

Implications of floral orientation for flight kinematics and metabolic expenditure of hover-feeding hummingbirds

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Summary

1. Nectar-bearing flowers are characterized by many different shapes, sizes and orientations, which may affect the way hummingbirds feed from them. Many hummingbird-pollinated flowers are oriented downwards, thereby requiring that trochilids feed while hovering with the bill oriented vertically upward.

2. We measured body orientations, wingbeat kinematics and hovering metabolic rates for Anna's Hummingbirds (*Calypte anna*) feeding from artificial flowers that were oriented horizontally, tilted 45° downwards and pointing vertically downwards.

3. When feeding from vertically oriented flowers, hummingbirds employed an upright body position combined with dorsal head flexion. Additional kinematic adjustments included an increased stroke plane angle relative to the longitudinal body axis and an increased stroke amplitude deriving from increases in the minimum positional angle of the wingbeat.

4. By contrast, wingbeat frequency, the stroke plane angle relative to horizontal, the ratio of the minimum to maximum positional angles of the wingbeat and the upstroke/downstroke ratio did not vary during feeding from different flower orientations.

5. Metabolic rates increased by an average (\pm SD) of 10.8 (\pm 8.8)% for feeding from vertically compared to horizontally oriented flowers.

6. Feeding from pendent flowers comes with a substantial metabolic cost that may influence floral selection by hummingbirds and thus the evolution of associated pollination syndromes.

Key-words: body angle, *Calypte anna*, head flexion, mass-specific metabolic rate, pendent flowers, respirometry, wingbeat kinematics

Introduction

Nectar-rich flowers exhibit varying shapes, sizes, colours and orientations as a result of long-term evolutionary processes shaping attractiveness to pollinators, and ultimately plant reproductive fitness. Floral attributes likely have important roles in filtering out non-pollinating nectar feeders (e.g. nectar robbers), in attracting legitimate ones, and in guiding pollinators to feed in specific ways. By so doing, pollination efficiency may increase and the rate of self-pollination may decrease, thereby enhancing plant reproductive fitness (Bertin 1982; Waser 1983; Howe & Lynn 1988; Vaknin, Yom-Tov & Eisikowitch 1996). Yet, the role of floral orientation relative to pollinator behaviour involves complicated, and to date largely unresolved, factors (Tadey & Aizen 2001; Aizen 2003; Fenster, Armbruster &

Dudash 2009). Although hummingbird-pollinated plants with flowers oriented vertically downwards are abundant in different geographical regions of the New World (van der Pijl 1961; Grant & Grant 1968; Stiles 1981; Bawa 1990; Fenster 1991; Proctor, Yeo & Lack 1996; Sazima, Buzato & Sazima 1996; Aizen 2003), the effects of variable floral orientation on feeding performance by hummingbirds have not yet been assessed (Tadey & Aizen 2001).

The highly diverse (~330 species) avian family Trochilidae is widely distributed throughout the Americas and pollinates substantial proportions of the angiosperm flora in various habitats (Grant & Grant 1968; Stiles 1981; Fenster 1991; Proctor, Yeo & Lack 1996; Aizen 2003). Hummingbird-pollinated flowers are typically characterized by a pendent orientation, as well as by red coloration, a tubular shape, absence of odour, and dilute, sucrose-based, nectar. Whereas the significance of floral coloration, shape and

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symmetry has been comprehensively demonstrated relative to hummingbird visitation (see Straw 1956; Grant & Grant 1968; Stanton, Snow & Handel 1986; Feinsinger & Busby 1987; Campbell 1989; Murcia 1990; Fenster 1991; Podolsky 1992, 1993; Hurlbert *et al.* 1996; Murcia & Feinsinger 1996; Temeles 1996; Campbell, Waser & Melendez-Ackerman 1997; Dafni & Kevan 1997; Melendez-Ackerman 1997; Melendez-Ackerman, Campbell & Waser 1997; Galen & Cuba 2001; Gómez, Perfectti & Camacho 2006; Gómez *et al.* 2008), the functional significance of flower orientation for the plants has remained elusive (Tadey & Aizen 2001; Aizen 2003; Fenster, Armbruster & Dudash 2009). It has been proposed that downward flower orientation minimizes nectar dilution and washout by rain and also prevents rain and solar radiation from damaging pollen (see Sprengel 1793; Schoen & Stewart 1986; Campbell 1989; Broyles & Wyatt 1990; Corbet 1990; Dudash 1991; Devlin, Clegg & Ellstrand 1992; Galen 1992; Bynum & Smith 2001; Tadey & Aizen 2001; Aizen 2003; Wang *et al.* 2010). Consequently, hummingbirds may select pendent flowers to ensure access to nectar not diluted by rain (Tadey & Aizen 2001), because these birds can discriminate among flowers by nectar concentration to select the most rewarding ones (Bené 1945; Hainsworth & Wolf 1976; Gut, Schlising & Stopher 1978; Sutherland & Gass 1995; Campbell, Waser & Melendez-Ackerman 1997; Melendez-Ackerman 1997; Gonzalez-Gomez, Bozinovic & Vasquez 2011).

Hovering is known to be one of the most demanding types of aerial locomotion as it requires considerably greater force and power production than most other aerial behaviours (see Dudley 2000; Clark & Dudley 2010). Hummingbirds are among the few avian taxa capable of sustained hovering and operate at or near functional limits for vertebrate aerobic capacity (e.g. Lasiewski 1963; Pennycuik 1968, 1969; Berger 1985; Suarez *et al.* 1991; Suarez 1992; Hochachka 1994; Chai & Dudley 1995, 1999; Dudley 2000). In hovering, the bird must produce enough vertical force to offset gravity, and externally imposed changes in body posture and wingbeat kinematics may further elevate the metabolic demands of flight. In Broad-tailed Hummingbirds (*Selasphorus platycercus*) and Rufous Hummingbirds (*Selasphorus rufus*), rates of oxygen consumption (\dot{V}_{O_2}) while hover-feeding increased by 5% when birds were forced to feed from flowers that limited their wingbeat amplitude (Wells 1993a). Under such settings, wingbeat frequency and stroke plane angle increased while wingbeat amplitude decreased. Given that so many hummingbirds pollinate downward-facing flowers, we hypothesized that such pendent flowers enhance floral visitation by reducing the cost of hovering when compared with hover-feeding from horizontally oriented flowers. To test this hypothesis, we measured flight metabolic rates (\dot{V}_{O_2}) and body and wingbeat kinematics of Anna's Hummingbirds (*Calypte anna*) feeding from artificial flowers that were oriented horizontally, tilted 45° downwards and oriented vertically (facing downwards) during still-air hovering.

Materials and methods

During June 2011–January 2012, we trapped six male Anna's Hummingbirds (mean \pm SD body mass: 4.52 g \pm 0.39 g) on the campus of the University of California, Berkeley, California, USA (37°52'N 122°15'W). Bird trapping and housing were carried out under permits from the United States Fish and Wildlife Service (permit no. MB054440-0) and California Department of Fish and Game (permit no. SC-006627). Birds were held for 1–7 weeks before being released back to the wild. All hummingbird husbandry and research were conducted in compliance with the University of California, Berkeley's Animal Use Protocol R282-0310.

To train hummingbirds to feed from vertical flowers, individual birds were presented with a single feeder positioned at one of three orientations (horizontal, tilted and vertical). The bird was allowed to feed for about 15 min before the feeder was switched to a different orientation, for a combined training duration of several hours during several days. The experiment started only after the birds were proficient with feeding from feeders positioned at different orientations. Hovering \dot{V}_{O_2} for all treatments on any given individual bird was measured over several hours in one day using mask respirometry within a 91.5 \times 91.5 \times 91.5 cm Plexiglas cube with a mesh-covered opening in one of its sides to allow the exchange of atmospheric air. Hovering birds fed from syringes filled with commercial avian nectar (Nektar-Plus, Nekton, Pforzheim, Germany) that were placed ~15 cm below the ceiling in the middle of the cube. \dot{V}_{O_2} was determined using an artificial feeder converted into a respirometry mask (see Berger & Hart 1972; Bartholomew & Lighton 1986; Clark & Dudley 2010; Welch 2011) made from the end of a 20-mL syringe, through which expired air was pulled by a dedicated airpump (UN73, Neuberger KNF, Freiburg, Germany). Air was subsampled from the main flow using a FoxBox (Sable Systems International, Las Vegas, NV, USA) following scrubbing of water vapour using Drierite (W.A. Hammond Drierite, Xenia, OH, USA). The rate of airflow through the mask was measured using a flowmeter (model 32446-33, Cole-Parmer, Vernon Hills, IL, USA), calibrated with a mass-flow calibrator (1E4-VCR-V-Q, DH Instruments Inc., Phoenix, AZ, USA). Mask airflow rate averaged 3.58 L min⁻¹ among all measurements.

By varying the air flow rate for this particular respirometry system, Clark & Dudley (2010) determined that an airflow rate of 3 L min⁻¹ was sufficient to capture respiratory gases from flying hummingbirds even at a high airspeed of 10 m s⁻¹. The relatively high air flow rate used during still-air hovering for the present study was hence more than sufficient to ensure that no respired gases could exit the mask during feeding. To validate this assumption, we used an approach different from that of Clark & Dudley (2010), namely the method of argon dilution following Lighton (2008; see also Welch 2011). We used a gas cylinder of pure argon connected to a flow regulator that maintained a constant flow rate, as continuously measured by the above-mentioned flowmeter. The flow regulator was connected to a tube, the other end of which was inserted into the respirometry mask. Due to the dilution of atmospheric air by argon influx, we could measure \dot{V}_{O_2} due to argon dilution and then compare it to the predicted \dot{V}_{O_2} calculated using the relevant equation of Lighton (2008, pp. 132). Measured \dot{V}_{O_2} due to argon dilution was, on average (\pm SD), 101.5% (\pm 9.3%) of the predicted value. We also found no significance difference between measured \dot{V}_{O_2} ($N = 6$ measurements) and the predicted \dot{V}_{O_2} (one-sample t-test with predicted \dot{V}_{O_2} set as the test's reference value, $t = 0.4$, $P = 0.71$) and consequently conclude that the respirometry system was properly calibrated.

We used three different feeder orientations (see Figs 1 and 2) in experiments: (i) horizontal, (ii) tilted 45° downwards (hereafter termed 'tilted') and (iii) vertical (facing downwards). An upward feeder orientation could not be used as birds simply perched on the feeder instead of hovering. For each bird,

two \dot{V}_{O_2} measurements were taken at each feeder orientation, followed by two measurements in a different and randomly chosen orientation. This sequence was repeated until six replicates were collected from each floral orientation for each bird. On a different day, two synchronized high-speed video cameras (X-PRI, AOS Technologies AG, Baden Daettwil, Switzerland) positioned laterally and above the Plexiglas cub were used to record (at 500 frames s^{-1}) the body positions and wing motions for the bird feeding from an artificial flower (outer diameter 25 mm; model no. 202-F, Perky-Pet, Woodstream Corporation, Lititz, PA, USA; Fig. 2), rather than from a respirometry mask connected to the syringe. As we used two different syringe-tip feeding devices, we could not measure bird metabolic rates and kinematics simultaneously. The use of an artificial flower rather than the respirometry mask was done to minimize possible aerodynamic interaction between the mask and the flapping wings. However, we note that, according to the study of Warrick, Tobalske & Powers (2005), the head of the birds and its surroundings where the artificial flowers and mask are positioned are not subjected to high vorticity, and as such cannot substantially interfere with wing aerodynamics. Moreover, wingtip vortices during hovering are not directed towards this part of the body, but are rather oriented downwards and backwards, as likely pertains to all hummingbird body positions elicited in the current study. Consequently, aerodynamic consequences of syringe and mask size and shape are probably small, and we accordingly neglect their possible effects on wingbeat kinematics and metabolic rates. To further examine whether aerodynamic interaction with the respirometry mask caused leakage of respired gas from the mask, we repeated validation of the system as specified above while the respirometry system was installed within a wind tunnel (see details in Sapir & Dudley 2012) operated at an airflow of 12 m s^{-1} . Under these conditions, the measured \dot{V}_{O_2} due to

argon dilution was 98.1% (\pm 4.8%) of the predicted value. We also found no significance difference between measured \dot{V}_{O_2} ($N = 6$ measurements) and predicted \dot{V}_{O_2} (one-sample t-test with predicted \dot{V}_{O_2} set as the test's reference value, $t = -0.95$, $P = 0.39$) under these conditions and conclude that indeed no substantial aerodynamic interaction with the mask can be detected even under strong airflows.

Total O_2 consumption in a feeding bout was calculated using EXPEData software (Sable Systems International, Las Vegas, NV, USA) that integrated, over time, the depletion of oxygen from the initiation of feeding to the return to atmospheric level. The instantaneous value of \dot{V}_{O_2} was calculated following Withers (1977):

$$\dot{V}_{O_2} = \dot{V}_E \left(\frac{F_{iO_2} - F_{eO_2}}{1 - F_{iO_2}} \right), \quad \text{eqn 1}$$

where \dot{V}_E is the mass flow of gas exiting the mask, F_{iO_2} represents the fractional concentration of O_2 entering the mask, and F_{eO_2} represents the fractional concentration of O_2 exiting the mask. The total volume of O_2 consumed during a feeding bout was divided by the total feeding time within the mask, as recorded via ExpeData using a photoresistor/LED pair attached to the feeder's opening (Bartholomew & Lighton 1986; Welch 2011; see Fig. 1) and monitored electronically via the FoxBox. The respiratory exchange ratio (RER) was assumed to be one, as determined empirically in a study involving this species following *ad libitum* feeding of carbohydrate-based diet (Welch, Altshuler & Suarez 2007).

Bird body mass was measured before and after daily experimental sessions, and the mean of these two measurements was used in subsequent calculations. Following Bartholomew & Lighton (1986), we excluded those \dot{V}_{O_2} measurements for which the feeding duration within the mask was too short for reliable measurements (i.e. <2 s). We further tested the effect of feeding duration on

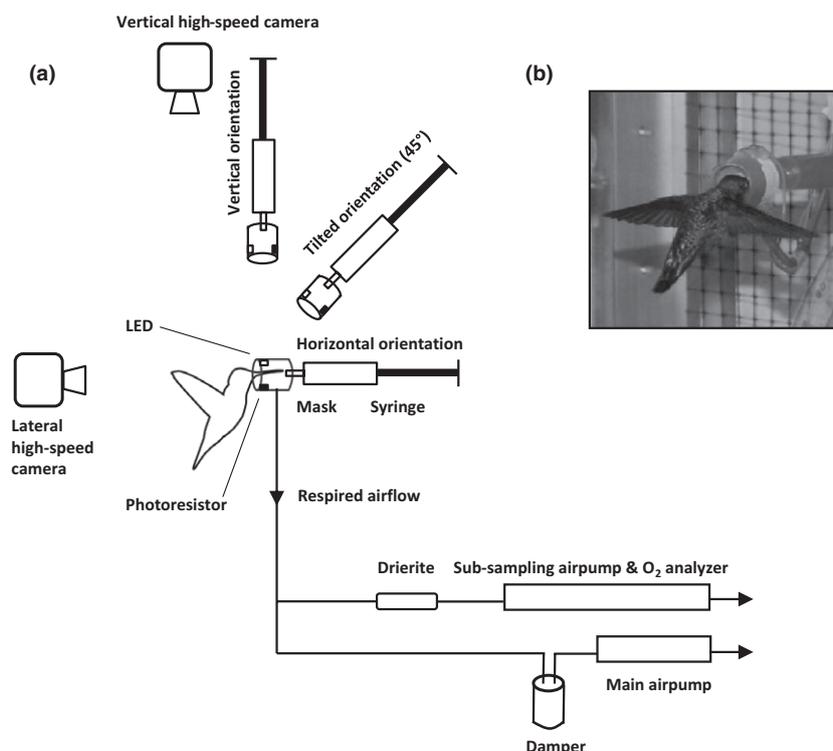


Fig. 1. Experimental set-up. (a) The configuration used to study Anna's Hummingbirds during hovering-feeding using respirometry and high-speed videography. Three feeder-mask and syringe orientations are illustrated; the mask was in the same approximate region for each orientation. (b) A photograph of a hummingbird feeding from respirometry mask.

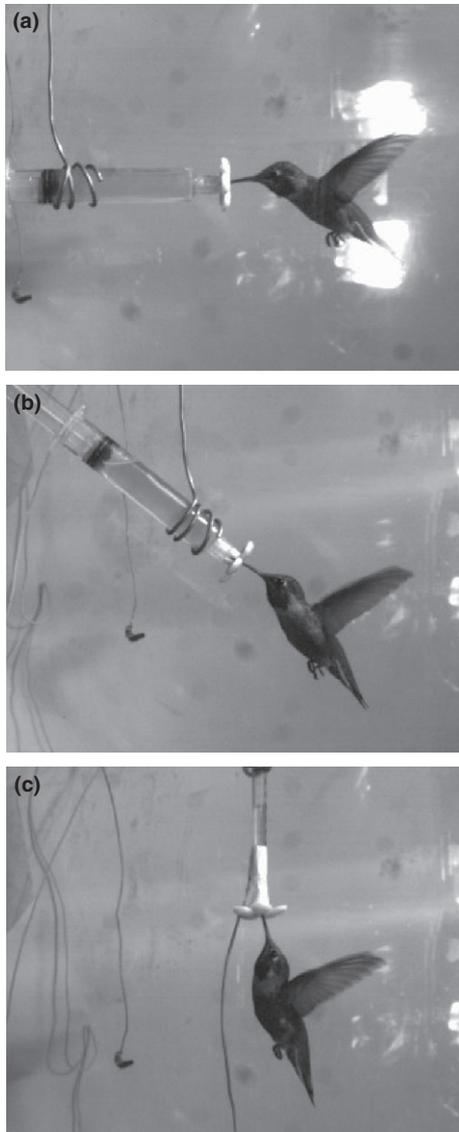


Fig. 2. Body posture of an Anna's Hummingbird when feeding from horizontal (a), tilted (b) and vertical (c) feeders.

measured \dot{V}_{O_2} for individual birds to evaluate potential bias of this assumption (see Bartholomew & Lighton 1986).

From sequences of high-speed video images, IMAGEJ software (version 1.43u, National Institutes of Health, Bethesda, MD, USA) was used to determine kinematic variables (Ellington 1984; Dudley 1992), namely body and wingstroke plane angle in relation to horizontal, ventral head flexion and wing stroke plane angle in relation to the longitudinal body axis, wingbeat amplitude, minimum and maximum position angles of the wingbeat, the ratio of the minimum to maximum positional angles of the wingbeat, wingbeat frequency and the ratio of upstroke to downstroke duration, for three individuals (Fig. 3). It is known that, similar to many flying insects, hummingbirds exhibit a relatively constant wingbeat frequency due to the mechanical resonance of their flight apparatus that operates optimally over a limited range of frequencies. Thus, no substantial variation in this kinematic feature is expected to be found in hummingbirds engaged in different flight conditions. Hummingbirds rhythmically move their wing nominally within a plane defined by patterns of flight muscle contraction and by wrist and shoulder rotations. This plane, termed the wing

stroke plane, can be defined relative to either horizontal or longitudinal body axis when viewed laterally (in which case it is sometimes called the anatomical wing stroke plane). Wingbeat amplitude corresponds to the angle formed between maximal and minimal wing positional angles when viewed perpendicularly to the horizontal wingbeat stroke plane. Hummingbirds are known to modulate their wing stroke plane angle, wingbeat amplitude, body angle and, to a lesser extent wingbeat frequency, to control flight speed and directionality, and to attain the required balance between vertical and horizontal forces. Changes in body angle may function indirectly to modulate the orientation of the stroke plane and thereby to regulate wing position under changing airspeeds and flight behaviours (see Dudley 2000; Tobalske *et al.* 2007; Sapir & Dudley 2012).

Values for \dot{V}_{O_2} and all kinematic variables were measured six and three times, respectively, for each treatment on each individual bird. For statistical analyses, we used SPSS (version 15.0, SPSS Inc., Chicago, IL, USA) with two-tailed tests and a critical α of 0.05. Although the metabolic hypothesis as stated above is one-tailed, we applied a more strict two-tailed test due to lack of

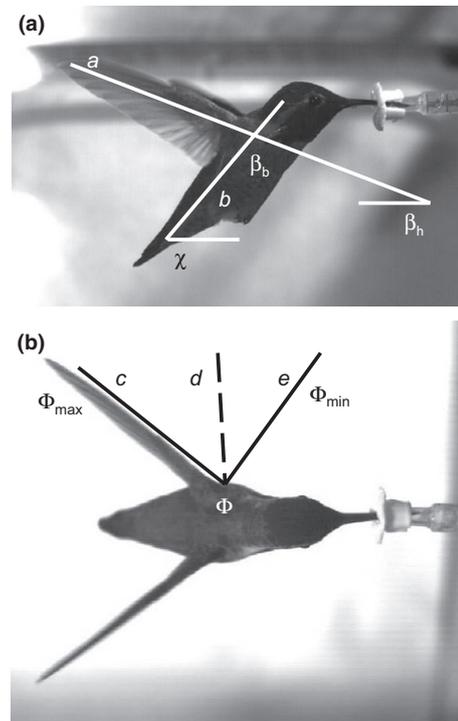


Fig. 3. Video frames of Anna's Hummingbird during hovering flight taken from lateral and vertically downward-directed (top) high-speed cameras. (a) Image of the lateral camera, with a depicting the line connecting the wing tip at the end of the upstroke (as can be seen in the photograph) and at the end of the downstroke (the other end of the line). The angle of this line relative to horizontal is the wing stroke plane in relation to horizontal (β_h). The wing stroke plane in relation to the longitudinal body axis (β_b) is given by the angle of this line in relation to b , the longitudinal body axis, which in relation to horizontal, is defined as the body angle (χ). (b) Image of the top camera, with c depicting the maximal wingbeat amplitude position that forms the angle Φ_{\max} , d depicting a line perpendicular the body that is used to measure the fore and rear parts of the wingbeat cycle, and e depicting the minimal wingbeat amplitude position that forms the angle Φ_{\min} . The wingbeat amplitude (Φ) equals $\Phi_{\max} - \Phi_{\min}$. All angles in (b) were calculated in relation to the horizontal stroke plane angle (see above) and not relative to horizontal.

previous studies supporting this hypothesis. We applied a two-way mixed ANOVA with treatment as an independent fixed factor, individual as an independent random factor and feeding duration as a dependent factor and also used a mixed ANCOVA with treatment as an independent fixed factor, individual as an independent random factor, feeding duration as an independent covariate and \dot{V}_{O_2} as a dependent factor. All kinematic variables (excluding upstroke/downstroke duration ratio) were evaluated using two-way mixed ANOVAs with treatment and individual as independent factors. All tests were followed by Bonferroni post hoc comparisons. For the upstroke/downstroke duration ratio, we used Kruskal–Wallis test because the data distribution significantly deviated from a normal distribution (Kolmogorov–Smirnov test, $Z = 1.384$, $P = 0.044$). All data are reported as mean values \pm SD, unless otherwise stated.

Results

Feeding durations did not significantly differ among the three flower orientation treatments (Table 2), with average values of 7.9 ± 3.7 s, 8.1 ± 2.7 s and 7.1 ± 2.5 s for feeding from horizontal, tilted and vertical flowers, respectively. Feeding duration did, however, vary significantly among individuals (see Table S1 in Supporting Information for data and Table 1 for statistical results). In addition, feeding duration (ranging from 3.03 to 18.68 s) had no significant effect on \dot{V}_{O_2} (Table 2). Mean values of \dot{V}_{O_2} differed significantly among the three treatments (Fig. 4, Table 2), with average values of 49.6 ± 4.9 mL O_2 h^{-1} g^{-1} , 52.4 ± 3.2 mL O_2 h^{-1} g^{-1} and 54.5 ± 5.1 mL O_2 h^{-1} g^{-1} for horizontal, tilted and vertical flower orientations, respectively. Given an energy equivalent for oxidation of a pure carbohydrate substrate (20.9 kJ L^{-1} O_2 ; see Walsberg & Wolf 1995), the estimated power inputs during hovering-feeding at horizontal, tilted and vertical flower orientations averaged 1.30 ± 0.10 watts, 1.38 ± 0.13 watts and 1.43 ± 0.14 watts, respectively. Hovering metabolic rate thus increased on average by about 10% when birds fed from pendent flowers. However, there was also a significant interaction between treatment and individual in the effect of flower orientation on hovering \dot{V}_{O_2} (Table 2), such that \dot{V}_{O_2} increased between 0 and 20% among different individuals feeding from vertical flowers, compared with that during feeding from horizontal flowers.

Table 1. Results of two-way mixed ANOVA testing the effects of treatment (independent fixed factor) and individual (independent random factor) on hummingbird feeding duration*

Source	d.f.	Sum of squares	Mean square	<i>F</i>	<i>P</i>
Intercept	1 (5)	6353.7 (258.1)	6353.7 (51.6)	123.1	<0.001
Treatment	2 (10)	21.4 (89.6)	10.7 (9.0)	1.2	0.343
Individual	5 (10)	258.1 (89.6)	51.6 (9.0)	5.8	0.009
Treatment \times Individual	10 (90)	89.6 (601.0)	9.0 (6.7)	1.3	0.221

*Error terms of each factor in the statistical model appear in parentheses.

For feeding from vertically oriented flowers, body angle relative to horizontal increased while ventral head flexion decreased, such that the birds flexed their heads backwards (Figs 2 and 5a). This combination of dorsal head flexion and upright body posture enabled the birds to direct their bills vertically. During feeding from horizontally oriented flowers, ventral head flexion and body angle in relation to horizontal were similar such that the birds directed their bills horizontally towards the feeder (Fig. 5a). In addition, the wing stroke plane relative to the longitudinal body axis increased during feeding from vertical flowers (Fig. 5b). A similar result was found for wingbeat amplitude, deriving largely from an increase in the minimum positional angle of the wingbeat (Fig. 5b), whereas the maximum positional angle remained unchanged (two-way mixed ANOVA, treatment $F_{2,4} = 4.34$, $P = 0.1$; individual $F_{2,4} = 14.25$, $P = 0.015$; treatment \times individual $F_{4,18} = 1.38$, $P = 0.280$). The remaining kinematic variables did not significantly differ among the different flower orientations: wingbeat frequency (treatment $F_{2,4} = 6.03$, $P = 0.062$; individual $F_{2,4} = 9.91$, $P = 0.028$; treatment \times individual $F_{4,18} = 2.75$, $P = 0.060$), wing stroke plane angle in relation to horizontal (treatment $F_{2,4} = 1.56$, $P = 0.32$; individual $F_{2,4} = 0.46$, $P = 0.660$; treatment \times individual $F_{4,18} = 19.00$, $P < 0.001$) and the ratio of the minimum to maximum positional angles of the wingbeat (treatment $F_{2,4} = 0.31$, $P = 0.75$; individual $F_{2,4} = 1.01$, $P = 0.443$; treatment \times individual $F_{4,18} = 5.49$, $P = 0.005$). The ratio of upstroke to downstroke durations also did not change among treatments (Kruskal–Wallis test, d.f. = 2, $\chi^2 = 4.77$, $P = 0.092$).

We also tested metabolic consequences of vertical floral orientation for the three birds for which kinematic variables were determined, but the results of this analysis were inconclusive (Table S2). Whereas the trend in \dot{V}_{O_2} in relation to floral orientation was nearly identical to that calculated for the larger sample size (Table S3), the effect of treatment was not significant, although the Bonferroni post hoc comparisons did find a significant ($P < 0.001$) difference between \dot{V}_{O_2} during feeding from vertical and horizontal flower orientations. Also, individual effects were not significant, whereas that of treatment \times individual was significant (see Table S2). Despite the similar trend (Table S3), the effect of treatment was not significant when data from only three birds were examined, and this is likely due to the lower power of the statistical test (power of 0.23 vs. 0.71 for the test with data from three and six birds, respectively).

Discussion

Feeding from pendent flowers is associated with a more upright body posture and dorsal flexion of the head, together with increases in the wing stroke plane angle in relation to the longitudinal body axis and in the wingbeat amplitude. Hovering while adopting this bizarre body posture (Fig. 2) also entailed a 10% increase in metabolic power input, suggesting that hummingbirds would obtain substantial energetic savings by electing to feed from

Table 2. Results of a mixed ANCOVA testing the effects of treatment (independent fixed factor), individual (independent random factor) and feeding duration (covariate) on hummingbird mass-specific metabolic rates during hovering*

Source	d.f.	Sum of squares	Mean square	F	P
Intercept	1 (30.2)	25 243.3 (574.6)	25 243.3 (19.0)	1328.6	<0.001
Feeding duration	1 (89)	0.2 (1156.3)	0.2 (13.0)	0.02	0.891
Treatment	2 (10)	420.3 (395.2)	210.1 (39.4)	5.3	0.026
Individual	5 (10.3)	391.7 (395.3)	78.3 (38.3)	2.0	0.154
Treatment × Individual	10 (89)	395.2 (1156.3)	39.5 (13.0)	3.0	0.002

*Error terms of each factor in the statistical model appear in parentheses.

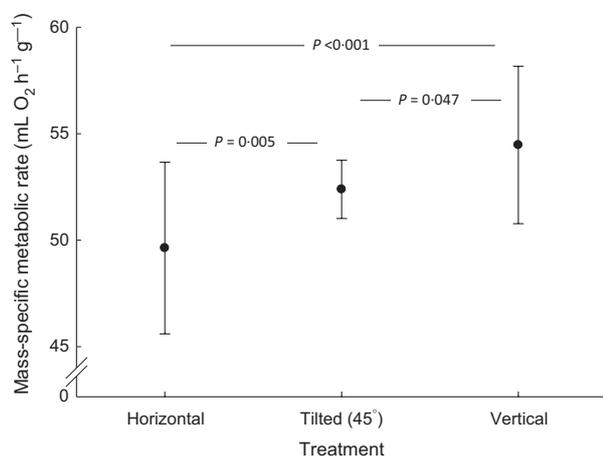


Fig. 4. Average (\pm SD) mass-specific metabolic rates of male Anna's Hummingbirds in relation to orientation of the respirometry mask and syringe. Significance levels are based on Bonferroni post hoc comparisons following the application of a General Linear Model in which mass-specific metabolic rate (dependent variable) was examined in relation to treatment (fixed categorical variable), bird identity (random categorical variable) and feeding duration (covariate). See Table 2 for results of the statistical analysis.

horizontal rather than vertical flowers. We originally hypothesized that pendent floral orientation might be associated with a reduction in hovering metabolic cost compared with that during feeding from horizontally oriented flowers. Our results however refute this hypothesis and instead suggest that pendent floral orientation is associated with an absolute increase in the metabolic cost of hovering. The downward orientation of many (but not all) hummingbird-pollinated flowers is thus puzzling, although the incentives of feeding from a nectar source potentially undiluted by rain may outweigh the elevated energetic demands associated with feeding from pendent flowers. For hummingbird-pollinated mistletoe flowers, orientation was not found to have any effect on nectar concentration, although exposure to rain did apparently dilute the nectar (Tadey & Aizen 2001). Floral orientation in the hummingbird-pollinated herb *Besleria sprucei*, by contrast, had a strong effect on nectar dilution, although this was primarily limited to the upward orientation (Aizen 2003). These findings on the functional significance of pendent orientation with respect

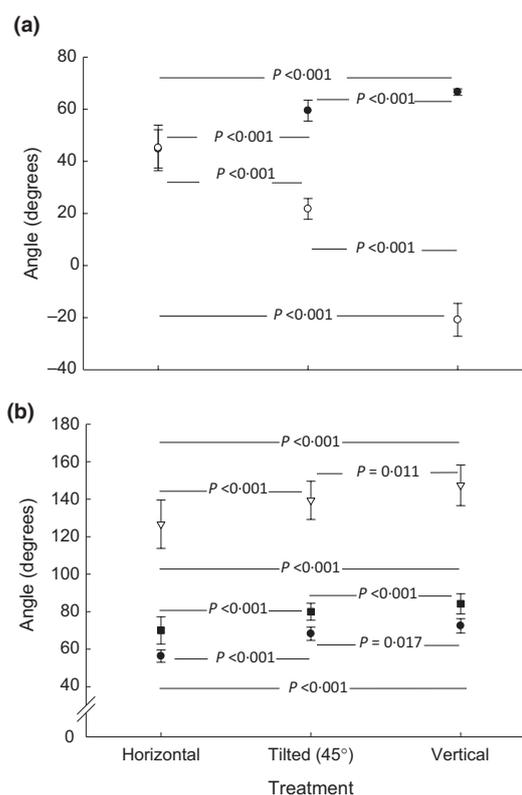


Fig. 5. Average (\pm SD) kinematic variables recorded for male Anna's Hummingbirds while hover-feeding at different flower orientations. (a) Longitudinal body axis in relation to horizontal (filled circles), and ventral head flexion in relation to the longitudinal body axis (open circles). (b) Wing stroke plane in relation to the longitudinal body axis (filled circles), wingbeat amplitude (open triangles) and the minimum positional angle of the wingbeat (filled squares). Significance levels are based on Bonferroni post hoc comparisons following the application of General Linear Models in which the kinematic variable (dependent variable) was examined in relation to treatment (fixed categorical variable) and bird identity (random categorical variable).

to precipitation may also derive from other flower-specific traits (e.g. diameter of nectar tubes) that can strongly affect nectar dilution (see Tadey & Aizen 2001; Aizen 2003).

Exposure to the elements is only one of several selective forces possibly influencing floral orientation. Floral conspicuousness, suggested to decrease in down-facing

flowers (Tadey & Aizen 2001), may strongly affect visitation by hummingbirds, although empirical evidence for the role of this factor is lacking (Tadey & Aizen 2001; Aizen 2003). Also, horizontal flowers may be more accessible to hummingbirds in the periphery of inflorescences compared with downward-facing flowers (Tadey & Aizen 2001). Flower orientation may thus represent the outcome of long-term evolutionary compromise among different and unrelated factors.

Here, floral orientation was shown to directly influence hummingbird flight energetics, which could presumably have a strong influence on patterns of floral evolution. As flower visitation and ultimately pollination derive from many interacting factors, including the metabolic cost of food acquisition, higher costs of hovering may reduce flower attractiveness and thus have direct consequences for plant fitness. Interestingly, the study of Tadey & Aizen (2001) that manipulated floral orientation also documented visitation rates by the hummingbird Green-backed Firecrown (*Sepehanoides sepehanoides*). This trochilid is common in the temperate forests of southern South America and is the exclusive pollinator of the mistletoe species studied (Ruffini 1992; Smith-Ramirez 1993). Tadey & Aizen (2001) predicted that downward-facing flowers would safeguard against nectar dilution and thus yield higher rates of trochilid visitation. Nonetheless, the overall orientation of hummingbird-visited flowers was statistically found to be more horizontal than that of random flower samples. Effects of nectar dilution may thus not pertain in this mistletoe species, as was corroborated by direct measurements of nectar concentration that found no significant effect of floral orientation, but that did find an effect of overall exposure to rain (Tadey & Aizen 2001). By contrast, a study of Ruby-throated Hummingbirds (*Archilochus colubris*) found no effect of orientation on visitation rates to artificial flowers (Fenster, Armbruster & Dudash 2009).

Our findings also have implications for methodological design of metabolic experiments on flying hummingbirds, as significant differences in both flight kinematics and metabolic rates were found at different feeder orientations. Studies of hummingbird flight energetics have usually used horizontal feeders (e.g. Tobalske *et al.* 2007; Warrick, Tobalske & Powers 2009; Clark & Dudley 2010), but others have employed deviations from horizontal (e.g. Bartholomew & Lighton 1986), or did not characterize feeder orientation (e.g. Wells 1993a,b). Methodological standardization through the use of horizontal feeder alignment would facilitate comparisons of metabolic rates obtained in different studies (see Welch 2011).

For hummingbird-pollinated flowers, it has been suggested that a pendent orientation may exclude some insects such as bees, and thus preventing visits by non-preferred pollinators (Grant & Grant 1968). However, downward-facing flowers have been subsequently documented in a number of insect-pollinated plants (Ushimaru & Hyodo 2005; Ushimaru, Kawase & Imamura 2006; Ushimaru *et al.* 2009; Rands, Glover & Whitney 2011). Nevertheless,

the prevalence of insect pollination of pendent flowers in different geographical regions is substantially lower than that of hummingbird pollination (Aizen 2003). Pendent flowers that are pollinated by insects might have also evolved due to similar selective processes, but there is a dearth of relevant empirical studies on the significance of flower orientation.

The trend of downward-oriented hummingbird-pollinated flowers seems to be fairly general in the New World. Aizen (2003) studied plant communities in a number of different South American regions, including temperate forests with variable rates of annual precipitation, and a tropical rain forest. This study found that, regardless of the region, hummingbirds pollinated more than 85% of all plant species possessing downward-facing flowers and that these plant species were abundant in their respective floras. Hummingbird-pollinated flowers oriented downwards seem also to be characteristic in Central and North America (see Grant & Grant 1968; Gentry 1982). In the Old World, the fraction of pendent flowers pollinated by such dedicated avian nectarivores as sunbirds (Nectariniidae) and honeyeaters (Meliphagidae) is unfortunately not known. Despite examples that resemble the hummingbird-pollinated floral syndrome, such as the red corolla and pendent floral orientation of the Red-hot Poker *Kniphofia laxiflora* (Asphodelaceae; see Brown, Downs & Johnson 2010), many Old World plants that are pollinated by avian nectarivores possess either horizontal- or upward-facing flowers that do not conform to the New World pattern for trochilid visitation (see Gill & Wolf 1975; Vaknin, Yom-Tov & Eisikowitch 1996; Kalinganire *et al.* 2001; Tandon, Shivanna & Ram 2003). The syndrome of bat-pollinated flowers in relation to flower orientation is also unknown, with examples of upward-facing (*Agave palmeri*; Ober & Steidl 2004), horizontal (*Amphitecna latifolia*, *Crescentia cujete*, *Vriesea gladioliflora*) and pendent flowers (*Merinthopodium neuranthum*; von Helversen, Holderied & von Helversen 2003). Hummingbirds are unique in their ability to sustain hovering flight compared with Old World avian nectarivores and, to a lesser extent, pollinating bats. Despite the 10% increase in their metabolic rate, they are able to feed from pendent flowers for long durations, as demonstrated in the present study, whereas similar feeding by the aforementioned groups is likely more restricted. We suggest that the prevalent pendent flower orientation evolved not because of the energetic consequences for pollinating hummingbirds, but rather to offset costs related to sun and rain damage, as well as to preclude pollination by unintended agents such as insects and bats.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Metabolic and kinematic data for individual hummingbirds.

Table S2. Statistical results of metabolic measurements for three individuals.

Table S3. Statistics of metabolic measurements under different treatments for three and six individuals.