

Allometry of maximum vertical force production during hovering flight of neotropical orchid bees (Apidae: Euglossini)

Michael E. Dillon^{1,*} and Robert Dudley^{2,3}

¹*Department of Biology, University of Washington, Seattle, WA 98195, USA*, ²*Department of Integrative Biology, University of California, Berkeley, CA 94720, USA* and ³*Smithsonian Tropical Research Institute, PO Box 2072, Balboa, Republic of Panama*

*Author for correspondence (e-mail: dillonm@u.washington.edu)

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Summary

The ability of orchid bees to generate vertical forces was evaluated using a load-lifting method that imposed asymptotically increasing loads during ascending flight, ultimately eliciting maximum forces while hovering. Among 11 orchid bee species varying by approximately an order of magnitude in body mass, the capacity to produce vertical forces expressed relative either to body weight or to flight muscle weight declined linearly with increased body mass. Allometric analysis of log-transformed data, by contrast, found maximum vertical force to scale isometrically with body mass, but also to exhibit a slightly negative allometry with respect to flight muscle mass. Maximum stroke amplitude at limiting loads averaged 140° and was remarkably constant among species, a result consistent with anatomical constraints of the

hymenopteran thorax on wing motions. By contrast, wing-beat frequencies during maximum performance declined with increasing body mass. Repeated lifting by individual bees reduced performance only when the number of consecutive lifts exceeded 15. Variation in linear mass density of the lifted load did not systematically alter performance estimates, although measurements on one species in two consecutive years at different thermal environments yielded significant differences in estimates of maximum force production. These findings suggest an adverse scaling of vertical force production at greater body mass even if flight muscle mass scales isometrically.

Key words: allometry, bee, euglossine, flight, force, hovering, performance.

Introduction

Flying animals modulate force production along the three orthogonal body axes yielding translational body accelerations critical for maneuvering in three dimensions. The capacity for force modulation, known as axial agility, is thus a major component of flight maneuverability and can critically influence the outcome of aerial interactions. However, few data are available on the accelerational capacities of flying insects, making both the allometry of force modulation and the biomechanical and physiological limits to axial agility unclear.

Body size strongly influences animal flight performance (Ellington, 1991; Norberg, 1990; Dudley, 2000), but the allometric correlates of axial agility have been limited to studies of vertical load-lifting. Through cumulative attachment of weights, Marden (1987) determined the maximum load that test animals would lift during takeoff. These data suggested that takeoff performance scales isometrically with flight muscle mass. Though a useful metric in its own right, the ability to takeoff is not equivalent to the ability to produce vertical forces. Takeoff from a surface requires initiation of wing flapping, involves vertical acceleration, and is influenced by ground effects (see Dudley, 2000; Rayner, 1991). By contrast, vertical force production in mid-air yields

acceleration of a mass in a direction opposite to gravitational acceleration, with no necessary ground effect. The relationship between takeoff performance and capacity for vertical force production is therefore unclear, leaving the empirical allometry of vertical force production and associated power expenditure unresolved for flying animals (see Dudley, 2000).

Flight with loads requires vertical forces often substantially in excess of body weight. In hummingbirds, the capacity for vertical force production is limited by the maximum stroke amplitude attainable by the wings (e.g. Chai and Dudley, 1995; Chai et al., 1997; Chai and Millard, 1997). However, the varied unsteady aerodynamic mechanisms associated with insect wing flapping (see Ellington et al., 1996; Dickinson et al., 1999; Sane and Dickinson, 2001, 2002) highlight the potential diversity of biomechanical constraints on flight performance. Kinematic and anatomical features of the flight apparatus, as well as physiological features of the flight muscle, may either individually or in concert act to limit either force or power output according to the particular context (Ellington, 1991; Dudley, 2000, 2002).

Although the ability to lift loads vertically is not necessarily equivalent to the capacity for whole-body acceleration in other

directions (cf. Marden, 1987; Berrigan, 1991; Petersson, 1995; Coelho, 1997), many insects carry either endogenous or exogenous loads supplemental to body mass. Studies of flight with artificially manipulated loads can therefore provide behavioral and ecological insights into functional consequences of organismal design (e.g. Marden, 1989; Kingsolver and Srygley, 2000; Srygley and Kingsolver, 2000).

Neotropical orchid bees (Apidae: Euglossinae) are superb hoverers (Casey et al., 1985; Dudley, 1995; Roubik and Ackerman, 1987), are easily collected using chemical baits that resemble the fragrances of their host flowers (Ackerman, 1983), and range in body mass from <50 mg to >1 g. These characteristics make them ideal for studying the allometry of flight performance. To avoid the potentially adverse consequences of cumulative load application in lifting studies, Chai et al. (1997) developed a beaded-string method that applies a monotonically but asymptotically increasing load to ascending animals. At the point of maximum load, animals transiently sustain their body mass and the added load of suspended string while remaining stationary in the air (i.e. while hovering). Here, we apply this method to evaluate the morphological and kinematic correlates of maximum hovering performance for eleven orchid bee species that vary by a factor of 15 in body mass. We also examine methodological features of asymptotic load-lifting, including effects of cumulative effort and of variation in the linear mass density of applied loads.

Materials and methods

We captured euglossine bees at chemical baits (cineole, methyl salicylate; see Ackerman, 1983) from 30 May–24 July 1997 on Barro Colorado Island (BCI), Republic of Panama, and from 25–30 July 1998 near the town of Gamboa,

approximately 10 km southeast of BCI. We included eleven species from four genera (*Eufriesea*, *Euglossa*, *Eulaema* and *Exaerete*; abbreviated subsequently as *Ef.*, *Eg.*, *El.* and *Ex.*, respectively) in the study, based on their availability and interspecific range of body masses (see Table 1). Chemical baits only attract male euglossines, and thus the study only included male bees. Typically within 20 min (maximum 58 min) of capture, we evaluated vertical flight capacity in a mesh chamber (30 cm × 30 cm × 30 cm) using the method of Chai et al. (1997). A beaded string was attached to the petiole of the bee, close to those abdominal segments within which the center of body mass is generally located for Hymenoptera (see Ellington, 1984a). Use of this location minimized pitching torque imposed on the bee while it lifted the string vertically. No beads were located on the first 3.5 cm of attached string, after which point a variable number of beads (depending on the mass of the species being tested, see below) were threaded together to yield a single bead group. This bead group and all other consecutively positioned bead groups were each separated by 2 cm of string.

The linear mass density of the beaded strings was based on bee size. We used two strings in 1997, one for which each of the two bead groups closest to the bee weighed 30±0.5 mg, whereas another nine consecutive bead groups each weighed 15±0.5 mg. For the second string in 1997, each consecutive bead group weighed 30±0.5 mg. In 1998, we also investigated the effects of string linear mass density on load-lifting by individual bees. One ‘thick’ string (linear density mass for the string alone of 2.1 mg cm⁻¹) and one ‘thin’ string (linear density mass density of 0.3 mg cm⁻¹) were used with each of two euglossine species (*Eg. imperialis*, *El. nigrita*). On each string type, the first bead group began 3.5 cm from the point of petiolar attachment, with subsequent bead groups

Table 1. Morphological parameters and maximum body weight-specific vertical force production for eleven species of euglossine bee

Taxonomic identification (<i>N</i>)	<i>m</i> (mg)	<i>m_w</i> (% <i>m</i>)	<i>R</i> (cm)	<i>AR</i>	<i>p_w</i> (N m ⁻²)	<i>FMR</i> (% <i>m</i>)	F_{vert}/mg
<i>Eufriesea pulchra</i> (11)	366.7±36.8	0.48±0.04	1.38±0.30	6.59±0.34	30.84±2.63	38.3±2.5	2.11±0.13 (<i>N</i> =10)
<i>Eufriesea schmidtiana</i> (1)	483.5	0.63	1.71	6.80	27.55	31.1 ^b	1.89
<i>Euglossa crassipunctata</i> (9)	68.1±7.0	n/a	0.81±0.02	6.39±0.26	16.15±1.52	32.8±1.2	2.06±0.09 (<i>N</i> =8)
<i>Euglossa imperialis</i> ^a (29)	165.9±22.9	0.45±0.07	1.11±0.04	6.80±0.35	22.57±2.37	32.1±2.3	2.01±0.12 (<i>N</i> =11)
<i>Euglossa sapphirina</i> (8)	56.6±4.3	n/a	0.73±0.02	6.65±0.54	17.49±1.30	32.9±1.4	2.04±0.10 (<i>N</i> =8)
<i>Euglossa tridentata</i> (10)	116.1±11.6	0.40±0.06	0.93±0.03	6.70±0.15	22.23±2.04	32.6±1.8	1.90±0.08 (<i>N</i> =10)
<i>Eulaema cingulata</i> (1)	545.4	0.55	1.76	6.88	29.70	34.8 ^b	2.07
<i>Eulaema meriana</i> (11)	817.1±95.4	0.77±0.05	2.17±0.06	6.28±0.15	26.83±3.07	33.7±3.5	1.77±0.11
<i>Eulaema nigrita</i> ^a (17)	401.6±53.6	0.57±0.07	1.55±0.06	6.45±0.29	26.37±2.80	33.9±3.7	2.13±0.25 (<i>N</i> =11)
<i>Exaerete frontalis</i> (12)	676.9±44.7	0.96±0.06	2.29±0.04	6.49±0.26	20.50±1.79	35.1±2.2	1.82±0.12 (<i>N</i> =10)
<i>Exaerete smaragdina</i> (3)	323.1±84.6	0.80±0.17	1.65±0.11	6.17±0.29	17.74±2.80	35.7±1.0 ^b	1.87±0.08 (<i>N</i> =2)

m, body mass; *m_w*, total wing mass; *m_{mus}*, flight muscle mass; *R*, wing length; *AR*, aspect ratio; *p_w*, wing loading; *FMR*, *m_{mus}*/*m*; **F_{vert}**, maximum vertical force; *g*, gravitational acceleration.

Values are means ± 1 s.d. Sample size *N* follows taxonomic identification unless otherwise noted.

n/a, not applicable.

^aMorphological data are pooled from 1997 and 1998 samples, whereas values of **F_{vert}/mg** refer to 1997 data only (see text).

^bValues for *FMR* were calculated using the regression of flight muscle mass on body mass for congeners (see text).

consecutively spaced at 2 cm intervals. Bead groups used with *Eg. imperialis* each weighed 15 ± 0.5 mg, whereas bead groups for *El. nigrita* weighed 30 ± 0.5 mg. We tested each bee with a thick and a thin beaded string and alternated string order in consecutive lifting bouts.

Following attachment of the beaded thread, we released bees within an experimental flight chamber (Fig. 1). Bees flew erratically at first, but generally within 1 min would hover steadily 12–15 cm above the chamber floor. From this position of submaximal hovering, bees would make short vertical forays, progressively lifting more bead groups along the attached string until no further weight could be sustained. This final moment of a lifting bout, usually lasting less than a second, represented maximum hovering flight with a supplemental load equal to the mass of the suspended region of the beaded thread. We included only those flights in which the bee hovered stably near the center of the chamber in the analysis. Bees then descended either to a lower height in the flight chamber and again vertically ascended, or sometimes descended to the chamber floor. To avoid transient behaviors at the start of a lifting trial, the first five vertical lifts of an individual were eliminated from consideration. The single highest lift obtained for any individual was assumed to represent its maximum hovering performance. Distance of the hovering bees to the chamber floor or sides was minimally 12 cm in all cases, corresponding to 4–5 wing lengths for the largest bees, and a minimum of 15 wing lengths for the smallest bees. These distances, together with the mesh

construction of the chamber sides and the open top of the chamber, preclude potential ground or boundary effects on hovering aerodynamics (see Rayner, 1991).

A video camera (Panasonic Omni-Minimovie) mounted above the flight chamber filmed lateral views of the hovering insect as projected from a mirror positioned at 45° to the chamber floor (see Fig. 1). A coordinate grid within this projected field of view permitted measurement of the absolute height of the flying bee (see Fig. 1). We determined maximum load from the measured length of lifted string, given known masses of individual bead groups together with the linear mass density of the thread alone. The vertical force required to sustain such maximally loaded hovering (F_{vert}) was calculated as the product of gravitational acceleration (g) and the sum of body mass and the maximum lifted load. By cycling through all recorded video images of a given sequence and relying on the phase mismatch between filming frequency (60 frames s^{-1}) and the wing-beat frequency, it was possible to identify those individual frames corresponding to extremes of wing motion within the stroke plane (see Dudley, 1995; Chai et al., 1997). Individual video frames were digitized using a QuickImage frame grabber and were analyzed using NIH Image. As the stroke plane angle of hovering euglossines is typically between 3° and 15° (Dudley, 1995), we took the horizontal projection of wing motions to indicate the actual stroke amplitude Φ (see Ellington, 1984b). We determined wing-beat frequency n by analyzing the audio track of the video tape *via* a peak-counting method implemented in Canary 1.2.

Individual bees were tested repeatedly in the flight chamber until flight performance deteriorated, at which point bees were placed in a closed plastic tube and were frozen. No more than 6 h later, body mass (m), thoracic mass (m_{th}), abdominal mass (m_{a}), and the mass of one ipsilateral wing pair were measured to within 0.01 mg. Total wing mass (m_{w}) was obtained by doubling this last measurement. Flight muscle mass (m_{mus}) for bees evaluated in 1998 was estimated as the difference between wet thoracic mass (minus legs and wings) and the mass of the dry thoracic exoskeleton following 24 h digestion in 0.5 mol l^{-1} NaOH. Correlation coefficients for linear regressions relating flight muscle mass to wet thoracic mass averaged 0.964 among species. Flight muscle mass was not measured on bees studied in 1997, but was instead estimated for these individuals according to the corresponding species regression derived in 1998. Three species (*Ex. smaragdina*, *Ef. schmidtiana*, *El. cingulata*) were studied in 1997 but were not subsequently captured. For these species, flight muscle mass was derived from thoracic mass data using regressions for congeneric species. One ipsilateral wing pair of each tested bee was saved for subsequent digital scanning and measurement with NIH Image to determine wing length R and wing area; total wing area S refers to the area of both wing pairs. Values of aspect ratio AR ($4R^2/S$), wing loading p_{w} (mg/S , where g is 9.81 m s^{-2}), and flight muscle ratio FMR (m_{mus}/m) were calculated for each bee.

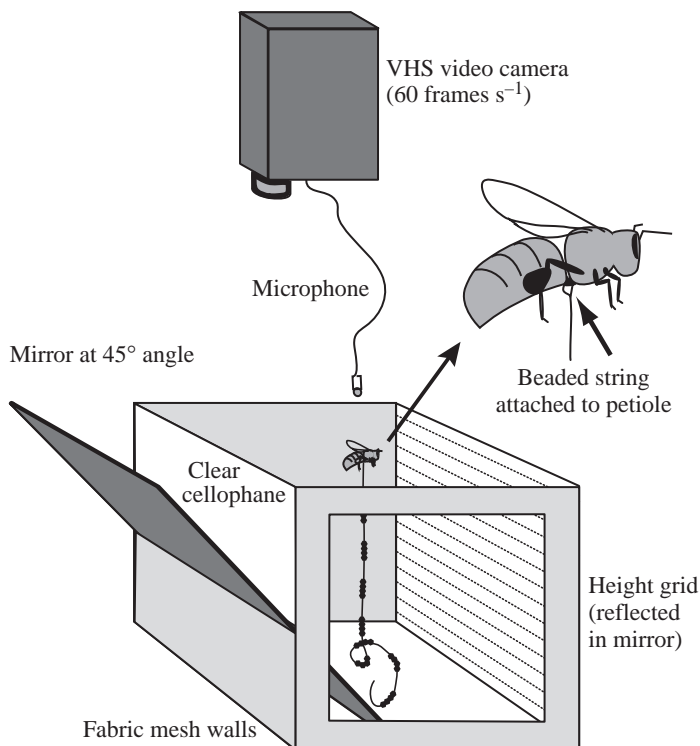


Fig. 1. Load-lifting assay used to determine maximum vertical force production. See text for detailed description.

Allometric relationships between morphology and flight performance were derived from species means. The random error in the predictor variables (morphological measurements) violates a primary assumption of ordinary least squares (OLS) regression and could cause significant error in the estimation of regression parameters, especially when the OLS correlation coefficients are low (Rayner, 1985). Therefore, we derived regression parameters for allometric relationships using reduced major axis (RMA) regression, which accounts for error in both the predicted and predictor variables (McArdle, 1988; Rayner, 1985). Single-factor and repeated-measures ANOVA were used to assess consequences of between year-variation among species and of multiple load-lifting trials by individual bees, respectively. Among-species comparisons are potentially confounded by the lack of statistical independence among data points (Felsenstein, 1985). Unfortunately, no species-level phylogeny for orchid bees is available at present (see Kimsey, 1987; Engel, 1999). Instead, a single factor ANOVA with body mass as a covariate was used to compare the two higher-order euglossine groupings of glabrous genera (*Euglossa*, *Exaerete*) and pubescent genera (*Eufriesea*, *Eulaema*), as based on taxonomic affinity and morphology (see Casey et al., 1985; Kimsey, 1987). Of the two euglossine species tested in both study years, one species (*Eg. imperialis*) yielded significantly lower maximum lifting values in 1998 (see below). Only the 1997 data for this species were used in subsequent analyses given our interest in maximum hovering performance. Statistical tests were performed using StatView 5.0 and R 1.6.1.

Results

Load-lifting methodology

The sometimes erratic behavior of bees when first placed in the flight chamber suggested that initial vertical forces might underestimate maximum lifting capacity. We used the 1997 data to test this possibility by first identifying individuals with six or more lifts. We then compared the average value of $F_{\text{vert}}/\text{mg}$ for the first five lifts with the average value for all subsequent lifts. We found no significant differences between initial and subsequent lifting performance either when individuals of the eight species under consideration were combined (Fisher's combined probabilities test, $\chi^2=15.8$, d.f.=16, $P=0.47$), or when each species was considered

individually (paired sign test, average $P=0.46$, range: 0.13–0.99). Additionally, we used a repeated-measures ANOVA to assess the effects on performance of repeated load lifting by individual insects (Table 2). Although maximum performance varied significantly among species for all four categories of cumulatively applied loads (significant species effect; see Table 2), the number of lifts only affected $F_{\text{vert}}/\text{mg}$ for those nine bees which had 16 or more lifts (Table 2). This decline in performance does not affect our estimates of allometry and performance limits, because we used the single highest lift as our metric of maximum hovering performance.

Using the total number of lifts per individual as a measure of cumulative effort, we asked whether the maximum vertical lifting force identified for each individual varied with effort by regressing log-transformed values of $F_{\text{vert}}/\text{mg}$ on the total number of lifts. Mass-specific maximum vertical force showed no significant correlation with cumulative effort when all individuals from the eleven species were pooled ($F=3.33$; d.f.=1,74; $P=0.07$). Within each of nine species (*El. cingulata* and *Ef. schmidtiana* each had only one individual bee and were thus excluded from this analysis), maximum lifting ability and cumulative effort were positively correlated only among individuals of *El. meriana* ($F=23.1$; d.f.=1,9; $P=0.001$) and of *El. nigrita* ($F=8.4$; d.f.=1,9; $P=0.018$). Overall significance of these regressions for the nine species under consideration was tested using a Fisher's combined probabilities test. This test yielded a marginally significant positive association between maximum lifting capacity and cumulative effort ($\chi^2=28.9$, d.f.=18, $P=0.0494$).

In 1998, we evaluated the effect of string linear mass density on maximum lifting performance for two species (*Eg. imperialis* and *El. nigrita*) using thick and thin bead strings. Variation in presentational order of the thick and thin strings had no significant effect on maximum lifting performance for either species (χ^2 goodness-of-fit test, 1 d.f., $P>0.75$). Two-way ANOVA was used to evaluate overall effects of species identity and string thickness on $F_{\text{vert}}/\text{mg}$ for these two species. Maximum lifting performance of *Eg. imperialis* was significantly lower than that of *El. nigrita* ($F=18.1$; d.f.=1,26; $P<0.001$), but effects of string type were non-significant ($F=1.79$; d.f.=1,26; $P=0.19$), as were interactions between string type and species identity ($F=0.10$; d.f.=1,26; $P=0.76$). No differences in mean morphological parameters were evident between the 2 years for either species (Table 3).

Table 2. Repeated-measures ANOVA testing for effects of species identity and cumulative number of lifts on maximum body weight-specific vertical force production

Number of lifts	N	P value					
		Species	d.f.	Lift no.	d.f.	Species × Lift no.	d.f.
1–5	56	<0.0001***	9,184	0.8384	4,184	0.8581	36,184
1–10	30	0.0007**	7,198	0.0786	9,198	0.4284	63,198
1–15	18	0.0093**	5,168	0.5535	14,168	0.7749	70,168
1–20	9	0.0062**	2,95	0.0003**	19,95	0.8657	38,95

N, number of individuals included in each category; d.f., degrees of freedom; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Table 3. Morphological parameters, air temperature and maximum vertical force production for two orchid bee species in each of 2 years, and results of an ANOVA evaluating between-year differences in the means

	<i>Euglossa imperialis</i>			<i>Eulaema nigrita</i>		
	1997	1998	<i>P</i> (d.f.=1,27)	1997	1998	<i>P</i> (d.f.=1,15)
<i>m</i> (mg)	166.3±24.5	165.5±21.8	0.93	414.1±62.5	378.7±20.2	0.20
<i>m</i> _{th} (mg)	63.7±7.3	64.1±6.8	0.88	162.6±20.2	163.2±14.0	0.95
<i>m</i> _a (mg)	44.2±12.0	46.2±10.8	0.66	140.9±47.6	111.6±16.0	0.17
<i>S</i> (cm ²)	0.368±0.02	0.350±0.031	0.09	0.753±0.05	0.733±0.07	0.52
<i>R</i> (cm)	1.11±0.04	1.10±0.03	0.39	1.56±0.07	1.54±0.04	0.54
<i>AR</i>	6.70±0.27	6.93±0.40	0.09	6.44±0.22	6.47±0.41	0.83
<i>p</i> _w (N m ⁻²)	22.1±2.6	23.2±2.0	0.24	26.9±3.1	25.4±2.2	0.33
<i>T</i> (°C)	24.9±1.5	28.8±0.7	<0.001***	26.4±1.7	28.3±0.5	0.015*
F _{vert} /mg	2.01±0.12	thin string: 1.49±0.33 thick string: 1.37±0.24	a a	2.13±0.25	thin string: 2.05±0.39 thick string: 1.85±0.34	a a

Values are means ± 1 s.d.

*m*_{th}, thoracic mass; *m*_a, abdominal mass; *S*, total wing area; *T*, air temperature in flight chamber; other symbols as in Table 1.

*See text for between-year and between-string comparisons.

P*<0.05; **P*<0.001.

However, *Eg. imperialis* exhibited a substantial and significant reduction in lifting performance in 1998 relative to that in 1997 (*F*=29.2; d.f.=1,27; *P*<0.001), whereas *El. nigrita* showed a similar but non-significant trend (*F*=2.0; d.f.=1,19; *P*=0.17). Also, ambient air temperatures in 1998 were 3–4°C higher than in 1997 (see Table 3) because of different experimental locations (1997: indoors laboratory on BCI; 1998: open air laboratory in Gamboa).

Morphology and flight performance

Maximum lifts were obtained from a total of 91 individual orchid bees representing 11 species (Table 1). On average, 8.3 individuals were studied from each species (range: 1–19), and an average of 10.0 lifts (range: 3–42) was obtained from each individual. Orchid bees typically sustained from 1.77–2.11 times their own body weight (Table 1). Among species,

maximum vertical force scaled isometrically with body mass (**F**_{vert}=15.4*m*^{0.97}; Table 4). Flight muscle mass scaled also isometrically with body mass (*m*_{mus}=0.298*m*^{1.02}, *P*<0.001, *r*²=0.997), with the exponent not significantly different from one (*P*=0.276, *t*=1.160, d.f.=9, two-tailed Student's *t*-test). OLS regression found the allometric exponent for the relationship between **F**_{vert} and flight muscle mass to be significantly less than one (**F**_{vert}=35.5*m*_{mus}^{0.95}; *P*=0.022, *t*=-2.351, d.f.=9, one-tailed Student's *t*-test; see Table 4); however, RMA regression could not distinguish the exponent from one (see Table 4). Nonetheless, linear regressions of both maximum body weight-specific and muscle-weight specific vertical forces declined significantly with body mass (Fig. 2).

Among these orchid bees, larger species had significantly larger wings than would be expected given isometric scaling.

Table 4. Correlation coefficients and allometric exponents for the regression of log transformed maximum vertical force production, **F**_{vert} (N), on log-transformed morphological parameters for eleven euglossine bee species

Parameter	<i>r</i>	<i>P</i> value	Ordinary least squares			Reduced major axis		
			Exponent	95% CL	<i>r</i> ²	Exponent	95% CL	<i>r</i> ²
<i>m</i> (kg)	0.993	<0.001***	0.97	0.93, 1.02	0.99	0.97	0.93, 1.02	0.99
<i>m</i> _{mus} (kg)	0.993	<0.001***	0.95	0.90, 0.99	0.99	0.95	0.90, 1.00	0.99
<i>R</i> (m)	0.957	<0.001***	2.25	1.85, 2.65	0.95	2.31	1.95, 2.75	0.95
<i>S</i> (m ²)	0.930	<0.001***	1.11	0.89, 1.33	0.94	1.14	0.95, 1.38	0.94
<i>AR</i>	-0.142	0.661	-2.70	-21.9, -16.5	0.01	-25.6	-51.3, 12.8	0.01
<i>p</i> _w (N m ⁻²)	0.613	0.022*	2.82	0.71, 4.94	0.50	3.98	2.39, 6.62	0.49
<i>FMR</i>	0.317	0.171	5.95	-4.87, 16.76	0.15	15.53	8.11, 29.7	0.15

P values are derived from a student's *t* test on the null hypothesis that there is no correlation between the log of **F**_{vert} and the log of the given parameter (*H*₀:*ρ*₀=0).

Symbols as in Table 3. CL, confidence limits; **P*<0.05, ****P*<0.001.

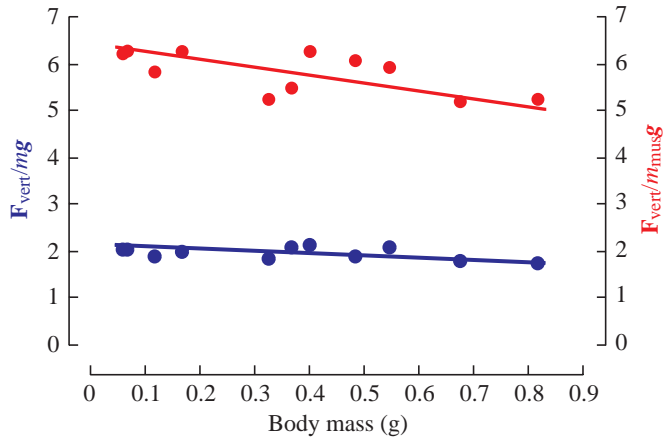


Fig. 2. Relationship between body mass m and either body weight-specific vertical force production (filled circles) or muscle weight-specific vertical force production (open circles) for eleven species of orchid bee. Regressions as follows: OLS, $F_{\text{vert}}/mg=2.06-0.244m$, $r^2=0.25$, $P=0.117$ (NS); $F_{\text{vert}}/m_{\text{mus}}g=6.22-1.10m$, $r^2=0.39$, $P=0.04$; RMA, $F_{\text{vert}}/mg=2.15-0.487m$, 99% CI for slope: -0.95 , -0.03 ; $F_{\text{vert}}/m_{\text{mus}}g=6.46-1.75m$, 99% CI for slope: -2.82 , -0.73 . Allometric exponents are given in Table 4. NS, not significant.

Wing length increased with $m^{0.41}$ ($R=0.135m^{0.41}$, $P<0.001$, $r^2=0.96$), with the scaling exponent significantly greater than 0.33 ($P=0.005$, $t=3.218$, d.f.=9, one-tailed Student's t -test). Wing area scaled with $m^{0.83}$ ($S=0.011m^{0.83}$, $P<0.001$, $r^2=0.95$), with the exponent significantly greater than 0.66 ($P=0.009$, $t=2.895$, d.f.=9, one-tailed Student's t -test). Maximum vertical force scaled isometrically with wing area, and with the square of wing length (see Table 4). Allometric scaling of wing area caused wing loading to increase more slowly with body mass than would be expected given isometry ($p_w=-0.02m^{0.17}$, $P=0.023$, $r^2=0.39$). Increased wing loading was significantly positively correlated with maximum vertical force production (Table 4). Neither aspect ratio nor FMR influenced maximum vertical force production (Table 4). The lack of correlation between FMR and F_{vert} is not explained by lack of variation in FMR , which ranged from 31.1–38.3% among euglossine species (Table 1). We could detect no effect of R , S , AR , p_w or FMR on maximum body weight-specific vertical force production ($P>0.10$ in all cases).

Single-factor ANOVA evaluating consequences of taxonomic association (glabrous or pubescent genera) on vertical force production, with body mass as a continuous covariate, demonstrated a significant taxonomic category effect ($F=65.9$; d.f.=1,72; $P<0.0001$), significant body mass effects ($F=2909.0$; d.f.=1,72; $P<0.0001$), and a significant interaction between taxonomic category and body mass ($F=35.4$; d.f.=1,72; $P<0.0001$). Individuals belonging to pubescent genera produced maximum vertical forces averaging 2.23 times higher than those of glabrous genera, but were also substantially heavier, by an average factor of 2.17.

Stroke amplitude during maximum loading averaged about 140° (Table 5), and among species was independent of body

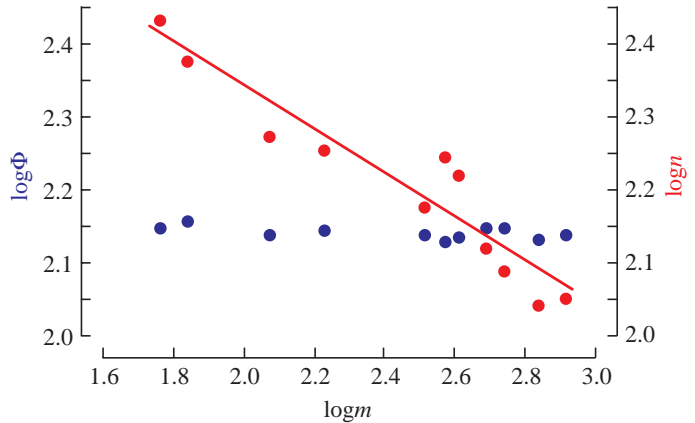


Fig. 3. Relationship between log body mass (m) and either log stroke amplitude (Φ ; filled circles) or log wing-beat frequency (n ; open circles) for eleven species of orchid bees. Linear regressions as follows: $\log\Phi=2.17-0.005\log m$, $r^2=0.35$, $P=0.055$ (NS); $\log n=2.94-0.13\log m$, $r^2=0.90$, $P<0.001$. NS, not significant.

mass (Fig. 3). By contrast, wing-beat frequency declined significantly, in proportion to $\text{mass}^{-0.30}$ (see Fig. 3).

Discussion

Methodological considerations

Maximum takeoff performance is a useful animal flight metric that is probably linked to the ability to escape from predators (Marden, 1987). Unfortunately, evaluating takeoff potentially conflates three distinct aerodynamic situations: the initiation of wing flapping, the ground effect, and vertical acceleration with concomitant ascent. The simultaneous occurrence of these three situations makes it difficult to assess the biomechanical limits to flight performance using the takeoff performance metric. By contrast, the load-lifting approach used here distinguishes between flight takeoff and maximum hovering performance attained subsequent to ascent. In these experiments, bees initially accelerated from rest, but then were progressively decelerated as continuously increasing loads were applied, resulting in transient but stationary bouts of hovering at maximum sustained load. Also, the height of the bee at this point exceeds the wing length by a factor of ten or more, rendering ground effects negligible (Rayner, 1991; Sane, 2001). We therefore consider these measurements to reliably indicate maximum vertical force production during hovering flight.

Coefficients of variation for maximum loads sustained consecutively by individual bees averaged about 5%, suggesting consistent performance within any given lifting bout. Decline in performance deriving either from physiological fatigue or reduced behavioral motivation was evident only for sequences comprising more than 15 lifts (i.e. vertical forays; see Materials and methods and Table 2). Similarly, only two of nine species demonstrated a significant inter-individual correlation between maximum relative sustained weight and the total number of lifts. These results

suggest that repeated sampling from individual bees following initial takeoff yields consistent estimates of maximum lifting capacity. Also, no systematic change in maximum performance was observed when the linear mass density of the load was changed for two species, suggesting that the dynamics of ascent with increasing load prior to stationary hovering (i.e. vertical acceleration followed by deceleration) bears no influence on the maximum sustainable load.

One of the two species (*Eg. imperialis*) examined in each of 2 consecutive years exhibited a substantial reduction (~30%; see Table 3) in maximum force production. The higher air temperatures in 1998 (Table 3) might, by contrast, have been predicted to yield greater rather than reduced lifting performance (e.g. Lehmann, 1999). Mechanisms underlying this reduction for *Eg. imperialis* in 1998 are unclear, particularly as no morphological differences characterized the species samples from the 2 years (Table 3). One possible factor is variable duration between capture and experimental measurements; although this period was always less than 1 h, any systematic change in time-dependent capacity or motivation to fly could influence subsequent estimates of maximum performance. Times between capture and measurement were, however, significantly shorter in 1998 ($P < 0.02$ for both species, Student's *t*-test). Nonetheless, *Eg. imperialis* in 1997 exhibited maximum lifting broadly comparable to that of other species (i.e. external loads approximately equal to body mass; Table 1), and only bees of this year were used in subsequent analyses of maximum performance by this species. A future methodological goal might be to determine the effects of measurement time course, and also more proximately of thoracic muscle temperature (see May and Casey, 1983), on experimentally determined flight abilities.

The allometry of vertical force production

Allometric analysis using log-transformed mass and force data demonstrated isometry in maximum vertical force production across a 12-fold range in euglossine body mass (Table 4). By contrast, use of non-log-transformed data found both body weight-specific and muscle weight-specific maximum vertical forces to decrease with increasing body mass (Fig. 2), in spite of isometric scaling of flight muscle mass. If muscle mass fraction remains constant among volant taxa, a systematic decline in wing-beat frequency with increased body mass under maximum conditions (Fig. 3) would yield a negative allometry in power availability and presumably in hovering capacity (see Pennycuik, 1975; Ellington, 1991; Dudley, 2000). A decline in the relative capacity to increase stroke amplitude may also act in concert with allometric decline in wing-beat frequency, further compromising maximum performance (see below).

Marden (1987) estimated takeoff forces to scale isometrically with muscle mass for a variety of volant animals ranging over four orders of magnitude in body mass (see also Marden and Allen, 2002). The method described in Marden (1987) differs substantially from that used here, and direct

Table 5. Stroke amplitude Φ and wingbeat frequency n during maximum load-lifting for eleven euglossine species

Taxonomic identification (<i>N</i>)	Φ (degrees)	n (Hz)
<i>Eufriesea pulchra</i> (10)	135.5±4.2	176.3±4.5
<i>Eufriesea schmidtiana</i> (1)	141.5	132.8
<i>Euglossa crassipunctata</i> (8)	143.9±2.4	238.6±15.5
<i>Euglossa imperialis</i> ^a (11)	139.9±2.3	180.0±4.1
<i>Euglossa sapphirina</i> (8)	141.7±3.6	271.4±11.5
<i>Euglossa tridentata</i> (10)	138.7±3.8	188.8±11.3
<i>Eulaema cingulata</i> (1)	140.9	123.3
<i>Eulaema meriana</i> (11)	137.8±4.3	112.7±4.2
<i>Eulaema nigrita</i> ^a (11)	137.5±3.3	167.0±6.1
<i>Exaerete frontalis</i> (10)	136.4±2.0	110.6±8.9
<i>Exaerete smaragdina</i> (2)	138.5±1.7	150.6±6.2

Values are means ± 1 s.d.
 Sample size *N* follows species identification.
^aValues refer to 1997 data only (see text).

comparison with the present results is not germane. The slight decline of force production relative to body mass and muscle mass suggested by this study (Fig. 2) may derive from the analysis of bees from within a single hymenopteran subfamily. Such a decline may be also lost statistically when mass data are log-transformed to yield allometric regressions ranging over only a single order of magnitude in body mass. Moreover, no existing study has evaluated the phylogenetic effect of statistical non-independence of species-level data points on conclusions from allometric studies. Future studies of the allometry of maximum flight performance would do well to incorporate the phylogenetic relatedness of taxa in question, as well as to evaluate as wide a body mass range as logistically possible

Generic relationships among euglossine bees are not well resolved (see Kimsey, 1987; Engel, 1999; Michener, 2000), and no subgeneric hypothesis of euglossine relatedness is presently available. The suprageneric categorization of glabrous and pubescent genera is, however, probably robust, given the general recognition that *Euglossa* and *Exaerete* are sister taxa (Dressler, 1982; Kimsey, 1987; Roubik, 1989). The approximately twofold difference in lifting capacity found here may be simply attributed to the fact that pubescent euglossine taxa tend have higher wing-beat frequencies for a given body mass relative to glabrous genera (Casey et al., 1985; see Tables 1 and 5) while the ratios in body mass and in muscle mass between the two categories are identical (see Table 1). Overall, both body mass and muscle mass are strong predictors of maximum vertical forces among euglossine species, as are the correlated size measures of wing length, wing area and wing loading. Maximum vertical force scaled with the square of wing length and with wing area to the first power, as predicted by aerodynamic theory (see Ellington, 1984c). Although flight muscle ratio is a strong predictor of takeoff performance (Marden, 1987), it is a poor predictor of maximum vertical forces in hovering flight (Table 4). Contrary to theoretical arguments (Pennycuik, 1968; Savile, 1957),

aspect ratio influenced neither takeoff ability (Marden, 1987) nor maximum vertical force production (Table 4), though it may affect induced power production (see Pennycuik, 1968).

Orchid bees attained maximum lifting performance at stroke amplitudes averaging 140°, independent of body mass (Fig. 3, Table 5). This constancy, which pertains for any hypothesis of phylogenetic relatedness among study species, suggests an anatomical limit to wing motions within the stroke plane that constrains hovering performance. Interestingly, a recent study of intraspecific limits to flight capacity in carpenter bees (S. P. Roberts, J. F. Harrison and R. Dudley, manuscript submitted for publication) found a similar limit of about 138° to stroke amplitude over a threefold range in body mass. Smaller carpenter bees hovering in hypodense but normoxic gas mixtures failed at lower air densities, a result attributable in part to the relatively greater thoracic muscle mass and presumably higher body mass-specific muscle power output characterizing smaller individuals (see S. P. Roberts, J. F. Harrison and R. Dudley, manuscript submitted for publication). Here, the absolute change in stroke amplitude from hovering to maximum performance systematically declined with increased body mass, but both small and large bees failed at the same limiting stroke amplitude. Such an interspecific decline in the capacity to increase stroke amplitude may, in part, be the mechanistic basis for the decline in relative force production at greater body mass (see Fig. 2). For neither carpenter bees nor orchid bees, however, does energetic performance at maximum hovering necessarily correspond to maximum power output of the flight muscle, as fast forward flight may require lower stroke amplitudes but also higher power outputs relative to hovering flight (see Dudley, 2000).

A limiting stroke amplitude near 140° may basally characterize all Hymenoptera, although it will be important to assess biomechanical constraints for a variety of flight behaviors, including hovering in hypodense or hypobaric gas mixtures, forward flight at maximum airspeed, and maximum accelerational capacity. For example, euglossine bees hovering in pure heliox exhibit stroke amplitudes below, albeit close to, the limiting values obtained here (Dudley, 1995). Hypobaric reductions of total pressure (possibly with hyperoxic enhancement to compensate for reduced oxygen availability; Dudley and Chai, 1996) may therefore be necessary to elicit failure air densities for hovering euglossines. The modulation of stroke amplitude to alter vertical force production is commonplace among hovering taxa (e.g. Chai and Dudley, 1995; Dudley, 1995; Lehmann and Dickinson, 1998; S. P. Roberts, J. F. Harrison and R. Dudley, manuscript submitted for publication), although changes in other kinematic features may also pertain. Recent work with robotic flapping wings at Re somewhat lower than those studied here has shown high sensitivity of transient and mean aerodynamic forces to the details of wing-beat kinematics, particularly the timing and velocity of wing rotation (Dickinson et al., 1999; Sane and Dickinson, 2001, 2002). Bumblebees in forward flight change wing rotational velocities systematically with airspeed (Dudley

and Ellington, 1990), but relevant high-speed videography has not been carried out for euglossines and represents an important direction for future studies of flight performance in this subfamily. Description of wing-beat kinematics in forward and rapid maneuvering flight of euglossines may also reveal limits to performance other than those imposed by a maximum stroke amplitude (see Chai and Dudley, 1999; Lehmann and Dickinson, 2001).

Particularly for hematophagous, nectarivorous and predatory insects, the capacity to lift loads vertically can be relevant to survival following resource acquisition (e.g. Hargrove, 1975; Wolf and Schmid-Hempel, 1989; Berrigan, 1991; Coelho and Hoagland, 1995; Coelho, 1997). Transient force augmentation in mid-air may be relevant in other behavioral contexts, particularly those of sexual selection (e.g. Marden, 1989; Petersson, 1995). Con- and heterospecific aerial interactions are likely to be associated with rapid modulation of both the magnitude and direction of the aerodynamic force vector, and vertical force production may be only a small part of the overall response repertoire. If muscle design overall is isometric, and the flight capacity for force production is systematically reduced at larger muscle mass, possibly *via* power limitation, then smaller taxa have a double advantage in maneuvering flight during competitive encounters: relative power availability is greater, and absolute capacities for both rotational and translational accelerations are higher than in larger forms.

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