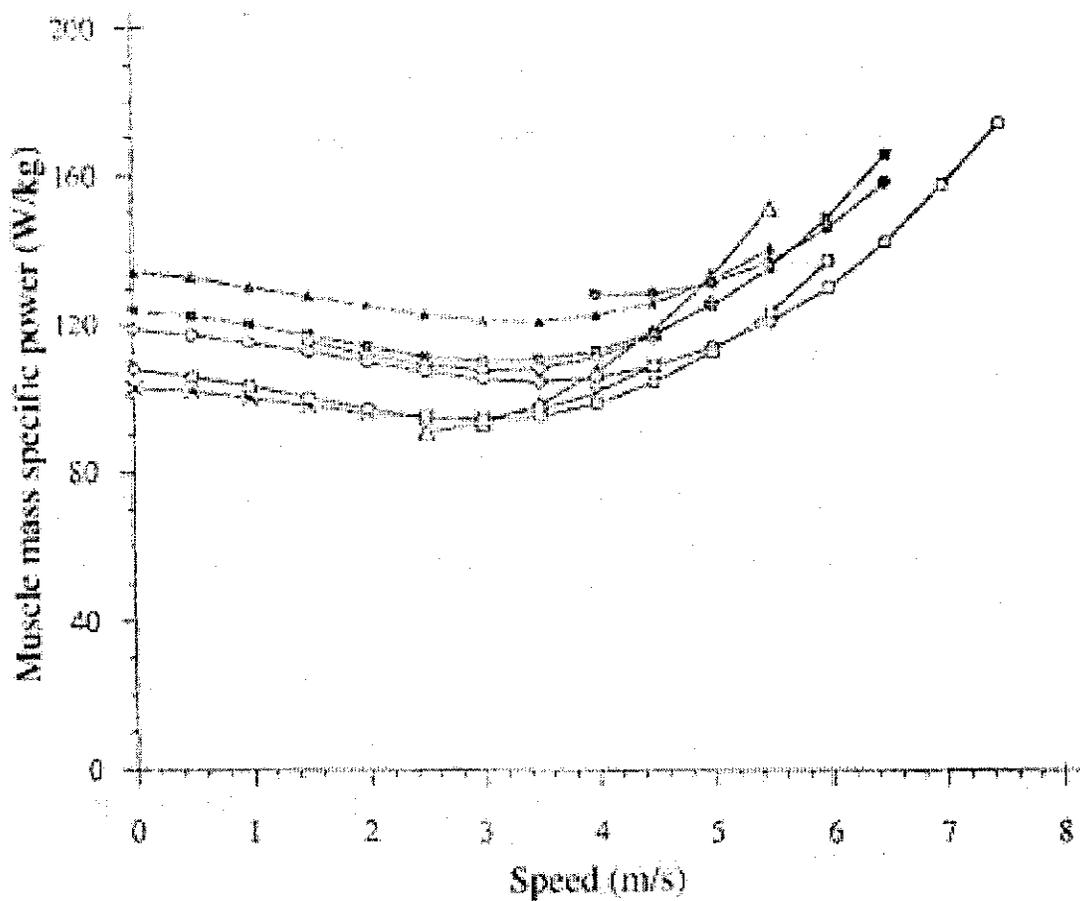


Figure 1: Power curve of bumble bee flight over various flight speeds from Cooper (1993). Symbols represent different individual bees.

However, the conventional calculation of lift and drag does not take into account differences in area between the two wings since total wing area encompasses both wing area and asymmetry (see e.g. Dudley and Ellington 1990a, Cooper 1993). Asymmetry has been addressed in birds and the increased costs of wing asymmetry have been quantified (Thomas 1993), but this has not been done in insects.

This Thesis

As organisms get older, their bodies change and begin to wear down and they must learn to deal with these changes in order to survive. All flying organisms suffer from loss in wing area at some time in their lives, and compensating for this loss in area is vital to survival. This is specifically true for insects whose wings are not renewable. Natural selection is acting on these organisms to balance out strength of their wings and usefulness of the individual. In many species this usefulness is related to reproductive output, but in foraging worker bumble bees who do not reproduce, their usefulness is in the amount of energy (nectar) they can return to their reproductive colony. Evolutionarily speaking, the bee must balance out energy put into making their wings stronger and the resulting energy returns for their colony. One must assume after millions of years of natural selection that the strength of the wings and compensatory effects to wing wear have been maximized to ensure the maximal energy return of the individual to the reproductive colony. So the question is, do bees compensate for their loss of wing area and risk other costs (e.g. energetic costs, foraging accuracy costs, survival costs) to maintain foraging efficiency or do wing worn bees forage less efficiently to ensure a longer survival or is it a combination of both options.

Wing wear is suspected to be associated with some costs, as is evident from a decreased survivorship rate for bees with higher wing area loss (Cartar 1992). Energetic costs of wing wear have not been detected (Hedenström et al. 2001), so perhaps the important costs are behavioural and not physiological. To address these behavioural effects of wing wear on flight performance, bumble bee workers (*Bombus* spp.) were followed flying between

fireweed flowers (*Chamerion angustifolium*). Bumble bee workers were used because they depend on flight and the lift generation capacity of their wings on a daily basis. Bees require their wings to forage on flowers, whether it be flight from the colony to the flower patch or flight between flowers. Some flowers allow bees to walk between inflorescences, but even so flight comprises much of a bumble bees foraging bout. Foraging worker bees are also relatively easy to train to forage on a set-up patch of flowers, and since they do not generally reproduce (some exceptions such as when the Queen has an early demise to exist), their only goal is to forage. It is expected that wing wear will decrease flight performance however it is unclear how exactly the bees will adjust.

The first study in this thesis addresses the effect of inter-flower distance on flight behaviour. Flower density could have an effect on bees with varying amount of mean wing loss. Higher flower densities require more manoeuvrability to negotiate between flowers and lower flower densities allow for more time for acceleration and deceleration along the flight. Flight performance was measured as travel time between the flowers, which potentially is a combination of both flight distance and flight speed. The second study was complimentary to the first study in that it expanded the flight performance variables into flight biomechanics (flight distance, velocity, acceleration and deceleration) and flight path (total displacement) at one of the previously used flower distances. Performance is expected to decrease, however how performance decreases is unknown. Wing wear was also expanded into two levels of wear, mean wing loss and wing asymmetry. Wing asymmetry is expected to add a new level of difficulty for a bee already trying to cope with losses in wing area.

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Chapter 2:

Travel times of bumble bees with artificially induced wing wear: the influence of inter-flower distance

Abstract

As flying insects age, their wings wear. Wing wear has been linked with decreased survivorship and flight performance in a number of flying insects, and moulting birds. Previous research has discounted the importance of energetic costs associated with wing wear in bumble bees. This study considers the time costs of foraging with wing wear. I relate experimentally induced wing wear to travel time (the time taken to fly between adjacent flowers) in foraging bumble bees (*Bombus* spp.) moving between flowers of fireweed (*Chamerion angustifolium*) set out at three inter-flower distances (10, 30, 50 cm). Bees were observed flying with their original wings, and then following two successive reductions in their wing area. Bees flying between close flowers (10 cm), slightly decreased their travel time under maximum wing loss. However, bees decreased travel time after wing reduction when moving between flowers spaced at 30 cm and 50 cm. Following maximum wing loss, bees were more likely to fall off flowers during take-off or landing. It is unclear why bees with reduced areas decreased travel time when moving between flowers spaced at intermediate (30 cm) and far (50-70 cm) distances. However, bees with smaller wing areas were clumsier, making behaviours such as landing and taking-off more difficult. Based on these effects of wing area on flight behaviour, it would seem that biomechanics, not physiology, might be the mechanism that links wing wear to fitness in foraging flying bumble bees.

Key Words: aging, asymmetry, biomechanics, *Bombus*, Bumble bees, flight performance, insect flight, inter-flower distance, travel time, wing wear.

Introduction

Flight is a relatively large component of a bumble bee's (genus *Bombus*) foraging trip, averaging 30% of the total foraging time spent flying, but varying widely depending on the flower species being visited (Heinrich 1973, Pleasants 1981, Cartar 1991). Not only is flying more energetically expensive than walking (reviewed in Heinrich 1979), it results in physical wear and tear of the wings, which itself can have detrimental effects on an individual's survival and foraging success.

Wing wear is related to age in a number of foraging insects (Ragland and Sohal 1973, Allsopp 1985, Mueller and Wolf-Mueller 1993, Visscher and Dukas 1997, Eltz et al. 1999, Burkhard et al. 2002). Wing wear accumulates as an individual gets older (Alcock 1996, Tofilski 2000, Higginson and Barnard 2004). In honey bees (*Apis mellifera*), foraging activity is negatively related with survival, but this result is not specifically related to the wear of the wings (Schmid-Hempel and Wolf 1988). Bumble bees do show evidence of senescence, with the older bees having a higher mortality rate than younger bees (Rodd et al. 1980). One study directly examined the effect of wing wear on mortality in bumble bees and found such a relationship in both natural and experimentally induced wing wear (Cartar 1992).

The more worn a wing becomes, the smaller its total surface area. This decreases lift generation per stroke (Hargrove 1975) and increases wing loading. To compensate for loss in wing area, a flying insect must alter its flight behaviour: by increasing the frequency or amplitude of its wingbeats. Other insects also increase their wingbeat frequency when their wings are clipped (tsetse flies: Hargrove 1975, stink bugs: Gopalakrishna et al. 1983,

butterflies: Kingsolver 1999). Bumble bees loaded to twice their initial weight had higher wingbeat frequencies than unloaded bees (Cooper 1993). However, increasing wingbeat frequency might be costly. Energy metabolism during flight is closely related to the wingbeat frequency, because mechanical power created by the muscle is related to wingbeat frequency (Casey and Ellington 1989). Wingbeat frequency and amplitude affect the power required to fly at different forward flight times. However, despite the above, bumble bees with 10% of their wing area removed increased their wingbeat frequency, but did not show an increase in energy costs (Hedenström *et al.* 2001).

Even though energetic costs of wing wear have not been detected, the important costs might be behavioural, not physiological. Male solitary bees with wing wear flew slower, flew less often, were less aggressive during territory defence, needed to thermo-regulate longer in the sun and in some cases were susceptible to breaking their wing veins and becoming flightless (Baltra 1994). Flycatchers (*Ficedula hypoleuca*) and starlings (*Sturnus vulgaris*) with a decreased wing area from moult had poorer take-off ability and manoeuvrability (Slagsvold and Dale 1996, Swaddle *et al.* 1996, Swaddle and Witter 1997).

Biomechanical changes resulting from wing wear can be expected to be particularly pronounced for foraging bumble bees, who spend much of their day flying short distances between flowers arranged in situations of high spatial complexity. Bees with wing wear could change their flight performance as a response or as a consequence of wing wear. One variable that could change is travel time between flowers. A loss in wing area may affect flight speeds (as seen in locusts Fischer and Kutsch 2000), in addition to success in executing intricate behaviours such as taking off from and landing on a flower. Another variable that might affect bees with worn wings differently is flower distance. Flowers that

are closely spaced have less time and space for acceleration and deceleration (and a greater proportion of the flight spent in acceleration/deceleration). Bees hindered with wing wear might not be able to manoeuvre efficiently around closer spaced flowers, and could take longer to forage between closer spaced flowers than bees with pristine wings.

This study addresses the effect of wing wear on the flight performance of bumble bee workers (*Bombus* spp.) flying between flowers arranged at different distances. Individual bees had their wings experimentally reduced, and their travel times while foraging on arrays of flowers were analysed in a repeated-measures design. Travel time of the foraging bee, which is related to foraging gain, should also reflect potential impacts of the loss of wing area. A wing worn bees' ability to forage at different flower densities was also determined. The bees' ability to take-off and land successfully was also noted as a measure of the manoeuvrability. I predict that wing wear will hinder bee flight, decrease in flight performance by increasing inter-flower travel time. Shorter inter-flower distance could also be more difficult for wing worn bees because they require more intricate manoeuvrability.

Methods

Wild bumble bee colonies (2 *Bombus bifarius*, 1 *B. flavifrons*, 1 *B. frigidus*) were obtained from nest boxes set up in west Bragg Creek, Alberta Canada (50° 57'N, 114° 34'W, elevation=1400 m). Colonies were moved to the R.B. Miller Kananaskis Field Station (50° 39'N, 114° 39'W, elevation=1500m). Individual worker bees were marked with two dots of coloured paint on their thorax, and their wings photographed at a distance of 2.5cm with a Nikon Coolpix 990 camera set in macro mode. Experiments were run in July and August

2003, with a mean air temperature (\pm SD) in the shade of 24°C (\pm 4°C). Experimental and control bees were run throughout the summer, and varied in age.

Bees were allowed to forage in a 4 m by 4 m by 2 m screen tent on arrays of individual fireweed (*Chamerion angustifolium*) flowers. The use of this foraging arena controlled other factors that could influence flight, such as strong winds, predators and obstacles between flowers. The array of flowers was arranged on a 1.5m by 1.5m piece of plywood placed 50 cm above the ground in the centre of the tent. Each flower was placed in a hole in the lid of a 4 cm tall by 2 cm wide plastic vial containing water, so that the flower was oriented horizontally. The colony was placed at one end of the flower array approximately 50 cm from the array edge. The observer remained beside the colony, allowing a single bee to exit the colony. Each trial was filmed using a hi-8 video camera located approximately 75 cm from the flower grid opposite the colony on a 1.25 m high tripod. The camera was angled downward towards the grid, which was only 50 cm off the ground.

Arrays of three inter-flower distances were used: 10 cm (total of 25 flowers), 30 cm (total of 16 flowers) and 50 cm (total of 9 flowers). While adjacent flowers were of a prescribed distance, the bee would also fly diagonally between the flowers, and on occasion even bypass the closest flowers. Only the flights at the prescribed flower distance were used, except for the 50 cm flower distance, where all flights were used to compensate for the low sample size at this distance (5% were of greater than 50 cm distance). More flight observations were provided from arrays with more flowers, such that 50% were 10 cm, 31% were 30 cm, and 14% were 50 cm (n= 5595 flights). For a given bee, three trials (single foraging trips) were run for each inter-flower distance, with the order of flower distances experienced randomly.

Following the initial learning trials, the bee's wings were clipped, whereby small amounts (estimated at 10% and 20% of the total wing area) of the posterior forewing margin were removed using fine scissors. Wings were then photographed for quantification of wing removal. Wing area removed from each wing was calculated by counting pixels using NIH Image V1.63 software (U.S. National Institutes of Health). This allowed for calculation of mean wing area removed and wing asymmetry (the difference in wing area between the wings). Average (\pm SD) mean wing loss for the first cut was 12.2% (\pm 4.5%) and 21.5% (\pm 5.7%) for the first and second cut respectively.

Bees then foraged in the flower arrangements as before, with three trials per flower distance. The process was then repeated with a second wing clipping. Since wing wear is not reversible, the three wing treatments were run in the same order. To control for the effect of age and/or experience, five bees (2 *B. bifarius*, 2 *B. flavifrons* and 1 *B. frigidus*) were handled the same way as the experimental bees, but without wing trimming. Fifteen experimental bees were analyzed, 5 *B. bifarius* (from 2 colonies), 7 *B. flavifrons* (from 1 colony) and 3 *B. frigidus* (from 1 colony).

Handling time per flower and time spent flying between flowers were measured from videotapes (to a resolution of 1/30s). The transition between handling and flying time was determined as the first sign of movement away from the flower at take-off, and flight ended when the bee changed its body orientation from semi-vertical to horizontal at landing. Travel time was calculated by dividing flying time by the distance between the flowers.

To control for the unbalanced design, the travel times of control and experimental bees were

analysed in separate mixed-model ANCOVAs. The terms in the ANCOVA of experimental bees were wing treatment (no cut, 1st cut, 2nd cut), distance between flowers (close, intermediate, far), species (*B. bifarius*, *frigidus*, *flavifrons*), colony number (A, B, C, D; nested within bee species), individual (random effect, nested within species and colony), air temperature in the shade (covariate), trial number (1, 2, 3), flower visit within each trial (covariate), whether the flower had been visited earlier in the trial (yes, no) and whether the flower was at the edge or in the middle of the grid (yes, no). The analysis of control bees was similar, except for it lacked the “wing treatment” term, which was replaced with an equivalent “order” term. A t-test with sequential Bonferroni correction was used to test differences between terms.

Due to the rarity of flight errors (problems with take-off or landing), a full nominal logistic model could not be fitted and instead a contingency analyses were performed on frequency of flight errors across the wing treatments, done separately for control and experimental bees. Berferonni’s adjustment was applied to experimental bees to determine significance of pairwise contrasts (i.e., $\alpha=0.05/2=0.025$).

Variables were transformed when necessary to ensure normality and homogeneity of the residuals, and the ANCOVA assumption of homogeneous slopes was verified. All statistical analyses were performed using JMP V5.0 (SAS Institute Inc. 2002).

Results

The travel times of control bees (ANCOVA, overall model $F_{18, 1799}=116.86$, $p<0.001$, $R^2=0.56$), travel times transformed by $\ln(\text{travel time})$, did not show a significant seasonal effect (ANCOVA, $F_{2,1632}=1.78$, $p=0.169$). Bees decreased travel time at larger between-flower distances (ANCOVA, $F_{2,1632}=664.92$, $p<0.001$), but there was no interaction between season and flower distance ($F_{4,1632}=1.98$, $p=0.095$). Bees decreased their flight time within a trial ($F_{1,1632}=21.83$, $p<0.0001$).

As with control bees, experimental bees (ANCOVA, overall model $F_{28, 5296}= 232.63$, $p<0.001$, $R^2= 0.55$) decreased travel time at large flower distances (Figure 2). However, how much they decreased travel time depended on wing treatment (wing treatment by distance interaction, Table 1). At each flower distance there is a significant effect of wing treatment (test on “slices” of the 3-way interaction: 10 cm, $F_{2, 5296}=9.38$, $p<0.001$; 30 cm, $F_{2, 5296}=19.78$, $p<0.001$; 50 cm, $F_{2, 5296}=4.90$, $p=0.008$, Figure 2). When the flowers were 10 cm apart, experimental bees after the second cut increased their travel time (≈ 0.735 s), while bees after only one cut flew the same as bees prior to wing clipping (≈ 0.774 s). At 30 cm flower distances, bees prior to cut flew about ≈ 1.343 s between flowers. Wing clipping decreased travel times to ≈ 1.272 s after the first cut and ≈ 1.167 s after the second cut. This decrease in travel time is also observed when flowers were more than 50 cm apart, however both the first and second wing clipping flew at the same time (≈ 1.641 s) while bees prior to cut flew ≈ 1.772 s.

Table 1: Mixed-model ANCOVA explaining travel times (ln-transformed) of experimental bumble bees (*Bombus* spp.) by wing treatment and distance between flowers. See methods for model details. The model error DF=5296.

	DF	Mean Square	F	P
Wing Treatment	2	1.16	10.09	<0.0001
Flower Distance	2	227.70	1985.99	<0.0001
Wing Treatment * Flower Distance	4	1.72	14.97	<0.0001
Temperature (covariate)	1	0.71	6.16	0.0131
Trial Number	2	1.59	13.89	<0.0001
ln (Flower Number) (covariate)	1	9.00	78.50	<0.0001
Revisit?	1	1.88	16.37	0.0001
Edge or Middle Flower	1	3.75	32.72	<0.0001
Species	2	7.54	3.18	0.0807
Colony [Species]	1	0.12	0.04	0.8470
Bee [Colony [Species]] (Random)	11	2.97	25.91	<0.0001

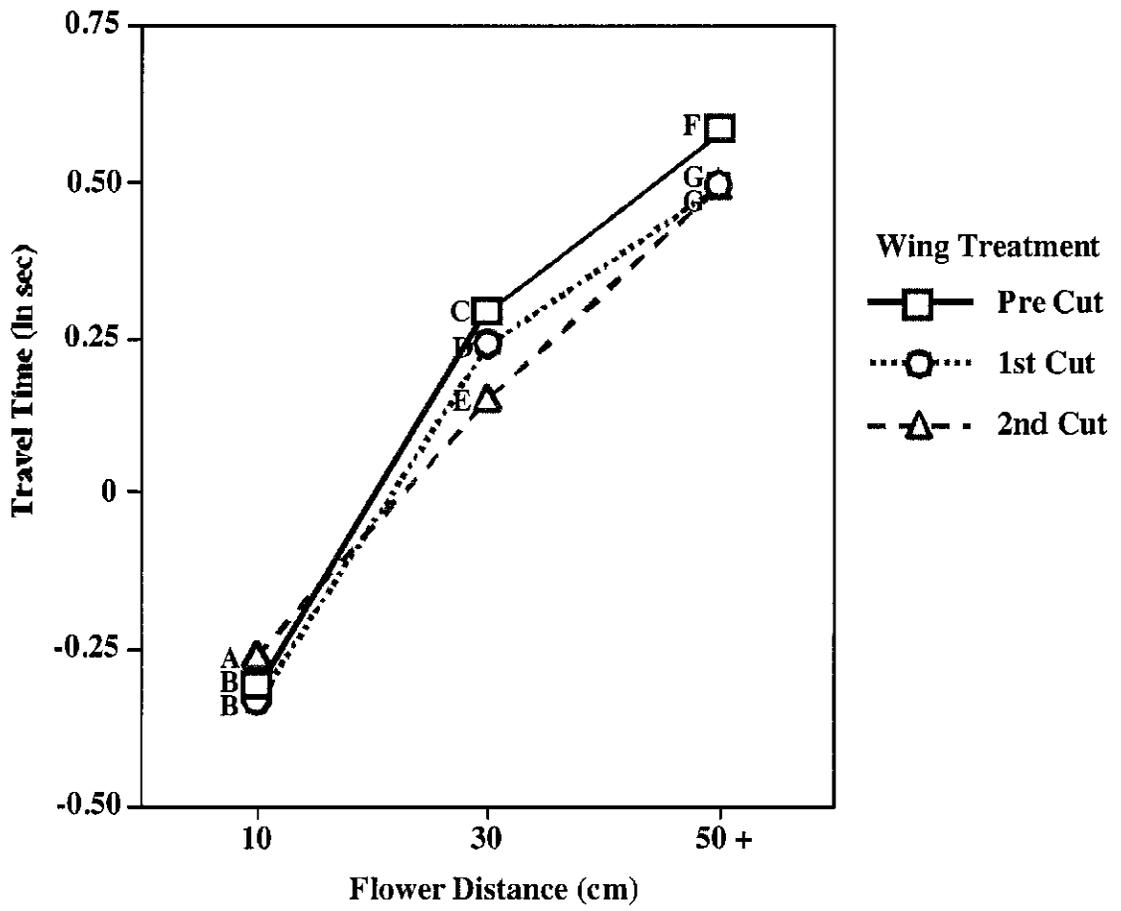


Figure 2: Effect of wing clipping on the travel time of experimental bumble bees (*Bombus* spp.) at three flower distances. Standard errors are present but too small to be visible. Letters show results of contrasts using sequential Bonferroni within each flower distance.

Of the 36 flight errors by bees with their wings clipped, 2 occurred prior to wing clipping, 6 occurred following to the first wing clip and 28 occurred following the second wing clip. Proportionally more flight errors occurred following the second wing cut ($\chi^2_1=41.64$, $p<0.0001$). There was no difference between the number of flight errors between the pre-cut and the 1st wing clipping ($\chi^2_1=1.22$, $p=0.27$). Control bees also had more flight errors later in the trials, with 1 in the first set of trials, none in the second set of trials and 4 in the final set of trials. More flights occurred later in the experiment (1st vs. 2nd vs. 3rd: $\chi^2_1=6.47$, $p=0.0393$; 1st vs. 2nd: $\chi^2_1=1.06$, $p=0.30$).

Discussion

Overall, removing 10 to 20% of a bumble bee's wings resulted in only moderate changes in flight behaviour. Changes to travel time were minimal and, manoeuvrability around this flower set-up was mostly maintained. This suggests that the biomechanical changes from wing wear, as detected in this simple and controlled setup, were minor. However, bees were not challenged by spatial complexity, obstacles, extreme weather, parasites, predators (e.g. crab spiders), or other challenges to flight normally encountered by wild-foraging bumble bees. Regardless, wing wear still influenced flight behaviour in some consistent ways.

When flowers were densely spaced (10 cm) bees with on average 20% of their whole wing area cut slightly increased the time they flew between flowers. A bee may need a certain minimum flying distance to accelerate and decrease its travel time, and also need enough time to be able to decelerate when it approaches the next flower. This could be particularly true for bees already burdened with a decrease in lift generation per wingstroke. At denser

flower arrays, high accelerating across such short distances is perhaps not possible for bees with wing wear. Also, a bee with high wing wear could be less manoeuvrable and could have more difficulty with precise flight patterns, such as taking off and landing on flowers that are closely spaced.

Bees with worn wings decreased the time they spent flying between flowers at intermediate (30 cm) to less dense (>50 cm) flower arrangements. Decreasing travel time between flowers should have the advantage of increasing foraging gain, allowing the bee to visit more flowers per unit time. However, foraging gain is also determined by foraging accuracy. Every flower has a different amount of reward (nectar quantity and concentration), and bees must access potential floral reward, often remotely (Marden 1984). Bees with worn wings may have to balance higher velocity with loss of foraging accuracy, and when flights are shorter, increasing travel time is just not feasible. This said, I measured a significant effect of wing wear on travel time, and a much smaller effect of wing wear on flight errors. In these circumstances, the benefits of flying faster would seem to outweigh the cost of reduced manoeuvrability. Given the above, we might predict that older bees, with more worn wings, could prefer less dense patches of flowers. At these patches, they may forage better, since they have lower travel times, than when the flowers are more closely spaced, where they have higher travel times.

Perhaps these decreases in travel time result from an increase in flight speed. As wing loading increases, the power curve for flight power (vs. flight speed) is translocated upwards along the power axis to include the added energetic cost of flying with this new burden (comparable to birds feeding young: Norberg 1981, and to nectar loading in bumble bees: Cooper 1993). This also increases the maximum range velocity (V_m), the time that

minimizes the power cost of flying a particular distance. However, such loading does not strongly change the overall shape of the power curve (Cooper 1993). The power curve for bumble bee flight is relatively flat from hovering to 4.5 m/s (Ellington et al. 1990), or a more J-shaped curve at the higher flight speeds (Cooper 1993). The breadth of the “flat” portion of the power curve is highly variable among individuals, and a slight change in power requirements might still be ecologically important. Since flying faster does not seem to have much energetic consequence, wing wear, rather than being a foraging cost, would counter-intuitively seem to be a benefit for foraging at flower densities with over a 30 cm distance between flowers. Perhaps, there is an unmeasured cost associated with faster travel time.

Why do bees not fly with the same travel time prior to wing clipping, if flying faster does not increase energetic costs? The answer may be that bees trading off flight speeds with accuracy in choosing rewarding flowers (Chittka et al. 2003). Bees with wing wear time up, presumably visiting more flowers in a shorter period of time, with little to no energy cost. However in the process they risk making sub-optimal flower decisions. Bees have been shown to assess floral rewards by hovering in front of a flower without landing (Marden 1984). For wing-worn bees, such remote assessment of flowers may not be an option. Wing-worn bees may also make more errors in landing: bees who had their wings clipped a second time made more flight errors. However, it should be noted that the overall rate of flight errors was small, with bees making near perfect landings most of the time (99.55%). The bees in my setup seemed to be able to compensate well for their loss of wing area. It is also possible that the fireweed flowers, by being placed horizontal rather than the natural vertical position and with greater stability than natural, made the visitation experience easier than those experienced by wing-worn bees in the nature. That is, this experimental setup

may be an inadequate test of the “manoeuvrability” costs of wing loss.

However, perhaps travel time actually reflects how far the bees fly between flowers rather than flight speed. Bees typically did not fly in a straight line between flowers, but rather they moved along an irregular trajectory. Bees that were followed using a three dimensional tracking system were found to fly between 1.6 to 1.9 times further than the actual distance between the flowers (Chapter 3). The decreases of travel time observed between flowers spaced more than 30 cm apart could be a decrease in flight distance or an increase in the directness of the flight. This would similarly to the increase in flight speed, increase the foraging efficiency of the bee, but could also mean the bees could have a decreased foraging accuracy. My calculation for travel time integrates both: actual distance travelled over the flight path, and flight speed. In this paper, the two effects are irrevocably confounded. Chapter 3 provides a more detailed view of how these variables separately are influenced by wing treatment.

The present paper suggests that more detailed analysis into the effect of wing wear is desirable. In particular, a more detailed look at flight path the bee takes between flowers would clarify whether bees are flying more directly or are flying faster between flowers.

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Chapter 3:

Flight performance of bumble bees with artificially induced wing wear: the influence of asymmetry and mean wing loss

Abstract

The more a flying organism uses its wings, the more its wings will suffer from wear and tear. This study looked at the effect of simulated wing wear - wing area reduction and asymmetry - on the flight behaviour of bumble bee workers (*Bombus flavifrons*) moving between flowers spaced 30 cm apart and arranged in a 2-dimensional grid. Flight behaviour was measured in 3-dimensions as: total flying distance, mean velocity, variability of velocity, maximum acceleration, maximum deceleration, time spent accelerating and displacement from a straight line between flowers. Bee biomechanics were largely unaffected by wing treatment. Small amounts of wing wear did slightly affect flight biomechanics; bees with small amount of wing clipping and little asymmetry accelerated and decelerated less. Also, when bees were burdened with both large asymmetry high loss of wing area, they flew further between flowers and specifically they had the highest maximum total displacement from a straight “bee-line” between the flowers. Bees are relatively resilient to changes in wing area and asymmetry, particularly when the worst-case values of these two traits are not jointly experienced. Biomechanical costs of wing wear could be related to a decreased manoeuvrability or predator evasion, which were not tested in this study.

Key Words: acceleration, aging, asymmetry, biomechanics, *Bombus*, Bumble bees, deceleration, flight path, flight performance, flight speed, flightless, wing area, wing wear.

Introduction

A consequence of flying is the inevitable wear and tear of the wings. This is particularly true for insects that, unlike their avian counterparts, cannot renew worn wings. Decreasing wing area lessens the amount of air moved by each wing stroke, thus decreasing the lift per stroke (Hargrove 1975). Such wing wear has been related to increased wingbeat frequency (Cooper 1993, Kingsolver 1999), decreased survivorship (Cartar 1992), and a change in flight performance (Baltra 1994, Fischer and Kutsch 2000, Chapter 2).

Loss of wing area is not the only problem faced by worn-winged organisms. Events that cause wing wear, such as failed predator attacks or collisions with solid objects, can result in asymmetric wings (butterflies: Carpenter 1937, Windig and Nylin 1999; solitary bees: Mueller and Wolf-Mueller 1993). Asymmetry adds difficulty to flight because an individual affected by wing loss must also compensate for the differential lift and thrust created by each wing. House flies with asymmetric wings were less able to avoid predators (barn swallows), had a higher rate of infection by a deadly fungus, and decreased mating success (Møller 1996). Tethered male midges with experimentally induced wing asymmetry had lower flight performance (decreased flight duration and wingbeat frequency, and increased wingbeat amplitude) (McLachlan 1997). It is possible that slight wing asymmetry may benefit some species, male butterflies with more wing asymmetry turned faster and more effectively defended their territories (Windig and Nylin 1999). Regardless, biomechanical calculations of lift and power generally ignore wing asymmetry (see e.g. Dudley and Ellington 1990, Cooper 1993).

Wing asymmetry in birds can result from partial moults, such as that in small passerine birds under time or stress constraints (Brommer *et al.* 2003). Asymmetry causes unequal lift generation by each wing resulting in a rolling action (Thomas 1993) and an increase in wingbeat frequency (Hambly *et al.* 2004). Birds with both experimentally and naturally occurring wing asymmetry had increased level flight speed (Swaddle 1997), decreased manoeuvrability (Swaddle *et al.* 1996, Swaddle and Witter 1998) and decreased survivorship (Brown and Brown 1998, Brommer *et al.* 2003). A change in flight cost was only observed in birds with symmetrical (vs. asymmetrical) wing reductions, and those birds also increased their wingbeat frequency (Hambly *et al.* 2004).

It would seem that both increased asymmetry and decreased wing area should negatively affect flight performance. A foraging bee flying between flowers may change its flight behaviour to compensate for the cost of flying with worn wings. For example, wing-clipped locusts flew more slowly than unclipped individuals (Fischer and Kutsch 2000). Bumble bees generally fly in an irregular path between the flowers (*pers. obs.*), perhaps balancing flight time with accuracy in choosing the next profitable flower (Chittka *et al.* 2003). A bee that is burdened with wing wear or asymmetry might fly in a more direct path, at the expense of choosing flight paths better suited to on-the-wing assessment of floral rewards. Another important measure of flight performance, acceleration and deceleration, might be compromised by worn wings.

This study addresses the flight performance of wing manipulated bumble bee workers (*Bombus flavifrons*) foraging on flowers of *Chamerion angustifolium* (common fireweed). In particular, it examines 3-dimensional detail of flight between flowers spaced at a distance

of 30 cm, the distance at which effects of wing loss on flight behaviour were maximally detected in an earlier study (Chapter 2). The previous study (Chapter 2) found effects of wing wear on flight behaviour but could not distinguish between changes in flight speed and flight distance. The present study makes this distinction, and measures flight using standard biomechanical variables (distance, velocity and acceleration) and flight path variables (displacement from bee-line). I predict that bees with wing wear will decrease their travel time as found in Chapter 2. Little asymmetry could increase manoeuvrability and thus decrease flight distance, but high levels of asymmetry are predicted to hinder the bee since it must compensate for the differential lift on each wing. This could increase flight distance, specifically the displacement of the bee from the straight bee-line between the two flowers.

Methods

Two bumble bee colonies (*Bombus flavifrons*) were obtained from nest boxes set up in west Bragg Creek, Alberta Canada (50° 57'N, 114° 34'W, elevation=1400 m). Colonies were moved to the R.B. Miller Kananaskis Field Station (50° 39'N, 114° 39'W, elevation=1500m). Individual worker bees were marked with two dots of coloured paint on the thorax and the wings photographed. Experiments were conducted during July and August 2004, with a mean air temperature (\pm SD) in the shade of 20°C (\pm 3°C).

Bees were allowed to forage in a 4 m by 4 m by 2 m screen tent on an array of individual fireweed flowers. Each flower was held in holes in the lids of small plastic specimen jars (see Chapter 2). Flowers were placed in a square grid 30 cm apart (total 16 flowers).

Factors that could influence flight performance, such as weather, predators, and obstacles,

were controlled in this environment. The colony was placed in the tent at one end of the flower array approximately 50 cm from the grid. The observer remained beside the colony, allowing only the current bee being observed to exit the colony. Ten trials (foraging trips) were run before wing clipping, to train bees in the foraging environment, and ten were run after wing clipping.

Foraging bees were videotaped using two JVC TK-C1380U video cameras, two Panasonic AG-750P video cassette recorders and two Horita TG-50 SMPTE time code generators. One video camera was placed to the left of the colony/observer and the second video camera perpendicular to the first camera to the right of the colony/observer. Each camera was on a tripod (1.25 m high) approximately 50 cm from and parallel to the flower array. Both cameras were focused in to include the nearest 9 flowers (nearest to the camera and colony). Nine flowers were observed to maximize sample size while ensuring ease of tracking bees during analysis of the videotapes.

To clip the wings, bees were placed into a freezer until they stopped moving (roughly 5 minutes). This was done to facilitate the cutting of the wing, and has been used in previous experiments (Cooper 1993, Mueller and Wolf-Mueller 1993, Higginson and Barnard 2004). The wings were clipped using fine scissors to induce variation (low and high) in two factors: mean wing loss (MWL) and asymmetry. A fifth group of unclipped bees served as controls. Each treatment group was replicated with 3 bees, but one bee from the wing treatment “small asymmetry : high MWL” died before completing 10 post-clipping foraging bouts. Amount removed from each wing was calculated from digital photos (see Chapter 2 for technique) using NIH Image V1.63. Natural levels (prior to cut) of asymmetry were on average (\pm SD) 2.9% (\pm 1.6%) of the total wing area. Wing clipping

resulted in a mean wing asymmetry of 1.9% (± 2.0) for small asymmetry and 23.2% ($\pm 5.9\%$) for large asymmetry. The resulting mean MWL for little cuts decreased the total wing area by an average 17.4% ($\pm 5.9\%$), while the large cuts decreased the wing area by 40.4% ($\pm 7.2\%$).

To test how much wing area a bee could lose before it suffered aerodynamic failure, a ‘flight test’ was conducted. Bees that had been used for the previous experiment and could fly on the test day (8 experimental and 2 control bees) had small portions of their wings removed. Following each cut a digital picture was taken and the bee allowed to fly. Successful flights were defined as when the bee could maintain a level flight, and could take off, a greater aerodynamic challenge than steady-state forward flight. Cuts continued until the bee could no longer fly. Some bees lost the ability to take-off but could still maintain level flight. All flight tests were done in a 3-hour period, on a single relatively cool autumn day (4 September 2004, air temperature between 14 and 16°C).

Videotapes were analyzed using Peak Motus Version 2000 (Peak Performance Technologies, Inc. 2001). Resulting flight coordinates were smoothed using a 5-point average (using 60 fps). Biomechanical variables, including flight distance, maximum acceleration, maximum deceleration, time spent accelerating and mean and variability velocity, were calculated from the resulting XYZ coordinates. Descriptive variables of the flight path were also calculated including vertical and horizontal displacement. Only complete flights between flowers were analyzed. Flights from two categories were analyzed: the first 15 flights after the wing cut and the last 15 flights after the wing cut.

A multivariate analysis of covariance (MANCOVA) was conducted to analyse the combined

effect of treatment on all six biomechanical variables: total flight distance, mean velocity, SD of velocity, maximum acceleration, minimum deceleration and percentage of flight in acceleration. The independent variables were: treatment (control, small asymmetry & low MWL, small asymmetry & high MWL, large asymmetry & low MWL, large asymmetry & high MWL), time since cut (early, late), individual bee (nested within treatment), trial (covariate), air temperature in the shade (covariate), and distance between the two flowers (covariate). The bees sometimes (7% of 600 flights) flew diagonally between flowers, a distance of 42 cm, so flower distance was added to the model as a covariate.

The MANCOVA produced canonical variables based on linear combinations of the original Y-variables. Canonical variables reduce dimensionality in the original variables, to summarize trends in group means. The canonical variables vary in strength, accounting for variation within the Y-variables (measured as eigenvalues). The mean and 95% confidence intervals of the treatments of the first two canonical variables were calculated. Contrasts were done between wing clipping treatments and control bees, and, to view their effects alone, for MWL and asymmetry. Following the planned contrasts, the 6 unplanned contrasts between wing clipping treatments were tested using t-test to which a sequential Bonferroni correction was applied. MANCOVA was conducted in accordance with the methods described in Scheiner (1993). Univariate ANCOVAs were also run for flying distance and mean velocity to help clarify the MANCOVA and results from Chapter 2.

To compare flight paths and allow for maximum flexibility of fits, 6th degree polynomial regressions were fitted through all the flight trajectories of bees flying between flowers spaced at 30 cm. Flight path was measured as a displacement of the bee from a straight line between the two flowers. Peak displacement and timing of the peaks were measured for

comparison between groups. 95% confidence intervals were fit to the polynomial fit using a $n=3$ bees.

Variables were transformed when necessary to ensure normality and homogeneity of the residuals, and multivariate normality. The MANCOVA assumption of homogeneous slopes was verified. All statistical analyses were performed using JMP V5.0 (SAS Institute Inc. 2002).

Results

Table 2 presents means for biomechanical and flight path variables, respectively. Bees had significantly lower maximum acceleration than maximum deceleration in all 5 treatments (paired t-test: control $t_{85}=2.67$, $p=0.0092$; small asymmetry & low MWL $t_{86}=2.93$, $p=0.0044$; small asymmetry & high MWL $t_{58}=2.24$, $p=0.0290$; large asymmetry & low MWL $t_{86}=2.76$, $p=0.0071$; large asymmetry & high MWL $t_{86}=2.06$, $p=0.0421$, Table 2). As a consequence, the bees spent more time accelerating over the foraging flight (1-sample t-test for mean=50: control $t_{85}=4.74$, $p<0.0001$; small asymmetry & low MWL $t_{86}=3.47$, $p=0.0008$; small asymmetry & high MWL $t_{58}=5.25$, $p<0.0001$; large asymmetry & low MWL $t_{86}=3.69$, $p=0.0004$; large asymmetry & high MWL $t_{86}=6.38$, $p<0.0001$, Table 2). The bees flew on average 33 to 35 cm/s between flowers, but fluctuated between 13 and 54 cm/s over the flight (Table 2). Bees did not take a direct route between flowers, flying between 1.6 to 1.9 times further than the actual distance and flying a maximum of 8 to 11 cm away from a straight line between the flowers (Table 2).

Table 2: Mean (\pm SE) of the biomechanical and flight path variables calculated for worker bees (*Bombus flavifrons*) flying between fireweed (*Chamerion angustifolium*) flowers. To control for the large effect of flower distance, values include only the flights between 30 cm flower distances. Values in each row are based on 3 bees. “Maximum displacement” is a measure from the bee-line between flowers.

Asym	MWL	N	Mean Velocity (cm/s)	SD Velocity (cm/s)	Max Acceleration (cm/s/s)	Max Deceleration (cm/s/s)	Time Spent Accelerating (%)	Flying Distance (cm)	Max Displacement (cm)
Small	Low	81	33.17 \pm 0.83	19.74 \pm 1.12	511.60 \pm 45.08	-544.33 \pm 43.87	51.33 \pm 0.47	50.42 \pm 1.38	9.13 \pm 0.44
Small	High	52	33.28 \pm 0.86	20.49 \pm 1.10	638.00 \pm 78.73	-662.57 \pm 73.65	53.25 \pm 0.65	47.57 \pm 1.66	8.23 \pm 0.40
Large	Low	83	33.27 \pm 0.79	18.93 \pm 0.58	507.08 \pm 28.71	-539.43 \pm 26.99	51.75 \pm 0.49	50.55 \pm 1.94	8.62 \pm 0.40
Large	High	80	34.88 \pm 0.66	18.67 \pm 0.56	475.96 \pm 23.31	-502.24 \pm 22.07	52.89 \pm 0.45	50.89 \pm 1.50	10.78 \pm 0.49
Control		81	34.47 \pm 0.82	19.69 \pm 1.00	558.55 \pm 43.13	-603.56 \pm 51.40	52.02 \pm 0.49	50.18 \pm 1.86	8.89 \pm 0.49

Bee biomechanics showed an effect of treatment (4 wing treatments and control) but their biomechanics did not differ according to time since cut (“Time” Table 3). The first two canonical variables CV 1 and CV 2 summarize 67% of the variation in the original six biomechanical variables. CV 1 is negatively correlated with flying distance and mean velocity, while CV 2 is positively correlated with mean velocity, maximum acceleration and maximum deceleration (Figure 3).

Table 3: MANCOVA explaining flight distance, mean velocity, variability of velocity, maximum acceleration, maximum deceleration (all ln-transformed) and proportion of time spent in acceleration for 5 treatments (combinations of 2 levels of MWL and asymmetry and control) of bees (*Bombus flavifrons*). Overall model Pillai’s Trace $F_{126, 2304}=2.49$, $p<0.001$. See Methods for model details. The F-values for Treatment, Treatment*Time and Bee [Treatment] are Pillai’s Trace.

	Numerator DF	Denominator DF	F	P
Treatment	24	1528	1.96	0.0036
Time since cut	6	379	1.71	0.1185
Treatment * Time since cut	24	1528	1.09	0.3467
Trial (covariate)	6	379	1.32	0.2492
Temperature (covariate)	6	379	1.45	0.1935
Flower Distance (covariate)	6	379	13.00	<0.0001
Bee [Treatment]	54	2304	2.47	<0.0001

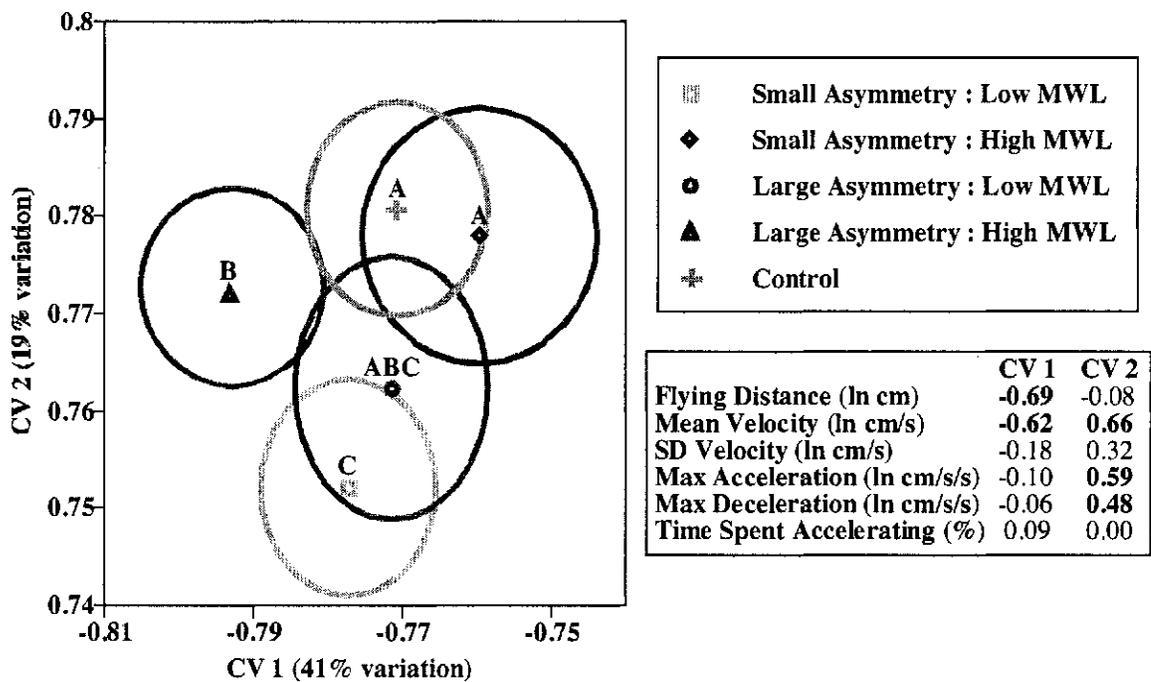


Figure 3: Multivariate differences among means of the 5 wing treatment groups, represented in the first two canonical variables (CVs) of the MANCOVA (Table 3). Ellipses represent 95% confidence intervals for each mean. Letters represent the results of post-hoc contrasts of means in 6-dimensional space (for the six biomechanical variables). Contrasts between control and wing treatments use a p value of 0.05. A sequential Bonferroni correction was applied to 6 unplanned contrasts (between wing treatments). The first two canonical variates explain 60% of the correlation among wing treatment groups. The table on the right shows correlation coefficients of each CV with each biomechanical variable. Important correlations are bolded.

Contrasts between treatment means are shown in Figure 3. Wing treatments that differed significantly from control were; bees with large asymmetry and high MWL (contrast: large asymmetry & high MWL vs. control $F_{6, 379}=2.15, p=0.0475$) and bees with small asymmetry and low MWL (contrasts: small asymmetry & low MWL vs. control $F_{6, 379}=2.11, p=0.0514$). Bees with large asymmetry and high MWL had a lower mean CV 1 (i.e., longer flying distance and higher velocity, Figure 3), while bees with small asymmetry and low MWL had a lower mean CV 2 (i.e., slower velocity, lower maximum acceleration and lower maximum deceleration, Figure 3). After using a sequential Bonferroni correction,

the other two wing treatments were not significantly different from controls (contrast: small asymmetry & high MWL vs. control $F_{6, 379}=0.86$, $p=0.52$; large asymmetry & low MWL vs. control $F_{6, 379}=0.84$, $p=0.54$; large asymmetry & high MWL vs. control $F_{6, 379}=2.15$, $p=0.0475$).

Asymmetry affected biomechanics but only when the MWL was high (contrasts: small asymmetry & high MWL vs. large asymmetry & high MWL $F_{6, 379}=3.70$, $p=0.0014$; small asymmetry & low MWL vs. large asymmetry & low MWL $F_{6, 379}=0.33$, $p=0.92$). Bees with high asymmetry and high MWL had a lower mean CV 1 (i.e., longer flying distance, higher velocity, Figure 3) than did bees with equivalently high MWL but low asymmetry (Figure 3). Asymmetry alone, independent of MWL, did not affect biomechanics (contrasts: low asymmetry vs. control $F_{6, 379}=1.01$, $p=0.14$; high asymmetry vs. control $F_{6, 379}=1.64$, $p=0.42$; large asymmetry vs. small asymmetry $F_{6, 379}=1.78$, $p=0.14$).

MWL affected biomechanics when asymmetry was low but not when asymmetry was high (contrasts: small asymmetry & low MWL vs. small asymmetry & high MWL $F_{6, 379}=3.45$, $p=0.0025$; large asymmetry & low MWL vs. large asymmetry & high MWL $F_{6, 379}=2.19$, $p=0.0437$). Bees with small asymmetry and low MWL had smaller CV 2 (i.e., lower velocity, lower maximum acceleration and lower maximum deceleration, Figure 3) and a slight decrease in mean CV1 (i.e., further flying distance and higher velocity, Figure 3) than did bees with small asymmetry but with high MWL (Figure 3). Biomechanics of bees with low MWL were significantly different from bees with high MWL but not significantly different from control bees (contrasts: low MWL vs. high MWL $F_{6, 379}=2.99$, $p=0.0072$; low MWL vs. control $F_{6, 379}=1.83$, $p=0.0929$). Bees with low MWL had a lower mean CV 2 (see above for interpretation) than did bees with high MWL. Bees with high mean wing

loss were not significantly different from control bees (contrast: high MWL vs. control $F_{6, 379}=0.42, p=0.87$).

Biomechanics of bees with small asymmetry and low MWL were significantly different from bees that had large asymmetry and high MWL (contrast: small asymmetry & low MWL vs. large asymmetry & high MWL $F_{6, 379}=2.77, p=0.0119$). Finally, bees with small asymmetry and high MWL and bees with large asymmetry and low MWL were not significantly different (small asymmetry & high MWL vs. large asymmetry & low MWL $F_{6, 379}=2.12, p=0.0499$).

Flying distance alone does not change with wing treatment (Univariate ANCOVA: Whole Model $F_{34, 373}= 2.72, p<0.0001$; Treatment $F_{4, 373}=0.33, p=0.86$; Treatment * Time since cut $F_{4, 373}=1.22 p=0.30$). Mean velocity did change wing treatment but it depended on the time since the wing was cut (Univariate ANCOVA: Whole Model $F_{34, 373}= 2.84, p<0.0001$; Treatment $F_{4, 373}=0.95, p=0.44$; Treatment * Time since cut $F_{4, 373}=2.67 p=0.0318$). Mean velocity changed with wing treatment but only immediately following wing clipping but not after some time since the wings were clipped (Univariate ANCOVAs: Early After Cut: Whole Model $F_{29, 174}=2.92 p<0.0001$; Treatment $F_{4, 174}=2.73 p=0.0305$; Later After Cut: Whole Model $F_{29, 174}=1.75 p=0.0153$; Treatment $F_{4, 174}=1.03 p=0.40$).

The flight paths of bees with simultaneously high levels of wing wear and asymmetry differed from the others (Figure 4). Based on comparison of 95% confidence intervals, the peak displacement from a straight line between flowers, ordered from smallest to largest were: large asymmetry & low MWL = control = small asymmetry & low MWL = small asymmetry & high MWL < large asymmetry & high MWL (Figure 4). The timing of the

maximum displacement for total displacement was unaffected by wing clipping (Figure 4). The general shape of the flight paths was the same among groups, with a unimodal peak (Figure 4).

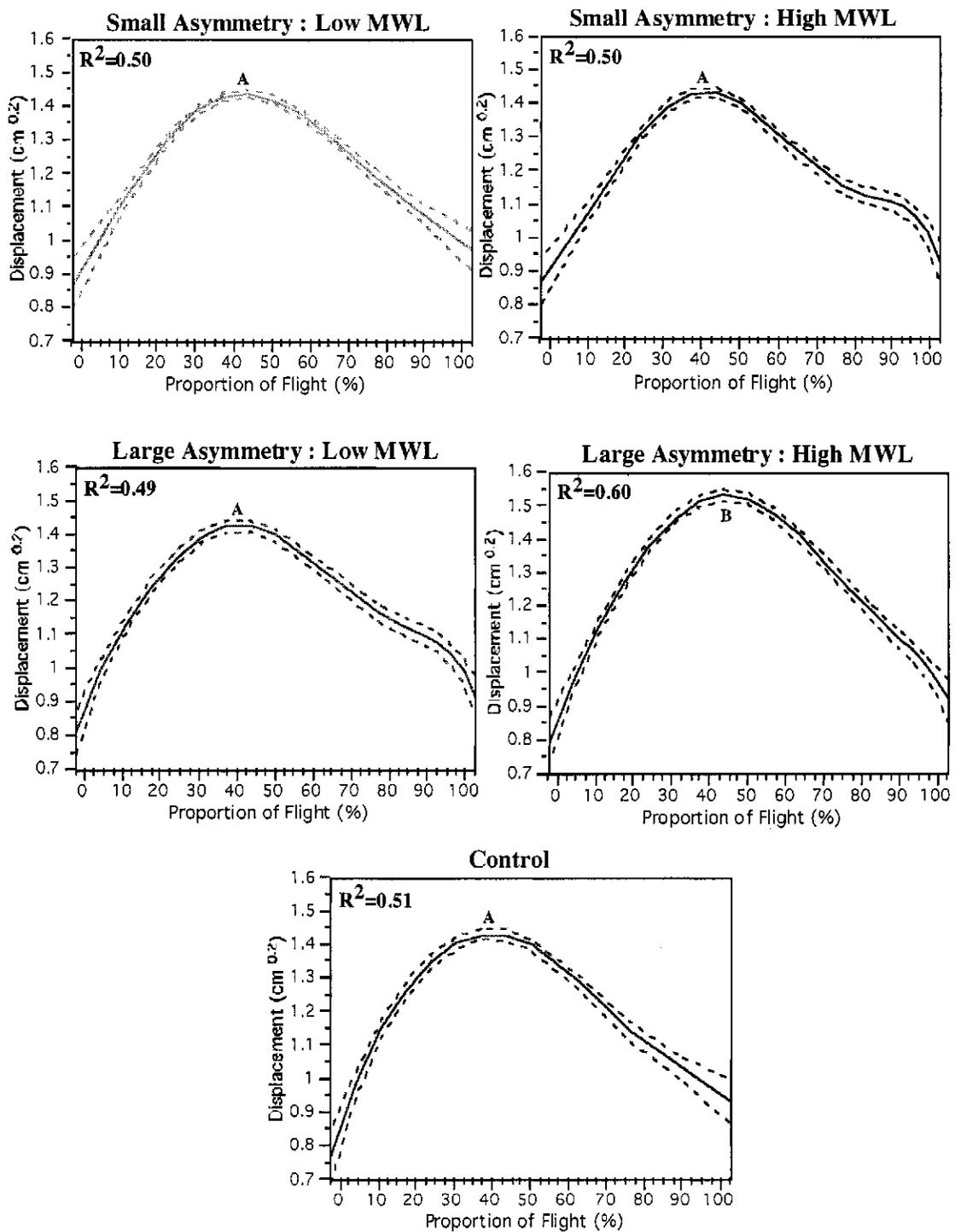


Figure 4: Total displacement (absolute value) along flight paths of bumble bees (*Bombus flavifrons*) flying 30 cm between fireweed flowers (*Chamerion angustifolium*) with varying amounts of MWL and wing asymmetry. Lines show 6th degree polynomial regression fits with 95% confidence intervals based on n=3 bees. Letters above peaks represent the result of a post-hoc analysis. See Table 2 for peak values.

The “flight test” showed that wing-reduced bees retained the ability to fly despite massive wing loss. The mean (\pm SD) wing area removed prior to the bee becoming flightless was 30.7% (\pm 8.3%) of the whole wing, or 50.7% (\pm 13.7%) of the forewing. The next cut, which resulted in aerodynamic failure, was 44.1% (\pm 7.5%) of the whole wing, or 72.9% (\pm 12.4%) of the forewing. When these values were compared to the total wing loss in the high MWL classification (average 40%), all bees in this treatment were above the highest measured wing loss prior to flightlessness (31%), while one bee was above the average wing loss measured when bees were flightless (44%). Yet, all bees in the high MWL treatment remained the ability to fly. Asymmetry was not specifically addressed in this test, as the asymmetries were low (prior to flightless: 5.72% \pm 4.48%; flightless 7.86% \pm 5.25).

Discussion

Bees with 41% of their total wings clipped or with wings of nearly 23% difference in wing area between the two wings, differed only slightly in biomechanics and flight path from bees with unmanipulated wings. These bees had their wings clipped to levels near aerodynamic failure; somewhere between 30% & 44% wing area loss was the flightless point for bumble bees. Yet the effect on flight biomechanics was relatively minimal (Figure 3, Table 3), nor is there much change in flight path until the bee experienced the most extreme wing treatment (Figure 4). Bumble bees seem to be very resilient to wing wear, showing only minor changes in their foraging flight performance. Presumably, this remarkable compensatory behaviour of bees to maintain flight behaviour has a cost, as could be seen ultimately by the increased mortality rate of bees with wing wear (Cartar 1992). As such, the biomechanical costs of wing wear still remain unclear.

Wing clipping treatment affected the biomechanics and flight paths of foraging bumble bees, but in a complex manner. Asymmetry had a large effect on biomechanics when coupled with high MWL: bees with asymmetrical wings and high flew further and less directly between flowers. Bees with little asymmetry and low MWL also altered their biomechanics, appearing to decrease their velocity, acceleration and deceleration over the flight. Treatment was not important in affecting consistency of velocity over the flight.

These results can be compared to those of Chapter 2 (Figure 2). The flight distance used in this experiment (30 cm) was equivalent to the intermediate flight distance used in the previous study. The wing treatment “small asymmetry & low mean wing loss” was approximately equivalent to the “1st cut” wing treatment of Chapter 2. The bees with small asymmetry and high mean wing loss were different from control bees as was seen in the equivalent bees in Chapter 2. However, indications are that in this study, those bees decreased in acceleration and deceleration along their path, something not directly correlated with travel time (as found in Chapter 2). To further clarify, the two variables that are comprised of travel time, flight distance and mean velocity, were tested separately. Mean velocity seemed to change the most with wing treatment, so it is expected that bees are speeding up with high wing clipping at flower distances exceeding 30 cm rather than taking a more direct path between flowers (as seen by the decrease in travel time in Chapter 2).

With high MWL, asymmetry seemed to hinder the bee’s flight. When wing area was reduced to near aerodynamic failure, bees flew differently. When the wear was symmetrical at high MWL, encumbered bees flew like bees with no wing wear. High asymmetry however resulted in an increased flight distance, and also a larger total displacement.

Perhaps when burdened with small wings incapable of producing high lift, bees lost the ability to compensate for the differential lift generation caused by asymmetry (Thomas 1993), and as such flew further and higher due to a decreased manoeuvrability (Swaddle et al. 1996, Swaddle and Witter 1998).

The overall sample size per treatment (n=3 bees) provided low power to detect effects of wing wear, and necessitated the use of a MANCOVA. The MANCOVA is more powerful than the alternative of individual ANCOVAs for each independent variable, in that it can detect multivariate effects of wing treatment on biomechanics that are undetectable univariately. Further study with a larger sample size would decrease the confidence intervals in Figure 3 and allow for more in-depth individual ANCOVAs of Y variables.

Overall bees were relatively resilient to wing wear with this flower set-up. As such, the costs of flying with wing wear are not clear. Further, bumble bees with a 10% wing cut did not have a detectable increase in energy costs for flight (Hedenström et al. 2001). It would seem that other factors need to be investigated in considering the cost of worn wings. These include ability to avoid predators and parasitoids, foraging gain, energetic costs of higher wing loss, manoeuvrability around obstacles, and flying between flowers of complex morphology or landing difficulty at simultaneously high levels of MWL and asymmetry. Consideration of other kinematics such as wing and body angle, wingbeat frequency and amplitude, may help to determine the true costs of wing wear.

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Chapter 4: General Discussion

This study addressed the behavioural consequences of wing wear in bumble bees. Energetic costs were not detected in bumble bees with 10% wing clipping (Hedenström et al. 2001) even so survival costs have been detected (Cartar 1992). It is possible that the costs of wing wear were behavioural rather than energetic. However, when bees had their wings clipped by 41% of their total area or asymmetry in wing area was created to nearly 23%, changes to flight performance were slight (Chapter 3). As such, at least for this flower set-up, bees seem to be relatively resilient to losses in wing area, and are able to compensate for losses in lift. Bees could be compensating by altering flight kinematics such as wingbeat frequency, wingbeat amplitude or stroke angle. However, a bumble bee foraging in the wild would not experience such a relatively easy flower set-up. A wing worn bee must also deal with obstacles between flowers, predators, parasites and complex flower morphologies, all of which might be more difficult to a bee with wing wear.

Some support for an increase in flight performance with increased wing asymmetry was found, but only when total wing area loss was minimal. At low MWL, symmetry between the wings resulted in a decrease in maximum acceleration and deceleration, while bees with equivalent MWL but high asymmetry flight biomechanics were not significantly different from control bees. However, bees with high MWL seemed hampered by high asymmetry. When bees suffered both from high MWL and large asymmetry they flew further and higher than bees with low asymmetry. At high MWL the bees were burdened with small wings could not adjust to the differential lift generation of asymmetry and thus it hindered flight performance (Thomas 1993, Swaddle et al. 1996, Swaddle and Witter 1998).

Bumble bees are social insects, so it is also important to remember that the fitness of each worker bee is a function of the reproductive output of the colony. A foraging worker bee can increase her fitness by helping to maintain an energy reserve for the whole colony. As such, wing wear not only affects the foraging performance of that individual worker bee, but also the reproductive output of her colony attributable to her foraging contributions. Colonies must balance survival of worker bees and the cost of creating new workers, which takes away energy that could be used to make more reproductives, young queens and males. At a certain point, maintaining a wing worn bee is no longer feasible for the colony.

Natural levels of wing wear can help determine what the natural threshold of wing loss and asymmetry wild bumble bees can endure. Also studying different species of bumble bee, and other insects, could also be of interest because different species have different wing morphology and flight patterns. Studying more complex flower morphologies, more obstacles for them to manoeuvre around and introducing difficulties like predators and parasites would also help determine how bees in the wild would actually alter their flight behaviours when suffering from wing wear. Also, studies of how bees change their flight kinematics (e.g. wingtip kinematics, wingbeat frequency and amplitude) with wing wear could help understand how bees are able to compensate for loss of wing area. Finally, addressing wing wear in other flying insects could be vital in further understanding wing wear. Every species of flying insect has a slightly different wing shape, some have 2 sets of wings, other have only 1 set, each species moves their wings in a unique fashion. As such, each species might have different way of compensating of wing wear, and the onset of senescence. Addressing these differences with a meta-analysis could help determine what the true behavioural consequences are of wing wear. Also, flying insects that are unlike the social bumble bee and not only are maximizing foraging efficiency, but also require energy

for reproduction, could differ in their reaction to wing wear.

A note on methodological design, cooling the bees in a freezer seemed to be a less obtrusive way of artificially inducing wing wear. In the first study, bees were held first by the legs with leather gloves and then with tweezers to separate the wings to make a cut. This resulted in a somewhat mobile bee, and the bee, on occasion, escaped. After cutting, some bees that were returned to the colony immediately hid, especially after the second wing clipping (pers. obs.). This was in contrast to the second study, where the bees were cooled then their wings clipped. The bees recuperated more quickly, and were ready to forage faster than bees that had been cut in the first study. On one occasion a bee that was cooled warmed up quickly and returned to foraging on the array within minutes (pers. obs.). Bees that were not cooled generally immediately hid under their colony and required more time to recuperate.

How organisms react to a loss of wing area is still a largely unknown field. Bumble bee workers seem to be relatively resilient to a loss of wing wear, having no significant increase in energy consumptions with a loss of 10% the total wing area and they show, at least in a simplified flower array, little change to their flight behaviour. However, logically, reductions in wing area must have some sort of disadvantage or there would not be higher in death rates of bees suffering from wing wear. Further research in this field will hopefully help us better understand wing wear, and its evolutionary consequences.

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