

## RESEARCH ARTICLE

# What causes wing wear in foraging bumble bees?

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### SUMMARY

**Flying is an ecologically important behaviour in many insects, but it often results in permanent wing damage. Although wing wear in insects is often used as a method to determine insect age, and is associated with an increased risk of mortality, the causes of wing wear are unresolved. In this paper, we examine whether wing use while foraging explains wing wear in bumble bees (*Bombus* spp.). Wing wear may result from three distinct flight characteristics during foraging: time spent in flight, flight frequency and frequency of wing collisions with vegetation. To test these hypotheses for causes of wing wear, we recorded digital video of individually marked bumble bees foraging in nature on 12 different plant species that result in variation in these flight characteristics, and recaptured these individuals to photograph their wings over time. Bumble bees with a higher frequency of wing collisions showed an increased loss of wing area, which became more severe over time. Neither time in flight nor flight frequency was uniquely and significantly associated with wing wear. Therefore, the collision frequency hypothesis best explained wing wear in bumble bees. We conclude that wing use during foraging in bumble bees results in wing wear. Wing wear reflects behaviour, not simply age. Because wing wear has elsewhere been shown to increase mortality, this study provides an important mechanism linking foraging behaviour with lifespan.**

Key words: *Bombus*, foraging behaviour, wear and tear, wing use.

### INTRODUCTION

Organisms undergo wear and tear of their bodies as they age, as illustrated by tooth erosion, hearing loss, appendage loss and ovipositor wear (reviewed by Finch, 1990; Lalonde and Mangel, 1994). An example common to many flying insects is wing wear. Insects have no mechanism to repair damage to their wings; therefore, as an insect ages and continues to use its wings, the amount of wing wear is cumulative and progressive (Alcock, 1996; Eltz et al., 1999; Hayes and Wall, 1999; Burkhard et al., 2002; Higginson and Barnard, 2004; Lopez-Uribe et al., 2008). Many studies have used wing wear to estimate relative insect age (Mueller and Wolf-Mueller, 1993; Kemp, 2000; Burkhard et al., 2002; Richards, 2003; Inoue and Endo, 2006; Peixoto and Benson, 2008). Wing wear has consequences, which include increased wingbeat frequency (Hargrove, 1975; Kingsolver, 1999; Hedenstrom et al., 2001), changed flight speed (Fischer and Kutsch, 2002), changed flight performance (Haas and Cartar, 2008; Jantzen and Eisner, 2008; Combes et al., 2010), changed foraging behaviour (Higginson and Barnard, 2004; Foster and Cartar, 2011) and increased risk of mortality (Cartar, 1992).

Many eusocial insects, particularly bees and wasps, rely on their wings to defend their nest from predators (Breed et al., 1990; Kastberger et al., 2009), maintain colony temperature and assure proper larval development (Heinrich, 1979a; O'Donnell and Foster, 2001), and acquire food for themselves and their colony (Heinrich, 1979a). Providing protection, care and food for the colony's young are the ways in which a non-reproductive forager increases its inclusive fitness. Therefore, wing wear may have important consequences for both individual and colony fitness.

Risk of mortality in worker bumble bees (*Bombus* spp.) increases with age (Brian, 1952; Garofalo, 1978; Rodd et al., 1980; Goldblatt

and Fell, 1987; Smeets and Duchateau, 2003), as it does in honey bees (*Apis mellifera*) (Dukas, 2008). Wing wear has been speculated to lead to an increased risk of mortality in honey bee drones (Rueppell et al., 2005) and tsetse flies (*Glossina morsitans*) (Allsopp, 1985). Bumble bees that either were wing-clipped or had high naturally occurring amounts of wear died earlier than did those with more pristine wings (Cartar, 1992). An increase in mortality risk with wing wear could result from a number of factors, all of which are supported only by speculation: decreased manoeuvrability, making it more difficult for a bee to escape from predators or severe weather conditions (Rodd et al., 1980), increased energy expenditure (Cartar, 1992) (but see Hedenstrom et al., 2001) and/or increased wingbeat frequency, which matters if the number of wingbeats is limited over a lifespan (Higginson and Gilbert, 2004). Regardless of how wing wear is linked with lifespan, the causes of wing wear have yet to be formally investigated in any insect.

Wing wear is associated with male intra-sexual competition (Alcock, 1996), mating attempts (Ragland and Sohal, 1973), failed predator attacks (Robbins, 1981) and foraging activity (Toth et al., 2009). Because flying workers of eusocial insect species do not compete for mates, do not mate and have few predators that might abrade wings, wing wear is most likely related to foraging activity in these individuals (Breed et al., 1990; Higginson and Gilbert, 2004; Toth et al., 2009); indeed, several studies have speculated that foraging activity leads to wing wear (Allsopp, 1985; Cartar, 1992; Higginson and Gilbert, 2004). In paper wasps (*Polistes metricus*), only workers that foraged exhibited wing wear (Toth et al., 2009). Similarly, bumble bees that perform duties within the colony exhibit little wear (R.V.C., unpublished) and live longer than do

their foraging counterparts (Brian, 1952; Garofalo, 1978). Honey bees that act as colony guards also incur less wing wear and fly less than their forager sisters (Breed et al., 1990). Although the role of foraging in wing wear has been acknowledged (Joyce and Schwarz, 2002; Toth et al., 2009), to date no study has investigated the mechanisms behind the causal relationship. Proposed causes include the amount of wing use (Cartar, 1992) and/or accidental collisions of the wings with vegetation while foraging (Wootton, 1992; Batra, 1994; Higginson and Gilbert, 2004). The dearth of information linking wing wear to wing use does not imply that understanding the cause(s) of wing wear is unimportant; rather, several studies have called for this research (Dudley, 2000; Burkhard et al., 2002; Peixoto and Benson, 2008).

Our study considers whether a bee's wing use during foraging influences wing wear. Bumble bees are eusocial insects whose workers forage on plant species that differ in floral morphology, distribution and the vegetation surrounding their flowers. These differences may influence the flight characteristics of the bees while foraging, such as the proportion of time a bee spends in flight, how frequently a bee initiates a flight within a given period (flight frequency) and how often a bee's wings collide with vegetation within a given period (collision frequency). Flying is roughly 10 times more energetically expensive than walking (Kammer and Heinrich, 1974; Heinrich, 1975). On average, bees spend roughly 25% of their time in flight when foraging in a flower patch. Plant morphology may sometimes allow bees to spend more time on flowers and less time flying, such as when they forage on the terminal head-like inflorescences of wild bergamot (*Monarda fistulosa*) (Cresswell, 1990) or on densely-clustered panicles, such as goldenrod (*Solidago canadensis*) (Heinrich, 1979a). While foraging on these plant species, bees have the option of walking along the inflorescences of an individual plant rather than flying. In contrast, bees foraging on more dispersed inflorescences, such as those of sticky purple geranium (*Geranium viscosissimum*), tall larkspur (*Delphinium glaucum*) or fireweed (*Chamerion angustifolium*), must use costly flight to reach the next flower on an inflorescence or another inflorescence (Foster and Cartar, 2011). Bumble bees also may forage on plant species that influence how often they must initiate flights within a given period. For instance, bees foraging on flowers in a patch of high density are likely to perform more flights within a given period compared with a bee foraging in a patch of low density. Flowers are also often surrounded by vegetation, which bees must manoeuvre around. In so doing, their wings may collide with the surrounding vegetation or flower parts (Batra, 1994; Higginson and Gilbert, 2004). Therefore, depending on the morphology of a plant species upon which a bee forages, its distribution and the vegetative clutter around the flowers, wing use may differ. These different flight characteristics (i.e. proportion of time in flight, flight frequency and wing-vegetation collision frequency) could independently affect wing wear.

To determine what causes loss of wing area in bumble bees, we tested three hypotheses involving wing use during foraging; that wing wear is influenced by: (1) the steady-state use of wings in keeping the bee airborne, (2) the stresses associated with flight initiation and cessation or (3) collisions that occur between wings and vegetation during flight. Wing wear could be related to steady-state wing use because of repeated distortion of the wing that occurs with each stroke; if this is the case we predict a positive relationship between the proportion of foraging time spent in flight and wing wear (H1). Flight initiation and cessation could result in wing wear if wing use during acceleration and/or deceleration is different from that during steady-state flight, and if wing kinematics during take-

off and landing results in wear. If wing wear is caused by flight initiation and cessation, we would predict a positive relationship between wing wear and the frequency of take-offs (flight frequency) during foraging (H2). Lastly, if wing wear is caused by wing collisions against vegetation during flight, then we would predict a positive relationship between the frequency of wing collisions and wing wear (H3). We tested these hypotheses in an observational study of wild-foraging, individually marked bumble bees.

## MATERIALS AND METHODS

### Site and plant species

This study was conducted between 1 July 1 and 31 August 2007 and 2008 in the vicinity of the R. B. Miller field station (50°38'50"N, 114°38'54"W, elevation 1490 m) in the Sheep River Wildlife Sanctuary, Kananaskis Country, southern Alberta, Canada. Bumble bees, *Bombus* spp. Latreille 1802, were observed foraging in an 11 km<sup>2</sup> area on 12 locally common plant species. These plant species were chosen because they present foraging bees with a variety of different floral morphologies, distributions and surrounding vegetation, resulting in a variety of flight characteristics during foraging (particularly proportion of time in flight, flight frequency and collision frequency). The plants were: *Chamerion angustifolium* (18.5% of all observed bees; N=139), *Cirsium arvense* (6.5%), *Delphinium glaucum* (7.9%), *Geranium viscosissimum* (18.0%), *Monarda fistulosa* (12.9%), *Solidago canadensis* (10.1%), *Oxytropis moticola* (3.6%), *O. splendens* (1.4%), *Trifolium hybridum* (3.6%) and *T. pratense* (2.9%). The remaining two plant species (*Melilotus alba* and *M. officinalis*, 15.1%) formed a functional pair, where the flight characteristics of a bee foraging on each species was comparable, and where bees often flew indiscriminately between co-occurring flowers of each species.

### Bee collection and digital video recording of foraging behaviour

Foraging was observed daily between 08:00 and 19:00 h in good weather (range 13–31°C, mean 21°C, not raining). Worker bumble bees were recorded on digital video while foraging in a patch of a focal plant species for ~2 min, using a hard disk camcorder (Everio GZ-MG555, JVC, Kanagawa, Japan) equipped with a directional microphone (ME66, Sennheiser, Wedemark, Germany). Each bee was filmed from a distance of ~1 m and then collected in a clear plastic vial (2×5 cm diameter×height). Vials containing the bees were placed into a cooler filled with ice packs, where they became torpid in ~30 min, and were then taken to a nearby laboratory for processing.

Torpid bees were weighed and marked by gluing a 2.8 mm diameter coloured and numbered plastic disk (queen marking tags; www.beeworks.com) weighing 1.97 mg (<3% of a bee's body mass) onto the upper thorax. Both forewings were then spread, clamped between two microscope slides and photographed in planar view using a digital camera (Coolpix 990, Nikon, Tokyo, Japan) from 2 cm. Bees were then placed back into their vial, cooled and returned to their location of capture. When marked bees were encountered on subsequent days on the same focal plant species, this process of videotaping, capturing and processing was repeated. Bumble bees are relatively plant-faithful; an individual bee generally forages only on one or two plant species (Heinrich, 1979b). Unsurprisingly, bumble bees captured while foraging on one plant species in this study continued to forage on it and were not recaptured on another. If a bee that had previously been recorded foraging on one plant species was found foraging on another, it would be removed from the data set. Time since first capture was calculated as the number

Table 1. Descriptive statistics for the six species of *Bombus* used in this study

| Species                | N  | Bee mass (g) | Loss of wing area per day (mm <sup>2</sup> day <sup>-1</sup> ) | Time in flight (%) | Flight frequency (flights min <sup>-1</sup> ) | Wing collision frequency (collisions min <sup>-1</sup> ) |
|------------------------|----|--------------|--|--------------------|---|--|
| <i>B. appositus</i>    | 10 | 0.221±0.043  | 0.256±0.136  | 30.0±12.6          | 17.8±8.61                                     | 73.1±67.9  |
| <i>B. bifarius</i>     | 56 | 0.102±0.016  | 0.102±0.070  | 24.9±17.7          | 12.7±10.1                                     | 50.5±44.1  |
| <i>B. californicus</i> | 6  | 0.185±0.039  | 0.151±0.088  | 18.9±3.0           | 9.4±3.0                                       | 95.8±27.6  |
| <i>B. flavifrons</i>   | 20 | 0.150±0.077  | 0.103±0.076  | 26.0±12.9          | 17.2±10.7                                     | 48.0±32.7  |
| <i>B. frigidus</i>     | 7  | 0.103±0.015  | 0.147±0.077  | 32.7±14.3          | 13.7±7.9                                      | 69.0±49.8  |
| <i>B. moderatus</i>    | 39 | 0.191±0.036  | 0.170±0.131  | 22.5±13.1          | 15.5±10.5                                     | 57.2±50.9  |

N, number of individuals.

Data are means ± 1 s.d.

of days between the first capture and subsequent recapture of a marked bee. In this calculation, we discounted poor weather days, in which foraging was not possible from time since first capture. Poor weather days occurred eight times during the 90 day study period, resulting in fewer than 10% of records for which the time since first capture was adjusted for non-foraging days.

Of 2439 bees that were individually tagged, 139 workers were recaptured at least once, and up to six times (median=1.0 recaptures; mean ± s.d.=1.50±0.82 recaptures). Table 1 shows the sample sizes and mean body mass of each species. To simplify the analysis, we previously excluded individuals from several rare species, which were: *B. sylvicola* (N=3), *B. nevadensis* (N=2), *B. mixtus* (N=1) and *B. occidentalis* (N=1).

#### Determining loss of wing area

Forewing images were cropped as detailed in Fig. 1. Wing area distal to this crop line was used in the analysis of wing wear, because wing damage to the forewing generally occurs only in this region (Mueller and Wolf-Mueller, 1993; Lopez-Urbe et al., 2008). The distal wing image was then extracted from the background of the cropped image using the magic wand tool in Adobe Photoshop CS2 (v9.0.2, Adobe Systems Inc., San Jose, CA, USA). To calculate wing area from pictures taken at different focal lengths, we first determined how many pixels were represented in 1 mm at different focal lengths from a fixed distance (2 cm) and then performed a regression relating pixels mm<sup>-1</sup> to focal length ( $R^2=0.996$ ,  $N=27$ ,  $P<0.0001$ ). For each wing photograph, we used focal length to estimate the number of pixels mm<sup>-1</sup>, and employed this scale information in ImageJ (v1.37, <http://rsb.info.nih.gov/ij/>) to calculate wing area (mm<sup>2</sup>). To determine mean loss of wing area, we measured the difference in distal wing image area of the left and right wings between the first capture and each subsequent recapture, and then took the mean of this value. For example, if a bee were captured three times ( $t$ ), we would calculate the wing area at  $t=2$

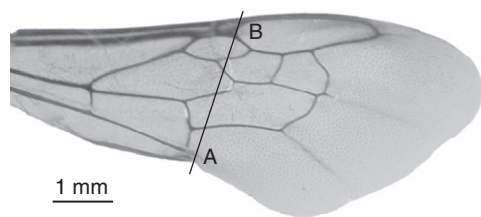


Fig. 1. Distal portion of the forewing of a worker bumble bee (*Bombus bifarius*). The line depicts the position at which the wing image was cropped, running from the centre of the notch on the trailing edge of the wing (A) to the proximal inside tip of the marginal cell (B). Wing area distal to this line was used in the analyses.

and subtract it from the wing area at  $t=1$ , then take the wing area at  $t=3$  and subtract it from  $t=1$ . We included individual bee as a random effect in the analysis, to recognize the repeated-measures nature of the data. Note that wing wear of the hind wing (roughly 25% of wing area) is not considered here, so our estimates of wing wear are conservative.

#### Measures of foraging behaviour

Video records of foraging bees were analyzed using the event recorder software JWatcher<sup>TM</sup> (v1.0, <http://www.jwatcher.ucla.edu>). Time in three states was measured: beating wings (flying or wing-assisted movement), not beating wings (stationary or walking on flower) and out of sight. Playback speed was slowed by a factor of three to permit detailed measurements of behaviours of the moving bees. We then calculated time spent in flight (time beating wings divided by total time in sight) and flight frequency (number of flights initiated divided by total time in sight).

To determine the frequency of wing collisions with vegetation (collision frequency), we used Soundtrack Pro 2 (v2.0.2, Apple Inc., Cupertino, CA, USA) to simultaneously view the video of the bee foraging and the corresponding frequency spectral graph. Bee wingbeat frequency is ~175 Hz (Hedenstrom et al., 2001), with harmonics ranging up to ~2000 Hz. Wing collisions were identified by a spike in the frequency spectral graph in the 16,000–19,000 Hz range (Fig. 2) that were audible in the audio and could be linked to

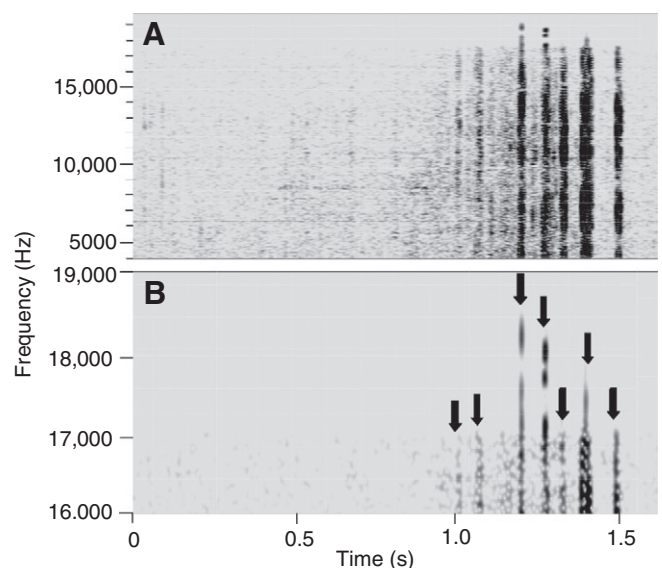


Fig. 2. Sound spectral analysis of a single audio track of a foraging *Bombus moderatus* on *Monarda fistulosa*, viewed using (A) a wide 4000–19,000 Hz and (B) a narrow 16,000–19,000 Hz frequency range. Arrows indicate bouts of wing collisions with vegetation.



the focal bee in the video. The duration of wing collisions was calculated by measuring the thickness of the spike at 16,000 Hz in the frequency spectral graph. We used Praat 5.1.44 (<http://www.fon.hum.uva.nl/praat/>) to find the wingbeat frequency of each bee, setting the pitch to a floor of 120 Hz and the ceiling to 250 Hz, with these frequencies exceeding the extremes of wingbeat frequencies of bumble bees reported in the literature (Hedenstrom et al., 2001; Joos et al., 1991). Because of high background noise, we were unable to calculate wingbeat frequencies of some bees used in this study. For these bees ( $N=16$ , or 11.5%), we estimated wingbeat frequency using bee mass using a second-order polynomial regression equation ( $R^2=0.55$ ).

The frequency of bee wing collisions ( $C$ ) on vegetation over a time period  $t_s$  was estimated as:

$$C = (F_{wb}t_D) / t_s, \quad (1)$$

where  $F_{wb}$  is wingbeat frequency,  $t_D$  is the duration (s) of a wing collision bout and  $t_s$  is time (min) in sight. Note that collision frequency differs from wingbeat frequency, as it is not measured in Hz (wingbeats  $s^{-1}$ ), but rather by the number of wing collisions with vegetation that occurred per minute. Wingbeat frequency is included in the equation of collision frequency because bumble bees beat their wings at  $\sim 175$  Hz and thus they collide their wings with vegetation repeatedly within a single collision bout, depending on their wing area. For bees that were videotaped more than once, the mean of these behaviours (time in flight, flight frequency and collision frequency) during wing use was used. Species-level means of these traits are reported in Table 1.

#### Statistical analysis

Variables were transformed as necessary using Box–Cox transformations to improve normality and homogeneity of the residuals. To reduce the problem of collinearity among variables, variance inflation factors were checked to ensure that all were  $<10$ . Statistical analyses were performed using JMP (v8.0.2, SAS Institute Inc., Cary, NC, USA).

To determine the effect of the flight characteristics on wing area loss, we used a mixed-model ANCOVA to assess how mean loss of wing area ( $mm^2$ ) [ $\ln(\text{wing area}+0.01)$ ] was related to bee species (fixed effect), bee individual (random effect; nested within bee species), year (fixed effect; two states) and six covariates: mean mass (g) of bee ( $-\text{mass}^{-0.5}$ ), time since first capture (d), time in flight (%), flight frequency [ $(\text{flights min}^{-1}+0.01)^{0.5}$ ], collision frequency [ $(\text{collisions min}^{-1}+0.01)^{0.5}$ ] and mean present wing area ( $mm^2$ ) [ $\ln(\text{wing area})$ ]. The presented model includes three two-way

interactions involving the three flight characteristics and time since first capture, which we included because we were interested in determining whether the damage to the wing by the flight characteristics changed over time. We tested for higher order covariate interactions but, as these were not significant, do not present them. We did not include plant species as a variable in the analysis because we did not want the term to statistically control variation from the flight characteristics in which we were interested (i.e. proportion of time in flight, flight frequency and collision frequency). That is, we were interested in using plant-induced variation in flight characteristics to explain wing wear, not in statistically controlling this variation by attributing it to plant species in a statistical model.

#### RESULTS

Loss of wing area did not vary significantly with any of the flight characteristics alone, and did not interact with time since first capture for proportion of time in flight and flight frequency (Table 2). However, collision frequency accelerated loss of wing area as time since first capture increased (time since first capture  $\times$  collision frequency; Table 2, Fig. 3). That is, loss of wing area was increased by high levels of wing hits expressed over longer time periods (particularly over periods greater than 4 days; Fig. 3). Loss of wing area differed by year (least square mean  $\pm$  s.e.m. area loss, 2007,  $-1.231 \pm 0.076$ ,  $N=156$ ; 2008,  $-1.960 \pm 0.149$ ,  $N=52$ ; Table 2); however, this could not be explained by a difference in the flight characteristics between years (i.e. no significant year  $\times$  wing use interactions).

Bees lost more wing area over longer time periods (time since first capture; Table 2) and bee species differed in their wing area loss. Present wing area did not influence subsequent wing wear (Table 2). Mass marginally influenced wing wear (Table 2), with heavier bees showing more wing loss.

#### DISCUSSION

This study found that the flight characteristics of a bee during foraging, particularly collision frequency, are indeed uniquely associated with loss of wing area. Wings collide with vegetation relatively often – roughly one strike every second (Table 1) – although these wing collisions occur in bursts rather than at a constant rate. A collision occurs when a bee is manoeuvring within vegetation, accidentally striking it with its wings (Batra, 1994; Higginson and Gilbert, 2004). Collisions also occur when a bee hits its wings on the inflorescence or flower upon which it is foraging during landing, take-off or wing-assisted movement (D.J.F.,

Table 2. Mixed-model ANCOVA predicting mean loss of wing area (ln transformed) in foraging bumble bees ( $N=206$ ,  $R^2=0.81$ )

| Source  | d.f.     | F     | P                 |
|---|----------|-------|-------------------|
| <b>Bee species</b>  | 5, 124.0 | 1.95  | 0.091             |
| <b>Year</b>   | 1, 140.7 | 17.63 | <b>&lt;0.0001</b> |
| <b>Time since first capture (days)</b>                                  | 1, 170.4 | 51.46 | <b>&lt;0.0001</b> |
| Mean mass (g; $-\text{mass}^{-0.5}$ )                                   | 1, 138.0 | 3.99  | <b>0.048</b>      |
| Mean current wing area [ $mm^2$ ; $\ln(\text{wing area}^2)$ ]           | 1, 140.7 | 2.24  | 0.137             |
| Time in flight (%)  | 1, 117.0 | 0.19  | 0.662             |
| Flight frequency [ $(\text{flights min}^{-1}+0.01)^{0.5}$ ]             | 1, 127.2 | 0.03  | 0.872             |
| Collision frequency [ $(\text{collisions min}^{-1}+0.01)^{0.5}$ ]       | 1, 131.3 | 0.35  | 0.555             |
| Time since first capture $\times$ Time in flight                        | 1, 148.7 | 0.23  | 0.632             |
| Time since first capture $\times$ Flight frequency                      | 1, 139.1 | 0.08  | 0.779             |
| <b>Time since first capture <math>\times</math> Collision frequency</b> | 1, 170.1 | 7.71  | <b>0.006</b>      |

Bee individual is a random effect (nested within bee species).

Terms that are statistically significant ( $P<0.05$ ) are in bold.

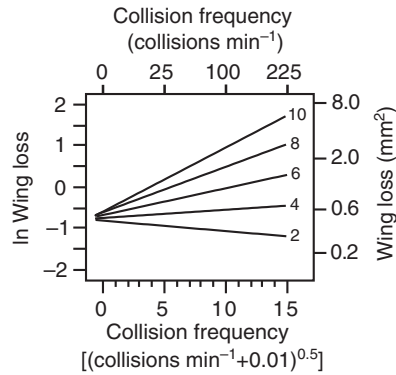


Fig. 3. Model fit of the interaction between collision frequency and time since first capture affecting mean loss of wing area in *Bombus* spp., after controlling for the other variables in the ANCOVA. Numbers to the right of each line represent the number of days since first capture. The fitted surface for >10 days is not presented because of small sample size.

unpublished). Wing collisions were related to greater amounts of damage as time since first capture increased (Fig. 3). An explanation for steeper collision–area-loss relationships over longer time periods could be because wing wear progresses from damaged sites along the wing edge (e.g. Burkhard et al., 2002). Damaged areas could be spread locally more easily when future wing collisions occur. Wing wear resulting from wing collisions could also increase over time if there was positive feedback, such that wings damaged by collisions resulted in a bee becoming less manoeuvrable when foraging, which may further increase the frequency of collisions. However, because wing wear did not accelerate based on present wing area (Table 2), this explanation is unlikely, but it may explain the accelerating wing wear observed in honey bees (Higginson and Barnard, 2004).

Bee mass was also a marginal contributor to wing wear. Bigger bees have bigger wings and thus would lose more wing area compared with smaller bees if the proportion of wing wear per day remained the same. However, this cannot explain why bigger bees experienced greater wing wear, as mass was still positively related to loss of wing area, even when present wing area was controlled for statistically. Larger bees may forage in cooler (Heinrich, 1979a) and darker conditions (Kapustjanskij et al., 2007) compared with their smaller counterparts; thus, they most likely spend more time foraging per day, or forage on days that smaller bees do not. This would result in them having more opportunities to damage their wings.

It is possible that loss of wing area could also be a result of underrepresented or unmeasured variables. We found that there was no relationship between time in flight and wing wear in bumble bees while foraging in a flower patch; however, the lack of a relationship is not evidence that the total time a bee spends in flight has no effect on wing wear. Bees must fly back and forth from their colony to their foraging sites multiple times a day over distances potentially up to 3 km away (Westphal et al., 2006), although the variability of flight radius from the colony is debated (Osborne et al., 2008; Wolf and Moritz, 2008; Charman et al., 2010). At our site, bee foraging trips take an average of approximately 50 min (R.V.C., unpublished). If their foraging patches are an average of 500 m from the colony and they fly at  $5 \text{ m s}^{-1}$ , then the time spent commuting to the patch is roughly 7% of the trip time. We were unable to film bumble bees flying between the nest and the foraging patch, potentially underrepresenting total time in flight, which could

have obscured a relationship between time in flight and wing wear. Unsuccessful predation attempts could also potentially influence wing wear. Bumble bee predators, and evidence of predation attempts (i.e. deeply torn wings), were rare at our field sites; therefore, predation seems unlikely to be a major contributor to wing area loss in bumble bees, although we cannot rule it out completely.

Wing wear appears to be primarily caused by the wings physically contacting an object (in our study, vegetation). This may explain why wing wear is not only related to foraging activity but also to intra-sexual competition (Alcock, 1996), failed predation attempts (Ragland and Sohal, 1973) and mating attempts (Robbins, 1981) in other insects; during these activities it is likely that the wings of these insects come into physical contact with the bodies or jaws of other individuals and/or the ground, resulting in wing damage.

Many studies have found that wing wear correlates weakly with relative age in insects, but that individuals do not incur wear at the same rate (Eltz et al., 1999; Burkhard et al., 2002; Lopez-Urbe et al., 2008), perhaps because individual behaviours leading to loss of wing area are ignored. An assumption of age grading is that wing wear should occur at a similar rate in individuals over a given time (Burkhard et al., 2002). In this study, this assumption is violated because wing wear is influenced not only by time, but also by the flight characteristics of a bee (Fig. 3). This suggests that the flight characteristics of a bee should also be considered to accurately age-grade flying insects, especially for social insects that use their wings while foraging.

Wing wear is associated with mortality in bumble bees (Cartar, 1992) and has been speculated to increase the risk of mortality in male honey bees (Rueppell et al., 2005) and tsetse flies (Allsopp, 1985). Individuals with a high frequency of wing collisions or high flight frequencies are more likely to suffer from wing wear over the long term, which could render them less manoeuvrable when escaping predators or flying between flowers in complex three-dimensional environments or unfavourable weather conditions. By determining the influence of flight characteristics during foraging on loss of wing area, we are one step closer to linking foraging behaviour to mortality in bumble bees, and potentially in other eusocial insects.

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