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The Foraging Behaviour of Bumble Bees, Mediated by the Costs of Wing-Worn Flight

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The Foraging Behaviour of Bumble Bees, Mediated by the Costs of Wing-Worn Flight

by

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A THESIS

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ABSTRACT

Bumble bees accumulate wing wear while foraging. If wing-worn workers face higher costs of flight, they should reduce their wing use, perhaps to increase their residual lifespan and net lifetime contribution to their colony. Measuring four behavioural variables (flight amount, flight frequency, choice of flower density, and distance travelled), I found that wing-worn workers sometimes used their wings less than pristine-winged bumble bees. Long-tongued, wing-worn workers were also more likely to forage on flowers with short corollas, which reduced their wing use. Wing use also depended on both past and present wing wear. Bees given experimental wing wear flew less, and bees with high natural wing wear used their wings less when feeding on *Cirsium arvense*. This study emphasizes the importance of wing wear in explaining the foraging behaviour of bumble bees. Wing-worn workers reduce their wing use in a manner predicted to offset higher flight costs of wing wear.

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Gregory Earle

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CHAPTER ONE: GENERAL INTRODUCTION

1.1 Wear and tear of body parts and their constraints on foraging behaviour

As an outcome of natural selection, foragers are predicted to optimize currencies (e.g., net rate of energetic intake) that optimize their foraging gains and thereby maximize fitness (Stephens and Krebs 1986; Davies *et al.* 2012). To this end, optimal foraging theory (OFT) is used to derive quantitative economic models based on the benefits and costs of foraging, predicting and explaining optimal behaviours (Pyke 1984). For individuals foraging in a patch, two well-supported foraging currencies are net rate of energetic intake (NREI; gain – cost / time; Pyke 1978; Charlton and Houston 2010) and efficiency (gain – cost / cost; Schmid-Hempel *et al.* 1985; Charlton and Houston 2010). Foragers maximizing either of these currencies should be sensitive to costs, particularly efficiency maximizers (Schmid-Hempel *et al.* 1985; Charlton and Houston 2010).

Though NREI and efficiency provide powerful predictions of foraging behaviour, care must be exercised to take into account the current physical and physiological state of an individual (e.g., its morphology). Limitations on foraging performance may be caused by wear and tear of body parts used while foraging, and it is important to consider current morphology when modelling costs of foraging, in particular.

Wear and tear of body parts is irreversible damage in insects, and has negative consequences for the organism (Finch 1990). Insects undergo considerable wear and tear which becomes more pronounced with age and the behaviours associated with use of worn body parts (Roitberg *et al.* 2005; Foster and Cartar 2011a). Wear and tear occurs in insect mandibles (Arens 1990; Roitberg *et al.* 2005; Scofield *et al.* 2011), wings (Cartar 1992a; Alcock 1996; Foster and Cartar 2011a), legs and tarsal claws (Morse 1981; Cherrill and Brown 1997; Harwood *et al.*

2013), and ovipositors (Lalonde and Mangel 1994; Papaj and Alonso-Pimental 1997). Wear and tear is the gradual degradation of insect body parts and may restrict an individual's foraging performance by imposing energetic, and/or biomechanical costs (e.g., reduced flight manoeuvrability caused by worn wings; speculated by Cartar 1992a; Papaj and Alonso-Pimental 1997; Roitberg *et al.* 2005; Johnson and Cartar 2014).

Egg-laying by female walnut flies (*Rhagoletis* spp.; Papaj and Alonso-Pimental 1997) illustrates the importance of wear and tear of body parts to explain behaviour as the preceding section explains. Female walnut flies lay egg-clutches in unparasitized or already parasitized walnuts. Egg-laying in parasitized walnuts has the cost of increased offspring competition, but the likely benefit of higher fecundity for the parent because of reduced handling time by not having to bore a hole into the walnut (Papaj and Alonso-Pimental 1997). Two additional explanations as to why females may lay their eggs in parasitized walnuts are to save energy (especially if energy is limiting), or reduce wear of their ovipositors subject to physical degradation, both from not having to bore a hole. Because a female walnut fly's ovipositor accumulates wear, I propose an alternative explanation: that worn-ovipositors increase handling time in unparasitized walnuts, and females lay eggs in pre-existing sites to save energy and/or prevent accumulation of future wear and tear.

Ovipositor wear in *Rhagoletis* spp. may have implications for egg-laying behaviour (i.e. choice of walnut) and demonstrates the need to account for the current state of an individual. To gain better insights and improve the predictions made by optimal foraging studies, it is important to consider the current morphology of characters attributed to that activity (e.g., feeding apparatus, wings, etc.) caused by wear and tear, as this may constrain adaptive behaviour and have implications for how an individual forages (Schofield *et al.* 2011).

1.2 Wing wear and its influence on insect flight

Wear of insect wings (wing wear) may have costs associated with wing use that can significantly alter behaviour. Insect wings generate high amounts of lift on downward wing-strokes (Liu *et al.* 1998; Ellington 1999). This is caused by the wing creating a leading edge vortex (LEV) on the downward wing-stroke during the brief translational movement (dynamic stall; Ellington *et al.* 1996). Thus, the LEV augments the lift force generated by producing an area of low pressure above the wing before being shed at the margins of the wing at the beginning of the next translational movement (Liu *et al.* 1998; Ellington *et al.* 1998). Wing wear occurring along the margins of the wing may affect the shedding of LEVs that can affect the generation of vertical forces required to maintain flight. Wing wear may impair flight performance by resulting in more erratic flight (Fischer and Kutsch 200; Jantzen and Eisner 2008; Combes *et al.* 2010), increase metabolism from an increased wing beat frequency to maintain lift (Kingsolver 1999; Hedenström *et al.* 2001), and reduce load-lifting ability (Buchwald and Dudley 2009; Johnson and Cartar 2014). In addition, changes in wing morphology caused by wing wear might also alter wing flexibility, a component crucial to enhance vertical force production (Mountcastle and Combes 2013).

1.3 Wing wear in bumble bees

Temperate eusocial bees (such as bumble bees and honey bees) rely on non-reproductive workers to provision the colony by collecting nectar and pollen – maximizing their inclusive fitness through colony survivorship and reproductive output (Schmid-Hempel *et al.* 1993; Goulson 2003). In bumble bees, workers provisioning the colony experience the greatest

metabolic costs while in flight (Heinrich 1975b; Ellington *et al.* 1990). Because workers typically optimize such cost-sensitive currencies, including (in order from least to most costly); NREI (Pyke 1978 and 1980; Best and Bierzychudek 1981; Hodges 1981), efficiency (Charlton and Houston 2010), and possibly foraging gain per wing beat [foraging gain – cost / number of wing beats; Higginson and Gilbert 2004], in addition to minimizing the risk of energy shortfall (Cartar and Dill 1990), the potential flight costs of wing wear in worker's may significantly influence a workers ability to maximize energetic returns to the colony per foraging trip.

Wing wear is caused by wing collisions with the vegetation while foraging (Foster and Cartar 2011a), and is associated with increased mortality rate in workers (Cartar 1992a; Dukas and Dukas 2011; Higginson *et al.* 2011). The proximate mechanisms underlying wing-worn mortality in workers are unclear. However, wing wear may result in higher metabolic, and/or biomechanical flight costs (i.e. reduced manoeuvrability and increased susceptibility to inclement weather or predation; Cartar 1992a). Wing wear also becomes more pronounced with age and cumulative foraging effort (Higginson and Barnard 2004; Foster and Cartar 2011a) and wing-worn mortality may reflect worker age. Studies investigating the ecological costs of wing wear in bees have found wing wear to influence flight performance (flight path less direct; Haas and Cartar 2004), reduce flower choosiness and foraging effort (Higginson and Barnard 2004; Higginson *et al.* 2011), reduce foraging rate (Dukas and Dukas 2011), influence choice of floral density (Foster and Cartar 2011b), and decrease lift capacity (Johnson and Cartar 2014).

It remains to be seen if wing wear increases the metabolic or biomechanical costs of flight under natural conditions. Under experimental conditions, wing-worn bumble bees have reduced lift capacity (Johnson and Cartar 2014) and the same might hold true in the wild having to carry nectar. Wing wear causes increased wing-loading, and a possible explanation for

reduced lift capacity is caused by higher metabolic costs to sustain flight (see Wolf *et al.* 1989 for wing-loading costs in honey bees).

Hedenström *et al.* (2001) found no metabolic cost of wing wear, but they only removed 10% of the forewing area in comparison to Cartar (1992a) who removed an average area of 18%, mimicking the upper limit of natural wing wear. Furthermore, Hedenström *et al.* (2001) did not account for additional weight carried by bumble bees that would reflect wing loading costs in the wild from a bee filling their crop with nectar. Their bees hovered in a calm, obstacle-free flight chamber, the perfect conditions under which not to express a metabolic cost of flight associated with wing wear. Air turbulence clearly increases metabolic costs of flight in hummingbirds (Ortega-Jimenez *et al.* 2014). Complex manoeuvres, wind, high wing wear, and loading constraints may account for higher energetic costs of wing wear that may have not been realized in a flight chamber (Wolf *et al.* 1999), and these require the study of wing wear in a natural setting.

If wing-worn bumble bees experience higher flight costs, then they should adjust their wing use consistent with optimizing foraging behaviour. Workers make foraging decisions primarily consistent with optimization of NREI (Pyke 1978 and 1980; Best and Bierzychudek 1981; Hodges 1981) and efficiency (Charlton and Houston 2010). Bumble bees also adjust their foraging effort based on the energetic requirements of the colony, by increasing their use of flight to increase immediate energetic gain, but reduce wing use otherwise (Cartar and Dill 1990). Similarly in honey bees, workers reduce wing use (fly less) when flying greater distances and carrying heavier loads by visiting fewer flowers and leaving the flower patch earlier (Schmid-Hempel *et al.* 1985; Schmid-Hempel 1986; Schmid-Hempel and Wolf 1988). Consistent with honey bees sensitivity of flight costs, it is hypothesized flight with worn wings is

more costly and wing-worn bumble bees should reduce their wing use to reduce flight costs of wing wear and maximize their foraging gain. Furthermore, given the importance of non-reproductive workers to the colony and the costs to produce them (Goulson *et al.* 2002; Goulson 2003), workers reducing the costs of wing-worn flight could prolong their life span, increasing colony survivorship and reproductive output (Schmid-Hempel *et al.* 1993).

1.4 How wing-worn bumble bees might reduce their wing use

Because bumble bees spend the majority of their time foraging for nectar and pollen they are an ideal organism to study the effects of wing wear on foraging behaviour. Wing-worn bumble bees should reduce their wing use in a flower patch by minimizing flight (Heinrich 1975b; Cartar and Dill 1990; Ellington *et al.* 1990). Bumble bees could either directly reduce their wing use by spending less time in flight and flying less frequently (Cartar and Dill 1990), or indirectly reduce their wing use by flying shorter distances (i.e. visiting closer inflorescences) and foraging in higher density flower patches (Foster and Cartar 2011b). Foraging in high density flower patches, with closer distances between adjacent inflorescences, should allow workers to fly shorter distances among inflorescences and walk between inflorescences.

Relative costs of flight might determine the distribution of bees with different levels of wing wear across flower patches of differing floral densities. Because all workers foraging in high density flower patches benefit from lower flight costs, high density flower patches should be attractive to bees of all classes of wing wear. However, disproportionate recruitment to high density flower patches will deplete resources in these patches, relative to flowers in low density patches. The ideal free distribution (IFD) model that predicts foragers match their foraging effort with the proportion of available resources (Fretwell and Lucas 1970). Based on the IFD, the

distribution of bumble bees among flower patches of different densities could in theory be mediated by the costs of wing wear (Cartar 2009), as a form of IFD adjusted to wing wear. If wing-worn bumble bees are more sensitive to the costs of flight than their pristine-winged competitors, they should forage more in high density flower patches (with their attendant lower flight costs), and cause disproportionate depletion of resources in these patches. In such circumstances, pristine-winged bumble bees, who can better afford the costs of flight, should forage in patches of lower floral density (and concomitantly receive more nectar per flower).

Bumble bees may also indirectly reduce their wing use by foraging on flower species with deeper corollas. Resource partitioning of floral resources in bumble bee species is typically based on differences in bumble bee tongue length; longer-tongued species forage on a wide range of flower species differing in corolla depth, while shorter tongued species forage mainly on species of flower with shallow corollas (Heinrich 1976; Inouye 1980; Ranta and Lundberg 1980). When tongue length matches corolla depth, workers maximize their foraging gain (Plowright and Plowright 1997). Furthermore, the time to probe a flower increases linearly with increasing flower depth, when the depth of the flower does not exceed the length of the bees tongue, and exponentially when it does (Harder 1983). Therefore, bees should spend a greater proportion of their foraging time handling deeper flowers that do not match their tongue length, than in flight. Because longer-tongued individuals are capable of visiting a wider range of corolla depths and are more capable of foraging on deeper flowers than short-tongued bees (Inouye 1980), long-tongued bees with wing wear should be more able to switch to deeper flower species than their short-tongued counterparts, thereby spending a greater proportion of their foraging time handling flowers than in flight (Cartar unpublished). Foraging on deeper flower species,

wing-worn workers should offset the costs of increased handling time on deeper flowers by reducing the costs of wing-worn flight.

1.5 Thesis Objectives

The first empirical part of this thesis (Chapter 2) examines whether wing wear influences bumble bee wing use and choice of flower species based on corolla depth in a natural setting. I use simple arguments based on OFT to explain why wing-worn bumble bees should forage differently from their pristine-winged counterparts. To this end, if wing-worn bumble bees reduce their wing use while foraging (relative to pristine-winged bees), this supports a cost of wing wear hypothesis (possible costs being metabolic and biomechanical). I test the importance of accounting for morphology (caused by wear of wings) relevant to foraging behaviour, to gain better insights into foraging behaviour.

The second empirical part of this thesis (Chapter 3) examines the relative importance of past and current wing wear. Because wing wear is correlated with worker age and foraging effort (Foster and Cartar 2011a), I trimmed bumble bee forewings varying in the extent of wing wear in a natural setting to decouple age and experience from wing wear, and examine changes in their foraging behaviour. If foraging behaviour is based on current wing wear, bumble bees should respond by reducing their wing use after wing area removal. If foraging behaviour reflects past experience based on past wing wear, then I expect the amount of wing wear an individual had before trimming to influence their wing use.

Overview:

- 1) Chapter 2: Examines the influence of wing wear on wing use in wild foraging bumble bees. I implement an observational study to measure worker foraging behaviour

influenced by varying degrees of wing wear. Furthermore, I examine the effects of wing wear, mediated by tongue length, on flower visitation.

- 2) Chapter 3: Examines the influence of past and present wing wear on foraging behaviour. I implement an experimental field study to test the effects of wing trimming on bumble bee foraging behaviour across a range of worker ages.
- 3) Chapter 4: I discuss my findings in terms of better understanding the costs of wing wear and the implications of wear and tear of body parts on foraging behaviour.

CHAPTER TWO: EFFECTS OF NATURAL WING WEAR ON FORAGING BEHAVIOUR

2.1 Abstract

Given that flight is the most energetically expensive foraging activity and that wing wear increases wing loading, I investigated whether wing-worn bumble bees reduce their wing use as predicted by the cost of wing wear (CWW) hypothesis. I found that wing-worn bumble bees flew less frequently than pristine-winged bees. However, other flight-related variables (flight time, distance flown, and floral density chosen), co-varied with wing wear depending on the species of flower. I also tested whether wing-worn bumble bees forage on deeper flower species to indirectly reduce their wing use. I found wing-worn, long-tongued bees were more likely to forage on shallow flowers and wing-worn, short-tongued bees tended to forage on deeper flowers, not supporting my prediction. However, long-tongued bumble bees used their wings less when handling shallow flowers, as was the case for short-tongued bees handling deep flowers, supporting the CWW hypothesis. Overall, bumble bee wing use and choice of flower species based on corolla depth support the cost of wing wear hypothesis, suggesting workers reduce their wing use to reduce both energy expenditure and the accumulation of further wear and tear.

2.2 Introduction

Wear of insect body parts impairs an organism's performance and reduces fitness by shortening lifespan (Finch 1990) and reducing fecundity (Skogland 1988). Foraging effort may also affect morphological senescence, as greater daily foraging effort is linked with reduced forager lifespan (Neukirch 1982; Schmid-Hempel and Wolf 1988; Wolf and Schmid-Hempel 1989). Temperate eusocial bees (mostly bumble bees and honey bees) rely on non-reproductive

workers to provision the colony, whose reproductive success determines their own (Schmid-Hempel *et al.* 1993; Goulson 2003). In-nest workers are less involved in high-metabolism activities, and have a lower rate of mortality than workers performing foraging tasks outside the colony (Katayama 1996), whose rate of mortality also increases with age (Rodd *et al.* 1980; Goldblatt and Fell 1986). Worker bees performing foraging tasks that exacerbate senescence may choose behaviours so as to maximize their net lifetime contribution to the colony, in part by reducing foraging costs so as to maximize net foraging benefits, thereby increasing colony survivorship and reproductive output (Schmid-Hempel *et al.* 1993).

As an organism ages, its body also wears, with negative consequences for the bearer (reviewed by Finch 1990). The body parts of insects undergo considerable wear and tear, and this wear becomes more pronounced with age and the behaviours associated with their use (Roitberg *et al.* 2005; Foster and Cartar 2011a). Wear and tear occurs in mandibles (Arens 1990; Roitberg *et al.* 2005; Scofield *et al.* 2011), wings (Cartar 1992a; Alcock 1996; Foster and Cartar 2011a), legs and tarsal claws (Morse 1981; Cherrill and Brown 1997; Harwood *et al.* 2013), and ovipositors of insects (Lalonde and Mangel 1994; Papaj and Alonso-Pimental 1997). Wear and tear of insect body parts is speculated to cause changes in the behaviours associated with their use, caused by greater energetic and/or biomechanical costs (Cartar 1992a; Papaj and Alonso-Pimental 1997; Roitberg *et al.* 2005).

Flight in bumble bees is the most metabolically expensive activity (Heinrich 1975b; Ellington *et al.* 1990), and foraging bees show a sensitivity to this high cost. For example, honey bees adjust foraging behaviour based on flight costs: they reduce wing use when faced with increased inter-flower distance by visiting fewer flowers (Schmid-Hempel *et al.* 1985), and return to the colony sooner when wing-loading is higher (Schmid-Hempel 1986). When faced

with lower colony energy needs bumble bees reduce their wing use by flying shorter distances, flying less often, and spending more time walking on, and probing inflorescences (Cartar and Dill 1990). The costs of flight with non-repairable wing damage (i.e., wing wear) may exacerbate these better-understood sensitivities to flight costs.

For eusocial temperate bees, wing wear has many detectable impacts. Workers of temperate insects rely on their wings to fly between their colony and flower patches and to move between flowers, collecting pollen and nectar to provision the colony. Wing wear is linked with higher mortality (Cartar 1992a; Dukas and Dukas 2011; Higginson *et al.* 2011), reduces flower choosiness and foraging effort (Higginson and Barnard 2004; Higginson *et al.* 2011), reduces foraging rate (Dukas and Dukas 2011), influences choice of flower density (Foster and Cartar 2011b), changes inter-floral flight trajectories (Haas and Cartar 2008), and decreases lift capacity (Johnson and Cartar 2014).

The proximate mechanisms linking wing wear to mortality in bumble bees remain unclear, but they likely involve metabolic and/or biomechanical costs (e.g., increased metabolism or reduced manoeuvrability and increased susceptibility to inclement weather or predation; speculated by Cartar 1992a). If wing-worn bumble bees experience high metabolic and biomechanical flight costs, then they should reduce their wing use to maximize their net lifetime contribution to the colony and maximize net foraging benefits, perhaps using similar strategies as honey bees and bumble bees sensitive to the costs of flight (Schmid-Hempel *et al.* 1985; Schmid-Hempel 1986; Cartar and Dill 1990).

Wing-worn bumble bees should reduce their wing use by flying shorter distances, flying less often, and spending more time walking on, and probing inflorescences (Cartar and Dill 1990). Furthermore, wing-worn bumble bees should reduce their wing use by foraging in higher

density flower patches, allowing them to walk between inflorescences and fly shorter distances (Foster and Cartar 2011b).

Another way bumble bees may reduce their wing use is by foraging on flower species with deeper corollas. Within- and among-bee species, workers visit floral resources based on tongue length and the depth of flower (corolla depth): longer-tongued species forage on a wide range of flower depths, but mostly deeper corollas, while shorter tongued species forage mainly on flowers with shallow corollas (Heinrich 1976; Inouye 1980; Ranta and Lundberg 1980), and foraging is most efficient when tongue length is close to corolla depth (Plowright and Plowright 1997). Because the time to probe a flower increases linearly when depth of the flower does not exceed the length of the bee's tongue, and exponentially when it does (Harder 1983), wing-worn bees should reduce their wing use by foraging on deeper flowers (whose depth does not exceed the length of their tongues) and spend a higher proportion of their time handling deep flowers, which require lower wing use (Cartar unpublished). Longer-tongued bees can also forage on a wider variety of corolla depths, so they are more likely to respond to wing wear by increasing their use of deeper flowers. Short-tongued bees have little physical capacity to forage on deeper flowers, so their response to wing wear should be to visit the same shallow flowers, but to adjust their behaviours to reduce wing use (described above). That is, I would expect the response of wing wear to depend on tongue length, where long-tongued bees have a greater diversity in potential responses.

In this study, I assume that wing wear has energetic and/or biomechanical costs, and ask: does wing wear affect foraging behaviour in bumble bees? Given that flight is energetically expensive (Heinrich 1975b; Ellington *et al.* 1990) and that wing wear increases wing-loading, wing-worn bumble bees should reduce their wing use while foraging by: spending less time in

flight (flying less), flying less frequently, flying shorter distances, foraging in flower patches of higher density, and foraging on deeper corollaed flower species (where tongue length permits). I observed wild-foraging bumble bees which varied naturally in their extent of wing wear. If the cost of wing wear (CWW) hypothesis is supported, I predict wing-worn bumble bees will do one or more of: fly less, fly less often, fly shorter distances, and forage in higher density flower patches relative to more pristine-winged bumble bees. I also predict long-tongued bumble bees with wing wear will feed more flower species with deeper corollas.

2.3 Methods

2.3.1 Study System

My study was conducted in the Sheep River Provincial Park, Kananaskis Country, Alberta (50.647°N, -114.648°W, elevation 1490 m) from July 1 – August 25, 2012 and 2013. Six species of bumble bees were observed during this study: *B. appositus*, *B. bifarius*, *B. californicus*, *B. flavifrons*, *B. mixtus*, and *B. moderatus* (Table A.1.1). Individuals vary within- and among-species in body size (estimated by marginal cell; refer to Figure 2.1) and prementum length (Table 2.1), a portion of the tongue that is strongly correlated with total tongue length (Morse 1977).

I observed bumble bees on different plant species at different times of the season, reflecting flowering phenologies. Workers were observed foraging on *Trifolium hybridum* blooming concurrently with *T. pratense*, *Chamerion latifolium*, and *Linaria vulgaris* in 2012 (Table A.1.1 and Table A.1.2). In both 2012 and 2013 I observed worker bumble bees foraging on *Geranium*, *Melilotus alba / officinalis*, *Monarda fistulosa*, and *Cirsium arvense* (Table A.1.1

and Table A.1.2) I treated the two species of *Melilotus* as one, because bees did not distinguish between them while foraging (see also Foster and Cartar 2011a).

The eight plant species on which I observed bumble bees foraging differed in floral characteristics in ways that allow useful contrasts in evaluating the effects of wing wear on foraging. Differences between flower species include average flower patch density, the number of flowers per inflorescence, and corolla depth, all of which affect wing use (i.e. time in flight; Table 2.2 and Table 2.3), and should reflect the impact of wing wear.

2.3.2 Floral Measurements

I characterized inflorescence size and corolla depth for each flower species. I chose thirty individual plants from each flower species by walking 100 m transects at peak phenology (Table A.1.2) and picking inflorescences haphazardly. I counted the number of flowers per inflorescence, and measured the corolla depth of 30 flowers (to the nearest 0.1 millimetre) using a Wiha dialMax dial Calliper (Schonach, Germany). The technique for measuring corolla depth differed according to flower species, but in all cases depth was measured from the floral constriction beyond which a bee's face cannot be inserted. *C. arvensis* was measured from the front edge of the corolla to the tip of the nectar spur, after controlling for flower curvature by pressing it flat. *L. vulgaris*, *M. alba/officinalis*, *M. fistulosa*, *T. hybridum*, and *T. pratense* all have fused petals into which bees extend their tongues. Therefore, I measured corolla depth in each of these species from the base of the flower to the base of the nectar spur. Both *C. latifolium* and *G. viscosissimum* feature no corolla tube so I measured flower depth from the point into which the bee extends its tongue (at the fused base of the anthers) to the nectary. I categorized flower species as being either shallow or deep (Table 2.3), to allow for analysis of tongue length as a nominal effect.

Table 2.1 Marginal cell and prementum lengths of six bumble bee species. Cells show mean \pm SD.

Bumble Bee Species	Marginal Cell Length (mm)	Premetum Length (mm)
<i>B. appositus</i>	3.69 \pm 0.316	4.46 \pm 0.468
<i>B. bifarius</i>	2.87 \pm 0.214	2.70 \pm 0.298
<i>B. californicus</i>	3.31 \pm 0.257	3.81 \pm 0.480
<i>B. flavifrons</i>	3.04 \pm 0.273	3.82 \pm 0.423
<i>B. mixtus</i>	2.78 \pm 0.216	2.67 \pm 0.164
<i>B. moderatus</i>	3.74 \pm 0.407	2.77 \pm 0.267

Table 2.2 Pooled floral density, distance flown, time in flight, and flight frequency (distance divided by year) for bumble bees foraging on eight flower species. Cells show mean \pm SD (n).

Flowering Plant Species	Floral Density (flowers/m ²)	Mean Distance (cm)		Flight Amount (%)	Flight Frequency (#Flights / Min)
		2012	2013		
<i>Cirsium arvense</i>	256.46 \pm 108.29 (64)	210.91 \pm 178.29 (26)	93.91 \pm 65.39 (38)	12.86 \pm 6.87 (64)	3.75 \pm 1.80 (64)
<i>Chamerion latifolium</i>	46.32 \pm 18.80 (19)	131.39 \pm 65.38 (19)	—	37.94 \pm 11.72 (19)	16.71 \pm 5.73 (19)
<i>Geranium viscosissimum</i>	7.25 \pm 2.42 (100)	469.96 \pm 130.31 (20)	242.78 \pm 102.75 (80)	35.44 \pm 8.52 (100)	13.72 \pm 3.36 (100)
<i>Linaria vulgaris</i>	129.26 \pm 79.78 (29)	212.95 \pm 128.39 (29)	—	20.31 \pm 9.72 (29)	6.08 \pm 3.00 (29)
<i>Melilotus alba / officinalis</i>	606.58 \pm 277.53 (118)	147.83 \pm 90.60 (53)	82.02 \pm 51.74 (65)	29.30 \pm 7.09 (118)	10.28 \pm 2.62 (118)
<i>Monarda fistulosa</i>	317.44 \pm 198.46 (83)	147.31 \pm 124.28 (47)	173.94 \pm 157.02 (36)	18.10 \pm 9.65 (83)	5.92 \pm 2.35 (83)
<i>Trifolium hybridum</i>	1535.95 \pm 977.16 (71)	131.13 \pm 102.09 (71)	—	28.69 \pm 9.16 (71)	7.63 \pm 2.03 (71)
<i>Trifolium pratense</i>	522.82 \pm 168.37 (49)	217.42 \pm 181.81 (49)	—	24.87 \pm 7.07 (49)	5.16 \pm 1.63 (49)

Table 2.3 Corolla depth (mean \pm SD) and corolla depth class for eight species of flowers (n=30 for all groups) Flower species were grouped as either Shallow or Deep based on their mean corolla depth.

Flowering Plant Species	Mean Corolla Depth Length (mm)	Corolla Depth Class
<i>Chamerion latifolium</i>	0.3 \pm 0.073	Shallow
<i>Melilotus alba / officinalis</i>	2.5 \pm 0.332	Shallow
<i>Trifolium hybridum</i>	3.5 \pm 0.341	Shallow
<i>Geranium viscosissimum</i>	5.5 \pm 1.517	Shallow
<i>Linaria vulgaris</i>	7.3 \pm 0.257	Deep
<i>Cirsium arvense</i>	8.9 \pm 0.820	Deep
<i>Trifolium pratense</i>	10.3 \pm 0.074	Deep
<i>Monarda fistulosa</i>	12.9 \pm 0.505	Deep

2.3.3 Observation of bumble bee behaviour

Worker bumble bees were observed foraging at flowers from 0800 to 1600 h in favourable weather conditions (temperature 9.5-33.0°C, mean 21.0°C; wind speed 0 to 19.0 km/h, mean 4.0 km / h; no rain). I timed individual bumble bees continuously for 4 minutes in 2012, and for 2 minutes in 2013 (refer to section 3.3). Following each timed bout, I netted the bumble bee and placed her in a clear plastic vial (2 cm diameter by 5 cm height) on ice to kill the bee and process her at the end of the day. During these observations, I stood at a distance of approximately one meter from the foraging bee so as not to disturb it.

During timed foraging bouts, I measured total time spent walking or handling flowers (i.e., non-flight) using a stop-watch, and frequency of flights with a counter. Time in flight (% flight) was calculated as 100 minus % non-flight, and flight frequency was calculated as the number of flights per minute. An assistant used a metal stake flag (stake length = 60 cm, flag dimensions 10 cm by 13 cm) to record the location of the inflorescence being visited by the bee at the start of the observation period, and at four equally spaced times over the bout (i.e., every minute in 2012, every thirty seconds in 2013), as signalled by a metronome. If the bee was in flight at the metronome signal, I marked the next inflorescence it visited. After the foraging bout,

the distance between the five marked inflorescences was measured to indicate the mean distance flown. For each of these marked inflorescences, a circular 1 m² hoop was positioned with the focal inflorescence at its centre, and the number of inflorescences (later adjusted to the number of flowers by multiplying by mean inflorescence size) was counted to estimate floral density (Table A.1.3).

2.3.4 Measurement of wing area

I weighed captured bees to the closest 1 mg using an Ohaus Adventurer Pro AV 53 balance (Ohaus, Ontario, Canada). Bumble bees collected in 2012 were frozen for later processing of their wing morphology. In the lab, the forewings of bees were removed, mounted between glass microscope slides, and photographed with an Olympus E-420 (10 megapixel) digital camera using a 2.5 X extension tube mounted on a Zeiss Stemi SV6 Dissecting Scope set at 0.85 power magnification. All photographs of bee forewings used the same focal length. Bumble bees collected in 2013 were put on ice for ten minutes to reduce activity and photographed in the field, after which I marked each bee on the thorax with different colour combinations using enamel paint (maximum of two colours) and then released them (see section 3.3.3). The forewings of torpid bumble bees were clamped between two microscope slides placed on a mount (5.5 cm distant from the camera), and photographed using a Panasonic DMC-FS7 Lumix Camera (10 megapixels) set at a focal length of 5.5 mm.

Marginal forewing area (Figure 2.1) was used to quantify wing wear, because wear and tear primarily occurs along the wing margins (Foster and Cartar 2011a). The image of the marginal forewing was cropped and extracted from the background using the magnetic lasso function in Adobe Photoshop CS5 (V 12.1). ImageJ (V1.47) was then used to threshold and quantify the marginal forewing area.

To quantify amount of wing wear of wing-worn bees whose original wing areas I did not observe, I needed to estimate area of a pristine marginal forewing and then subtract the observed marginal forewing area. I used the marginal cell (Figure 2.1) to predict marginal areas of pristine-winged bees using linear regression of ln-transformed variables (Figure 2.2). The interaction between bumble bee species and marginal cell was initially included in the model, but I removed the interaction as it was non-significant ($p = 0.46$). Removing bumble bee species from the model ($N = 107$, $p < 0.0001$, $R^2=0.95$) lowered the variation accounted for by one percent. The length of the marginal cell also predicts bee body mass ($n=414$, $p < 0.0001$, $R^2=0.91$) and larger bees have a longer marginal cell. Because the length of the marginal cell varies less than bee mass (given variation in the load sizes of bees), I used length of the marginal cell in all statistical analyses as a proxy for bee mass.

2.3.5 Measurement of prementum length

Measuring tongue length of a dead bee is difficult, but the prementum is an inflexible element that is easily measured, and is strongly correlated with the whole length of the tongue (Medler 1962; Morse 1977). Therefore I used prementum length as a proxy for tongue length. I was only able to measure the prementums of preserved bees collected in 2012 (I did not collect bees in 2013) so for 2013 bees I used a reference portion of the bee's wing to predict prementum length. Because bees of different body sizes and species have different tongue lengths (Harder 1985; Peat et al. 2005) I used the length of the marginal cell as the reference portion to predict prementum length (Morse 1977). I used an ANCOVA to predict prementum length (ln[mm]) from marginal cell length (ln[mm]) and bee species (nominal effect) (Overall model $F_{6,50} = 88.21$, $p < 0.0001$, $R^2=0.91$; Figure 2.3). There was no interaction between bee species and

marginal cell length, so the slope of the body size-tongue length relationship was the same for all species.

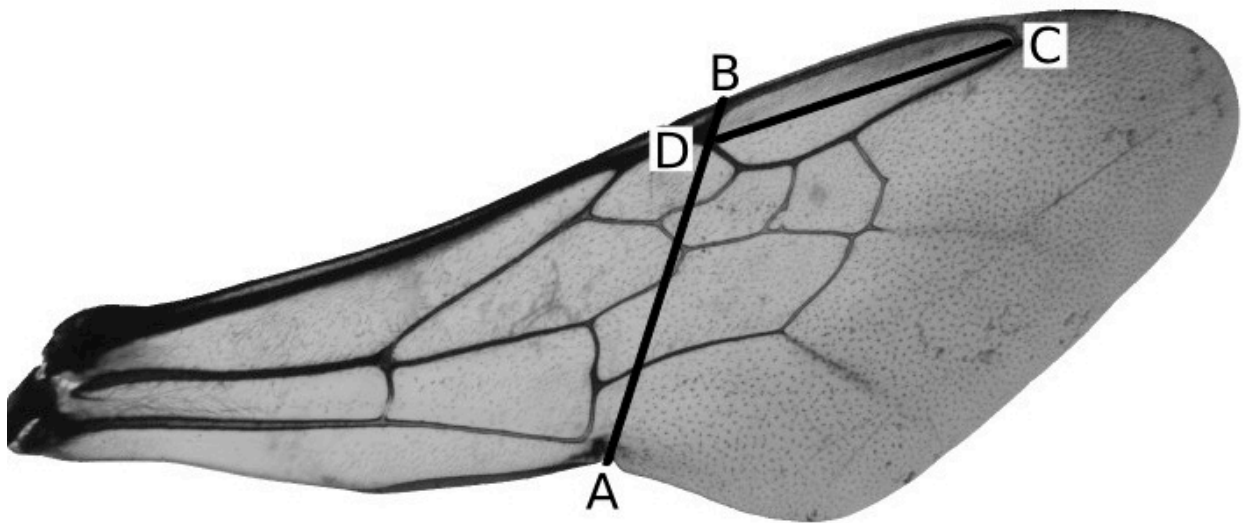


Figure 2.1 Bumble bee (*Bombus bifarius*) forewing. The marginal forewing is distal to the line passing through the center of the wing notch (A) and the base of the marginal cell (B). The length of the marginal cell is depicted by the line between the cell's tip (C) and its base (D)

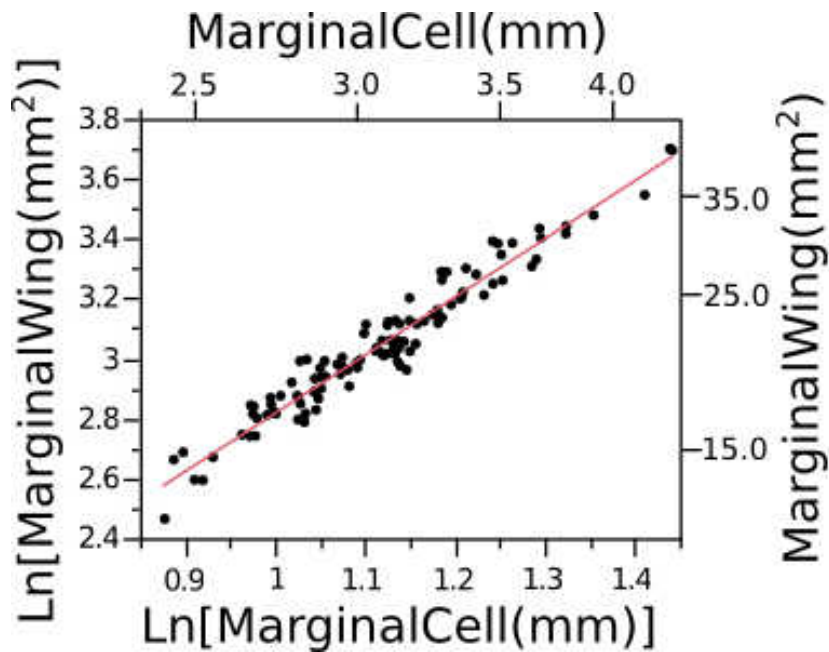


Figure 2.2 Prediction of Marginal Wing Area ($\ln[\text{mm}^2]$) from Marginal Cell Length ($\ln[\text{mm}]$) ($N = 107$, $p < 0.0001$, $R^2=0.94$). The regression coefficient (\pm SE) is 1.94 ± 0.046 .

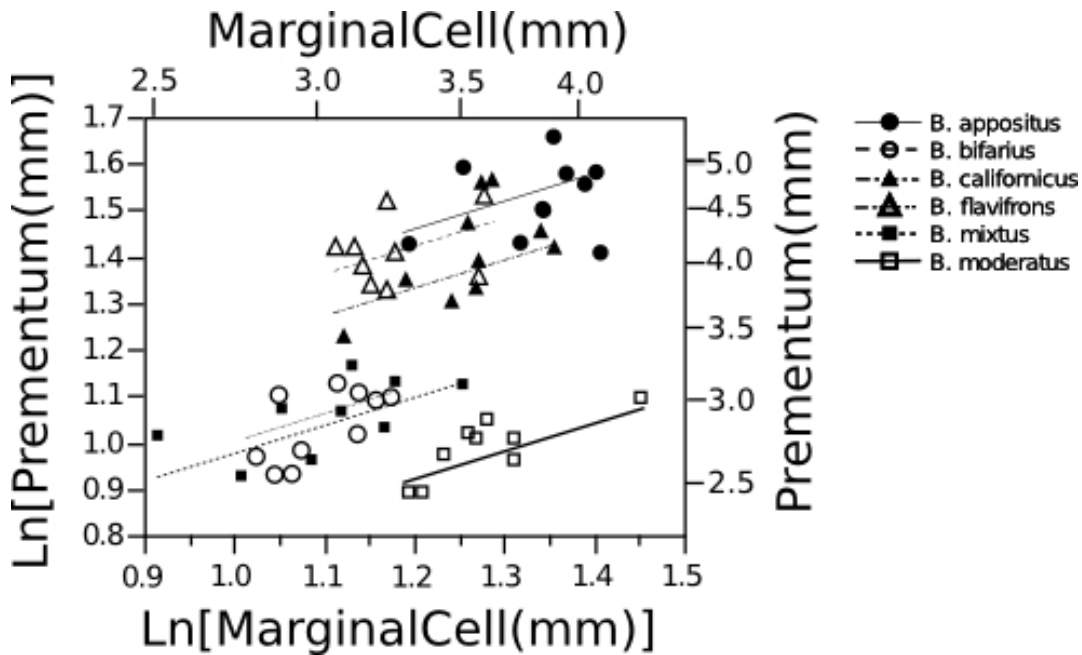


Figure 2.3 Prediction of prementum length ($\ln[\text{mm}]$) from marginal cell length ($\ln[\text{mm}]$) and bee species ($N = F_{6,50} = 88.21$, $p < 0.0001$, $R^2=0.91$).

2.3.6 Statistical Analysis

2.3.4.1. Influence of wing wear on bumble bee foraging behaviour

I used Generalized Linear Mixed Models to analyze bumble bee foraging behaviour in response to naturally occurring wing wear. The four variables of bumble bee foraging behaviour were % flight, flight frequency (#/min), mean distance flown (cm), and floral density (flowers/m²). I used a gamma distribution with a log link function to analyze % flight, flight frequency, and mean distance flown, and a negative binomial distribution with a log link function to analyze floral density.

All four Generalized Linear Mixed Models considered the independent variables: flower species visited (FSV; fixed effect, 8 levels), year (fixed effect, 2 levels), length of marginal cell (LMC; covariate), % wing wear (% WW; covariate), % WW*FSV, and bee species (random effect). I also checked for all possible interactions, except those involving random effects. Non-significant interactions were removed to increase error degrees of freedom.

2.3.4.2 Influence of wing wear on choice of flower species (measured as corolla depth class)

Analysis of bumble bee visitation with respect to class of corolla depth, shallow (LS mean \pm SE; 3.56 ± 0.102) and deep (10.44 ± 0.119) was conducted using a Multinomial Logistic Model. Plant species was not nested in corolla depth. The model explained corolla depth (Shallow, Deep) from the effects: prementum length (PL; covariate), % WW (covariate), and the interaction between PL*% WW. Year (fixed effect, 2 levels), % flight (covariate), flight frequency (covariate), distance flown (covariate), floral density (covariate) were included in the model to account for differences between species of flower and bumble bee foraging behaviour on different species of flowers. I also included julian day (covariate) of observation, nested within year to account for seasonal effects. I also included julian day squared (covariate) in my

original model to allow for control of non-linear seasonal effects, but removed the variable from the model as it did not improve model fit. Because marginal cell length was used to predict prementum length, I excluded marginal cell from the analysis to avoid issues of multicollinearity.

I used JMP (V10.1) to fit all models except for the generalized linear models, for which I used the GLIMMIX procedure of SAS (V9.3). The residuals of all model fits were examined to verify their normality and homogeneity. Variables were transformed with Box-Cox transformations as needed, to ensure normality and homogeneity of the residuals. Results for p-values between 0.5 and 0.1 were considered marginally significant in my analysis.

2.4 Results

2.4.1. Flight Behaviour of Bumble Bees

The percent of time that bumble bees spent in flight while foraging (% flight, or flight amount) in response to wing wear depended on the species of flower (Table 2.4). The CWW hypothesis predicts that bees with worn wings use their wings less. Support for this hypothesis from flight amount was equivocal. Bumble bees with higher wing wear spent more time in flight when foraging on *C. arvensis*, and less time when foraging on *M. fistulosa* (Table A.1.4; Figure 2.4). Wing wear also marginally increased flight amount on *T. hybridum* (Table A.1.4; Figure 2.4). The CWW hypothesis was supported by flight frequency. Bumble bees with higher wing wear decreased their flight frequency, regardless of the flower species on which they were foraging (Table 2.5; Figure 2.5). The flight frequency of bumble bees differed between years: bees in 2012 had a higher flight frequency (mean \pm SD; 2.055 ± 0.034) than bees in 2013 (1.976 ± 0.041 ; Table 2.5).

Table 2.4 Effect of wing wear on % flight in foraging bumble bees (N = 528). A generalized linear mixed-model was fit, with bumble bee species as a random effect. Terms that are statistically significant are in boldface. Error degrees of freedom were 510.

Source	Directionality	Numerator df	F	P
% Wing Wear	Decrease	1	0.10	0.7577
Flower Species	N/A	7	32.46	< 0.0001
Marginal Cell Length	Decrease	1	4.63	0.0319
Year	N/A	1	0	0.9449
% Wing Wear * Flower Species	N/A	7	4.40	< 0.0001

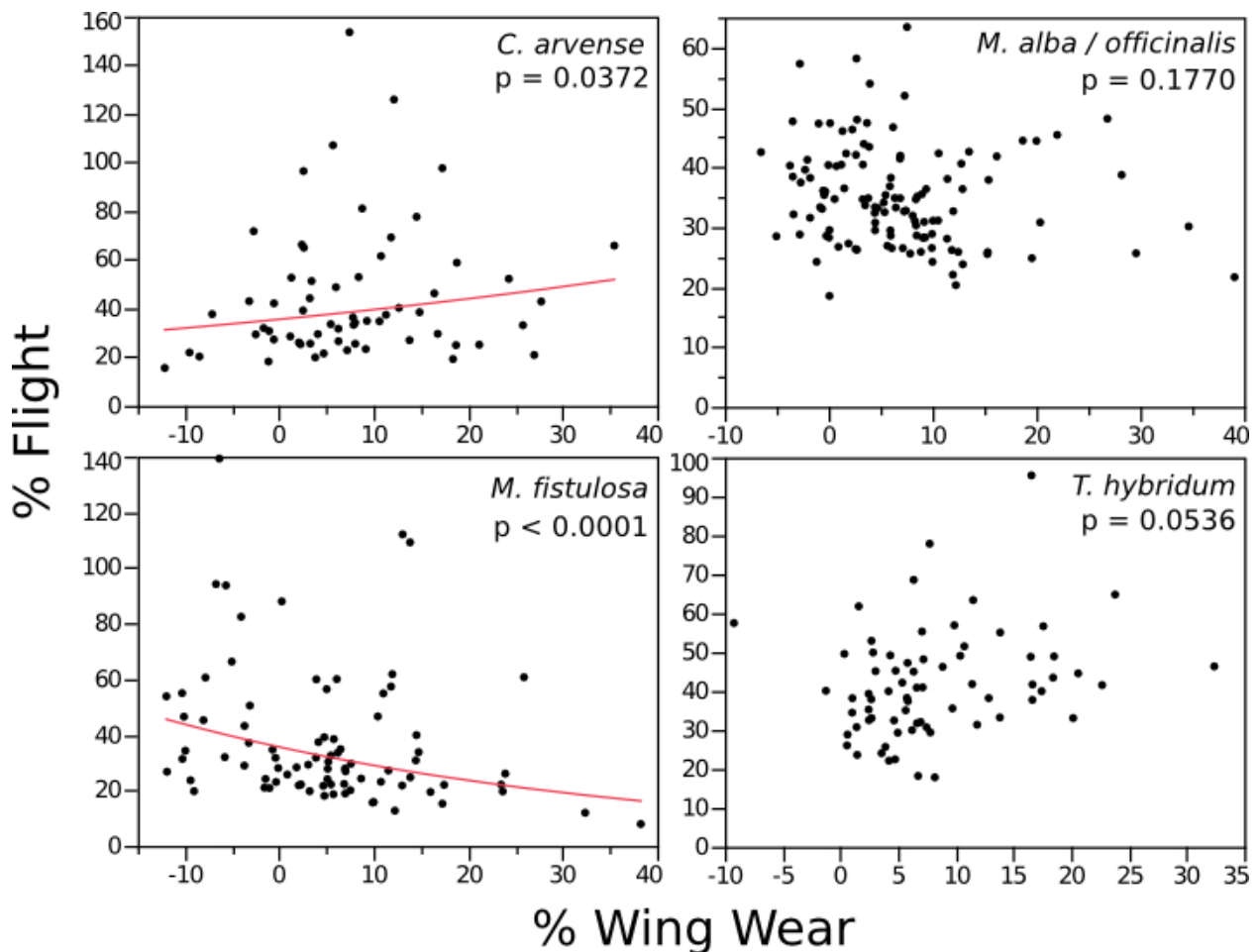


Figure 2.4 Partial regression visualization of the generalized linear model fit predicting % flight from % wing wear separately for flower species. Model details are presented in Table 2.4. The 4 flower species whose regression P values exceeded 0.2 are not graphed.

Table 2.5 Effect of wing wear on flight frequency (#flights / min) in foraging bumble bees (N = 528; generalized linear mixed-model bumble bee species is a random effect). Terms that are statistically significant are in boldface.

Source	Directionality	Numerator df	Denominator df	F	P
% Wing Wear	Decrease	1	463.6	6.13	0.0137
Flower Species	N/A	7	355.3	96.69	< 0.0001
Marginal Cell Length	Increase	1	70.15	7.18	0.0092
Year	N/A	1	486.8	4.40	0.0364

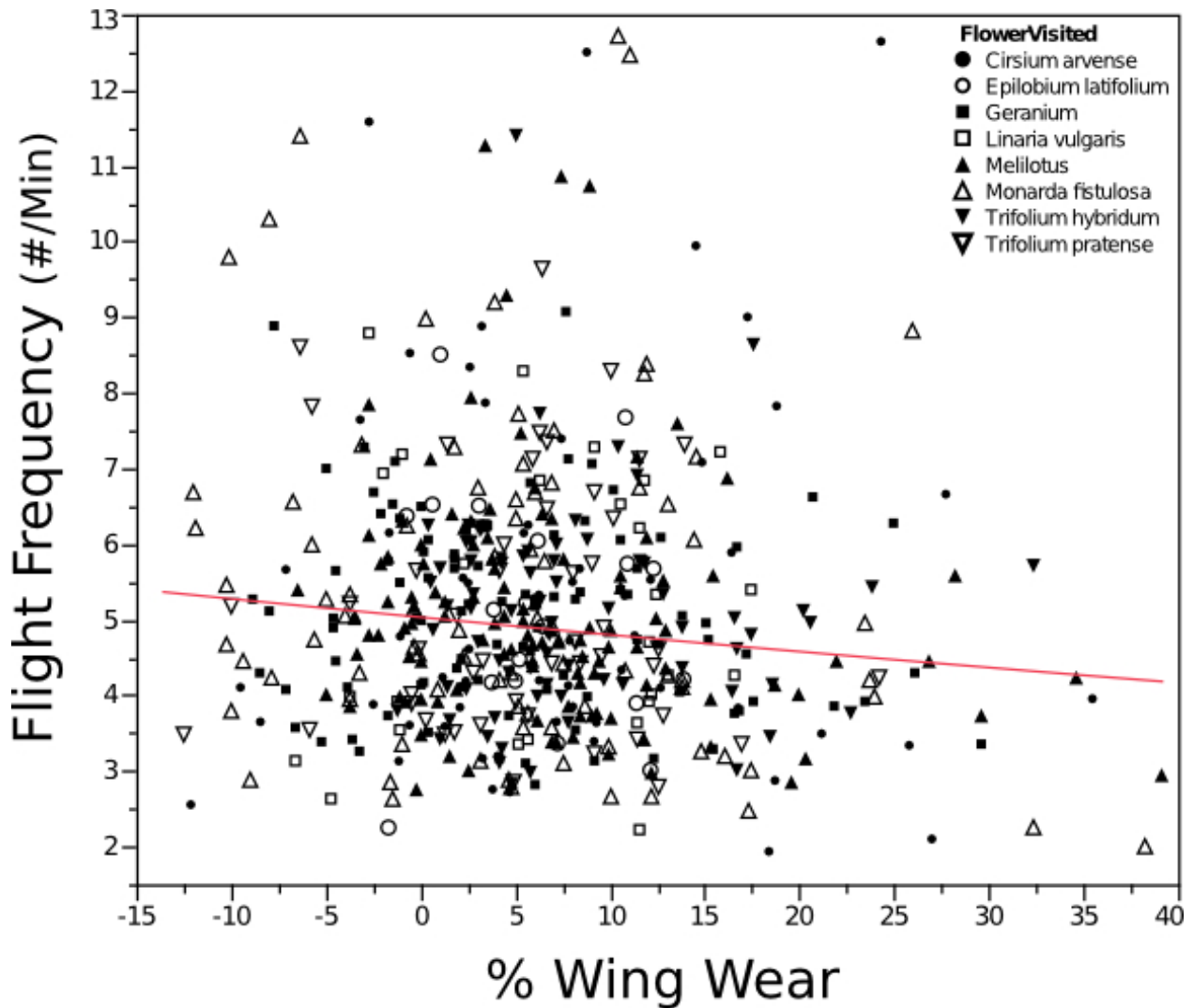


Figure 2.5 Partial regression visualization of the model fit predicting flight frequency (#flights / min) from % wing wear. Table 2.5 shows the fitted model

Support for the CCW hypothesis was marginal for distance flown. The mean distance flown by foraging bees [measured every sixty (2012) and thirty (2013) seconds] differed according to flower species (Table 2.6). Wing-worn bumble bees flew shorter distances on *M. fistulosa* (Table A.1.5; Figure 2.6). Bumble bees flew further in 2012 (mean \pm SD; 5.290 ± 0.069) than they did in 2013 (4.730 ± 0.084 ; Table 2.6), as expected when distance was measured over different time intervals in the 2 years. Among year differences in distance flown likely reflect an artefact of data collection, but the inclusion of the Year term in the statistical model corrects for this artefact, and allows for simultaneous examination of the ecological variables.

Table 2.6 Effect of wing wear on mean distance flown (cm) every 30 or 60 s in foraging bumble bees (N = 528, generalized linear mixed-model, bumble bee species is a random effect). Terms that are statistically significant are in boldface.

Source	Directionality	Numerator df	Denominator df	F	P
% Wing Wear	Decrease	1	476.8	0.27	0.6035
Flower Species	N/A	7	185.7	20.07	< 0.0001
Marginal Cell Length	Increase	1	79.43	3.94	0.0506
Year	N/A	1	49.23	49.23	< 0.0001
%Wing Wear *Flower Species	N/A	7	465.2	2.24	0.0297

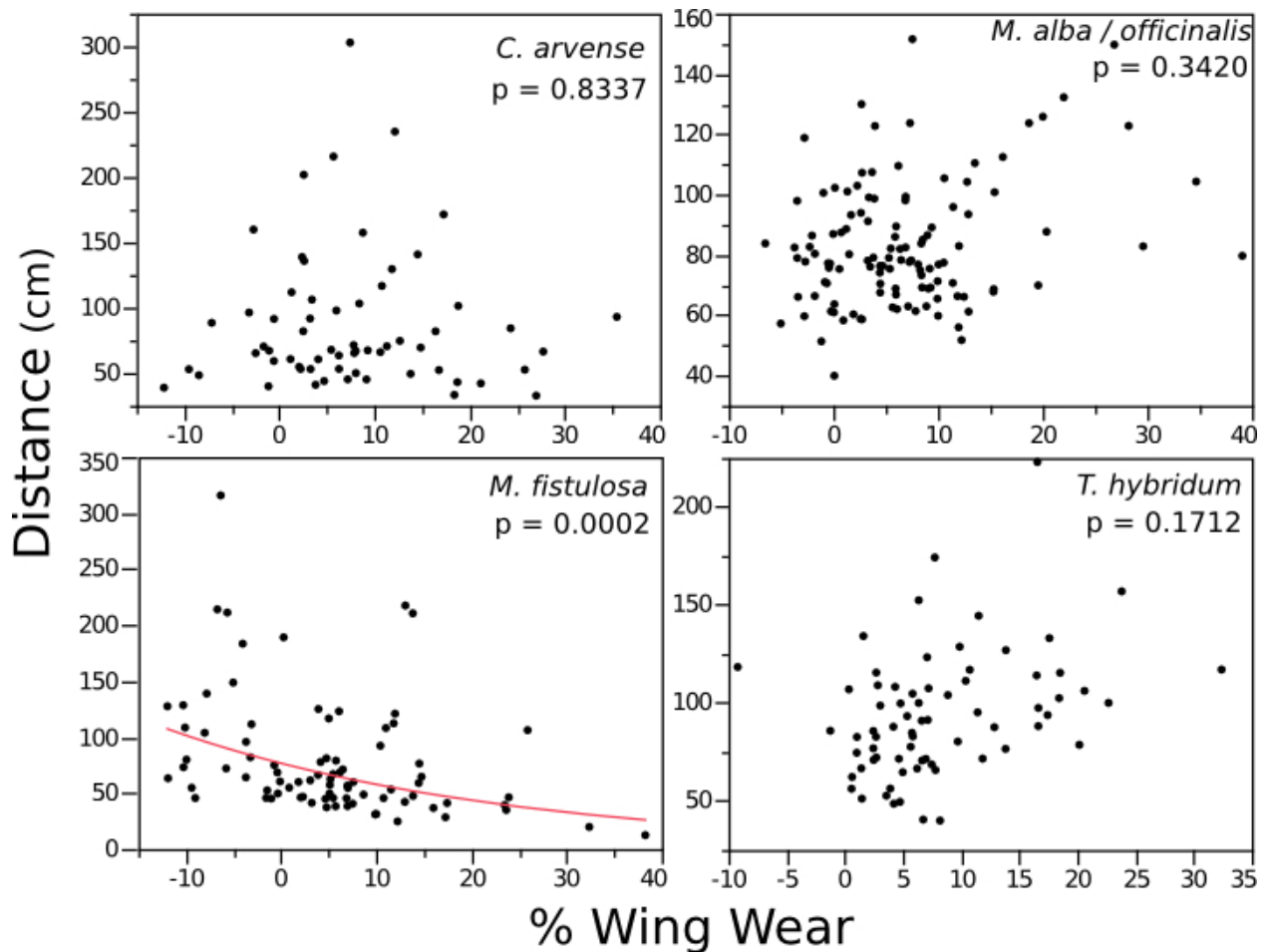


Figure 2.6 Partial regression visualization of the generalized linear model fit predicting mean distance flown (cm) from the interaction between % wing wear and flower species. Model details are presented in Table 2.6. The 4 flower species whose regression P values exceeded 0.2 are not graphed.

Support for the CCW hypothesis was equivocal for choice of floral density. Bumble bees foraging on *C. arvensis* and *M. fistulosa* foraged at higher floral densities as their wing wear increased (Table A.1.6; Figure 2.7), but were marginally likely to forage at lower floral densities on *M. alba / officinalis*, and *T. hybridum* (Table A.1.6; Figure 2.7). Bumble bees in 2013 foraged at higher floral densities (mean \pm SD; 5.362 ± 0.054) than did bumble bees in 2012 (5.202 ± 0.033 ; Table 2.7). However, I controlled for year in the model so the partial effects shown in Figure 2.7 are adjusted to account for differences between years.

Table 2.7 Effect of wing wear on mean density (#flowers / m²) in foraging bumble bees (N = 528, generalized linear mixed-model, bumble bee species is a random effect). Terms that are statistically significant are in boldface. Error degrees of freedom were 510.

Source	Directionality	Numerator df	F	P
% Wing Wear	Decrease	1	0.15	0.6956
Flower Species	N/A	7	439.43	< 0.0001
Marginal Cell Length	Decrease	1	0.00	0.9840
Year	N/A	1	4.88	0.0275
% Wing Wear *Flower Species	N/A	7	510	0.0009

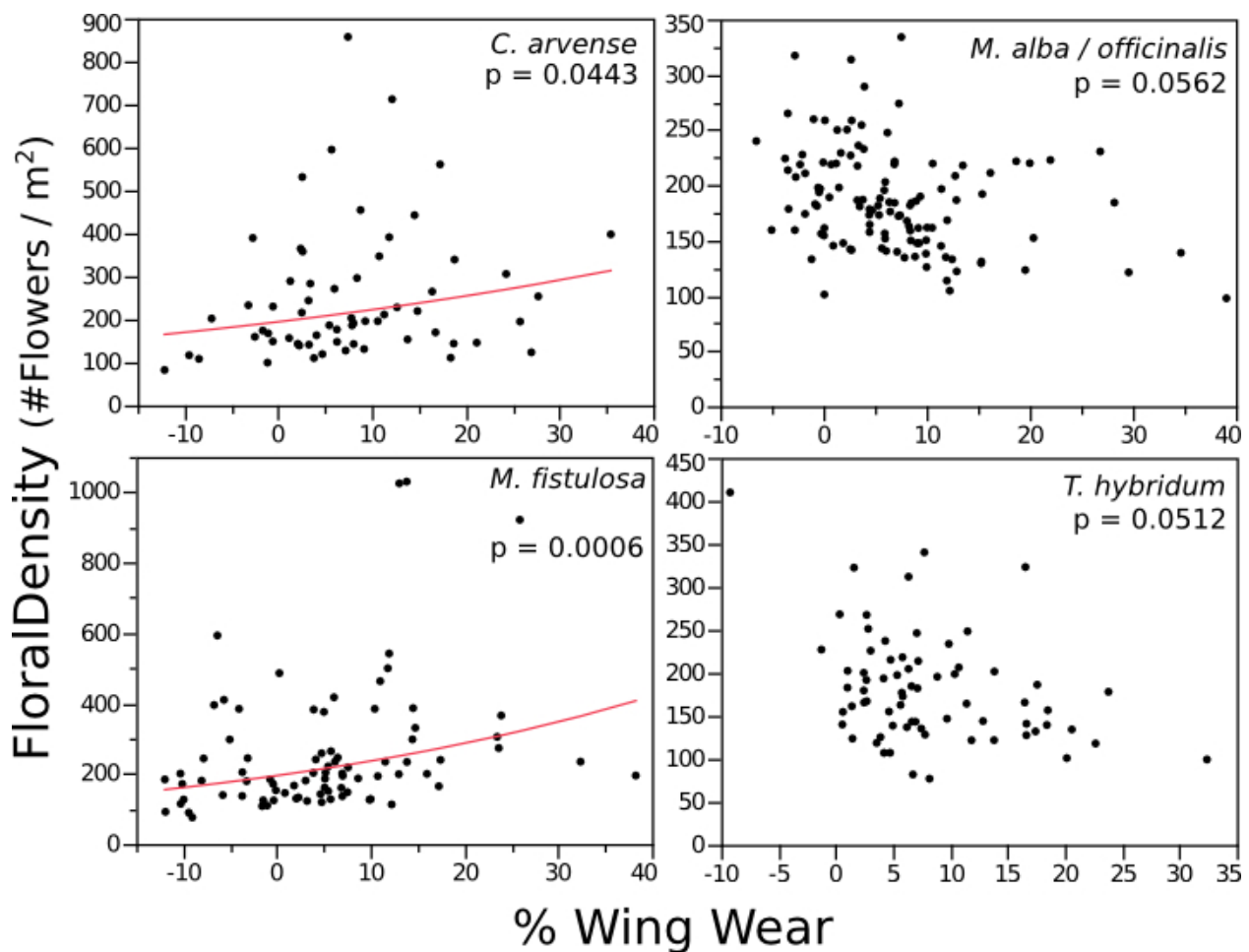


Figure 2.7 Partial regression visualization of the generalized linear model fit predicting mean floral density (#flowers / m²) from the interaction between %wing wear and flower species. Model details are presented in Table 2.7. The 4 flower species whose regression P values exceeded 0.2 are not graphed.

Table 2.8 Support of the data on the cost of wing wear hypothesis for each wing use variable.

	Cost of Wing Wear Hypothesis
Flight Time	Equivocal
Flight Frequency (#/min)	Supported
Mean Distance (cm)	Marginal
Floral Density (#/m ²)	Equivocal

2.4.2. Bumble Bee Choice of Flower Species

I predicted that wing-worn bees should increase their use of flower species with deep corollas, particularly if they have the longer tongues needed to successfully forage on these flowers. Corolla depth of flowers chosen by foraging bumble bees was marginally predicted by an interaction between prementum length and % wing wear (Table 2.9), but in a manner opposite to what I predicted. Long-tongued bumble bees visited deep flowers, but with more wing wear they were more likely to visit species with shallow flowers (Table 2.10). Short-tongued bumble bees visited shallow flowers, but with more wing wear they were more likely to visit deep flowers (Table 2.11). Bumble bees foraging on deep flowers were also more likely to fly for shorter periods of time, fly less often, and fly greater distances (Table 2.9).

Table 2.9 Effect of wing wear on choice of flower species based on corolla depth (fixed effect, 2 levels) in foraging bumble bees. In this model, a positive sign is associated with choice of shallow corollas. A multinomial logistic model was fit (N = 514, $p < 0.0001$, $R^2 = 0.78$) and terms that are statistically significant are in boldface.

Source	Choice (Deep / Shallow)	Estimate	Standard Error	ChiSquare	Prob > ChiSq
Julian[Year]	N / A	0.215	0.308	0.49	< 0.0001
Year	N / A				0.0087
Flight Time	+	-0.168	0.037	21.06	< 0.0001
Flight Frequency ^ (1/2)	+	-4.419	0.635	48.39	< 0.0001
Distance ^ (1/2)	-	0.365	0.075	23.47	< 0.0001
Ln(Floral Density)	+	-0.125	0.180	0.48	0.3796
Prementum Length	-	0.011	0.002	40.73	< 0.0001
%Wing Wear	+	-0.032	0.028	1.29	0.8004
%Wing Wear*Prementum Length		-0.0004	0.0002	4.63	0.0592

Table 2.10 Likelihood of visiting shallow flowers, as jointly influenced by % wing wear and prementum length. Model details are presented in Table 2.8. Probabilities are expressed for small (2.5%), median (50%), and large (97.5%) levels of each factor.

		Prementum Length		
		Short	Medium	Long
%Wing Wear	Low	0.999	0.935	0.000
	Medium	0.991	0.902	0.006
	High	0.908	0.834	0.419

Table 2.11 Likelihood of visiting deep flowers, as jointly influenced by % wing wear and prementum length. Model details are presented in Table 2.8. Probabilities are expressed for small (2.5%), median (50%), and large (97.5%) levels of each factor.

		Prementum Length		
		Short	Medium	Long
% Wing Wear	Low	0.001	0.065	1.00
	Medium	0.009	0.098	0.994
	High	0.092	0.166	0.581

To determine if long-tongued bumble bees use their wings less on flowers with shallow corollas and short-tongued bumble bees use their wings less on flowers with deep corollas, I tested the effects prementum length and corolla depth had on % flight and flight frequency. I found long-tongued bumble bees flew shorter times and less frequently on shallow flowers than did short-tongued bumble bees (Figure 2.8 and Figure 2.9). Short-tongued bumble bees flew less and less frequently on deep flowers than did long-tongued bumble bees (Figure 2.8 and Figure 2.9).

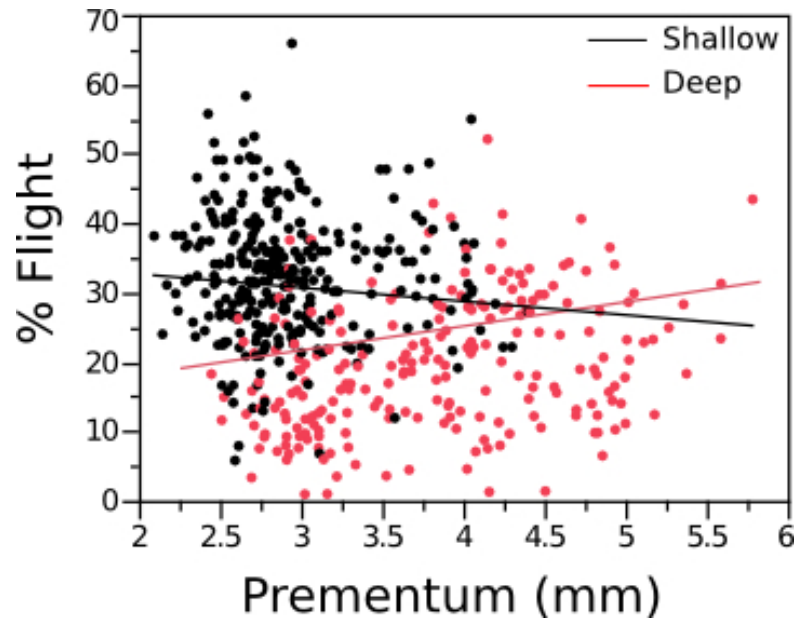


Figure 2.8 Partial regression visualization of the general linear model fit predicting % flight ($N = 535$, $R^2 = 0.41$) from the interaction between prementum length and corolla depth (2 levels; $p < 0.0001$). Bumble bee species is included in the model as a random effect.

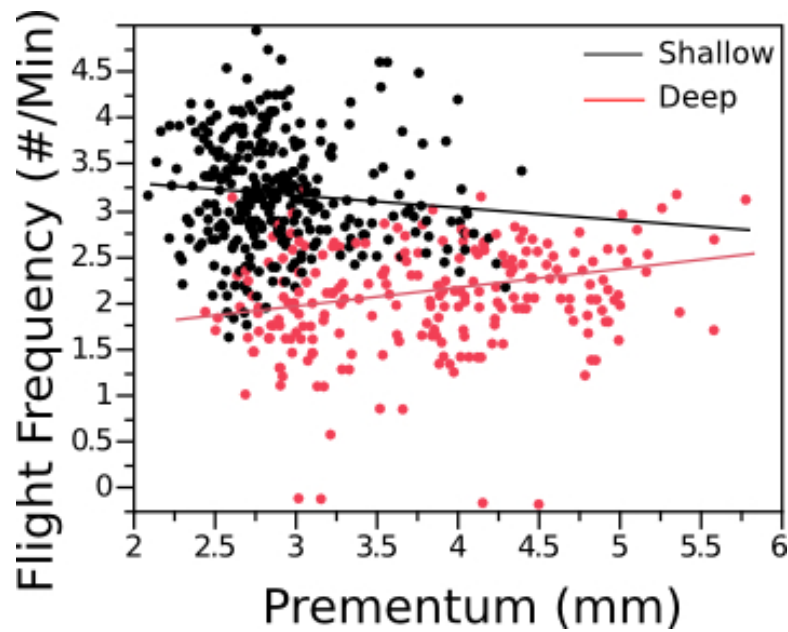


Figure 2.9 Partial regression visualization of the general linear model fit predicting flight frequency (# / min; $N = 535$, $R^2 = 0.50$) from the interaction between prementum length and corolla depth (2 levels; $p = 0.0030$). Bumble bee species is included in the model as a random effect.

2.5 Discussion

2.5.1 Influences of wing wear on wing use and same-species choices

Overall, the prediction that wing-worn bumble bees would have reduced flight times relative to pristine-winged bees was poorly supported (Table 2.8). Wing-worn bumble bees flew less on *M. fistulosa* but more on *C. arvensis* and marginally more on *T. hybridum*. There was no detectable effect of wing wear on flight amount for bumble bees foraging on *C. latifolium*, *G. viscosissimum*, *L. vulgaris*, or *T. pratense*. That is, depending on the plant species, the hypothesis received support (1 species), rejection (2 species), and lack of support (4 species). Considering all effects together (weighting partial regression coefficients by their t value), flight amount overall was not influenced by wing wear (mean coefficient \pm SE; -0.00025 ± 0.00402).

It is unclear why wing-worn bumble bees foraging on *C. arvensis* and *T. hybridum* flew more than pristine-winged bees. One possible explanation is that the amount of wing use varies on different flower species. Workers foraging on *C. arvensis* have short flight amounts (Table 2.3) and therefore should incur minor elevations in their flight costs from using their wings more, such that the costs of wing wear in terms of flight amount may be negligible. If this is the case, then I would expect to see no effect of wing wear on flight amount for *C. arvensis*. However, workers flew more with increasing wing wear. I used partial correlations to test if wing wear was correlated with flight amount controlling for the other wing use variables (i.e. flight frequency, distance, floral density) and marginal cell length. Wing wear was not correlated with flight amount for *C. arvensis* ($p = 0.208$), suggesting that wing-worn bumble bees are not flying more on *C. arvensis*. Similarly, when considering all wing use variables and marginal cell, flight amount did not increase with wing wear on *T. hybridum* ($p = 0.93$).

Another possibility is that bumble bees accumulate less wing wear over time on *C. arvensis* and *T. hybridum*. Inflorescences of *C. arvensis* and *T. hybridum* are presented upright away from surrounding vegetation that might impede between-flower flight (pers. obsv). Because bumble bees acquire wing wear from hitting their wings against the vegetation while foraging (Foster and Cartar 2011a), they incur less wing wear on *C. arvensis* and *T. hybridum* (Foster unpublished). Therefore, workers foraging on these two species of flower may only incur minor elevations in wing-worn flight costs caused by small amounts of wing wear, such that the costs of wing wear are negligible.

Wing-worn bumble bees flew less frequently, supporting the CWW hypothesis (Table 2.8). It is likely that wing-worn foraging has greater energetic costs. Hedenström et al. (2001) measured the metabolic rate of bumble bees with artificial wing wear (10% reduction in wing area) induced to hover in a flight chamber. Metabolic rate was unrelated to wing wear, though wing-beat frequency increased with wing wear. However, bumble bees forage in complex environments that are markedly different from a flight chamber. Bumble bees must maintain appropriate thoracic temperatures that are usually higher than ambient temperature (Heinrich 1972), compensate for the weight of their nectar load (bees storing nectar have a 3°C higher thoracic temperature (Heinrich 1975a), manoeuvre between vegetation obstacles and through wind gusts in the flower patch, and possibly adjust to changes in air turbulence (see Ortega-Jimenez *et al.* 2014 for hummingbirds). These factors may account for higher metabolic costs in complex environments to sustain continuous flight that may not be realized in a flight chamber (Wolf *et al.* 1999; Ortega-Jimenez *et al.* 2014). Therefore, workers that experience higher wing-loading as a result of wing wear may need to increase their metabolic rate to sustain continuous foraging in complex environments. It would be of interest for future research to measure

metabolic rates of bumble bees foraging in the wild using the doubly labelled water technique (Wolf 1999), in relation to wing wear.

Perhaps wing-worn bumble bees reduced their flight frequency to minimize future wing wear. Bumble bees acquire wing wear from hitting their wings against the vegetation while foraging (Foster and Cartar 2011a), including during take-off. Bumble bees may reduce the amount of wear and tear to reduce future loading costs of wing wear, because each 1% loss of wing area results in a decrease in load size of 5% (Johnson and Cartar 2014) and would mean smaller nectar load sizes per foraging trip. Wing wear also changes flight performance by reducing manoeuvrability (Haas and Cartar 2004), and workers may reduce acquisition of wing wear to maintain manoeuvrability in the flower patch, especially if the incidence of predation is higher (Cartar 1992a). While I cannot test this idea in the present study, which would involve repeated measurements of individuals, it would be of interest in future studies to investigate whether less wing-use leads to less wing wear.

As with flight amount, the two variables indirectly related to wing use (i.e. distance flown and choice of flower density) were equivocal in their support of the hypothesis that wing wear is costly while foraging (Table 2.8). Wing worn bees flew shorter distances than pristine-winged bees in *M. fistulosa*, and foraged at higher floral densities in *C. arvensis* and *M. fistulosa*. However, wing-worn bees also flew marginally longer distances and foraged at lower flower densities in *M. alba / officinalis*, and *T. hybridum*. Using partial correlation to consider the other wing use variables and marginal cell length, workers flew greater distances and foraged at lower floral densities on *M. alba / officinalis* ($p = 0.0020$ and $p = 0.0127$ respectively), but showed no response on *T. hybridum* ($p = 0.4438$ and $p = 0.1247$ respectively) with wing wear. The different responses to wing wear depend on a few flower species; however, distance flown and floral

density are not overly influenced by wing wear (weighting partial regression coefficients by their t values; mean coefficient \pm SE; distance flown: -0.00286 ± 0.00492 ; flower density -0.00164 ± 0.00426). This weaker influence may be because bumble bees only indirectly reduce their wing use by flying shorter distances (Table A.1.7), and foraging in higher density flower patches (Table A.1.8), when direct effects (flight amount, flight frequency) may matter most.

2.5.2 Influence of wing wear on bumble bee choice of flower species

The prediction that long-tongued bumble bees with wing wear would shift to deeper flower species, while short-tongued bumble bees with wing wear will be less likely to make the shift to deeper flowers, was not supported. Instead, long-tongued bumble bees with wing wear were more likely to visit flower species with shallow corollas, and short-tongued bees with wing wear were more likely to visit flower species with deep corollas.

Why might long-tongued, wing-worn bumble bees have selected shallow flowers? Long-tongued bumble bees forage less efficiently on shallow flowers relative to their short-tongued competitors (Plowright and Plowright 1997). Therefore, if long-tongued bumble bees with wing wear forage on shallow corolla flowers, there must be some factor to compensate them for the reduced foraging efficiency, perhaps involving a reduction in wing use. Long-tongued bumble bees should fly for shorter times and fly less often foraging on shallow corolla flowers based on an increased handling time from their tongue of inefficient length (Plowright and Plowright 1997). In support of this speculation, long-tongued bumble bees flew less and less frequently on shallow flowers than did short-tongued bumble bees. The situation was reversed on deep flowers: long-tongued bumble bees flew more and more often on deep flowers than short-tongued bumble bees. Future studies should test if there is a trade-off between handling

efficiency and wing use to see if wing-worn bumble bees experience a reduced handling efficiency and offset this by reducing the costs of wing-worn flight.

2.6 Conclusions

I found wing-worn bumble bees differed in their foraging behaviour relative to pristine-winged bees, but not always in ways predicted by the CWW hypothesis. Wing-worn bumble bees flew less often, however, my results for flight times, distances, and floral density were ambiguous (response to wing wear was based on flower species). Overall, flight frequency supports the CWW hypothesis, and flight, distance, and floral density moderately supports the CWW hypothesis, but only once post hoc partial correlation analyses were performed. I also found wing-worn bees forage on shorter and deeper corolla flowers based on the length of their tongue, resulting in a reduction in wing use and further lending support to the CWW hypothesis, but again, this conclusion results from post hoc analyses following the non-supportive logistic model. It is yet to be documented if the incidence of predation is higher in wing-worn bumble bees (Cartar 1992a). I speculate that wing-worn foragers reduce their wing use to also minimize the accumulation of wear and tear, particularly if collisions with vegetation are more likely on take-off and landing, which would explain why wing-worn bees reduced flight frequency, but not flight time. Comparisons of foraging behaviour in wing-worn bumble bees relative to pristine-winged bees in terms of energy expenditure and minimizing the accumulation of further wear and tear merits further attention.

CHAPTER THREE: EFFECTS OF ARTIFICIAL WING WEAR ON WING USE BY WILD-FORAGING WORKERS

3.1 Abstract

Changes in foraging behaviour caused by wing wear may reflect present flight costs (concurrent wing wear hypothesis; ConWW hypothesis), or past wing wear (past wing wear hypothesis; PWW hypothesis). By artificially trimming bumble bee forewings that varied naturally in initial wing wear and therefore age of workers, I experimentally decoupled age and experience from current wing wear, to examine their joint effects on foraging behaviour. I found that bumble bees flew shorter distances after removal of wing area, whereas changes in flight frequency related to loss of wing area depended on the species of flower (supports ConWW hypothesis) and past wing wear (supports both hypotheses). Reduced wing use after bees had their wings trimmed supported the concurrent wing wear hypothesis, as effects of wing wear were independent of past wing wear and therefore worker age and/or experience. But past wing wear also affected wing use: bumble bees with high past wing wear flew less and less far on *C. arvensis*, supporting the PWW hypothesis. Overall, I found support for both hypotheses (concurrent and past wing wear), with a stronger signal from ConWW, suggesting foraging performance depends on both past and current state.

3.2 Introduction

Behaviour changes with experience, and such changes are ubiquitous in insects (reviewed by Papaj and Prokopy 1989; Dukas 2008). For temperate eusocial bees (i.e., bumble bees and honey bees) that rely on workers to provision the colony with resources and thereby obtain fitness (Schmid-Hempel *et al.* 1993; Goulson 2003), and whose workers experience a spatially

and temporally complex environment, learned foraging behaviour is particularly common. Bumble bees demonstrate improved foraging performance based on experience. Workers enhance their foraging rate after a learning period of at least 30 foraging trips (Peat and Goulson 2005), visit the most profitable flowers on successive foraging trips (Cartar 2004; Burns and Thomson 2005), and depart from foraging patches based on past experience (Biernaskie *et al.* 2009). Bumble bees also follow foraging routes which become more repeatable and efficient (Ohashi *et al.* 2007; Saleh and Chittka 2007); resulting in experienced foragers returning to flowers at regular intervals, travelling faster between flowers, and increasing their rate of nectar intake (Ohashi *et al.* 2008).

Current state also affects foraging performance. Worker bumble bees must maintain thoracic temperatures that are usually higher than ambient to fly while foraging (Heinrich 1972). Foraging is also influenced by competitors (Goulson *et al.* 1998), energetic state of the colony (Cartar 1992b), and morphology, particularly the match between a bumble bee's tongue length and floral depth (Harder 1983; Plowright and Plowright 1997). In addition, wear of bumble bee wings influences wing use (Foster and Cartar 2011b; see Chapter 2), and has an effect on bumble bee foraging performance.

Wing wear likely increases rate of mortality (Cartar 1992a; Higginson *et al.* 2011; Dukas & Dukas 2011) and influences foraging behaviour (Higginson & Barnard 2004; Foster and Cartar 2011b; see Chapter 2). Flight is the most energetically expensive foraging activity that bumble bees perform (Heinrich 1975b; Ellington *et al.* 1990), and workers appear to reduce their wing use when faced with potentially higher costs of wing-worn flight (Foster and Cartar 2011b; See Chapter 2). The costs of wing wear leading to mortality remain unclear, however, wing wear likely leads to increased metabolic (speculated by Cartar 1992a), biomechanical (speculated by

Cartar 1992a; Haas and Cartar 2004), and loading (i.e., for every 1 % loss of wing area, 5 % loss of load lifted; Johnson and Cartar 2014) costs. Honey bees integrate information about their current foraging bout (i.e. wing-loading and distance between inflorescences) and, in the face of greater flight costs, reduce their wing use (Schmid-Hempel *et al.* 1985) and return to the colony sooner (Schmid-Hempel 1986). If wing-worn bumble bees experience higher flight costs, then like honey bees, they should reduce their wing use. Bumble bees whose colony is less needy of nectar reduce their wing use by flying shorter distances, flying less often, and spending more time walking on and probing inflorescences (Cartar and Dill 1990). Bumble bees with wing wear also reduce their wing use by foraging in higher density flower patches, allowing them to walk between inflorescences and fly shorter distances (Foster and Cartar 2011b).

Given that wing wear becomes more pronounced with age and cumulative foraging effort (Higginson and Barnard 2004; Foster and Cartar 2011a), changes in foraging behaviour in the face of wing wear may independently reflect past experience, including age [past wing wear (PWW) hypothesis] or present flight costs [concurrent wing wear (ConWW) hypothesis]. For the PWW hypothesis, individuals base present behaviour on past foraging investment (i.e., past wing use), akin to committing the “Concorde Fallacy” where current behaviour is based solely on how much an individual has already invested (Dawkins and Brockmann 1980). But, more generally, past experience over a lifetime is expected to inform present decisions (Dukas and Visscher 1994). However, past investment may also influence future investment such that past behaviour should affect present behaviour in individuals with a finite lifetime energy budget (see Fagerström 1982 for lifetime reproductive budget). If bumble bees are limited by their energy budget, then past wing use based on past wing wear should influence future foraging investment by having an effect on their current wing use. This would especially be the case if bumble bees

adopted cost-sensitive currencies that have a finite lifetime energy budget, such as foraging gain per wing beat (foraging gain per wing beat [foraging gain – cost / lifetime number of wing beats; Higginson and Gilbert 2004]). Based on this, wing-worn bumble bees that have a higher wing beat frequency (Hedenström *et al.* 2001) would exacerbate senescence and should choose behaviours that reduce wing use, thereby reducing foraging costs to maximize net foraging benefits for the colony (an idea developed in Chapter 2).

This study investigates the question: do foraging responses to experimentally induced wing wear reflect present and/or past wing wear (amount of wing wear prior to manipulation)? Given bee sensitivity to flight costs reviewed above, I expect present costs of wing wear to influence foraging behaviour. If wing wear reflects past experience and age, then I expect past wing wear to influence foraging behaviour. To distinguish these hypotheses (ConWW and PWW respectively), I experimentally trimmed bumble bees' forewings (mimicking natural wing wear) to experimentally decouple age and experience from wing wear, and observed changes in foraging behaviour. If wing wear reflects concurrent costs (ConWW hypothesis), I expect workers to reduce their wing use after wing trimming, particularly by decreasing their flight time, flying less frequently, flying shorter distances, and/or by choosing higher density flower patches. If wing wear reflects past costs (PWW hypothesis), I expect workers to use their wings based on the amount of wing wear prior to trimming. I test these hypotheses with worker bumble bees foraging in subalpine meadows, observing changes in their foraging behaviour after trimming their wings.

3.3 Methods

3.3.1 Study System

This study was conducted in the Sheep River Provincial Park, Kananaskis Country, Alberta (50.647°N, -114.648°W, elevation 1490 m) from July 1 – August 25, 2013. Six species of bumble bee were studied: *Bombus appositus*, *B. bifarius*, *B. californicus*, *B. flavifrons*, *B. mixtus*, and *B. moderatus* (Table A.2.1). Bumble bees were observed foraging on flowering *Cirsium arvense*, *Geranium viscosissimum*, *Melilotus alba / officinalis*, and *Monarda fistulosa* (refer to Section 2.3.1 and Table A.2.1 for details). The four plant species on which I observed bumble bees foraging differ in floral characteristics in ways that allow useful contrasts for the study of wing-worn foraging (refer to Section 2.3.1).

3.3.2 Bumble Bee Observations and Collection

I observed worker bumble bees foraging daily from 0800 to 1600 in favourable weather conditions (range 9.5-31.0°C, mean 20.0°C; wind speed 0.0 – 19.0 km / h, mean 3.5 km / h; no rain). I timed individual bumble bees continuously for two minutes before netting and placing them individually in a clear plastic vial (2 cm diameter by 5 cm height) to be processed in the field immediately.

During timed foraging bouts, I measured bumble bee foraging behaviour (see Section 2.3.2). On the same and subsequent days, I observed processed bees within the same flower patch, to test for changes in their foraging behaviour. On average, bees foraging behaviour was measured again in four days (re-sightings; Table A.2.2).

3.3.3 Processing of Individuals

Captured bees were put on ice for ten minutes to reduce activity. Their forewings were clamped between two microscope slides, placed on a mount (5.5 cm distant from the camera),

and photographed using a Panasonic DMC-FS7 Lumix Camera (10 megapixels) set at a focal length of 5.5 mm. I then removed bees from the clamp and trimmed both forewings using a pair of fine scissors (area removed mean \pm SD; 14.95 ± 5.88 %) to mimic natural wing wear (Figure 3.1). Bumble bees with high amounts of wing wear prior to wing trimming had a small portion of wing area removed (17.28 ± 4.75 %), in comparison to bees with small amounts of initial wing wear (14.04 ± 6.39 %). After trimming, I photographed forewings a second time to calculate % area loss based on a contrast between the first and second photographs. Bees were then marked on the thorax with colour patterns unique to each individual using Testors Enamel Paint (maximum of two colours), allowing individual recognition.

Every fourth bumble bee served as a control, processed identically to the wing loss treatment, except that no wing area was removed. After experimental and control bees were processed, they were allowed to warm up in the sun before being released to resume foraging activities.

3.3.3 Measurement of Wing Area and Wing Wear Score

I measured wing area using the procedures described in Chapter 2 for 2013 bees. Marginal forewing area (Figure 2.1) was used to quantify wing wear, because wear and tear primarily occurs along the wing margins (Foster and Cartar 2011a). I also quantitatively scored natural wing wear on a scale from 0 to 3 (Figure 3.2), with no wing wear = 0 (mean \pm SD area loss; -3.08 ± 3.94 %; $n = 39$), small nicks along the margins of their wings = 1 (area loss -1.51 ± 5.45 %; $n = 131$), at least one wing with continuous ragged margin = 2 (area loss 5.35 ± 6.58 %; $n = 121$), and deep gouges in the wing margin = 3 (area loss 21.61 ± 7.42 %; $n = 45$). Because of the low sample size in wear class 3, I grouped bumble bees with an initial wing wear score of 2

and 3 (area loss $9.81 \pm 9.95 \%$; $n = 166$) together for analysis of the effects wing area removal has on foraging behaviour.

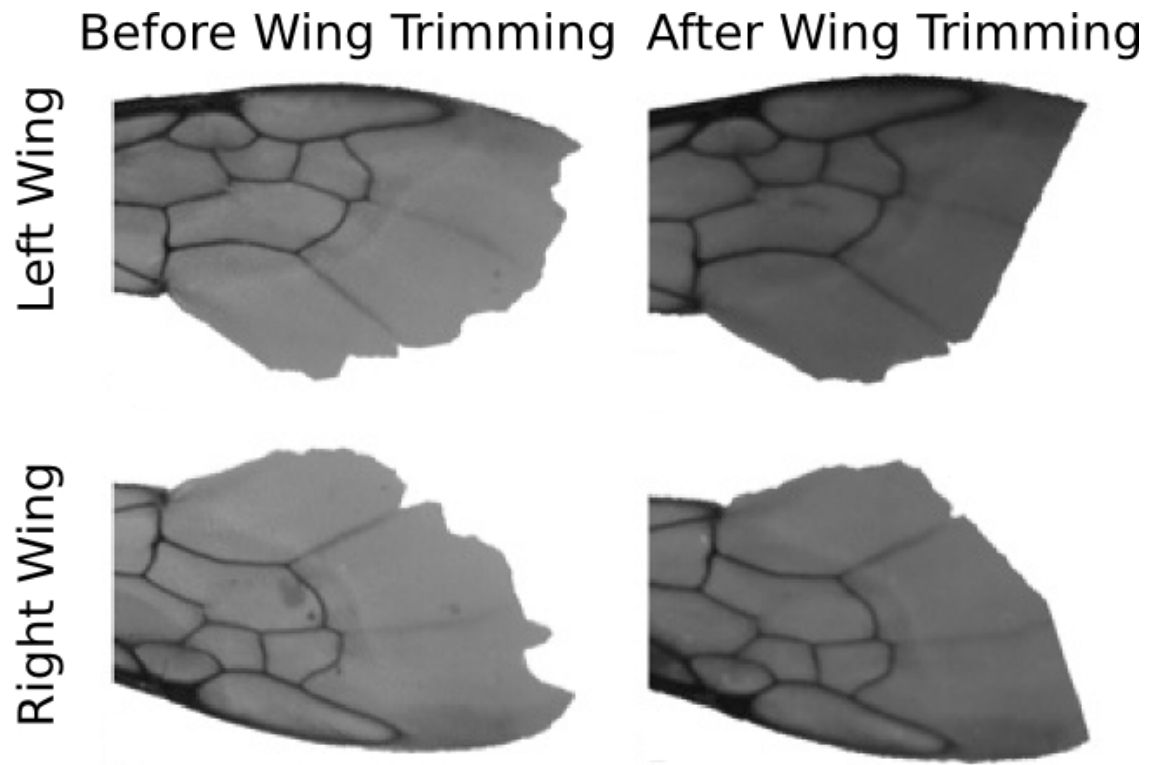
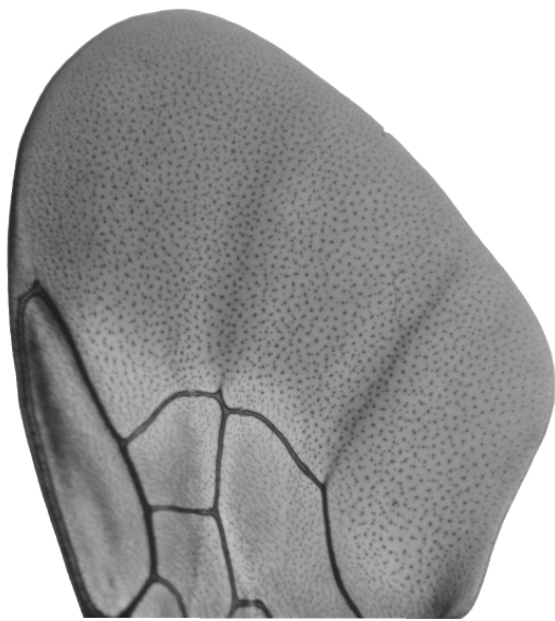
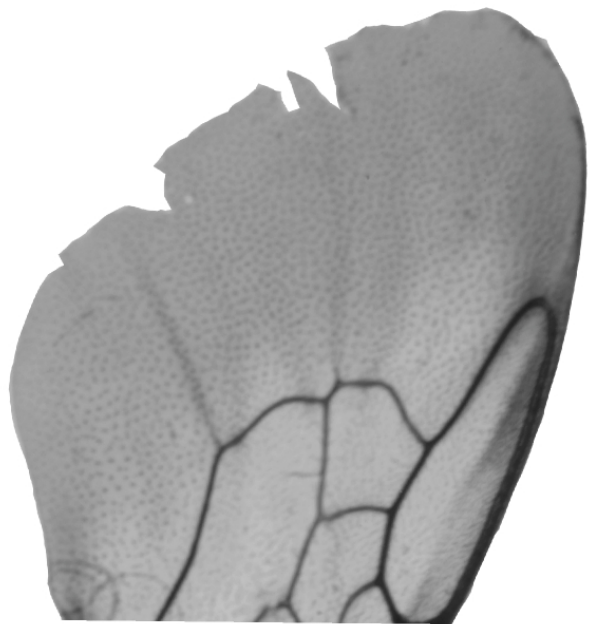


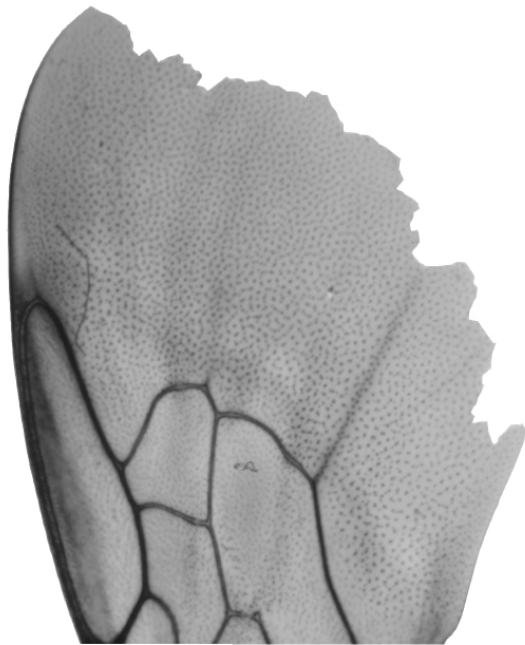
Figure 3.1 Bumble bee (*Bombus melanopygus*) left and right forewings before and after wings were cut.



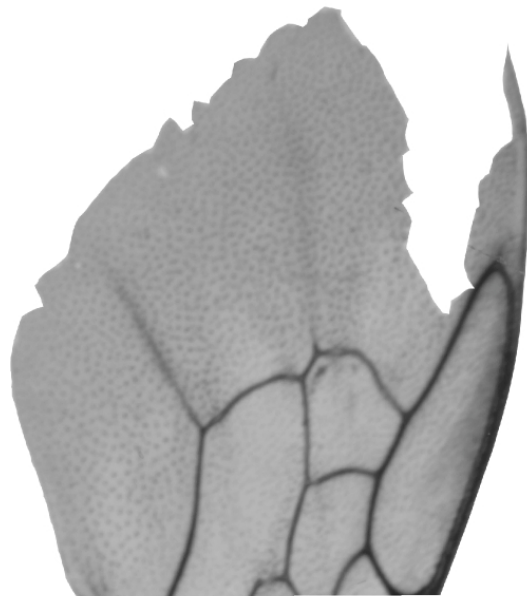
WWS: 0



WWS: 1



WWS: 2



WWS: 3

Figure 3.2 Initial wing wear scores of bumble bee forewings.

3.3.4 Statistical Analysis

3.3.4.1. Influence of area wing removal on foraging behaviour

I used Generalized Linear Mixed Models to analyze bumble bee foraging behaviour in response to artificially induced wing wear. The four variables of bumble bee foraging behaviour were % flight, flight frequency (# / min), mean distance flown (cm), and floral density (# flowers / m²). I used a gamma distribution with a log link function to analyze % flight, flight frequency, and mean distance flown, and a negative binomial distribution with a log link function to analyze floral density. The Kenward-Roger degrees of freedom approximation was implemented to account for repeated measures of individuals (Kenward and Roger 1997).

All four Generalized Linear Mixed Models considered the effects: flower species visited (FSV; fixed effect, 4 levels), initial wing wear score (IWWS; fixed effect; the principal measure of the past wing wear hypothesis), length of marginal cell (LMC; covariate), % area loss or wing area removed (% AL; covariate; the principal metric of the contemporary wing wear hypothesis), % AL*FSV, IWWS*FSV, IWWS*% AL, bee species (random effect), and individual nested within bee species (repeated measures effect). The % AL*FSV and IWWS*FSV terms allow for the assessment of how the costs of present and past wing wear respectively affect foraging behaviour on different species of flower. The IWWS*% AL term allows for assessment of how past and present wing wear might combine to influence behaviour. I specified an exponential covariance structure in the analysis of % flight, flight frequency, and mean distance flown, and a power covariance structure for mean floral density to account for unequal repeated measures of individuals from the day since first capture and individual-specific covariance. I also checked for all possible interactions between non-random effects. Non-significant interactions were removed to increase error degrees of freedom. Day since first captured (DSFC; covariate) was initially

included in the model, but was non-significant and removed from the analysis. Based on this, the covariate % area loss can be considered as a two-state variable for each individual (before or after wing area removal), allowing me to test how workers respond to different amounts of % area loss (ConWW hypothesis).

3.3.4.2. Influence of processing on bumble bee foraging behaviour

The procedure of trimming bumble bee's wings required a large amount of handling (refer Section 3.3.3), which can be stressful on the bee and potentially influence foraging behaviour. Therefore it was necessary to test bumble bee's foraging response after processing, while not removing any wing area. To investigate the effects processing had on foraging behaviour, I used the same models (refer to section 3.3.4.1), except % area loss was replaced with processing (fixed effect; 2 levels; before or after) for control bees. I checked for all possible interactions between non-random effects. Non-significant interactions were then removed to increase error degrees of freedom.

I used the GLIMMIX procedure in SAS 9.3 to fit all Models. The deviance residuals of all model fits were examined to verify their normality and homogeneity. SAS 9.3 was also used to obtain Least-Squares (LS) means for experimental and control bumble bees, and I used a Tukey HSD Test used to compare LS means, controlling for the family-wise error rate. Results for p-values between 0.5 and 0.1 were considered marginally significant in my analysis. F-values from significant effects were transformed to compare the effect sizes as outlined by Mullen (1989) using the following formula:

$$r = \left[\frac{F}{F + df} \right]^{\frac{1}{2}}$$

3.4 Results

3.4.1. Effect of Wing Removal on Bumble Bee Behavioural Response

The ConWW hypothesis was supported by flight amount. Individuals reduced their flight amount after wing area removal (Table 3.1; Figure 3.3), but wing wear score was unimportant (Table 3.3). Support for the PWW hypothesis was equivocal. Bumble bee flight amount was influenced by initial wing wear score differently for the flower species (Table 3.3). Bumble bees foraging on *C. arvensis* with an initial wing wear score of 2 had a lower flight time than bumble bees with a score of 0 and 1 (Figure 3.4).

Table 3.1 Effect of wing area removal (before and after) on % flight in foraging bumble bees (N = 268). Terms that are statistically significant are in boldface. Wing Wear Score*% Area Loss was non-significant and not included in the model.

Source	Directionality	Numerator df	Denominator df	F	P
Wing Wear Score	N/A	2	22.6	0.65	0.5295
% Area Loss	Decrease	1	173.8	10.42	0.0015
Flower Species	N/A	3	65.2	63.42	< 0.0001
Marginal Cell Length	Increase	1	110.8	3.00	0.0585
Wing Wear Score*Flower Species	N/A	6	57.27	4.63	0.0007

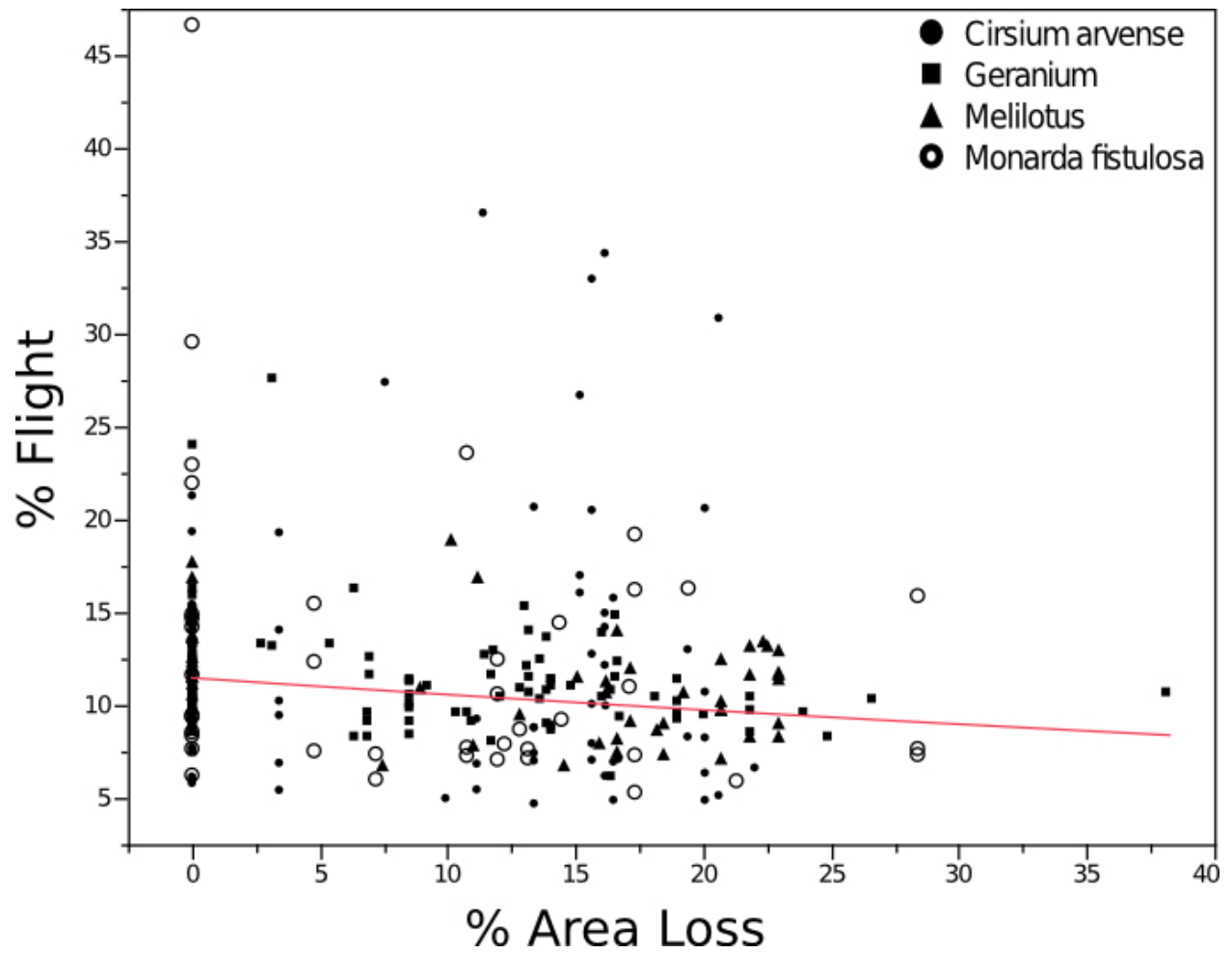


Figure 3.3 Partial regression visualization of the model fit predicting % flight from % area loss. Table 3.1 shows the fitted model.

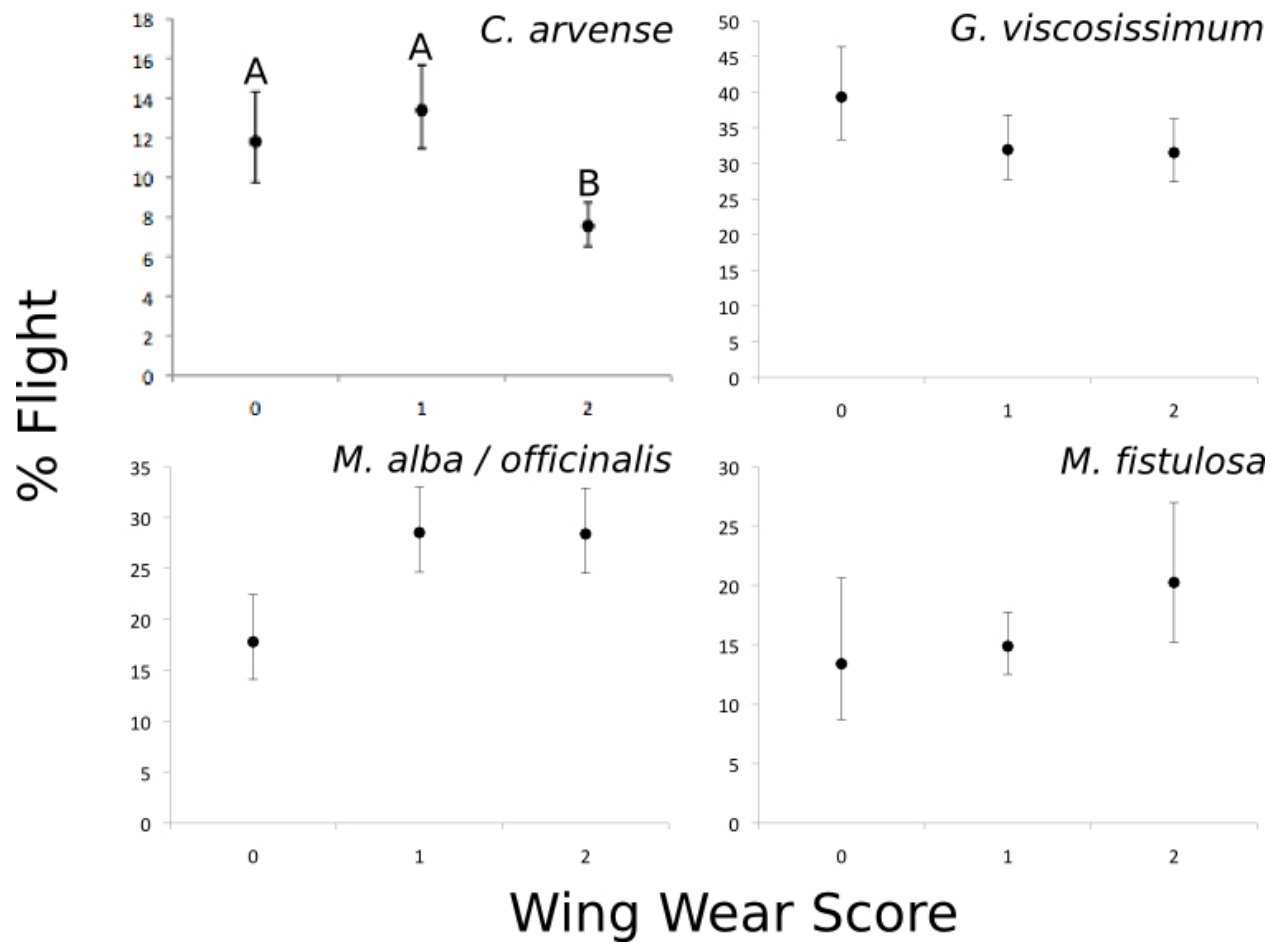


Figure 3.4 Visualization of the generalized linear mixed-model predicting % flight from the interaction between initial wing wear score and flower species. Letters show significant differences between means (Tukey HSD, $P < 0.05$). Model details are presented in Table 3.1. Least-square means (\pm standard error) are shown.

The ConWW hypothesis was supported for flight frequency measured on 2 flower species. Flight frequency was influenced by % area loss differently according to flower species (Table 3.2). Bumble bees with reduced wing areas flew less on *C. arvense* and *M. fistulosa* (Figure 3.5 and Table A.2.3). The effect of removal of wing area on flight frequency also depended on initial wing wear score (Table 3.2). Individuals with an initial wing wear score of 0 and 2 lowered their flight frequency after wing area removal (Figure 3.6 and Table A.2.4). This was not the case for bees with a wing wear score of 1 (Figure 3.6), providing support for both the ConWW and PWW hypotheses.

Table 3.2 Effect of wing area removal (before and after) on flight frequency (# / min) in foraging bumble bees (N = 268). Terms that are statistically significant are in boldface.

Source	Directionality	Numerator df	Denominator df	F	P
Wing Wear Score	N/A	2	206.1	0.49	0.6155
% Area Loss	Decrease	1	83.33	15.25	0.0002
Flower Species	N/A	3	166	61.98	< 0.0001
Marginal Cell Length	Increase	1	139.1	12.38	0.0006
% Area Loss*Wing Wear Score	N/A	2	150.4	4.20	0.0168
% Area Loss*Flower Species Visited	N/A	3	52.4	3.48	0.0175

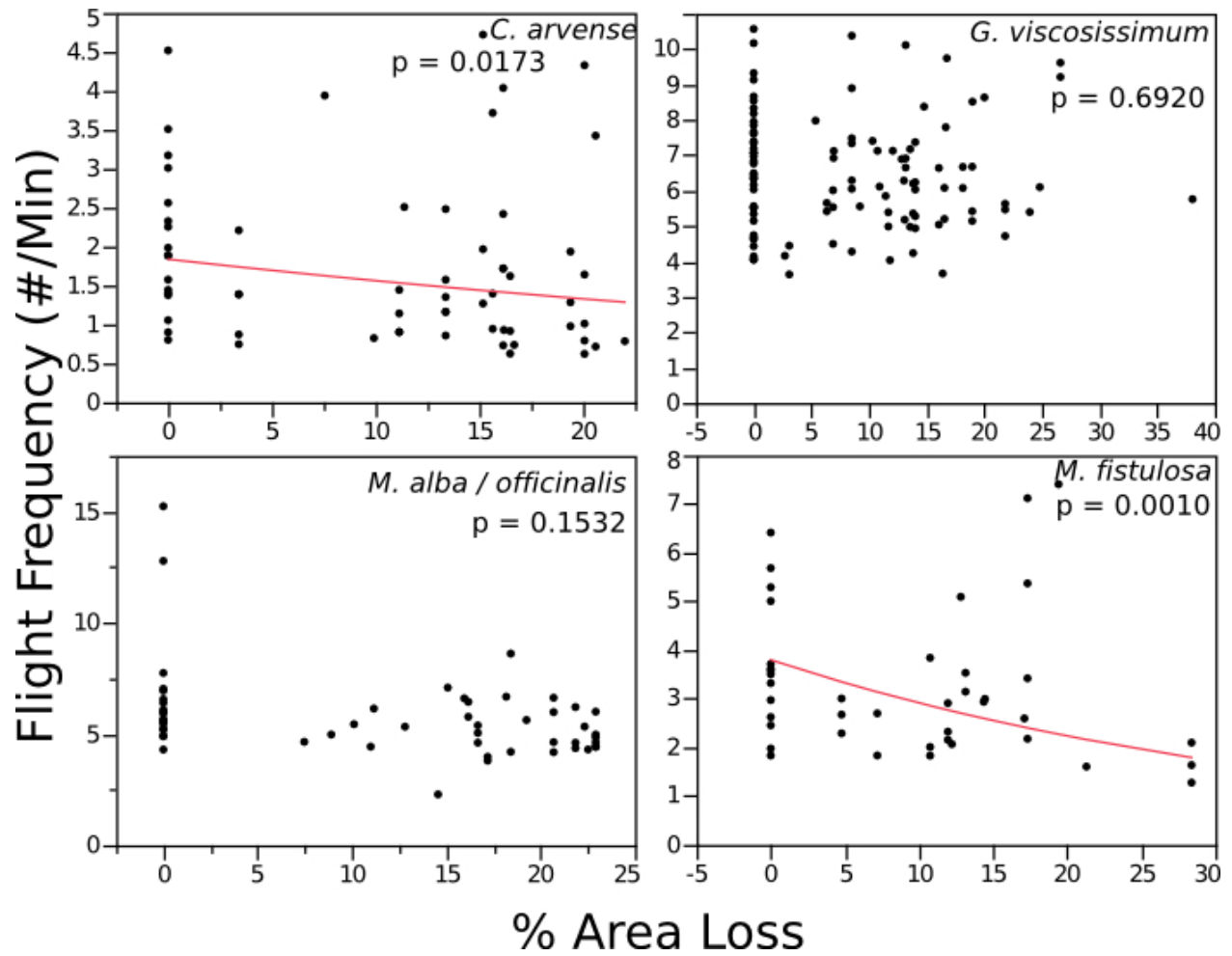


Figure 3.5 Partial regression visualization of the generalized linear mixed-model predicting flight frequency (# / min) from the interaction between % area loss and flower species. Model details are presented in Table 3.2.

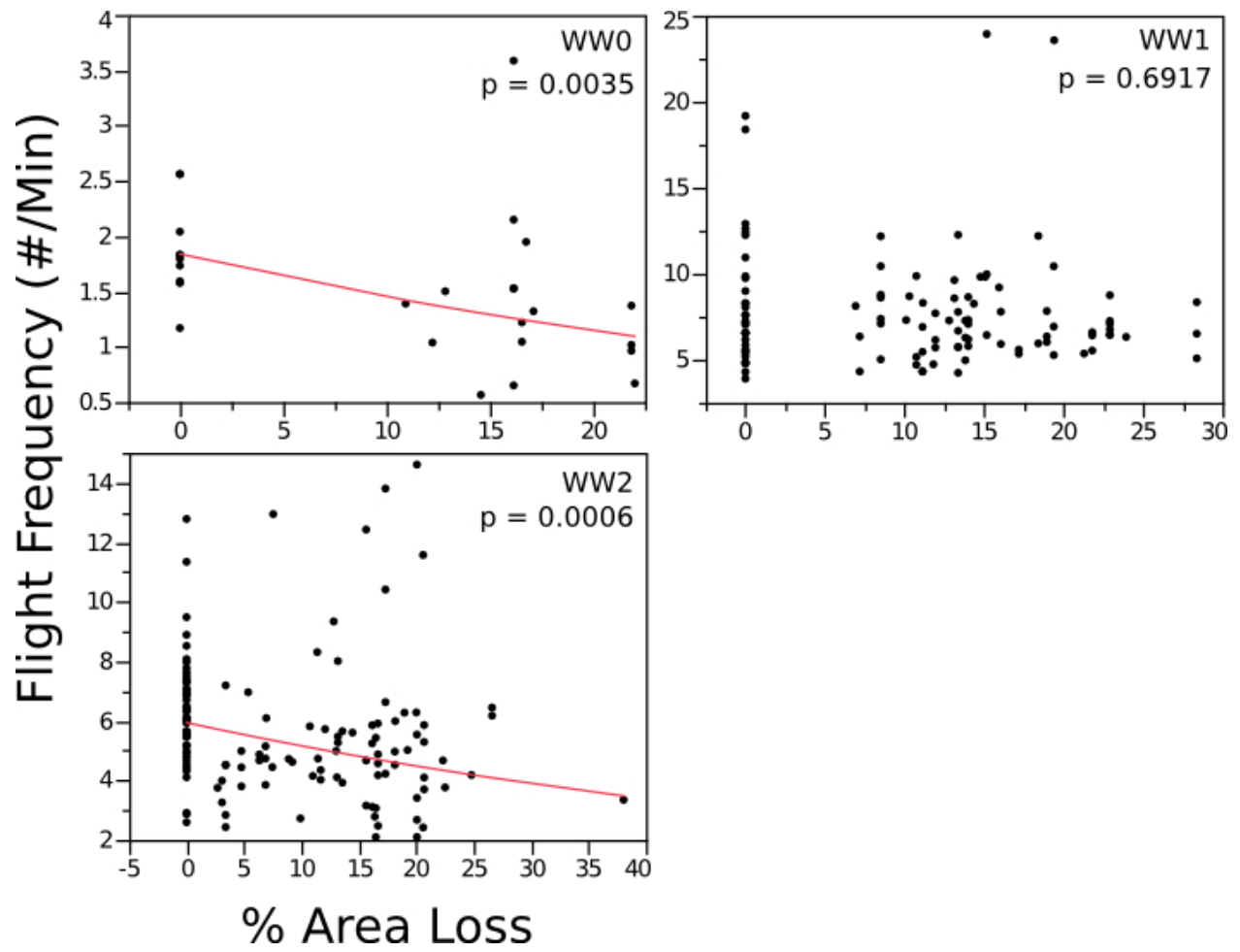


Figure 3.6 Partial regression visualization of the generalized linear mixed-model predicting flight frequency (# / min) from the interaction between % area loss and initial wing wear score. Model details are presented in Table 3.2.

The ConWW hypothesis was supported by distance flown. Individuals flew shorter mean distances after removal of wing area (Table 3.3), and mean distance flown decreased as wing area removal increased (Figure 3.7). Support for the PWW hypothesis was equivocal. Initial wing wear score affected mean distance flown, but differently for different flower species (Table 3.3; Figure 3.8). Bumble bees foraging on *C. arvensis* with an initial wing wear score of 2 flew shorter distances than bumble bees with a score of 0 and 1 (Figure 3.8). Removal of wing area and the amount of wing wear a bee had prior to wing trimming both had no effect on the mean floral density chosen by bumble bees (Table 3.4).

Table 3.3 Effect of wing area removal (before and after) on distance flown (cm) in foraging bumble bees (N = 266). Terms that are statistically significant are in boldface. Wing Wear Score*% Area Loss was non-significant and not included in the model.

Source	Directionality	Numerator df	Denominator df	F	P
Wing Wear Score	N/A	2	25.31	0.17	0.85
% Area Loss	Decrease	1	226.9	4.44	0.0362
Flower Species	N/A	3	46.68	24.14	< 0.0001
Marginal Cell Length	Increase	1	90.82	2.09	0.15
Flower Visited*Wing Wear Score	N/A	6	45.59	3.65	0.0049

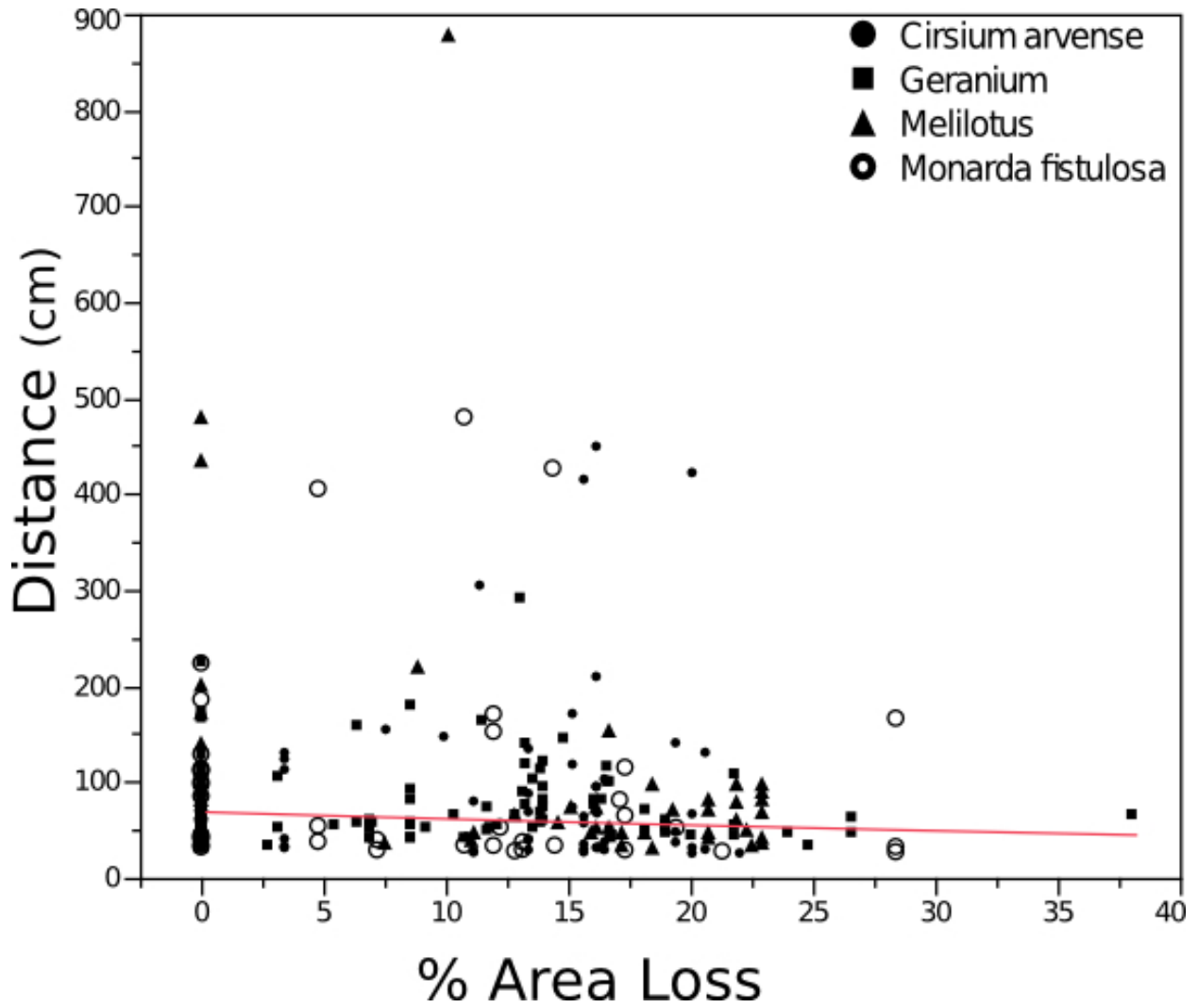


Figure 3.7 Partial regression visualization of the model fit predicting distance flown (cm) from % area loss. Table 3.3 shows the fitted model.

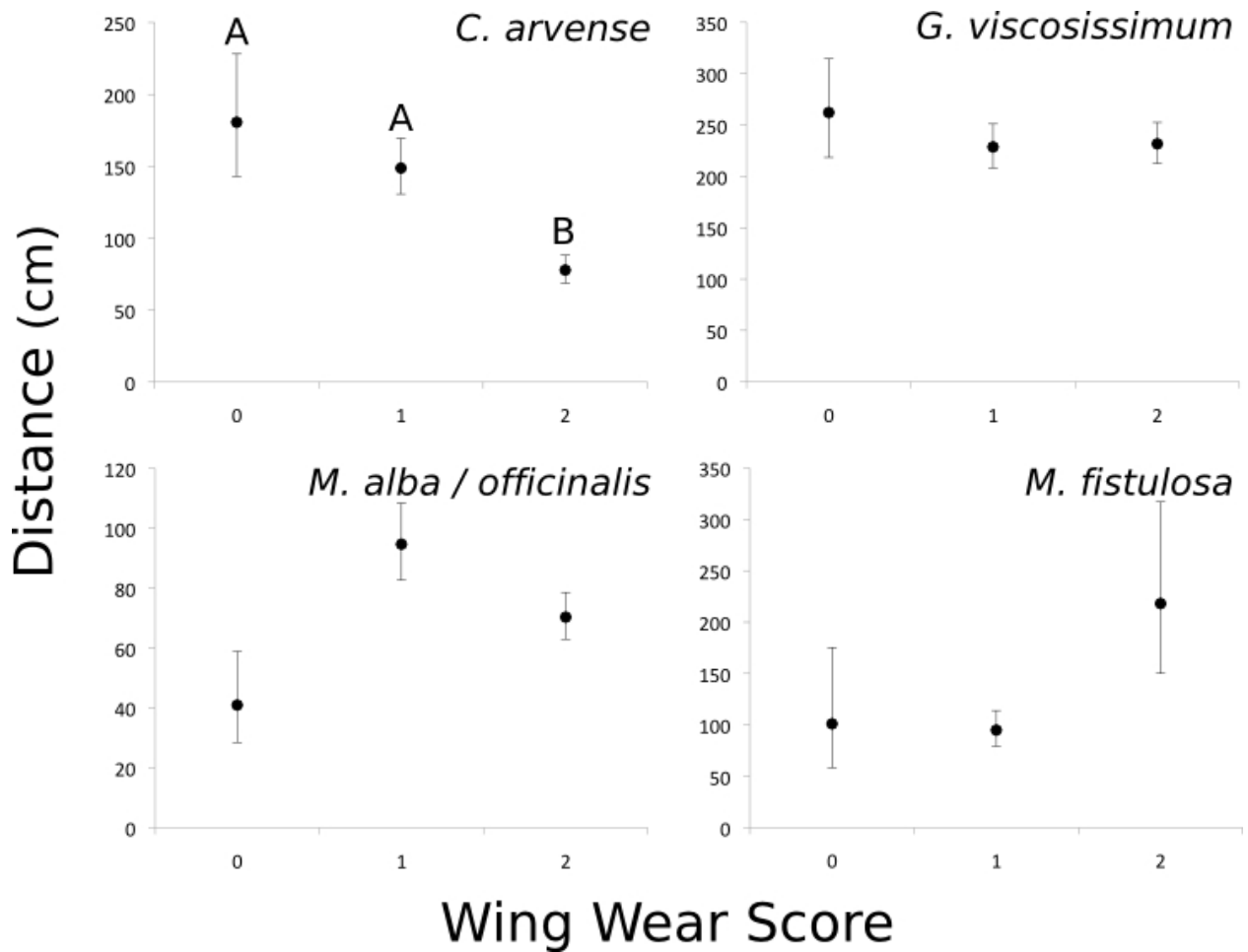


Figure 3.8 Visualization of the generalized linear mixed-model predicting distance flown (cm) from the interaction between initial wing wear score and flower species. Letters show significant differences between means (Tukey HSD, $P < 0.05$). Model details are presented in Table 3.3. Least-square means (\pm standard error) are shown.

Table 3.4 Effect of wing area removal (before and after) on floral density (#flowers / m^2) in foraging bumble bees ($N = 271$). Terms that are statistically significant are in boldface. WWS*% AL was found to be non-significant and not included in the model.

Source	Directionality	Numerator df	Denominator df	F	P
Wing Wear Score	N/A	2	74.09	0.07	0.9291
% Area Loss	Increase	1	206.4	1.05	0.3074
Flower Species	N/A	3	54.6	1682.39	< 0.0001
Marginal Cell Length	Increase	1	14.52	7.37	0.0163

Table 3.5 Support for the wing wear hypotheses for each wing use variable.

	Concurrent Wing Wear Hypothesis	Past Wing Wear Hypothesis
Flight Time	Not Supported	Marginal
Flight Frequency (#/min)	Half Supported	Marginal
Mean Distance (cm)	Supported	Marginal
Floral Density (#/m ²)	Not Supported	Not Supported

3.4.2. Effect of Bee Processing on Behaviour

I did not analyze control bees (i.e. those with no wing area removed) in the preceding analyses because their lack of % area loss precluded their inclusion in the analysis. But it is worth establishing that the patterns described for experimental bees did not also occur in bees which suffered no experimental loss of wing area. Control bees flew less after being processed (Table 3.6 and Figure 3.9). The effect sizes of % area loss and processing in experimental and control bees respectively were not different (Table 3.10), suggesting lower flight time is a result of handling bees and not wing area removal. Support for the PWW hypothesis was equivocal. Flight amount for these bees was influenced by initial wing wear score, but differently according to flower species (Table 3.6). Bumble bees foraging on *C. arvensis* with an initial wing wear score of 2 had a lower flight amount than bumble bees with a score of 0 and 1. The effect size for control and experimental bees are similar (Table 3.10) and indicates the importance past wing wear had for % flight on *C. arvensis*.

Table 3.6 Effect of handling (before and after) on % flight in control foraging bumble bees (N = 266). Terms that are statistically significant are in boldface.

Source	Directionality	Numerator df	Denominator df	F	P
Wing Wear Score	N/A	2	22.24	0.52	0.5996
Processing	N/A	1	111.7	8.51	0.0043
Flower Species	N/A	3	63.28	59.44	< 0.0001
Marginal Cell Length	Increase	1	85.64	1.42	0.2370
Wing Wear Score*Flower Species Visited	N/A	6	54.28	4.42	0.0010

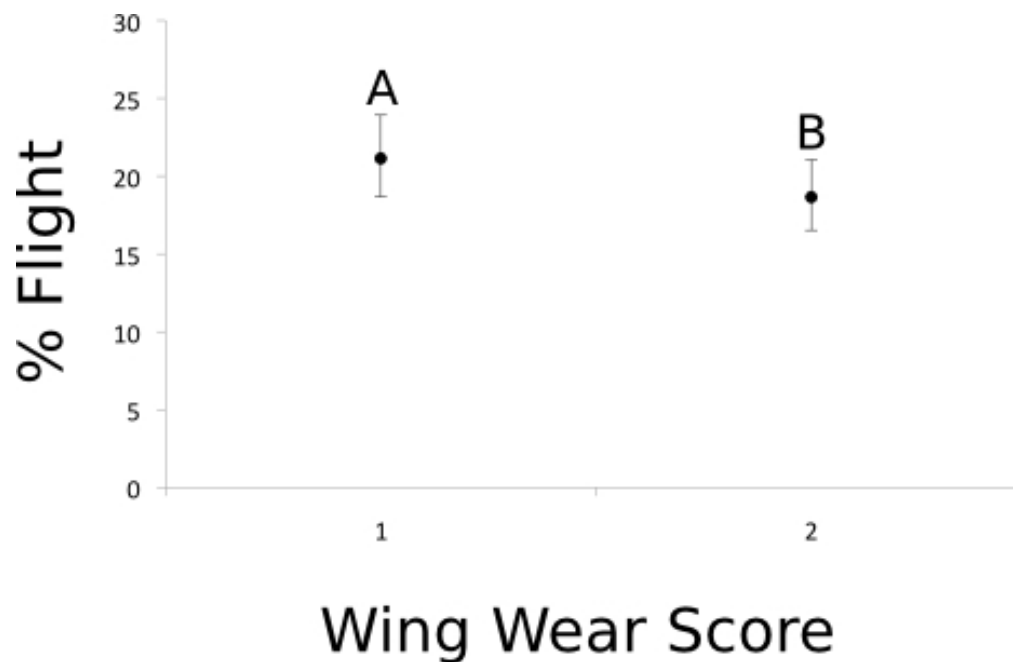


Figure 3.9 Visualization of the generalized linear mixed-model predicting %flight from bumble bee handling (before and after) for control bees. Model details are presented in Table 3.5. Least-square means (\pm standard error) shown.

Bumble bees flew less frequently after being handled (Table 3.7 and Figure 3.10). The effect size of bee processing is smaller than the effect sizes for % area loss on *M. fistulosa* and % area loss with an initial wing wear score of 0 and 2 (Table 3.10). This suggests % area loss affects flight frequency, and the effect that processing bees had on flight frequency is marginal.

Table 3.7 Effect of handling (before and after) on flight frequency (# / min) in foraging control bumble bees (N = 266). Terms that are statistically significant are in boldface.

Source	Directionality	Numerator df	Denominator df	F	P
Wing Wear Score	N/A	2	192.5	2.76	0.0660
Processing	N/A	1	164.6	7.29	0.0077
Flower Species	N/A	3	151.5	162.40	< 0.0001
Marginal Cell	Increase	1	145.6	10.65	0.0014

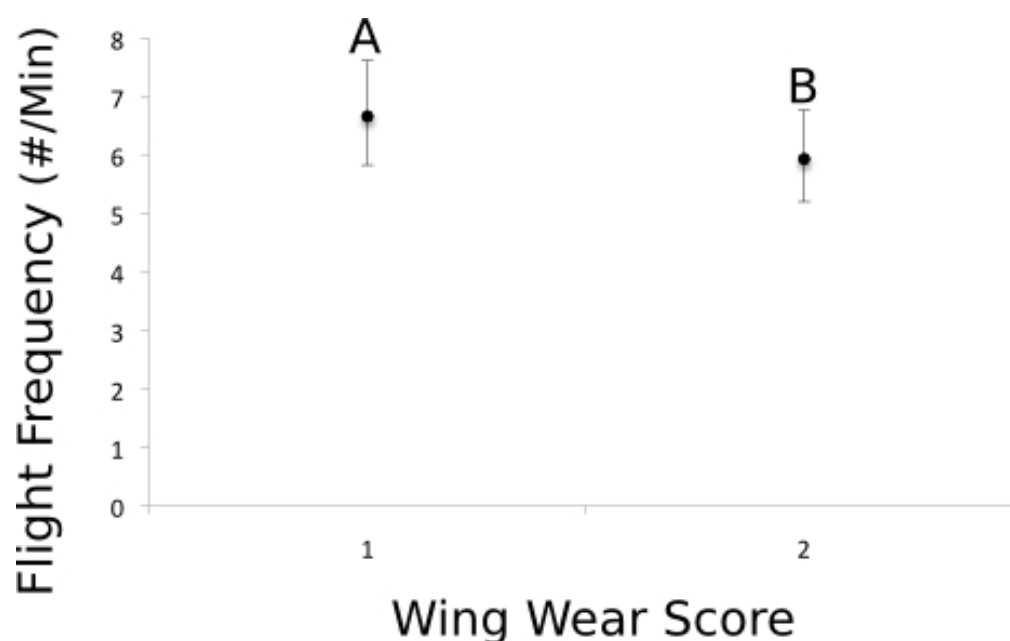


Figure 3.10 Visualization of the generalized linear mixed-model predicting flight frequency (# / min) from bumble bee handling (before and after) for control bees. Model details are presented in Table 3.6. Least-square means (\pm standard error) are shown.

Support for the PWW hypothesis was equivocal for distance flown. Initial wing wear score altered mean distance flown, but differently according to flower species (Table 3.8). Bumble bees foraging on *C. arvensis* with an initial wing wear score of 2 flew shorter distances than bumble bees with a score of 0 and 1. The effect sizes between experimental and control bees are similar (Table 3.10) and indicates the importance past wing wear has for distance flown on *C.*

arvense. Processing had no effect on the distance flown (Table 3.8) or choice of floral density (Table 3.9).

Table 3.8 Effect of handling (before and after) on distance flown (cm) in foraging control bumble bees (N = 266). Terms that are statistically significant are in boldface.

Source	Directionality	Numerator df	Denominator df	F	P
Wing Wear Score	N/A	2	23.97	0.14	0.8715
Processing	N/A	1	222.9	1.86	0.1745
Flower Species	N/A	3	49.29	25.94	< 0.0001
Marginal Cell	Increase	1	87.98	1.41	0.2382
Flower Visited * Wing Wear Score	N/A	6	45.19	3.96	0.0046

Table 3.9 Effect of handling (before and after) on choice of floral density (# flowers / cm²) in foraging control bumble bees (N = 269). Terms that are statistically significant are in boldface.

Source	Directionality	Numerator df	Denominator df	F	P
Wing Wear Score	N/A	2	169	0.09	0.9182
Processing	N/A	1	185.1	1.21	0.2722
Flower Species	N/A	3	71.8	2478.51	< 0.0001
Marginal Cell	Increase	1	13.65	10.47	0.0061

Table 3.10 Effect sizes of wing use variables, % flight, flight frequency (# / min), and distance (cm), for experimental and control bumble bees (N = sample size).

Wing Use Variable	Experimental Analysis	Control Analysis
% Flight	% Area Loss: 0.238 (271)	Processing: 0.266 (61)
Flight Frequency (# / Min)	Flower Visited*Wing Wear Score: <i>C. arvense</i> : 0.977 (69) Flower Visited*% Area Loss: <i>C. arvense</i> : 0.176 (69) <i>M. fistulosa</i> : 0.504 (41)	Flower Visited*Wing Wear Score: <i>C. arvense</i> : 0.978 (20) Processing: 0.206 (61)
Distance Flown (cm)	Wing Wear Score*% Area Loss: WW0: 0.335 (27) WW2: 0.258 (135) % Area Loss: 0.139 (271)	Processing: 0.0806 (61)
	Flower Visited*Wing Wear Score: <i>C. arvense</i> : 0.954 (69)	Flower Visited*Wing Wear Score: <i>C. arvense</i> : 0.953 (20)

3.5 Discussion

Overall, the Concurrent WW hypothesis was supported by data on flight time and distance flown as workers reduced their flight amount and flew shorter distances after wings were trimmed (Table 3.5). Response to wing wear based on flight amount and distance flown appears to be based on the current state of the bees' wings (ConWW hypothesis), and not worker age or experience (PWW hypothesis). Bumble bees appear to adjust their foraging behaviour in the face of higher flight costs associated with wing wear to prolong worker lifespan and maximize returns to the colony, speculated from observational data (Chapter 2).

The effects of post-processing on flight amount in control bees were similar to those of % area loss in experimental workers. Because the effect sizes between experimental and control bees were similar, I can conclude that workers did not lower their flight amounts in response to % area loss (Table 3.5). Rather, the effects of processing confound time in flight, and workers reduce their flight times in response to processing. Removal of wing area appears only to affect the distance flown.

The PWW hypothesis was supported by time in flight and distance flown on *C. arvensis* (Table 3.5). Bumble bees with high amounts of initial wing wear flew less, and flew shorter distances when foraging on *C. arvensis*. Bees foraging on *C. arvensis* appear sensitive to the initial costs of wing wear after wing area removal, indicating that foraging behaviour depends on both current and past wing wear. Control bees with high amounts of past wing wear also flew less, and shorter distances on *C. arvensis*. The effect sizes between experimental and control bees were similar lending additional support to the PWW hypothesis (Table 3.5).

Why might past wing wear have an independent effect on current behaviour? Such behaviour would qualify as “Concordian” (Dawkins and Brockmann 1980), whereby past

investment determines present allocation of costly resources. However, there may be situations in which past investment affects future investment, such that past behaviour should affect present behaviour. Fagerström (1982) argued that past reproductive effort must influence current reproductive effort when there is a finite lifetime number of progeny. The relevant empirical question is therefore: is there any evidence of lifetime limits to reproductive performance? In bumble bees, reproductive decisions can be translated into decisions about current foraging effort based previous foraging effort and past wing wear; a similar question obtains: is there any evidence of a limited lifetime foraging effort? Because *C. arvensis* blooms at the end of the season (Table A.2.5) and workers on this flower have high wing wear (mean % wing wear \pm SD; 6.81 ± 11.97 ; Table A.2.5), it is a suitable candidate to observe the effects past foraging effort and wing wear have on current foraging effort, as the majority of bumble bees at this point are seasoned foragers with few new workers. Workers with a high amount of wing wear on *C. arvensis* would reduce their current wing use based previous foraging effort based and past wing wear, ultimately influencing their future investment or foraging effort (if energy is limiting [i.e. foraging gain per wing beat]).

Overall, the ConWW hypothesis, bumble bees would fly less frequently after wing area removal was supported in half the plant species (Table 3.5). Bumble bees flew less frequently on *C. arvensis* and *M. fistulosa* after wing area removal and there was no effect of % area loss for bumble bees foraging on *G. viscosissimum* and *M. alba / officinalis*. Considering all the effects together, flight frequency was not influenced by % area loss (weighting partial regression coefficients by their t value; mean coefficient \pm SE; -0.013095 ± 0.005356).

It is unclear why bumble bees responded to removal of wing area by flying shorter distances, but did not fly less often (on all species of flowers). Workers responded to natural wear and tear

by flying less often, so it is expected that bumble bees would fly less frequently after wing area removal. Bumble bees lose an average (\pm SD) of approximately $0.15 \pm 0.096 \text{ mm}^2$ per day (Foster and Cartar 2011a) which amounts to an average (\pm SD) loss of $1.58 \pm 2.56 \text{ mm}^2$ of wing surface in the wild (Chapter 2), corresponding to a reduction in maximum load lifted (Johnson and Cartar 2014). That is, wing loss is gradual. The responses to wing trimming in my study represent a sudden and large loss of wing area that do not mimic natural responses to wing wear as I removed an average (\pm SD) of $14.95 \pm 5.88\%$ (high end) of the bumble bee's forewings in workers that already varied in their extent of natural wing loss. Bumble bees flew shorter distances based on a sudden and high amount of wing area loss. In contrast, as workers gradually acquire wing wear that accumulates over time, workers fly less frequently to lower their wing use (Chapter 2).

Thus far, I have discussed the separate effects of past (initial score) and present (before/after removal) wing wear on flight behaviour. Flight frequency was influenced by their combined effects (Table 3.5). Bees with low and high amounts of initial wing wear (initial score of 0 and 2) flew less after wing area removal, but bees with intermediate wing wear (score of 1) showed no flight frequency response to wing loss (Figure 3.6). Wing wear is correlated with age in workers and bees accumulate more wear with wing use (Foster and Cartar 2011a). I use this observation to speculate about why wear class 1 differed from the other two. Bumble bees with a wing wear score of 0 (wing area loss mean \pm SD; $-3.08 \pm 3.94\%$) are likely to be new foragers that have little foraging experience, but are vigorous (start of their foraging life). Bumble bees with a wing wear score of 2 ($9.81 \pm 9.95\%$) have substantial foraging experience, but lack vigour relative to new workers (end of their foraging life). Bumble bees with a wing wear score of 1 ($-1.51 \pm 5.45\%$) are likely of intermediate foraging experience and foraging life. Workers that are either

experienced or vigorous (not both) may be more sensitive to the costs of wing wear as they are either naïve about the flower patch or have little vigour remaining to maintain wing use in the face of higher flight costs.

Behavioural responses after wing area removal were immediate and did not change over the period of observation (analysis not shown). Day since first capture was unrelated to flight amount, flight frequency, and distance flown over a few days (mean \pm SD; 2.28 ± 2.71 days). Many bumble bees were observed the same day their wings had been trimmed (15 individuals) and the day following (41 bees), suggesting bees readily respond to wing wear. However, because of the sudden loss in wing area workers experienced, they may be forced to use their wing's less.

Trimming bumble bees' wings had no effect on choice of floral density (Table 3.5). I predicted bumble bees would adjust for the increased flight costs of wing wear by foraging at higher floral densities (as found by Foster and Cartar 2011b), this non-result is not necessarily surprising. Bumble bees with natural wing wear (see Chapter 2) indirectly reduce their time in flight and flight frequency by foraging in higher density flower patches (Table A.1.8).

3.6 Conclusions

Overall, the results support the cost of wing wear hypothesis (see Chapter 2 for an explanation), and the strongest effects of wing wear were independent of worker age and experience (supporting the ConWW hypothesis). Bumble bees responding to wing area removing by flying shorter distances and flying less frequently on *C. arvensis* and *M. fistulosa*. In addition to this, I found behavioural responses after wing area removal were immediate and workers did not gradually adjust to loss of wing area (at least over the subsequent few days). Foraging

behaviour was also influenced by initial wear before trimming. Bumble bees with high amounts of wing wear flew less and for shorter distances, when foraging on *C. arvensis*. It may be that current foraging effort is influenced by previous foraging effort, as reflected by past wing wear (PWW hypothesis). The time taken for workers to respond to natural wing wear and the effects that past wing wear has on current foraging effort merits further attention. The importance that bumble bees place on flight costs for past and current wing wear appear to both be important, but were only found to simultaneously matter for flight frequency. Past wing wear by itself was only found to matter for workers foraging on *C. arvensis* and suggest that the current costs of wing wear (ConWW hypothesis) have greater influence on foraging behaviour. Studies investigating the importance of past and current wing wear, in addition to circumstances where they simultaneously matter (i.e. flight frequency) merits further attention.

CHAPTER FOUR: IMPLICATIONS OF FORAGING WITH WING WEAR

4.1 Wing wear changes foraging behaviour

In chapters 2 and 3, I found that workers responded to natural and artificial wing wear by reducing their wing use, but how this was achieved was surprisingly different. I found bumble bees with high natural wing wear reduced their wing use by lowering their flight frequency relative to pristine-winged bees, but changes in flight time, distance flown (cm), and choice of floral density (#flowers / m²) depended on flower species, and in the case of flight amount, were not always consistent with the CWW hypothesis. Wing-worn bumble bees foraging on *C. arvense* and *T. hybridum* flew more than pristine-winged bees. Using partial correlations to test if flight time is correlated with wing wear while accounting for marginal cell length and the other wing use variables, wing wear is not correlated with flight time on *C. arvense* or *T. hybridum*. It is likely that wing-worn bumble bees are not flying more on these two species of flower, and their non-responsiveness to wing wear may reflect two possibilities. First, low flight times on *C. arvense* (Table 2.3) may result in workers incurring only minor elevations in wing-worn flight costs. Second, bees foraging on *C. arvense* and *T. hybridum* may accumulate less wing wear over time, and only incur minor elevations in wing-worn flight costs

Because workers responded to natural wing wear by flying less frequently, I expected a similar response after trimming bee's wings, decoupling age and experience from wing wear. Interestingly, I found bees responded to removal of wing area by flying shorter distances, but changes in flight frequency were based on the species of flower. Overall, these results support the cost of wing wear hypothesis and contemporary wing wear hypothesis (Chapter 2 and Chapter 3 respectively). However, wing wear is linked with forager age and foraging effort

(Foster and Cartar 2011a) and there remains the possibility that trimming bees' forewings results in workers thinking they have suddenly aged. Responses to wing trimming may be based on increasing worker age and not wing wear, and it remains to be tested if bumble bees use wing wear as a proxy for their age.

Why might bumble bees respond differently to natural wing wear and artificial wing wear? Bumble bees accumulate small amounts of wing wear each day, resulting in an average (\pm SD) daily area loss of $0.15 \pm 0.096 \text{ mm}^2$ (Foster and Cartar 2011a), or $1.58 \pm 2.56 \text{ mm}^2$ average wing area in the wild (personal obsv). Bumble bees respond to small amounts of wing wear accumulated over their lifetime (approximately two weeks; Goulson 2003) by flying less frequently. In contrast, workers responded to sudden and large loss of wing area by flying shorter distances. This is a puzzling result that clearly requires further investigation, as workers may be responding differently to gradual and sudden loss of wing area. I have no speculations to offer to account for this difference.

Bumble bees also indirectly reduced their wing use by shifting visitation of flower species based on corolla depth and the length of the bee's tongue. Because handling time increases with depth of flower (Harder 1983), and deeper flowers require less flight in their visitation, I predicted wing-worn, long-tongued bumble bees to feed on deeper flower species that were unavailable to shorter-tongued species. I found long-tongued, wing-worn bees were more likely to forage on shallow flowers and short-tongued, wing-worn bees tended to forage on deeper flowers, not supporting my prediction. Long-tongued bumble bees forage less efficiently on shallow flowers (Plowright and Plowright 1997), and also spend less time in flight and fly less frequently than shorter-tongued bumble bees (Figure 2.8 and Figure 2.9). Counter-intuitively, wing-worn, long-tongued bumble bees appeared to reduce their wing use by visiting

shallow flowers and the converse seems to have been the case for wing-worn, short-tongued bees (Figure 2.8 and Figure 2.9). In a strange twist, my specific prediction related to tongue length was unsupported, but the patterns observed supported the more general CWW prediction that wing worn bees reduce their wing use.

Overall, these results support the cost of wing wear hypothesis. Future studies should test if wing-worn bumble bees offset reduced handling efficiency when foraging on different flower depths than predicted on the basis of the match between tongue length and corolla depth (Heinrich 1976; Inouye 1980; Ranta and Lundberg 1980), by lowering their flight costs. I documented species of flower bumble bees were foraging on at the time of capture and assumed workers were foraging on flower depths based on their amount of wing wear, by accounting for co-available flower species in my model. Future studies should test if wing-worn bumble bees switch species of flowers based on flower depth, by repeatedly observing individuals foraging on concurrently blooming flowers and observing switches in species of flower. Studies should also take into account the nearest flower patch to the colony to test if wing-worn bumble bees are visiting flower species based on the closest flower patch available.

4.2 How do bees respond to past and current wing wear?

Though wing wear accumulates with age and foraging effort (Foster and Cartar 2011a), the behavioural responses to wing wear (Foster and Cartar 2011b; Chapter 2) are independent of worker experience (Chapter 3). Individual bumble bees that varied in their extent of wing wear responded to wing trimming by reducing their wing use, supporting the contemporary wing wear hypothesis (see previous section). Responses to loss of wing area were almost immediate (at least within the same or following day), suggesting bumble bees rapidly integrate information about

their current wing morphology and make behavioural changes to reduce their wing use. Foraging effort was also influenced by past wing wear. Workers with high amounts of initial wing wear had lower flight amounts and flew shorter distances foraging on *C. arvensis*. In bumble bees there may be situations in which past foraging effort based on previous wing wear affects future foraging effort, thereby past behaviour and wing wear affect current wing use (see Fagerström 1982 for theoretical argument based on reproductive effort). If workers have a lifetime limited energy budget (i.e., optimizing foraging gain per wing beat), it is likely previous foraging effort and wing wear influence current wing use. *C. arvensis* blooms at the end of the season, and the majority of workers collecting nectar at this time are old foragers (Table A.2.5). This may explain why the effects of initial wing wear on flight time were only detected on *C. arvensis* because workers had already acquired sufficient wing wear from past foraging experience (Table A.2.5).

Past and current wing wear combined to influence flight frequency, but only for bees with low and high amounts of initial wing wear. It is possible that bumble bees with intermediate wing wear are experienced and vigorous foragers, relative to naïve or older workers. Naïve bumble bees have little knowledge of the flower patch and may be less able to maintain wing use in the face of higher flight costs, as are older bees that have little energy remaining. Bumble bees with intermediate wing wear have knowledge of the flower patch and enough energy to afford the costs of wing wear.

Overall, these results support both the past and contemporary wing wear hypotheses (Chapter 3). Past wing wear influences mortality (Cartar 1992a) and the effects of past wing wear by itself were only detected on *C. arvensis*. It is likely that the current costs of wing wear

(ConWW hypothesis) matters most, as this was found to have the greatest influence of bumble bee wing use. The importance of past and current wing wear merits further attention.

4.3 What are the costs of wing wear?

Wing wear in bumble bees is linked to mortality (Cartar 1992a; Dukas and Dukas 2011) and responses to wing trimming are independent of worker experience (Chapter 3). The proximate mechanisms accounting for the effect of wing wear on mortality remain unclear, but the costs are likely biomechanical and/or metabolic (Cartar 1992a). Biomechanical costs could be the result of reduced manoeuvrability, increasing susceptibility to inclement weather or predation. Reduced manoeuvrability is unlikely to account for high mortality in wing-worn bumble bees. Wing-worn bumble bees exhibit little change in their flight performance (Haas and Cartar 2004). Furthermore, bumble bees forage in suitable weather conditions (i.e. no rain, low wind speed) and are mostly preyed upon by crab spiders on inflorescences (Morse 1986), changes in flight performance may not contribute to mortality caused by inclement weather and aerial predation.

Metabolic costs seem a more likely candidate to account for wing-worn mortality in wild foraging bumble bees. Wing-worn bumble bees experience higher wing-loading costs (Johnson and Cartar 2014) and increased wing-loading caused by wing wear is likely to increase the metabolic costs to maintain hovering flight (Ellington *et al.* 1990). Hedenström *et al.* (2001) found no metabolic costs in wing-worn bumble bees flying in a flight chamber. However, bumble bees must maintain a high thoracic temperature (Heinrich 1974), compensate for the weight of their nectar load (Heinrich 1975a) and loading costs associated with nectar load size (Johnson and Cartar 2014), as well as manoeuvre between vegetation obstacles and through wind

gusts. All of these factors are likely to account for greater metabolic costs in wing-worn bumble bees foraging in a natural setting that are not realized in a flight chamber (Wolf *et al.* 1999; Ortega-Jimenez *et al.* 2014). I assumed bumble bees reduced their wing use (Foster and Cartar 2011b; Chapter 2) to account for higher metabolic or biomechanical costs. Measurements of metabolism using the doubly labelled water technique in wing-worn bumble bees foraging in a complex environment is required to resolve the question.

4.4 Implications of wing wear for adaptive foraging behaviour

Bumble bees forage in complex environments where there are many factors that influence wing use. The density of competitors foraging in the flower patch (Goulson *et al.* 1998), energetic state of the colony (Cartar 1992b), and morphology (i.e., match between a bumble bee's tongue length and floral depth; Harder 1983; Plowright and Plowright 1997) all influence how much a bumble bee uses her wings. This would explain the deviation between observations and predicted wing use based on the amount of wing wear in my models (see Chapter 2 and Chapter 3), as wing use is clearly influenced by factors other than just wing wear. That being said, wing wear still affects wing use.

Bumble bees respond to wing wear by reducing their wing use (Foster and Cartar 2011b; Chapter 2), independent of worker age and experience (Chapter 3). I assumed bumble bees lowered their wing use to account for metabolic or biomechanical costs (Cartar 1992a). Independent of the costs of wing wear, this study demonstrates the importance of using wing wear to better explain bumble bee foraging behaviour.

Natural selection assumes foragers to optimize currencies that increase their energetic gains and maximize fitness (Stephens and Krebs 1986; Davies *et al.* 2012). In bumble bees, non-

reproductive workers increase their energetic gains foraging in a flower patch to increase their inclusive fitness (Schmid-Hempel *et al.* 1993; Goulson 2003). Workers performing foraging tasks that exacerbate senescence are expected to maximize net lifetime contribution to the colony, in part by adjusting foraging behaviour in light of changed costs (Schmid-Hempel *et al.* 1993). Wing-worn bumble bees reduce their wing use, probably to reduce the costs of wing-worn flight, influencing adaptive foraging behaviour predicted by optimal foraging theory (Pyke 1984). To gain better insights and improve the predications made by optimal foraging studies, limitations on foraging performance caused by wear and tear of body parts should be accounted for, as this likely constrains adaptive behaviour.

APPENDIX A

A.1 Chapter two tables and results

Table A.1.1 Frequency of individual bumble bee observations of six bumble bee species foraging on eight plant species.

Plant Species	All bees	<i>B. appositus</i>	<i>B. bifarius</i>	<i>B. californicus</i>	<i>B. flavifrons</i>	<i>B. mixtus</i>	<i>B. moderatus</i>
<i>Cirsium arvense</i>	64	6	39	0	10	0	9
<i>Chamerion latifolium</i>	19	0	0	0	7	12	0
<i>Geranium viscosissimum</i>	100	0	55	0	0	45	0
<i>Linaria vulgaris</i>	31	17	0	6	0	0	8
<i>Melilotus alba / officinalis</i>	118	0	73	0	18	16	11
<i>Monarda fistulosa</i>	83	17	8	10	30	18	0
<i>Trifolium hybridum</i>	71	0	47	0	19	0	5
<i>Trifolium pratense</i>	49	0	0	9	40	0	0
Total	535	40	222	25	124	91	33

Table A.1.2 Period bumble bees were observed on species of flower, providing an estimate of the range for the different flowering phenologies.

Plant Species	2012	2013
<i>Cirsium arvense</i>	August 22 – August 24	August 17 – August 24
<i>Chamerion latifolium</i>	July 27 – July 29	
<i>Geranium viscosissimum</i>	July 7 – July 18	July 2 – July 19
<i>Linaria vulgaris</i>	August 17 – August 21	
<i>Melilotus alba / officinalis</i>	August 12 – August 16	July 21 – August 6
<i>Monarda fistulosa</i>	July 31 – August 5	August 10 – August 16
<i>Trifolium hybridum</i>	July 5 – August 6	
<i>Trifolium pratense</i>	July 5 – August 6	

Table A.1.3 Inflorescence size (#Flowers / Inflorescence) of the different species of flower. Cells show mean \pm SD.

Plant Species	# Flowers / Inflorescence
<i>Cirsium arvense</i>	36.9 \pm 16.72
<i>Chamerion latifolium</i>	2.3 \pm 0.84
<i>Geranium viscosissimum</i>	1.47 \pm 0.62
<i>Linaria vulgaris</i>	6.8 \pm 3.49
<i>Melilotus alba</i> / <i>officinalis</i>	19.85 \pm 6.13
<i>Monarda fistulosa</i>	20.73 \pm 8.54
<i>Trifolium hybridum</i>	23.6 \pm 10.24
<i>Trifolium pratense</i>	30.9 \pm 13.16

Table A.1.4 Partial regression coefficients predicting %flight by the interaction between wing wear and flower species. Table 2.4 presents the fitted model. Terms that are statistically significant appear in boldface.

Source	Estimate	Standard Error	df	t	P
<i>Cirsium arvense</i>	0.01605	0.005100	510	2.09	0.0372
<i>Chamerion latifolium</i>	-0.00422	0.01793	510	-0.25	0.8056
<i>Geranium viscosissimum</i>	-0.00308	0.004399	510	-0.70	0.4848
<i>Linaria vulgaris</i>	0.001961	0.009658	510	0.20	0.8392
<i>Melilotus alba</i> / <i>officinalis</i>	-0.00593	0.004388	510	-1.35	0.1770
<i>Monarda fistulosa</i>	-0.02062	0.004231	510	-4.87	< 0.0001
<i>Trifolium hybridum</i>	0.01241	0.006413	510	1.93	0.0536
<i>Trifolium pratense</i>	0.001398	0.007511	510	0.19	0.8524

Table A.1.5 Partial regression coefficients predicting mean distance (cm) flown by the interaction between wing wear and flower species. Table 2.6 presents the fitted model. Terms that are statistically significant appear in boldface.

Source	Estimate	Standard Error	df	t	P
<i>Cirsium arvense</i>	-0.00127	0.009082	418.4	-0.14	0.8887
<i>Chamerion latifolium</i>	-0.01524	0.03160	488.3	-0.48	0.6298
<i>Geranium viscosissimum</i>	-0.00456	0.007829	488.1	-0.58	0.5606
<i>Linaria vulgaris</i>	-0.00149	0.01704	473.1	-0.09	0.9304
<i>Melilotus alba</i> / <i>officinalis</i>	0.007774	0.007873	480.5	0.99	0.3240
<i>Monarda fistulosa</i>	-0.02818	0.007603	393.2	-3.71	0.0002
<i>Trifolium hybridum</i>	0.01732	0.01264	485.7	1.37	0.1712
<i>Trifolium pratense</i>	0.002718	0.01392	412.8	0.20	0.8453

Table A.1.6 Partial regression coefficients predicting mean floral density (#flowers / m²) by the interaction between wing wear and flower species. Table 2.7 presents the fitted model. Terms that are statistically significant appear in boldface.

Source	Estimate	Standard Error	df	t	P
<i>Cirsium arvense</i>	0.01347	0.006679	490.1	2.02	0.0443
<i>Chamerion latifolium</i>	-0.00613	0.02467	510	-0.25	0.8038
<i>Geranium viscosissimum</i>	-0.00428	0.007387	510	-0.58	0.5622
<i>Linaria vulgaris</i>	-0.00204	0.01211	502.4	-0.17	0.8663
<i>Melilotus alba / officinalis</i>	-0.01083	0.005881	484.9	-1.84	0.0662
<i>Monarda fistulosa</i>	0.01929	0.005613	487	3.44	0.0006
<i>Trifolium hybridum</i>	-0.01649	0.008436	472.4	-1.95	0.0512
<i>Trifolium pratense</i>	-0.00609	0.009899	477.3	-0.62	0.5384

Table A.1.7 Mean distance flown (cm) in foraging bumble bees. A general linear model with bumble bee species as a random effect was fit (N = 514, R² = 0.42). Terms that are statistically significant appear in boldface.

Source	Directionality	df	F	P
Ln(Marginal Cell)	Increase	136.8	0.4877	0.4861
Flower Species	N/A	457.8	24.8902	< 0.0001
Flight	Increase	501.2	61.657	< 0.0001
Flight Frequency ^(1/2)	Increase	500.2	32.1339	< 0.0001

Table A.1.8 Floral density (#flowers / m²) in foraging bumble bees. A general linear model with bumble bee species as a random effect was fit (N = 532, R² = 0.30). Terms that are statistically significant appear in boldface.

Source	Directionality	df	F	P
Ln(Marginal Cell)	Increase	323.3	4.3688	0.0374
Flight	Decrease	524.3	0.3837	0.5359
Flight Frequency ^(1/2)	Decrease	526.5	38.4046	< 0.0001

A.2 Chapter three tables and results

Table A.2.1 Frequency of re-sightings of bumble bees of six species foraging on four different plant species. Bumble bees were observed foraging for two minutes before being captured and all repeated observations were made on the same plant species.

Plant Species	Total # Re-sightings	<i>B. appositus</i>	<i>B. bifarius</i>	<i>B. californicus</i>	<i>B. flavifrons</i>	<i>B. mixtus</i>	<i>B. moderatus</i>
<i>Geranium viscosissimum</i>	80	0	48	0	0	32	0
<i>Melilotus alba / officinalis</i>	65	0	28	0	14	12	11
<i>Monarda fistulosa</i>	35	17	0	9	9	0	0
<i>Cirsium arvense</i>	38	6	19	0	9	0	4
Total	218	23	95	9	32	44	15

Table A.2.2 Frequency of bumble bee re-sightings from the day since first captured (0.5 is the same day as initial capture). Six species of bumble bee were observed foraging for two minutes on the plants *Cirsium arvense*, *Geranium viscosissimum*, *Melilotus alba / officinalis*, and *Monarda fistulosa*. Re-sightings were all made on the same flower species. Some individuals were re-sighted more than once (mean re-sight frequency = 3.57, SD = 3.05).

Day since first captured	Total # Recaptured / day	<i>B. appositus</i>	<i>B. bifarius</i>	<i>B. californicus</i>	<i>B. flavifrons</i>	<i>B. mixtus</i>	<i>B. moderatus</i>
0.5	15	1	5	0	5	3	1
1	53	6	21	2	12	8	4
2	48	1	19	3	9	11	5
3	17	3	10	0	1	2	1
4	21	4	9	1	3	2	2
5	13	2	2	1	4	1	3
6	15	3	3	2	4	1	2
7	18	0	11	0	2	3	2
8	9	0	7	0	1	1	0
9	4	0	3	0	1	0	0
11	3	0	2	0	0	1	0
12	2	0	0	0	1	1	0
13	1	0	0	0	0	1	0
15	1	0	0	0	1	0	0
16	1	0	1	0	0	0	0
Total	221	20	93	9	44	35	20

Table A.2.3 Partial regression coefficients predicting flight frequency (# / min) by the interaction between %wing area removal and flower species. Table 3.4 presents fitted model. Terms that are statistically significant appear in boldface.

Source	Estimate	Standard Error	df	t	P
<i>Cirsium arvense</i>	-0.01620	0.006748	181.1	-2.40	0.0173
<i>Geranium viscosissimum</i>	-0.00164	0.004127	144.2	-0.40	0.6920
<i>Melilotus alba / officinalis</i>	-0.00809	0.005645	209.3	-1.43	0.1532
<i>Monarda fistulosa</i>	-0.02645	0.007413	37.38	-3.57	0.0010

Table A.2.4 Partial regression coefficients predicting flight frequency (# / min) by the interaction between %wing area removal and initial wing wear score. Table 3.4 presents fitted model. Terms that are statistically significant appear in boldface.

Source	Estimate	Standard Error	df	t	P
Wing Wear Score 0	-0.02354	0.007792	72.16	-3.02	0.0035
Wing Wear Score 1	-0.00170	0.004283	208.4	-0.40	0.6917
Wing Wear Score 2	-0.01405	0.004018	172.3	-3.50	0.0006

Table A.2.5 Days (mean \pm SD) and % wing wear (mean \pm SD) for bumble bees observed foraging on species of flowers.

Flower Species	Julian Day (mean \pm SD)	% Wing Wear (mean \pm SD)
<i>Geranium viscosissimum</i>	193.05 \pm 3.76	4.72 \pm 8.16
<i>Melilotus alba / officinalis</i>	209.49 \pm 5.77	4.45 \pm 7.34
<i>Monarda fistulosa</i>	223.46 \pm 2.29	-2.20 \pm 8.72
<i>Cirsium arvense</i>	231.30 \pm 2.60	6.81 \pm 11.97

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