

Kinematics of hovering hummingbird flight along simulated and natural elevational gradients

Douglas L. Altshuler^{1,*} and Robert Dudley^{1,2,†}

¹*Section of Integrative Biology, University of Texas at Austin, Austin, Texas 78712, USA* and ²*Smithsonian Tropical Research Institute, PO Box 2072, Balboa, Republic of Panama*

*Author for correspondence at present address: Bioengineering, Mail Code: 138-78, California Institute of Technology, 1200 East California Boulevard, Pasadena, CA 91125, USA (e-mail: doug@caltech.edu)

†Present address: Department of Integrative Biology, University of California, Berkeley, CA 94720, USA

Accepted 12 June 2003

Summary

Hovering flight is one of the most energetically demanding forms of animal locomotion. Despite the cost, hummingbirds regularly hover at high elevations, where flight is doubly challenging because of reduced air density and oxygen availability. We performed three laboratory experiments to examine how air density and oxygen partial pressure influence wingbeat kinematics. In the first study, we experimentally lowered air density but maintained constant oxygen partial pressure. Under these hypodense but normoxic conditions, hummingbirds increased stroke amplitude substantially and increased wingbeat frequency slightly. In the second experiment, we maintained constant air density but decreased oxygen partial pressure. Under these normodense but hypoxic conditions, hummingbirds did not alter stroke amplitude but instead reduced wingbeat frequency until they could no longer generate enough vertical force to offset body weight. In a final combined experiment, we decreased air density but increased oxygen availability, and found that the wingbeat kinematics were unaffected by supplemental oxygen.

We also studied hovering and maximally loaded flight performance for 43 hummingbird species distributed along a natural elevational gradient in Peru. During free hovering flight, hummingbirds showed increased stroke amplitude interspecifically at higher elevations, mirroring the intra-individual responses in our first laboratory experiment. During loaded flight, hummingbirds increased both wingbeat frequency and wing stroke amplitude by 19% relative to free-flight values at any given elevation. We conclude that modulation of wing stroke amplitude is a major compensatory mechanism for flight in hypodense or hypobaric environments. By contrast, increases in wingbeat frequency impose substantial metabolic demands, are only elicited transiently and anaerobically, and cannot be used to generate additional sustained lift at high elevations.

Key words: air density, elevation, flight, hummingbird, hyperoxia, hypoxia, kinematics, load lifting, oxygen concentration, wingbeat frequency, stroke amplitude.

Introduction

Animal flight at high elevations presents a double physiological challenge: reduced air density demands higher lift and mechanical power output, whereas reduced oxygen availability can constrain metabolic power input. Two of the most important mechanisms for generating higher lift in hovering flight are (1) to increase the horizontal area swept out by the wings and (2) to increase the wingbeat frequency (Ellington, 1984). However, muscular activity and correlated wingbeat kinematics can also be adversely affected by reduced oxygen availability. How do animals satisfy the conflicting requirements of flight at high elevations? The purpose of this study was to determine how hummingbirds modulate wingbeat kinematics in both reduced air density and reduced oxygen partial pressures. We present results from laboratory and field experiments that measure both sustained and burst kinematic

performances of hovering hummingbirds under different conditions of hypodense, hypoxic and hypobaric air.

Hummingbird flight has been previously examined in a suite of experimental studies that evaluated hovering in normoxic but hypodense air (Chai and Dudley, 1995), in hypoxic and hypodense air (Chai and Dudley, 1996), in hyperoxic and hypodense air (Chai et al., 1996), and during maximal load-lifting (Chai et al., 1997; Chai and Millard, 1997). The principal conclusion from these experiments is that when challenged by low-density air, hummingbirds exhibit considerable modulation in stroke amplitude, ultimately reaching a constraint on flight performance in low-density air when the stroke amplitude reaches a geometric limit near 180° (Chai and Dudley, 1995). In contrast, hummingbirds increase wingbeat frequency only marginally in hypodense air, and then

only if the air is normoxic. Here, we present kinematic data for these and other similar experiments that isolate the effects of air density and oxygen partial pressure, with specific reference to elevational gradients in the field. Specifically, we determined wingbeat kinematics for hummingbirds hovering in two experimental conditions that mimic effects of increasing altitude: (1) decreased air density, attained by replacing normal air with normoxic heliox, and (2) hypoxia, attained by replacing normal air with pure nitrogen. In addition, we performed a third experiment to test metabolic performance with reference to elevation, by filming birds in hypodense hyperoxia, which we accomplished by replacing normal air with hyperoxic heliox.

The hummingbird family is species-rich, comprising over 320 species found exclusively in the New World (Schuchmann, 1999); however, comparative data on hummingbird wingbeat kinematics are confined to data sets on body mass and wingbeat frequency (Greenewalt, 1975). Curiously, the allometry of hummingbird wingbeat frequencies follows a different scaling relationship relative to that of insects. In a non-phylogenetic analysis, insect wingbeat frequencies scale with $\text{mass}^{-0.24}$, whereas hummingbird wingbeat frequency scales significantly differently, with $\text{mass}^{-0.61}$ (Dudley, 2000). Thus, relative to insects, hummingbirds wingbeat frequencies decline more rapidly with increased body mass.

Hummingbirds occur at almost all elevations in the Americas where there are flowering plants (Schuchmann, 1999), and some Andean hummingbirds subsist on flowers at elevations as high as 5000 m (Carpenter, 1976). Thus, the Andes provide a natural context for studying hummingbird flight across elevational gradients. We filmed hovering and load-lifting for an assemblage of hummingbirds over a ~4000 m elevational range in southeast Peru. The kinematics of both types of flight were examined with reference to both elevation and body mass.

Materials and methods

Hummingbird flight experiments were performed using chambers sufficiently small to allow for bird restraint and filming but large enough to prevent boundary effects. Specifications and sizes for the flight chambers in each of the experiments are given below. In all experiments, a video camera (Sony Video 8 CCD-TR44) was used to record wingbeat kinematics at 60 frames s^{-1} with a high-speed shutter of 1/4000 s. The camera filmed a mirror positioned at 45° above the flight chamber to obtain horizontal wing projections and flight kinematics. Horizontal projection of wing motions yielded accurate measurements of wing positional angles because the stroke plane angle of hummingbird wings is close to zero (e.g. Chai and Dudley, 1996; Stolpe and Zimmer, 1939). Because the camera filmed at 60 frames s^{-1} and hummingbird wingbeat frequencies varied from 14 to 75 Hz, it was not possible to film multiple frames per wing stroke for most of the hummingbirds. Instead, we analyzed kinematics

over many wing stroke cycles corresponding to periods of 2 s for all experiments, except for load-lifting (see below).

In frame-by-frame analysis of video films, we measured two features of wingbeat motion in each of the experiments: wingbeat frequency and wing stroke amplitude (Chai and Dudley, 1996). Wingbeat frequency (N) is the number of complete wingbeats per second (Hz), and is determined from the interaction frequency between the wingbeat frequency and the filming rate (60 frames s^{-1}) of the video camera. For a hummingbird hovering with a wingbeat frequency of 60 Hz, the wing would appear stationary. When the wingbeat frequency exceeds 60 Hz, the wing appears to move forward in consecutive frames, whereas wingbeat frequencies less than 60 Hz result in a film sequence in which the wing lags behind. Thus an average wingbeat frequency can be calculated by counting the number of apparent wingbeat cycles completed over the course of 1 s, and then by adding this number to 60 if the wing appears to move forward, or subtracting it from 60 if the wing appears to move backwards.

The stroke amplitude (Φ) is the angular extent of wing motion within the stroke plane, and was determined from the subset of frames within the sequence used to calculate wingbeat frequency. At the end of either halfstroke, the wings appear as stationary thin lines when viewed from above. For these moments, wings were oriented vertically and thus were either in the middle of pronation or supination. The angular extents of wing motion were measured using a protractor, with the angle between the midpoints of pronation and supination termed the wing stroke amplitude.

Decreased air density trials

Density reduction experiments were performed at two elevations in the Colorado Rocky Mountains, USA. The low-elevation site was located in Cheyenne Canyon Park outside Colorado Springs, CO (1875 m), and was characterized by an average air density of 0.987 kg m^{-3} and an average oxygen partial pressure of 128.3 mmHg (1 mmHg=133.3 Pa). The high-elevation site was the Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO (2900 m), characterized by an air density of 0.862 kg m^{-3} and an oxygen partial pressure of 112.1 mmHg. These elevations are close to the lower and upper elevation limits, respectively, of co-occurrence for both rufus *Selasphorus rufus* and broad-tailed *S. platycercus* hummingbirds, during the breeding season of the latter.

Hummingbirds at both sites were captured in mist nets or feeder traps, and were then immediately transported to a field laboratory for flight trials. Density-reduction trials were performed on a total of 24 individuals comprising 12 individuals at each site: four male *S. platycercus* Swainson, four male *S. rufus* Gmelin and four female *S. rufus*. Females of *S. platycercus* were not used because the study occurred during their nesting season. Furthermore, long-term studies of hummingbird population dynamics were ongoing at the RMBL, and thus it was not permissible to hold breeding females for sufficient time to complete all flight experiments.

Birds were placed individually in an airtight Perspex

cylinder (0.5 m diameter; 1 m high) within which normodense air at the local ambient pressure was gradually replaced with normoxic heliox ($\rho=0.41 \text{ kg m}^{-3}$ at sea level), thus maintaining constant oxygen partial pressure while gradually lowering air density. Birds rested on a retractable perch that was retracted every few minutes, thus forcing the birds to hover. Trials progressed until birds could no longer sustain hovering flight. At this point, the chamber was flooded with normodense air. Air temperature and humidity were measured directly within the flight chamber; local barometric pressure was obtained from climatic data collected at each site. These data, together with a directly measured change in acoustic frequency of a resonant whistle located within the chamber, enabled calculation of air density following heliox infusion (Dudley, 1995).

Data were analyzed using a repeated-measures analysis of variance (ANOVA) with air density as the repeated independent measure. The species/gender class of the hummingbird and the starting elevation of the experiment were the other independent variables. Dependent variables in two separate analyses were wingbeat frequency and stroke amplitude.

Hypoxia trials

After flight trials in heliox and following a period of feeding, rest and recovery, the same individual hummingbirds served as subjects for flight trials in hypoxia. Protocols and analysis were identical to previously described heliox manipulations except that pure nitrogen was infused into the flight chamber and gradually replaced both the nitrogen and oxygen of unmanipulated air. Because the density of nitrogen ($\rho=1.165 \text{ kg m}^{-3}$ at sea level) is very similar to that of normal air, this manipulation permitted a reduction in oxygen concentration at a near-constant air density. In addition to measurements of humidity and temperature, we also recorded instantaneous oxygen concentration of the mixture within the flight chamber using an Oxygen Monitor (GC Industries GC 501, Poulsbo, WA, USA).

Hyperoxia trials

We studied flight performance in hypodense hyperoxic air in a series of experiments between 1995 and 1997 (Altshuler et al., 2001; Chai et al., 1996). Here we further analyze for comparative purposes the kinematic data from those hyperoxia experiments. Our previous articles contain a complete description of the methods and only a brief description will be given here.

All experiments were conducted with captive ruby-throated hummingbirds *Archilochus colubris* L. Hovering flight was studied in a large plexiglas chamber (90 cm×90 cm×90 cm) in 1995 and 1996, and in a smaller chamber (60 cm×60 cm×60 cm) in 1997. In the primary experiment, normal air was replaced with hyperoxic heliox (35% oxygen/65% helium; $\rho=0.57 \text{ kg m}^{-3}$ at sea level). For comparison, the same birds were also tested in normoxic heliox of similar or equivalent air density (see Altshuler et al., 2001).

Effects of gender, oxygen concentration and air density on wingbeat kinematics were tested using repeated-measures ANOVA, with air density as the repeated independent measure.

Free hovering of Andean hummingbirds

Our comparative studies on hummingbird flight kinematics were carried out between June 1997 and August 2000. We visited 11 field sites in the Departments of Cusco and Madre de Dios in southeastern Peru that spanned elevations from 400 m to 4300 m. Hummingbirds were captured in mist nets and were then brought to a field laboratory for measurements and flight trials, after which they were released. Body mass (m) was determined to within 0.001 g using an Acculab Digital Scale (Model #PP-2060D, Edgewood, NY, USA) or to within 0.1 g using a hanging spring balance (Avinet, Dryden, NY, USA).

Free-flight trials began by releasing a hummingbird into a nylon-mesh flight chamber (0.9 m high×0.45 m×0.45 m) with a Perspex top. Most birds initially tried to fly through the Perspex, but quickly learned that it was impassable. Thereafter, the birds hovered in the chamber for several minutes before perching on the walls of the chamber. We filmed this hovering flight and acquired kinematic variables from the video films using the methods described above. For sexually dimorphic species, kinematic data were pooled by gender and averaged. Species averages were then calculated as the average of values for males and females. In species without easily distinguishable sexes, only one average among individuals was calculated per species.

This study involves the comparative analysis of interspecific data. It is now widely appreciated that such analyses require an explicit phylogenetic framework. Felsenstein (1985) showed that data points representing species values should not be treated as independent observations because of the potential confounding effects of phylogenetic relatedness. We incorporated such phylogenetic influences by using standardized independent contrasts (Felsenstein, 1985) as calculated by the program CAIC (Purvis and Rambaut, 1995). Species data were log-transformed prior to computing contrasts to remove heterogeneity of variance and correlation between node values and independent contrasts (Garland et al., 1992). The phylogeny contains 73 hummingbird taxa and was generated using Bayesian phylogenetic analysis (Huelsenbeck and Ronquist, 2001). Two nuclear genes (AK1, Beta-fibrinogen intron 7) and one mitochondrial gene (ND2) were sequenced and analyzed using a general time-reversible (GTR) plus site-specific gamma model of evolution (J. A. McGuire and D. L. Altshuler, unpublished data). Independent contrasts were calculated by setting all branch lengths to one, which gave equal weight to each contrast and provided the best method of standardization for regression of these data (Garland et al., 1992). Data were analyzed using multiple regressions of independent contrasts with two independent variables: body mass and elevation. All regressions of independent contrasts were constrained to go through the origin (Garland et al., 1992).

Maximum load lifting trials with Andean hummingbirds

In conjunction with free flight trials, Andean hummingbirds were also tested for maximum load-lifting performance. Complete protocols for asymptotic load-lifting in hummingbirds are available elsewhere (Chai et al., 1997; Chai and Millard, 1997), and only a brief account will be given here. A rubber harness connected to a thread with color-coded beads was placed over the head of each hummingbird. Hummingbirds were released on the floor of the nylon-mesh flight chamber (0.9 m high×0.45 m×0.45 m). Because the natural escape response of a hummingbird is to fly directly upwards, we were able to obtain films of vertical ascent. The typical behavior of a hummingbird during load-lifting is to fly as high as possible, and then to hover briefly (~0.5 s) before descending laterally towards the chamber wall. In addition to a video camera positioned above the flight chamber that filmed wingbeat kinematics, a second, synchronized camera (Video 8XR CCD-TRV16; Sony) filmed the floor of the chamber to determine simultaneously the colors of the remaining beads, and thus by subtraction, the total weight lifted by the bird. After hummingbirds had made multiple ascending flights and started to tire, the weight chain was removed and the trial was ended.

From video films, the maximum weight lifted by each bird was determined and three flight sequences exhibiting maximum lifting performance were analysed. Thus, kinematic data were averaged over three bouts of maximum lifting for 1–2 s of total analysed flying time. Interspecies comparisons were made using phylogenetic controls, as described above.

Results*Decreased air density trials*

Morphological and kinematics parameters of the *Selasphorus* hummingbirds at the two study sites are given in Table 1. Wingbeat frequency increased as air density decreased ($F_{2,34}=3.793$, $P<0.05$), although the differences

were slight (Fig. 1A). The different classes of hummingbirds differed in wingbeat frequency ($F_{2,17}=119.395$, $P<0.0001$). Short-winged *S. rufus* males exhibited higher wingbeat frequencies than did *S. rufus* females with intermediate wing lengths, which in turn had higher frequency than long-winged *S. platycercus* males.

As air density was decreased, *Selasphorus* hummingbirds also increased stroke amplitude (Fig. 1B; $F_{2,34}=51.630$, $P<0.0001$) up to approximately 180°, at which point the birds were unable to hover and exhibited aerodynamic failure (*sensu* Chai and Dudley, 1995). The average stroke amplitudes at failure for *S. platycercus* males, *S. rufus* males and *S. rufus* females were 182°, 182° and 177°, respectively. Values of stroke amplitude greater than 180° can be reached because the wings are separated by the small distance of the body width. The hummingbird species/gender classes differed significantly in their stroke amplitudes across air densities ($F_{2,17}=11.677$, $P<0.001$), and a significant interaction with air density was also detected ($F_{4,34}=3.347$, $P<0.05$). As air density was decreased, males of *S. rufus* increased their stroke amplitude less than did males of *S. platycercus*, because the stroke amplitude of *S. rufus* males was higher than that of other hummingbirds at both elevations in the study.

Hypoxia trials

As the partial pressure of oxygen was decreased, hummingbirds decreased wingbeat frequency ($F_{2,26}=10.853$, $P<0.001$), although the overall differences were slight (Fig. 2A). Hummingbirds ceased hovering when ambient air reached a minimum oxygen concentration (aerobic failure), and then gradually descended to the bottom of the chamber. The species/gender classes also differed in wingbeat frequency ($F_{2,13}=295.164$, $P<0.0001$), showing the same patterns with wing size as for flight in low-density air. The average decrease in wingbeat frequency within a site and across partial pressures of oxygen from the start of the experiment to the point of aerobic failure for *S. platycercus* males, *S. rufus* males and *S.*

Table 1. Mean morphological and kinematic values for *Selasphorus* hummingbirds in the Colorado Rocky Mountains

Gender	Elevation	<i>m</i> (g)	<i>R</i> (mm)	<i>n</i> (Hz)	Φ (degrees)	WDL (g m ⁻²)
<i>S. platycercus</i>						
Females	1875	3.375	56.398	34.078	146.431	0.042
	2900	3.592	57.790	37.669	151.742	0.041
Males	1875	3.332	54.816	37.181	149.348	0.043
	2900	3.360	54.355	37.890	151.999	0.043
<i>S. rufus</i>						
Females	1875	3.331	45.238	44.747	144.998	0.065
	2900	3.800	47.711	47.623	161.085	0.060
Males	1875	3.404	42.754	54.066	163.893	0.066
	2900	3.601	42.496	55.563	167.573	0.071

Stroke amplitude (Φ) and wingbeat frequency (*n*) were measured during free flight at each site.

Body mass (*m*), wing length (*R*), and Φ were used to calculate the wing disc loading, WDL.

Free-flight wingbeat kinematics were attained for *S. platycercus* females although these birds were not included in flight trials.

rufus females was 1.1 Hz, 2.0 Hz and 2.4 Hz, respectively. In addition to the comparisons within sites, wingbeat frequencies also differed between sites. Overall, wingbeat frequencies were higher at 2900 m than at 1875 m ($F_{1,13}=18.962$, $P<0.001$), even though less oxygen was available at the high elevation site. Combining the results of the air density and the hypoxia experiments indicates that these differences in wingbeat frequency between elevations were caused by differences in air density rather than oxygen partial pressure. Furthermore, *S. platycercus* males did not significantly increase wingbeat frequency at high elevations, resulting in a significant species/gender \times elevation effect for wingbeat frequency ($F_{2,13}=8.464$, $P<0.005$). These hummingbirds also have the longest wings of the hummingbirds sampled, underscoring the fact that lower density required higher wingbeat frequency, with longer-winged *S. platycercus* males being less sensitive to such changes.

Unlike wingbeat frequency, stroke amplitude exhibited no specific relationship with oxygen concentration ($P>0.25$), although values were quite erratic (Fig. 2B). However, the species/gender classes differed in stroke amplitude ($F_{2,13}=9.874$, $P<0.005$): *S. rufus* males had higher stroke amplitudes than did *S. rufus* females, which in turn exhibited

stroke amplitudes greater than those of *S. platycercus* males. This pattern again conforms to differences in wing length. At higher elevations, all hummingbirds exhibited higher stroke amplitudes than those hovering at low elevation ($F_{1,13}=45.564$, $P<0.0001$), a pattern clearly associated with differences in air density between the two sites.

Hyperoxia trials

Kinematics of hovering performance in hyperoxia were compared to those in normoxia at equivalent air densities (Fig. 3). Supplemental oxygen had no effect on either wingbeat frequency or stroke amplitude (all trials characterized $P>0.80$), however, indicating that oxygen partial pressure *per se* was not limiting to hummingbirds, even when flying at very low air densities near aerodynamic failure.

These hyperoxia experiments also allowed for further comparison between sexes and across air densities. With respect to intersexual differences, *Archilochus colubris* males have much shorter wings and also much higher wingbeat frequencies than do females ($F_{1,14}=182.295$, $P<0.0001$). As air density decreased in hyperoxia, all *A. colubris* increased both wingbeat frequency ($F_{4,56}=21.61$, $P<0.0001$) and wing stroke amplitude ($F_{4,56}=56.208$, $P<0.0001$).

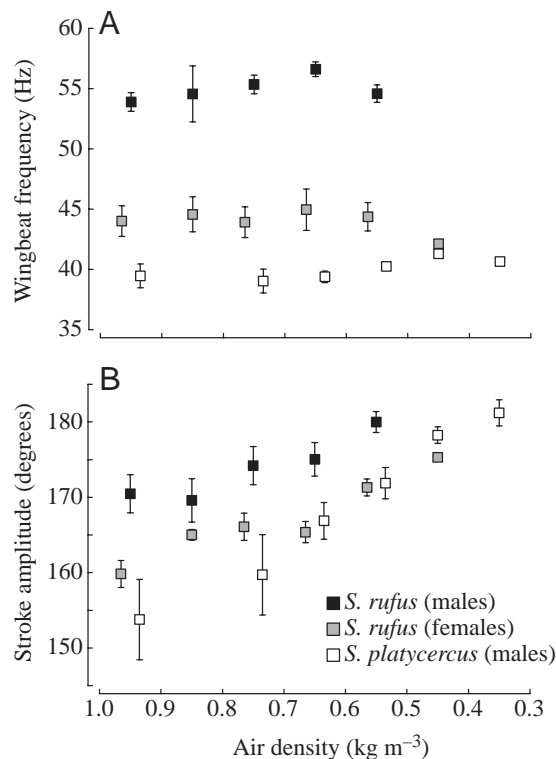


Fig. 1. Hovering kinematics in hypodense air. As ambient air was replaced with normoxic heliox, air density decreased but the partial pressure of oxygen remained constant. The data depicted here were from the experiments performed at 1875 m, although the same trends were evident at 2900 m. (A) Wingbeat frequency increased slightly with decreasing air density. (B) Stroke amplitude increased substantially with decreasing density to a limit near 180°. Values are means \pm S.E.M.

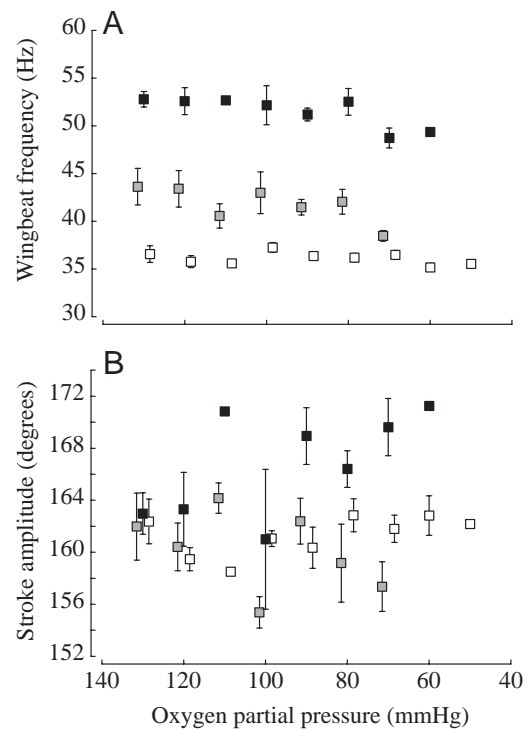


Fig. 2. Hovering kinematics in hypoxic air. As ambient air was replaced with pure nitrogen, the partial pressure of oxygen declined but the air density varied only slightly. Otherwise, conditions were identical to that of the first experiment described in Fig. 1. (A) The wingbeat frequency decreased slightly but significantly as oxygen partial pressure decreased (see text). (B) Stroke amplitude varied considerably but exhibited no clear pattern with changing partial pressure of oxygen. Values are means \pm S.E.M. All symbols as in Fig. 1. 1 mmHg=133.3 Pa.

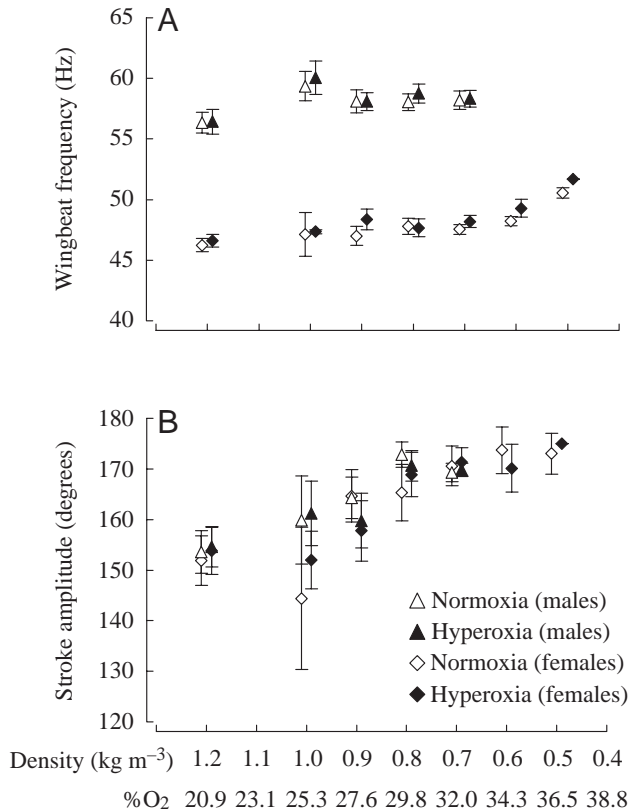


Fig. 3. Kinematics in hyperoxic air. As ambient air was replaced with hyperoxic heliox, air density decreased and oxygen concentration increased simultaneously. During normoxia trials, ambient air was replaced with normoxic heliox so that air density decreased but oxygen concentration remained at 21%. Hummingbird kinematics in hyperoxia were equivalent to those in normoxia. Values are means \pm S.E.M. See text for details. %O₂ is the oxygen concentration at each density under hyperoxia.

Free hovering of Andean hummingbirds

Video recordings of free hovering flight were made for 347 individual hummingbirds from 43 species. Of these species, 38 were represented in the present phylogeny. Thus, the raw species analyses (Fig. 4) consisted of 43 data points whereas the phylogenetically controlled analyses (Fig. 5C,D) are based on 37 (i.e. $N-1$) contrasts.

In a multiple regression, wingbeat frequency was uncorrelated with elevation ($P > 0.10$ in all cases), but was inversely related to body mass considering both raw interspecies data (Fig. 4A; $y = 39.3 - 1.6x$, $P < 0.0001$) and phylogenetically controlled contrasts (Fig. 5C; $y = -0.466x$, $P < 0.0001$).

Wing stroke amplitude increased systematically with elevation (Fig. 4B), mirroring the results of the density-reduction experiments using heliox. This trend was not significant for the raw interspecies data ($y = 152.773 + 0.002x$, $P = 0.067$), but was significant for phylogenetically controlled contrasts (Fig. 5D; $y = 0.036x$, $P < 0.05$). Wing stroke amplitude showed no systematic relationship with body mass ($P > 0.20$ in both analyses).

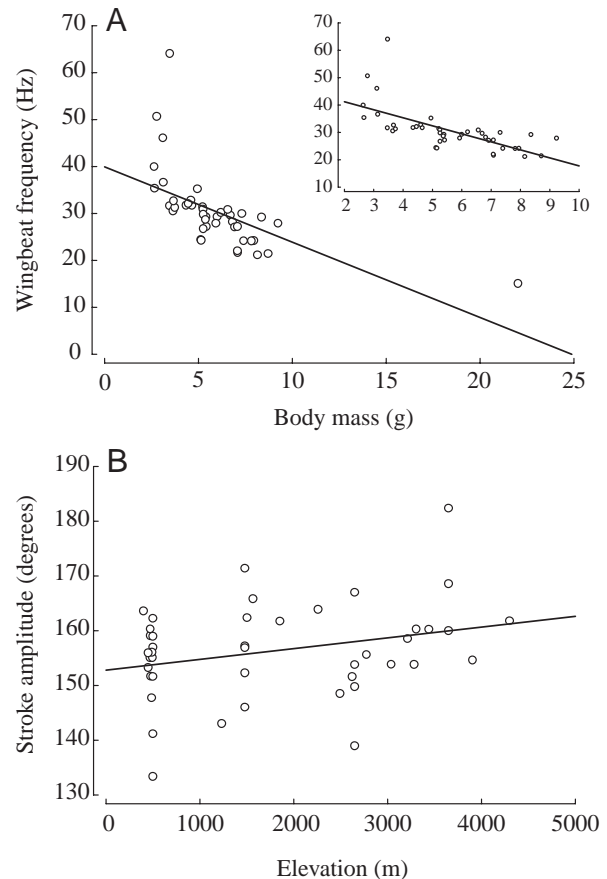


Fig. 4. Wingbeat kinematics during hovering across a natural elevational gradient in the Peruvian Andes. Data are species mean for 43 species of hummingbirds. (A) Wingbeat frequency decreased with increasing body mass. The largest hummingbird is the giant hummingbird *Patagona gigas*, which is substantially larger than all other trochilid taxa and is considered an outlier. However, the decrease in wingbeat frequency with body mass is found even if *P. gigas* is removed from the analysis (inset; all $P < 0.001$; see text). (B) Stroke amplitude increased with increasing elevation, mirroring the results of experiment 1 (Fig. 1). See text for regression equations.

Maximum load lifting trials with Andean hummingbirds

Results of load-lifting experiments, along with the kinematic parameters for free hovering flight, are plotted in Fig. 5 as functions of both body mass and elevation. Both raw species data and phylogenetically controlled independent contrasts are included.

As in free hovering, wingbeat frequency was unrelated to elevation during maximum load-lifting (all $P > 0.15$), but was negatively correlated with body mass for both raw interspecific data and for the independent contrasts (Fig. 5C; $y = -0.43x$, $P < 0.0001$). Wingbeat frequency during load-lifting was considerably higher than during free flight; hummingbirds increased wingbeat frequencies among species and across elevations by 19%, on average, relative to free hovering flight (Fig. 5A). Thus, hummingbirds possess the ability to modulate frequency upwards over very short time spans, but do not use

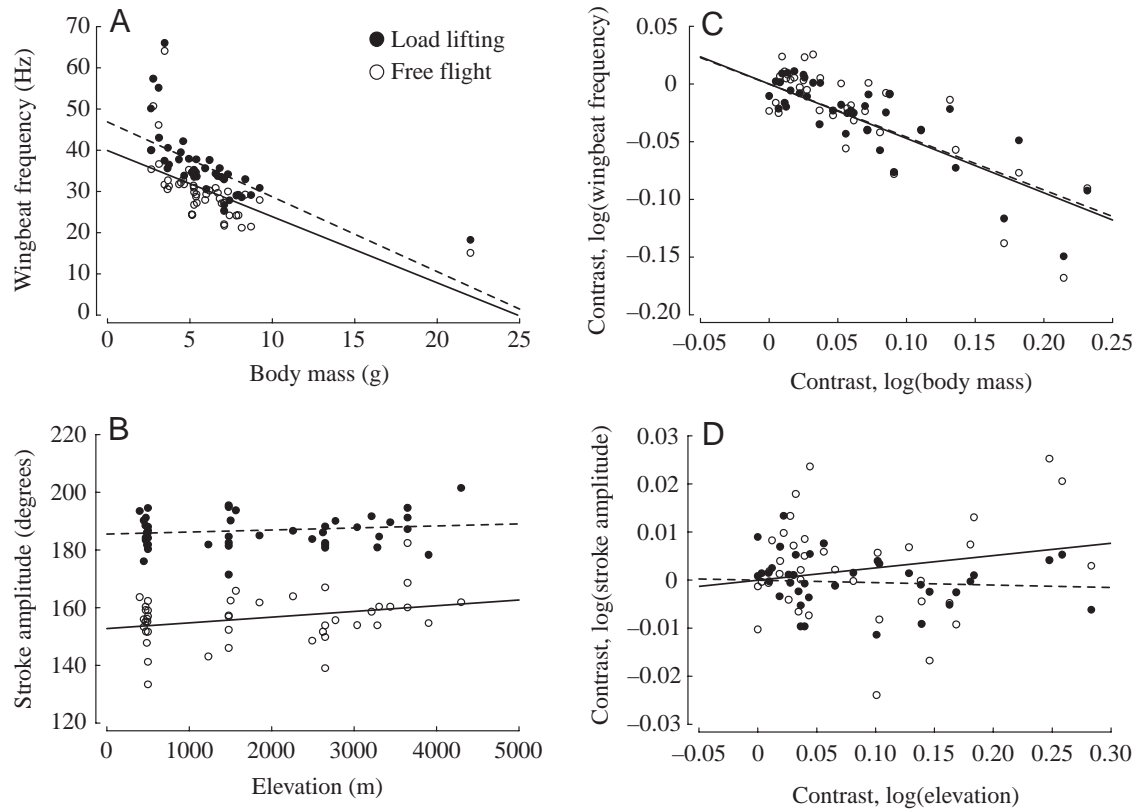


Fig. 5. Wingbeat kinematics during free hovering and load-lifting in Peruvian hummingbirds. (A,B) Raw species data, (C,D) phylogenetically corrected independent contrast data. Solid lines, free flight; broken lines, load-lifting. Wingbeat frequency decreased with increasing body mass during both load-lifting and free hovering flight. Stroke amplitude increased with increasing elevation during free flight, but not with elevation during load-lifting, because all hummingbirds reached a maximum stroke amplitude of approximately 180° at the point of maximum lifting. See text for regression equations.

this ability during sustained hovering, as was required during the density-reduction experiments.

Stroke amplitude increased with elevation during free flight, but was independent of elevation during load lifting for both raw species data and phylogenetically controlled contrasts (Fig. 5B,D; $P > 0.15$ in both cases). Furthermore, stroke amplitude during maximal load lifting reached a geometric limit between 176° and 201° . Thus hummingbirds were unable to increase stroke amplitude with increasing elevation because all hummingbirds used the maximum stroke amplitude near 180° during maximum loading (Fig. 5B). On average, hummingbirds increased stroke amplitude by 19% in loading relative to free flight. As for free flight, loaded stroke amplitude was independent of body mass ($P > 0.2$ in both cases).

Discussion

Kinematic mechanisms employed to augment lift production vary with both oxygen availability and air density. During sustained flight in hypodense air, hummingbirds increase lift primarily *via* modulation of wing stroke amplitude, with relatively constant wingbeat frequency. When generating transiently high vertical forces, however (probably *via* anaerobic pathways; Chai et al., 1997), hummingbirds also

significantly increase wingbeat frequency. Sustained increases in wingbeat frequency would require additional and probably limiting oxygen delivery to the flight muscles, whereas stroke amplitude can be increased substantially under normoxic and even hypoxic hypobaric conditions. These patterns were demonstrated both in laboratory experiments and in the comparative field study of Andean hummingbirds.

Hummingbirds, like other birds, are clearly resistant to low oxygen partial pressures. In hypoxia, hummingbirds exhibited only slight decreases in wingbeat frequency and were able to fly at oxygen partial pressures equivalent to the hypobaric hypoxia of 6000 m or more. Diverse morphological and physiological adaptations of hummingbirds in particular, and of many birds in general, enhance oxygen delivery under such conditions (Altshuler et al., 2001).

During anaerobic burst performance, hummingbirds increased wingbeat frequency by 19% on average, which is the same percentage increase as exhibited in modulation of stroke amplitude. Thus, the kinematic potential to increase lift *via* modulation of wingbeat frequency is substantial but, in conjunction with concurrent increases in stroke amplitude and metabolic demand, probably reaches a constraint on rates of oxygen delivery at some stage of the cardiovascular or respiratory system. Because hummingbirds did not increase

their wingbeat frequency when supplied with supplemental oxygen during a hypodense challenge (Chai et al., 1996), diffusion limitations within the pathway for oxygen are unlikely to pertain. Many birds exhibit multiple exchanges of air in the lung per inspiration (Dubach, 1981), although no pulmonary adaptations specific to hummingbirds have been identified. Convective limitations either in the lungs or the cardiovascular system (Bishop, 1999) probably limit oxygen delivery during the maximum hovering performance of hummingbirds, but the precise nature of such limits remains an open question.

Although air density and oxygen concentrations were monitored during the gas infusion trials, we did not measure changes in the partial pressure of CO₂. Disruption of normal CO₂ levels can alter chemoreceptor reflexes in birds, but these are typically detected at concentrations much higher (2–5%) than ambient (0.03%) (e.g. Butler and Stephenson, 1988). If anything, CO₂ concentration decreased during gas infusion trials to potentially half of the ambient concentration. Thus, we cannot exclude the possibility that hypocapnia adversely affected hummingbird performance.

Although stroke amplitude is a major mechanism for increasing lift production during sustained hovering, different hummingbird taxa are not equal in their ability to modulate it. For *Selasphorus* hummingbirds in Colorado, the birds with the higher wing disc loading (*S. rufus* males) exhibit higher stroke amplitude during free hovering. Thus, they are unable to increase stroke amplitude as much as hummingbirds with lower wing disc loadings and stroke amplitudes, and consequently exhibit aerodynamic failure at higher air densities. Another intra-site comparison is available for a set of three hummingbird species from southeast Arizona at an elevation of 1676 m (Chai and Millard, 1997). Here, the two heavier species had much higher stroke amplitudes than did the lighter species. Finally, Andean hummingbird species at higher elevations also exhibited a decreased ability to increase stroke amplitude. In each case, the ceiling is set by the geometric limit near 180°, beyond which angle the contralateral wings interfere with one another, either aerodynamically or physically (Chai and Dudley, 1995).

Wingbeat frequency is highly correlated with body mass as shown for the Peruvian hummingbirds, but this is likely to be an indirect correlation arising from associations with wing inertia. Body mass of flying animals is itself generally correlated with wing length, which is a strong predictor of wingbeat frequency (Greenewalt, 1962, 1975). For the Colorado *Selasphorus* hummingbirds, each of the species/gender classes is of similar body mass, but the wingbeat frequencies segregate according to differences in wing length (Figs 1, 2; Table 1). The body sizes of North American hummingbirds, however, are quite similar relative to the wide range of size variation present in tropical hummingbird communities. For example, one of the smallest vertebrate endotherms is the Cuban bee hummingbird *Mellisuga hellenae*, with individuals as small as 1.8 g. The largest hummingbird is the giant hummingbird *Patagona gigas*, which reaches a body

mass of 24 g. The hummingbird assemblage from southeast Peru studied here includes most of this mass range; the smallest individual studied was a 2.35 g reddish hermit *Phaethornis ruber* and the largest the giant hummingbird. After incorporating phylogenetic relatedness of species, it was determined that wingbeat frequency scaled as body mass^{-0.466} during free hovering, and as body mass^{-0.429} during maximal load-lifting for this assemblage. Although these values cannot be compared with phylogenetically controlled estimates for insects, it is likely that this decline in wingbeat frequency with increasing body mass is still much steeper in hummingbirds, for reasons as yet unclear but likely to relate to the positive allometry of hummingbird wing area (Dudley, 2000).

It is now clear that hummingbirds are capable of considerable modulation of wingbeat frequency and stroke amplitude, although the magnitude of such responses can vary according to morphological features and elevational occurrence. Nonetheless, little is known about modulation of detailed wingbeat kinematics, including such features as angle of attack, torsion along the wing, wing rotational velocities, and temporal changes in wing area related to positional changes of the feathers (Altshuler and Dudley, 2002). Although future studies of hummingbird adaptation to the aerodynamic challenges of hypobaria should consider a wider range of kinematic parameters, it is nonetheless striking that the behavioral responses to low-density air exhibited by individual hummingbirds are mirrored in evolutionary time by hummingbird taxa adapted to flight at varying elevations.

We thank Peng Chai for deep insights into hummingbird flight performance. We also thank Peter Baik, Jeff Chen, Jeremy Goldbogen, Holly Hughes and Andrea Smith for assistance with video analysis. Laboratory and field experiments were supported by grants from the Earthwatch Institute, the Explorers Club, the Graduate Program in Zoology at the University of Texas, the Institute for Latin American Studies at the University of Texas, the National Science Foundation (IBN 9817138, IBN 992155 and DEB 0108555), Sigma Xi, and the Graduate School of the University of Texas at Austin.

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