

# Contractile activity of the pectoralis in the zebra finch according to mode and velocity of flap-bounding flight

Bret W. Tobalske\*, Lisa A. Puccinelli and David C. Sheridan

Department of Biology, University of Portland, 5000 N. Willamette Boulevard, Portland, OR 97203, USA

\*Author for correspondence (e-mail: tobalske@up.edu)

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## Summary

We studied flying zebra finch (*Taeniopygia guttata*,  $N=12$ ), to provide a new test of a long-standing 'fixed-gear' hypothesis that flap-bounding birds use only intermittent non-flapping phases, instead of variation in muscle activity, to vary mechanical power output in flight. Using sonomicrometry and electromyography, we measured *in vivo* fascicle length and neuromuscular recruitment in the pectoralis as the birds flew in different flight modes (level, ascending, descending; mean velocity  $1.6 \pm 0.3 \text{ m s}^{-1}$ ) and across velocities in a new, variable-speed wind tunnel ( $0\text{--}12 \text{ m s}^{-1}$ ). Synchronized high-speed digital video (250 Hz) provided a record of wing kinematics. Flight mode had a significant effect upon pectoralis strain, strain rate, fractional shortening and the relative timing of muscle activity (onset, offset and duration). Among flight velocities, we observed significant variation in pectoralis strain, fractional lengthening and shortening, strain rate, relative electromyographic (EMG) amplitude, and EMG duration and offset. In particular,

variation in strain rate and relative EMG amplitude indicates that the fixed-gear hypothesis should be rejected. Instead, it appears that zebra finch vary work and power output within wingbeats by modulating muscle contractile behavior and between wingbeats using intermittent bounds. Muscle activity patterns and wing kinematics were similar between free flight and wind tunnel flight at similar speeds. Comparing flights with and without surgically implanted transducers and electrodes, zebra finch exhibited a reduction in maximum velocity (from  $14$  to  $12 \text{ m s}^{-1}$ ) and a significant increase in wingbeat frequency and percent time flapping. This identifies a potential limitation of *in vivo* flight measurements, and similar studies of bird flight should, therefore, include measurements of the extent to which flight performance is compromised by experimental protocol.

Key words: zebra finch, *Taeniopygia guttata*, intermittent, flight, muscle.

## Introduction

Flap-bounding is a widespread form of intermittent flight exhibited by relatively small birds (Rayner, 1985; Rayner et al., 2001; Tobalske, 2001). This flight style consists of flapping phases interrupted by bounds during which the wings are held motionless and flexed against the body. Mathematical models indicate that the flight style may offer an aerodynamic advantage compared with continuous flapping, particularly at relatively fast flight speeds and if the bounding bird generates weight support during bounds using lift from the body and tail (Csicsàky, 1977; DeJong, 1983; Rayner, 1985; Rayner et al., 2001; Tobalske et al., 1999; Tobalske, 2001).

An alternative, long-standing explanation for the use of flap-bounding has been previously described as a 'fixed-gear' hypothesis (Rayner, 1977, 1985; Rayner et al., 2001; Tobalske, 2001). Small birds tend to either have a single fiber type or single myosin isoform in the fibers of their primary downstroke muscle, the pectoralis (Rosser and George, 1986; Rosser et al., 1996). As a given fiber type is expected to have an optimum velocity for efficient contraction, work and power output (Hill, 1950; Nelson et al., 2004), the fixed-gear hypothesis predicts

that a physiological constraint of a single fiber type limits the range of efficient contractile velocities available to small birds. Furthermore, early work (Aulie, 1970) suggested that small birds have only a single motor unit in their pectoralis, and this condition should preclude variation in motor-unit recruitment as a mechanism for modulating force among wingbeats (Rayner, 1977, 1984). Thus, the pectoralis of small birds is predicted to be 'geared' for maximal mechanical power output during activities such as acceleration, ascent or hovering. According to the fixed-gear hypothesis, intermittent non-flapping phases represent the only way to vary average power below maximal value without incurring a loss of efficiency due to variation in contractile velocity (Rayner, 1985).

Indirect evidence from flap-bounding zebra finch (*Taeniopygia guttata* Vieillot) casts doubt upon the validity of the fixed-gear hypothesis because the birds exhibit variation in angular velocity of their wing when flying at different velocities (Tobalske et al., 1999). Also, other small birds that use intermittent flight, such as the budgerigar (*Melopsittacus undulatus*), vary motor-unit recruitment in a manner that

appears to be associated with variation in force production in their pectoralis (Tobalske and Dial, 1994; Tobalske, 2001; Hedrick et al., 2003). In the present study, we seek to directly test the fixed-gear hypothesis using *in vivo* measures of muscle length change and neuromuscular recruitment. Explicitly, if the fixed-gear hypothesis is correct, the zebra finch should exhibit no significant variation in two variables in the pectoralis: (1) strain rate ( $L s^{-1}$ ) and (2) relative electromyographic (EMG) amplitude.

Although commonly used for studies of animal flight in the laboratory, wind tunnels represent an artificial environment, and relatively few data are available for comparing wind tunnel and free flight (e.g. Tobalske et al., 1997; Liechti and Bruderer, 2002). Moreover, virtually nothing is reported of the effects of surgical implantation of transducers and electrodes upon flight performance. Thus, we also compare flight performance among modes of flight with performance across flight speeds and include tests for effects of surgery upon intermittent flight kinematics.

## Materials and methods

### *Birds and experimental design*

We obtained zebra finch ( $N=12$ ; three males and nine females) from a commercial supplier (Bird Hut, Portland, OR, USA). The University of Portland Institutional Animal Care and Use Committee (IACUC) approved all housing and experimental protocols. Differences between genders were not significant, so we pooled the data. We measured morphology of the zebra finch using standard techniques (Pennycuick, 1989; Tobalske et al., 1999; Table 1). Body mass (g) was measured using a digital balance, immediately before starting experiments involving surgery. Wing measurements were made with the wings spread as during mid-downstroke. Linear measurements (cm) were obtained using digital calipers or a metric rule, and areas ( $cm^2$ ) were measured using digitized

photographs with a known scale for pixel:metric conversion. One bird died during captivity, and this bird was dissected to obtain measures of single pectoralis mass (g) and maximal pectoralis fascicle length (mm) in the sternobrachialis portion of the muscle (Tobalske and Dial, 2000).

A subset of the birds ( $N=7$ ) were trained to fly in a flight cage, 1 m wide  $\times$  2 m long  $\times$  2 m high, constructed of 2.5 cm plastic pipes and covered with 1.36 cm nylon mesh. Four 650 W halogen lights (Lowel Tota-light, Lowel-Light Manufacturing, Inc., Brooklyn, NY, USA) were distributed around the cage to continuously illuminate the field for video recording. The birds were trained to fly between two perches in response to a hand signal. For level flight, these perches were 1.5 m apart and 1 m in height. Alternatively, the perches were configured to provide an average flight path of 1.5 m in length and ascending at  $+60^\circ$  or descending at  $-60^\circ$ .

A different subset of the birds ( $N=5$ ) was trained to fly in a variable-speed wind tunnel over the full range of flight speeds for which they were willing to fly ( $0-14 m s^{-1}$  prior to surgery and  $0-12 m s^{-1}$  after surgery). Training protocols followed Tobalske et al. (1999). Three 650 W halogen lights (Lowel Tota-light) were used to illuminate the flight chamber.

### *Wind tunnel*

The wind tunnel was designed for studies of avian flight at the University of Portland. It is generally similar in design to the Harvard-CFS tunnel described in Hedrick et al. (2003); Barlow et al. (1999) and original sources therein were used in the design of the tunnel. The tunnel is an open circuit with a closed jet, featuring a 6:1 contraction ratio. Total length is 6.1 m. The working section in which the bird flies is square in cross-section,  $60 \times 60 \times 85$  cm inner diameter at the inlet, with clear lexan walls, 6 mm thick, used to provide views inside the working section. The flight chamber increases to a  $61.5 \times 61.5$  cm outlet to accommodate boundary-layer thickening. Air is drawn through the tunnel using a 7.5 kW (10 horsepower) direct current motor and a 0.75 m-diameter fan assembly (AFS-75 Series; SMJ Incorporated, Grand Junction, CO, USA). During experiments, velocity is selected as equivalent air velocity rather than true air velocity, as recommended by Pennycuick et al. (1997).

To describe the general quality of airflow within the flight chamber, we sampled profiles with the tunnel nominally set at equivalent airspeeds of 6, 10 and  $18 m s^{-1}$ . The profiles were obtained in the mid-plane of the working section using a pitot-static probe placed at 36 locations in a 10 cm spaced grid pattern (Hedrick et al., 2003). Mean equivalent velocities ( $\pm$ S.D.) at the three settings were:  $5.9 \pm 0.2$ ,  $9.9 \pm 0.3$  and  $18.1 \pm 0.5 m s^{-1}$ . Maximum absolute deviations from the mean were always less than 10% of the nominal equivalent velocity (8.6%, 9.1% and 7.0% at 6, 10 and  $18 m s^{-1}$ ). A velocity traverse in the mid-plane, mid-height indicated that the local boundary layer thickness was  $<1$  cm. Measurements in the mid-plane, mid-traverse using a 30 cm turbulence sphere indicated a percent turbulence of 1.2% (Barlow et al., 1999).

Table 1. *Morphological data for the zebra finch (Taeniopygia guttata)*

Variable	Mean value
Body mass (g)	$13.3 \pm 1.7$
Single wing length (cm)	$7.2 \pm 0.3$
Wing span (cm)	$16.1 \pm 1.3$
Mean wing chord (cm)	$3.9 \pm 0.3$
Aspect ratio	$4.2 \pm 0.6$
Single wing area ( $cm^2$ )	$26.5 \pm 2.3$
Combined area of both wings and body ( $cm^2$ )	$62.8 \pm 5.2$
Wing loading ( $N m^{-2}$ )	$20.8 \pm 2.6$
Disc loading ( $N m^{-2}$ )	$3.7 \pm 0.4$
Pectoralis mass (g)	0.9
Pectoralis maximum fascicle length (mm)	15.0

Values are means  $\pm$  S.D. ( $N=12$ , except pectoralis data, where  $N=1$ ).

### Kinematic analysis

We measured wing and body kinematics during non-implanted and implanted flights using digital video. A Redlake PCI-2000 (San Diego, CA, USA) provided a lateral view (250 Hz, PCI-2000, stored using PCI-R v.2.18 software), and a Redlake Motionscope 250 (250 Hz) provided a dorsal view for correction of lateral-view parallax and to confirm wing posture during non-flapping phases. Analog output from the Motionscope was imported to computer and stored using Quicktime v.3.5 software (Apple, Inc.). For both cameras, we used a shutter speed of 1/1000 s.

The birds were marked with dots of non-toxic black ink to identify anatomical landmarks when viewing digital images: shoulder, base of tail and distal tip of wing at the 9th primary. Approximate center of mass was calculated as a point halfway between the shoulder and the base of the tail. We digitized these anatomical landmarks using Didge v.2.07 (A. Cullum, Creighton University, Omaha, NE, USA).

From the video, we measured wingbeat frequency (Hz) as the number of wingbeats within a flapping phase divided by the duration of the flapping phase (in s). Non-flapping intervals consisted of bounds during which the wings were held flexed against the body for periods of 8 ms or more (two instances, 0.2% of total, featured glides). From the observed duration of flapping and non-flapping phases, we calculated the percentage of time spent flapping. We analyzed 111 flights within the flight cage (37 for each flight mode: level, ascending and descending) and 868 flapping/non-flapping phases in wind tunnel flight.

### Sonomicrometry and electromyography

Following unimplanted experiments, we surgically implanted electromyography (EMG) electrodes and sonomicrometry transducers into the left pectoralis of the zebra finch and repeated the experiments (Hedrick et al., 2003; Tobalske et al., 2003). To accomplish the implantation, birds were anesthetized using isoflurane inhalant and maintained at a surgical plane. Feathers were removed from the upper back as well as the left pectoralis. An alcohol solution was used to cleanse the skin. A 1 cm incision was made on the ventral surface over the pectoralis, and a 0.5 cm incision was made along the back. Sonomicrometry transducers (Sonometrics, London, Ontario, Canada; omnidirectional, 1.0 mm, 37 Gauge copper wire) and electromyography electrodes (twisted pair, bipolar 0.5 mm exposed tips, 100  $\mu$ m silver wire) were moved underneath the skin from the dorsal incision to the incision over the pectoralis. Two holes, 8 mm apart, were placed parallel to the longest muscle fascicles of the sternobrachialis portion of the pectoralis (Tobalske and Dial, 2000), and the sonomicrometry crystals were placed 3 mm deep into the holes. The crystals were sutured in place using 6-0 braided silk. A 25 Gauge hypodermic needle was used to implant the electromyography electrodes immediately between the sonomicrometry crystals. All wires were sutured to the intervertebral ligaments, and then all incisions were sutured

closed. The total mass of all recording equipment carried by the bird was 1.05 g (7.5% of body mass). The animal was allowed to fully recover from surgery, with experiments beginning within 4 h and lasting approximately 2 h. Following the experiments, a recovery surgery was performed in which all recording equipment was removed from the bird. All of the experimental animals recovered from the experiments were later donated to private individuals.

Electromyographic signals were amplified (1000 $\times$ ) and filtered (60 Hz notch, 100 Hz low pass, 5000 Hz high pass) using an AM Systems (Carlsborg, WA, USA) Model 1700 Differential AC amplifier. Sonomicrometry signals were created and recorded using a Sonometrics TRX Series 4 Digital Ultrasonic Measurement System and Sonosoft 3.2.1 software. Synchronization between the video and pectoralis data was obtained by sending a trigger pulse from the video camera to a separate channel on the Sonometrics A/D converter.

Subsequent analysis of the recorded signals was accomplished using IGOR 3.6 (Wavemetrics, Inc., Lake Oswego, OR, USA). EMG signals remained as recorded in volts, while sonomicrometry data were calibrated to strain ( $L/L_{rest}$ ) using the resting, perched values for crystal separation as  $L_{rest}$ . EMG bursts were identified as continuous sequences of spikes with rectified amplitude at least twice the amplitude of baseline electrical noise. For each contractile cycle (= wingbeat cycle), we measured the duration of EMG activity in the pectoralis (in ms) from onset to offset and also calculated the percentage of the wingbeat cycle in which the muscle was active. Start of downstroke was defined using the sonomicrometry trace at the onset of pectoralis shortening. Fractional lengthening (%) and fractional shortening (%) were the proportions of pectoralis strain in which the muscle was longer than or shorter than resting length, respectively. Strain rate (muscle  $L s^{-1}$ ) was pectoralis strain divided by the duration of time between maximum and minimum length as the muscle shortened during a contractile cycle. We also measured shortening duration (%), the time interval from maximum to minimum muscle length, relative to total cycle time. We analyzed 704 wingbeats including at least 10 from each speed for each bird.

### Statistical analysis

For each variable, we computed the mean value within each bird at each mode or speed. We then tested for a significant effect of flight mode or speed upon each variable using a univariate repeated-measures analysis of variance. To evaluate the effect of implanting recording equipment upon flight kinematics, we analyzed wingbeat frequency and the percent time spent flapping using repeated-measures ANOVA with experimental condition (implanted *versus* non-implanted) as a between-subjects factor. Values are presented as means  $\pm$  S.D.

### Results

While engaged in intermittent bounds, the pectoralis was inactive, and muscle length ( $L/L_{rest}$ ) was  $1.05 \pm 0.04$  (105% of

resting length; Fig. 1), indicating that the muscle was always at a greater length during bounds than it was during perching, even though the external wing posture is the same as in a perched bird (Tobalske et al., 1999). During level flight, either in the flight cage or wind tunnel, the birds gained altitude prior to a bound and lost altitude thereafter. Mean flight velocity in the flight corridor was  $1.6 \pm 0.3 \text{ m s}^{-1}$  and did not vary significantly among flight modes or due to surgical implantation of transducers and electrodes. As an example of typical flight behavior, Fig. 1 presents kinematics from 0.75 s of flight at an equivalent airspeed of  $12 \text{ m s}^{-1}$  in the wind tunnel. Two bounds lasting 104 ms and 80 ms, respectively, were interspersed between three flapping phases. Within the interval shown, one complete flapping and non-flapping cycle started at 0.25 s and ended at 0.64 s. This intermittent flight cycle included eight wingbeats, and the percentage of time spent flapping was 77.9%. Altitude, expressed relative to mean altitude during the interval, varied from  $-4.7 \text{ cm}$  at the beginning of the interval, during a flapping phase, to a maximum of  $4.1 \text{ cm}$  during the first bound.

Flight mode and velocity had significant effects ( $P < 0.05$ ; Table 2) upon muscle contractile dynamics (Figs 2, 3), the timing of wing movements (Fig. 4), and the relative magnitude and timing of muscle activity during the wingbeat cycle (Fig. 5). Among flight modes, the finch exhibited greater muscle strain ( $20.8 \pm 5.6\%$ ), fractional lengthening ( $14.5 \pm 5.0\%$ ), fractional shortening ( $-6.3 \pm 6.4\%$ ), strain rate ( $9.2 \pm 2.8 \text{ L s}^{-1}$ ) and relative EMG amplitude ( $0.9 \pm 0.3$ ) during ascending flight compared with level and descending flight. Minimal values for these variables were measured at intermediate flight velocities in the wind tunnel. While strain rate ( $6.6 \pm 2.3 \text{ L s}^{-1}$ ) and muscle strain ( $17.5 \pm 5.6$ ) were lowest

Table 2. Statistical significance of observed differences among means for pectoralis data in zebra finch (*Taeniopygia guttata*) flying in different modes in a wind tunnel ( $N=7$ ) and across different velocities in a wind tunnel ( $N=5$ )

Variable	Flight mode (d.f.=6,2) <i>P</i> -value	Flight velocity (d.f.=4,6) <i>P</i> -value
Strain rate ( $L \text{ s}^{-1}$ )	0.0013	<0.0001
Strain ( $\Delta L/L_{\text{rest}}$ )	0.0007	<0.0001
Fractional lengthening ( $\Delta L/L_{\text{rest}}$ )	0.1316	0.0004
Fractional shortening ( $\Delta L/L_{\text{rest}}$ )	0.0021	<0.0001
Relative amplitude of EMG	0.1246	0.0248
Shortening phase of wingbeat (%)	0.2982	0.3242
EMG onset (%)	0.0380	0.1492
EMG offset (%)	0.0330	0.0161

Values are from repeated-measures analysis of variance.

during flight at  $4 \text{ m s}^{-1}$ , relative EMG amplitude ( $0.5 \pm 0.1$ ) was lowest during flight at  $8 \text{ m s}^{-1}$ . Among flight velocities, flight at  $12 \text{ m s}^{-1}$  elicited maximum strain ( $23.7 \pm 4.5$ ) and strain rate ( $9.9 \pm 2.0 \text{ L s}^{-1}$ ), whereas relative EMG amplitude ( $0.6 \pm 0.2$ ) was greatest during hovering flight ( $0 \text{ m s}^{-1}$ ).

There was a significant effect of surgical treatment upon wingbeat frequency and percent time flapping ( $P < 0.002$  for both variables; Fig. 4). Surgery decreased wingbeat frequency by an average of  $2.0 \pm 0.1 \text{ Hz}$  among flight modes. By contrast, among flight velocities, post-surgery wingbeat frequencies were  $3.4 \pm 0.7 \text{ Hz}$  greater than pre-surgery flights. Post-surgery flights always featured a higher percentage of time spent flapping; the mean increase was  $20.4 \pm 9.8 \text{ Hz}$  among flight modes and  $17.0 \pm 8.0 \text{ Hz}$  among flight velocities. Wingbeat frequency was highest during ascending flight prior to surgery ( $29.5 \pm 1.3 \text{ Hz}$ ) and lowest ( $20.7 \pm 2.2 \text{ Hz}$ ) during pre-surgery wind tunnel flight at  $4 \text{ m s}^{-1}$  (Fig. 4A,B). By contrast, the variation in percentage of time spent flapping was greatest among velocities in the wind tunnel, with a minimum of  $62.6 \pm 9.6\%$  during pre-surgery flights at  $8 \text{ m s}^{-1}$  and a maximum of  $98.7 \pm 1.1\%$  during post-surgery flight at  $0 \text{ m s}^{-1}$  (Fig. 4C,D).

The percentage of a wingbeat cycle that the pectoralis spent shortening

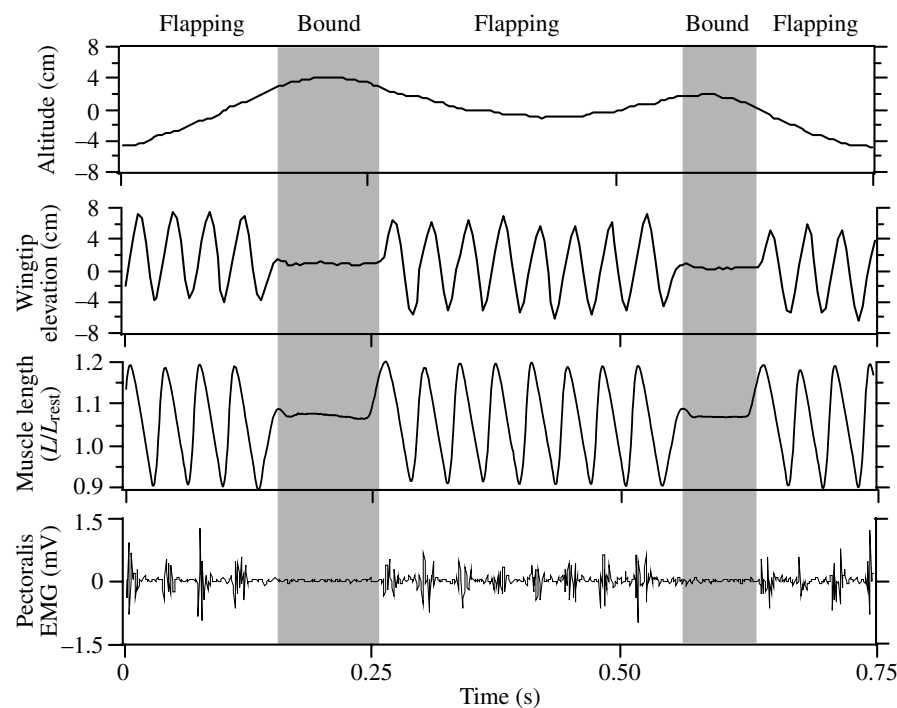


Fig. 1. Kinematics and muscle activity during zebra finch (*Taeniopygia guttata*) flight at an equivalent airspeed of  $12 \text{ m s}^{-1}$  in the wind tunnel. Two bounds and 13 wingbeats are included in this 0.75 s interval of flight. Altitude is for the estimated center of mass relative to average altitude during the flight interval.

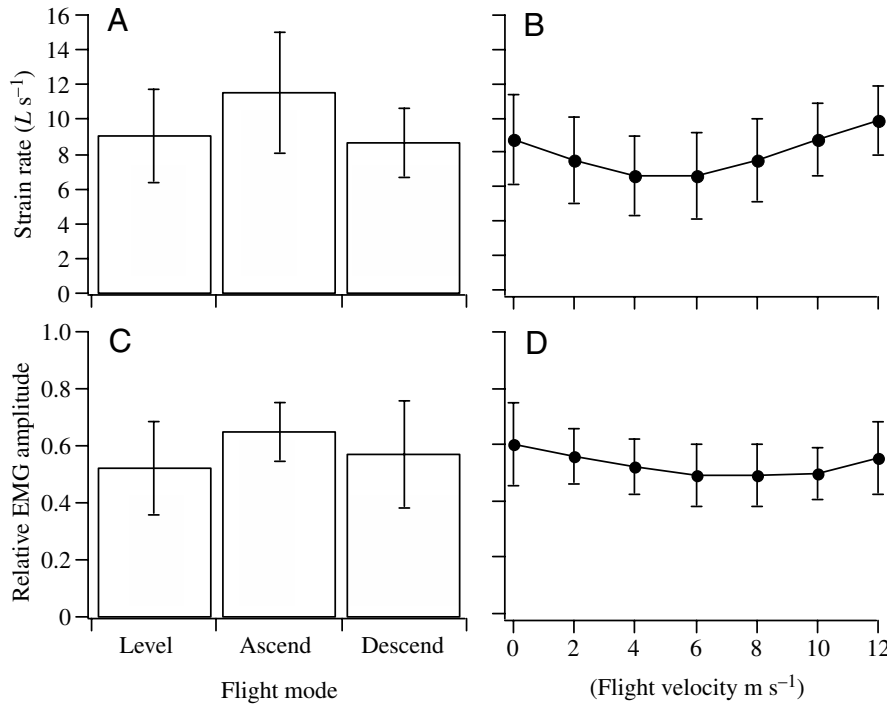


Fig. 2. Strain rate ( $L s^{-1}$ ) and relative electromyographic (EMG) amplitude in the pectoralis muscle of zebra finch (*Taeniopygia guttata*). (A) Strain rate in different modes of flight. (B) Strain rate across a range of velocity in the wind tunnel. (C) Relative EMG amplitude in different modes of flight. (D) Relative EMG amplitude at different equivalent airspeeds in the wind tunnel.

( $66.9 \pm 2.9\%$ ) did not change significantly among flight modes ( $P=0.2$ ) or velocities ( $P=0.3$ ; Fig. 5; Table 2), but mode and velocity caused significant variation in the timing of electrical activity within the wingbeat cycle. Burst duration of the EMG signal was greatest at intermediate velocities ( $19.7 \pm 3.7$  ms) and during level free flight ( $14.5 \pm 1.6$  ms) and was least during flight at  $12 m s^{-1}$  ( $16.0 \pm 1.8$  ms) and during ascending free flight ( $11.0 \pm 1.8$  ms). As may be observed in Fig. 5, the relative offset of EMG activity varied among flight modes ( $P=0.03$ ) and velocities ( $P=0.03$ ). Relative onset also appeared to change among flight modes ( $P=0.04$ ) but not flight velocities ( $P=0.1$ ).

### Discussion

Two key results lead us to reject the fixed-gear hypothesis: zebra finch exhibited significant variation in (1) strain rate and (2) relative EMG amplitude in their pectoralis (Fig. 2). The observed variation suggests that flap-bounding birds have the

capacity to modulate force, work and power output within wingbeats. Further variation is accomplished between wingbeats using intermittent bounds. Our results confirm predictions from previous studies of wing kinematics and neuromuscular recruitment (Tobalske et al., 1999; Tobalske, 2001). The U-shaped variation we observed in strain rate across flight speeds is consistent with previously reported U-shaped curves observed for angular velocity of the wings over the same range of speeds (Tobalske et al., 1999).

Likewise, the pattern of variation in EMG amplitude is similar to that reported for other species flying in different modes (Dial, 1992) and across velocities (Tobalske, 2001). The small size of the humerus in the zebra finch precluded surgical implantation of strain gauges for measuring pectoralis force production; therefore, our interpretation is an inference from recent study in the cockatiel (*Nymphicus hollandicus*; 84 g; Hedrick et al., 2003) that demonstrates that (1) strain rate is positively correlated with power output and (2) EMG amplitude, presumed to represent temporal and spatial summation of motor units, is positively correlated with force, work and power output (also see Dial and Biewener, 1993; Dial et al., 1997; Williamson et al., 2001).

In a comparative, biological context, variation in strain rate in the pectoralis of zebra finch was more substantial than variation in motor unit recruitment. Data for strain rate across flight velocities are available from the cockatiel (Hedrick et al., 2003), a species that does not use flap-bounding flight. As in the finch, the cockatiel exhibits a U-shaped curve of strain rate as a function of flight speed, ranging from  $5.19 L s^{-1}$  during flight at  $5 m s^{-1}$  to  $6.73 L s^{-1}$  at  $13 m s^{-1}$ . The coefficient of variation (CV, expressed as a percentage) for strain rate of the pectoralis in the cockatiel is 9.3% whereas it was

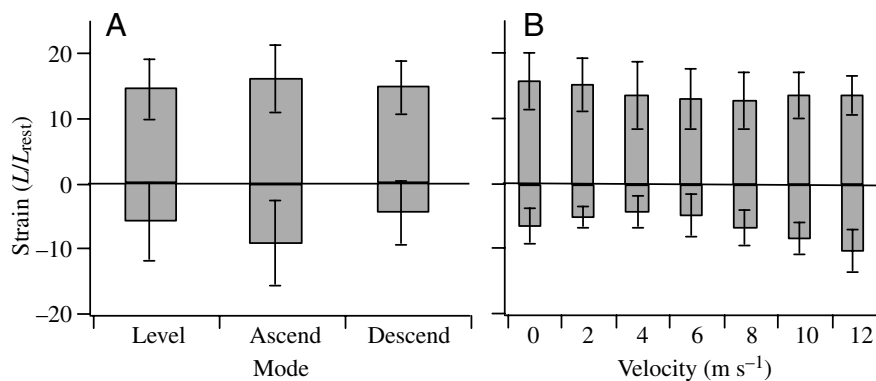


Fig. 3. Muscle strain ( $\Delta L/L_{rest}$ ) in the pectoralis muscle of zebra finch (*Taeniopygia guttata*). (A) Strain in different modes of flight. (B) Strain rate ( $L s^{-1}$ ) across a range of velocity in the wind tunnel.

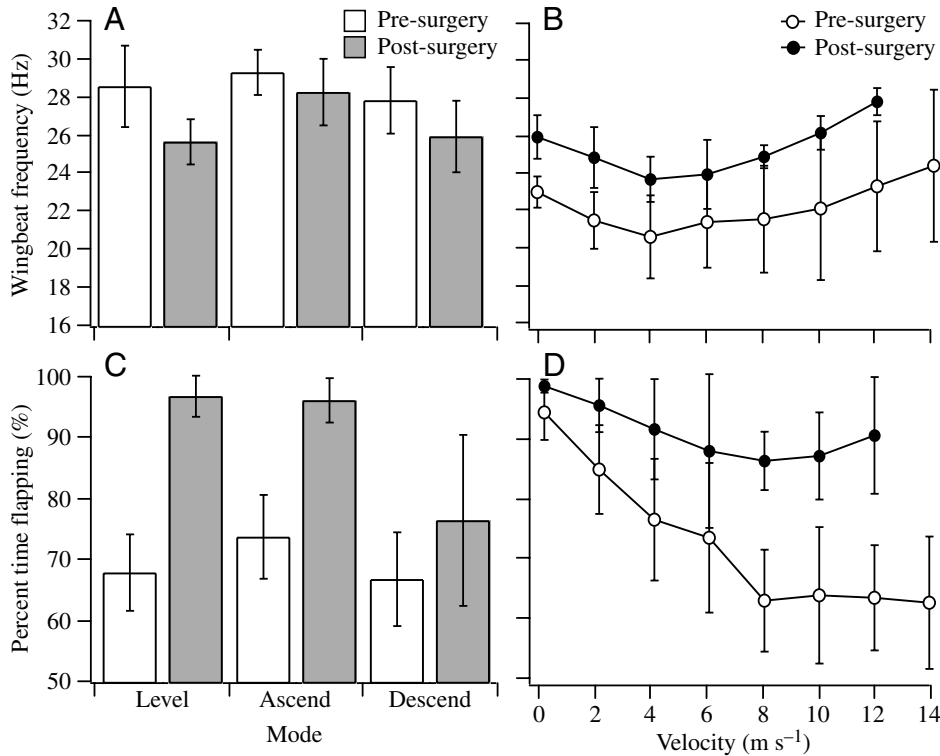


Fig. 4. Wingbeat frequency (Hz) and percent time flapping (%) in the zebra finch (*Taeniopygia guttata*). (A) Wingbeat frequency in different modes of flight. (B) Wingbeat frequency across a range of velocity in the wind tunnel. (C) Percent time flapping in different modes of flight. (D) Percent time flapping across a range of velocity in the wind tunnel.

15.3% among flight velocities in the zebra finch. Likewise, among flight modes, pectoralis strain rate in pigeons (*Columba livia*; 649 g; B. W. Tobalske, R. J. Hicks, D. C. Stark and

A. A. Biewener, unpublished) exhibits a CV of 12.8% versus the 16.1% we observed in the zebra finch. By contrast, at 7.7%, the CV for relative amplitude of EMG in the zebra finch across velocities is less than the CV of 50.0% in black-billed magpies (*Pica hudsonica*; 150 g; Tobalske et al., 1997) and 27% in the cockatiel (Hedrick et al., 2003).

The zebra finch is presently the smallest bird species for which *in vivo* pectoralis strain data are available.

Average strain rates in this species during flight in the wind tunnel ( $7.9 \pm 1.2 L s^{-1}$ ) are slightly higher than those exhibited by the cockatiel ( $5.8 \pm 0.5 L s^{-1}$ ; Hedrick et al., 2003), and the strain rate exhibited by the finch during ascent ( $11.3 L s^{-1}$ ) is well above values exhibited by galliform birds engaged in ascent ( $4.3\text{--}6.3 L s^{-1}$ ; 0.043–5.2 kg; Tobalske and Dial, 2000; Askew and Marsh, 2001). Also, the peak strain rates observed in the zebra finch pectoralis are near 90% of  $V_{max}$  on force–velocity curves for the lateral gastrocnemius and peroneus longus of turkeys (*Meleagris gallopavo*; Nelson et al., 2004). These data suggest that strain rate for a given activity may scale negatively with increasing body mass in birds. This prediction awaits further comparative study, as  $V_{max}$  for muscles from a wide size range of vertebrates (0.03–4.5 kg) is independent of body size (Nelson et al., 2004).

Peak strains of 23.7% in the pectoralis (Fig. 3) are considerably less than the 32–35% strains reported for pigeons (Biewener et al., 1998), black-billed magpies (Warrick et al., 2001), mallards (1 kg; *Anas platyrhynchos*; Williamson et al., 2001) and cockatiels (Hedrick et al., 2003). The zebra finch strains are more similar to those exhibited by small galliform birds (0.043–1 kg) engaged in take-off (Tobalske and Dial, 2000; Askew and Marsh, 2001). Thus, it appears that smaller birds may utilize less strain and higher strain rate to develop power for flight compared with larger birds. Doubtless, these patterns are related to phylogeny and ecology, with, for example, mallards and chukars (*Alectoris chukar*) having similar body mass (1 kg) but quite different strain rates during ascending flight (Tobalske and Dial, 2000; Williamson et al., 2001).

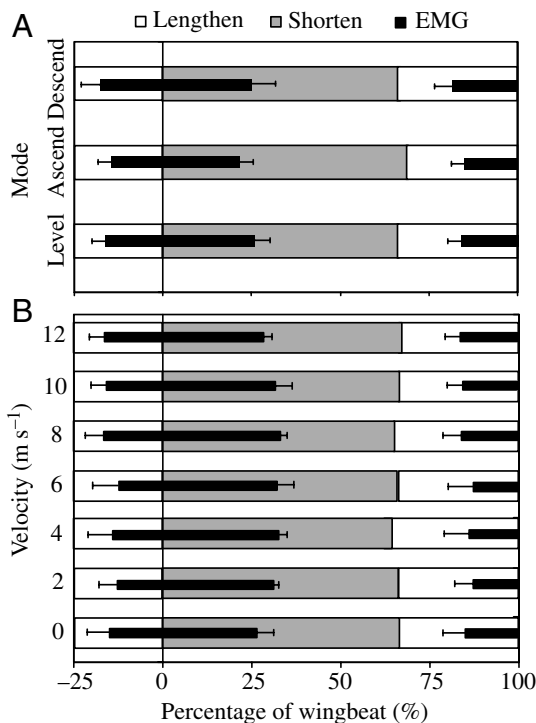


Fig. 5. Relative timing of pectoralis activity and muscle shortening during wingbeats in the zebra finch (*Taeniopygia guttata*). One wingbeat included a single shortening and lengthening phase in the pectoralis as measured using sonomicrometry. (A) Timing according to flight mode. (B) Timing according to flight velocity.

The variation we observed for the relative timing of the shortening phase of the pectoralis contraction cycle, as well as the duration, onset and offset of EMG activity, may signal additional mechanisms for modulating work and power output per wingbeat (Hedrick et al., 2003). Askew and Marsh (1998) provide important insight into how strain trajectories and muscle activation–deactivation modulate power output in mammalian muscle. A similar *in vitro* study of muscle contractile dynamics of zebra finch pectoralis would aid greatly in understanding the functional consequences of the variation we have reported in this study.

Few studies have compared animal flight performance in wind tunnels with performance in free flight (Tobalske et al., 1997; Liechti and Bruderer, 2002), and no study, to our knowledge, has evaluated the effect of surgical implantation on flight performance. Liechti and Bruderer (2002) report that effective wingbeat frequency (wingbeat  $s^{-1}$ ; including non-flapping phases) was less in barn swallows (*Hirundo rustica*) and house martins (*Delichon urbica*) during flight outdoors compared with in a wind tunnel, and it appears that our observations of pre-surgery percentage time spent flapping (Fig. 4C) are consistent with their study. Although wingbeat frequency in zebra finch during the short-duration flights in the flight corridor was higher than wingbeat frequency at a comparable speed (2 m  $s^{-1}$ ) in the wind tunnel, the percentage time spent flapping was considerably less.

Our observation that surgery and the transport of recording transducers and electrodes increased wingbeat frequency and percent time flapping is perhaps not surprising, but it does provide new and sobering insight that confirms that the process of experimenting with flying animals alters their flight performance. In light of the change in performance pre- and post-surgery (Fig. 4A,B), our data must be interpreted with caution. Future *in vivo* experiments with flying birds should also seek to evaluate the effects of the experimental procedure on locomotor performance; in this way, we might refine experimental design to minimize ‘observer-induced’ error.

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