

Effects of flight speed upon muscle activity in hummingbirds

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SUMMARY

Hummingbirds have the smallest body size and highest wingbeat frequencies of all flying vertebrates, so they represent one endpoint for evaluating the effects of body size on sustained muscle function and flight performance. Other bird species vary neuromuscular recruitment and contractile behavior to accomplish flight over a wide range of speeds, typically exhibiting a U-shaped curve with maxima at the slowest and fastest flight speeds. To test whether the high wingbeat frequencies and aerodynamically active upstroke of hummingbirds lead to different patterns, we flew rufous hummingbirds (*Selasphorus rufus*, 3 g body mass, 42 Hz wingbeat frequency) in a variable-speed wind tunnel (0–10 m s⁻¹). We measured neuromuscular activity in the pectoralis (PECT) and supracoracoideus (SUPRA) muscles using electromyography (EMG, $N=4$ birds), and we measured changes in PECT length using sonomicrometry ($N=1$). Differing markedly from the pattern in other birds, PECT deactivation occurred before the start of downstroke and the SUPRA was deactivated before the start of upstroke. The relative amplitude of EMG signal in the PECT and SUPRA varied according to a U-shaped curve with flight speed; additionally, the onset of SUPRA activity became relatively later in the wingbeat at intermediate flight speeds (4 and 6 m s⁻¹). Variation in the relative amplitude of EMG was comparable with that observed in other birds but the timing of muscle activity was different. These data indicate the high wingbeat frequency of hummingbirds limits the time available for flight muscle relaxation before the next half stroke of a wingbeat. Unlike in a previous study that reported single-twitch EMG signals in the PECT of hovering hummingbirds, across all flight speeds we observed 2.9 ± 0.8 spikes per contraction in the PECT and 3.8 ± 0.8 spikes per contraction in the SUPRA. Muscle strain in the PECT was $10.8 \pm 0.5\%$, the lowest reported for a flying bird, and average strain rate was 7.4 ± 0.2 muscle lengths s⁻¹. Among species of birds, PECT strain scales proportional to body mass to the 0.2 power ($\propto M_b^{0.2}$) using species data and $\propto M_b^{0.3}$ using independent contrasts. This positive scaling is probably a physiological response to an adverse scaling of mass-specific power available for flight.

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Key words: Rufous hummingbird, *Selasphorus rufus*, electromyography, sonomicrometry, flight.

INTRODUCTION

The function of muscle during animal flight is of particular interest because flying requires higher power output than other forms of locomotion (Schmidt-Nielsen, 1972). Efforts to measure mechanical work and power produced by the primary downstroke muscle in birds, the pectoralis (hereafter PECT), have involved surgical implantation of sonomicrometry and strain-gauge transducers (Biewener et al., 1998; Hedrick et al., 2003; Tobalske et al., 2003b; Soman et al., 2005; Tobalske and Biewener, 2008) or sonomicrometry coupled with ergometry (Askew and Ellerby, 2007). From these studies, it is clear that mechanical power output varies with flight speed according to a U-shaped curve (Tobalske et al., 2003; Askew and Ellerby, 2007). To modulate work and power across flight speeds, birds may vary total muscle strain (proportional length change), contractile velocity, and the timing and magnitude of motor unit recruitment (Hedrick et al., 2003; Tobalske et al., 2005; Askew and Ellerby, 2007; Tobalske and Biewener, 2008). In cockatiel (*Nymphicus hollandicus*), work and power in the PECT is modulated primarily using motor-unit recruitment (Hedrick et al., 2003).

Due to their small size, hummingbirds (Trochilidae) are generally hypothesized to represent an extreme among extant birds for their adaptations for powered flight (Altshuler and Dudley, 2002). They are the smallest flying vertebrates, and they are the only birds capable of sustained hovering. Their hovering ability is due, at least in part, to their use of an aerodynamically active, lifting upstroke (Stolpe and Zimmer, 1939; Warrick et al., 2005; Tobalske et al., 2007; Warrick et al., 2009). Consistent with this, their primary upstroke muscle, the supracoracoideus (hereafter, SUPRA) is proportionally larger than it is in other bird species (Stolpe and Zimmer, 1939; Greenewalt, 1962).

The available evidence for neuromuscular function during hummingbird flight, revealed using electromyography (EMG), suggests that hovering hummingbirds exhibit a unique twitch contraction in their PECT (Hagiwara et al., 1968), consisting of a single, alternating current EMG spike per wingbeat. By contrast, other bird species exhibit EMGs in their flight muscles that consist of multiple spikes per contraction (Hagiwara et al., 1968; Dial, 1992a; Tobalske and Dial, 1994; Tobalske, 1995; Tobalske et al., 1997; Tobalske and Dial, 2000; Tobalske et al., 2005; Hedrick et

al., 2003; Ellerby and Askew, 2007). To accomplish fast forward flight, Hagiwara et al. (Hagiwara et al., 1968) report that hummingbirds increase PECT recruitment: the amplitude of EMG increases and the number of spikes per burst increases to 2–5. However, flight velocity was not measured, so it is not possible to interpret this observation in relation to existing models of flight costs (Rayner, 1979) or to empirical measures of metabolic power input (Berger, 1985; Clark and Dudley, 2009).

It is possible that the small number of spikes per burst is due to the limited time for muscle activation and deactivation during high-frequency wingbeats. Hagiwara et al. report 8 ms time to peak force during isometric contraction in hummingbirds with wingbeat frequencies near 40 Hz (Hagiwara et al., 1968). Assuming downstroke lasts 50% of a wingbeat (Tobalske et al., 2007), these birds would have only 12.5 ms to develop tension and relax their primary flight muscles unless some tension is present in each muscle at wing turnaround (Tobalske and Biewener, 2008).

Another unique aspect of hummingbird flight is that they do not regularly engage in intermittent flight. Other small birds routinely use intermittent bounds, glides or both (Tobalske, 2001; Tobalske et al., 2007). It has been hypothesized that intermittent pauses are a mechanism for modulating power output in lieu of varying contractile velocity in the flight muscles (Rayner, 1985). Although sonomicrometry has revealed that contractile velocity varies significantly in the PECT of flap-bounding birds (Tobalske et al., 2005), the magnitude of this variation has not been compared with an alternative model such as the hummingbird that does not use (or only rarely uses) intermittent flight.

Given the small size and unusual flight morphology and style of the hummingbird, we undertook the present study to test whether patterns of neuromuscular recruitment and contractile behavior in hummingbirds were different from those observed in larger bird species.

MATERIALS AND METHODS

Birds and experimental design

We measured muscle activity during flight in five female rufous hummingbirds, *Selasphorus rufus*, Gmelin 1788 (body mass 3.4 g, wingspan 10 cm). We previously reported on the three-dimensional wing and body kinematics along with additional morphological data for the same study subjects (Tobalske et al., 2007). We caught the animals in the wild under permits from the US Fish and Wildlife Service and the Oregon Department of Fish and Wildlife. All housing and experimental protocols were approved by the University of Portland Institutional Animal Care and Use Committee. During captivity, birds were housed in 1 m × 1 m × 1 m flight cages with *ad libitum* access to food and water in the form of Nektar-Plus (NEKTON®; Günter Enderle, Pforzheim, Baden-Württemberg, Germany) or a 20% sucrose solution (mass:volume).

We flew the birds in an open-circuit, variable-speed wind tunnel – the properties of which have been described previously (Tobalske et al., 2005). The working section of the tunnel is 85 cm in length, square in cross section, 60 cm × 60 cm at the inlet and increases to 61.5 cm × 61.5 cm at the outlet to accommodate boundary-layer thickening. Maximum deviations in velocity within a cross-section are <10% of the mean, the boundary layer is <1 cm thick and turbulence is 1.2%. Wind speed during experiments and for reporting in this paper is equivalent air velocity rather than true air velocity (Pennycuick et al., 1997).

Birds were fully acclimated to the flight chamber because we completed unimplanted three-dimensional kinematic studies (Tobalske et al., 2007) prior to beginning the EMG and

sonomicrometry experiments that are described herein. We allowed the birds to rest on a perch between trials, and the birds were allowed to feed periodically from a 1 ml syringe containing 20% sucrose solution. Although unimplanted rufous hummingbirds can easily sustain flight in the tunnel at 12 m s⁻¹ (Tobalske et al., 2007), the birds exhibited diminished performance following implantation of electrodes and transducers (see below) so that the maximum speed was 10 m s⁻¹ during EMG experiments (achieved in 3 out of 4 birds) and 8 m s⁻¹ during sonomicrometry experiments.

To measure the timing of wing motion in relation to muscle activity, and to limit our samples to phases of flight during which velocity and altitude were held approximately constant, we used two synchronized Redlake cameras, a PCI-2000 and PCI-500 (Redlake MASD LLC, San Diego, CA, USA) sampling at 500 frames s⁻¹ and with a shutter speed of 1/2500 s.

Flights were illuminated from outside the flight chamber using four 650 W halogen lights (Lowel Tota-light, Lowel-Light Manufacturing, Inc., Brooklyn, NY, USA).

Digitizing and three-dimensional reconstruction were accomplished as in Tobalske et al. (Tobalske et al., 2007) using custom M-files (Hedrick, 2008) (available: <http://www.unc.edu/~thedrick/>) in MATLAB v.7.6.5 (The Mathworks, Inc., Natick, MA, USA). Subsequent analyses of wingtip and wrist trajectories and chord angle (deg) relative to the frontal plane of the body were accomplished using MATLAB and IGOR Pro. (v.6.12a, Wavemetrics, Inc., Beaverton, OR, USA). Downstroke started with wrist depression and upstroke began with wrist elevation. Wing chord was a line connecting the wrist and the distal tip of the first secondary, and chord angle was the cranially oriented acute angle formed between this lead line and the mid-frontal plane of the bird.

Electromyography and sonomicrometry

We measured electromyographic activity from the PECT and SUPRA muscles in four birds. In a different bird, we measured PECT strain (length/resting length; L/L_{rest}) using sonomicrometry (Tobalske and Biewener, 2008) and EMG activity. The tiny size of the birds precluded the use of sonomicrometry with multiple EMGs.

To accomplish implantation, birds were anesthetized using isoflurane inhalant and maintained at a surgical plane. Feathers were removed from the skin over the left PECT.

An alcohol solution was used to clean the skin. EMG electrodes (Cooner Wire, Inc., Chatsworth, CA, USA; twisted pair, bipolar 0.5 mm exposed tips, 100 μm silver wire) and sonomicrometry transducers (Sonometrics, London, Ontario, Canada; omnidirectional, 0.7 mm, 42 gauge insulated copper wire) were implanted through the skin and into the muscles. The EMG electrodes were implanted percutaneously using a 25 gauge hypodermic needle to push the EMG electrodes into the sternobrachialis (SB) portion of the PECT and through the SB portion of the PECT into the mid-belly of the SUPRA. For sonomicrometry, two holes, 6 mm apart, were made through the overlying skin into the muscle, parallel to the longest muscle fascicles of the SB of the PECT (Tobalske et al., 2005; Welch and Altshuler, 2009). The sonomicrometry crystals were placed ~1.5 mm deep into the holes. We sutured all wires near the point of exit from the PECT to superficial fascia of the muscle and to the skin using using 6-0 braided silk.

Electrode and transducer leads were woven together and extended 60 cm from the animal before being connected to insulated recording cables using a miniature connector. The total mass of all EMG and transducer leads carried by the birds during experiments was ~0.2 g (6% of body mass).

The animal was allowed to recover from surgery, with experiments beginning within 1.5 h and lasting approximately 30 min. Following the experiments, a recovery surgery was performed in which all recording equipment was removed from the bird. All of the animals fully recovered from the experiments.

Electromyographic signals for the combined PECT and SUPRA measurements were amplified (1000 \times) and filtered (60 Hz notch, 100 Hz low pass, 5000 Hz high pass) using an AM Systems (Carlsborg, WA, USA) Model 1700 differential AC amplifier. These analog signals were imported to computer using a Digidata 1322 16-bit A/D converter sampling at 5000 Hz and Axoscope v.10 software (Axon Instruments, Union City, CA, USA). Video was synchronized using a trigger pulse from the cameras sent to the Digidata converter. Sonomicrometry signals were created and recorded at 1050 Hz using a Sonometrics TRX Series 4 Digital Ultrasonic Measurement System and Sonosoft 3.2.1 software (London, Ontario, Canada). Synchronization between the sonomicrometry, video and EMG data was obtained by sending a trigger pulse from the video cameras and voltage output from the AM Systems amplifier to a separate channels on the Sonometrics A/D converter.

Subsequent analysis of the recorded signals was accomplished using IGOR software. EMG signals remained as recorded in volts, and sonomicrometry data were calibrated to strain (L/L_{rest}) using the resting, perched values for crystal separation as L_{rest} . EMG bursts were identified as one or more spikes with rectified amplitude at least twice the amplitude of baseline electrical noise (Tobalske et al., 2005; Tobalske and Biewener, 2008). For each contractile cycle (=wingbeat cycle), we measured the amplitude (mV) and duration (ms) of EMG activity in the PECT and SUPRA from onset to offset. Average EMG amplitude was obtained by dividing the EMG by duration of activity. We counted the number of spikes within each bout of muscle activity as individual peaks of raw EMG signal that exceeded baseline. We calculated the percentage of the wingbeat cycle in which each muscle was active, with the start of downstroke defined using the onset of wrist depression as in Tobalske et al. (Tobalske et al., 2007). Lastly, we computed relative EMG amplitude as in previous studies (Tobalske et al., 2005; Tobalske and Biewener, 2008): average amplitude for each burst within a given bird and muscle was divided by the maximum value exhibited for the muscle.

Fractional lengthening (%) and fractional shortening (%) were the proportions of PECT strain in which the PECT was longer than or shorter than resting length, respectively. Average strain rate (muscle lengths s^{-1}) was PECT strain divided by the duration of time between maximum and minimum length as the muscle shortened during a contractile cycle.

We analyzed 287 wingbeats for EMG data from the combined measurements of PECT and SUPRA activity and 82 wingbeats from the combined measurements of PECT fascicle strain and EMG activity. This represented at least 10 wingbeats from each speed for each bird used in an experiment.

Statistical and comparative analysis

For each variable, amplitude of EMG, duration of EMG, relative offset of PECT, relative onset and offset of SUPRA and spike number per EMG burst, we computed the mean value within each bird for each flight speed. We then tested for a significant effect of speed upon each variable using a univariate repeated-measures analysis of variance (StatView version 5.0.1, SAS Institute, Inc., Cary, NC, USA). Values throughout are presented as means \pm s.d.

To place the effects of velocity upon relative amplitude of EMG in the rufous hummingbird within a comparative context, we used

coefficients of variance (c.v.=among-flight speed mean/s.d.). These data were compared with EMG data from a variety of larger bird species that have also been studied over comparable ranges in speed in a wind tunnel [zebra finch *Taeniopygia guttata*, 13 g (Tobalske et al., 2005; Ellerby and Askew, 2007); budgerigar *Melopsittacus undulatus*, 45 g (Ellerby and Askew, 2007); cockatiel *N. hollandicus*, 78.5 g (Hedrick et al., 2003); black-billed magpie *Pica hudsonica*, 82 g (Tobalske et al., 1997)]. For this analysis, we divided relative amplitude of EMG exhibited by a given bird and muscle at each flight speed by the maximum value exhibited for that bird and muscle among flight speeds.

We also added our present data to data from earlier studies to explore the scaling of PECT strain and strain rate among species. We computed means from the species that have been studied using sonomicrometry over a wide range of speeds in a wind tunnel or in different flight modes including take-off, level flight and landing (see Table S1 in supplementary material): zebra finch (Tobalske et al., 2005; Ellerby and Askew, 2007); budgerigar (Ellerby and Askew, 2007); cockatiel (Hedrick et al., 2003); black-billed magpie (Warrick et al., 2001); blue-breasted quail *Coturnix chinensis*, 44 g (Askew et al., 2001); northern bobwhite *Colinus virginianus*, 200 g; chukar *Alectoris chukar*, 492 g; ring-necked pheasant *Phasianus colchicus*, 943 g; and wild turkey *Meleagris gallopavo*, 5.3 kg (Tobalske and Dial, 2000); rock dove *Columba livia*, 605 g (Biewener et al., 1998; Soman et al., 2005; Tobalske and Biewener, 2008); and mallard *Anas platyrhynchos*, 1 kg (Williamson et al., 2001). If a species was studied more than once, we used means among studies. The data we selected were always from the anterior sternobranchialis portion of the PECT.

We log-transformed these data and tested for a significant effect of body size upon strain and strain rate using reduced-major axis regressions of independent contrasts (Felsenstein, 1985; Garland et al., 1992; Garland et al., 2005) in the PDAP package (Midford et al., 2008) for Mesquite (v.2.5) (Maddison and Maddison, 2008). Contrasts were made positive prior to regression. We developed a phylogenetic tree (see Fig. S1 in supplementary material) using trees based on molecular data in Hackett et al. (Hackett et al., 2008) for among-family relationships and Kimball and Braun (Kimball and Braun, 2008) for relationships within the Phasianidae. We set all branch lengths=1 (i.e. punctuated equilibrium model), and we confirmed that the contrasts were adequately standardized (Garland et al., 2005). We also confirmed that our conclusions regarding scaling exponents and statistical significance were robust when changes in the phylogeny were introduced using alternative trees based on molecular (Ericson et al., 2006) or anatomical (Livezey and Zusi, 2007) evidence. To aid in visual interpretation of the scaling patterns, we include graphs of species (tip) data along with graphs of independent contrasts.

RESULTS

The PECT and SUPRA each exhibited a multi-spike burst of EMG activity per wingbeat (Figs 1 and 2). There were significantly more spikes in the SUPRA signal (3.8 ± 0.2 , range 1–8) compared with the PECT (2.9 ± 0.2 bursts, range 1–6; paired *t*-test, $P=0.02$, d.f.=3).

Although SUPRA EMG duration was slightly longer (5.4 ± 0.6 ms) than PECT EMG duration (4.9 ± 0.7 ms) the difference was not significant ($P=0.11$). These durations represented $21 \pm 2\%$ and $23 \pm 2\%$ of the wingbeat cycle, respectively (Fig. 3A). Flight speed did not have a significant effect upon spike number per EMG (repeated-measures ANOVA, $P=0.95$ for PECT and $P=0.7593$ for SUPRA, d.f.=2,5 per test) or duration of EMG ($P=0.31$ for PECT and $P=0.19$ for SUPRA, d.f.=2,5).

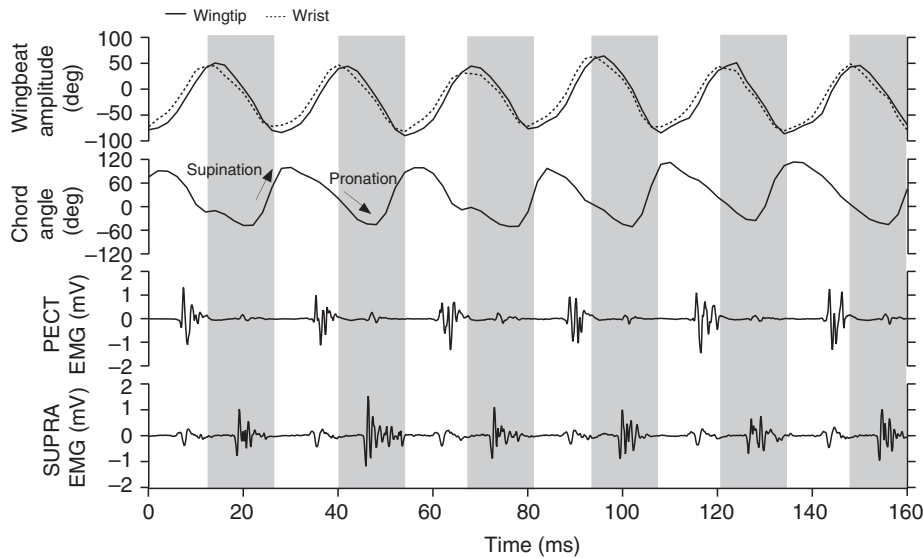


Fig. 1. Wing kinematics (amplitude of wingtip and wrist, chord angle at mid-wing) and electromyographic (EMG) activity in the pectoralis (PECT) and supracoracoideus (SUPRA) of a rufous hummingbird (*Selasphorus rufus*) during hovering flight in a wind tunnel. The shaded areas represent downstrokes defined using motion of the wrist. Arrows indicate supination and pronation as measured at mid-wing.

In the bird for which we measured PECT length using sonomicrometry (Fig. 2), average strain was $10.8 \pm 0.5\%$. Fractional lengthening (relative to perched, resting length) was $4.4 \pm 1.6\%$ and fractional shortening was $-6.4 \pm 1.8\%$ (Fig. 4). Average strain rate during PECT shortening was 7.4 ± 0.2 muscle lengths s^{-1} (see Fig. S2 in supplementary material). Although total strain and strain rate did not exhibit consistent trends with flight speed, there was a tendency for fractional lengthening to decrease, and fractional shortening to increase, as speed increased (Fig. 4). For example, fractional lengthening was $6.9 \pm 1.2\%$ during hovering and 2.6 ± 0.7 during flight at $6 m s^{-1}$.

The onset and offset of EMG activity in the PECT occurred during kinematic upstroke, prior to depression of the wrist relative to the frontal plane of the body, and EMG activity in the SUPRA occurred during kinematic downstroke (Figs 1–3). These patterns were different from those in other birds in which PECT activation extends into kinematic downstroke and SUPRA activation extends into kinematic upstroke (Fig. 3B) (Tobalske and Biewener, 2008). In the hummingbird, PECT activity was in the

middle of pronation phase of the mid-wing chord relative to the frontal plane of the body, and SUPRA activity was concurrent with a transition from pronation to supination of the wing chord (Figs 1 and 2). Average wingbeat frequency was 42 ± 2 Hz, so wingbeat duration was 24 ± 1 ms. The transition from downstroke to upstroke occurred at $51 \pm 5\%$ of the wingbeat cycle. PECT onset preceded the onset of depression of the wrist by $29 \pm 4\%$ of the cycle and offset of PECT EMG preceded wrist depression by $9 \pm 2\%$. SUPRA onset and offset preceded wrist elevation by $39 \pm 3\%$ and $16 \pm 2\%$ of cycle duration. The only significant effect of flight speed upon these variables was for relative onset of SUPRA activity, which was earliest during flight at 0 and $2 m s^{-1}$ (38% of cycle) and increased with increasing flight speed to be at 43% of cycle during flight at 8 and $10 m s^{-1}$ (repeated-measures ANOVA, $P=0.002$, $d.f.=2,5$).

There was a time lag between wrist movement and tip movement potentially due to inertial or aerodynamic loading of the wing (Fig. 1), and there was also a time lag between the onset of PECT shortening and the onset of wrist depression relative to the frontal

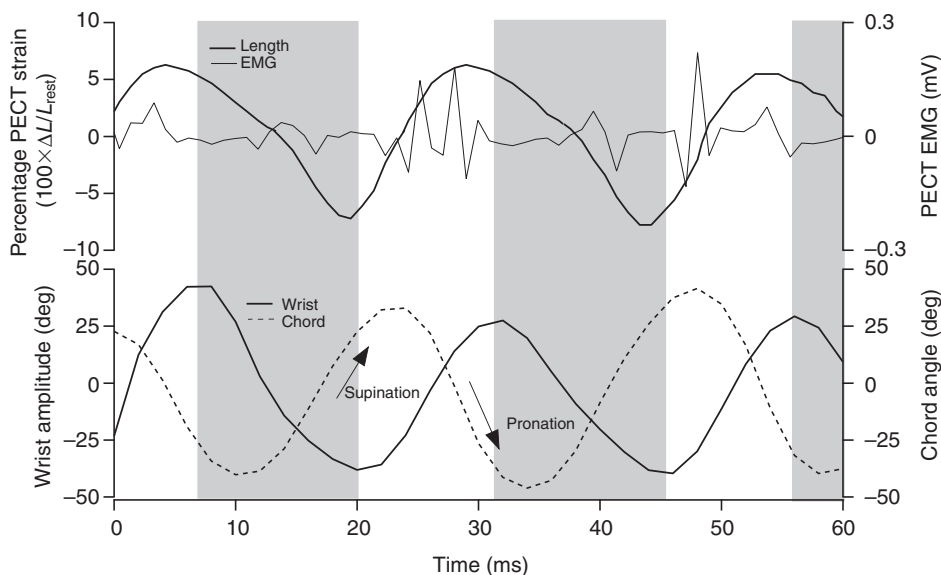


Fig. 2. Contractile activity in the pectoralis (PECT) and wing kinematics (wrist amplitude and chord angle of mid-wing) of a rufous hummingbird (*Selasphorus rufus*) during flight at $6 m s^{-1}$ in a wind tunnel. Muscle length was measured using sonomicrometry. The shaded areas represent downstrokes as indicated by wrist motion. Wrist amplitude and chord angle are with reference to the frontal plane of the body. Arrows indicate the direction of supination and pronation as measured at mid-wing. The electromyography (EMG) recorded with sonomicrometry was limited to a 1050 Hz recording frequency and was not typical of the EMG data from other birds (e.g. Fig. 1).

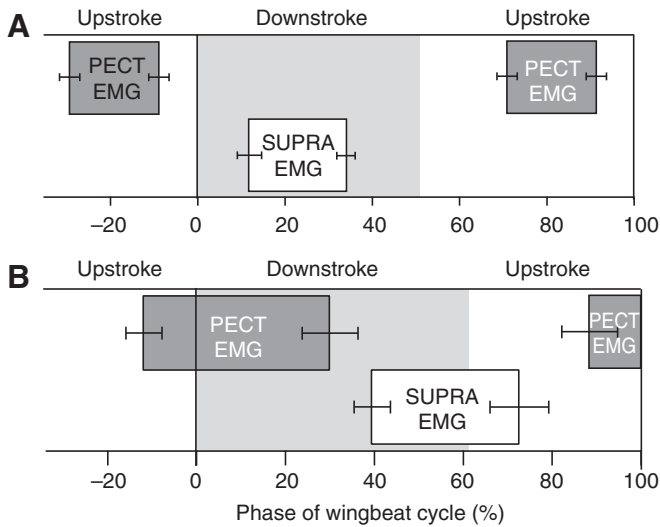


Fig. 3. Relative timing of activation in the pectoralis (PECT) and supracoracoideus (SUPRA) of (A) rufous hummingbirds (*Selasphorus rufus*, $N=4$) and (B) comparable data from rock dove *Columba livia* (from Tobalske and Biewener, 2008). Data are pooled from all flight velocities ($0\text{--}10\text{ m s}^{-1}$) in hummingbirds and among flight modes (ascending, level and descending) in pigeons. Downstroke is defined using wrist motion for the hummingbird and pectoralis length in the pigeon. EMG, electromyography.

plane of the body (Fig. 2). The duration of these lags was near or less than the time resolution of our 500 Hz video (i.e. $\pm 2\text{ ms}$). For example, average lag time between wrist depression and wingtip depression in the wingbeats in Fig. 1 was $2\pm 1\text{ ms}$, and average lag time between PECT shortening and wrist depression for the wingbeats in Fig. 2 was $3.3\pm 0.3\text{ ms}$ (14% of wingbeat cycle).

The relative amplitude of EMG signal from the PECT and SUPRA varied according to a U-shaped curve with flight speed (Fig. 5A). Values for relative amplitude of EMG were strongly correlated between the two muscles ($R=0.99$). The effect of flight speed was statistically significant for the relative amplitude of EMG in the SUPRA ($P=0.014$, d.f.=2,5) and marginally non-significant

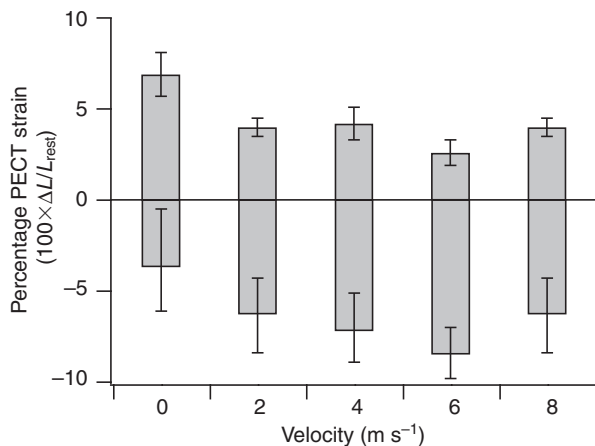


Fig. 4. Muscle strain and fractional length changes in the pectoralis (PECT) according to flight velocity. Resting length is indicated by the origin. Values are means \pm s.d. among wingbeats ($N>10$ per velocity) for one rufous hummingbird (*Selasphorus rufus*). L , length; L_{rest} , resting length.

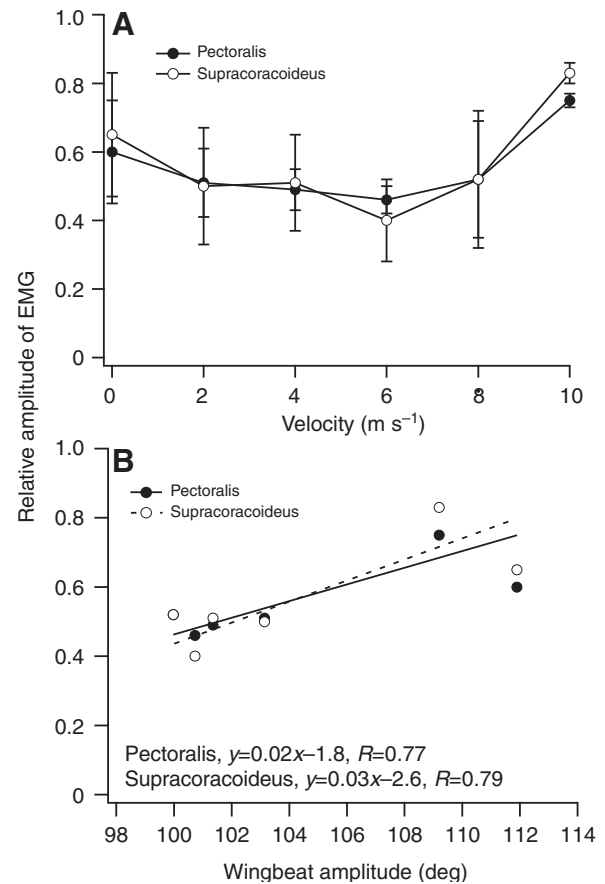


Fig. 5. Electromyographic (EMG) activity in the pectoralis (PECT) and supracoracoideus (SUPRA) of rufous hummingbirds (*Selasphorus rufus*, $N=4$) flying at velocities of $0\text{--}10\text{ m s}^{-1}$. (A) Relative amplitude of EMG as a function of flight velocity and (B) relative amplitude of EMG as a function of wingbeat amplitude (Tobalske et al., 2007). Formulas are for reduced-major axis regressions. Filled circles=PECT, open circles=SUPRA.

in the PECT ($P=0.059$, d.f.=2,5). For both muscles, the largest relative amplitude of EMG was exhibited during flight at 10 m s^{-1} (PECT= 0.75 ± 0.02 , SUPRA= 0.83 ± 0.03), and the smallest amplitude was during flight at 6 m s^{-1} (PECT= 0.46 ± 0.04 , SUPRA= 0.4 ± 0.1).

We observed a positive correlation between relative amplitude of EMG and average wingbeat amplitude (deg) (Tobalske et al., 2007) (Fig. 5B) and relative amplitude of EMG and average angular velocity of the wing (rad s^{-1}) (Tobalske et al., 2007). For wingbeat amplitude and PECT EMG amplitude, $R=0.77$ and, for SUPRA EMG amplitude, $R=0.79$. Trends were weaker for regressions on angular velocity of the wing. We compared PECT EMG with angular velocity during downstroke and SUPRA EMG with angular velocity during upstroke (Tobalske et al., 2007). Correlations were 0.49 for PECT EMG and 0.70 for SUPRA EMG.

Comparing the effects of flight speed upon relative amplitude of EMG in the PECT, it is clear that the range of variation exhibited by hummingbirds is in the middle of the range exhibited by other bird species (Fig. 6A). Coefficient of variance (c.v.) for the hummingbird PECT was 25%. This is similar to the c.v. for the 79 g cockatiel [28% (Hedrick et al., 2003)]. By contrast, c.v. for the 13 g zebra finch is less [8% (Tobalske et al., 2005); 11% (Ellerby and Askew, 2007)] and c.v. in the 182 g black-billed magpie is greater (44%). Fewer comparative data are available for the SUPRA

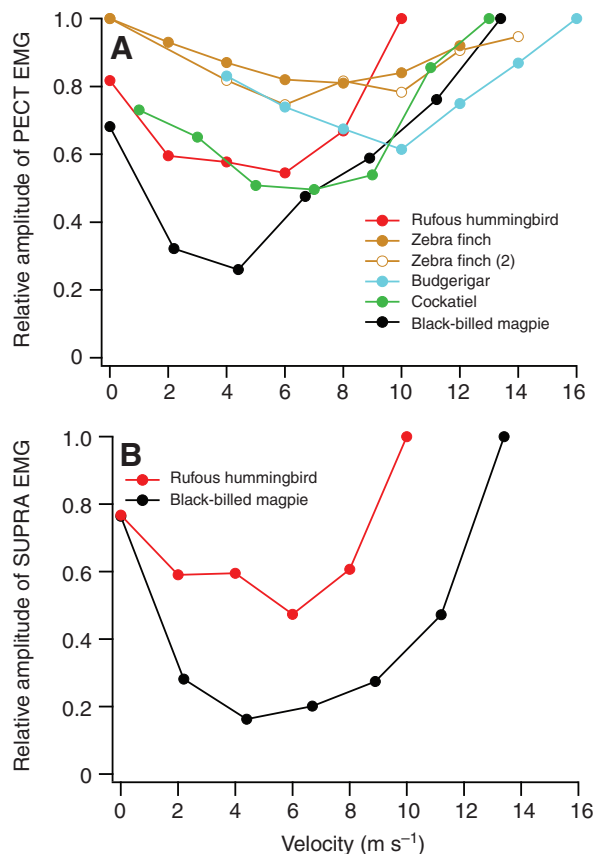


Fig. 6. Comparisons among bird species for relative amplitude of electromyography (EMG) in (A) pectoralis (PECT) and (B) supracoracoideus (SUPRA) as a function of flight speed in a wind tunnel. Values shown are means. Red=rufous hummingbird *Selasphorus rufus* (this study); orange=zebra finch *Taeniopygia guttata* (Tobalske et al., 2005) and zebra finch (2) (Ellerby and Askew, 2007); blue=Budgerigar *Melopsittacus undulatus* (Ellerby and Askew, 2007); green=cockatiel (Hedrick et al., 2003); black=black-billed magpie *Pica hudsonica* (Tobalske et al., 1997).

(Fig. 6B) but, consistent with the pattern for the PECT, c.v. for the SUPRA in the black-billed magpie is greater (70%) than in the hummingbird (27%).

Also in a comparative context (Fig. 7), we observed a significant effect of body mass upon PECT strain and strain rate. For the log-transformed species (tip) data shown in Fig. 6, strain scales with body mass to the 0.2 power (strain $\propto M_b^{0.2}$), $R=0.69$, and strain rate $\propto M_b^{-0.13}$, $R=-0.59$. Statistical analyses using independent contrasts of log-transformed data showed PECT strain $\propto M_b^{0.26}$, $R=0.78$, $P=0.003$, d.f.=10, and average PECT strain rate $\propto M_b^{-0.13}$, $R=-0.63$, $P=0.04$, d.f.=9.

DISCUSSION

Our results provide novel insight into the effects of small body size upon neuromuscular activity and muscle strain during bird flight. It was surprising that rufous hummingbirds exhibited multiple spikes per EMG burst during hovering (Fig. 1), that timing of PECT and SUPRA activity preceded the half stroke of the wingbeat for which the muscles are shortening (Figs 1–3), and that PECT strain, at 10.8%, was much less than PECT strain in other species (Figs 2 and 7).

A previous study (Hagiwara et al., 1968) reported single spikes of EMG activity in the PECT per wingbeat for three different species

of hummingbird during hovering, although a recording from an Allen's hummingbird (*Selasphorus sasin*) exhibited some multiple-spike EMG bursts along with single-spike bursts immediately preceding a transition from hovering to forward flight. The rufous hummingbirds in our study exhibited multiple spikes per EMG burst in the PECT and SUPRA at all flight speeds (Figs 1 and 2). Moreover, while data from the other species showed the birds increased the number of spikes per wingbeat to accomplish forward flight, our results did not indicate a significant effect of flight speed in rufous hummingbirds. The species studied by Hagiwara et al. (Hagiwara et al., 1968) are 1.2–1.3 times larger than the rufous hummingbirds in our study (4–4.5 g versus 3.4 g), which may indicate an effect of body size on neuromuscular recruitment. Given our small sample size, the statistical power for our tests was often low, so any lack of significant effect of flight speed upon spike number must be interpreted with caution. For example, the power for our test of an effect of flight speed upon spike number per PECT burst was 0.081 and for the SUPRA it was 0.132. Thus, these differences among species need to be tested using comparative analysis (e.g. Altshuler et al., 2004) probably involving wind tunnel measurements to accomplish flight over a range of speeds.

Regardless of differences among hummingbird species, our study supports a general conclusion that activity of the flight muscles of hummingbirds features fewer spikes per EMG burst than are exhibited in other birds (Tobalske and Dial, 1994; Tobalske, 1995; Tobalske et al., 1997; Tobalske and Dial, 2000; Hedrick et al., 2003; Tobalske et al., 2003; Tobalske et al., 2005; Soman et al., 2005; Ellerby and Askew, 2007; Tobalske and Biewener, 2008). As in other birds, however, the presence of multiple spikes per burst reveals temporal or spatial variation in motor-unit recruitment (Loeb and Gans, 1986). Raw EMG signals from species within the Phasianidae (pheasants and allies) suggest that spike number may decrease as body size decreases (Tobalske and Dial, 2000). Collectively, these data appear consistent with a hypothesis that there is a positive relationship between body size, time available for neuromuscular activation and spike number.

Our results indicate that hummingbirds, due to their high frequency of wingbeat, are approaching a limit for the time necessary for contraction and relaxation of the fast oxidative-glycolytic fibers that make up their flight muscles (Welch and Altshuler, 2009). We observed an offset between PECT and SUPRA activity and kinematic downstroke and upstroke, respectively (Figs 1–3). Sonomicrometry shows that the PECT is deactivated approximately concurrent with the onset of PECT shortening whereas EMG activity is present during the first third of PECT shortening in many larger birds including the pigeon (Tobalske and Biewener, 2008) (Fig. 3B). In isometric stimulation (Hagiwara et al., 1968) hummingbird muscle has a time to peak force of 8 ms and a decay time of 20 ms or more. These values may be different during cyclical contractions (e.g. Askew and Marsh, 1998) but their magnitude relative to the 12 ms duration of each half stroke during the hummingbird wingbeat helps explain why neuromuscular deactivation precedes wing motion (Figs 1–3).

Although superficially suggested by the EMG activity and kinematics, it is unlikely that the electrical activity and muscle force development are completely out of phase. The beginning of wrist elevation and depression almost certainly do not represent the onset of all kinematic or biomechanical effects of the PECT and SUPRA, because, as in other birds, it is likely that these muscles do more than elevate and depress the wing. The forces from the PECT and SUPRA are used to decelerate the wing, stabilize the shoulder and effect long-axis rotation of the humerus at transitions between half

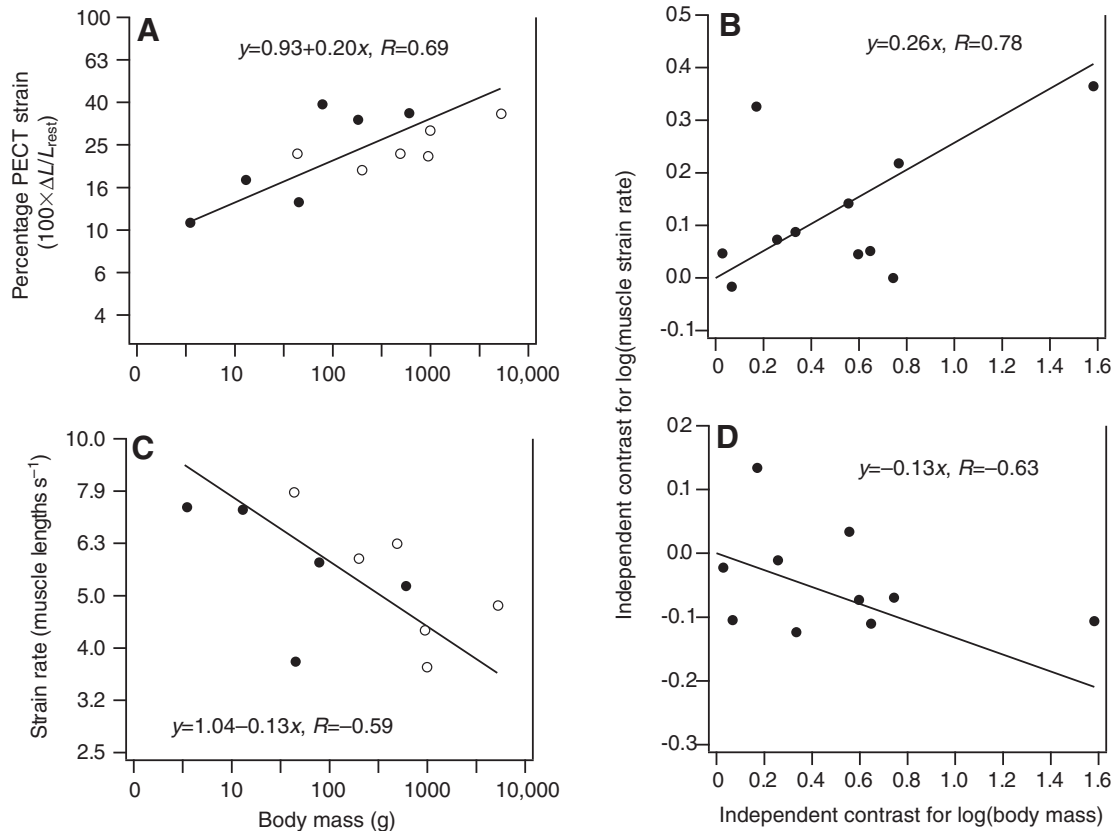


Fig. 7. Reduced-major axis regressions and correlation coefficients describing the effects of body mass upon (A and B) strain in the pectoralis (PECT) and (C and D) strain rate in the PECT. Variables are log-transformed. A and C show species (tip) data and B and D are for independent contrasts. For A and C, open circles=species engaged in take-off and escape, filled circles=species engaged in level flight, a range of flight modes or velocities.

strokes (Poore et al., 1997; Tobalske and Biewener, 2008). Furthermore, at least during hovering, hummingbirds produce rotational circulation and lift at wing turnaround using long-axis pronation and supination of the wing (Warrick et al., 2009).

The delay in relative onset of activity in the SUPRA with increasing flight speed was probably related to changes in the magnitude of wing supination during upstroke. During hovering, chord angle (supination) is 93 deg, and this angle decreases with each increase in forward flight speed to reach 23 deg during flight at 10 ms^{-1} (Tobalske et al., 2007). With less supination, then, it appears that the SUPRA is recruited later in the cycle. The decrease in upstroke supination with increasing flight speed may also help account for decreasing fractional lengthening of the PECT as speed increases (Fig. 4).

Our data from hummingbirds extend the range of body mass that supports an emerging trend for the scaling of muscle strain with body mass in flying birds (Figs 2 and 7). Within the Phasianidae, Tobalske and Dial (Tobalske and Dial, 2000) observed that muscle strain scales $\propto M_b^{0.23}$ but data from a small species, blue-breasted quail, was contradictory to this trend (Askew et al., 2001). Placing data from hummingbirds in a broad comparative context (including the blue-breasted quail) again suggests that PECT strain scales positively with increasing body mass. The observed positive scaling of strain with body size probably represents a response to an adverse scaling of mass-specific power available from the flight muscles for sustained flight (Hill, 1950; Pennycuik, 1975; Tobalske and Dial, 2000). Assuming isometric scaling of other muscle quantities

and wingbeat frequency $\propto M_b^{-0.33}$, a scaling of muscle strain $\propto M_b^{0.33}$ would be sufficient to keep mass-specific power output constant under these classical models. However, given the inter-relationship between strain rate and stress, scaling of one quantity with no change to the others is unlikely and the observed scaling probably represents a compromise among various factors. A negative scaling of strain rate with increasing body mass ($\propto M_b^{-0.13}$) also merits further study (Fig. 7C,D). The pattern suggests a departure from dynamic similarity in the use of the PECT.

It is important to note that the largest species in our comparative analysis (e.g. mallard, phasianids, including the ring-necked pheasant and wild turkey) were measured during take-off or escape flight (Tobalske and Dial, 2000; Williamson et al., 2001) (Fig. 7A,C; see Table S1 in supplementary material), and our sample from the literature therefore probably includes some bias toward higher-power forms of flight in the larger birds. Nonetheless, when comparing average PECT strain in birds that were flown over a wide range of speeds, the trend of an increase in strain with increasing body mass remains. The 10.8% strain we observed in hummingbirds is much less than the 33% and 39% strains reported for black-billed magpies and cockatiels when flying over a wide range of speeds (Warrick et al., 2001; Hedrick et al., 2003).

The humeral morphology in hummingbirds probably contributes to their ability to accomplish flight using relatively small strain in the PECT. Their humerus is hypothesized to be specifically adapted for hovering flight (Stolpe and Zimmer, 1939; Karhu, 1992; Mayr, 2003). Hummingbirds have a relatively short humerus with a

proportionally massive deltopectoral crest and a medially directed humeral head with a medio-cranial-oriented condyle; this anatomy is thought to permit a strongly adducted humeral posture compared with the posture in other birds (Stolpe and Zimmer, 1939; Karhu, 1992). A prediction that follows is that much of wing sweep (amplitude) is accomplished using long-axis rotation of the humerus in hummingbirds whereas the same movement is accomplished using humeral elevation and depression in other species (Stolpe and Zimmer, 1939; Karhu, 1992). It is also thought that the medio-cranial condyle facilitates sweep of the humerus to accomplish pronounced wing supination during upstroke when hovering or flying slowly (Stolpe and Zimmer, 1939; Karhu, 1992; Mayr, 2003). These anatomical features of hummingbirds may create proportionally small moment arms for the PECT and SUPRA so that small muscle strains generate large amplitude wingbeats.

A lack of significant variation in spike number and duration of EMG burst might suggest limited scope in hummingbirds for modulating muscle activity compared with other species of small birds such as zebra finch (Tobalske et al., 2005) but hummingbirds exhibit greater variation in EMG amplitude than do zebra finch and budgerigar (Tobalske et al., 2005; Ellerby and Askew, 2007). The other small species regularly use intermittent flight styles, with flap bounding observed in both species and flap gliding in the budgerigar (Tobalske and Dial, 1994; Tobalske et al., 1999). All three species have PECT muscles made up of the same type of fast oxidative-glycolytic fibers (Rosser and George, 1986; Welch and Altschuler, 2009). Thus, the pattern in hummingbirds, which typically use continuous flapping, may indicate the magnitude of modulation of motor unit recruitment that is required to fly over a range of speeds without using intermittent flight.

Relative amplitude of EMG is positively correlated with muscle force, work and power in cockatiels (Hedrick et al., 2003). If the same correlation is present in hummingbirds, our measurements of EMG amplitude from the PECT and SUPRA indicate that the hummingbird's minimum power speed (V_{mp}) (Rayner, 1979; Hedenström and Ålerstam, 1995) is 6 m s^{-1} and maximum range speed (V_{mr}) is 10 m s^{-1} (Figs 4 and 5). Of course, such conclusions are speculative and require further study. However, recent metabolic measurements of Anna's hummingbirds (*Calypte anna*, controls with intact tails) exhibit the same V_{mp} at 6 m s^{-1} (Clark and Dudley, 2009).

As amplitude of EMG is positively correlated with wingbeat amplitude (Fig. 5B), it is also tempting to conclude that the pattern supports the 'mechanical-oscillator' hypothesis – a model for hummingbird wing motion (Greenewalt, 1960) in which it is predicted that muscle force and strain are correlated. However, among flight speeds in the one bird for which we measured muscle strain using sonomicrometry, we observed a negative correlation between EMG amplitude and muscle strain ($R=-0.57$) as well as between muscle strain and wingbeat amplitude ($R=-0.20$). This is quite different from the positive correlation between direct measurements of force, strain and EMG amplitude in the cockatiel (Hedrick et al., 2003).

The skeletal anatomy of the hummingbird wing may potentially account for this discrepancy. In addition to the hypothesized contribution of the humerus to wing sweep and long-axis rotation described above, it is thought that relatively open or flat articular surfaces at the elbow and wrist facilitate long-axis rotation even when the wing is extended (Stolpe and Zimmer, 1939). By contrast, in most other birds, the forearm is thought to represent a four-bar linkage with limited long-axis rotation (Dial 1992b; Vazquez, 1994), and ridges and grooves in the articular surfaces at the wrist are hypothesized to serve as a locking mechanism, preventing rotation of the distal wing when the wing is extended (Vazquez, 1992). Thus,

if hummingbirds vary wrist and distal wing excursion, in part, using rotation at the elbow and twist in the forearm, some modulation in wingbeat amplitude could be accomplished without direct variation in humeral rotation and PECT strain.

Caution is necessary in interpreting this result from one hummingbird. In addition to uncertainties due to a sample $N=1$, the bird may have been relatively fatigued due to the more-extensive surgery required for implantation of sonomicrometry crystals. Compared with the other birds that were implanted only with EMG electrodes, the bird implanted with sonomicrometry crystals would not fly at 10 m s^{-1} and, in general, its flights were of shorter duration.

LIST OF ABBREVIATIONS

EMG	electromyography
<i>L</i>	length
L_{rest}	resting length
M_b	body mass
PECT	pectoralis
SB	sternobrachialis
SUPRA	supracoracoideus

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REFERENCES

- Altschuler, D. L. and Dudley, R. (2002). The ecological and evolutionary interface of hummingbird flight physiology. *J. Exp. Biol.* **205**, 2325-2336.
- Altschuler, D. L., Dudley, R. and McGuire, J. A. (2004). Resolution of a paradox. Hummingbird flight at high elevation does not come without a cost. *Proc. Natl. Acad. Sci. USA* **101**, 17731-17736.
- Askew, G. N. and Ellerby, D. J. (2007). The mechanical power requirements of avian flight. *Biol. Lett.* **3**, 445-448.
- Askew, G. N. and Marsh, R. L. (1998). Optimal shortening velocity (V/V_{max}) of skeletal muscle during cyclical contractions: length-force effects and velocity-dependent activation and deactivation. *J. Exp. Biol.* **201**, 1527-1540.
- Askew, G. N., Marsh, R. L. and Ellington, C. P. (2001). The mechanical power output of the flight muscles of blue-breasted quail (*Coturnix chinensis*) during take-off. *J. Exp. Biol.* **204**, 3601-3619.
- Berger, M. (1985). Sauerstoffverbrauch von Kolibris (*Colibri coruscans* und *C. thalassinus*) beim Horizontalflug. In *Biona-Report 3, Bird Flight* (ed. W. Nachtigall), pp. 307-314. Stuttgart: G. Fischer.
- Biewener, A. A., Corning, W. R. and Tobalske, B. W. (1998). *In vivo* pectoralis muscle force-length behavior during level flight in pigeons (*Columba livia*). *J. Exp. Biol.* **201**, 3293-3307.
- Clark, C. J. and Dudley, R. (2009). Flight costs of long, sexually selected tails in hummingbirds. *Proc. R. Soc. Lond. B. Biol. Sci.* **276**, 2109-2115.
- Dial, K. P. (1992a). Activity patterns of the wing muscles of the pigeon (*Columba livia*) during different modes of flight. *J. Exp. Zool.* **262**, 357-373.
- Dial, K. P. (1992b). Avian forelimb muscles and nonsteady flight: can birds fly without using the muscles of their wings? *Auk* **109**, 874-885.
- Ellerby, D. J. and Askew, G. N. (2007). Modulation of pectoralis muscle function in budgerigars (*Melopsittacus undulatus*) and zebra finches (*Taenopygia guttata*) in response to changing flight speed. *J. Exp. Biol.* **210**, 3789-3797.
- Ericson, P. G. P., Anderson, C. L., Britton, T., Elzanowski, A., Johansson, U. S., Källersjö, M., Ohlson, J. I., Parsons, T. J., Zuccon, D. and Mayr, G. (2006). Diversification of Neoaves: integration of molecular sequence data and fossils. *Biol. Lett.* **2**, 543-547.
- Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.* **125**, 1-15.
- Garland, T., Jr, Harvey, P. H. and Ives, A. R. P. (1992). Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* **41**, 18-32.
- Garland, T., Jr, Bennett, A. F. and Rezende, E. L. (2005). Phylogenetic approaches in comparative physiology. *J. Exp. Biol.* **208**, 3015-3035.
- Greenewalt, C. H. (1960). The wings of insects and birds as mechanical oscillators. *Proc. Am. Philos. Soc.* **104**, 605-611.
- Greenewalt, C. H. (1962). Dimensional relationships for flying animals. *Smithsonian Miscellaneous Collections* **144**, 1-46.
- Hackett, S. J., Kimball, R. T., Reddy, S., Bowie, R. C. K., Braun, E. L., Braun, M. J., Chojnowski, J. L., Cox, W. A., Han, K.-L., Harshman, J. et al. (2008). A phylogenomic study of birds reveals their evolutionary history. *Science* **320**, 1763-1768.
- Hagiwara, S., Chichibu, S. and Simpson, N. (1968). Neuromuscular mechanisms of wing beat in hummingbirds. *Z. Vergl. Physiol.* **60**, 209-218.
- Hedenström, A. and Ålerstam, T. (1995). Optimal flight speed of birds. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **348**, 471-487.
- Hedrick, T. L. (2008). Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspir. Biomim.* **3**, 034001.
- Hedrick, T. L., Tobalske, B. W. and Biewener, A. A. (2003). How cockatiels (*Nymphicus hollandicus*) modulate pectoralis power output across flight speeds. *J. Exp. Biol.* **206**, 1363-1378.

- Hill, A. V. (1950). The dimensions of animals and their muscular dynamics. *Sci. Prog.* **38**, 209-230.
- Karhu, A. (1992). Morphological divergence within the order Apodiformes as revealed by the structure of the humerus. In *Papers in Avian Paleontology Honoring Pierce Brodkorb* (ed. K. E. Campbell), 379-384. *Nat. Hist. Mus. Los Angeles Co. Sci. Ser.* **36**, 379-384.
- Kimball, R. T. and Braun, E. L. (2008). A multigene phylogeny of Galliformes supports a single origin of erectile ability in non-feathered facial traits. *J. Avian Biol.* **39**, 438-445.
- Livezey, B. C. and Zusi, R. L. (2007). Higher-order phylogeny of modern birds (Theropoda, Aves: Neornithes) based on comparative anatomy. II. Analysis and discussion. *Zool. J. Linn. Soc.* **149**, 1-95.
- Loeb, G. E. and Gans, C. (1986). *Electromyography for Experimentalists*. Chicago: University of Chicago Press.
- Maddison, W. P. and Maddison, D. R. (2008). Mesquite: a modular system for evolutionary analysis. Version 2.5. <http://mesquiteproject.org>.
- Mayr, G. (2003). Phylogeny of early tertiary swifts and hummingbirds (Aves: Apodiformes). *Auk* **120**, 145-151.
- Midford, P. E., Garland, T., Jr and Maddison, W. (2008). PDAP:PDPTREE package for Mesquite, version 1.12. http://mesquiteproject.org/pdap_mesquite/
- Pennycuik, C. J. (1975). Mechanics of flight. In *Avian Biology*, Vol. 5 (ed. D. S. Farner and J. R. King), pp. 1-75. New York: Academic Press.
- Pennycuik, C. J., Alerstam, T. and Hedenström, A. (1997). A new low turbulence wind tunnel for bird flight experiments at Lund University, Sweden. *J. Exp. Biol.* **200**, 1441-1449.
- Poore, S. O., Ashcroft, A., Sanchez-Haiman, A. and Goslow, G. E., Jr (1997). The contractile properties of the M. supracoracoideus in the pigeon and starling: a case for long-axis rotation of the humerus. *J. Exp. Biol.* **200**, 2987-3002.
- Rayner, J. M. V. (1979). A new approach to animal flight mechanics. *J. Exp. Biol.* **80**, 17-54.
- Rayner, J. M. V. (1985). Bounding and undulating flight in birds. *J. Theor. Biol.* **117**, 47-77.
- Rosser, B. W. C. and George, J. C. (1986). The avian pectoralis: histochemical characterization and distribution of muscle fiber types. *Can. J. Zool.* **64**, 1174-1185.
- Schmidt-Nielsen, K. (1972). Locomotion: energy cost of swimming, running and flying. *Science* **177**, 222-228.
- Soman, A., Hedrick, T. L. and Biewener, A. A. (2005). Regional patterns of pectoralis fascicle strain in the pigeon *Columba livia* during level flight. *J. Exp. Biol.* **208**, 771-786.
- Stolpe, V. M. and Zimmer, K. (1939). Der Schwirrflyug des Kolibri im Zeitlupenfilm. *J. Ornithol.* **87**, 136-155.
- Tobalske, B. W. (1995). Neuromuscular control and kinematics of intermittent flight in European starlings (*Sturnus vulgaris*). *J. Exp. Biol.* **198**, 1259-1273.
- Tobalske, B. W. (2001). Morphology, velocity, and intermittent flight in birds. *Am. Zool.* **41**, 177-187.
- Tobalske, B. W. and Biewener, A. A. (2008). Contractile properties of the pigeon supracoracoideus during different modes of flight. *J. Exp. Biol.* **211**, 170-179.
- Tobalske, B. W. and Dial, K. P. (1994). Neuromuscular control and kinematics of intermittent flight in budgerigars (*Melopsittacus undulatus*). *J. Exp. Biol.* **187**, 1-18.
- Tobalske, B. W. and Dial, K. P. (2000). Effects of body size on take-off flight performance in the Phasianidae (Aves). *J. Exp. Biol.* **203**, 3319-3332.
- Tobalske, B. W., Olson, N. E. and Dial, K. P. (1997). Flight style of the black-billed magpie: variation in wing kinematics, neuromuscular control and muscle composition. *J. Exp. Zool.* **279**, 313-329.
- Tobalske, B. W., Peacock, W. L. and Dial, K. P. (1999). Kinematics of flap-bounding flight in the zebra finch over a wide range of speeds. *J. Exp. Biol.* **202**, 1725-1739.
- Tobalske, B. W., Hedrick, T. L., Dial, K. P. and Biewener, A. A. (2003). Comparative power curves in bird flight. *Nature* **421**, 363-366.
- Tobalske, B. W., Puccinelli, L. A. and Sheridan, D. C. (2005). Contractile activity of the pectoralis in the zebra finch according to mode and velocity of flap-bounding flight. *J. Exp. Biol.* **208**, 2895-2901.
- Tobalske, B. W., Warrick, D. R., Clark, C. J., Powers, D. R., Hedrick, T. L., Hyder, G. A. and Biewener, A. A. (2007). Three-dimensional kinematics of hummingbird flight. *J. Exp. Biol.* **210**, 2368-2382.
- Vazquez, R. J. (1992). Functional osteology of the avian wrist and the evolution of flapping flight. *J. Morph.* **211**, 259-268.
- Vazquez, R. J. (1994). The automating skeletal and muscular mechanisms of the avian wing (Aves). *Zoomorphology* **114**, 59-71.
- Warrick, D. R., Tobalske, B. W., Biewener, A. A. and Dial, K. P. (2001). Sonomicrometry and kinematic estimates of the mechanical power of bird flight. *Bull. Mus. Comp. Zool.* **156**, 257-268.
- Warrick, D. R., Tobalske, B. W. and Powers, D. P. (2005). Aerodynamics of the hovering hummingbird. *Nature* **435**, 1094-1097.
- Warrick, D. R., Tobalske, B. W. and Powers, D. R. (2009). Lift production in the hovering hummingbird. *Proc. R. Soc. Lond. B. Biol. Sci.* **276**, 3747-3752.
- Welch, K. C. and Altshuler, D. L. (2009). Fiber type homogeneity of the flight musculature in small birds. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **152**, 324-331.
- Williamson, M. R., Dial, K. P. and Biewener, A. A. (2001). Pectoralis muscle performance during ascending and slow level flight in mallards (*Anas platyrhynchos*). *J. Exp. Biol.* **204**, 495-507.