

## Research article

# Contractile properties of the pigeon supracoracoideus during different modes of flight

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### Summary

The supracoracoideus (SUPRA) is the primary upstroke muscle for avian flight and is the antagonist to the downstroke muscle, the pectoralis (PECT). We studied *in vivo* contractile properties and mechanical power output of both muscles during take-off, level and landing flight. We measured muscle length change and activation using sonomicrometry and electromyography, and muscle force development using strain recordings on the humerus. Our results support a hypothesis that the primary role of the SUPRA is to supinate the humerus. Antagonistic forces exerted by the SUPRA and PECT overlap during portions of the wingbeat cycle, thereby offering a potential mechanism for enhancing control of the wing. Among flight modes, muscle strain was approximately the same in the SUPRA (33–40%) and the PECT (35–42%), whereas peak muscle stress was higher in the SUPRA (85–126 N m<sup>-2</sup>) than in the PECT (50–58 N m<sup>-2</sup>). The SUPRA mainly shortened relative to resting length and the PECT mainly lengthened. We estimated that elastic energy storage in the tendon of the SUPRA contributed between 28 and 60% of the net work of the SUPRA and 6–10% of the total net mechanical work of both muscles. Mechanical power output in the SUPRA was congruent with the estimated inertial power required for upstroke, but power output from the PECT was only 42–46% of the estimated aerodynamic power requirements for flight. There was a significant effect of flight mode upon aspects of the contractile behavior of both muscles including strain, strain rate, peak stress, work and power.

Key words: flight, muscle, supracoracoideus, force, work, power, stress, strain.

### Introduction

The supracoracoideus (SUPRA) is the second largest muscle of the avian wing. It is the major antagonist to the larger pectoralis (PECT), which is the primary downstroke muscle for bird flight (Dial, 1992a). The SUPRA is active in all modes and speeds of flight (Dial 1992a; Tobalske, 1995), yet, with practice, birds may take off without use of the muscle (Degernes and Feduccia, 2001; Sokoloff et al., 2001). Electromyographic (EMG) data suggest that the muscle decelerates the wing during late downstroke and reaccelerates the wing during the beginning of upstroke (Dial, 1992a). Regardless of its capacity to elevate the wing, an *in situ* study of the function of the SUPRA indicated that the principal role of the muscle is supination of the humerus during the transition from downstroke to upstroke (Poore et al., 1997). The need for supination explains the activation of the SUPRA during faster flight when lift should presumably function to elevate the wing independent of muscle activation (Rayner, 1985; Poore et al., 1997; Hedrick et al., 2002; Hedrick et al., 2004).

The mechanical properties of the SUPRA *in vivo* are, unfortunately, unknown. The vast majority of the variation observed in avian wingbeat kinematics occurs during upstroke (Scholey, 1983; Tobalske, 2000; Tobalske et al., 2003a) and this variation appears to correspond with changes in aerodynamic function (Rayner, 1995; Spedding et al., 2003). In contrast, considerable insight is now available on the contractile behavior of the PECT in flying birds.

The PECT is largely designed to generate work and power (Biewener, 1998; Biewener and Roberts, 2000). Power output in the PECT varies with flight mode and speed (Dial and Biewener, 1993; Hedrick et al., 2003; Tobalske et al., 2003b; Tobalske et al., 2005). The large size and complex architecture of the PECT (Sokoloff et al., 1998) is accompanied by significant heterogeneity in regional activation patterns (Boggs and Dial, 1993) and muscle strain (Biewener et al., 1998; Soman et al., 2005).

The anatomy of the SUPRA differs from that of the PECT (Baumel et al., 1993; Poore et al., 1997). Although both muscles are bipinnate, the SUPRA is narrow, with a long tendon of insertion. The PECT is broad, with a short tendon of insertion and a substantial region of parallel fibers in the anterior *pars sternobrachialis*. Interpreted in the light of muscle function during terrestrial locomotion, the anatomy of the SUPRA would suggest that the muscle is used to produce force rather than work and also to exploit elastic energy storage and recovery (Biewener and Baudinette, 1995; Roberts et al., 1997; Biewener, 1998; Biewener and Roberts, 2000). Release of stored energy reduces the metabolic cost of terrestrial locomotion (Alexander, 1988; Biewener and Roberts, 2000). Such storage has been identified as a potential function of the avian furcula (Jenkins et al., 1988), but it has not been documented for any muscles of the wing.

As power costs for flight are high (Harrison and Roberts, 2000), it is generally assumed that selective pressures in evolution optimized the avian wing for metabolic efficiency. A

competing selective pressure is likely for wing control, particularly during maneuvers (Warrick et al., 2002). For maneuvering, it is thought that distal muscles of the wing are relatively more important than large proximal muscles such as the PECT and SUPRA (Dial, 1992a; Dial, 1992b). However, using EMG recordings and *in situ* rates of force development in the PECT and SUPRA, Poore et al. (Poore et al., 1997) hypothesized that there should be antagonistic force development in the PECT and SUPRA to facilitate control.

During slow flight in most birds, weight support and thrust are produced only during downstroke and upstroke appears to be aerodynamically inactive (Spedding et al., 1984; Tobalske, 2000; Hedrick et al., 2004; Usherwood et al., 2005). Therefore, in slow flight, we expect PECT power to match the aerodynamic requirement for flight while SUPRA power should match the inertial power required for upstroke. Inertial work produced by the PECT to accelerate the wing during downstroke is expected to be transformed into aerodynamic work at the end of downstroke (Van den Berg and Rayner, 1995; Hedrick et al., 2004).

Much of the information presently available about PECT function in flying birds is from the pigeon *Columba livia* (Gmelin 1789), so we selected this species for investigating SUPRA function. We began with four predictions from prior research. Given the expected role of the SUPRA in supination of the wing (Poore et al., 1997), we hypothesized (1) that peak force in the muscle would occur at the transition from downstroke to upstroke rather than at mid-upstroke. Anatomy led us to predict that (2) it would operate with little length change and store elastic energy in its tendon (Biewener, 1998; Biewener and Roberts, 2000). A need for control of the wing and joint stability would result in (3) overlap in force production with the PECT (Poore et al., 1997). Finally, given the present evidence that upstroke produces little or no lift during slow flight (Tobalske, 2000), we hypothesized that (4) power output in the SUPRA should equal inertial power in upstroke, whereas power output in the PECT should match aerodynamic power required for slow flight.

## Materials and methods

### Birds and experimental design

We obtained pigeons ( $N=7$ , including five white carneau and two king, body mass  $561.9 \pm 94.9$  g, means  $\pm$  s.d., Table 1) from commercial suppliers. An additional three white carneau pigeons ( $562.3 \pm 8.2$  g) (Soman et al., 2005) were used for 3-D kinematic analysis and estimation of inertial power ( $P_{\text{iner}}$ ). The birds were housed in a  $2 \text{ m} \times 8 \text{ m} \times 2 \text{ m}$  outdoor aviary at the Concord Field Station, Harvard University (Bedford, MA, USA) and had access to food and water *ad libitum*. The Institutional Animal Care and Use Committee at Harvard University approved all housing and experimental protocols (accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International). The birds were trained to fly between platforms ( $30 \text{ cm} \times 40 \text{ cm}$ ) and horizontal poles (diameter 2.5 cm) to accomplish one of three modes of flight: level, ascent and descent. Level flights ( $3.9 \pm 0.5 \text{ m s}^{-1}$ ) were between platforms supported 1.5 m above the floor and spaced 7–9 m apart. Ascending ( $1.4 \pm 0.5 \text{ m s}^{-1}$ ) and descending ( $1.8 \pm 0.3 \text{ m s}^{-1}$ ) flights were from a platform placed on the ground and a horizontal pole placed 1.2 m horizontally and 2 m vertically from the platform to effect flight paths  $\sim 60^\circ$  relative to horizontal. Flight speeds varied from  $0 \text{ m s}^{-1}$  (hovering, when the bird was unwilling to ascend) to  $2 \text{ m s}^{-1}$ . All training and experiments took place in a hallway  $1.9 \text{ m} \times 4.2 \text{ m} \times 12 \text{ m}$ .

Table 1. Morphological data for pigeons (*Columba livia*)

Variable	Mean value
Body mass (g)	562 $\pm$ 95
Single wing length (cm)	33 $\pm$ 2
Wing span (cm)	74 $\pm$ 4
Average wing chord (cm)	11.3 $\pm$ 0.8
Aspect ratio	6.6 $\pm$ 0.2
Single wing area (cm <sup>2</sup> )	359 $\pm$ 45
Aerodynamic area of both wings and body (cm <sup>2</sup> )	837 $\pm$ 104
Wing loading (N m <sup>-2</sup> )	66 $\pm$ 12
Disc loading (N m <sup>-2</sup> )	13 $\pm$ 2
Single SUPRA mass (g)	10 $\pm$ 2
SUPRA average fascicle length (mm)	24 $\pm$ 2
SUPRA fascicle angle (degrees)	24 $\pm$ 7
SUPRA cross-sectional area (cm <sup>2</sup> )	3.7 $\pm$ 0.9
Single SUPRA tendon mass (mg)	46 $\pm$ 24
SUPRA tendon length (mm)	25 $\pm$ 13
SUPRA tendon cross-sectional area (mm <sup>2</sup> )	1.5 $\pm$ 0.4
Single PECT mass (g)	53 $\pm$ 10
PECT fascicle length (mm)	47 $\pm$ 6
PECT fascicle angle (degrees)	19 $\pm$ 2
PECT cross-sectional area (cm <sup>2</sup> )	10 $\pm$ 2

PECT, pectoralis; SUPRA, supracoracoideus. Values are means  $\pm$  s.d.,  $N=7$ .

Morphometric data (Table 1) were obtained after the completion of experiments. Mass ( $M$ ) was obtained for single muscles, and 10 measurements of fascicle length ( $L$ ) and fascicle angle ( $\alpha$ ) were obtained for each surface, superficial and deep. Muscle cross-sectional area ( $A$ ) was calculated as  $M/\rho L$  assuming a muscle density ( $\rho$ ) of  $1060 \text{ kg m}^{-3}$ . Measurements of tendon length and mass from the SUPRA were used to estimate tendon cross-sectional area assuming a tendon density of  $1120 \text{ kg m}^{-3}$  (Ker, 1981).

Additional wing and body measurements were obtained with the wings spread as in mid-downstroke using standard techniques (Tobalske and Dial, 1996; Tobalske et al., 1999).

### Video recording and modeling of power requirements

We obtained synchronized 2-D kinematic data during implanted flights ( $N=7$  birds) to estimate the aerodynamic power requirements of the PECT during flight. To estimate the inertial power requirements of the SUPRA during upstroke, we also obtained 3-D kinematic data from unimplanted level and ascending flights in three birds affiliated with Soman et al. (Soman et al., 2005). Mathematical modeling was accomplished using Igor Pro version 4.0.6 (Wavemetrics, Inc., Beaverton, OR, USA) and Matlab version 6.5 (The MathWorks Inc., Natick, MA, USA).

During implanted flights, we used a Redlake PCI-500 (San Diego, CA, USA) to obtain a lateral-view video (250 Hz, shutter speed 0.5 ms, stored using PCI-R version 2.18 software) with a pixel:metric scale corrected for parallax using a synchronized Panasonic AG-450 S-VHS camera (60 Hz, shutter speed 1000 Hz) that offered cranio-caudal views of the flight path. We adapted the methods of Hedrick et al. (Hedrick et al., 2003) and Tobalske et al. (Tobalske et al., 2003b) in which 3-D kinematic data were applied to the aerodynamic models of Rayner (Rayner, 1979a; Rayner, 1979b), Pennycuick (Pennycuick, 1975) and Wakeling and Ellington (Wakeling and Ellington, 1997). In the 3-D analysis, fully described in equations 2–7 in Hedrick et al. (Hedrick et al., 2003), separate estimates of induced, profile, parasite and climb power were summed for each video frame, and the sums were then integrated over an entire wingbeat cycle. Lacking the resolution of 3-D data, we instead calculated the component aerodynamic

powers for each half of the wingbeat cycle and then integrated over the full cycle ( $P_{\text{aero}}$ ).

From earlier work (Tobalske and Dial, 1996; Tobalske et al., 2003a), we assumed that the wings were fully extended throughout downstroke and that wing span at mid-downstroke was always the same as the morphological wing span of a given bird (Table 1). We then scaled mid-upstroke wing span according to the upstroke:downstroke span ratio within a given wingbeat. Finally, we assumed that wing length did not change throughout upstroke.

Our results were sensitive to our assumptions of wing length, particularly for downstroke. For example, during level flight a 10% decrease in wing length at mid-downstroke increased our estimate of  $P_{\text{aero}}$  by 13% (18% increase for induced power, 17% decrease for profile power), and a 10% increase in wing length decreased  $P_{\text{aero}}$  by 11% (15% decrease for induced power and 20% increase for profile power). In comparison, 10% changes in wing length during upstroke caused the estimated profile power and  $P_{\text{aero}}$  to vary by <1%.

We later obtained additional high-speed video cameras, which permitted us to measure 3-D kinematics and, thereby, estimate  $P_{\text{iner}}$  during upstroke (Hedrick et al., 2004). All video recording, camera calibration, data filtering and measurements of mass distribution were accomplished as in Hedrick et al. (Hedrick et al., 2004). We calculated  $P_{\text{iner}}$  required for upstroke as the change in kinetic energy of the wing from the start of the upstroke to its maximum divided by wingbeat duration.

#### Surgical procedure

Following training, we surgically implanted EMG electrodes and sonomicrometry (SONO) transducers into the SUPRA and PECT muscles by adapting standard methods used for the PECT (Biewener et al., 1998; Tobalske et al., 2005). We also attached two strain gauges (FLE-1, Tokyo Sokki Kenkyujo, Ltd, Tokyo, Japan) to the dorsal surface of the deltopectoral crest adjacent to, and parallel with, the insertion of each muscle (Biewener et al., 1998; Soman et al., 2005) (Fig. 1). One pair of 2.0 mm SONO crystals (Sonometrics, Inc., London, ON, Canada) and a fine-wire bipolar EMG electrode (0.5 mm bared tips with 2 mm spacing; California Fine Wire, Inc., Grover Beach, CA, USA) were implanted parallel to the fascicle axis of the mid-anterior region of the sternobrachial portion of the PECT (Fig. 1A). Another pair of SONO crystals and an EMG electrode were implanted through the PECT and into the mid-anterior SUPRA.

The SONO crystals were implanted at a depth of about 4 mm beneath the superficial fascia of the muscle and at a distance of 8–12 mm apart. For implantation into the SUPRA, two other openings were made through the PECT using fine-tip scissors and watchmaker's forceps. The crystals in the SUPRA were directed through the openings using Silastic tubing, which we subsequently removed. Once each SONO crystal had been inserted and aligned to ensure a maximum signal quality, all openings were sutured with 4-0 silk. A 4-0 silk suture was used to tie down all electrodes a few millimeters away from the exit point on the superficial fascia of the PECT to eliminate movement artifacts. As all tie-down sutures were superficial to the PECT, there was a relatively greater risk that slippage may have occurred in the crystals implanted in the SUPRA. Crystal spacing was approximately 10 mm, so our measurements of muscle length would change by ~0.1% for 1% slippage.

After the experiments, the pigeons were killed using an intravenous overdose of sodium pentobarbital (100 mg kg<sup>-1</sup>) for

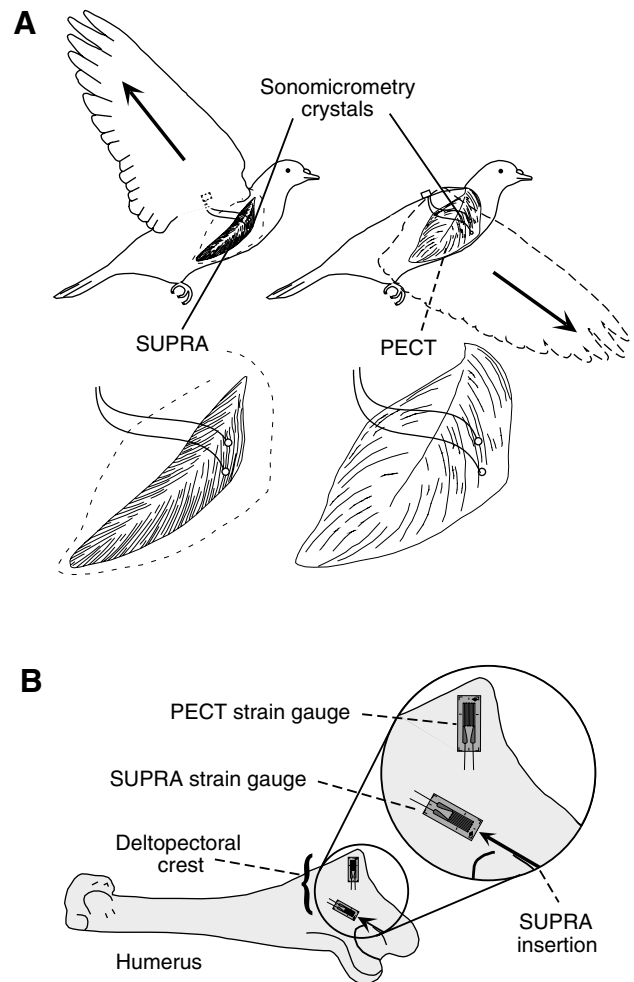


Fig. 1. Placement of (A) sonomicrometry crystals for measuring fascicle length in the the supracoracoideus (SUPRA) and the pectoralis (PECT), and (B) strain gauges on the dorsal surface of the deltopectoral crest of the left humerus for measuring bone strain, calibrated to estimate SUPRA and PECT force, in a pigeon (*Columba livia*).

verification of electrode placement, calibration of strain gauges and measurement of morphology (Table 1).

#### *In vivo* muscle recordings and calibrations

Recordings were made by connecting the bird to a 12 m shielded cable, which the animal dragged as it flew (the suspended portion of cable weighed ~80 g). Qualitatively, there was no apparent effect of these elements upon flight kinematics in the pigeons, but we did not obtain a sufficient sample of non-implanted flights to use for a statistical test of this hypothesis. The cable was connected to strain gauge bridge amplifiers (Vishay 2120, Micromeritics, Inc., Raleigh, NC, USA), a sonomicrometry amplifier (Triton 120.2, Triton Technology, Inc., San Diego, CA, USA) and EMG amplifiers (Grass P5-11, Grass Telefactor, West Warwick, RI, USA). All signals were recorded at 5 kHz onto a Pentium II computer running Windows NT (Microsoft, Inc., Redmond, WA, USA) using a Digidata 1200 A/D converter (Axon Instruments, Union City, CA, USA) at 5 kHz. For analysis of SONO and EMG signals, we used the methods of Hedrick et al. (Hedrick et al., 2002) and Tobalske et al. (Tobalske et al., 2005).

Sonomicrometry signals were corrected to represent the instantaneous average  $L$  of the muscle in which the crystals were implanted (Fig. 1A, Table 1). To treat analysis for the PECT and SUPRA equally, we assumed  $\Delta L$  was uniform throughout the fascicle and throughout the muscle. This assumption merits caution as evidence from a different vertebrate indicates that heterogeneity of strain is apparent even within single fascicles (Ahn et al., 2003). Other studies in pigeons suggest our method may have caused a slight overestimate of muscle strain ( $\epsilon$ ) for the PECT as a whole because the region we implanted includes fibers that exhibit the greatest  $\Delta L$  within the muscle (Biewener et al., 1998; Soman et al., 2005). No information is available for regional heterogeneity of contractile behavior in the SUPRA. The measured distance between the sonomicrometry crystals was increased by 2.7% to account for the velocity of sound in muscle ( $1540 \text{ m s}^{-1}$ ) (Goldman and Heuter, 1956) relative to the value of  $1500 \text{ m s}^{-1}$  assumed by the Triton 120.2 amplifier. This value was then increased by 0.74 mm to account for the higher velocity of sound through the epoxy lens of the 2 mm electrodes relative to muscle tissue (Biewener et al., 1998). We also corrected for a 5 ms phase delay and a frequency-dependent attenuation in the amplitude of the sonomicrometry signals, both of which were due to the 100 Hz linear phase filter inherent to the Triton 120.2 amplifiers (Tobalske and Dial, 2000). Resting length ( $L_{\text{rest}}$ ) was measured during perching, with the wings folded and the PECT inactive, and  $\epsilon = \Delta L L_{\text{rest}}^{-1}$ .

To calibrate strain in the bone of the humerus into units of muscle force, we performed pull calibrations (Dial and Biewener, 1993; Biewener et al., 1998; Soman et al., 2005). Silk suture (OO) was secured around the anterior portion of the PECT 2 cm from the insertion on the deltopectoral crest (DPC) or the emergent tendon of the SUPRA immediately adjacent to the belly of the muscle. The other end of the suture was attached to a calibrated force transducer (Kistler 9203, Amherst, NY, USA). The anatomy of the DPC (Fig. 1B) resulted in cross-talk between strain-gauge channels arising from principal strains transmitted when forces were exerted by either muscle on the DPC. Tension produced by either muscle resulted in a compressive principal strain acting perpendicular to the tensile strain and, thus, generally in line with the other muscle's tendon of attachment. In other words, tension in one muscle artificially inflated tension measured for the other muscle. This cross-talk was approximately 50% from the PECT channel to the SUPRA channel and 5% from the SUPRA channel to the PECT channel.

We corrected for cross-talk from the PECT to the SUPRA channel and chose to ignore cross-talk in the opposite direction because of circularity in the underlying argument. Corrections were applied to the SUPRA channel at the stage of raw voltages. Using data obtained during PECT pull calibrations, we regressed SUPRA force upon PECT force. For *in vivo* data from a given bird, the scaling factor from this regression was multiplied by observed PECT force and added to the uncorrected SUPRA force to provide a corrected SUPRA force. The correction factor affected measurements throughout the wingbeat cycle because residual tension was always present in the PECT during flight. We calculated muscle stress ( $\sigma_m$ , in kPa) as force (N) divided by  $A \cos \alpha$  (Alexander, 1983) (Table 1).

We measured work (mJ) and power (W) for each muscle using the work loop technique (Josephson, 1985; Biewener et al., 1998). A work loop 'shape factor' (Hedrick et al., 2003) was calculated as the observed area of a work loop relative to the area of a rectangle with the same range of stress and strain. Net work per wingbeat

duration yielded muscle power ( $P_{\text{mus}}$ ), and mass-specific power ( $\text{W kg}^{-1}$ ) was calculated as  $P_{\text{mus}}$  divided by  $M$  (Table 1). Tendon elastic energy recovery ( $U_{\text{rec}}$ , in J) was calculated following Biewener and Baudinette (Biewener and Baudinette, 1995):

$$U_{\text{rec}} = 0.5(\sigma_t^2/E)V_t \times 0.93,$$

where  $\sigma_t$  is tendon stress (in MPa),  $E$  is elastic modulus and  $V_t$  is tendon volume (in  $\text{m}^3$ ). We used an estimate of 1.0 GPa for  $E$  and 0.93 for tendon resilience based on observed ranges of data reported by Bennett et al. (Bennett et al., 1986), Ker (Ker, 1981) and Shadwick (Shadwick, 1990). Our estimated tendon elasticity could be inaccurate because the data in these references are from different tendons in mammals; however, tendon properties are generally similar across the species studied.

We analyzed contractile properties and timing for 242 wingbeats using the onset of PECT shortening to identify the start of individual wingbeats and the onset of PECT lengthening to identify the start of upstroke.

#### Statistical analysis

For each variable, we computed the mean value within each bird for each flight mode. We then tested for a significant effect of mode upon each variable using a univariate repeated-measures analysis of variance (StatView version 5.0.1, SAS Institute, Inc., Cary, NC, USA). Values are presented as means  $\pm$  s.d.

#### Results

During flight, the SUPRA and PECT muscles exhibited contraction cycles that alternated with each other and were relatively uniform during most of the flight sequence (Fig. 2). The first one or two wingbeats during take-off and last several wingbeats prior to landing featured lower amplitude muscle strain, stress and EMG voltage. Wingbeat frequency averaged  $8.6 \pm 0.2$  Hz (wingbeat duration = 116 ms) and did not vary significantly ( $P = 0.0926$ ) among flight modes. Likewise, flight mode did not have a significant effect upon the relative timing of most of the contractile events in the wingbeat cycle (Fig. 3). There were two exceptions. First, the SUPRA started shortening earlier during ascending flight ( $48 \pm 8\%$ ) relative to level ( $49 \pm 7\%$ ) and descending ( $50 \pm 8\%$ ) flight ( $P = 0.0313$ ). Second, the relative offset of SUPRA EMG activity occurred later during ascending ( $77 \pm 5\%$ ) compared with level ( $70 \pm 7\%$ ) and descending ( $71 \pm 11\%$ ) flight ( $P = 0.0172$ ).

Peak stress ( $\sigma_m$ ) in the SUPRA occurred at  $65 \pm 8\%$  of the wingbeat cycle, immediately after the transition between downstroke and upstroke (lag time averaged 4% of the wingbeat cycle). Peak  $\sigma_m$  in the PECT occurred during the middle of downstroke. For either muscle, a peak  $\sigma_m$  occurred as the muscle was shortening. Shortening in the SUPRA lasted  $54 \pm 7\%$  of the wingbeat cycle and shortening in the PECT lasted  $62 \pm 4\%$ . Neuromuscular activation preceded the onset of shortening in both muscles with a relative lead time of  $10 \pm 7\%$  in the SUPRA and  $12 \pm 4\%$  in the PECT. The duration of EMG activity in the SUPRA was shorter than in the pectoralis, at  $33 \pm 3\%$  and  $58 \pm 5\%$ , respectively, relative to cycle duration. Consequently, a considerable fraction of force development by both muscles lasted beyond EMG offset (SUPRA,  $29 \pm 9\%$ ; PECT,  $39 \pm 7\%$ ). The pigeons consistently exhibited overlap in force production by the SUPRA and PECT muscles (Figs 2–4). An interval of simultaneous, antagonistic force took place during late downstroke, as force was declining in the PECT, and another occurred during late upstroke, as force was declining in the SUPRA. When positive

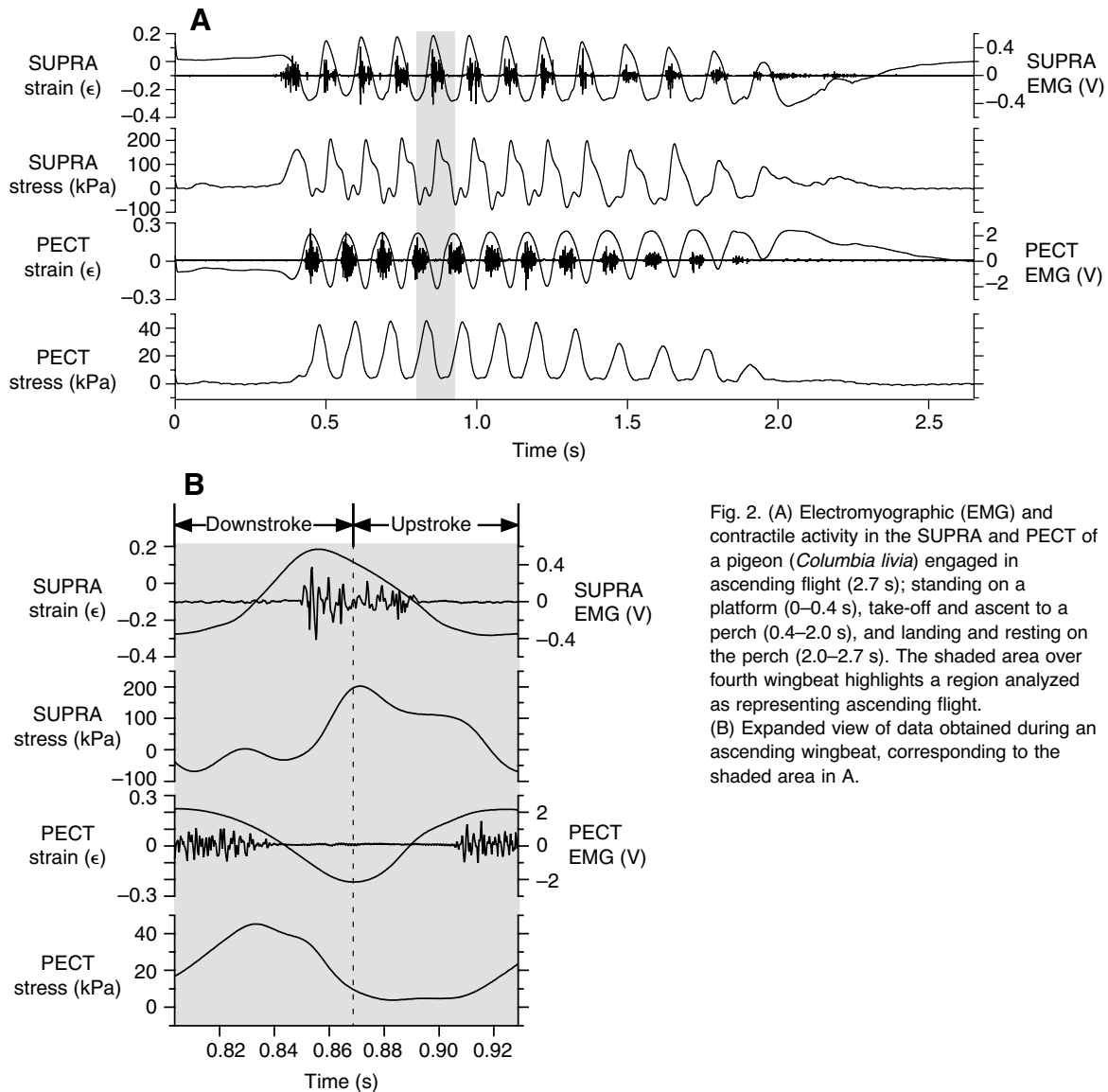


Fig. 2. (A) Electromyographic (EMG) and contractile activity in the SUPRA and PECT of a pigeon (*Columbia livia*) engaged in ascending flight (2.7 s); standing on a platform (0–0.4 s), take-off and ascent to a perch (0.4–2.0 s), and landing and resting on the perch (2.0–2.7 s). The shaded area over fourth wingbeat highlights a region analyzed as representing ascending flight. (B) Expanded view of data obtained during an ascending wingbeat, corresponding to the shaded area in A.

$\sigma_m$  was present in its antagonist muscle, negative work absorbed by the SUPRA was  $-16 \pm 16$  mJ and by the PECT was  $-48 \pm 7$  mJ. These values were 9% of the net work performed by either muscle (Table 2). The magnitude of overlap in antagonistic force was increased by our method of correction of cross-talk from the PECT for  $\epsilon$  measured by the SUPRA strain gauge; nevertheless, overlap remained apparent even when uncorrected (raw) signals from the SUPRA strain gauge were evaluated relative to the strain-gauge signal from the PECT (Fig. 4).

Many of the contractile properties that we measured varied significantly according to flight mode (Table 2). Overall,  $\epsilon$ ,  $\sigma_m$ , work and power reached maximum values during ascending flight, were least during descending flight, and were intermediate during level flight. The only exception to this pattern was fractional lengthening in the PECT, which remained nearly the same ( $P=0.3016$ ) during ascent ( $28 \pm 7\%$ ) and level flight ( $28 \pm 7\%$ ).

Stress ( $\sigma_m$ ) was greater in the SUPRA than in the PECT (Fig. 5A), and there was a significant effect of flight mode upon the peak  $\sigma_m$  exhibited by the SUPRA ( $P=0.0463$ ) and PECT ( $P=0.0017$ ; Table 2). In the SUPRA, peak  $\sigma_m$  varied from  $85 \pm 30$  kPa during descent to  $125 \pm 65$  kPa during ascent. Peak  $\sigma_m$

during descent in the PECT was  $50 \pm 12$  kPa and it was  $58 \pm 15$  kPa during ascending flight.

Our measurements of peak  $\sigma_i$  in the tendon of the SUPRA averaged  $24 \pm 14$  MPa, which provided an estimate of recovered energy of  $58 \pm 27$  mJ among flight modes. This represented  $33 \pm 5\%$  of the net work performed by the SUPRA and  $8 \pm 2.2\%$  of the net work performed in sum by the PECT and SUPRA. Although  $U_{rec}$  in the SUPRA tendon during ascending flight was over twice the amount estimated for descending flight ( $88 \pm 85\%$  versus  $36 \pm 29\%$ , Table 2), substantial variance among birds resulted in a marginally non-significant effect of flight mode upon  $U_{rec}$  ( $P=0.0548$ ).

Muscle strain ( $\epsilon$ ) during wingbeats was generally similar in the two muscles (SUPRA:  $36 \pm 3\%$ ; PECT:  $38 \pm 4\%$ ). During flight, the SUPRA tended to operate over a range of  $L$  that was less than  $L_{rest}$ , whereas the PECT operated over lengths greater than  $L_{rest}$  (Fig. 1 and Fig. 5A). Flight mode did not have a significant effect upon fractional shortening in the SUPRA ( $P=0.0762$ ) or fractional lengthening in the PECT ( $P=0.3015$ ). In contrast, the observed variation associated with flight mode was significant for  $\epsilon$  in the SUPRA ( $P=0.0039$ ), fractional lengthening in the SUPRA

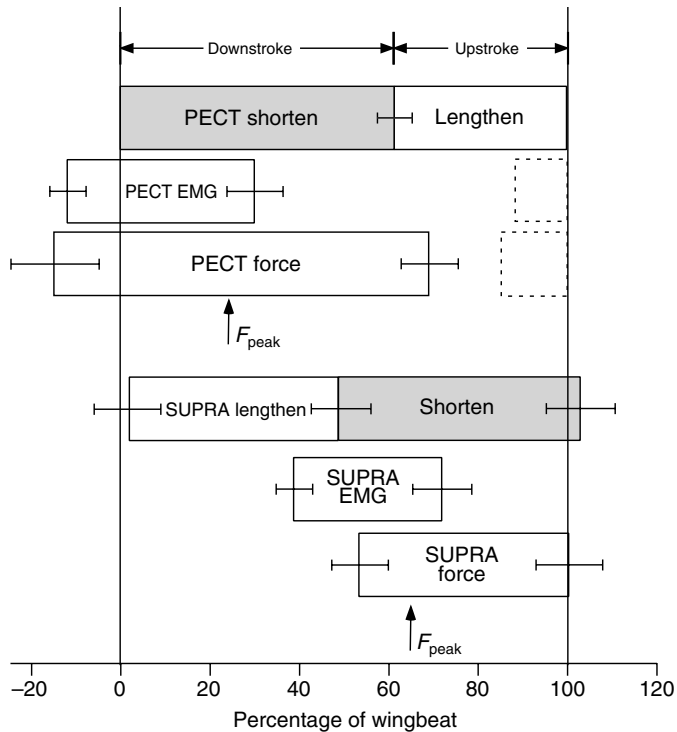


Fig. 3. Relative timing of length change, activation and force in the SUPRA and PECT of flying pigeons (*Columba livia*,  $N=7$ ). Data from different modes of flight were pooled to create this figure. Values are means  $\pm$  s.d. Broken lines indicate data for a subsequent wingbeat.

( $P=0.0105$ ),  $\epsilon$  in the PECT ( $P=0.008$ ) and fractional shortening in the PECT ( $P=0.0452$ ).

The comparatively brief duration of SUPRA shortening (Fig. 3) resulted in the SUPRA having higher average strain rates compared with the PECT (Table 2). Strain rate in the SUPRA varied between  $5 \pm 2$  and  $7 \pm 2$   $L s^{-1}$ , and from  $5 \pm 1$  to  $6 \pm 1$   $L s^{-1}$  in the PECT. Strain

rate also varied significantly among flight modes for both muscles (SUPRA,  $P=0.0042$ ; PECT,  $P=0.0023$ ).

Work loops differed in shape factor between the two muscles and among modes of flight (Fig. 6). For example, in Fig. 6, the shape factors of the work loops in the PECT were  $18 \pm 4\%$  greater than the shape factors for work loops in the SUPRA. This difference in shape factor is consistent with the comparatively steeper shoulders of the SUPRA work loops on either side of peak  $\sigma_m$ .

Among flight modes, net work of the PECT was  $3.2 \pm 0.5$  times greater than that of the SUPRA (mean  $535 \pm 77$  versus  $172 \pm 54$  mJ). Although flight mode had a significant effect upon positive and net work in the SUPRA and the PECT (all  $P < 0.03$ ), negative work, or absorption of external energy by the muscles, did not vary significantly with mode ( $P=0.3381$ , SUPRA;  $P=0.2297$ , PECT; Table 2). The amount of negative work relative to positive work was similar in the two muscles, representing  $16 \pm 3\%$  in the SUPRA and  $19 \pm 2\%$  in the PECT.

SUPRA mass-specific power varied from  $106 \pm 50$   $W kg^{-1}$  during descent to  $194 \pm 98$   $W kg^{-1}$  during ascent ( $P=0.0178$ ; Table 2). Expressed as  $P_{mus}$  and doubled to represent the output from both muscles, the mean among flight modes was  $3 \pm 1$  W (Fig. 7). These measurements of  $P_{mus}$  were 2.5 and 2.3 times greater than our estimates of  $P_{iner}$  required from the SUPRA muscles for upstroke during level and ascending flight, respectively. However, the estimated  $P_{iner}$  required from the muscles was within 1 s.d. of  $2P_{mus}$  (Fig. 7).

In contrast, the  $P_{mus}$  in the PECT, doubled to represent both muscles, was much less ( $44 \pm 3\%$ ) than our estimates of  $P_{aero}$  (Fig. 7). PECT mass-specific power was also  $36 \pm 9\%$  less than mass-specific power in the SUPRA (Table 2). Mass-specific power in the PECT varied among flight modes ( $P=0.0124$ ) and was maximal during ascent at  $105 \pm 14$   $W kg^{-1}$ .

## Discussion

Most aspects of our four hypotheses pertaining to the comparative function of the SUPRA and PECT were supported by our results: (1) Peak  $\sigma_m$  in the SUPRA occurred at the transition between

Table 2. Contractile properties of the SUPRA and PECT muscles in pigeons (*Columba livia*) during different modes of flight

Variable	Level	Ascending	Descending	<i>P</i>
SUPRA peak stress (kPa)	96 $\pm$ 48	125 $\pm$ 65	85 $\pm$ 30	0.0463
SUPRA strain ( $\epsilon$ )	35 $\pm$ 10	40 $\pm$ 14	33 $\pm$ 10	0.0039
SUPRA fractional lengthening (%)	7 $\pm$ 6	12 $\pm$ 8	6 $\pm$ 5	0.0105
SUPRA fractional shortening (%)	-27 $\pm$ 7	-27 $\pm$ 6	-27 $\pm$ 6	0.0760
SUPRA strain rate ( $L s^{-1}$ )	6 $\pm$ 1	7 $\pm$ 2	5 $\pm$ 2	0.0042
SUPRA net work (mJ)	153 $\pm$ 78	234 $\pm$ 144	130 $\pm$ 52	0.0145
SUPRA positive work (mJ)	180 $\pm$ 93	273 $\pm$ 167	164 $\pm$ 66	0.0152
SUPRA negative work (mJ)	-26 $\pm$ 32	-39 $\pm$ 53	-33 $\pm$ 31	0.3381
SUPRA mass-specific power ( $W kg^{-1}$ )	127 $\pm$ 57	194 $\pm$ 98	106 $\pm$ 50	0.0178
SUPRA energy recovery (mJ)	51 $\pm$ 62	88 $\pm$ 85	35.8 $\pm$ 29	0.0548
PECT peak stress (kPa)	53 $\pm$ 15	58 $\pm$ 15	50 $\pm$ 12	0.0017
PECT strain ( $\epsilon$ )	36 $\pm$ 8	42 $\pm$ 8	35 $\pm$ 9	0.0008
PECT fractional lengthening (%)	28 $\pm$ 7	28 $\pm$ 7	26 $\pm$ 8	0.3016
PECT fractional shortening (%)	-8 $\pm$ 3	-14 $\pm$ 4	-9 $\pm$ 5	0.0452
PECT strain rate ( $L s^{-1}$ )	5 $\pm$ 1	6 $\pm$ 1	5 $\pm$ 1	0.0023
PECT net work (mJ)	531 $\pm$ 208	614 $\pm$ 133	460 $\pm$ 133	0.0267
PECT positive work (mJ)	642 $\pm$ 228	748 $\pm$ 181	587 $\pm$ 155	0.0293
PECT negative work (mJ)	-110 $\pm$ 97	-134 $\pm$ 105	-127 $\pm$ 85	0.2297
PECT mass-specific power ( $W kg^{-1}$ )	87 $\pm$ 25	105 $\pm$ 14	75 $\pm$ 24	0.0124

SUPRA, supracoracoideus; PECT, pectoralis. Values are means  $\pm$  s.d.,  $N=7$  (except ascending  $N=6$ ), *P* value calculated using repeated measures ANOVA (d.f.=5,2).

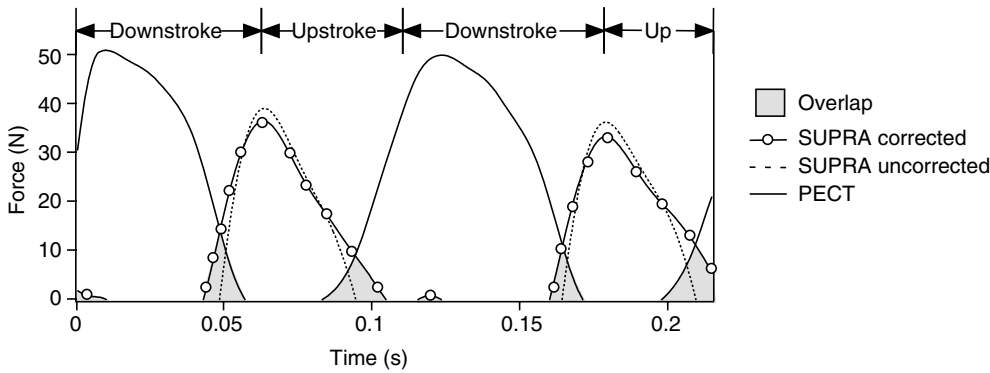


Fig. 4. Strain gauge recordings from the SUPRA and PECT of a pigeon (*Columba livia*) illustrating antagonistic force development at the end of downstroke and the end of upstroke. Overlap of force production was apparent even when SUPRA recordings were not corrected for cross-talk from the PECT.

downstroke and upstroke (Figs 2–4); (2) the SUPRA stored substantial elastic energy, ranging from 36 to 88 mJ (Table 2, Fig. 5A); (3) there was overlap of antagonistic force at the end of each half stroke (Figs 2–4); and (4)  $P_{\text{mus}}$  in the SUPRA was close to the estimated  $P_{\text{iner}}$  for upstroke (Fig. 7). However, two inconsistencies with our predictions were apparent. Contrary to hypothesis (2),  $\epsilon$  in the SUPRA was over 30% and only slightly less than in the PECT. Also, contrary to (4), power output in the PECT was less than half of the estimated  $P_{\text{aero}}$ .

These discrepancies with our predictions emphasize the role of proximal muscles as producers of work and indicate that independent methods are needed to further explore  $P_{\text{mus}}$  for the pectoralis and  $P_{\text{aero}}$  in bird flight.

Our *in vivo* experiments show that a major role of the SUPRA is for supination of the humerus at the end of downstroke, entirely consistent with the *in situ* experiments of Poore et al. (Poore et al., 1997). Peak  $\sigma_m$  occurred at wing turnaround (Fig. 2B, Figs 3 and

4), and the shapes of the work loops generated by the SUPRA (Fig. 6) revealed that stress declined rapidly during mid- and late upstroke when wing elevation was occurring (Tobalske and Dial, 1996; Tobalske, 2000). Poore et al. (Poore et al., 1997) argued that long-axis rotation of the humerus was a critical step during the evolution of flapping flight. Nevertheless, our data show that the SUPRA also elevates the wing, as  $\sigma_m > 0$  Pa throughout the upstroke (Figs 2–4).

Given the complexity of the musculature of the avian wing (Dial, 1992a; Baumel et al., 1993), it is sobering that our predictions of function from anatomy were only partially correct. This suggests that *in vivo* studies are required to adequately understand muscle function. It would be more convenient if all muscles could be neatly categorized as either force producers or work producers; however, such a simple dichotomy is often unlikely to be the case. Given the morphology of the SUPRA (Fig. 1A) and patterns exhibited by terrestrial animals with leg muscles featuring long tendons of insertion [e.g. tammar wallabies, *Macropus eugenii* (Biewener and Baudinette, 1995)], we expected low  $\epsilon$  in the muscle. Our measurements of relatively large  $\epsilon$  (Table 2, Fig. 5) are consistent with a compromise in muscle design that permits the SUPRA to generate work and power to match  $P_{\text{iner}}$  for upstroke (Fig. 7), while at the same time favoring economical force generation (Biewener and Roberts, 2000). As the SUPRA is a proximal muscle, our results are similar to those of experiments in mammalian terrestrial locomotion in which greater  $\epsilon$  is exhibited by proximal muscles of the limb compared with distal muscles (Gregersen et al., 1998; Gillis and Biewener, 2001).

Elastic energy recovered from the SUPRA tendon is a novel result for muscles of the avian wing. Aside from the role of the furcula (Jenkins et al., 1988), elastic energy storage is not presently recognized as a mechanism available to flying birds (Harrison and Roberts, 2000). Storage and recovery of energy in tendons saves energy during terrestrial locomotion (Taylor and Heglund, 1982; Alexander, 1988; Biewener and Baudinette, 1995; Baudinette and Biewener, 1998; Biewener and Roberts, 2000). Energy recovery ( $U_{\text{rec}}$ ) of the order of 8% relative to the combined work output of the SUPRA and PECT (Table 2) should be included when estimating the efficiency of bird flight. At 32% of the net work of the SUPRA,  $U_{\text{rec}}$  approached the lower end of the range of recovery reported for the extensor tendons in the legs of tammar wallabies (38–52%) (Biewener and Baudinette, 1995). The capacity for energy storage merits further study in other muscles of the wing that exhibit long tendons of insertion, including the tensor propatagialis longus and flexors and extensors of the distal wing (Dial, 1992a; Baumel et al., 1993).

It is widely recognized that co-activation of antagonist muscles provides stability about a musculoskeletal joint, such as the knee

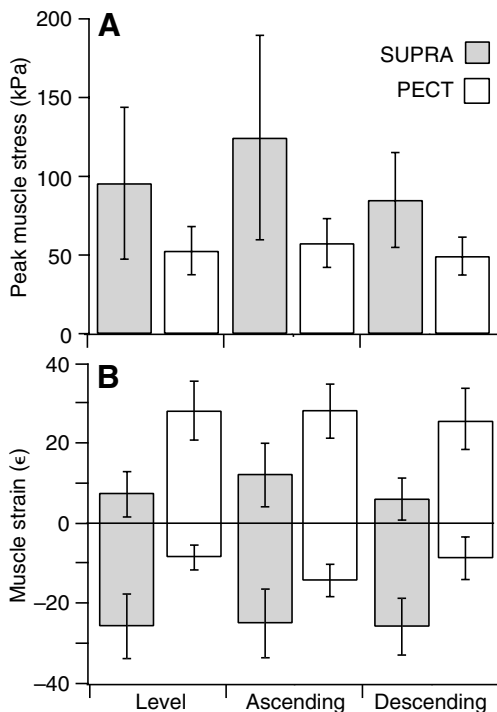


Fig. 5. (A) Peak stress ( $\sigma_m$ ) in the SUPRA and PECT of pigeon (*Columba livia*) during different modes of flight. (B) Fractional length changes in the SUPRA and PECT according to mode of flight. Resting length is indicated by the origin. Values are means  $\pm$  s.d.,  $N=7$  (except ascending  $N=6$ ).

(Baratta et al., 1988; Kellis, 1998), and improves the accuracy of arm movements (Suzuki et al., 2001; Gribble et al., 2003). However, Poore et al. (Poore et al., 1997) were the first to suggest that antagonistic forces of the SUPRA and PECT might function to improve wing control during the rapid wing oscillations that occur during bird flight. Our results provide the first direct evidence for such a role (Figs 2–4). Whereas the two muscles are activated simultaneously during gliding flight (Tobalske, 1995; Tobalske, 2000), neuromuscular activation of these muscles does not overlap during flapping flight. Instead, because the decay of force within

each muscle substantially lags the offset of EMG activity (Fig. 3), a significant overlap of antagonistic force occurs at wing turnaround.

The SUPRA generated work and power sufficient to meet the inertial requirements of wing upstroke (Fig. 7), which were within 1 s.d. of combined  $P_{\text{mus}}$ . Based on kinematic inferences, there has been some debate over the aerodynamic function of upstroke during slow flight in pigeons and other birds with wings of relatively high aspect ratio (Tobalske, 2000). Our experiments provide new evidence that the upstroke is largely an aerodynamically inactive

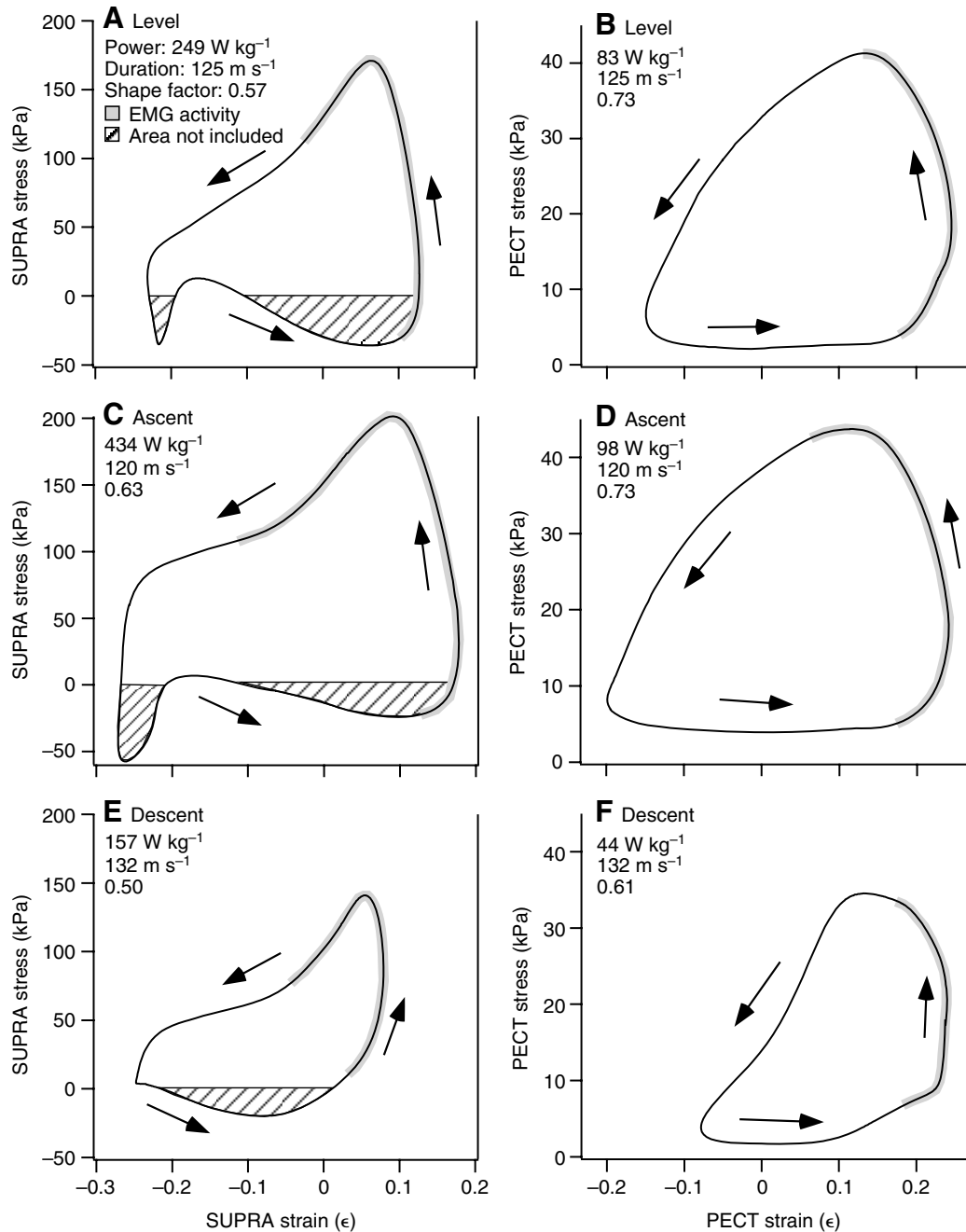


Fig. 6. Representative work loops from the SUPRA and PECT muscles of a pigeon (*Columba livia*) engaged in (A,B) level, (C,D) ascending and (E,F) descending flight. Arrows indicate direction of contraction; bold lines indicate EMG activity. The hatched areas feature artificial negative stress ( $\sigma_m$ ) due to compression of the SUPRA strain gauge by cross-talk from PECT force that remained even after a correction factor was applied; these areas were not included in the analysis.



recovery; additional evidence includes the wake analysis of Spedding et al. (Spedding et al., 2003) on a thrush nightingale (*Luscinia luscinia*) and pressure measurements made about the wings of pigeons by Usherwood et al. (Usherwood et al., 2005). The small discrepancy between observed SUPRA  $P_{\text{mus}}$  and estimated  $P_{\text{iner}}$  (Fig. 7) suggests that any induced or profile power requirements of upstroke are <10% of  $P_{\text{aero}}$  for slow flight.

Our measurements of PECT  $P_{\text{mus}}$ , at 44% of estimated  $P_{\text{aero}}$ , are enigmatic. We reported similar results previously (Biewener et al., 1998), wherein mass-specific power from the PECT was  $70.2 \text{ W kg}^{-1}$  in level flight, slightly less than the  $87 \text{ W kg}^{-1}$  we observed here (Table 2). In contrast, Soman et al. (Soman et al., 2005) recently obtained measurements of  $207 \text{ W kg}^{-1}$  for level flight of pigeons under similar conditions. Their analysis used positive work rather than net work to calculate  $P_{\text{mus}}$ . Calculated in this way, our measurement of mass-specific power during level flight (Table 2) would be  $105 \text{ W kg}^{-1}$ , and  $108 \text{ W kg}^{-1}$  in an earlier study (Biewener et al., 1998). Mean PECT  $\epsilon$  during level flight is similar among these studies: 36.2% in the present study (Table 2), 32% in Biewener et al. (Biewener et al., 1998) and 31.9% in Soman et al. (Soman et al., 2005). Likewise, morphology, wingbeat frequency and general work loop shapes are similar among the studies. Thus, differences in  $P_{\text{mus}}$  are due to calibrations of the strain gauges used to calculate PECT force.

Uncertainties over pull calibrations were previously reported for other experiments (Hedrick et al., 2003; Tobalske et al., 2003b), in which pull calibrations were abandoned and aerodynamic models were instead used to calibrate force. We hypothesize that our measurements of PECT force in pigeons were low because superficial, cranial PECT fibers adjacent to the DPC exerted a disproportionately large bending moment on the crest during our pull calibrations. In contrast, pull calibrations of the SUPRA appeared less sensitive to the location along the tendon at which we pulled. The insertion of the SUPRA is restricted in area, and the tendon passes through a foramen triosseum, which restricts the line of action upon the humerus (Baumel et al., 1993). Nevertheless, the potential inaccuracy of the pull calibrations means that caution is necessary when interpreting our reported  $\sigma_m$ , work and  $P_{\text{mus}}$  for both muscles.

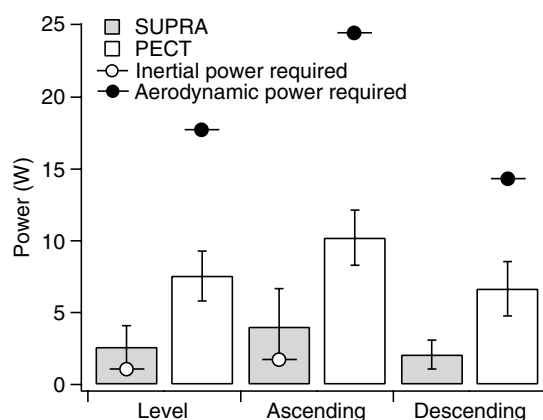


Fig. 7. Mean muscle power output ( $P_{\text{mus}}$ ) measured in the SUPRA and PECT muscles of the pigeon (*Columba livia*) during different modes of flight compared with the estimated inertial power requirement ( $P_{\text{iner}}$ ) for upstroke and aerodynamic power requirement ( $P_{\text{aero}}$ ) assuming that all lift is provided during downstroke. Values for  $P_{\text{mus}}$  are doubled to represent output from paired left and right muscles. Values are means  $\pm$  s.d.,  $N=7$  (except ascending  $N=6$ ).

The accuracy of aerodynamic models of slow flapping flight is uncertain because unsteady effects may dominate the local flow field and make quasi-steady models inaccurate (Spedding, 1993; Dickson and Dickinson, 2003). Other empirical studies, independent of sonomicrometry and strain gauge technology, also show deficits in power output relative to required power. Early flow measurements identified only 60% of the necessary momentum in the wake to account for weight support in the pigeon (Spedding et al., 1984). Higher resolution of the flow field may eliminate this measurement deficit (Spedding et al., 2003), but attempts have not yet been undertaken using pigeons. Differential pressure transducers on the wings and tails of pigeons yield measurements of power output sufficient to support 82% of body weight, with an estimated mass-specific power of  $273 \text{ W kg}^{-1}$  under similar level, slow flight conditions (Usherwood et al., 2005).

It appears promising that *in vitro* measurements of  $P_{\text{mus}}$  in the PECT of blue-breasted quail (*Coturnix chinensis*) exceed  $P_{\text{aero}}$  predicted using quasi-steady aerodynamic models (Askew and Marsh, 2001; Askew et al., 2001). These measurements use an ergometer to measure force in isolated fascicle bundles that are stimulated and strained according to *in vivo* EMG and sonomicrometry data but, even so, neglect large negative work components measured *in situ* that are judged to be artifacts of the technique (Askew and Marsh, 2001). With integration of *in vitro* work loop techniques (Askew et al., 2001), higher resolution analysis of wake dynamics (Spedding et al., 2003; Warrick et al., 2005) and novel measurements of local pressure (Usherwood et al., 2005), together with continued development of DPC strain-force recordings, an improved understanding of the aerodynamics of flight in birds should emerge. Nevertheless, our combined force, length change and activation recordings of the PECT and SUPRA reveal novel functions of these two muscles that depend on *in vivo* observations of muscle function.

#### List of symbols and abbreviations

$\alpha$	fascicle angle
$\epsilon$	muscle strain
$\rho$	muscle density
$\sigma_t$	tendon stress
$\sigma_m$	muscle stress
$A$	muscle cross-sectional area
$E$	elastic modulus
$L$	fascicle length
$L_{\text{rest}}$	fascicle length at rest
$M$	muscle mass
$P_{\text{aero}}$	aerodynamic power
$P_{\text{iner}}$	inertial power
$P_{\text{mus}}$	muscle power
$U_{\text{rec}}$	elastic energy recovery
$V_t$	tendon volume

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#### References

- Ahn, A. N., Monti, R. J. and Biewener, A. A. (2003). *In vivo* and *in vitro* heterogeneity of segment length changes in the semimembranosus muscle of the toad. *J. Physiol.* **549**, 877-888.
- Alexander, R. McN. (1983). *Animal Mechanics* (2nd edn). London: Blackwell Scientific.
- Alexander, R. McN. (1988). *Elastic Mechanisms in Animal Movement*. Cambridge: Cambridge University Press.

- Askew, G. N. and Marsh, R. L. (2001). The mechanical power output of the pectoralis muscle of blue-breasted quail (*Coturnix chinensis*): *in vivo* length cycle and its implications for muscle performance. *J. Exp. Biol.* **204**, 3587-3600.
- 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.
- Baratta, R., Solomonow, M., Zhou, B. H., Letson, D., Chuinard, R. and D'Ambrosia, R. (1988). Muscular coactivation. The role of the antagonist musculature in maintaining knee stability. *Am. J. Sports Med.* **16**, 113-122.
- Baudinette, R. V. and Biewener, A. A. (1998). Young wallabies get a free ride. *Nature* **395**, 653-654.
- Baumel, J. J., King, A. S., Breazile, J. E. and Evans, H. E. (ed.) (1993). *Handbook of Avian Anatomy: Nomina Anatomica Avium*. Cambridge, MA: Publications of Nuttall Ornithological Club 23.
- Bennett, M. B., Ker, R. F., Dimery, N. J. and Alexander, R. McN. (1986). Mechanical properties of various mammalian tendons. *J. Zool. Lond.* **209**, 537-548.
- Biewener, A. A. (1998). Muscle function *in vivo*: a comparison of muscles used for elastic energy savings versus muscles used to generate mechanical power. *Am. Zool.* **38**, 703-717.
- Biewener, A. A. and Baudinette, R. V. (1995). *In vivo* muscle force and elastic energy storage during steady-speed hopping of tammar wallabies (*Macropus eugenii*). *J. Exp. Biol.* **198**, 1829-1841.
- Biewener, A. A. and Roberts, T. J. (2000). Muscle and tendon contributions to force, work, and elastic energy savings: a comparative perspective. *Exerc. Sport Sci. Rev.* **28**, 99-107.
- 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.
- Boggs, D. F. and Dial, K. P. (1993). Neuromuscular organization and regional EMG activity of the pectoralis in the pigeon. *J. Morphol.* **218**, 43-57.
- Degernes, L. A. and Feduccia, A. (2001). Tenectomy of the supracoracoideus muscle to deflect pigeons (*Columba livia*) and cockatiels (*Nymphicus hollandicus*). *J. Avian Med. Surg.* **15**, 10-16.
- 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.
- Dial, K. P. and Biewener, A. A. (1993). Pectoralis muscle force and power output during different modes of flight in pigeons (*Columba livia*). *J. Exp. Biol.* **176**, 31-54.
- Dickson, W. B. and Dickinson, M. H. (2004). The effect of advance ratio on the aerodynamics of revolving wings. *J. Exp. Biol.* **207**, 4269-4281.
- Gillis, G. B. and Biewener, A. A. (2001). Hindlimb muscle function in relation to speed and gait: *in vivo* patterns of strain and activation in a hip and knee extensor of the rat (*Rattus norvegicus*). *J. Exp. Biol.* **204**, 2717-2731.
- Goldman, D. E. and Heuter, T. F. (1956). Tabular data of the velocity and 1378 absorption of high-frequency sound in mammalian tissues. *J. Acoust. Soc. Am.* **28**, 35-37.
- Gregersen, C. S., Silverton, N. A. and Carrier, D. R. (1998). External work and potential for elastic storage at the limb joints of running dogs. *J. Exp. Biol.* **201**, 3197-3210.
- Gribble, P. L., Mullin, L. I., Cothros, N. and Mattar, A. (2003). Role of cocontraction in arm movement accuracy. *J. Neurophysiol.* **89**, 2396-2405.
- Harrison, J. F. and Roberts, S. P. (2000). Flight respiration and energetics. *Annu. Rev. Physiol.* **62**, 179-205.
- Hedrick, T. L., Tobalske, B. W. and Biewener, A. A. (2002). Estimates of circulation and gait change based on a three-dimensional kinematic analysis of flight in cockatiels (*Nymphicus hollandicus*) and ringed turtle-doves (*Streptopelia risoria*). *J. Exp. Biol.* **205**, 1389-1409.
- 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.
- Hedrick, T. L., Usherwood, J. R. and Biewener, A. A. (2004). Wing inertia and whole-body acceleration: an analysis of instantaneous aerodynamic force production in cockatiels (*Nymphicus hollandicus*) flying across a range of speeds. *J. Exp. Biol.* **207**, 1689-1702.
- Jenkins, F. A., Jr, Dial, K. P. and Goslow, G. E., Jr (1988). A cineradiographic analysis of bird flight: the wishbone in starlings is a spring. *Science* **241**, 1495-1498.
- Josephson, R. K. (1985). Mechanical power output from striated muscle during cyclical contraction. *J. Exp. Biol.* **114**, 493-512.
- Kellis, E. (1998). Quantification of quadriceps and hamstring antagonist activity. *Sports Med.* **25**, 37-62.
- Ker, R. F. (1981). Dynamic tensile properties of the plantaris tendon of sheep (*Ovis aries*). *J. Exp. Biol.* **93**, 283-302.
- 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.
- 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. (1979a). A new approach to animal flight mechanics. *J. Exp. Biol.* **117**, 47-77.
- Rayner, J. M. V. (1979b). A vortex theory of animal flight. 2. The forward flight of birds. *J. Fluid Mech.* **91**, 731-763.
- Rayner, J. M. V. (1985). Mechanical and ecological constraints on flight evolution. In *The Beginnings of Birds: Proceedings of the International Archaeopteryx Conference* (ed. M. K. Hecht, J. H. Ostrom, G. Viohl and P. Wellnhofer), pp. 279-288. Eichstatt: Freunde des Jura-Museums Eichstatt.
- Rayner, J. M. V. (1995). Dynamics of the vortex wakes of flying and swimming vertebrates. In *Symposia of the Society for Experimental Biology XLIX* (ed. C. P. Ellington and T. J. Pedley), pp. 131-155. Cambridge: The Company of Biologists.
- Roberts, T. J., Marsh, R. L., Weyand, P. G. and Taylor, C. R. (1997). Muscular force in running turkeys: the economy of minimizing work. *Science* **275**, 1113-1115.
- Scholey, K. D. (1983). *Developments in vertebrate flight: climbing and gliding of mammals and reptiles, and the flapping flight of birds*. PhD thesis, University of Bristol, UK.
- Shadwick, R. E. (1990). Elastic energy storage in tendons: mechanical differences related to function and age. *J. Appl. Physiol.* **68**, 1033-1040.
- Sokoloff, A. J., Ryan, J. M., Valerie, E., Wilson, D. S. and Goslow, G. E., Jr (1998). Neuromuscular organization of avian flight muscle: morphology and contractile properties of motor units in the pectoralis (*pars thoracicus*) of the pigeon (*Columba livia*). *J. Morphol.* **236**, 179-208.
- Sokoloff, A. J., Gray-Chickering, J., Harry, J. D., Poore, S. O. and Goslow, G. E., Jr (2001). The function of the supracoracoideus muscle during takeoff in the European starling (*Sternus vulgaris*): Maxheinz Sy revisited. In *New Perspectives on the Origin and Early Evolution of Birds: Proceedings of the International Symposium in Honor of John H. Ostrom* (ed. J. Gauthier and L. F. Gall), pp. 319-332. New Haven: Peabody Museum of Natural History.
- 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.
- Spedding, G. R. (1993). On the significance of unsteady effects in the aerodynamic performance of flying animals. *Contemp. Math.* **141**, 401-419.
- Spedding, G. R., Rayner, J. M. V. and Pennycuik, C. J. (1984). Momentum and energy in the wake of a pigeon (*Columba livia*) in slow flight. *J. Exp. Biol.* **111**, 81-102.
- Spedding, G. R., Rosén, M. and Hedenström, A. (2003). A family of vortex wakes generated by a thrush nightingale in free flight in a wind tunnel over its entire natural range of flight speeds. *J. Exp. Biol.* **206**, 2313-2344.
- Suzuki, M., Shiller, D. M., Gribble, P. L. and Ostry, D. J. (2001). Relationship between cocontraction, movement kinematics and phasic muscle activity in single-joint arm movement. *Exp. Brain Res.* **140**, 171-181.
- Taylor, C. R. and Heglund, N. C. (1982). Energetics and mechanics of terrestrial locomotion. *Annu. Rev. Physiol.* **44**, 97-107.
- Tobalske, B. W. (1995). Neuromuscular control and kinematics of intermittent flight in European starlings (*Sternus vulgaris*). *J. Exp. Biol.* **198**, 1259-1273.
- Tobalske, B. W. (2000). Biomechanics and physiology of gait selection in flying birds. *Physiol. Biochem. Zool.* **73**, 736-750.
- Tobalske, B. W. and Dial, K. P. (1996). Flight kinematics of black-billed magpies and pigeons over a wide range of speeds. *J. Exp. Biol.* **199**, 263-280.
- 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., Hedrick, T. L. and Biewener, A. A. (2003a). Wing kinematics of avian flight across speeds. *J. Avian Biol.* **34**, 177-184.
- Tobalske, B. W., Hedrick, T. L., Dial, K. P. and Biewener, A. A. (2003b). Comparative power curves in bird flight. *Nature* **421**, 363-366.
- 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., 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.
- Usherwood, J. R., Hedrick, T. L., McGowan, C. P. and Biewener, A. A. (2005). Dynamic pressure maps for wings and tails of pigeons in slow, flapping flight, and their energetic implications. *J. Exp. Biol.* **208**, 355-369.
- Van den Berg, C. and Rayner, J. M. V. (1995). The moment of inertia of bird wings and the inertial power requirement for flapping flight. *J. Exp. Biol.* **198**, 1655-1664.
- Wakeling, J. M. and Ellington, C. P. (1997). Dragonfly flight. III. Lift and power requirements. *J. Exp. Biol.* **200**, 583-600.
- Warrick, D. R., Bundle, M. W. and Dial, K. P. (2002). Bird maneuvering flight: blurred bodies, clear heads. *Integr. Comp. Biol.* **42**, 141-148.
- Warrick, D. R., Tobalske, B. W. and Powers, D. R. (2005). Aerodynamics of the hovering hummingbird. *Nature* **435**, 1094-1097.