Shape of wing wear fails to affect load lifting in bumble bees with experimental wing wear

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Shape of wing wear fails to affect load lifting in bumble bees with experimental wing wear

Jordan C. Roberts & Ralph V. Cartar

Author for correspondence

Jordan C. Roberts, Department of Biological Sciences, University of Calgary
2500 University Drive N.W., Calgary, AB, Canada, T2N 1N4
Phone: (403) 220-3555
Fax: (403) 289-9311
jcrobert@ucalgary.ca

Ralph V. Cartar, Department of Biological Sciences, University of Calgary
2500 University Drive N.W., Calgary, AB, Canada, T2N 1N4
cartar@ucalgary.ca
Abstract

Wing wear reflects the accumulation of irreversible damage to an insect’s wings over its lifetime, and this damage should influence flight performance. In the case of bumble bees, flight seems robust to variation in wing area asymmetry and air pressure, but not to loss of wing area. However, how the pattern of wing wear affects flight performance remains unstudied. In nature, wing wear typically occurs in a ragged and haphazard pattern along the wing’s trailing margin, a shape strikingly different from the straight cut applied in past studies. In this study, we test if shape of wing wear (implemented as 4 distinct treatments plus a control) affects maximum load lifting capabilities and wingbeat frequency of worker bumble bees (*Bombus impatiens* Cresson 1863). We found that shape of wing wear of 171 mg bees had no detectable effect on maximum load lifting capability (detectable effect size = 18 mg) or on wingbeat frequency (detectable effect size = 15 Hz), but that loss of wing area reduced load lifting capability and increased wingbeat frequency. The importance of wing area in explaining the load lifting ability of bumble bees is reinforced in this study. But, paradoxically, shape of wing wear did not detectably affect lift generation, which is determined by unsteady aerodynamic forces in these lift-reliant insects.

Key words: *Bombus impatiens*; bumble bees; insect flight, insect wing; load lifting; wing shape; wing wear.

Header:

Jordan C. Roberts & Ralph V. Cartar, Load-lifting capability in bumble bees is insensitive to shape of wing wear
Unlike birds, which can preen misaligned feather barbs or regrow damaged feathers, insects lack a mechanism for wing repair. This means that damage resulting from collisions (Higginson and Gilbert 2004), fighting (Alcock 1996), predation attempts (Benson 1972), and prolonged use (Allsopp 1985) will accumulate through an insect’s life. For bumble bees (Hymenoptera: *Bombus* spp.), wing wear is primarily caused by collisions with foliage during foraging bouts (Foster and Cartar 2011a). Experimentally-induced wing wear in bumble bees results in increased mortality (Cartar 1992) and decreased load lifting (Johnson and Cartar 2014), but with little effect on metabolic rate (Hedenstrom et al. 2001) or on flight trajectories while traveling between flowers with unobstructed flight paths (Haas and Cartar 2008).

Experimental studies of wing wear and flight capabilities of bees have typically simulated wing wear by clipping a straight strip along the trailing edge of the forewing (Cartar 1992; Hedenstrom et al. 2001; Haas and Cartar 2008; Johnson and Cartar 2014; Vance and Roberts 2014), or by removing the hind wing (Buchwald and Dudley 2010). In nature, wing wear occurs more stochastically, often in a haphazard and ragged pattern along the distal edge of the forewing or involving small punctures, not a clean shear (Mueller and Wolf-Mueller 1993; Mountcastle and Combes 2014; Fig. 1). It is therefore possible that inferences about effects of wing wear on flight and mortality that use a straight cut might be limited in applicability to wing wear in nature. In this study, we test the hypothesis that the shape of wing wear affects the flight capability of bumble bees, in addition to area and asymmetry.

Natural ragged and artificially straight-clipped wing edges might differ in their aerodynamic performance. One mechanism for diminution of flight performance in naturally-worn wings is the presence of “flaps” (i.e., protruded tips) unsupported by adjacent wing
membrane. While spanwise flexion of wings may be important in the production of lift during bumble bee flight (Mountcastle and Combes 2013), the “flaps” of a ragged edged, naturally-worn wing (Fig. 1) may be too flexible and generate little or no lift during flight, partially through the loss of induced camber, and may be affected by any supporting wing veination (e.g., Fig. 2E, arrow). Wing flexion is important in insect flight (Wootton 1992), with induced camber being particularly important for generating lift (Young et al. 2009). A wing edge with flaps might therefore generate less lift than expected simply on the basis of area, particularly if the flaps are not braced by wing veination.

A ragged edge may also increase the turbulent vorticity experienced by a wing in flight, increasing drag. However, to our knowledge, no studies have examined shape of wing wear and aerodynamics in insects. To stretch the comparison, we have drawn on knowledge from bird moulting and battle damage in aircrafts as a source of comparison. In simulations of bird flight using theory for fixed-wing aircraft, wing shape mattered: lift to drag ratios were lower in moulting rectangular wings than in intact rectangular wings, and gap size and position affected performance (Hedenstrom and Sunada 1999). Similarly, aircraft with simulated battle damage at mid-length distances on the airfoil, at angles of attack > 8°, suffered major decreases in their coefficient of lift and increases in their coefficients of drag (Irwin 1999). Despite differences in Reynolds number and in kinematics (fixed-wing aircraft vs. bees with a semi-circular wingbeat at ~180 Hz), damage-induced disruption of airflow patterns may to some extent be shared between worn bee wings and moult- and battle-damaged plane wings, and suggest that irregular wing shape can be detrimental to generation of lift. Thus, deep valleys of ragged wing wear and holes in the wing may disturb airflow over the wing, lowering lift to drag ratios.
Among bees, wing wear is manifested in several qualitatively different shapes that we attempt to independently simulate in this paper: flaps (Fig. 1A,B), valleys (Fig. 1A,B) (supported by distal venation and unsupported), holes (Fig. 1B,C), and raw area loss (Fig. 1D). The valleys of wing wear can produce deep span-wise damage from the trailing wing edge. Raggedness produces flaps (supported or unsupported by wing veination). Holes can also produce damage at many points along the length and chord of the wing, but generally only involve the loss of a small amount of wing membrane. We compare the performance of wings damaged according to these classes of wear shape (Fig. 2A,B,C) with the performance obtained from straight clipping along the wing margin (Fig. 2D) used in previous experiments (Cartar 1992; Hedenstrom et al. 2001; Haas and Cartar 2008; Vance and Roberts 2009; Johnson and Cartar 2014), or no wear (controls; Fig. 2E). We measure flight performance as the maximum load lifting capabilities of bees during hovering flight (Dillon and Dudley 2004; Buchwald and Dudley 2010).

In challenging flight circumstances such as following experimentally induced wing wear, bees may attempt to increase their lift production by altering their wingbeat kinematics. Two ways this could be accomplished are through an increased wingbeat frequency, or an increased stroke amplitude. In low pressure conditions simulating high altitude, bees compensate by increasing their stroke amplitude, rather than increasing their wingbeat frequency (Altshuler et al. 2005; Dillon and Dudley 2014). However, honey bees *Apis mellifera* (Linnaeus 1758) with asymmetrical and symmetrically induced wing wear (of the straight edge variety) increased both their stroke amplitude and (to a lesser extent) their wing beat frequency (Vance and Roberts 2014). Carpenter bees *Xylocopa varipuncta* (Patton 1879) hovering in lower density gas mixtures also use both strategies to generate lift (Roberts et al. 2004). We therefore also examine how wing beat frequency varies with shape of wing wear. The typical outcome of loss of wing area is
an increase in wingbeat frequency, so we expected an increase in wingbeat frequency at higher levels of wear. We also expected a greater increase in stroke amplitude in the treatments hypothesized to have the largest impact on flight performance (i.e., unsupported flaps and holes), but could not test this hypothesis, as we did not measure stroke amplitude.

In this experiment, we examine how shape of simulated wing wear affects maximum load lifted and wingbeat frequency of flying bumble bees. If location of wear affects flight performance, then each treatment tests a particular mechanism which may affect lift producing capabilities, related to length of the intact wing (the valley treatment), support from adjacent wing membrane (the flapped treatment), and air pressure between top and bottom wing surface (the hole treatment). We test these hypotheses with bumble bees, using the asymptotic loading method for estimating maximum capacity for load lifting (Buchwald and Dudley 2010).

**Materials and methods**

The experiment was conducted on bees from a commercially raised colony of *Bombus impatiens* (Cresson 1863), obtained from BioBest (Leamington, ON, Canada). Assessments were carried out in November 2013 and December 2013. The colony was provided with a nectar solution and a pollen paste *ad libitum*.

Prior to wing alteration, bees were anesthetized in a -20°C freezer for (mean ± SD) 5.97 ± 1.45 min, until the subjects were unable to flap their wings. Three bees that were unable to fly or resume coordinated movement following being removed from the freezer were excluded from analysis, as their behaviour suggested they suffered damage from the anesthesia. Time in freezer was not significant in statistical models explaining load lifting or wingbeat frequency, and therefore not included in fitted models.
Several types of wing shape were experimentally applied (Fig. 2A-E) using a fine pair of scissors. Bees with intact wings (Fig. 2E) served as controls. Control bees were handled in an equivalent manner to those with wing manipulations applied. Straight-clipped wing treatments were cut in a similar fashion to those of previous experiments: a thin strip was cut parallel to the distal edge of the wing (Fig. 2D; Cartar 1992; Hedenstrom et al. 2001; Haas and Cartar 2008). To produce a flapped wing edge, two V-shaped areas were cut from the distal edge of the wing, placed such that the area between formed a triangular flap (Fig. 2A). In the hole treatment, wings were pierced with a 1 mm diameter probe, scraping against the wing in a circular arc to remove area, rather than simply puncturing the membrane (Fig. 2B). The hole treatment was used to determine the effect of wear at a specific point, with minimal impact on area loss, as well to allow wing damage away from the wing’s trailing edge, a wear feature that occurs naturally (Fig. 1B, C). In the valley treatment (with one “V” shape removed), we removed a singular wide “V”, with its apex approximately at mid chord (Fig. 2C).

Bumble bees used in this study (n=57) ranged in mass from 90 to 288 mg (mean ± SD: 171.4 ± 44.1 mg) with mean marginal cell length (± SD) of 2.71 ± 0.25 mm. For the treatments with major area loss (i.e., not the control or hole treatment), the mean area losses (%) were: flapped treatment 11.2 ± 3.8 % (n=19), valley treatment 12.5 ± 3.3 % (n=9), and straight cut treatment 23.4% ± 4.4 % (n=10). Bees with the straight cut treatment had the most wing area removed, with 3 distinct wing loss groupings (Tukey HSD P < 0.05 contrasts: control = hole < flapped = valley < straight cut) flapped and valley were statistically indistinguishable). Lengths of mid-flaps of the flapped treatment, measured from the flap centre, were (mean ± SD) 1.11 ± 0.438 mm. For the valley treatment, the depths of the removed areas (mean ± SD) were 1.53 ± 0.31 mm.
We used an asymptotic lifting mechanism (Chai et al. 1997; Dillon and Dudley 2004; Buchwald and Dudley 2010; Johnson and Cartar 2014) to determine the maximal load lifting capabilities of bumble bees. A beaded string was tied with a noose knot around the petiole, which is near their centre of mass (Dudley and Ellington 1990). The beads were placed in sets of two at known distances and weights. Bees were compelled to vertically ascend by an overhead 13 W ultraviolet light (Blue Planet, Trileaf Distribution, Trifeuil, Toronto, ON, Canada) positioned 48 cm above the test arena. Each bee rose to a height at which it could not lift the next bead set. The most proximal bead sets were placed approximately 5 cm from the noose and following this set, bead sets were placed 2 cm apart. The average bead set weighed (mean ± SD) 15.9 ± 4.8 mg. The maximum load lifted was calculated as the total bead and string weight lifted by the bee. Bees completed flight trials in a small clear plexiglas box (29x29x20 cm) open at its top and filmed directly overhead by a Panasonic Lumix-DMC-FZ200 at 30 fps mounted 65 cm above the chamber. A mirror was placed at a 45° angle at the bottom of the flight chamber, and a vertical scale placed on the wall opposite to the mirror, so height could be determined from the overhead recording. The maximal load lifted during the 15-minute flight trial was the flying bout where the bee lifts the most bead sets. Flying bouts where bees lifted off towards the wall of the experimental arena within 2 cm, or where bees ascended while within 2 cm of the walls were not counted. This was done to avoid the complication of boundary effects on flight (Rayner and Thomas 1991). During the trials bees were largely left alone. However, if bees were consistently lethargic, they were encouraged to fly by a gentle puff of human breath, or by gentle prodding. In cases where the noose knot of the beaded string was clearly impeding movement, the knot was rearranged to a more favourable position.
Following each trial, bees were sacrificed (by freezing), weighed and their forewings removed and photographed (Olympus E-420 Zuiko Digital). Wing area loss was determined using ImageJ v 1.47 (Rasband, NIH, MD, USA, 2014). Trials occurred in temporal blocks (n=10) with every treatment occurring in random order in each block. Bees were haphazardly selected from the colony to participate. Each bee received only one treatment as repeated anesthesia affects a bee’s flight, independent of wing alteration (personal observation).

To explore the compensation mechanisms of bees with wing wear, the wingbeat frequencies of bees were determined from the sound files associated with the flight trials. Audio tracks were exported from a short period of flight using SimplemovieX v 3.12 (Aero Quartet, Spain, 2010). Pitch was quantified as the dominant frequency assumed to correspond to the wing beat frequency of the bee. We examined for a dominant frequency in the range 150 to 250 Hz for each bee using Praat v 5.3.56 (Boersma and Weenink, Institute of Phonetic Sciences, 2005). Audio was selected from the trials for clarity and consistency, using steady (non-erratic) flight bouts, to avoid Doppler effects of rapid bee movements and changes to wingbeat frequency due to maneuvering. Several flight trials (n=10) contributed no data on dominant frequency. These tended to be when bees only achieved short flight periods, or with high background noise. We extracted frequencies from the “voiced” frames in Praat, which are the audio frames in which a dominant frequency can be determined.

Statistical analyses were completed using JMP v11.0 (SAS institute Inc, Cary, NC, USA). ANCOVAs were fit explaining maximum load lifted (g^{0.5}) and wingbeat frequency (Hz) from treatment (5 states), area loss (%), and marginal cell length (a proxy measurement for bee size; Fig. 2E). Maximum loads lifted were squareroot transformed to meet the assumptions of our analyses (i.e., normal and homogeneous residuals). Marginal cell length is commonly used as
a measure of bee size (e.g., Plowright and Jay 1968; Goldblatt and Fell 1987; Owen 1988).

Marginal cell length was used as a measurement of size in preference to bee mass, since nectar crops were not cleared prior to trials and mass may therefore be a less reliable measurement of bee size. Marginal cell length in the subjects used in this experiment was strongly correlated with intact wing area ($r = 0.982$), and wing area is strongly correlated with bee mass, at least interspecifically (Bullock 1999). As well, we found marginal cell length ($\ln[\text{mm}]$) and bee mass ($\ln[\text{g}]$) were strongly correlated in our subjects ($r = 0.904$). We expect the loss of precision is due to nectar load in our subjects. Therefore the use of marginal cell length is likely an appropriate size measurement for bees. Area loss (%) and the treatments were highly correlated (variance inflation factors $>8$), so these two variables were analyzed in separate models as well (i.e., new models either included treatment or area loss). None of the “nuisance” variables (time in freezer, block, or specific beaded string used) were significant in the models explaining lift and they were therefore excluded from the models presented. In the analysis of wingbeat frequency, block was included as a random effect, as it explained a small portion of the variance (11.3% in the saturated model, 4.0% in the treatment model, and 7.3% in the area loss model).

**Results**

The saturated model explaining maximum load lifted with all of the explanatory variables (treatment (5 states), area loss (%), and marginal cell length) showed area loss and marginal cell length had a significant effect on maximum load lifting capability (Table 1). Larger bees lifted larger loads, and those with greater area loss lifted less. Wing shape did not affect load lifting ability (Table 1). The same conclusion is obtained from models in which wing area loss and treatment are considered in isolation, along with bee size. For the treatment model, all treatments (including controls) still had statistically indistinguishable load lifting abilities (Fig. 3a; Table 1),
but larger bees lifted bigger loads (Fig. 3b; Table 1). In contrast, area loss again reduced
maximal load lifting capability (Fig. 4a; Table 1), while larger bees still lifted bigger loads (Fig.
4b; Table 1). Taking these three model fits together, load-lifting was affected by area loss, not by
treatment.

The saturated mixed effects model explaining wing beat frequency found larger bees had
lower wing beat frequencies, but that, area loss and treatment were insignificant. But results
were different when wing area loss and treatement were considered in isolation, along with bee
size. In the treatment model of wingbeat frequency, wingbeat frequency was unaffected by
treatment (Fig. 5a; Table 1), but larger bees had lower wingbeat frequencies (Fig. 5b; Table 1).
In the area loss model, area loss (%) had a significant positive effect on wingbeat frequency (Fig.
6a; Table 1), and bigger bees had lower wingbeat frequencies (Fig. 6b; Table 1). Hence,
wingbeat frequency was affected by area loss, not treatment. Taking these three model fits
together, wing beat frequency is affected by wing area loss, but not by shape of wing damage
(i.e., treatment).

The detectable effect size for the load lift capabilities using the treatment model, with a
power of 0.95, an \( \alpha \) of 0.05, \( n=57 \), and the observed sample variance, was 0.135 \( g^{0.5} \) or \(~18.2 \) mg.
That is, our conclusion of no effect of wing shape on load lifting means that differences among
treatments in load lifting that were within 18.2 mg qualify for us as having “no effect”. For
wingbeat frequency, we calculated detectable effect size using a ANCOVA (power analysis is
not possible in a mixed effects model. In the resulting model, using a power of 0.95, \( \alpha = 0.05, n \)
= 47, and the observed sample variance, the detectable effect size was 15.5 Hz. Hence, our
conclusion of no effect of wing shape on wingbeat frequency can be qualified in that treatments
that were no more different than 15.5 Hz (i.e., ~8% of an average control bee’s wingbeat frequency of 190 Hz) are statistically indistinguishable.

Discussion

In analyses of both maximum load lifting capability and wingbeat frequency, treatments that varied the shape of wing wear had no detectable effect on the outcomes. For bumble bees, the shape of area loss is apparently inconsequential to load lifting capability. Area loss however is a major determinant of load lifting capability. Hence, the mechanisms by which we proposed different shapes of wing wear to be affecting aerodynamic capabilities are likely not having a major impact on lift-producing capabilities.

The detectable effect size of these results on load-lifting capabilities (roughly 18 mg) is approximately 1/5 of of a 170 mg bumble bee worker’s hourly foraging rate (Spaethe and Weidenmuller 2002), and roughly 1/3 of a 162 mg bumble bee worker’s foraging load size (Johnson and Cartar 2014). Thus, if there are indeed differences in load-lifting ability among wing wear shapes, they likely reflect a fraction of an individual’s foraging capabilities. A bigger sample size and a more controlled means of adjusting wing shape could distinguish more subtle differences in load lifting that may exist between shapes of wing wear.

Our results are congruent with the noneffect of asymmetry of wing wear on flight capability (Johnson and Cartar 2014). However, they are in some ways contrary to those of Hedenstrom and Sunata (1999), who modeled flight capabilities of moulting birds, and found the location of missing feathers important to lift capabilities. Hedenstrom and Sunata (1999) used steady-state aerodynamics in their model, which might poorly reflect unsteady state dynamics acting on an insect wing in flight (Dickinson et al. 1999, Hedrick et al. 2014). To our knowledge, there has
been no analysis of worn-winged insect flight that uses unsteady state mechanisms, and from the current study these mechanisms (reviewed by Hedrick et al. 2014) would appear to be necessary to explain our results. Through the use of modeling and particle image velocimetry, it should now be feasible to study the unsteady aerodynamics involved in a damaged insect wing during flight (Hedrick et al. 2014).

It also appears that bees do not compensate for wing wear shape by changing wingbeat frequency. Either this is because there is truly little effect of wing wear shape on aerodynamic performance, or because bees increase their stroke amplitudes (not measured by us) in compensating for the changes in shape (Altshuler et al. 2005; Dillon and Dudley 2014; Vance and Roberts 2014). Bees increase their wingbeat frequency at higher levels of wear. This likely has to do with the decreased inertia in flapping smaller, lighter wings (Vance and Roberts 2014). It is difficult to say whether the increased wingbeat frequency in bees with higher area loss serves as the primary aerodynamic compensation, without knowing the stroke amplitudes of these bees. Honey bees with worn wings (straight cut) increased their stroke amplitude with increased wing wear (Vance and Roberts 2014).

Maximal lift capability is but one factor of a bee’s flight. Others aspects of flight may be more impacted by changes to shape of wing wear. In very simple foraging scenarios, bumble bees suffer minor losses in flower-to-flower flight capabilities with major wing wear (Haas and Cartar 2008). However, in more complex foraging scenarios, where a bee must fly between flowers in an inflorescence, or change altitude in order to land on the next set of flowers, more complex and difficult maneuverability must be implemented (speculated by Higginson and Barnard 2004). Bumble bees make foraging choices in the field that depend on their wing wear (Foster and Cartar 2011b). As such, it is worthwhile to examine the effect of shape of wing wear
in natural circumstances, to determine the effect of wing shape on maneuverability. Similarly, Lepidoptera with experimentally removed hindwings have severely reduced maneuverability, while still being able to fly (Jantzen and Eisner 2008). Among Drosophila, the complex maneuvers involved in predator avoidance are controlled by subtle changes to the wing kinematics (Muijres et al. 2014). Maneuverability, again, may depend on wing shape.

Bees use some mechanisms for avoiding wear during flight, which likely involve the spanwise flexion and flexural stiffness of the wing (Mountcastle and Combes 2014). Regardless of the mechanisms, our study suggests bees are likely more dependent on preventing loss of wing area, rather than conserving wing shape. Along with providing support, the venation of the wing appears to be more resilient to wing wear. But the trailing, distal edge of bee wings lacks venation and closed cells. This may indicate the mass and rigidity associated with venation is more costly to flight than is wing wear in the distal portions of the wing.

Overall, the shape of wing wear appears not to be important in affecting a bee’s load lifting capabilities. This research adds wear shape to the category of traits to which a bee’s flight capabilities are resilient, along with bees’ ability to forage with wing wear, resist the pitfalls of asymmetric damage, and fly at low air pressures (Haas and Cartar 2008; Johnson and Cartar 2014; Dillon and Dudley 2014). Clearly, bumble bees are capable of accommodating a wide array of wing injuries during flight: apparently all but loss of wing area.

Acknowledgements

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References


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Tables

Table 1. Results of ANCOVAs regressions explaining maximum load lifted and wing beat frequency using all variables (Saturated model), and one variable at a time (Treatment and Area loss models). The analyses of wingbeat frequencies used the blocking variable order as a random effect. The first 4 rows show overall model fit. A (+) or (-) following the \( p \) values indicate the direction of the relationship. Variance Inflation factors for wing area removed in the saturated models are 8.2 for load lifted, and 9.0 for wingbeat frequency, suggesting that the “saturated models” should be regarded with skepticism.

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Figure Captions

Fig. 1. Examples of natural wing wear in wild-foraging bumble bees in SW Alberta, Canada. Flaps are denoted with thick arrows, and valleys with thin arrows. A) Valley & flapped (*B. mixtus*), B) Valley and flapped and hole, (*B. flavifrons*), C) Hole and area loss (*B. perplexus*), D) Area loss (*B. mixtus*).

Fig. 2. Treatments applied to bee wings (*B. impatiens*). A. Flapped; B. Hole; C. Valley; D. Straight cut; E. Control, indicating length of the marginal cell (solid line) and the distal wing-thickening that potentially provided support for marginal flaps (arrow).

Fig. 3. Relationship between maximum load lifting capability and: A. wing treatment (control (n=10), straight cut (n=10), flapped (n=19), hole (n=9), and valley (n=9)), B. bee size (marginal cell length). Least square means (± 1 SE) and leverage plots are based on the model reported in Table 1 (column 3).

Fig. 4. Relationship between maximum load lifting capability and: A. wing area removed, B. bee size (marginal cell length). Leverage plots are based on the model reported in Table 1 (column 5).

Fig. 5. Relationship of wingbeat frequency and: A) wing treatment (control (n=8), straight cut (n=9), flapped (n=14), hole (n=8), and valley (n=8)), B) bee size (marginal cell length). Least square means (± 1 SE) and leverage plot are based on the mixed effects model reported in Table 1 (column 4).

Fig. 6. Relationship of wingbeat frequency and: A) wing area removed, B) bee size (marginal cell length). Leverage plots are based on the mixed effects model reported in Table 1 (column 6).
Figure 1.
Figure 2

A

B

C

D

E

Figure 3

Figure 4
Figure 5

Figure 6