Effects of load type (pollen or nectar) and load mass on hovering metabolic rate and mechanical power output in the honey bee *Apis mellifera*

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Summary

In this study we tested the effect of pollen and nectar loading on metabolic rate (in mW) and wingbeat frequency during hovering, and also examined the effect of pollen loading on wing kinematics and mechanical power output. Pollen foragers had hovering metabolic rates approximately 10% higher than nectar foragers, regardless of the amount of load carried. Pollen foragers also had a more horizontal body position and higher inclination of stroke plane than measured previously for honey bees (probably nectar foragers). Thorax temperatures ranked pollen > nectar > water foragers, and higher flight metabolic rate could explain the higher thorax temperature of pollen foragers. Load mass did not affect hovering metabolic rate or wingbeat frequency in a regression-model experiment. However, using an analysis of variance (ANOVA) design, loaded pollen and nectar foragers (mean loads 27% and 40% of body mass, respectively) significantly increased metabolic rate by 6%. Mean pollen loads of 18% of body mass had no effect on wingbeat frequency, stroke amplitude, body angle or inclination of stroke plane, but increased the calculated mechanical power output by 16–18% (depending on the method of estimating drag). A rise in lift coefficient as bees carry loads without increasing wingbeat frequency or stroke amplitude (and only minimal increases in metabolic rate) suggests an increased use of unsteady power-generating mechanisms.

Key words: flight metabolism, loading, power output, wing kinematics, insect, honey bee, *Apis mellifera*.

Introduction

Many flying insects transport loads that substantially increase body mass. Load carriage requires an increase in induced power, the component of aerodynamic power that creates lift (Ellington, 1984c). These increases in induced power can be generated by increased wing stroke amplitude, wingbeat frequency and/or the mean lift coefficient averaged over the stroke, which together require an increase in metabolic rate, assuming constant total efficiency of force production (cf. Lehmann, 2001). In this study we investigate the effect of load carriage on metabolic rates (mW), thorax temperatures, wing kinematics and mechanical power output of flying honey bees, *Apis mellifera*.

The loads carried by honey bee foragers range widely and can approach their maximal lifting capacity. Honey bees typically carry pollen and nectar loads representing 20% and 35% of body mass, respectively, maximally reaching 80% of body mass (Winston, 1987). This load-lifting capacity is what would be expected from the ratio of thorax to body mass of 0.4–0.5 (Marden, 1987).

Currently, there is a conflict in the literature regarding the effect of load carriage on flight metabolic rate in honey bees. Wolf et al. (1989) found that metabolic rates during hovering and slow forward flight were positively correlated with nectar load, increasing by approximately 40% when carrying loads equal to 75% of body mass. In contrast, Balderrama et al. (1992) and Moffat (2000) found that metabolic rates of honey bees were unrelated to load size (for loads up to 30% of body mass), but were linearly related to the reward rate of experimental flowers. These authors suggested that behavioral activation may be a more important determinant of flight metabolic rate than increases in power requirements during loading (Balderrama et al., 1992; Moffat, 2000).

The prior studies of load-lifting in honey bees have all examined nectar carriage. Flight metabolic rate of honey bees can be affected by the sugar content of hemolymph and the crop (Gmeinbauer and Crailsheim, 1993), so it is conceivable that the results of Wolf et al. (1989) were confounded

by variation in hemolymph sugar levels. However, the Balderrama et al. (1993) and Moffat (2000) studies can be criticized because metabolic rates were averaged over periods including flight and non-flight (during which bees sat and fed on nectar). We address this controversy in two ways. First, we compare loading effects on metabolic rates in pollen and nectar foragers. We can directly compare our nectar-loading data to prior studies. In addition, because pollen foragers do not consume their load, there should be no confounding effects of variation in hemolymph sugar levels when comparing loaded and unloaded pollen foragers. Second, we examine the effect of load carriage on wing kinematics and calculated mechanical power output in hovering pollen foragers. In bumblebees, loading causes similar increments in metabolic rate and mechanical power output (Cooper, 1993); it seems reasonable that honey bees should show a similar pattern.

The type of load carried (pollen or nectar) may also affect the metabolic cost of load carriage during flight. First, pollen is carried externally on the pollen baskets of the legs, whereas nectar is carried internally in the abdominal honey stomach, potentially causing the center of mass of the animal to differ with the two load types. Changes in the center of mass may alter body angles, wing positions and angles of attack, or may require correctional changes in the flight movements to prevent such changes in body angle. We measured wing kinematic patterns in pollen foragers, and compare these to previous data (Ellington, 1984a,b,c).

Secondly, honey bees tend to specialize on either pollen or nectar and such specialization is partially genetically based (Calderone and Page, 1989; Robinson and Page, 1989; Fewell and Page, 1993). Flight metabolic rates differ among genotypic lineages within a colony (Harrison et al., 1996a), leading to the question of whether genetic differences between pollen and nectar foragers are also associated with metabolic variation. To partially address this possibility, we tested for genetic differences between our pollen and nectar foragers using variation in allozymes of malate dehydrogenase-1 (MDH-1), which has been linked to varation in flight metabolism in honey bees (Coehlo and Mitton, 1988; Harrison et al., 1996a).

Thirdly, variation in metabolic rates between pollen and nectar foragers could be generated by variation in cooling capacities during flight. Nectar foragers can extrude their crop contents for evaporative cooling of the body, while pollen foragers lack this option (Heinrich, 1979; Cooper et al., 1985). Thoracic temperatures affect flight force output and metabolic rate (Feller and Nachtigall, 1989; Nachtigall et al., 1989; Coelho, 1991; Hrassnigg and Crailsheim, 1999; Harrison and Fewell, 2002). With our respirometry chambers, we could not obtain thoracic temperatures quickly enough to ensure that the measured temperature would be similar to that during flight. Therefore, to test for an effect of load type on thermal balance in flying honey bees, we compare the thorax temperatures of pollen, nectar and water foragers while returning to the hive across a range of ambient temperatures.

Materials and methods

Animals

These experiments were conducted with European honey bee *Apis mellifera ligustica* Spin. colonies, maintained in the courtyard of the Life Sciences complex at Arizona State University. Colonies were two-story hives, with naturally mated queens. For the thoracic temperature and malate dehydrogenase experiments, bees from a single three-story hive were studied. For the loading experiments, bees from five colonies were used. All experiments were run in spring and summer.

Thoracic temperature of pollen, nectar and water foragers in the field

We measured thorax temperatures of 512 pollen, nectar and water foragers returning to a single hive in the field. After measurement of thorax temperature, all the bees were frozen for later measurement of malate dehydrogenase (MDH-1) phenotype. Testing for these effects within a single colony was critical as this allowed us to control for variation among colonies.

Returning foragers were collected from 07.00 to 09.00 h, 12.00 to 14.00 h and 17.00 to 19.00 h over 4 days in mid-June. Shaded air temperatures near the colony entrance were 24–38°C; all temperatures were measured in shade, though the bees had generally just flown in the sun. 24 pollen and non-pollen foragers were collected during each period using forceps insulated with styrofoam. We measured their thoracic temperature within 5 s of capture using a Physitemp MT-29/T hypodermic needle microprobe (29 gauge, time constant=0.025 s) and a Physitemp BAT-10 thermocouple thermometer. Shaded ambient temperature was recorded at 30 min periods throughout the sampling period using another thermocouple. Bees with visible pollen loads on their legs were classified as pollen foragers. To determine whether non-pollen foragers were nectar or water foragers, we separated the thorax and abdomen with a razor blade, and removed the crop contents by gently squeezing the abdomen. We determined the solute concentration of the crop contents with a Fisher hand refractometer (Santa Clara, CA, USA). We classified bees with crop solute content concentrations of less than 5% as water foragers. Bees with less than 5 µl of liquid in their crops were discarded. For this and all subsequent experiments, statistical analysis was performed using SYSTAT version 9, with $P<0.05$ considered significant.

Effect of load type on malate dehydrogenase-1 variation

Each bee used in the thorax temperature experiment was tested for malate dehydrogenase-1 (MDH-1) phenotype using cellulose acetate electrophoresis (Helena Laboratories system). We crushed the head in 100 µl of extraction buffer (*B*-nicotinamide adenine dinucleotide phosphate and DL-dithiothreitol: 0.13 and 6.5 mmol l^{-1} respectively, 0.00001× Triton X-100). We used a discontinuous buffer system; cellulose plates were soaked with tris-glycine soaking buffer, and gels were run on a tris-malate running buffer (Hebert and

Beaton, 1989). Homogenates were run for 22 min at 250 V, stained for MDH-1 (Hebert and Beaton, 1989), and scored for the presence of three possible alleles, which we recorded as 'slow', 'medium' or 'fast'.

Effect of loading on hovering metabolic rate and wingbeat frequency

Experimental design

We collected workers foraging from artificial resource stations set up several meters away from the colonies. The resource stations consisted of dishes with sponges soaked in 40% sugar solution or with freshly ground pollen. Pollen and nectar were replenished throughout the day. Bees that arrived at the pollen dish and began to collect pollen were considered pollen foragers; those bees landing on the nectar dish and extending their proboscis for nectar uptake were considered nectar foragers.

In one experiment (designated the Regression experiment), load mass varied continuously. We collected 86 bees that had spent varying amounts of time on the resource dishes. In this case, data were analyzed with an analysis of variance (ANOVA) design in which behavior (pollen *versus* nectar load type) was considered an independent categorical factor and total mass (bee + load) was a covariate. In a second experiment (termed the ANOVA experiment), load mass varied discontinuously. 20 unloaded foragers were collected as they arrived at the resource dishes, and 20 foragers were also collected when they showed signs of departing from the feeding station (preening, retraction of proboscis in nectar foragers, or fast walking). For this second experiment, data were analyzed by a two-way ANOVA, with behavior (pollen *versus* nectar) and loading status (unloaded *versus* loaded) as categorical factors. Data for these two experiments were analyzed separately for statistical purposes, but all other techniques were identical.

Carbon dioxide emission during hovering

Bees were placed in the respirometry chamber within 2 min of capture from the foraging stations. We measured metabolic rate indirectly using flow-through carbon dioxide respirometry at 24±1°C in a clear lucite cylinder, 50 mm in diameter and 14 cm long. Air free of CO_2 and H_2O flowed through the chamber at $3.70\,l\,min^{-1}$, monitored by an Omega FMA-5613 mass flow meter (Stamford, CT, USA). Calculated flow velocity in the chamber was $3.6\,m\,s^{-1}$. To obtain CO_2- and H_2O-free air, room air flowed through a Balston 75-52 FT-IR purge gas generator (Haverhill, MA, USA) and was further scrubbed by a column of Drierite and ascarite just prior to entering the flight chamber. The excurrent air passed through a column of magnesium perchlorate to remove the water vapor before the CO_2 content was measured with a Licor LI-6252 CO_2 analyzer (Lincoln, NE, USA), interfaced with Sable Systems hardware and software (Las Vegas, NV, USA) and a computer.

To encourage bees to hover in the center of the tube, flight trials were conducted in a dark room, and a light (battery-powered steady white light) was used to illuminate the chamber from above and induce bees to hover in one area. Metabolic rates were only calculated during periods in which bees hovered spontaneously with little vertical or horizontal movement. Because the effort of flight decreases near the chamber's edge (Raynor and Thomas, 1991), we also discarded data from bees that flew within a 1 cm of the chamber wall. Hovering periods typically lasted 3–4 min, and all but five of the hovering periods were at least 40 s in length, with the majority representing at least 1.5 min of continuous hovering. Hovering metabolic rates were repeatable within individuals (data not shown).

Wingbeat frequency

We recorded wingbeat frequencies onto audio tape during hovering flight in the respirometry chamber using an optical tachometer (constructed at Arizona State University) (Unwin and Ellington, 1979). The tape was later analyzed using Sound Edit™ on a Macintosh computer by measuring the duration of 10–15 wingbeats at five intervals throughout the trial and averaging these intervals.

Optical tachometers are potentially subject to errors associated with counting the fore and hind wings separately, and with counting pulses associated with wing tip fluctuations. We were particularly concerned with these possibilities as our measurements of wingbeat frequency substantially exceeded those reported by Ellington (1984a,b,c) for honey bees. Therefore, we induced several honey bees to fly in a glass cylinder and measured their wingbeat frequency using the optical tachometer while simultaneously recording their hovering with hi-speed video photography at 3000 frames s^{-1}. Using NIH Image, we digitized the video and measured the wingbeat frequency by counting the frames required per wingbeat. The two methods gave identical measurements of wingbeat frequency.

Mass measurements

After metabolic measurements, we froze bees in liquid nitrogen and transferred them to a –70°C freezer. We later thawed and weighed them to obtain total mass (bee + load) using a Mettler AE 240 analytical balance (sensitivity ±0.01 mg). Pollen loads were removed from the legs and weighed. We estimated load mass of nectar foragers by subtracting the average unloaded bee mass from the total mass of the loaded bee. For the one bee in which load calculated in this manner was (slightly) negative, we used a load mass of zero. We also measured thorax mass (including wings and legs without pollen loads).

Respiratory exchange rates and calculation of metabolic rates

Calculation of metabolic rates from CO_2 emission requires knowledge of the ratio of CO_2 produced to O_2 consumed (respiratory exchange rate, RER), which depends on the substrate catabolized. Although reported RERs are generally 1.0 for honey bees (Rothe and Nachtigall, 1989), prior studies have not reported whether the bees measured were pollen or nectar foragers. Potentially, substrate utilization might differ

among pollen and nectar foragers if they consume and metabolize part of the loads they carry. Therefore, we measured the RERs of ten flying pollen and nectar foragers using closed system respirometry.

We collected loaded pollen and nectar foragers from resource dishes and placed them in a 500 ml Erlenmeyer flask. After adding the bee, the flask was flushed for 1 min with air lacking CO_2 and water. The flask was then sealed, and the bee was mildly agitated to fly for 10 min at 24.7±0.1°C. We only analyzed data from bees that flew for at least 9.5 min during the 10 min trial. We extracted a 60 ml gas sample from the flask *via* a septum. The gas sample was then injected into a stream of dry, CO_2-free air and drawn sequentially through a magnesium perchlorate column (to remove water), the CO_2 analyzer (Anarad AR-411, Santa Barbara, CA, USA), an ascarite column (to remove CO_2), the oxygen analyzer (Ametek S-3A, Pittsburg, PA, USA) and an Ametek R-1 flow controller. Output of the CO_2 and O_2 analyzers was digitized and analyzed with Sable Systems Datacan V hardware and software. The RER was calculated as the ratio of the volume of CO_2 emitted relative to the volume of O_2 consumed.

Effect of pollen loading on wing kinematics and mechanical power output

Honey bees have a pattern of periodic stationary hovering while collecting pollen. We induced pollen foragers to exhibit this stationary hovering by luring them to collect pollen off a spoon. During the foraging interval, pollen foragers exhibited virtually perfect hovering, only briefly darting to the spoon to collect 1–2 mg of pollen before hovering again while they packed the pollen onto their pollen baskets. Bees repeated the foraging–packing cycle 10–15 times until fully loaded, at which point we captured them with forceps. The spoon was positioned within a filming stage, which consisted of a Panasonic VHS video recorder (Los Angeles, CA, USA) simultaneously filming the top and side views of the bee using a mirror positioned at a 45° angle to horizontal. After capture, we froze the bees in liquid nitrogen and later weighed their pollen loads and bodies. In this case, thorax mass was measured after removing both pairs of wings and all of the legs.

The wingbeat frequency of individual foragers were measured from the moment of arrival until the bee showed signs of departure, using an optical tachometer recording onto the videotape. Then wingbeat frequency was analyzed at 5 s intervals throughout the loading trial using Sound Edit™.

We used these data to calculate the mechanical power output for 17 pollen foragers hovering in the unloaded and maximally loaded state. Wingbeat frequencies were averaged for three different sequences at the beginning and at the end of the trial. Videotape images were analyzed by capturing an image in Apple Video recorder and importing into NIH Image where stroke amplitude, inclination of stroke plane, and body angle were measured (defined as in Dudley, 1995). The horizontal movement of the wings can be observed as a blur when viewing the hovering bee from the top. Stroke amplitude was measured as the angle between two lines drawn parallel to the top of outer edges of this top-view wing blur. The wing blur can also be seen in the side view and inclination of stroke plane was measured as the angle between this side-view wing blur and true horizontal (90° to a suspended, weighted string in the viewing frame). By convention (Dudley, 1995), positive inclinations of stroke planes correspond to angles above horizontal. A line parallel to the body axis was drawn and the angle between this line and true horizontal was used as the body angle; by convention, positive body angles correspond to angles below horizontal.

Wings were removed from filmed bees and weighed to within 0.1 µg using a Cahn C-31 microbalance (Cerritos, CA, USA) after rehydration for 24 h by placing wings in a chamber with water-saturated air. Wing area and length was determined by filming one pair of wings for each bee and analyzing images using NIH Image.

We calculated mean lift coefficients averaged over the stroke and power output for the filmed bees at the beginning using Ellington's model (1984a,b,c) by substituting our morphological and kinematic parameters into a Matlab program, written by Lance Tammero and Erica Feuerbacher (University of California, Berkeley, USA). The wingbeat frequency, stroke amplitude, body angle and body mass were all substituted directly into the model. Power outputs g^{-1} flight muscle were calculated, assuming that muscle mass constitutes 75% of the thorax mass (Heinrich, 1980). We calculated aspect ratio $Æ\!R$ using the formula $4R^2S^{-1}$ (Ellington, 1984a), where R is wing length and S is the surface area of one set of wings (i.e. right wings or left wings). Wing moments were obtained from averages for honey bees reported by Ellington (1984a). Traditionally, profile power outputs during insect flight have been calculated assuming that drag=$7/Re^{1/2}$, where Re is Reynold's number (Ellington, 1984a,b,c); we term this the E Drag Estimate. Recently, much higher estimates of drag and profile power have been made for insect flight. In *Drosophila melanogaster* the lift to drag ratio=0.55 (Sane and Dickinson, 2001), so we also calculated profile power assuming this ratio applies to honey bees, which we term the SD Drag Estimate.

Results

Effects of load type (pollen versus nectar)

Thoracic temperature

Thorax temperature increased with air temperature, and load type significantly affected the relationship between thorax temperature and air temperature (Table 1). Load type had small effects on the slopes and intercepts for regression lines relating thorax temperature to air temperature (Table 1). However, for all three paired comparisons among load types, there was a significant interaction between air temperature and load type effects on thorax temperature (general linear model; nectar and pollen foragers: $F=21.2$, d.f.=1, 420, $P<0.001$; nectar and water foragers: $F=20.6$, d.f.=1, 259, $P<0.001$; pollen and water foragers: $F=114$, d.f.=1, 342, $P<0.001$). The predicted thorax temperature of pollen foragers was approximately 0.6°C higher than nectar foragers at 24°C air temperature, rising to 0.8°C

Table 1. *Linear regressions relating thorax temperature to air temperature for nectar, pollen and water foragers*

Load type	Intercept	Slope	F	d.f.	P
Nectar	26.6±1.08	0.43±0.040	153	1, 168	<0.001
Pollen	27.0±0.660	0.44±0.020	465	1, 251	<0.001
Water	26.5±1.35	0.41±0.040	99	1, 90	<0.001

Values are means ± S.E.M.

The response of thorax temperature to air temperature differed for all pairwise comparisons between load types (significant air temperature × load type interaction term in linear regression analyses, see text for details).

higher at 38°C. Predicted thorax temperatures for water foragers were 0.7°C below nectar foragers at 24°C air temperature and 0.9°C lower at 38°C.

Malate dehydrogenase phenotype

Bees carrying different types of load had different MDH-1 phenotypes (Fig. 1). MDH-1 phenotype differed significantly between nectar and pollen foragers (*G* test: *G*=57.2, *P*<0.001), between nectar and water foragers (*G*=6.4, *P*<0.05), and between pollen and water foragers (*G*=27.4, *P*<0.001).

Body and load sizes

Mean body mass of unloaded bees was approximately 73 mg (Fig. 2). Mean pollen loads were 24% and 27% of unloaded body mass in the regression and ANOVA experiments, respectively. Mean nectar loads were 32% and 40% of unloaded body mass in the two experiments (Fig. 2). Some nectar loads nearly equalled mean bee mass (the largest load was 70 mg).

There was some inconsistent evidence that pollen foragers are smaller than nectar foragers. Pollen foragers had significantly smaller thoracic mass than nectar foragers in the ANOVA but

not the Regression experiment (Fig. 2, Tables 2, 3). Pollen and nectar foragers did not differ significantly in head width (pollen foragers: 3.76±0.015 mm; nectar foragers 3.77±0.013, *t*-test, Table 2). There was also a tendency for nectar foragers to carry heavier loads (Fig. 2, Table 3, significant Behavior×Load status interaction effect). There was evidence that larger bees carry heavier loads, as total mass significantly (positively) affected thorax mass and head width (Table 3).

Flight metabolism

Pollen and nectar foragers did not significantly differ in respiratory quotient (RQ) values (pollen foragers, RQ=1.00±0.014, *N*=10; nectar foragers, RQ=1.01±0.025, *N*=10; *t*-test, *t*=0.781, *P*=0.40), suggesting that both pollen and nectar foragers utilize only carbohydrate for catabolism during
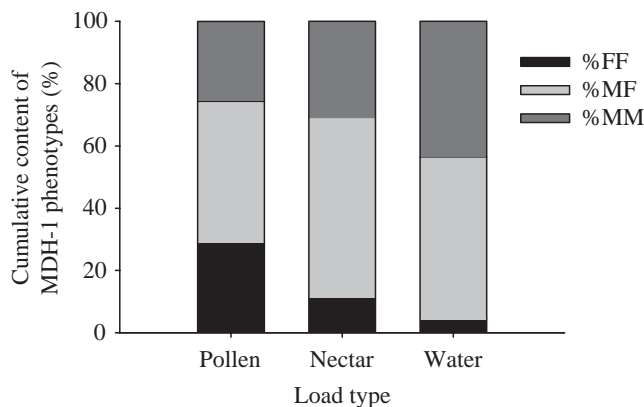

Fig. 1. Stack graph showing the cumulative content (%) of the foragers of each type being scored as FF (2 fast alleles), MF (1 medium, 1 fast allele) or MM (2 medium alleles) for malate dehydrogenase-1 phenotype. All pair-wise comparisons between load types were significant (*G* tests). F, fast; M, medium.
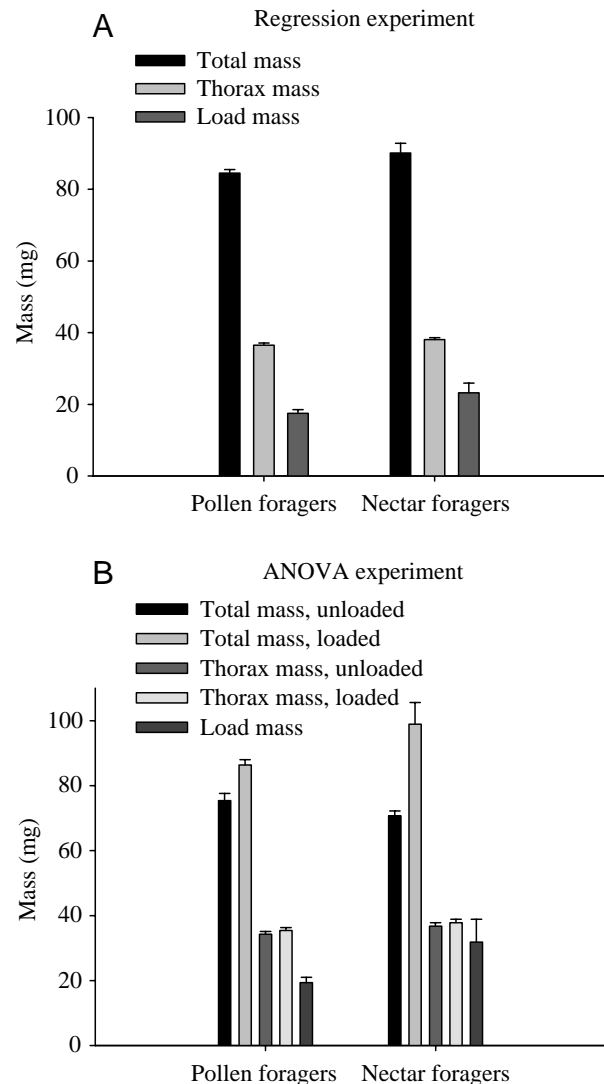

Fig. 2. Average total (bee + load) mass, thorax mass, and load mass of pollen (*N*=37) and nectar (*N*=49) foragers in the Regression experiment (A) and in the ANOVA experiment (B; *N*=10 for each forager type-status classification). In this and all subsequent figures, values are means ± S.E.M.

Table 2. F *values for the Regression experiment, in which behavior (pollen versus nectar load type) was considered a categorical factor and total mass a covariate*

Variable	Behavior	Total mass	N
Metabolic rate (mW)	12.9***	2.00	86
Thorax mass (mg)	2.32	5.56*	86
Head width (mm)	0.02	6.06*	75
Wingbeat frequency (Hz)	0.13	0.19	75
Load (mg)	2.54	69.85***	86

***$P<0.001$; **$P<0.01$; *$P<0.05$.

Table 3. F *values for the ANOVA experiment, in which behavior (pollen versus nectar) and load status (unloaded versus loaded) were considered categorical factors in a two-way ANOVA*

Variable	Behavior	Load status	Behavior×Load status interaction
Metabolic rate (mW)	14.89***	5.60*	0.01
Thorax mass (mg)	6.42*	1.39	0.01
Total mass (mg)	1.12	27.80***	5.29*
Load (mg)	1.09	27.63***	5.35*

***$P<0.001$; **$P<0.01$; *$P<0.05$.
$N=40$ for all variables.

flight. Therefore, we converted CO_2 emission rates to metabolic rates (in mW) assuming 21.4 J ml CO_2^{-1} (Gordon, 1982).

Pollen foragers had higher metabolic rates than nectar foragers. In the Regression experiment, pollen foragers had a significantly higher metabolic rate per bee than nectar foragers (averaging across all loads; pollen foragers: 60.5±0.79 mW; nectar foragers: 56.5±0.86 mW, ANOVA, Fig. 3, Table 2). Similarly, in the ANOVA experiment, unloaded pollen foragers had a metabolic rate approximately 10% higher than unloaded nectar foragers (Fig. 4, Table 3). Load status (loaded *versus* unloaded) did not affect the difference in hovering metabolic rates between pollen and nectar foragers (Fig. 4, Table 3; no significant behavior×load status interaction).

Wingbeat frequencies

Average wingbeat frequencies did not differ significantly between pollen and nectar foragers (Regression experiment, averaging across all loads; 234±1.5 Hz and 233±1.9 Hz, respectively; Fig. 5A, Table 2).
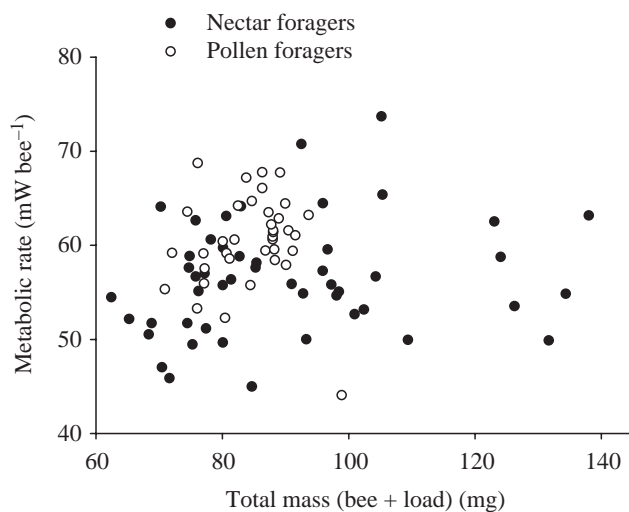
Effects of load mass
Flight metabolism

There was no significant effect of load mass on metabolic rate in the Regression experiment for either pollen or nectar foragers (Fig. 3, Table 2). However, metabolic rates significantly increased in loaded foragers by 6% in the ANOVA experiment for both pollen and nectar foragers (Fig. 4, Table 3).

Wingbeat frequencies

In the Regression experiment, wingbeat frequencies did not significantly vary with total mass in either pollen or nectar foragers (Fig. 5A, Table 2). The wingbeat frequency decreased significantly with increasing thorax mass in both pollen and nectar foragers (Fig. 5B).

During the mechanical power output measurements, bees varied in the amount of time they spent loading (average 146 s, range 83–272 s) and so we examined how wingbeat frequencies changed as a function of the percentage of total flight time at the resource station. Mean final pollen load size in this study was 13.2±1.39 mg (18% of mean body mass, $N=19$), range 5.2–26.4 mg (6–35% of mean unloaded body mass). The mean

Fig. 3. Metabolic rates during hovering of pollen (open circles) and nectar (filled circles) foragers (Regression experiment) *versus* total mass (bee + load). Pollen foragers had significantly higher total metabolic rates than nectar foragers, but there was no significant effect of total mass on metabolic rate (see Table 2).
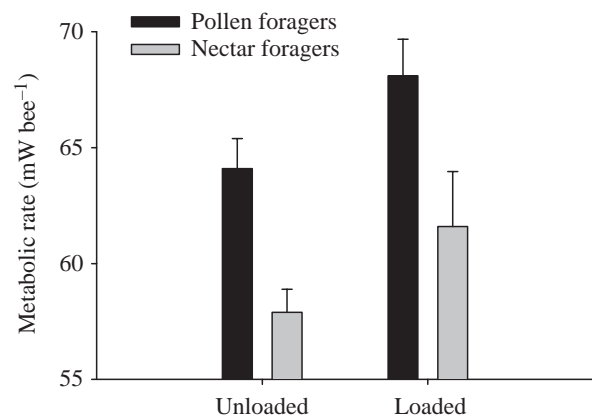

Fig. 4. Metabolic rates during hovering of loaded and unloaded pollen and nectar foragers from the ANOVA experiment. Both behavior (pollen *versus* nectar) and load status (unloaded *versus* loaded) affected hovering metabolic rate (see Table 3).
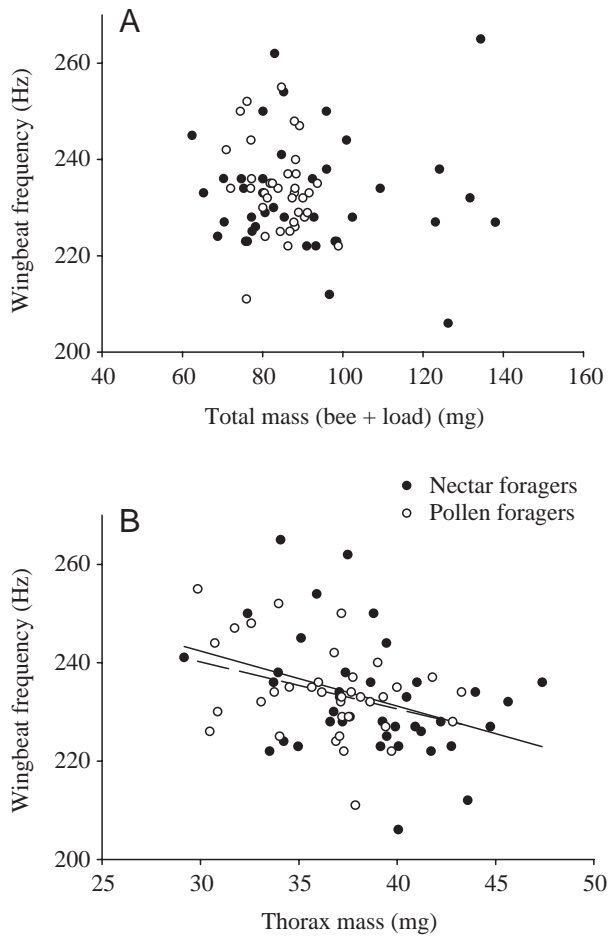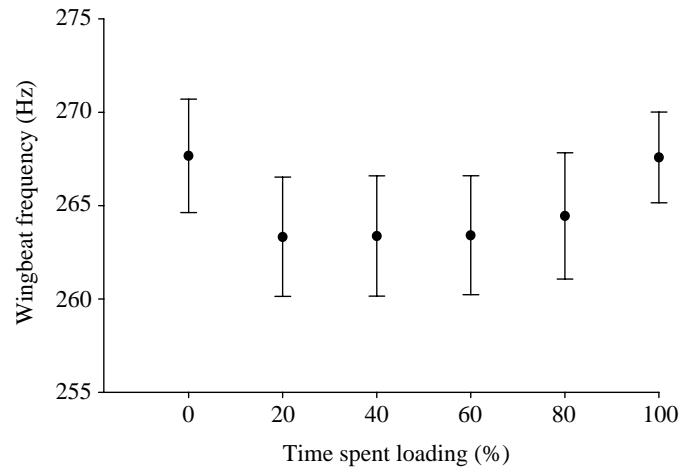
Fig. 6. The effect of time (which is correlated with load amount) at the resource station on average wingbeat frequency for bees during the mechanical power output measurements. The increase in time represents a change from near zero to near-maximal pollen load. Wingbeat frequency did not significantly change with time (linear regression, N=24, P>0.05).

Fig. 5. (A) The relationship between wingbeat frequency (wingbeat frequency) and total mass (bee + load, in mg) in pollen (open circles) and nectar (filled circles) foragers hovering in the Regression experiment. There was no significant effect of behavior or total mass on wingbeat frequency (see Table 2). (B) wingbeat frequency *versus* thorax mass in Regression experiment. For pollen foragers (solid line): wingbeat frequency=269–0.96 (thorax mass), r^2=0.124, N=37, P=0.035; for nectar foragers (broken line): wingbeat frequency=276–1.12 (thorax mass), r^2=0.130, N=49, P=0.025. The slopes differed significantly (analysis of covariance, $F_{3,7\ 1}$= 3.56, P=0.020).
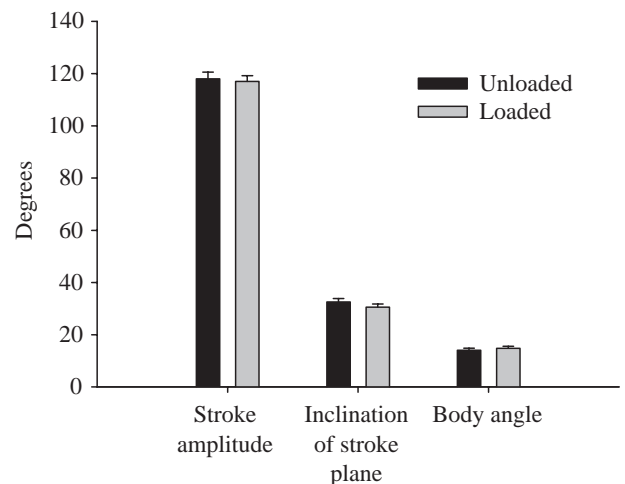

Fig. 7. Stroke amplitude, inclination of stroke plane and body angle of unloaded and loaded (mean load=18% of body mass) pollen foragers. There were no significant differences between unloaded and loaded animals (t-tests, N=17, P>0.05).

wingbeat frequency did not vary significantly with time spent loading (Fig. 6, regression, P>0.05), nor did it vary significantly during pollen loading for 19 of 24 pollen foragers tested (linear regressions on the data for individuals). Of the five that did show significant changes in wingbeat frequency, four had significantly lower wingbeat frequencies with increasing load size. Mean wingbeat frequencies of pollen foragers were higher in the field (during the mechanical power output measurements) than in the respirometry chamber (268.0±2.13 Hz and 233.3±1.24 Hz, respectively; ANOVA, F=134.2, P<0.0001).

Stroke amplitude and angles of the wing and body

Stroke amplitude, inclination of stroke plane and body angle did not differ significantly between bees with zero load and those carrying their maximal load before capture (Fig. 7, paired t-tests; stroke amplitude: T=0.29, P>0.75; inclination of stroke plane: T=1.48, P>0.15; body angle: T=0.59, P>0.50). Mean stroke amplitude was 118±2.4°, mean inclination of stroke plane was 31±1.3°, and mean body angle was 14±0.8°. The mean final pollen load for the 17 bees successfully filmed for these parameters was 14.1±0.88 mg, 19% of the mean unloaded body mass of foragers.

Mechanical power output

Induced power (power used to generate lift) significantly increased from the unloaded to the loaded state, regardless of

Table 4. *Components of flight muscle mass-specific rate, and coefficients of drag, mean lift coefficients and efficiencies for loaded and unloaded pollen foragers in hovering flight*

Variable	Unloaded	Loaded
Induced power	71±2.1	94±3.5*
Coefficient of drag		
using E estimate	0.17±0.002	0.17±0.002
using SD drag estimate	1.27±0.048	1.47±0.041*
Profile power		
using E drag estimate	42±2.7	38±1.8
using SD drag estimate	289±10.7	323±11.9*
Inertial power	510.7±25.5	496±22.7
Total power		
using E drag estimate	112±2.8	132±3.9*
using SD drag estimate	361±11.3	417±13.6*
Lift coefficient	0.68±0.028	0.80±0.023*
Metabolic rate	2680	2849*
Efficiency		
using E drag estimate	0.042	0.046
using SD drag estimate	0.135	0.146

Power and metabolic rate, $W kg^{-1}$ flight muscle; other parameters are unitless.

Mean lift coefficients are averaged over upstroke+downstroke.

Power output parameters were calculated using Ellington's power model (Ellington, 1984b; E drag estimate) or assuming that the drag to lift ratio equals 0.55 (Sane and Dickinson, 2001; SD drag estimate).

*A significant difference between the unloaded and loaded states (t-test, $P<0.05$, $N=17$).

which estimate of drag we used (Table 4). The Reynold's number Re for the wings was calculated as 1669±38.9 for unloaded bees and 1664±37.3 for loaded bees. The coefficient of drag and the profile power (power used to overcome drag on the wing) did not significantly differ between loading states using the E drag estimate (Table 4). Using the SD drag estimate, the coefficient of drag is proportional to the coefficient of lift, so both the coefficient of drag and profile power significantly increased when calculated using the SD drag estimate (Table 4). In neither case did inertial power (power used to accelerate and decelerate the wings) significantly increase with loading state. Estimates of profile power and total power were approximately threefold greater when we used the SD drag estimate compared to when the E estimate of drag was used (Table 4). The substantial disparity between these two methods for calculating profile and total power illustrates the need for further research in this area.

Total power was calculated assuming perfect elastic storage of inertial power, an assumption generally considered to be nearest to reality (Dickinson and Lighton, 1995). Total power increased significantly by 16–17% regardless of the method of estimated drag and profile power (Table 4). This increase is quite similar to the 18% increase in body mass due to loading that these bees experienced. The lift coefficient (lift per wing stroke) also increased significantly from unloaded to loaded status (18% increase; t-test, $T=4.1$, $P=0.002$), regardless of the manner in which drag was estimated.

We calculated efficiency (power output/power input) for unloaded and loaded pollen foragers since the body masses and maximal loads were similar during the ANOVA experiment and the power output measurements (Table 4). Efficiencies were threefold higher when calculated using the SD drag estimates, and tended to be higher for loaded bees. However, we could not test these trends statistically as the power outputs and metabolic rates were measured on different bees.

Discussion

We found two surprising, but clear, results in this study. First, pollen foragers have higher hovering metabolic rates than nectar foragers, regardless of loading status. Second, loading increased the mechanical power output required to fly by a fraction similar to the increase in total mass, yet honey bees were able to carry these loads without any increase in wingbeat frequency or wing stroke amplitude, and with relatively small increases in metabolic rate.

Effects of load type (pollen versus nectar foragers)

Pollen foragers had metabolic rates during hovering that averaged 10% greater than nectar foragers (Figs 3, 4; Tables 2, 3). Differences in flight metabolic rates between pollen and nectar foragers were similar whether loaded or unloaded bees were compared (Fig. 4, Table 3). Thus, the higher hovering metabolic rates of pollen foragers cannot be explained by biomechanical effects of the load type such as differences in drag or center of mass.

These metabolic differences may reflect physiological or behavioral plasticity. For example, pollen foragers might be more behaviorally activated (excited or motivated) by their resource than nectar foragers. In support of this hypothesis, hovering metabolic rate is correlated with sugar reward rate in honey bees (Moffat, 2000). Pollen may be considered a more rewarding resource by these bees.

The metabolic differences between pollen and nectar foragers could also be due to genetically based variation in muscle, wing morphology or neuroendocrine properties which influence hovering metabolism and/or wing kinematics. This possibility is supported by the differences we found in malate dehydrogenase phenotype, and the many papers which have documented genetic effects on foraging behavior in honey bees (Calderone and Page, 1988; Robinson and Page, 1989; Fewell and Page, 1993).

Bees specializing on pollen *versus* nectar collection might use different wing kinematics; such differences could be genetically based or due to phenotypic plasticity. The only kinematic parameter that we measured for both pollen and nectar foragers was wingbeat frequency, and the mean wingbeat frequency did not differ between pollen or nectar foragers.

However, comparison of our data for pollen foragers with the data reported for honey bees by Ellington (1984b) suggests that pollen foragers may fly with their abdomens much nearer to the horizontal plane, and with a more inclined stroke plane than nectar foragers. The bees studied by Ellington were probably nectar foragers, as these are the most common type of forager. In our study, pollen foragers had a mean inclination of stroke plane of 31°, compared to a mean inclination of stroke plane of 11° in the Ellington study, while our pollen foragers had body angles of 14° compared to 56° in the Ellington study. Note that the absolute angles between the wing and body were identical (45°) in both studies, indicating that the pollen foragers in this study are simply rotated forward along the pitch axis relative to the bees studied by Ellington. Perhaps a more horizontal body angle facilitates the packing of pollen into the corbicula. A more inclined stroke plane directs less of the lift generating air vortices vertically downward, such that less lift is generated for the same power output. Thus, the higher metabolic rates during hovering of pollen foragers could be due to pollen foragers requiring more power output to generate the same vertical lift as nectar foragers.

There was also some evidence that pollen foragers had smaller thoraxes than nectar foragers; since mass-specific metabolic rates tend to decrease with size, these mass differences could contribute to the differences in metabolic rates between pollen and nectar foragers. However, unloaded body mass and head width did not differ between pollen and nectar foragers (Tables 2, 3), and thorax mass was not correlated with flight metabolic rate (Pearson test, $P>0.05$), all of which suggest that body size differences are unlikely to explain the differences in flight metabolism between pollen and nectar foragers. Wingbeat frequency of both pollen and nectar foragers were negatively correlated with thorax mass (Fig. 5B), but wingbeat frequency was not correlated with metabolic rate (Pearson test, $P>0.05$). Wingbeat frequency and body mass are also negatively correlated in euglossine bees (Casey et al., 1985).

Differences in the thoracic temperatures of free-flying pollen and nectar foragers appear to be at least partially mediated by differences in flight metabolic rates. As in the study of Cooper et al. (1985), pollen foragers had significantly higher thoracic temperatures than nectar foragers. We also found that water foragers had the coolest thoracic temperatures. Nectar foragers extrude a droplet of nectar from their crops into their mouthparts during flight at high temperatures to evaporatively cool (Heinrich, 1979; Cooper et al., 1985). Thus, the observed differences in thoracic temperature among pollen, nectar and water foragers could be due variation in the ability of foragers to evaporatively cool themselves, as nectar and water foragers may have greater liquid crop content. Another possible explanation for higher body temperatures in pollen foragers is that pollen may add drag during forward flight, slowing forward speed and convective cooling. However, if heat loss rates are similar for nectar and pollen foragers, the 5% difference in thorax and air temperatures (at 24°C) between pollen and nectar foragers (Table 1) can be completely accounted for by the greater metabolic rates of pollen foragers. This suggests another possible evolutionary hypothesis for the higher flight metabolic rates in pollen foragers: since pollen foraging is more likely to occur at cooler morning air temperatures (J. Fewell, personal observations), pollen foragers may possess higher flight metabolic rates to aid thermoregulation during flight.

Effects of load mass

Load mass appears to increase flight metabolic rate, but only slightly. Loaded pollen and nectar foragers had significantly higher metabolic rates than unloaded pollen and nectar foragers in the ANOVA experiment (Fig. 4, Table 3), but the increase in metabolism (6%) was small compared to the 27% (pollen) and 40% (nectar) increases in body mass. We found no effect of load on metabolic rates in the Regression experiment (Fig. 3, Table 2). It is likely that this second result simply stems from the fact that the effect of load on metabolic rate was so small that it was obscured by individual variation; in other words, the regression experiment lacked sufficient statistical power despite reasonably large sample sizes (N=86). The coefficients of variation for hovering metabolic rate were 7.9% and 10.6% for pollen and nectar foragers, respectively, in the Regression experiment, both of which were greater than the change in metabolic rate with load of 6% observed in the ANOVA experiment.

Thus our results are intermediate between those of Wolf et al. (1989) who reported a stronger effect of loading on metabolic rate and those of Balderrama et al. (1992) and Moffat (2000), who reported no effect of load on flight metabolism. The regression equations provided by Wolf et al. (1989) predict that the mean loads observed in our ANOVA experiment would lead to an increase in mean metabolic rates of 25% for nectar foragers and 14% for pollen foragers. The comparison of the results of our Regression experiment (no effect of load) with the results of Wolf et al. (1989) is quite striking, since some of the bees in the Regression experiment carried loads as great as the maximum carried in the study by Wolf et al. (1989), while having metabolic rates similar to unloaded bees (Fig. 3). One possible explanation for the differences between our results and those of Wolf et al. is that their data were based primarily on repeated measures of individuals after consuming different amounts of nectar, while our data were based on measures of different individuals who carried different masses of nectar or pollen. In other words, inter-individual variation may have obscured the loading effect in our study. Arguing against this possibility is the observation that wing kinematic variables did not differ with loading, even within individuals (Figs 5–7).

An alternative possibility is that the difference between these studies reflects the different air temperatures used during the metabolic measurements (Wolf et al., 1989, 32°C; this study, 24°C). The major difference between our results and those of Wolf et al. (1989) are the metabolic rates measured for unloaded bees. Our unloaded nectar foragers had metabolic rates 36% higher (57 *versus* 42 mW bee^{-1}) than hovering bees in Wolf et al. (1989), while our maximally loaded nectar foragers had metabolic rates virtually identical to those of Wolf

et al. Unloaded hovering honey bees have lower metabolic rates and wingbeat frequencies at higher air temperatures, which aids in stabilization of thoracic temperature (Harrison et al., 1996a,b; Roberts and Harrison, 1999). Perhaps unloaded bees hovering in cooler air (as in this study) are generating excess power and heat (relative to that required to hover) for thermoregulatory purposes, but are using steering mechanisms to maintain position. Bees flying in cool air could then use this excess power to carry loads without increasing metabolic rate, thereby apparently increasing efficiency. In contrast, bees flying at warmer air temperatures (as in the Wolf et al., 1989 study) may reduce metabolic heat production and power output toward the minimum required for bees to hover (approximately 0.3 W g^{-1}, Nachtigall, 1989; Harrison and Fewell, 2002), and thus must increase their power output and metabolism in order to carry loads. This hypothesis could be tested by examining the interactive effects of load mass and air temperature on hovering metabolic rate and wing kinematics.

In the filmed pollen foragers, total power output significantly increased by 16–18% (depending on the drag estimate used), correlating well with the 18% increase in body mass of loaded foragers (Table 4). This increase in total power output for loaded foragers is greater than the 6% increase in metabolic rate, suggesting an increase in muscle efficiency (power output/power input) during loading. Increases in efficiency with loading have also been reported for bumblebees (Cooper, 1993).

Honey bees were able to generate this increased mechanical power output without any detectable changes in wing kinematics (Figs 5–7). Long-eared bats increased their wingbeat frequency and stroke amplitude with increasing load (Rayner and Thomas, 1991). However, in honey bees, wingbeat frequency of loaded foragers flying in the metabolic chamber remained constant with increasing load size. These laboratory data were corroborated by the data from the power output measurements in which pollen foragers in the field did not increase their wingbeat frequency while loading. The consistency in the laboratory and field studies indicates that loaded foragers in the respirometry chamber did not preferentially utilize air currents near the chamber wall (Rayner and Thomas, 1991) in order to carry loads without added cost, since there were no surfaces within several cm of the bees being filmed. Similarly, stroke amplitude, inclination of stroke plane and body angle all remained constant for hovering pollen foragers, as load increased from 0 to 18%. In contrast, bumblebees with a load equal to 18% of body mass (this was the mean load of the filmed pollen foragers) increased their metabolic rate by 15%, their wingbeat frequency by 5% and their stroke amplitude by 4% (estimated from equations provided by Cooper, 1993). Changes in wingbeat frequency and stroke amplitude have been shown to be tightly coupled to changes in metabolic rate during tethered flight in flies (Dickinson and Lighton, 1995). The observation that hovering honey bees can carry substantial loads without increasing wingbeat frequency or stroke amplitude may explain the relatively small effects of loading on metabolic rate.

How are honey bee foragers able to carry significant loads without changing wingbeat frequency, stroke amplitude or inclination of stroke plane? In loaded individuals, the mean lift coefficient averaged over the stroke significantly increased by an amount similar to the increase in induced power within individuals (Table 4). The rise in mean lift coefficient averaged over the stroke is likely to have occurred due to increased use of unsteady power generation mechanisms such as have been demonstrated for *Manduca sexta* and *Drosophila melanogaster* (Ellington et al., 1996; Dickinson et al., 1999). The hypothesis that the hovering pollen foragers utilize such aerodynamic mechanisms is also supported by the extraordinary body and inclination of stroke planes of hovering pollen foragers. Only the hoverfly has been reported to fly with such an inclined stroke plane (Ellington, 1984c). Hoverflies utilize variable and complex wing movements whose aerodynamic and energetic consequences remains unclear (Ellington, 1984c). Pollen foragers of *Apis mellifera* should provide an easily available and tractable model for future studies of the aerodynamics of hovering flight at high stroke planes.

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