The Journal of Experimental Biology 210, 3789-3797
Published by The Company of Biologists 2007
doi:10.1242/jeb.006296

Modulation of pectoralis muscle function in budgerigars *Melopsitaccus undulatus* and zebra finches *Taeniopygia guttata* in response to changing flight speed

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Accepted 20 August 2007

Summary

Flight power varies in a U-shaped relationship with flight speed, requiring the modulation of flight muscle power in order to meet these changing power demands. The power output of the pectoralis muscle can potentially be modulated by changing strain trajectory and the relative timing and intensity of muscle activity. Pectoralis muscle length change and activity patterns were recorded in budgerigars *Melopsitaccus undulatus* and zebra finches *Taeniopygia guttata* at a range of flight speeds using sonomicrometry and electromyography (EMG). The pectoralis muscles in these species contain a single muscle fibre type. Therefore, the power output is entirely determined by muscle activity and strain trajectory, rather than recruitment of motor units with different contractile properties as in many other vertebrate muscle systems. Relative EMG intensity, wingbeat frequency and muscle strain varied in an approximately U-shaped relationship with flight speed. The shape of the length trajectory varied with flight speed in budgerigars, with the proportion of the cycle spent shortening being lowest at intermediate flight speeds. In zebra finch pectoralis muscle the shape of the length trajectory did not vary significantly with flight speed. In both species the observed changes in muscle recruitment and length trajectory are consistent with meeting flight power requirements that vary in a U-shaped pattern with speed. Both species utilised intermittent flight, tending to spend relatively less time flapping at intermediate flight speeds. This supports the idea that intermittent flight is used as a simple power modulation strategy. However, the idea that intermittent flight serves to maintain a 'fixed gear' is over-simplistic and fails to recognise the plasticity in performance at the level of the muscle. Intermittent flight is only one component of a complex power modulation strategy.

Key words: flight, power, modulation, strain, recruitment, intermittent flight.

Introduction

All animals tailor the mechanical power output of their muscles to the changing demands of locomotion. Where locomotion involves the transfer of momentum to a surrounding fluid, as in flying and swimming, mechanical power requirements change with speed. Avian flight power has a U-shaped relationship with speed (Rayner, 1999; Askew and Ellerby, 2007) and during swimming, the mechanical power requirements increase exponentially with speed (Alexander, 2005). During terrestrial locomotion, where the net work and power output per stride are zero (Cavagna et al., 1977), a link between power and speed is less clear. However, during accelerations (Roberts and Scales, 2002), changes in potential energy due to incline locomotion (Gabaldon et al., 2004) and load carrying (Ellerby and Marsh, 2006), muscle mechanical power output must be increased.

A number of mechanisms can vary muscle power output. First, muscles or motor units that that have different physiological properties can be recruited in a task-specific manner. Muscles differ widely in the stress that they can generate, the rate at which they are able to shorten and the degree of curvature of their force–velocity relationships (Josephson, 1993). All of these factors affect the power that can be generated, resulting in a wide range of measured skeletal muscle power outputs, from 0.5 W kg^{-1} in eel slow muscle (Ellerby et al., 2001) to 390 W kg^{-1} in blue-breasted quail pectoralis muscle (Askew and Marsh, 2001). In essence, power output can be increased by recruiting faster contracting muscles or motor units (Altringham and Johnston, 1990; Jayne and Lauder, 1994; James et al., 1995; Wakeling, 2004). Second, the number of motor units recruited within a given muscle may be varied as a means of modulating muscle force output. For example, in the lateral gastrocnemius muscle in running turkeys, motor unit recruitment progressively increases with steeper inclines (Roberts et al., 1997). Third, where an organism moves through a fluid, the muscular power source can simply be turned off periodically to control average power output. This occurs in fish during 'burst and coast' swimming, and birds during intermittent flight. These strategies may have an added benefit by reducing overall power requirements relative to those associated with constant power output (Videler and Weihs, 1982; Rayner, 1985). Fourth, fascicle strain trajectory and its

Table 1. *Numbers, mass and dimensions of experimental animals*

	Zebra finch (*N*=6)	Budgerigar (*N*=7)
Body mass (g)	12.7±0.9	45.0±2.1
Wing span (mm)	178±3	292±5
Total pectoralis muscle mass (g)	2.3±0.2	6.6±0.2

relationship with muscle activity, key determinants of muscle mechanical performance (Marsh, 1999a; Askew and Marsh, 2001), may also be changed to control muscle power output. The strain–activity relationship determines whether the muscle acts as (1) a power source, primarily being active during fascicle shortening, as in the power-producing muscles of swimmers and fliers (Altringham and Ellerby, 1999; Biewener et al., 1992); (2) an economical force producer, acting isometrically as in the distal limb muscles of many terrestrial animals (Roberts et al., 1997); or (3) is active while being stretched by an external load and absorbs mechanical energy acting as a brake or stabiliser (Marsh, 1999b). In power-producing systems, more subtle manipulations of strain trajectory can modulate the power output of the muscle (Askew and Marsh, 1997; Askew and Marsh, 1998). Within limits, increasing strain amplitude increases the work done by the muscle per strain cycle (Askew and Marsh, 1998). Changing the strain cycle frequency can change the rate at which that work is done (Askew and Marsh, 1997; Askew and Marsh, 1998). Changes in the relative proportion of the strain cycle spent shortening can also affect power output. An asymmetrical strain cycle, in which a greater proportion of the cycle is spent shortening than lengthening, generates a greater power output than a symmetrical cycle (Askew and Marsh, 1997; Girgenrath and Marsh, 1999). Ultimately, however, the scope for changing strain trajectory is limited by the intrinsic physiological properties of the muscle concerned.

The mechanical power for avian flight is primarily supplied by the pectoralis muscles. The power output of these muscles must be modulated to meet speed-related changes in flight power requirements. Some birds are constrained in terms of the mechanisms they can employ to modulate pectoralis muscle power output. Many smaller bird species, including the zebra finch and budgerigar, have homogeneous pectoral muscles consisting of a single fibre type, fast oxidative glycolytic (FOG) (Rosser and George, 1986). This prevents them from recruiting motor units with different intrinsic properties. Intermittent flight strategies and/or manipulations of the relationship between muscle strain and activity are therefore the only power modulation mechanisms that are available to them. The extent to which these different mechanisms are used to modulate pectoralis muscle power output has not been quantified.

The primary aim of this study was to determine the relative importance of different power modulating mechanisms in budgerigars *Melopsitaccus undulatus* and zebra finches *Taeniopygia guttata* across a range of flight speeds. We measured muscle *in vivo* pectoralis fascicle strain and activity during wind tunnel flight by sonomicrometry and electromyography (EMG), respectively. Kinematic data obtained simultaneously by high-speed video recordings were also used to examine intermittent flight strategies. As well as indicating power modulation strategies at the muscle level, the strain and activation data obtained are used in the companion paper (Ellerby and Askew, 2007) to determine pectoralis power output *in vitro* using the work-loop technique (Josephson, 1985). We will test the hypothesis that the speed-related changes in power requirements can be met by modulating the activity and strain pattern of the pectoralis muscle.

Materials and methods

Experimental animals and flight training

Zebra finches *Taeniopygia guttata* Vieillot 1817 and budgerigars *Melopsittacus undulates* Shaw 1805 were purchased from commercial suppliers as juveniles approximately 1–2 months of age. They were housed in wire cages with *ad libitum* access to a commercial seed mix and water under a 12 h:12 h light:dark cycle. Supplemental vitamin-enriched, high-protein-egg food was also provided once a day. Simultaneous EMG, sonomicrometry and kinematic data were obtained from six individuals of each species. EMG and kinematic data only were obtained from a seventh budgerigar (Table 1).

Training regimens were similar for both species. We exercised the birds in the wind tunnel once a day. The amount of time spent in the wind tunnel was increased incrementally until the birds could sustain at least 15 min of continuous, steady flight at 10 m s^{-1} (zebra finch) or 12 m s^{-1} (budgerigars). This typically took 2–3 weeks. Once this level of endurance had been reached, the birds were exposed to a wide range of speeds from 4 up to 14 m s^{-1} (zebra finch) or 16 m s^{-1} (budgerigars). The speed range was dictated by the level of endurance of the birds at its extremes. At the extremes of this range the birds could not sustain steady flight for more than 30 s without becoming fatigued and landing on the bottom of the working section. A small hole in the side of the wind tunnel allowed a perch to be introduced into the front of the working section. The birds were trained to land on the perch when it was presented to them and to start flying, then land on the bottom of the working section when it was withdrawn.

Design and implantation of EMG electrodes and sonomicrometry crystals

For each experiment we constructed a set of implants consisting of two 1.0 mm sonomicrometry transducers, two bipolar EMG electrodes and a single ground wire. These were soldered to a female, double row, eight-way PCB socket (1.27 mm pitch, zebra finch connector; 2 mm pitch, budgerigar connector). The soldered terminals were sealed with epoxy resin. The total mass of the implant was 0.7 g for zebra finches and 1.5 g for budgerigars.

Prior to surgery the birds were given a dose of analgesic (Vetergesic, Reckitt Benckiser Healthcare (UK) Ltd, Hull, UK; 20 μg buprenorphine hydrochloride kg^{-1} body mass, by intramuscular injection into the gastrocnemius muscle) and

antibiotic (Baytril, Bayer Healthcare, Bury St Edmunds, UK; 10 mg enrofloxacin kg^{-1} body mass, by injection into the gastrocnemius muscle). Transducers and electrodes were implanted under isoflurane anaesthesia, induced at 3% concentration in oxygen and maintained at 1% concentration. Feathers were removed from small (1.5 cm×1.5 cm) areas of skin overlying the pectoralis and the dorsal surface of the pelvis. The exposed areas were swabbed with betadeine antiseptic and isolated with sterile drapes. A 1 cm skin incision was made with a scalpel at each site. A path between the two incision sites was opened by blunt dissection of the sub-cutaneous connective tissue. The transducer and EMG wires were passed along this path from the dorsal to the pectoral incision site.

To enable secure anchoring of the sonomicrometry transducers, 1.5 mm side arms were attached to the transducer wires with epoxy resin. These were made from a bent stainless steel insect pin, and oriented perpendicular to the wire 2.5 mm from the transducers. To insert a transducer into the muscle a 0.5 mm cut was made in the muscle fascia, parallel to the long axis of the fascicles, using the cutting edge of a hypodermic needle. The fascicles were separated using fine forceps, the transducer pushed into the resulting hole and the side arms sutured to the muscle fascia (using 6.0 silk suture). A second transducer was inserted in the same manner, approximately 8 mm from the first, along the same muscle fascicle.

The EMG electrodes were constructed from twisted pairs of 0.075 mm diameter Teflon coated silver wire (Goodfellow Cambridge Ltd, Cambridge, UK). The bared tips were 1 mm in length and separated by an offset of 2 mm. These were inserted into the pectoralis muscle, parallel to the muscle fascicles and adjacent to the sonomicrometry transducers, using a 25-gauge hypodermic needle with a blunted internal cutting edge, then secured by suturing to the muscle fascia (using 6.0 silk suture).

The skin incisions were closed with 5.0 silk suture. The PCB connector was left protruding from the dorsal site, anchored firmly to the skin with suture. Budgerigars were fitted with a 'tunic' made from Vetrap bandage (3M, Neuss, Germany). This covered both incision sites and the connector while allowing complete freedom of movement during recovery. After cessation of anaesthesia the birds regained consciousness within 5 min. They were left to recover for between 24 and 48 h before performing the flight experiments.

The wind tunnel

The wind tunnel had a working section of 52 cm×52 cm×95 cm (width×height×length). It was constructed from transparent Perspex^{TM}. The back wall (viewed laterally) was spray-painted matt black to avoid generating reflected images during filming. Vertical nylon line (0.4 mm diameter) spaced 15 mm apart upstream and 7.5 mm downstream, restricted birds to the working section of the wind tunnel.

Camera setup and kinematic analysis

The birds were filmed using a Kodak Motion Corder high-speed camera (model SR500; San Diego, CA, USA) at a frame rate of 125 Hz. The camera was sited lateral and perpendicular to the working section of the wind tunnel. A mirror was mounted at a 45° angle above the working section of the tunnel, providing a simultaneous lateral and top view of the bird. Birds were filmed the day before implantation of transducers and EMG electrodes, and during collection of *in vivo* data post-implantation. The camera was triggered using an external TTL input generated with a signal generator (Wavetek, San Diego, CA, USA). This was recorded as an additional analogue data channel input along with the *in vitro* data, allowing synchronisation of these data with the video images.

The stroke amplitude, stroke plane angle relative to the horizontal, relative duration of time spent flapping, and the frequency with which non-flapping intervals occurred were quantified in the birds while uninstrumented, and post-instrumentation during collection of sonomicrometry and EMG data. The onset of non-flapping phases in finches can be easily determined as the wings are fully withdrawn and held against the body. In budgerigars, intermittent flight behaviour is more complex as the wings are held partly outstretched between bouts of flapping. Despite this, the distinction between the flapping phases, with high amplitude wing tip movements, and the non-flapping phases with little or no wing tip movement and partial wing retraction, is clear.

Experimental protocols

Instrumented birds were placed in the wind tunnel while wrapped in a soft cloth to prevent them from reaching the wires and connectors. After connection of the dorsal connector to the data cable the bird was released and allowed to sit on the perch at the front of the working section. The wind tunnel was set to 10 m s^{-1} and the bird flown for a short time to allow adjustment of sonomicrometer triggering levels. Data were subsequently collected at 2 ms^{-1} intervals over a range of speeds, from 4 to 16 m s^{-1} in budgerigars, and at 0 and from 4 to 14 m s^{-1} in zebra finches. Flight was initiated by withdrawing the perch. At each speed we waited until the bird was flying steadily, then initiated saving of the *in vivo* data and triggered the camera. At 125 Hz frame rate the camera could store 18 s of video images in its internal memory. Once the data were saved the perch was reintroduced and the bird was allowed to rest for approximately 2 min while the video segment was saved onto digital video tape. After completion of data collection the birds were euthanized by cervical dislocation. The placement of the transducers and EMG electrodes in the pectoralis muscle was confirmed by dissection.

Signal processing and data collection

The implants in the bird were linked to the external equipment *via* a lightweight data cable. This was 1.5 m long and consisted of a twisted pair of insulated 42-gauge copper wires linked to each sonomicrometer transducer, a pair of 0.07 mm enamelled copper wires linked to each EMG electrode, and a single 0.07 mm enamelled copper wire acting as a ground wire. These were soldered to a male PCB connector that was attached to the connector on the bird. The total mass of the cable and proximal connector was 0.65 and 1.37 g for zebra finch and budgerigars, respectively. The individual wires were kept bundled together with cyanoacrylate adhesive applied at intervals along its length. The cable exited the working section *via* a small dorsal hole and connected to a junction box placed on top of the wind tunnel. This was linked to pre-amplifiers and

the sonomicrometer *via* shielded cables. The junction box and all the peripheral equipment were linked to a common ground. EMG signals were recorded *via* pre-amplifiers (DAM50, WPI, USA) at a gain of 1000 and a 10 kHz low-pass filter applied. The two EMG channels were collected as additional analogue inputs using the A to D boards of the sonomicrometer system (TRX Series 8, Sonometrics, Ontario, Canada) at a sampling rate of 2.1 kHz. All data were stored on the hard drive of a PC.

Data handling and analysis

The *in vivo* data were exported as text files using Sonometrics SonoLAB software (Version 3.4.25, Sonometrics, Ontario, Canada). All subsequent data handling and analysis was carried out using Igor Pro (Version 5.0.1.0, WaveMetrics, USA). A digital high-pass filter, designed with the Igor IFDL filter design package, was applied to the EMG data to remove movement artefacts. Sonomicrometry data are subject to two main types of error: those caused by high-frequency electrical noise riding on the transducer signals, and level shifts caused by changes in transducer signal strength. We were fortunate that the environment in which we carried out the experiments was largely free of electrical noise. Level shifts, as the name suggests, are apparent instantaneous changes in length. These are a consequence of the way in which the sonomicrometer measures length by triggering of the 'ringing' signal created by a high-frequency voltage pulse applied to the piezoelectric transducer. A series of voltage peaks is generated by the oscillation of the transducer. Ideally, length is measured by triggering off the first peak. Changes in signal strength occasionally result in a shift in triggering to a subsequent voltage peak. These artefacts were removed using an Igor macro (designed by R. L. Marsh) that moved the portion of trace after the level shift back down to the pre-shift level.

Only segments of steady flying in the centre of the working section were selected for analysis. For each individual and speed, 16 wingbeats were analysed to extract basic EMG and strain parameters. We determined EMG onset and offset times for these wingbeats. Filtered EMG waves were rectified and the integral over the EMG burst calculated. This was divided by burst duration to determine EMG intensity. To enable comparison between individuals, EMG intensity was scaled relative to the mean intensity at the highest flight speed, where the maximum intensity was typically recorded. We also determined the time and muscle segment length at the maximum and minimum of each strain cycle. These data were used to calculate strain amplitude, strain cycle frequency, and the relative durations of muscle lengthening and shortening within each cycle.

All statistical analyses were carried out using SPSS (Version 11.0, SPSS Inc., USA). A general linear model (GLM) was used to test for changes in EMG and strain parameters with flight speed. A GLM was also used to test for changes in flight performance as a result of surgical implantation by comparing pre- and post-surgical flight kinematics. In all models speed was treated as an independent variable, and an identifier of each individual bird included as a random factor. For muscle function analyses, the dependent variables were relative EMG intensity, EMG timing relative to peak muscle length, EMG duty cycle (EMG duration expressed as a proportion of strain cycle duration), strain cycle frequency, strain amplitude, and the proportion of the strain cycle spent shortening. For kinematic analyses, the dependent variables were wingbeat frequency, amplitude and stroke plane angle, and the proportion of flight time spent flapping the wings. All proportional data were arcsine-transformed prior to analysis. Where significant speed effects were detected by the GLM, Scheffé's *post-hoc* test was used to perform pairwise comparisons between mean values. This test was selected as it is relatively conservative in terms of declaring measured differences as being statistically significant.

Results

Muscle recruitment patterns

Both zebra finches and budgerigars used intermittent flight strategies. Zebra finches used bounding flight. This was characterised by an absence of pectoralis muscle EMG activity during the bounding phase and the wings being held close to the body, although with the pectoral muscle fascicles at a greater length than during resting on a perch (Fig. 1). Budgerigars showed greater individual variation in terms of intermittent flight behaviour, and true bounding was rarely observed. The wings were typically partially outstretched during the non-flapping phases, and EMG activity was not always absent in these phases as in the zebra finches (Fig. 2).

In both species, EMG activity started before peak muscle strain in all individuals and at all speeds (Figs 1, 2). There was no significant change in the timing of EMG onset relative to peak muscle strain with flight speed in zebra finches (GLM, F=1.43, P=0.236) or budgerigars (GLM, F=0.892, P=0.515). The overall mean values were 6.0±0.2 and 8.8±0.8 ms before peak strain (mean ± 1 s.d.) in zebra finches and budgerigars, respectively. No significant changes in EMG duty cycle (EMG duration as a proportion of the strain cycle) with flight speed were detected in zebra finches (GLM, F=1.62, P=0.177, Fig. 3A; overall mean ± 1 s.d.=0.41±0.08). In budgerigars there was a significant change in EMG duty cycle with speed (GLM, F=3.37, P=0.014, Fig. 4A). In this species, EMG duty cycle was lowest at the lowest and highest speeds and increased to a maximum at intermediate speeds (Fig. 4A). We did not detect changes in the absolute duration of muscle activity in either species (GLM, F=1.572, P=0.189, zebra finches; F=2.431,

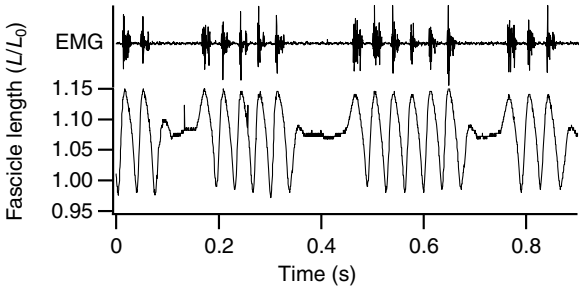

Fig. 1. *In vivo* zebra finch pectoralis fascicle strain and EMG activity. Traces show representative data obtained at a flight speed of 10 m s^{-1}. Fascicle segment length was measured *in situ* by sonomicrometry. Muscle activity was measured using a bipolar EMG electrode (see text). Fascicle length is shown relative to resting length L_0 measured with the bird resting on a perch.

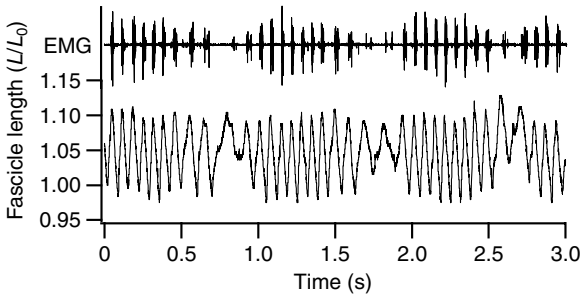

Fig. 2. *In vivo* budgerigar pectoralis fascicle strain and EMG activity. Traces show representative data obtained at a flight speed of 12 m s^{-1}. Fascicle segment length was measured *in situ* by sonomicrometry. Muscle activity was measured using a bipolar EMG electrode (see text). Fascicle length is shown relative to resting length L_0 measured with the bird resting on a perch.

P=0.06, budgerigars). A significant change in the level of relative EMG intensity with flight speed was detected in both zebra finches (GLM, F=2.87, P<0.025, Fig. 3B) and budgerigars (GLM, F=13.36, P<0.001, Fig. 4B). Relative EMG intensity was lowest at intermediate speeds. This pattern is different to that previously measured in budgerigars, which showed an increase in intensity with speed from 7 to 13 m s^{-1} (Tobalske and Dial, 1994). The narrower speed range used in the earlier study may explain the failure to detect a U-shaped relationship. Tobalske et al. detected changes in EMG intensity with flight speed in zebra finches (Tobalske et al., 2005), although the relative magnitude of speed related changes was lower.

Muscle strain modulation with flight speed

A significant change in the overall pectoral muscle strain with flight speed was detected in both zebra finches (GLM, F=8.26, P<0.001, Fig. 3C) and budgerigars (GLM, F=4.85, P=0.002, Fig. 4C). Strain was lowest at intermediate speeds in both species. In both species the range of strain measured across the range of speeds studied was similar: 0.12–0.15 in budgerigar and 0.13–0.16 in zebra finch. We also detected significant changes in strain cycle frequency (and concomitant changes in wingbeat frequency) with flight speed in both species (GLM, F=8.14, P<0.001, zebra finches, Fig. 3D; GLM, F=3.35, P=0.014, budgerigar, Fig. 4D). Wingbeat frequency in both species was highest (16 Hz in budgerigar and 30 Hz in zebra finch) at the highest flight speed and had a minimum at an intermediate speed. The degree of asymmetry of the strain cycle in terms of the relative amounts of time spent lengthening and shortening was also quantified. We detected no significant changes in relative shortening duration in in zebra finches (GLM, F=1.71, P=0.154, Fig. 3E), but did detect a significant change in budgerigars (GLM, F=3.26, P=0.016, Fig. 4E). In zebra finches the overall mean relative shortening duration was 0.67±0.10 (mean ± 1 s.d.), whereas in budgerigars the range was 0.56–0.69.

Flight kinematics

Kinematic data were collected from both species before implantation of sonomicrometry transducers and EMG electrodes, and subsequently in the same individuals after implantation during collection of pectoralis muscle fascicle strain and activity data. We detected no effect of instrumenting the birds on stroke amplitude in zebra finches (GLM, F=0.211, P=0.665, Fig. 5A), but detected a significant change in budgerigars (GLM, F=7.36, P=0.03, Fig. 6A). Stroke amplitude changed significantly with speed in zebra finches (GLM, F=27.8, P<0.001, Fig. 5A), but not in budgerigars (GLM, F=0.80, P=0.578, Fig. 6A). No effect of instrumentation on stroke plane angle was detected in either species (GLM, F=0.02, P=0.901, zebra finches, Fig. 5B; GLM, F=0.61, P=0.462, budgerigars, Fig. 6B). Stroke plane angle changed significantly with speed in both species (GLM, F=14.10, P<0.001, zebra finches, Fig. 5B; F=57.97, P<0.001, budgerigars, Fig. 6B).

Instrumenting the birds affected intermittent flight behaviour in both species. Significant changes in relative flapping duration, the proportion of the total flight time spent flapping, were detected (GLM, F=54.49, P<0.001, zebra finches, Fig. 5C; GLM, F=21.31, P<0.001, budgerigars, Fig. 6C). We also detected a significant effect of instrumentation on the frequency of non-flapping phases (GLM, F=8.95, P=0.036, zebra finches, Fig. 5D; GLM, F=20.72, P=0.002, budgerigars, Fig. 6D). Significant changes in relative flapping duration with speed were detected in both species (GLM, F=6.32, P<0.001, zebra finches, Fig. 5C; GLM, F=5.24, P<0.001, budgerigars, Fig. 6C). The frequency of non-flapping phase changed

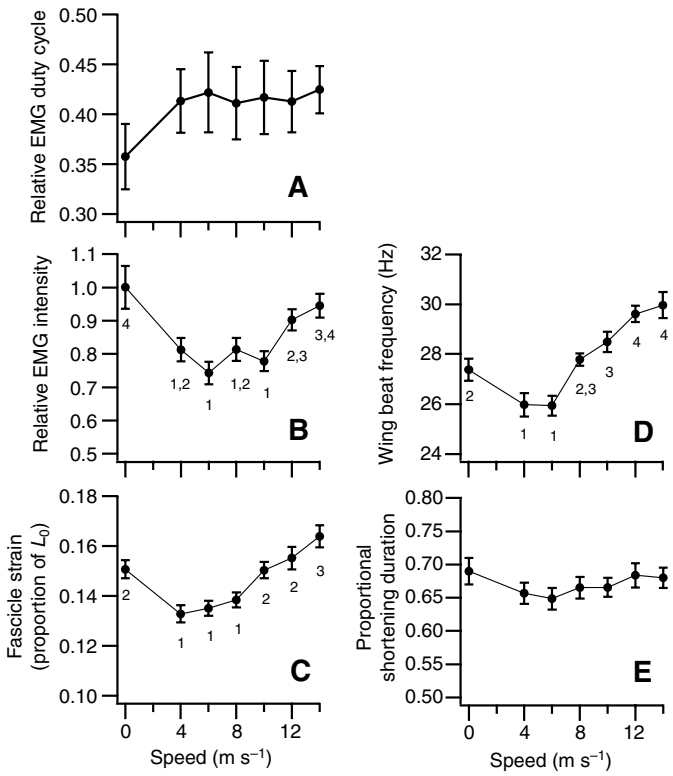

Fig. 3. The relationship between zebra finch pectoralis function and flight speed. (A) EMG duty cycle. (B) Relative EMG intensity. (C) Fascicle strain. (D) Wingbeat frequency. (E) Proportional shortening duration. The numbers adjacent to the data points denote homogenous subsets between which no significant differences were detected using a Scheffé *post-hoc* test (P>0.05). Values are means ± s.e.m. (N=6).

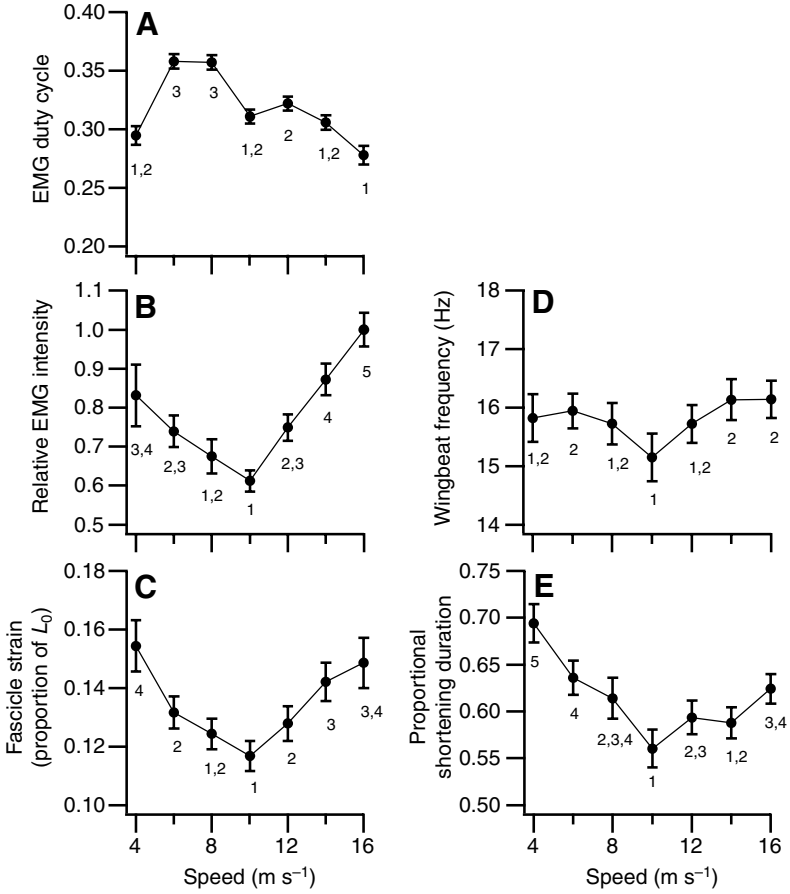

Fig. 4. The relationship between budgerigar pectoralis function and flight speed. (A) EMG duty cycle. (B) Relative EMG intensity. (C) Fascicle strain. (D) Wingbeat frequency. (E) Proportional shortening duration. The numbers adjacent to the data points denote homogenous subsets between which no significant differences were detected using a Scheffé *post-hoc* test (P>0.05). Values are means ± s.e.m.; N=7 (A,B); N=6 (C,D).

significantly with speed in zebra finches (GLM, F=2.64, P=0.045, Fig. 5D), but not budgerigars (GLM, F=0.95, P=0.475, Fig. 6D). Overall the type and magnitude of the effects are similar to those recorded elsewhere (Tobalske et al., 2005) in similarly instrumented birds.

Because all the individual zebra finches bounded with the wings held against the body, at all speeds, wing kinematics during the non-flapping phase was not quantified in this species. Budgerigars typically held their wings partially outstretched in the non-flapping phase. As there was potential for speed- or instrumentation-related changes in this behaviour we quantified the relative wingspan (non-flapping span, relative to maximum wingspan) during this phase. We detected no significant changes due to instrumentation (GLM, F=0.02, P=0.886), or speed changes (GLM, F=1.18, P=0.323) in the relative wingspan of the budgerigars during the non-flapping phase (Fig. 6E). The absence of speed-related changes is in contrast to previous work with this species that showed a progressive retraction of the wings during the non-flapping phase with increasing speed (Tobalske and Dial, 1994).

Discussion

Contrasting strategies for power modulation

We undertook this study to test the hypothesis that the power output of muscles with a homogeneous fibre composition can be varied by modulation of recruitment and strain trajectory. We selected two species of bird with pectoralis muscles composed of a single muscle fibre type and used electromyography and sonomicrometry to measure the degree of variation in muscle strain and activity pattern with flight speed. While both species showed changes in muscle recruitment and fascicle strain trajectory across a range of flight speeds, they differed in the type and scope of these changes. To allow comparison between the two species we have calculated the scope for changing the various recruitment and strain trajectory parameters across the range of flight speeds. In this case we define scope for each measure of pectoralis muscle function as the ratio of the highest to the lowest measured values for each parameter. The comparison is only meaningful if both species have reached either the upper limit of flight performance, or a similar proportion thereof. In the absence of data concerning the maximal level of aerobic metabolism this cannot be definitely established. However, the inability of the birds to sustain flight for more than 30 s at the extremes of the speed ranges suggests that upper limits to performance were reached or being approached in both species. The budgerigars did not hover in the working section of the wind tunnel, so the scope during forward flight is reported for this species. For zebra finches, which do hover, the scope during forward flight as well as during hovering is reported.

In terms of speed-related changes in EMG intensity, budgerigars show a greater scope (1.60-fold) than zebra finches (1.34-fold overall, 1.27-fold, forward flight). The detailed neuromuscular organization of the pectoralis muscles in these species is unknown. As the pectoralis muscles in both species consist of a single muscle fibre type (Rosser and George, 1986), EMG intensity changes likely indicate changes in the level of motor unit recruitment in the muscle across the speed range, rather than the progressive recruitment of increasingly fast fibre types seen in the mixed fibre type muscle systems of most other vertebrates. If all other factors that determine muscle power and force production remained unchanged, then this would indicate simple modulation of muscle force, with consequent changes in muscle work output per wingbeat. Changes in fascicle strain trajectory in concert with those in EMG intensity prevent a straightforward link between EMG intensity and muscle force being established for the pectoralis muscles of either species.

Both budgerigars and zebra finches modulated fascicle strain. In budgerigars, the observed scope for changing strain amplitude was 1.32, relative to 1.23 in zebra finches. Beyond this, the strain modulation patterns of the two species diverge. The shape of the strain cycle, in terms of relative time spent shortening, did not change significantly with speed in zebra finch (Fig. 3E), whereas the scope for change was greater in

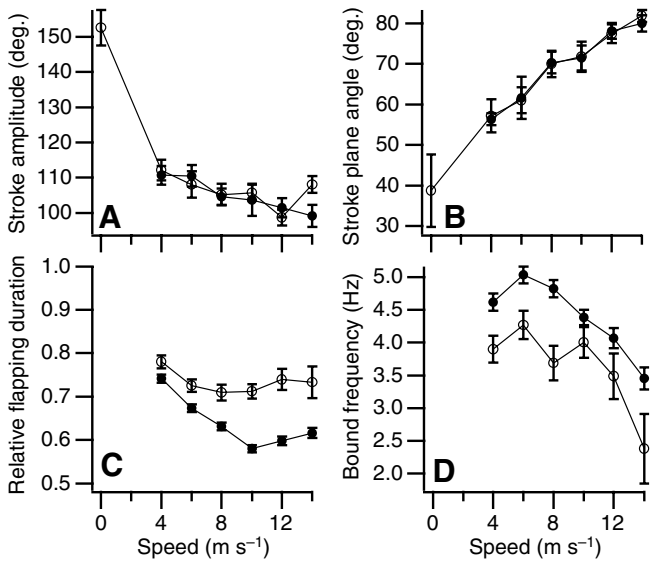

Fig. 5. Flight kinematics of zebra finches across a range of speeds. (A) Stroke amplitude. (B) Stroke plane angle relative to the horizontal plane. (C) Relative proportion of time spent flapping. (D) Bound frequency. Closed symbols, data from uninstrumented birds; open symbols, data from instrumented birds. Values are means ± s.e.m. (*N*=6).

budgerigars (scope 1.24, Fig. 4E). The only parameter in which zebra finches showed greater scope for change than budgerigars was strain cycle frequency, where the scope was 1.16, relative to 1.07 in budgerigars.

In both species, the observed changes in recruitment and fascicle strain trajectory are consistent with meeting flight power requirements that change in a U-shaped pattern with speed (Askew and Ellerby, 2007; Rayner, 1999; Ellerby and Askew, 2007). EMG intensities, synonymous with increased force production, are highest at the extremes of the speed ranges (Fig. 3B, Fig. 4B). Muscles have distinct optima for the strain and frequency at which power output is maximal, as shown by *in vitro* physiological studies (Stokes and Josephson, 1988; Josephson and Stokes, 1989; Askew and Marsh, 1997) and mathematical models (Weis-Fogh and Alexander, 1977; Josephson and Stokes, 1989; Josephson, 1989). The proportion of the strain cycle spent shortening has also been shown to be an important determinant of muscle power output, with power increasing as the proportion of the cycle spent shortening increases (Askew and Marsh, 1997). Where differences in strain amplitude, strain cycle frequency and asymmetry were detected, the conditions most likely to maximise power output occurred at the upper and lower extremes of the speed range (Figs 3, 4). In some muscles the relationship between strain trajectory and activation recorded *in vivo* during activities that demand high mechanical powers (such as sound production or escape locomotion) have been shown *in vitro* to correspond to those that are optimal for maximising power output (Askew and Marsh, 2001; Girgenrath and Marsh, 1999). In flying vertebrates, it seems reasonable to hypothesise that at the upper and lower extremes of the speed range, where flight power requirements are highest, the flight muscles operate under conditions that are optimal for maximising power output. At intermediate speeds, the reduced power requirements can be met by the flight muscles operating under sub-optimal conditions. Our measurements on the pectoralis muscle in budgerigars and zebra finches support this hypothesis, in that where these parameters are modulated, strain cycle amplitude, asymmetry and frequency are reduced at intermediate flight speeds (Figs 3, 4).

In budgerigars, the minima for EMG intensity, strain, and relative shortening duration all occurred at 8–10 m s^{-1} (Fig. 4B,C,E), suggesting that this may be the minimum power speed. This corresponds closely to the speed at which metabolic power, as measured by respirometry, is lowest in this species (Tucker, 1968; Bundle et al., 2007). No metabolic power-speed data are available for zebra finches; however our *in vivo* muscle strain and activity data suggest a minimum power speed lying between 4 and 8 m s^{-1} (Fig. 3). For both species, the strain and activity parameters that show variation show minima at flight speeds that closely correspond to the speed at which mechanical power is lowest, as measured *in vitro* and as estimated from an aerodynamic analysis (Askew and Ellerby, 2007; Ellerby and Askew, 2007).

The largely U-shaped relationships between relative EMG intensity and flight speed observed in budgerigars and zebra finches in the present study, and a previous study on zebra finches (Tobalske et al., 2005), are absent in some other studies. In European starlings *Sturnus vulgaris*, relative EMG intensity

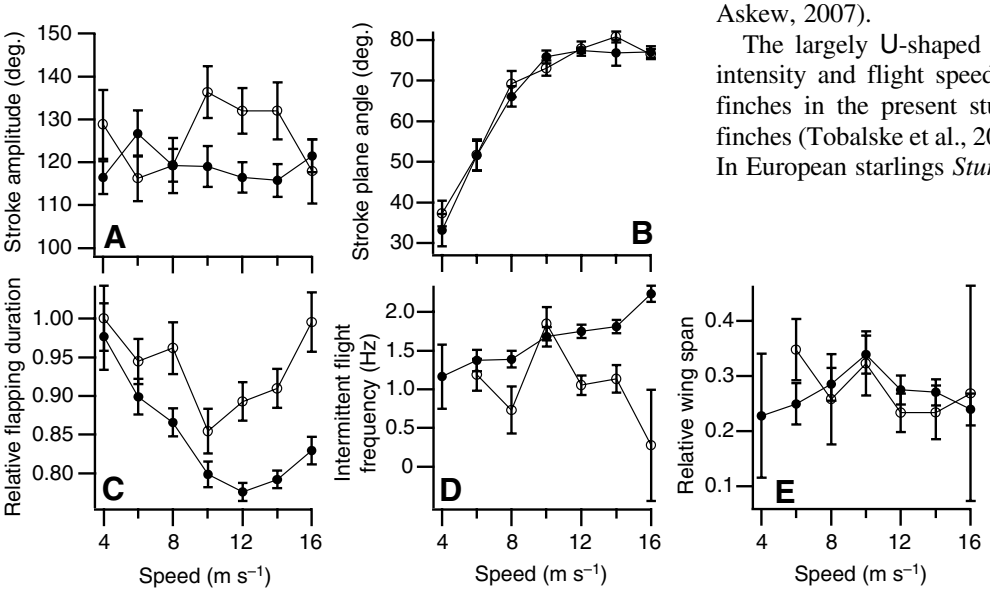

Fig. 6. Flight kinematics of budgerigars across a range of speeds. (A) Stroke amplitude. (B) Stroke plane angle relative to the horizontal plane. (C) Relative proportion of time spent flapping. (D) Frequency of non-flapping phase. (E) Relative wing span during non-flapping phase. Closed symbols, data from uninstrumented birds; open symbols, data from instrumented birds. Values are means ± s.e.m. (*N*=7).

increased 1.4-fold with increasing speed between 8 and 16 m s^{-1} (Tobalske, 1995). In budgerigars, relative EMG intensity increased 1.4-fold with increasing speed between 7 and 13 m s^{-1} (Tobalske and Dial, 1994). The different patterns may be due to the narrower range of flight speeds used in these previous studies. It is likely that 8 m s^{-1} in starlings is close to the minimum power speed (Ward et al., 2001) and 7 m s^{-1} in budgerigars is only a little below the minimum power speed (Ellerby and Askew, 2007). Therefore it might be expected that relative EMG intensity and wingbeat frequency will increase at speeds below the minima investigated. The only comparable muscle strain and activity data in the literature that cover a broad range of flight speeds are from the cockatiel [*Nymphicus hollandicus* (Hedrick et al., 2003)], where the scope for changing strain cycle amplitude, relative shortening duration and frequency was 1.29, 1.24 and 1.24, respectively. EMG intensity also was also correlated with measured muscle force in this species, indicating power modulation through changes in motor unit recruitment in addition to changes in strain trajectory.

Where data are available, it is clear that birds are manipulating strain trajectory and motor unit recruitment to modulate pectoralis power output across a range of flight speed. There is, however, a high degree of interspecific variation in terms of the scope for change in the various parameters that can be changed to modulate power output at the muscle level. Even the budgerigar and cockatiel, which are both in the family Psittacidae, show different patterns of strain trajectory change. The extent to which both species can change strain amplitude (scope 1.29 cockatiel, 1.32 budgerigar) and relative shortening duration (scope 1.24 cockatiel, 1.24 budgerigar) are very similar, but wingbeat frequency changes much more in cockatiels (scope 1.24) than in budgerigars (scope 1.07). Given the limited data available for comparison, any patterns relating to phylogeny or scaling are currently impossible to discern.

While our data are indicative of power modulation with speed at the muscle level, it is difficult to draw conclusions about the relative importance of changes in recruitment and strain trajectory without making direct measurements of muscle mechanical performance. Unless all other factors that influence power output remain equal, changes in one parameter cannot meaningfully be related to likely changes in muscle power output. The primary determinants of muscle power output are the intrinsic physiological properties of the muscle, the time-course of muscle activation, the level of motor unit recruitment and the strain trajectory of the muscle (Josephson, 1999). The shortening or lengthening velocity of the muscle is determined by the length trajectory and is therefore affected by the shape of the length trajectory, the cycle frequency and strain. A simple change in the level of motor unit recruitment, indicated by changes in EMG intensity would be indicative of changes in muscle force production. However, where this occurs in concert with any change in the muscle's length trajectory, this link can no longer be established. Changes in the length trajectory alter where the muscle operates on the length–force relationship (strain and mean muscle length) and force–velocity curve (strain, frequency, and shape), and change the relative importance of mechanisms such as stretch activation and shortening deactivation (Askew and Marsh, 1998; Josephson, 1999). This means that attempting to discern the relative importance of different power modulation mechanisms by component analysis (e.g. Hedrick et al., 2003) is ultimately fruitless because of the complex inter-relationship between the various factors affecting muscle force production.

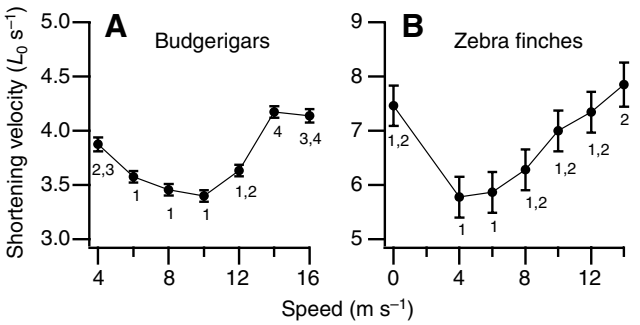

Fig. 7. Mean in-flight shortening velocities of (A) budgerigar and (B) zebra finch pectoralis muscles. The numbers adjacent to the data points denote homogenous subsets of data between which no significant differences were detected using a Scheffé *post-hoc test* (P>0.05). Values are means ± s.e.m. (N=6).

Is there a fixed gear?

Rayner hypothesised that the constraints of having a single pectoral muscle fibre type may be a factor in the adoption of intermittent flight strategies (Rayner, 1985). Without the ability to recruit muscle fibres with a range of intrinsic physiological properties to match changing power requirements, flapping intermittently provides a means of modulating average power output.

Fixed gear models of this type are underpinned by the basic force–velocity relationship of muscles *in vitro*. Under conditions of isovelocity shortening the power output of most muscles is maximised at a shortening velocity of approximately one-third of the maximal shortening velocity (V_{max}). Therefore, to preserve optimum muscle function in terms of maximising power output, it is argued that fascicle shortening velocity should be maintained within narrow limits across a range of flight speeds. This does not necessarily imply that the overall strain trajectory should remain constant, but that the cumulative effects of changing cycle frequency, amplitude, and shape should preserve the shortening velocity of the fascicles when active.

A more detailed examination of the physiological properties of muscle leaves this line of reasoning open to question for a number of reasons. (1) Even under conditions of simple, isovelocity shortening, the velocity range at which power output is close to maximal is actually quite broad. In the mouse soleus muscle the optimal shortening velocity to maximise power output is $0.22V_{max}$, but 90% of maximum power output is achieved over a range of velocities from approximately 0.15 to $0.35V_{max}$ (Askew and Marsh, 1998). (2) Data obtained from isovelocity measurements are difficult to relate to complex, cyclically operating systems. This is particularly true where shortening velocity is not constant, as in the avian pectoralis muscle (Askew and Marsh, 2001) (see Figs 1, 2). (3) *In vitro* force–velocity measurements are typically made in fully activated muscle. In the avian pectoralis, the fascicles are stretched while being activated, and shorten while deactivating

(Figs 1, 2). Changes in the rate of stretch and shortening will also affect stretch activation and shortening deactivation (Askew and Marsh, 1998). For example, increasing the velocity of shortening during relaxation facilitates deactivation. (4) Where the proportion of the cycle spent shortening changes, as in the budgerigar, optimal V/V_{max} also changes (Askew and Marsh, 1998). So, while muscles are clearly constrained by their intrinsic contractile properties, we should expect those constraints to be broader than suggested by standard force–velocity relationships. This is borne out by the fact that the average shortening velocity showed considerable plasticity across the speed range in both species (Fig. 7), changing significantly with speed (F=3.31, P=0.015, budgerigars; F=3.81, P=0.006, zebra finches), the scope for changing shortening velocity being 1.23 and 1.36 in budgerigars and zebra finches, respectively. Thus the argument that intermittent flight may allow average muscle power output to be modulated while preserving an optimal V/V_{max} is over simplistic. This does not rule out intermittent flight behaviour as a power modulation mechanism. Both budgerigars and zebra finches tended to spend proportionally less time flapping their wings at intermediate speeds where power requirements are lowest (Fig. 5C, Fig. 6C). This is consistent with the use of intermittent flight as a simple power modulation strategy by periodically turning off the mechanical power source to control the average power output over time. Overall, however, the idea that intermittent flight serves to maintain a 'fixed gear' fails to recognise the plasticity in function at the muscle level, and intermittent flight should be seen as being only one component of a complex power modulation strategy in both species.

Supported by a grant from the Biotechnology and Biological Sciences Research Council (24/S15677). The work complied with The Animals (Scientific Procedures) Act 1986 and was carried out under license from the British Home Office and with approval from the local ethical review committee.

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