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# Regional patterns of pectoralis fascicle strain in the pigeon *Columba livia* during level flight

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## Summary

**Regional fascicle strains were recorded *in vivo* from the pectoralis of carneau pigeons using sonomicrometry during level slow flight, together with regional electromyography (EMG) and deltopectoral crest (DPC) strain measurements of whole muscle force. Fascicle strain measurements were obtained at four sites within the pectoralis: the anterior (Ant), middle (Mid) and posterior (Post) sternobrachium (SB), and the smaller thoracobrachium (TB). Strains were also recorded along the intramuscular aponeurosis of the pectoralis to assess its 'in-series' compliance with respect to strains of Post SB and TB fascicles. In-series segment strains were also obtained along Ant SB and Mid SB fascicles, which insert directly on the DPC without attaching to the intramuscular aponeurosis.**

**In-series segment strains differed from 2% to 17.2%, averaging differences of 6.1% at the Ant SB site and 1.4% at the Mid SB site. Temporal patterns of in-series fascicle segment strain were similar at both sites. Regional fascicle strains also exhibited similar temporal patterns of lengthening and shortening and were most uniform in magnitude at the Ant SB, Mid SB and TB sites (total strain: 33.7%, 35.9% and 33.2% respectively), but were smaller at the Post SB site (24.4%). Strains measured along the aponeurosis tracked the patterns of contractile fascicle strain but were significantly lower in magnitude (19.1%). Fascicle lengthening strains (+25.4%) greatly exceeded net shortening strains (–6.5%) at all sites.**

**Much of the variation in regional fascicle strain patterns resulted from variation of *in vivo* recording sites among individual animals, despite attempts to define consistent regions for obtaining *in vivo* recordings. No significant variation in EMG activation onset was found, but deactivation of the Ant SB occurred before the other muscle sites. Even so, the range of variation was small, with all muscle regions being activated midway through lengthening (upstroke) and turned off midway through shortening (downstroke). While subtle differences in the timing and rate of fascicle strain may relate to differing functional roles of the pectoralis, regional patterns of fascicle strain and activation suggest a generally uniform role for the muscle as a whole throughout the wingbeat cycle. Shorter fascicles located in more posterior regions of the muscle underwent generally similar strains as longer fascicles located in more anterior SB regions. The resulting differences in fiber length were accommodated by strain in the intramuscular aponeurosis and rotation of the pectoralis insertion with respect to the origin. As a result, longer Ant and Mid SB fascicles were estimated to contribute substantially more work per unit mass than shorter Post SB and TB fascicles. When the mass fractions of these regions are accounted for, our regional fascicle strain measurements show that the anterior regions of the pectoralis likely contribute 76%, and the posterior regions 24%, of the muscle's total work output. When adjusted for mass fraction and regional fascicle strain, pectoralis work averaged 24.7±5.1 J kg$^{-1}$ (206.6±43.5 W kg$^{-1}$) during level slow (~4–5 m s$^{-1}$) flight.**

Key words: muscle, pectoralis, strain, work, flight, bird, sonomicrometry.

## Introduction

The manner in which muscles change length when they contract is critical to how they function. Muscles may either shorten to produce energy or lengthen to absorb energy, or they may limit their length change to generate force more economically and hold position (Biewener and Roberts, 2000; Dickinson et al., 2000; Marsh, 1999; Roberts et al., 1997). In each of these roles, studies examining the length changes of muscles under a variety of locomotive conditions often assume either that fiber length changes during a contraction are uniform throughout the muscle, or that their fractional length changes are similar. Only when all of a muscle's fibers are equal in length are both conditions possible (Gans and DeVree, 1987). By assuming that strain (or fractional shortening) is uniform throughout a muscle, whole muscle work and power

can be calculated based on an average or localized measure of muscle strain. For muscles with variable fiber or fascicle lengths, an assumption of uniform strain can also be used to calculate total muscle work and power based on the muscle's mean fascicle length. If a muscle displays varying recruitment patterns across different motor activities, it also makes them easier to interpret if the strains are uniform. However, an assumption of uniform strain likely oversimplifies regional differences in neuromuscular organization, architecture, recruitment pattern and muscle–tendon dynamics.

Recent cine-MRI imaging studies of the human biceps brachii (Pappas et al., 2002) and soleus (Finni et al., 2003) muscles and X-ray imaging of the rat soleus muscle (Monti et al., 2003), demonstrate non-uniform patterns of fascicle and/or muscle–tendon aponeurosis strain when the human muscles shortened at low to moderate forces and the rat soleus contracted isometrically *in situ*. *In vivo* measurements of strain in human soleus and gastrocnemius muscles using ultrasound also show the compliant nature of aponeurosis and tendon during walking and counter-movement exercises (Fukunaga et al., 2001; Kawakami et al., 2002; Narici et al., 1996). In their study of the human biceps brachii, Pappas et al. (2002) found that distal fascicles near the biceps tendon strained less than those located centrally within the muscle. Similarly, a recent study (Ahn et al., 2003) of a less complex muscle, the semimembranosus of the American toad (*Bufo americanus*, after Linneaus: source for all other taxonomic identifications reported), also showed heterogeneous strain patterns when fascicle segments along the muscle's length were recorded using sonomicrometry under both *in vivo* and *in vitro* conditions. Ahn et al. found that proximal and central fascicle semimembranosus segment strains were much larger and differed substantially in pattern compared with strains recorded in the distal region, similar to the pattern observed in the human biceps brachii (Pappas et al., 2002). Heterogeneous strains in the toad semimembranosus occurred despite the muscle segments exhibiting similar electromyographic (EMG) patterns of activation. The growing evidence that strain heterogeneity may occur along fascicles and between different regions on a whole muscle level reinforces patterns observed previously in single isolated muscle fibers (e.g. Edman and Flitney, 1982; Edman and Reggiani, 1984; Kawakami and Lieber, 2000). Nevertheless, it is noteworthy that other studies have found homogeneous patterns of strain along single fibers (Cleworth and Edman, 1972; Mutungi and Ranatunga, 2000), consistent with general assumptions made in past studies of whole muscle function.

In general, architecturally complex muscles are believed to allow for regional specialization of a muscle's function. Compartmentalization of a single muscle into functionally distinct neuromuscular regions has been described for several larger mammalian muscles, such as the cat gastrocnemius and plantaris (English, 1984; English and Letbetter, 1982), the cat biceps femoris and semitendinosus (Chanaud and MacPherson, 1991; English and Weeks, 1987), the pig masseter (Herring et al., 1979, 1989), and the rat gluteus maximus (English, 1990). Neuromuscular compartments are defined by having discrete motor unit territories and fiber type characteristics, and their presence suggests that the functional role of a muscle may be varied based on regionally differential recruitment. To date, functional specialization within a muscle has largely been based on fiber architecture and histochemical features, motor unit territory, and differential patterns of EMG activation. When combined with recent observations of heterogeneous *in vivo* strain patterns within skeletal muscles, these studies reinforce the view that regional differences in fascicle strain may be correlated with recruitment of distinct neuromuscular compartments to provide varying functional roles for a single muscle.

In light of these observations, the goal of our present study was to investigate regional patterns of *in vivo* fascicle strain and EMG in the pectoralis muscle of the pigeon *Columba livia* in greater detail than has been done previously. The avian pectoralis is a large and architecturally complex muscle, with fibers differing in both orientation and length. In addition to providing the major downward motion of wing for aerodynamic lift, the pectoralis may also rotate (pronate) the wing at the shoulder. The muscle may therefore contract differentially to depress and rotate the wing during different phases of the downstroke, as well as in relation to differing modes of flight (Dial et al., 1988).

The pectoralis muscle of birds is divided into sternobrachial (SB) and thoracobrachial (TB) regions, based on their origins from the body and their attachment to a large central aponeurosis (APON), or membrana intramuscularis, which runs from the muscle's tendinous insertion on the deltopectoral crest (DPC) of the humerus for a distance of about two thirds of the muscle's length (Fig. 1; Baumel, 1993). Whereas the more posterior fibers of the SB and all of the TB fibers insert onto the central aponeurosis, the more anterior SB fibers insert directly onto the DPC. Kaplan and Goslow (1989) showed that the pigeon pectoralis is innervated by two main nerve branches: a rostral branch that innervates the anterior SB and a caudal branch that innervates the posterior SB and TB. This led Boggs and Dial (1993) to hypothesize a rostro-caudal pattern of activation and functional recruitment from more anterior SB to more posterior SB and TB motor units of the muscle (Sokoloff et al., 1998). Boggs and Dial (1993) observed that EMG recordings obtained from multiple regions of the pectoralis SB and TB during take-off, level slow flight, and landing flight appeared to support this hypothesis, with TB fibers being activated later than the most anterior SB fibers.

In a related study, Biewener et al. (1998) examined *in vivo* fascicle strains in the anterior and middle SB regions of the pigeon pectoralis (termed 'anterior' and 'posterior' in their study) in relation to DPC measurements of muscle force obtained under similar conditions of slow level flight. Significant differences in the magnitude and pattern of fascicle length change relative to muscle force (i.e. 'work loop' behavior) were observed, in part due to a difference of 6% strain between the two sites (relative to a total fascicle strain range of 32%). Temporal patterns of EMG activation relative

to fascicle strain were uniform. Although this earlier study provided an initial assessment of fascicle strain patterns at these two sites, it did not assess broader patterns of regional muscle strain, particularly in more posterior regions of the pectoralis. The goal of the present study was to expand our analysis of regional *in vivo* fascicle strain patterns within the avian pectoralis, in addition to assessing 'in-series' segment strains along individual fascicles. As before, measurements of regional strain and activation pattern are related to the muscle's role in producing motions of the wing at the shoulder associated with generating aerodynamic power for flight.

In carrying out this analysis, we test two hypotheses. The first is that in-series segment strains are uniform along the length of a fascicle. This is generally assumed in studies of whole muscle function. The second hypothesis is that regional fascicle strains within the pigeon pectoralis vary inversely with fascicle length. That is, we hypothesize that short fascicles undergo larger strains than long fascicles. Our second hypothesis arises from the fact that, despite its extremely broad origin (arising from the thoracic ribs, enlarged sternal carina and furcula), the pectoralis has a short focal tendinous insertion on the ventral surface of the DPC (Baumel, 1993), requiring that fascicles of different length must accommodate (i.e. produce) similar whole muscle length changes. This suggests that shorter, more posterior SB and TB fascicles may undergo greater strains than longer fascicles located in the anterior and middle SB regions. Alternatively, it is possible that variation in the series elastic compliance among fascicles within the muscle and rotation of the muscle, as a whole, may allow shorter fascicles to strain similarly to long fascicles. Because shorter TB and posterior SB fascicles insert along the length of the muscle's intramuscular aponeurosis, the muscle's aponeurosis likely represents a significant component of the series elastic compliance of these posterior fascicles. This component is lacking with respect to more anterior SB fibers, which do not insert along the aponeurosis. Hence, our alternative hypothesis is that regional fascicle strains are uniform despite differences in fascicle length. To explore this we made *in vivo* strain recordings along the aponeurosis, in addition to those made along the fascicles.

Hypotheses of uniform strain along the length of a fascicle and among different regions within a muscle not only assume uniform strain within sarcomeres of individual fibers, but also between fibers that are arranged in-series, or overlap, and do not traverse the entire length of the fascicle(s). This is the case for fibers within the avian pectoralis (Gaunt and Gans, 1993; Trotter et al., 1992), as well as for other long fibered, strap-like muscles in certain mammals and amphibians (see Trotter et al., 1995, for a review). Consequently, whereas force transmission *via* shear transfer between adjacent contracting muscle fibers and their surrounding connective tissue matrix may ensure more uniform strain and effective force transmission along the muscle's length, an in-series fiber arrangement raises the possibility that heterogeneous patterns of strain may arise along the length of a muscle's fascicles. Assessing the nature and magnitude of strain along a fascicle's length and among different regions of a muscle are of considerable significance for calculating the work and power that a muscle performs. Often this is based either on an indirect assessment of total muscle length change or, more recently using sonomicrometry, one or two localized measures of *in vivo* fascicle strain (Askew et al., 2001; Biewener and Corning, 2001; Biewener et al., 1998; Daley and Biewener, 2003; Gabaldón et al., 2004; Hedrick et al., 2003; Roberts et al., 1997; Tobalske et al., 2003), from which whole muscle length change is calculated assuming uniform strain throughout the muscle. If fascicle strains vary inversely with their length (hypothesis 2), then a localized measurement may be prone to underestimating or overestimating total muscle work and power.

The avian pectoralis is an ideal muscle for examining in-series and regional fascicle strain patterns using sonomicromety because its superficial fascicles are accessible to study. Given the muscle's complex architecture and central role in flight, quantification of its regional pattern of fascicle strain not only offers an important test of strain uniformity on a whole muscle level and allows fascicle contractile strain patterns to be related to intramuscular series elastic compliance, but provides insight into whether neuromuscular recruitment patterns may be linked to the pectoralis' role in the control of wing motion for maneuvering and unsteady flight, as well as serving as the main flight motor for generating aerodynamic power.

## Materials and methods

### *Animals and training*

Six white carneau pigeons *Columba livia* (body mass 556.1±25.1 g, mean ± S.D.) were purchased from the Palmetto Pigeon Plant (Sumter, SC, USA) and housed in a 2 m×8 m×2 m outdoor aviary at the Harvard University Concord Field Station (Bedford, MA, USA). The birds were provided with food and water *ad libitum*. All training and experimental procedures were approved by the Harvard University Institutional Animal Care and Use Committee. The birds were trained to fly between two wooden platform perches (0.3 m×0.4 m) over a distance that varied from 3.5 m to 6 m. The perches were positioned 1.0 m above the floor and located within a hallway measuring 1.9 m×4.2 m×12 m, width×height×length. Training occurred in 1 h long sessions 2–3 times per week, beginning 2 weeks prior to surgery and recording. Netting placed at a height of 2.5 m prevented the birds from landing upon other possible perches. Clapping typically sufficed to encourage the birds to fly between the perches. The flight path within the hallway allowed the birds to achieve steady slow flight of ~5 m $s^{-1}$ midway over each trial.

### *Surgical procedures*

After training was complete sterilized sonomicrometry (SONO) crystals and electromyography (EMG) electrodes were implanted in different regions of the pectoralis and a

strain gauge was attached to the deltopectoral crest of the humerus. The pigeons were anesthetized using isoflurane administered *via* a mask (3% for induction and 2–3% during surgery, at a flow rate of 0.4 l $min^{-1}$). Once an appropriate level of anesthesia was induced, the feathers over the left shoulder, left pectoralis and upper back were plucked and removed. Surgical areas were isolated by taping back surrounding feathers and scrubbed with betadine solution. A 2–3 cm incision was then made in the skin over the pigeon's back between its wings, and a 6 cm incision was made along the ventral surface of the left pectoralis (near the base of the sternum). Ten 2.0 mm sonomicrometry crystals (38 AWG, SonoMetrics Corp., London, Canada) and four fine-wire bipolar EMG electrodes were then passed from the back, subcutaneously beneath the wing, to the opening over the pectoralis.

The SONO crystals were inserted within the pectoralis along fascicles in four defined regions of the muscle, identified as: anterior sternobrachialis (Ant SB, *N*=5), middle SB (*N*=5), posterior SB (*N*=4), and thoracobrachialis (TB, *N*=4) (Fig. 1). In three of the animals sonomicrometry crystals were implanted along the intramuscular aponeurosis (APO) – also known as the membrana intramuscularis, instead of the Post SB or TB sites. In these cases, the EMG electrode was implanted in muscle fascicles inserting onto the aponeurosis from the posterior SB region of the muscle. In addition to *regional* fascicle strain measurements made at these five sites, we also obtained *in-series* fascicle strain recordings at the Ant SB and Mid SB sites by implanting three crystals along the fascicle, with the central 'pinging' crystal emitting a sound pulse that was received by the more proximal and distal crystals adjacent to it (Fig. 1A).

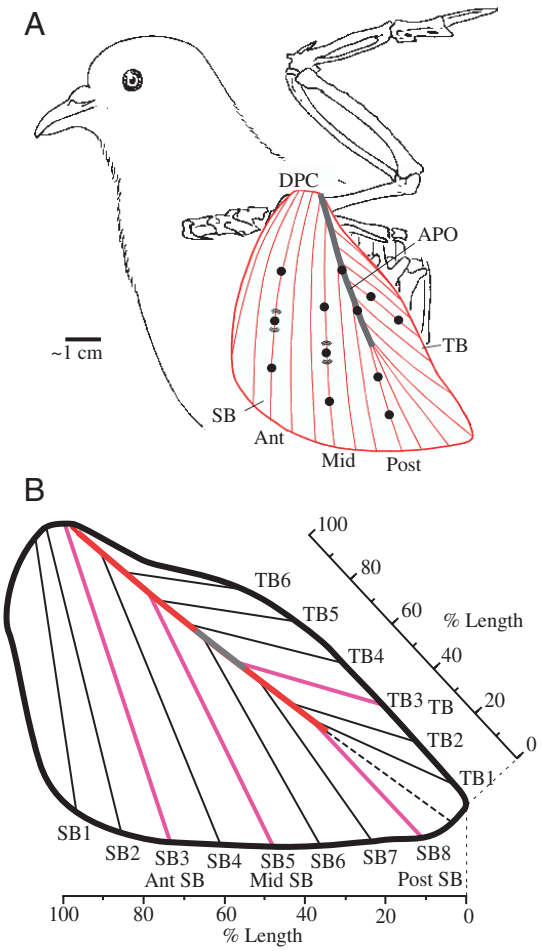
All crystal pairs were implanted at a depth of ~4 mm beneath the superficial fascia of the muscle and at a distance of ~10–12 mm apart. The crystals were implanted in small openings made parallel to the fascicles by puncturing the muscle surface and gently spreading with small sharp-pointed scissors. After being inserted into the muscle, crystal pairs were aligned to ensure maximum signal quality that was displayed on an oscilloscope during the surgery. The openings were then closed using 4-0 silk suture. Suture ties were also made to anchor the sonomicrometry crystal lead wires to the pectoralis ~3–5 mm away from the implantation site to prevent movement artifacts in the recorded signals. Silver fine-wire (0.1 mm diameter, California Fine Wire, Grover Beach, CA, USA), 1 mm bared tip, twisted offset hook, bipolar EMG electrodes were inserted adjacent to the crystal sites using a 23-gauge hypodermic needle and anchored to the pectoralis with 3-0 silk suture. Throughout the procedure, sterile saline was used to keep the muscle moist. After the SONO crystals and EMG electrodes were implanted, the ventral opening over the pectoralis was sutured.

The animal was then positioned to attach a strain gauge to the DPC of the humerus to obtain *in vivo* bone strain recordings of muscle force. The dorsal surface of the DPC was first exposed by making a ~15 mm incision over the left shoulder and retracting the overlying deltoid muscle. A small single-element metal foil strain gauge (1×2 mm active grid, type FLA-1-11; Tokyo Sokki Kenkyujo Co., Ltd., Tokyo, Japan) was then passed subcutaneously from the opening over the back to the opening over the shoulder. Next the periosteum of the DPC was removed by lightly scraping with a scalpel, and the bony surface dried using a cotton applicator dipped in methyl-ethyl ketone. After this, the strain gauge was bonded to the dorsal surface of the DPC, perpendicular to the humeral shaft, using a self-catylizing cyanoacrylate adhesive. During the downstroke, the DPC is pulled ventrally by the contracting pectoralis, so that the dorsal surface develops a principal axis of tensile strain that is nearly perpendicular to the long axis of the humerus (Dial and Biewener, 1993). This makes the strain gauge sensitive to forces produced by the pectoralis but not to other muscle or aerodynamic forces transmitted by the bone between the elbow and the shoulder. This approach has been used in our previous studies (Dial and Biewener, 1993; Biewener et al., 1998; Tobalske et al., 2003) to record *in vivo* flight forces from calibrated DPC bone strain recordings. After bonding the strain gauge to the DPC, a miniature 'back plug', consisting of three Microtech (3xGM-6, Boothwyn, PA, USA) connectors to which the SONO, EMG and strain gauge lead wires were pre-soldered and insulated with epoxy, was secured to thoracic vertebral ligaments over the pigeon's back using 2-0 silk suture. The remaining skin incisions were then sutured with 3-0 silk and the pigeons were allowed a 24 h period for recovery prior to carrying out experimental flight recordings.

### *Experimental recordings*

Multiple (15–26) flight trials were recorded from each bird over the period of the following day, and 5–7 representative trials were selected from each individual for analysis. In each trial, 11 wingbeats representing the three main phases of flight were studied. Based upon the sonomicrometry recordings of muscle fascicle length change, the first three wingbeats of a trial were considered to represent the 'take-off' period of flight, the five middle wingbeats of the trial as 'level flight', and the last three recorded wingbeats as representative of the 'landing' period of flight.

All signals were transmitted from the bird to the recording equipment *via* a lightweight 4.5 m long multi-lead shielded cable (4×6-lead cable, type NMJF6/30-4046SJ, Cooner Wire, Chatsworth, CA, USA) from the back plug connector on the bird. The weight of the unsupported portion of the cable that trailed behind the animal was estimated to be 33.5 g, about 6% of the birds' mean body mass. Outside the flight corridor, the lightweight cable was distributed *via* heavier, shielded cable to the recording amplifiers (one Micromeasurements Vishay 2120A strain gauge bridge amplifier, Raliegh, NC, USA; four Grass P-511 EMG amplifiers, West Warrick, RI, USA; and two 4-channel Triton 120.2 sonomicrometry amplifiers, San Diego, CA, USA). The outputs from these amplifiers were sampled by an A/D converter (Axon Instruments, Digidata 1200, Union City, CA, USA) at 5 kHz and stored on a computer for subsequent analysis. The EMG signals were amplified 1000×




| Site | Position (% length from caudal tip) |
|---|---|
| Ant SB | 75.7±9.2 |
| Mid SB | 43.9±7.2 |
| Post SB | 8.2±19.5 |
| TB | 52.9±14.0 |
| APO | 88.1±8.7 |

Fig. 1. (A) Recording sites within the pigeon pectoralis, showing the locations of the sonomicrometry (SONO) crystals used to record in-series fascicle strain in the anterior (Ant) and middle (Mid) regions of the sternobrachialis (SB) portion of the pectoralis, together with SONO measurements obtained in the posterior (Post) SB and thoracobrachial (TB) portion of the pectoralis to assess regional patterns of fascicle strain. Electromyography (EMG) electrodes were implanted immediately adjacent to the SONO crystal pairs. The middle SONO crystal at the Ant and the Mid sites was used to 'ping' the two opposing receiving crystals to obtain adjacent in-series measurements of fascicle segment strain at these two locations. Additionally, in three birds measurements of aponeurosis (APO) strain were obtained to examine series-elastic stretch of the aponeurosis resulting from forces transmitted to the deltopectoral crest (DPC) from the TB and more posterior regions of the SB. Measurements of overall muscle force were obtained from a strain gauge attached to the dorsal aspect of the DPC, the ventral surface to which the pectoralis has a tendinous insertion. See text for further details. (B) Map of the pectoralis muscle based on digital images obtained from the muscles of the six birds, showing the fascicle architecture and average recording locations (magenta fascicles) for each muscle region. Measurements of fascicle length and pinnation angle relative to the aponeurosis (shown in red) were determined from the images and referenced to a coordinate system at eight defined points along the SB and six points along the TB. To compare across animals, the positions of the recording sites were normalized as a percentage of SB and TB length relative to the caudal tip of the muscle. The locations (mean ± S.D.) of the fascicle recordings sites is shown below.

and filtered (60 Hz notch, 100–3000 Hz bandpass) before sampling. Sampled EMG signals were subsequently bandpass filtered from 40 to 1500 Hz, using a 4th order zero-lag digital butterworth filter. Sonomicrometer and strain gauge recordings were also filtered after sampling, using a 4th order zero-lag digital Butterworth 40 Hz low pass filter to remove high frequency noise introduced by the multiple sonomicrometry leads in the cable.

### *DPC force calibration and morphometrics*

Following the flight trials, the pigeons were euthanized with an overdose of sodium pentabarbitol delivered intravenously *via* the brachial vein. The distal portion of the pectoralis muscle was then exposed beneath its attachment to the ventral surface of the DPC and a 00 silk suture made around the entire muscle. The silk suture was then attached to a force transducer (Kistler 9203, Amherst, NY, USA), and a series of pull calibrations were performed with the bird held by hand and tension applied along the muscle's main axis to the DPC. A linear regression of the rise and fall of DPC strain *versus* measured force, sampled *via* the computer's A/D converter, was used to determine a dynamic calibration for pectoralis force (Biewener et al., 1998).

After calibrating the DPC strain gauge, the entire pectoralis was then exposed to verify EMG electrode and SONO crystal placement. In all cases, the SONO crystals were aligned within 2° of the fascicle axis, so that errors due to misalignment were <0.1%. The pectoralis muscle was then dissected free from its skeletal attachments and photographed using a 5.2 megapixel digital camera, with ruler in view for length calibration. Measurements of pectoralis mass, fascicle length (Table 1) and fascicle orientation relative to each sonomicrometry crystal pair were then obtained using digital calipers and a digital balance. Measurements of fascicle length, pinnation angle, and the coordinate positions of the sonomicrometry recording sites were also determined from the digital images using using NIH ImageJ$^{TM}$ (Fig. 1B). Fascicle length measurements agreed closely with those measured using calipers directly on the muscle. A tracing obtained from the digital image of the pectoralis from pigeon 5 was also used to construct a two-dimensional (2D) geometric model to assess how measured patterns of fascicle and aponeurosis strain related to overall length and shape changes of the muscle during a wingbeat cycle. The model predicted the instantaneous position of the SONO crystals from their resting position and the instantaneous fascicle and aponeurosis strains. Computations for the geometric model were carried out in MATLAB R13 (The MathWorks, Natick, MA, USA). Coordinates for the sonomicrometry recording locations of each muscle were

Table 1. *Morphometric data*

| Pigeon no. | Body mass (g) | Pectoralis mass (g) | Fractional mass | | | | Fascicle length (mm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ant SB | Mid SB | Post SB | TB | Ant SB | Mid SB | Post SB | TB |
| 1 | 576.0 | 54.4 | 0.40 | 0.28 | 0.12 | 0.21 | 87.0 | 58.5 | 52.5 | 46.0 |
| 2 | 543.7 | 46.6 | 0.37 | 0.20 | 0.17 | 0.26 | 83.0 | 76.0 | 60.0 | 41.0 |
| 3 | 548.4 | 52.9 | 0.36 | 0.26 | 0.16 | 0.22 | 89.5 | 94.5 | 54.5 | 39.2 |
| 4 | 553.9 | 48.5 | 0.30 | 0.33 | 0.19 | 0.18 | 83.0 | 77.0 | 63.5 | 40.3 |
| 5 | 521.5 | 55.8 | 0.28 | 0.31 | 0.20 | 0.20 | 73.3 | 66.0 | 52.0 | 45.5 |
| 6 | 592.7 | 54.0 | 0.33 | 0.23 | 0.16 | 0.22 | 78.0 | 70.1 | 54.0 | 42.0 |
| Mean ± S.D. | 556.1±25.1 | 52.0±3.6 | 0.34±0.04 | 0.28±0.05 | 0.17±0.03 | 0.21±0.03 | 82.3±5.9 | 73.7±12.3 | 56.1±4.6 | 42.3±2.8 |

SB, sternobrachial portion of pectoralis; TB, thoracobrachial portion of pectoralis.

referenced to the caudal base of the muscle in order to determine average coordinates for each defined recording site (Ant SB, Mid SB, Post SB, TB and APO) and to assess the variation in recording sites among animals (Fig. 1B).

For the purposes of estimating regional contributions of fascicle strain to overall pectoralis work output, fractional masses of the different pectoralis regions, from which sonomicrometry measurements of fascicle strain had been obtained (Fig. 1), were then determined by sectioning the muscle through its depth midway between the adjacent SONO recording sites and recording the separate masses of the muscle regions on a balance. Fractional masses of the different regions reported in Table 1 were then used to calculate the fractional work component of each region to total muscle work. Even so, this approach relies on using the same time-varying measurement of whole muscle force obtained from our DPC strain recording applied to the individual regional fascicle strain recordings to estimate regional contributions of muscle work.

### *Sonomicrometry*

Sonomicrometry techniques and analysis to measure *in vivo* fascicle length changes followed those of Biewener and Corning (2001) and Biewener et al. (1998). Signals from the Triton 120.2 amplifier were adjusted for the 5 ms delay introduced by the Triton's filter and were corrected for offset errors (underestimate of length: 0.82 mm for the 2.0 mm crystals) introduced by the greater speed of sound through the epoxy coating of each crystal compared with the muscle, as well as a 2.7% correction to account for the greater speed of sound through skeletal muscle (1540 m $s^{-1}$; Goldman and Hueter, 1956) *versus* water (1500 m $s^{-1}$). Fractional length changes, or fascicle strains ($\Delta L_{seg}/L_0$), were calculated based on segment length changes measured between the crystals ($L_{seg}$) relative to their resting length ($L_0$), measured when the birds stood quietly at rest on the perch.

To confirm that the fascicle strains measured during the course of this investigation were representative of particular regions of the muscle, we assessed the repeatability of our measurements by also collecting a second set of SONO measurements from adjacent sites of the anterior and mid SB regions of pigeon 5. In this bird, measurements were first recorded from the Ant SB, Mid SB, Post SB, and the TB regions, after which the crystals in the Ant SB and Mid SB regions were re-implanted ~5 mm rostrally (toward the furcula) relative to their original positions. Following a second 24 h recovery period, experimental recordings were again made for the same flight conditions to compare the adjacent sets of strain measurements obtained at the Ant SB and Mid SB sites.

### *Statistical analysis*

Two-factor analysis of variance (ANOVA) was used to test for differences in regional strain measurements, for strain differences between in-series fascicle strain measurements at the Ant SB and Mid SB sites. The factors considered in the ANOVA analyses were the bird and the fascicle location; we report $F$ and $P$ values for the location factor. Data were considered independent at the trial level, values for a particular trial were determined by computing the mean for all wingbeats in that trial. Results were considered significant at $P<0.05$. All computations were performed using the MATLAB R14 Statistics Toolbox (The MathWorks, Natick, MA, USA).

## Results

### *In-series fascicle strain patterns*

*In vivo* recordings of in-series fascicle segment strains in the anterior and middle sternobrachial (SB) regions of the pigeon pectoralis during level slow flight exhibited generally uniform patterns of strain *versus* time over the course of the wingbeat cycle (Fig. 2). The timing of maximum and minimum strain was similar ($P>0.05$) for all comparisons of in-series fascicle strain at these two sites within the muscle. Whereas strain in the proximal region of the Ant SB site was significantly greater than strain in the distal region ($P<0.007$), both regions strained similarly at the Mid SB site ($P=0.137$; Table 2). Differences in total in-series fascicle strain (lengthening plus shortening) within individual animals ranged from 2.0 to 17.2% strain, averaging 6.1% ($N=6$) between proximal and distal segments at the Ant SB site, and 1.4% ($N=6$) at the Mid SB site across individuals (Fig. 3). In 8 of 12 cases across both sites in the six individuals, proximal segment strains exceeded distal segment strains (Table 2).

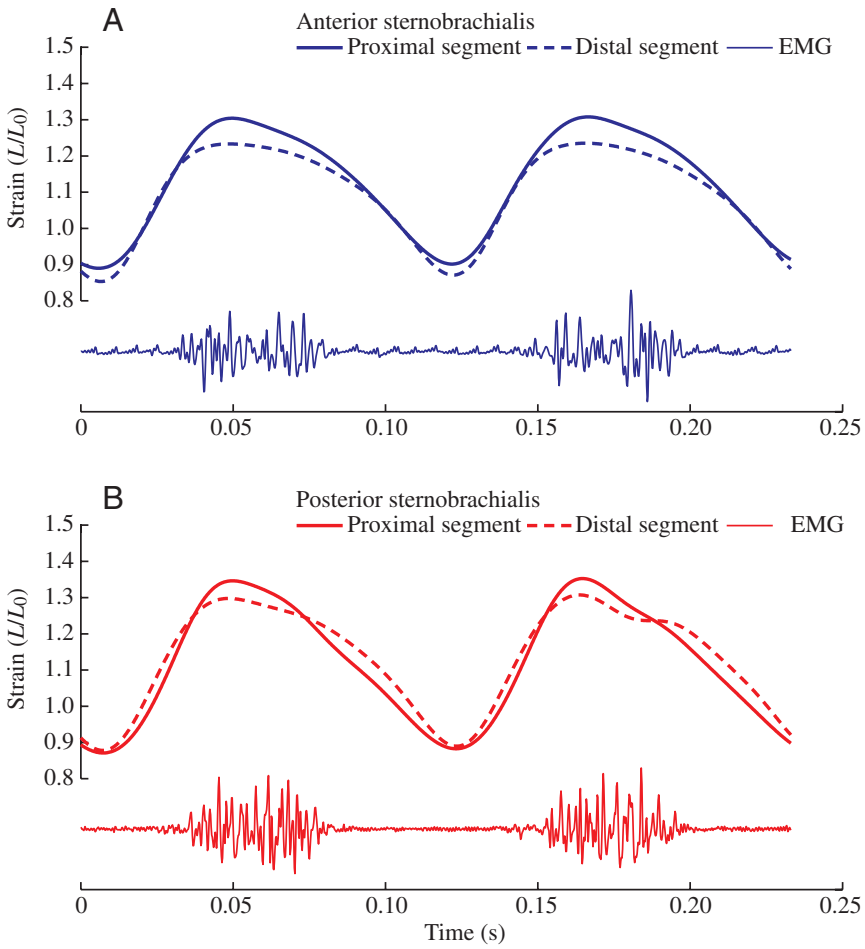



Fig. 2. Representative in-series fascicle segment strain recordings obtained at the anterior and middle SB pectoralis sites for two wingbeats during slow (~4–5 m $s^{-1}$) level flight, together with the EMG recorded from the distal location in each instance. EMG signals were bandpass filtered from 40 to 1500 Hz and sonomicrometer fascicle strain recordings were low-pass (40 Hz) filtered using a 4th order zero-lag digital Butterworth filter. In general, the pattern of fascicle strain ($P$>0.05 for time of maximum and minimum strain) and timing of EMG activation ($P$>0.05) were consistent across proximal and distal sites and at both locations among the birds sampled. In-series fascicle strain magnitude was also generally similar (in the cases shown for pigeon 3, distal fascicle strain averaged 3.5% less than that recorded from the proximal site), with differences in strain magnitude averaging less than 3.8% when proximal and distal sites were compared across all individuals at both locations sampled (see Table 2).

### *Regional strain patterns*

Regional *in vivo* fascicle strain recordings obtained from different sites distributed throughout the pectoralis also exhibited consistently similar patterns of strain with respect to the timing of muscle activation and force development (Fig. 4). Although differing sites exhibited variation in the magnitude of lengthening and (net) shortening strain (Figs 4 and 5; Table 3), all sites lengthened and shortened at generally similar times. Similar temporal patterns of strain also corresponded to uniform patterns of EMG activation (Figs 4A,B and 6). Fascicles within the TB region generally showed the largest difference in strain pattern, exhibiting a slight delay in the onset of fascicle shortening. In addition, lengthening strains recorded from the Post SB region were lower in magnitude than lengthening strains recorded at the other fascicle sites, resulting in lower overall Post SB strains. Strains measured along the intramuscular aponeurosis (Figs 4B,C and 5) were substantially less than those recorded along fascicles at all muscle sites. Figs 4C and 6 also show that APO strains are slightly delayed in timing; i.e. shortening begins and ends slightly later than in the fascicles. However, the temporal pattern of APO lengthening and shortening generally mirrored fascicle strain patterns and was not

Table 2. *In-series total fascicle strain data based on level flight measurements*

| | Anterior sternobrachialis | | | | Middle sternobrachialis | | | |
|---|---|---|---|---|---|---|---|---|
| Pigeon no. | Distal | Proximal | d.f. | $P$ | Distal | Proximal | d.f. | $P$ |
| 1 | 0.285±0.023 | 0.455±0.049 | 64 | <0.0001 | 0.297±0.027 | 0.356±0.029 | 64 | <0.0001 |
| 2 | 0.334±0.061 | 0.357±0.040 | 92 | 0.0353 | 0.367±0.039 | 0.280±0.031 | 92 | <0.0001 |
| 3 | 0.367±0.028 | 0.400±0.021 | 62 | <0.0001 | 0.439±0.037 | 0.457±0.039 | 72 | 0.0562 |
| 4 | 0.362±0.044 | 0.190±0.023 | 58 | <0.0001 | 0.310±0.039 | 0.360±0.050 | 58 | 0.0001 |
| 5 | 0.228±0.029 | 0.381±0.041 | 66 | <0.0001 | 0.370±0.047 | 0.350±0.038 | 66 | 0.0571 |
| 6 | 0.280±0.023 | 0.436±0.067 | 84 | <0.0001 | 0.420±0.037 | 0.318±0.044 | 72 | <0.0001 |
| Mean ± S.D. | 0.309±0.054 | 0.370±0.095 | 10 | 0.2056 | 0.367±0.057 | 0.353±0.059 | 10 | 0.6876 |

Measurements (32<$N$<47 wingbeats per individual) were obtained from the five middle wingbeats of each flight trial (see Materials and methods).

Results are individual means ± S.D., with statistical comparisons made between proximal and distal fascicle sites within individuals and the group means ($N$=6) based on two-tailed $t$-tests.

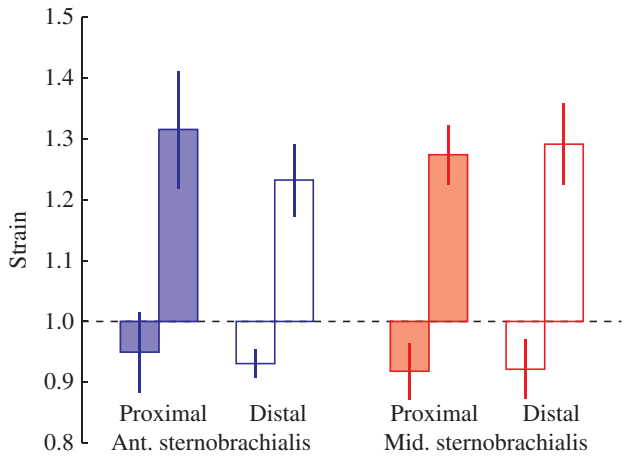



Fig. 3. Summary histogram showing in-series fascicle strains (mean ± S.D.) measured at the anterior and middle SB regions of the pectoralis among the five birds sampled.

significant different. Significant variation ($P$<0.0001) in regional pectoralis strain magnitude was observed (Table 3). When compared across all animals for which recordings were obtained at each muscle site, total strains at the Ant SB, Mid SB and TB sites were generally uniform, averaging 33.7±4.2%, 35.9±4.6%, and 33.2±5.8%, in contrast to 24.4±6.0% at the Post SB site and 19.1±3.9% along the aponeurosis. For all sites, the magnitude of lengthening strain (mean for all sites: +25.4±4.7%) greatly exceeded net shortening strain (–6.5±0.9%) relative to the muscle's resting length (Figs 4, 5).

### *Implications for pectoralis shape change*

Based on the average strains recorded at each fascicle site and along the aponeurosis among the six pigeons, we constructed a two-dimensional geometric model of the pectoralis to assess how the recorded patterns of muscle strain related to its overall length and shape changes during a wingbeat cycle. Variation among animals in the recording locations of the regional fascicle strain measurements determined from the digital images was substantial (Fig. 1B), and likely contributed to some of the inter-individual variation in strain magnitudes recorded at each site. Fig. 7 shows the results of our model for three time points during the wingbeat cycle: maximum strain (for the mid SB site, start of downstroke), maximum pectoralis force (measured from the DPC strain gauge), and minimum strain (for the mid SB site, end of downstroke). At maximum strain (Fig. 7A), the displacement of the more anterior fascicles, relative to their resting position, and their distal attachment to the DPC is mainly in a superior direction, consistent with the humerus and wing being in their maximally elevated position at this time. Additionally, there is evidence of a posterior displacement of the Ant and Mid SB fascicles, suggesting humeral retraction in combination with elevation during the upstroke. Strain in the TB fascicles also results in an upward but more anterior displacement, whereas strain of the post SB region is mainly in the anterior direction. The strain patterns and resulting displacements suggested by the 2D muscle model indicate that significant fascicle rotation occurs, in addition to lengthening strain. Strains and displacements of the muscle at the time of peak pectoralis force (Fig. 7B) are consistent with past observations in pigeons and other birds that the pectoralis develops peak force when it remains stretched near to its maximum length approximately one-third through the downstroke. At minimum strain (Fig. 7C), the displacement of the fascicles is generally posterior to their resting positions. In all but the TB site, the displacements are also in a ventral direction, consistent with maximal depression of the humerus and arm wing at this time. The TB site suggests a relative dorsal movement, but this (and the modeled 'wrinkling' of the aponeurosis along its length) is likely the result of our simple 2D model of the muscle, which ignores out-of-plane strains that are certain to affect the muscle's overall geometry. Displacements at minimum strain are small relative to those at maximum strain, consistent with fascicle lengthening contributing fourfold more than net fascicle shortening (Fig. 5) to overall fascicle shortening during the downstroke.

### *Fascicle strain versus resting fascicle length*

Least-squares regression (Fig. 8) showed no significant ($r^2$=0.14; $P$>0.05) relationship between total fascicle strain and a fascicle's resting length. Indeed, rather than an inverse relationship being observed, the trend suggests a positive though weak correlation between strain and fascicle length.

### *Timing of regional muscle activation and fascicle strain relative to force*

Regional EMG patterns of pectoralis activation were highly uniform. No significant differences ($P$>0.05, two-way ANOVA considering bird and implant location) were observed among individuals in terms of EMG onset, but EMG offset differed significantly within the muscle, with the anterior sternobrachialis offset occurring before the rest of the muscle ($P$<0.05, two-way ANOVA and Tukey–Kramer *post-hoc* test, Fig. 6). Similarly, EMG onset times varied by less than 2 ms and EMG offset times by less than 3 ms in those individuals for which the most significant regional differences were observed. Within all individuals and at all muscle locations the pectoralis was activated midway through lengthening during the upstroke and was deactivated about midway through shortening during the downstroke, EMG activation generally preceded the onset of muscle force by 3–8 ms and ended 5–15 ms after the muscle had developed peak force (Fig. 6). EMGs recorded from fascicles immediately adjacent to the intramuscular aponeurosis were generally delayed 5–10 ms relative to the timing of EMGs recorded along fascicles at sites further from the aponeurosis.

### *Regional muscle work-loop patterns*

Fig. 9 shows regional work loops generated by different regions of the pectoralis muscle in two pigeons that displayed

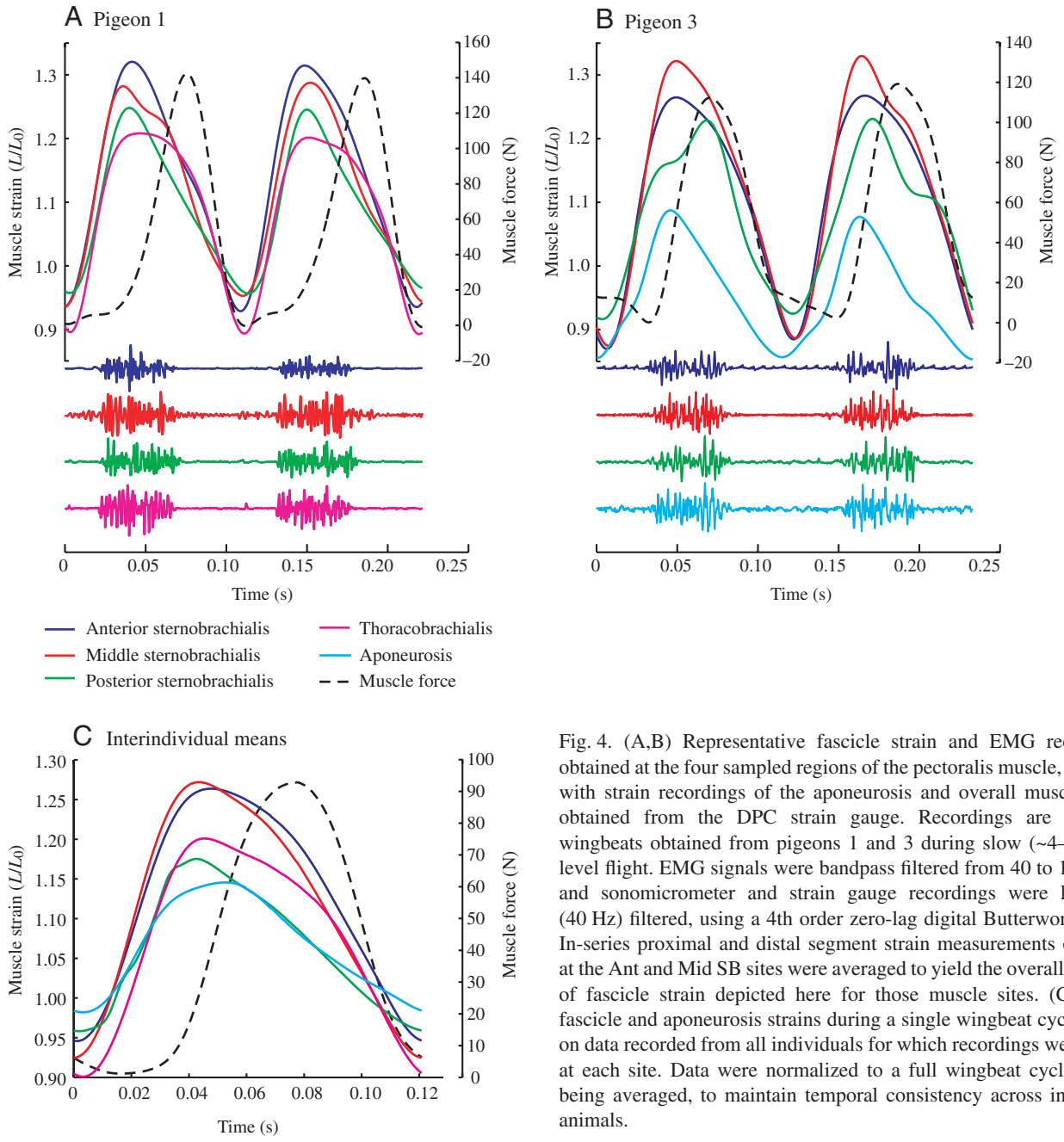



Fig. 4. (A,B) Representative fascicle strain and EMG recordings obtained at the four sampled regions of the pectoralis muscle, together with strain recordings of the aponeurosis and overall muscle force obtained from the DPC strain gauge. Recordings are for two wingbeats obtained from pigeons 1 and 3 during slow (~4–5 m $s^{-1}$) level flight. EMG signals were bandpass filtered from 40 to 1500 Hz, and sonomicrometer and strain gauge recordings were low-pass (40 Hz) filtered, using a 4th order zero-lag digital Butterworth filter. In-series proximal and distal segment strain measurements obtained at the Ant and Mid SB sites were averaged to yield the overall patterns of fascicle strain depicted here for those muscle sites. (C) Mean fascicle and aponeurosis strains during a single wingbeat cycle based on data recorded from all individuals for which recordings were made at each site. Data were normalized to a full wingbeat cycle before being averaged, to maintain temporal consistency across individual animals.

the greatest variation in work loop pattern. Work loops are plotted with fascicle length expressed as both strain (Fig. 9A,C) and length (Fig. 9B,D). Fascicle strains recorded at all sites relative to pectoralis force resulted in work loops that are entirely counter-clockwise. Note, however, that variation in work loop pattern among sites is wholly dependent on variation in the magnitude and pattern of fascicle length change relative to muscle force recorded at the DPC strain gauge site (the actual work loop behavior of individual fascicles would require independent isolated intramuscular force measurements, which are not possible). Because the more posterior regions (Post SB and TB) of the muscle have both shorter fascicles and reduced strains, their contribution to the mechanical work output of the muscle, as a whole, was substantially reduced compared to more anterior SB regions (Figs 9 and 10; Table 4). Net mechanical work (loop area) among sites varied by over twofold in pigeon 1 and 1.7-fold in pigeon 5. The other pigeons (not shown) exhibited similar variation in the net work output among different fascicle regions (Table 4). For the trials shown in Fig. 9, cycle duration was 0.114 s, resulting in power outputs that ranged from 20.5 to 7.9 W. Overall, the pigeons' wingbeat frequency averaged 8.47±0.4 Hz (0.118 s cycle duration).

To estimate the fractional contributions of the different regions to total muscle work and power output, we related the net work and power output measured at individual fascicle recording sites (Fig. 9) to the fractional muscle mass represented by each site (Table 1). Fig. 10 shows the inter-

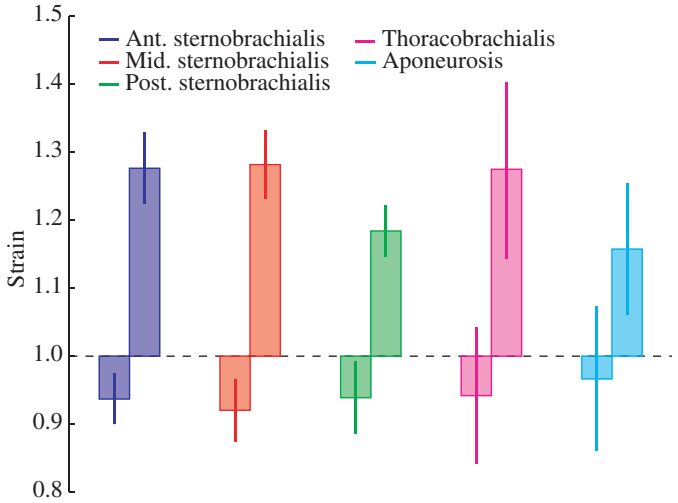



Fig. 5. Summary histogram showing regional patterns of fascicle strain (mean ± S.D.) measured at five sites (see Fig. 1) within the pectoralis. Note that strains measured along the intramuscular aponeurosis represent passive length changes associated with series-elastic stretch of the aponeurosis resulting from forces transmitted by the TB and posterior SB fibers that insert on the aponeurosis.

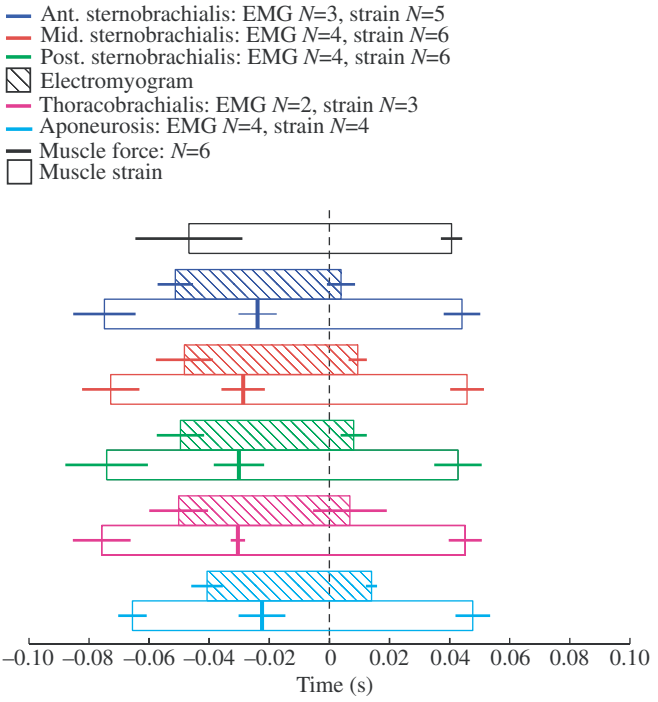



Fig. 6. Summary histogram of the timing (mean ± S.D.) of muscle activation and strain recorded from the different regions of the pigeon pectoralis, together with total muscle force measured at the DPC. Time zero is defined relative to when the pectoralis exerts peak force, with the force histogram spanning the period of force development by the muscle. EMG histograms span from the mean onset to the mean offset of the EMG. Strain histograms span from the onset of muscle lengthening to the end of muscle shortening. The central bar within the strain histograms represents the time the muscle region reaches its peak length.

individual means (± S.E.M.) of the fraction of total pectoralis mass estimated for each region (open bars) together with the fraction of mass-weighted work that each region generated (shaded bars). These data are also reported in Table 4, which shows the regional muscle work estimated for each site and mean total muscle work performed by the pectoralis as a whole (1.282 J), together with the fractional work estimated for each region. Fractional mass (34%) and work output (43%) was largest in the Ant SB region, followed by the Mid SB (28% mass, 33% work). Because strains at these two sites were greater than those recorded at the Post SB site, and Ant and Mid SB fascicles are much longer than fascicles in the Post SB and TB sites, the Ant and Mid SB regions of the muscle were estimated to produce a disproportionate amount of work compared with the Post SB (17% mass, 11% work) and TB (21% mass, 13% work) regions. Thus, regional fascicle strains indicate that the anterior regions of the pectoralis contributed 76%, and the posterior regions 24%, of the muscle's total work output. When adjusted for mass fraction and regional fascicle strain, pectoralis work for the six pigeons averaged 2.564 J (for the left and right pectoralis combined), or 24.7±5.1 J $kg^{-1}$ muscle. This corresponds to a power output of 21.7 W based

Table 3. *Regional fascicle strain data with ANOVA comparisons made among sites within individuals as well as based on group means, derived from measurements obtained during level flight*

| Pigeon no. | Ant SB | Mid SB | Post SB | TB | APO | *F* | *P* |
|---|---|---|---|---|---|---|---|
| 1 | 0.363±0.033 | 0.326±0.027 | 0.267±0.030 | 0.251±0.045 | | 86.47 | <0.0001 |
| 2 | 0.341±0.048 | 0.324±0.034 | 0.161±0.024 | 0.374±0.103 | | 231.91 | <0.0001 |
| 3 | 0.381±0.024 | 0.447±0.038 | | | 0.214±0.032 | 70.81 | <0.0001 |
| 4 | 0.275±0.033 | 0.337±0.045 | | 0.329±0.032 | 0.145±0.015 | 27.81 | <0.0001 |
| 5 | 0.299±0.035 | 0.359±0.042 | 0.301±0.046 | 0.375±0.056 | | 28.78 | <0.0001 |
| 6 | 0.367±0.046 | 0.360±0.040 | 0.249±0.034 | | 0.214±0.039 | 103.56 | <0.0001 |
| Mean ± S.D. | 0.337±0.042 | 0.359±0.046 | 0.244±0.060 | 0.332±0.058 | 0.191±0.039 | 4.56 | 0.0172 |

Measurements (32<*N*<47 wingbeats per individual) were obtained from the five middle wingbeats of individual flight trials (see Materials and methods).

Results are individual means ± S.D.

For an explanation of abbreviations, see Fig. 1.

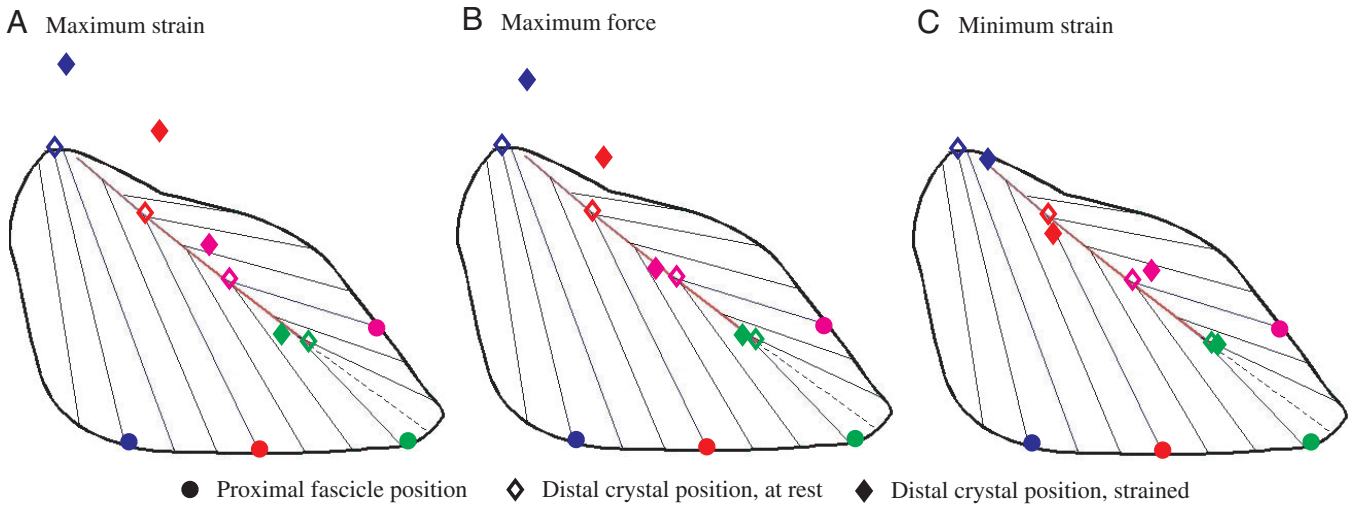



Fig. 7. Map of pectoralis fascicle architecture and geometry at three time points during the downstroke: (A) maximum (Mid SB) strain at start of downstroke, (B) maximum DPC pectoralis force, approximately one-third through downstroke, and (C) minimum (Mid SB) strain at end of downstroke. Distal end displacements of the defined fascicle regions shown as solid colored diamonds are referenced to their resting position (open diamonds), proximal ends were fixed in position and are shown as solid coloured circles. Displacements were determined using a 2D model of the pectoralis derived from digital images of the muscles that were referenced to a calibrated coordinate system, based on fascicle lengths and angles measured at eight defined points along the SB and six defined points along the TB portions of the muscle. Fascicle displacements reflect the average inter-individual strains recorded at each site, combined with their average coordinate locations (see Fig. 1B).

on the mean cycle duration across individuals (0.118 s), or 206.6±41.5 W $kg^{-1}$ muscle during level slow (~4–5 m $s^{-1}$) flight.

### *Repeated strain measurements in adjacent fascicles*

Strains recorded on sequential days in adjacent parallel sites (~5 mm apart) in the anterior and mid SB regions of the pectoralis muscle of pigeon 5 resulted in strains that differed by as little as 1.1±0.7% (proximal Mid SB) to as much as 10.3±0.9% (proximal Ant SB), with an average difference of 3.7±0.9% among the four adjacent sites sampled ($P$<0.05; d.f.=7; $F_{ant}$=20.0; $F_{mid}$=43.3). Although not shown, no discernible differences in the temporal pattern of strain between adjacent sites were observed.

## Discussion

Recordings of *in vivo* fascicle strain and EMGs in five regions of the pigeon pectoralis during slow level flight reveal uniform temporal patterns of regional fascicle length change and muscle activation, as well as significant series elastic compliance of the muscle's intramuscular aponeurosis. Compliance of the aponeurosis enables shorter, more posterior fascicles to contract with smaller strain than longer, more anterior fascicles, to accommodate the overall length excursion of the pectoralis muscle as it depresses the humerus during flight. Although our EMG results appear to differ from those of Boggs and Dial (1993), our sampling of muscle sites was less broadly distributed within the muscle than theirs. Even so, the temporal shift in EMG activation reported by Boggs and Dial was small, with EMG onset times for regions similar to those that we sampled occurring between 7% and 10% of the wingbeat cycle (representing a delay of only 3 ms for a cycle duration of 0.118 s). In their study, Boggs and Dial (1993) found that the most anterior region of pectoralis, where fibers originate from the furcula to insert on the DPC, and the most superior TB region appeared to show the most distinct difference in EMG activation timing. These sites have the shortest fascicles and represent very small portions of the pectoralis. They are also less amenable to obtaining recordings of fascicle length change. Consequently, we cannot address whether changes in EMG timing at these sites result in differences in fascicle strain pattern. For the four regions of the

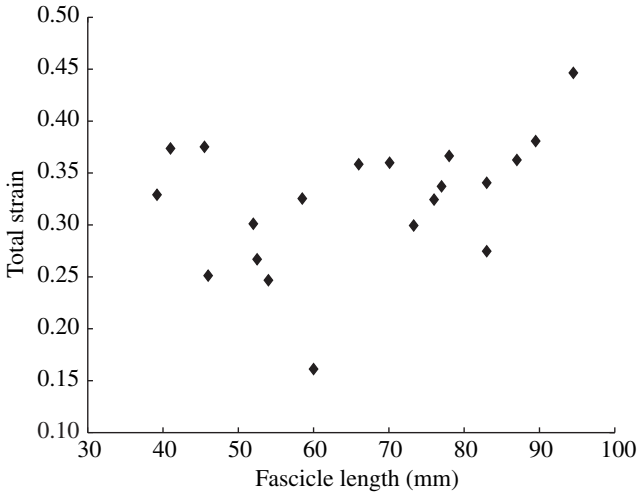



Fig. 8. Total fascicle strain plotted *versus* fascicle length for level flight, based on measurements obtained for all recordings sites in six pigeons. No significant relationship between total strain and fascicle length was observed (least squares regression: $r^2$=0.144, $P$>0.05).

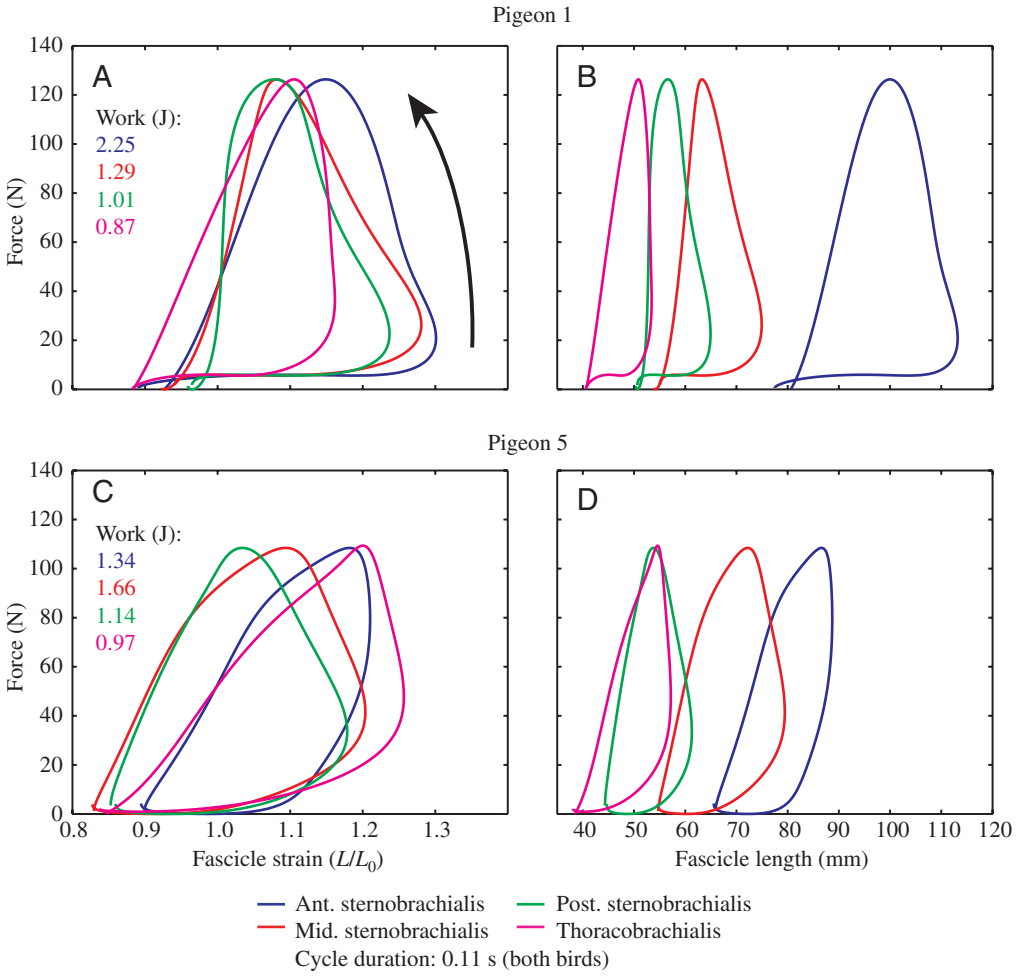



Fig. 9. Work loops obtained from different regions of the pectoralis muscle in two pigeons plotted with fascicle length expressed as both strain (A,C) and length (B,D). The black arrow denotes the counter-clockwise path of the work loop, indicating the positive net work (area contained within the work loop in B,D) performed during the muscle's fascicles over the course of the wingbeat cycle. Note that the more posterior regions of the muscle have both shorter fascicles and reduced strains (A,C), leading to reduced mechanical work output. Cycle duration was 0.11 s for both pigeons, giving power outputs ranging from 7.9 to 20.5 W. All loops are taken from the 5th wingbeat of one trial from each bird.

muscle that we sampled here, there was no evidence of a significant temporal delay in EMG activation, resulting in relatively minor differences in the temporal pattern of fascicle strains at these sites.

### *In-series fascicle strain and strain heterogeneity*

Recordings of in-series fascicle segment strains in the Ant SB and Mid SB regions of the pectoralis showed frequent instances (9 of 12 cases) in which significant differences in strain magnitude were observed along individual fascicles, with strain differences ranging from 2.0 to 17.2% strain. When averaged across animals the differences were 6.2% between proximal and distal Ant SB segments and only 1.4% between proximal and distal Mid SB segments. Although the in-series strain measurements within individual animals contradict our hypothesis that fascicles strain uniformly along their length, when averaged across animals the results provide stronger support for this hypothesis. Heterogeneous strain patterns along individual fascicles have recently been observed in the toad semimembranosus under both *in vivo* and *in vitro* contractile conditions (Ahn et al., 2003). Ahn et al. found what when central and proximal semimembranosus fascicle segments shortened during hopping, the distal segment was initially stretched before shortening to a much smaller extent than shortening of the central and proximal segments. Under *in vitro* isometric conditions, central and proximal semimembranosus segments also shortened by stretching the distal segment of the fascicle. Such radically different patterns of fascicle segment strains observed in the toad semimembranosus were not observed here. Although the magnitude of strain often varied between Ant SB and Mid SB proximal and distal fascicle segments, the overall in-series strain pattern of pectoralis fascicle shortening and lengthening during the wingbeat cycle was quite uniform (Fig. 2). Our repeated measurements of in-series fascicle strain in pigeon 5 also indicate that up to 4% of the variation in strain magnitude observed at any given recording site may be attributable to variation in (at least our) experimental technique owing to differences in crystal alignment during implantation and motion relative to the fascicles to which the crystals are anchored.

Recent analyses of myofascial force transmission within and between muscles (Huijing, 1999) show that forces developed within a muscle can be transferred laterally to other muscles. That muscle fibers do not operate as independent force generators is reinforced by the work of Purslow and Trotter (1994), who showed that structural linkages between muscle fibers and their endomysium and perimysium likely function to provide lateral force transmission *via* shear transfer from the fiber to its connective tissue matrix rather than solely along the muscle fiber's (and fascicle's) axis. Consequently, localized fascicle strains may not necessarily be uniform along a fascicle's length. Recent studies using cine-MRI, ultrasound,

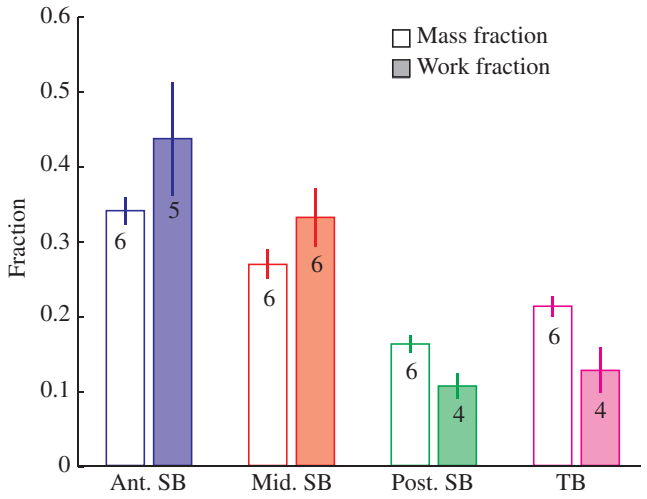



Fig. 10. Histogram showing the mass-fractions (open bars) and work-fractions (shaded bars, inter-individual mean ± S.E.M.) of muscle work estimated for the four muscle regions (SB: Ant, Mid, Post and TB; see Fig. 1) from which *in vivo* fascicle strains were sampled. Mass-fraction data were obtained for all six pigeons, whereas work-fractions were obtained for those animals and regions in which reliable work loops (Fig. 9) were obtained over multiple wingbeat cycles. Mass-weighted work fractions were calculated by multiplying the net work from each region by the fractional mass (Table 1), then dividing by the summed averages for all regions. The average summed work was 1.28 W and average duration was 0.12 s. Individual bird values were based on a minimum sample of 8 wingbeats, with an average sample size of 40 wingbeats (Table 4).

and x-ray imaging have also found regional differences in fascicle strain that may in part arise from in-series strain heterogeneity (Pappas et al., 2002; Finni et al., 2003; Monti et al., 2003). Consequently, based on these findings and the results observed here, it is clear that assumptions of uniform in-series fascicle strain at the whole muscle level need re-examination and deserve further study. Nevertheless, the generally consistent in-series fascicle strain patterns that we observed when averaged across individual birds suggest that interpretations of pectoralis muscle function in pigeons, and likely other birds, based on a localized measurement of fascicle strain are qualitatively robust and likely to yield reliable quantitative measurements of whole muscle work and power.

### *Regional patterns of fascicle strain in relation to intramuscular series elastic compliance*

Although different regions of the pectoralis showed little difference in the timing of fascicle shortening and lengthening and EMG activation, significant variation in the pattern and magnitude of fascicle strain was observed among the four sites (Ant SB, Mid SB, Post SB and TB) sampled. Regional differences in the magnitude of fascicle strain among these sites were generally consistent across individual animals. As a result, significant differences remained when strains for the four sites were averaged across the six birds (Table 3). However, at least some of the variation in regional strain pattern among individuals likely resulted from experimental variation in the specific sites that were targeted for implantation during surgery to quantify strain patterns for different regions of the muscle (Fig. 1B). The greatest difference in fascicle strain magnitude occurred within the posterior SB region. Although strains within the Ant SB and Mid SB differed by an average of only 6% (average strain difference=0.022), strains within the Post SB were 30% less (average strain difference: 0.104) than the average for the Ant SB and Mid SB sites.

The much greater contribution of fascicle lengthening (+25.4±4.7%) *versus* net fascicle shortening (–6.5±0.9%) to the total 32% shortening strain of the pectoralis was also generally consistent for the four sampled regions, and consistent with our earlier results for Silver King pigeons flying under similar conditions (+20±5% and –12±4%; Biewener et al., 1998). A similar pattern of relative fascicle length change associated with shortening work has also been

Table 4. *Regional muscle work estimated within the pigeon pectoralis (32<N<47 wingbeats per individual)*

| | Muscle work (J) | | | | |
|---|---|---|---|---|---|
| Pigeon no. | Ant SB | Mid SB | Post SB | TB | Total |
| 1 | 0.752±0.103 | 0.309±0.044 | 0.091±0.014 | 0.139±0.031 | |
| 2 | 0.734±0.179 | 0.362±0.095 | 0.150±0.034 | 0.269±0.110 | |
| 3 | | 0.648±0.095 | | | |
| 4 | 0.322±0.050 | 0.350±0.075 | | 0.086±0.015 | |
| 5 | 0.325±0.053 | 0.461±0.069 | 0.188±0.031 | 0.169±0.032 | |
| 7 | 0.652±0.104 | 0.406±0.061 | 0.112±0.026 | | |
| Mean ± S.D. | 0.557±0.217 | 0.423±0.122 | 0.136±0.043 | 0.166±0.077 | 1.282±0.264 |
| Fractional mean ± S.D. | 0.434±0.156 | 0.330±0.113 | 0.106±0.036 | 0.130±0.044 | |

Results are individual means ± S.D. Note that differences in regional and fractional muscle work are entirely based on differences in fascicle strain recorded within local regions and not due to muscle force, which was measured for the muscle as a whole.

Regional muscle work (J) was averaged across all animals for which reliable measurements were obtained at each site, and the means for each site used to determine total muscle work. Fractional values of regional muscle work are dimensionless.

observed in the pectoralis of cockatiels *Nymphicus hollandicus* and doves *Streptopelia risoria* flying over a range of speeds in a wind tunnel (+25±6% and –7±4%, total: 32%; Hedrick et al., 2003) and for mallard ducks *Anas platyrhynchos* during ascending flight (+22±4% and –8±3%, total: 30%; Williamson et al., 2001). Although this might suggest a robust pattern for the avian pectoralis during flight, Askew and Marsh (2001) found a more uniform pattern in blue-breasted quail *Coturnix chinensis* during vertical take-off flight (+11±2% and –12±2%, total: 23%), with even more limited lengthening (+8±2%) *versus* net shortening (–14±2%) during horizontal flight. In their study of take-off flight in four different-sized Phasianid species, Tobalske and Dial (2000) also observed symmetric lengthening relative to shortening strains in the smallest species (Northern bobwhite *Colinus virginianu*s: +10±2% and – 9±3%, total: 19%) compared with the largest species (wild turkey *Meleagris gallopavo*: +21±1% and –14±8%, total: 35%). In addition to showing a scaling effect on total muscle shortening strain, Tobalske and Dial's results also suggest that the relative contributions of lengthening *versus* shortening strain may be size-dependent within the Phasianidae, consistent with the results of Askew and Marsh (2001) for quail. However, as cockatiels and ring-neck doves are generally intermediate in size relative to both quail species, lengthening *versus* shortening strain patterns of pectoralis almost certainly reflect other factors as well, such as wing loading and wing shape, which are likely to affect pectoralis muscle strain and wing stroke amplitude and which differ considerably between these species.

When pooled across all recording sites and animals, regional fascicle strains within the pigeon pectoralis showed no evidence of an inverse relationship with fascicle length (Fig. 8). Although counter to our second hypothesis, this likely results from series elastic compliance within the muscle that allows shorter fascicles to strain more similarly to long fascicles and rotation of the fascicles about their origin. Strains measured along the aponeurosis averaged 19.1%, with APO shortening and lengthening occurring slightly after fascicle length change (Figs 4 and 6). This allows shorter, posterior fibers of the SB and all of the fibers of the TB that insert along the aponeurosis to undergo strains generally similar to (or even less than) the long anterior fibers of the SB (Fig. 1), as force is transmitted to the deltopectoral crest of the humerus.

A simple two-dimensional geometric model of the pectoralis, based on average strains recorded in the aponeurosis and at the different fascicle regions (Fig. 6), shows how overall length and shape changes of the pectoralis in relation to local fascicle strains are accommodated *via* compliance of the aponeurosis and fascicle rotation. The results of this analysis demonstrate that more anterior SB fascicles undergo substantial elevation as well as retraction at maximum strain, which is likely mediated *via* fascicle rotation. Rotation, in addition to linear strain, of fascicles during muscle contraction is well documented in ultrasound imaging studies of human soleus and gastrocnemius muscles (e.g. Fukunaga et al., 2001; Narici et al., 1996). Strain displacements of more posterior fascicles are more anteriorly and superiorly oriented. However, it is important to note that our model, and the superficial fascicle strain measurements on which it is based, do not account for out-of-plane forces and strains of the muscle, which are likely to affect the relative magnitude and timing of aponeurosis strain relative to those of the muscle's fascicles. As a result, distortions in the shape change of the muscle (e.g. along the aponeurosis relative to the TB fascicle) based on averaged relative strains for each region are observed, but are unlikely to occur. Nevertheless, by mapping measurements of regional muscle strain from localized sonomicrometry recordings onto a simple geometric model of the muscle, we are able to show better how the pectoralis changes length and shape over the course of a contraction cycle and how regional differences in length change are accommodated by the muscle's internal compliance.

### *Implications for muscle work and power*

Our results show that normalizing local fascicle strain recordings to an average fascicle length for the muscle, as a whole, provides the most reliable assessment of whole muscle work and power. This has been the most common approach for analyzing muscle work and power output under *in vivo* conditions for birds in flight (Biewener et al., 1998; Hedrick et al., 2003; Tobalske et al., 2003; Williamson et al., 2001) and for evaluating *in vivo* muscle function during terrestrial locomotion (Biewener and Corning, 2001; Biewener et al., 2004; Daley and Biewener, 2003; Gabaldón et al., 2004; Roberts et al., 1997). Because all fascicles contracted with similar strains, it is likely that more posterior fascicles of the pigeon pectoralis contributed a much smaller share to whole muscle work than the muscle's longer, more anterior fibers. This cannot be stated with full certainty because local forces within the fascicles were not measured. Nevertheless, our results indicate that by applying a localized measure of fascicle strain to the muscle's *average* fascicle length, regional differences in muscle work can be accounted for with reasonable accuracy.

Because no difference in the timing of activation was observed among fascicle regions, regional differences in muscle work are unlikely to reflect regional differences in the role of the pectoralis in controlling motions of the humerus and wing. Instead, the differing orientation and length of fascicles in the pectoralis appear to be linked to an overall coordinated movement of the humerus and wing, at least during level flight. Whether more distinct patterns of regional muscle activation and strain within the pectoralis occur during non-steady and maneuvering flight remains to be determined. Our findings reinforce the view of the pectoralis as the principal motor for flight, which produces a coordinated depression and (some degree of) pronation of the wing during the downstroke for aerodynamic lift, whereas other, principally more distal, wing muscles serve to adjust the wing's shape and orientation during maneuvering and landing (Dial, 1992). As a result, differences in the timing of pectoralis activation and force development relative to other muscles controlling the wing are likely most

important for adjusting movements of the wing to differing flight requirements, rather than differential recruitment within the pectoralis itself.

Our measurement of 207±41.5 W kg$^{-1}$ muscle for the Carneau pigeon pectoralis observed here based on more detailed examination of regional muscle strain patterns is more than twofold higher than our earlier measurement of 108±29.6 W kg$^{-1}$ (calculated as mass-specific *positive* power, not net power, for equivalence here) obtained for the pectoralis of Silver King pigeons (Biewener et al., 1998) using similar methods but based on strain recordings at only two sites (roughly corresponding to Ant SB and Mid SB). In both studies average total pectoralis strains were similar (30–35%). Consequently, most of the difference in muscle work and power must reflect differences in pectoralis force measurement at the DPC. In both cases, our measurements of pectoralis force and fascicle strain yield much lower values than Usherwood et al. (2005), who recently found a value of 273 W kg$^{-1}$ using direct wing pressure recordings to estimate aerodynamic lift for smaller wild-type pigeons flying under similar conditions. However, the coefficient of variation of these measurements is high and Usherwood et al. do not attempt to evaluate the variation of their estimate. In a study of cockatiels and ringed turtle doves flying at 4–5 m s$^{-1}$ in the CFS wind tunnel, Tobalske et al. (2003) observed ~100 W kg$^{-1}$ for cockatiels and ~150 W kg$^{-1}$ for doves (again, reported here as total positive power) based on *in vivo* pectoralis force and strain recordings. These compare favorably with earlier measurements of ~85 W kg$^{-1}$ pectoralis positive power for black-billed magpies *Pica pica* obtained by Dial et al. (1997) during wind tunnel flight at the same speed. Nevertheless, magpies have lower aspect ratio wings and similar wing loading as cockatiels, and so would be expected to have higher, not lower, power costs at a similar speed.

These differences in mass-specific muscle power output certainly reflect differences and variation in experimental technique, as well as interspecific differences in wing shape and wing kinematics. Tobalske et al. (2003) note that calibration of the DPC strain gauge may lead to some underestimate of muscle force. However, the magnitude of this is difficult to assess. Achieving reliable estimates of the mechanical power output of flight in different avian species and across different flight conditions is of considerable interest. The consistency of our regional measurements of pectoralis strain indicates that estimates of whole muscle strain are likely more reliable than estimates of whole muscle force. Consequently, refinement and improvements in force measuring technique, combined with the application of novel techniques (Hedrick et al., 2004; Usherwood et al., 2005; Spedding et al., 2003) and comparison of these techniques and approaches, including aerodynamic modeling, are needed to improve our understanding of how the mechanical power requirements of flight vary among species and flight conditions.

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