



SYMPOSIUM

Ontogeny of Flight Capacity and Pectoralis Function in a Precocial Ground Bird (*Alectoris chukar*)

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Synopsis Flight is the defining characteristic of birds, yet the mechanisms through which flight ability develops are only beginning to be understood. Wing-assisted incline running (WAIR) and controlled flapping descent (CFD) are behaviors that may offer significant adaptive benefits to developing birds. Recent research into these forms of locomotion has focused on species with precocial development, with a particularly rich data set from chukar partridge (*Alectoris chukar*). Here we briefly review the kinematics and aerodynamics of flight development in this species. We then present novel measurements of the development of pectoralis contractile behavior during the ontogenetic transition toward powered flight. To obtain these new empirical data, we used indwelling electromyography (EMG) and sonomicrometry and tested WAIR and CFD in seven age classes of chukar ($n=2-4$ birds per age) from 5 days post hatching (dph) to adult (300+ dph). For each age class, we measured muscle activity during maximal performance, which was WAIR at 65° in birds 5 dph, CFD in birds 9 dph, WAIR at 80° in birds 14 dph, level flight in birds 25–61 dph, and ascending flight in adults. We also measured muscle activity during sub-maximal performance in all age classes. Flapping chukar chicks use near-continuous activation of their pectoralis at relatively low electromyography amplitudes for the first 8 days and progress to stereotypic higher-amplitude activation bursts by Day 12. The pectoralis undergoes increasing strain at higher strain rates with age, and length trajectory becomes more asymmetrical with greater variation in contractile velocity within the shortening phase of individual contractions. At 20–25 days (12–15% adult chukar mass), pectoralis activity and locomotor performance approaches that of adults, although strain rate exhibits a temporary decrease at 61 dph concurrent with using newly-replaced primary feathers. To better understand how these patterns relate to the evolution of life-history strategy and locomotion, we encourage future efforts to explore these behaviors in altricial and semi-altricial bird species.

Introduction

The juvenile stage of life history in many animals is understood to be under intense predation pressure (Martin 2015), and it is hypothesized that over evolutionary time this pressure has led to the rapid development of locomotor capacity in precocial species (Carrier 1995; Herrel and Gibb 2006; Martin 2015). Birds in particular experience a locomotor metamorphosis, transitioning from obligate bipedal runners to facultative fliers, but the development of their locomotor apparatus is only beginning to be revealed. During the past 15 years, considerable effort has been

applied to revealing the kinematics, mechanics and aerodynamics of the ontogenetic onset of flight capacity in several species of precocial birds. A model species for this research has been the chukar partridge (hereafter “chukar”, *Alectoris chukar*, Dial 2003a, 2003b; Dial et al. 2006; Tobalske and Dial 2007; Jackson et al. 2009; Heers et al. 2011, 2015). Some comparative data are available for other precocial species including sister taxa in the Galliformes (Australian brush turkey, *Alectura lathami*, Dial and Jackson 2011; blue peafowl, *Pavo cristatus*, Heers and Dial 2014; and chickens, *Gallus domesticus*,

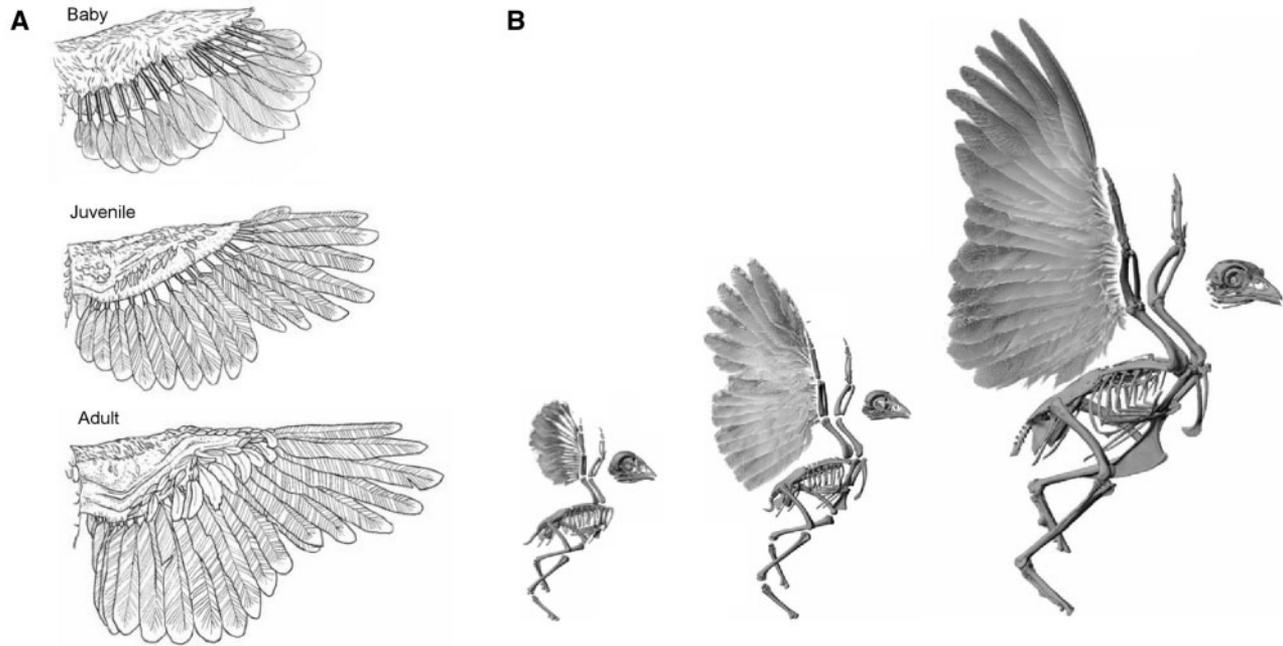


Fig. 1 Morphology in different age classes of chukar. **(A)** Wing morphology in babies (6–8 dph), juveniles (25–28 days), and adults (45+ days; Dial et al. 2006). The wings are scaled so that the musculoskeletal portions are the same length. **(B)** skeletal morphology obtained using micro-computed tomography (μ CT) scanning (Heers et al. 2016).

Kozak et al. 2016; LeBlanc et al. 2016) and one precocial species in the Anseriformes (mallards, *Anas platyrhynchos*, Dial et al. 2012). Only recently has research begun to explore the onset of flight capacity in altricial birds, thus far in Passeriformes (Dial et al. 2016; Cornell et al. 2017; Crino et al. 2017).

Herein, we briefly review of what is presently understood about the development of flight capacity in chukar, and we describe new empirical *in vivo* measures of pectoralis function during ontogeny in this species.

Wing morphology

Chukar chicks exhibit a sigmoidal growth curve, hatching at ~ 10 g in body mass and achieve adult mass (~ 500 g) after approximately 120 days (Dial et al. 2006). Chukar forelimb and hindlimb “modules” (Gatesy and Dial 1996) develop concurrently, whereas other precocial species including mallards and peafowl exhibit trade-offs between hindlimb and forelimb function (Dial and Carrier 2012; Heers and Dial 2015). Chukar young will walk away from their nest within 1–2 days post hatching (dph). Their wings are initially covered primarily with downy feathers although each shaft (rachis) of the flight feathers is apparent. By 4 dph, the wing planform is capable of deflecting air and producing minor levels of aerodynamic force when it is dried, spread, and spun as a propeller (Heers et al. 2011) even though the rachis of each flight feather is encased in a sheath so that there are gaps between

which air can easily move (Heers et al. 2011, Dial et al. 2012). At 6 dph, chukar flight feathers are paddle-shaped, with stiff shafts and a round terminus due to free distal barbs outside the main sheath (Fig. 1A). The feather barbs emerge progressively from distal to proximal as the rachis continues to grow, thus increasing planform area and decreasing whole-wing transmissivity at 8 dph (Heers et al. 2011; Fig. 1A), yet the feather barbules retain symmetry. At this stage of development (6–8 dph), the keel of the sternum is not ossified; the ends of the long bones of the wing are also primarily cartilaginous (Heers et al. 2016; Fig. 1B).

Subsequent development of the flight feathers (e.g., 14–23 dph; Dial et al. 2006) includes progressive elongation of the rachis, loss of the sheath surrounding the barbs, and emergence of bilateral asymmetry between the leading and trailing vanes of the primaries (distal flight feathers; Dial et al. 2006). During this time, the keel and ends of the long bones remain unossified (Heers et al. 2016). By 32 dph, the flight feathers and coverts have grown to the point where the wing surface is complete, thus reducing macro-scale transmissivity of the wing compared with the condition due to gaps caused by the feather sheaths in younger wings (Dial et al. 2012). During development, the feathers increase in barbule and barbicular density which function to progressively reduce micro-scale transmissivity (Heers et al. 2011; Dial et al. 2012).

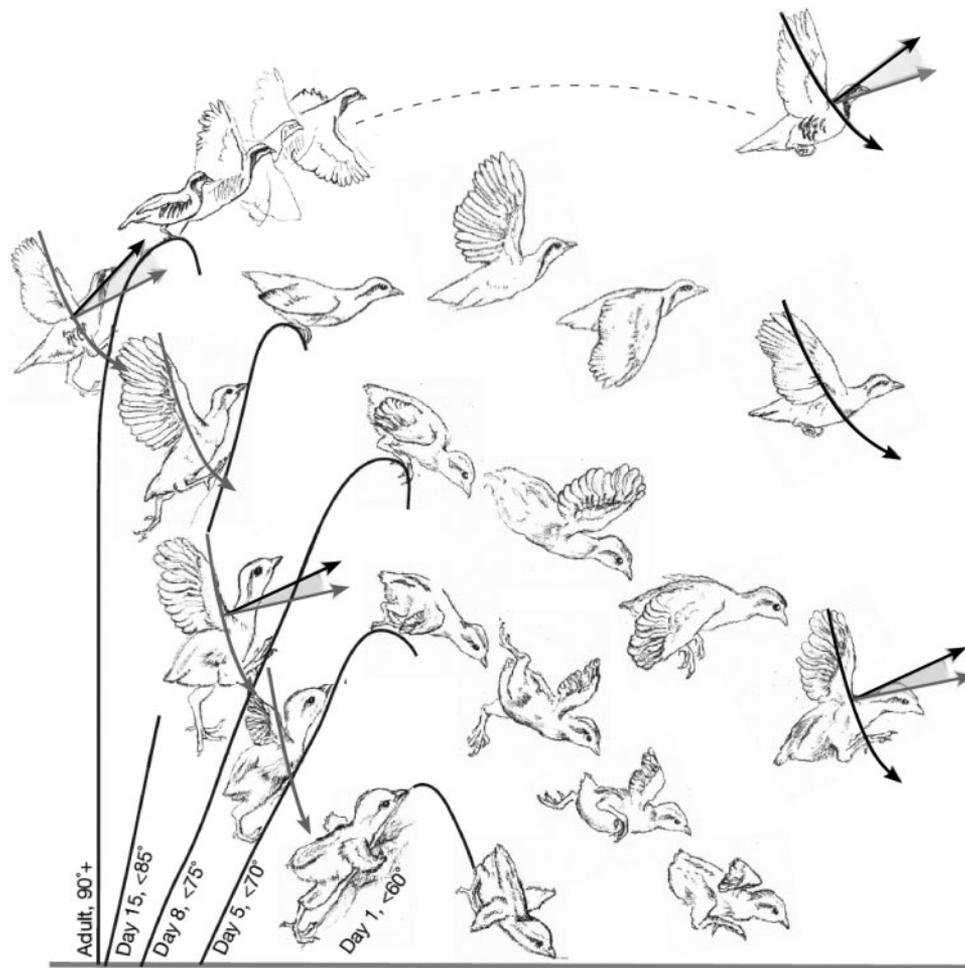


Fig. 2 Locomotor development during ontogeny in the chukar from hatching to adulthood. Beginning at 5 dph fledglings use their wings to contribute to ascend inclines in wing-assisted incline running (WAIR) and to slow their fall from an elevation (controlled flapping descent, CFD). Stroke curves represent the trajectory of the wing. Vectors indicate average lift during WAIR and estimated lift during slow level flight and descent (Dial et al. 2008).

Wing area increases linearly with age until 45 dph, and the manner in which this varies with body mass yields minimum wing loading (weight divided by surface area of flight apparatus) in juveniles ≤ 30 dph (Dial et al. 2006). Lower wing loading yields significant benefits to early flight performance (Dial et al. 2006; Dial and Carrier 2012; Heers et al. 2016), and, conversely, higher wing loading leads has been shown to reduce climbing and flight ability in adult brush turkey (Dial and Jackson 2010). By 100 dph, the skeleton is fully ossified (Heers et al. 2016; Fig. 1B).

Locomotor capacity and patterns of wing movement

Two recently described behaviors offer an opportunity to observe the use of the flight apparatus in developing birds before they can fly. During wing assisted incline running (WAIR), birds produce aerodynamic forces with their wings that assist their legs in climbing up an incline to escape to a refuge

(Bundle and Dial 2003; Tobalske and Dial 2007). In controlled flapping descent (CFD), the birds flap their wings and generate forces that reduce their acceleration toward the ground (Dial et al. 2008) and may also assist in righting their orientation due to inertial redistribution of mass (Evangelista et al. 2014). WAIR and CFD are ubiquitous among avian species and may offer significant adaptive benefits to developing birds (Dial et al. 2008).

Chukars exhibit a generally increasing trend in locomotor performance (here defined as maximal ascent angle) with increasing age (Fig. 2); similar increases are apparent in other precocial species, but the timing of the onset of flight capacity depends upon forelimb-hindlimb tradeoffs during development (Dial and Carrier 2012; Heers et al. 2016). Detailed kinematic analysis leads to the identification of three stages of development in locomotion (Jackson et al. 2009; Fig. 3). In early Stage I

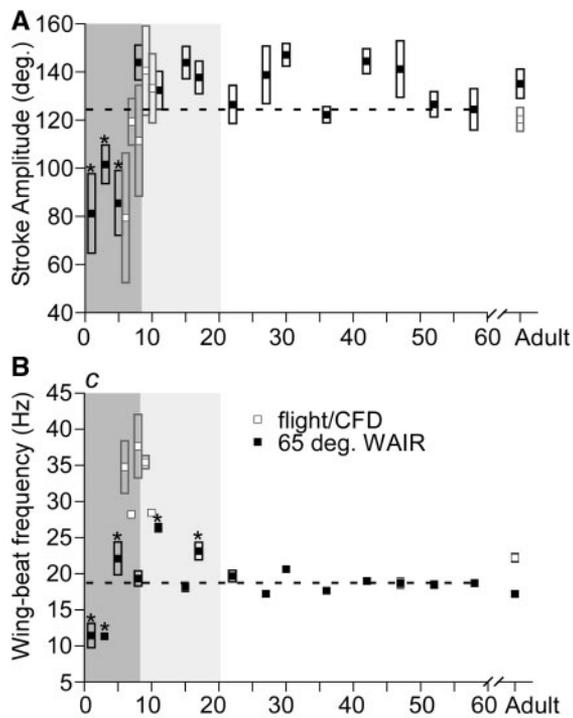


Fig. 3 Kinematic measurements related to aerodynamic force production during development in chukar. Both stroke amplitude (**A**) and wingbeat frequency (**B**) during wing assisted incline running (WAIR) reach or surpass 58 dph values by 8 dph. Background shading indicates stage of development (dark gray, Stage I, light gray, Stage II, white, Stage III). Open squares, flight or controlled flapping descent (CFD); filled squares, 65° WAIR (Jackson et al. 2009).

(1–6 dph) the birds will use quadrupedal “walking” to climb up a slope, and their flapping does not reduce acceleration during CFD. Stage II is from 7 to 19 dph; the birds use symmetric wing movement during flapping in WAIR and CFD, and they reduce acceleration and reorient their body during CFD (Jackson et al. 2009; Evangelista et al. 2014). Stage III begins at 20 dph and from this age through adulthood the birds are capable of vertical WAIR and level flight (Dial et al. 2008; Jackson et al. 2009; Fig. 2). For comparison, mallards, in which wing development lags significantly behind hindlimb development, can swim immediately but do not achieve flight capacity until 45 dph (Dial and Carrier 2012). During younger ages, mallards use their wings and legs in “steaming” or wing-assisted surface swimming (Dial and Carrier 2012); over their development, performance also linearly increases with age.

External wing kinematics appear to suddenly “crystallize” or converge on adult form during the transition from Stage I to Stage II (8–10 dph; Jackson et al. 2009; Fig. 3). For example, prior to this crystallization event, stroke amplitude is smaller (80–100°) compared to birds 10+ dph (~120°; Fig. 3A). Wingbeat frequency is also less (~12 Hz) in

birds 5 dph and younger engaged in WAIR compared with birds 20+ dph in which it is ~18 Hz (Fig. 3B). Wingbeat frequency during WAIR and CFD temporarily increases (9–16 dph) and is particularly high in CFD during the crystallization phase (~35 Hz) before reducing to the values observed in birds 20+ dph (Jackson et al. 2009; Fig. 3B). Other kinematic variables including stroke angle and angle of attack of the wing converge upon adult values at approximately 10 dph (Jackson et al. 2009).

Skeletal kinematics are generally similar between juveniles and adult birds engaged in the same level of locomotor activity, but some differences are apparent (Baier et al. 2013; Heers et al. 2016). In contrast with external measures of wing amplitude (Jackson et al. 2009; Fig. 3A), younger birds (7–18 dph) exhibit higher stroke amplitude of the humerus because they depress and retract their humerus more than adults. Juveniles also keep their wings relatively more extended during downstroke, more flexed during upstroke, and use greater angles of attack (Heers et al. 2016) compared with adults.

Aerodynamics

With increasing age in chukars engaged in WAIR, *in vivo* aerodynamic forces from the wings increase in magnitude (Tobalske and Dial 2007; Fig. 4). This is undoubtedly due to concurrent maturation of the feathers, skeletal elements (Fig. 1), as well as neuromuscular control and muscle contractile properties. One way to isolate the contributions of other variables from the feather maturation is to dry and spread the wings and then spin them as a propeller to emulate the middle of downstroke (Heers et al. 2011; Dial et al. 2012; Fig. 5).

Downstroke is the primary phase of aerodynamic force production during the chukar wingbeat, and individual downstrokes generate circulation that, when shed into the wake of the bird, create discrete vortex rings shed once per downstroke (Tobalske and Dial 2007; Fig. 4). These rings, and the associated induced air flow, can be used to estimate the magnitude and direction of average aerodynamic forces. During WAIR, average forces are oriented upwards and toward the substrate, thus confirming that the wings are being used to improve traction by the hindlimbs as well as elevate the center of mass. Comparable evidence is provided by force-plate measures (Bundle and Dial 2003). Adjusted for wing size, circulation did not vary among three age classes (6–8 dph, 25–28 dph and 45+). Absolute differences in wing size therefore mean that lift from the wings is estimated to be 6.6% of body weight in birds 6–8 dph

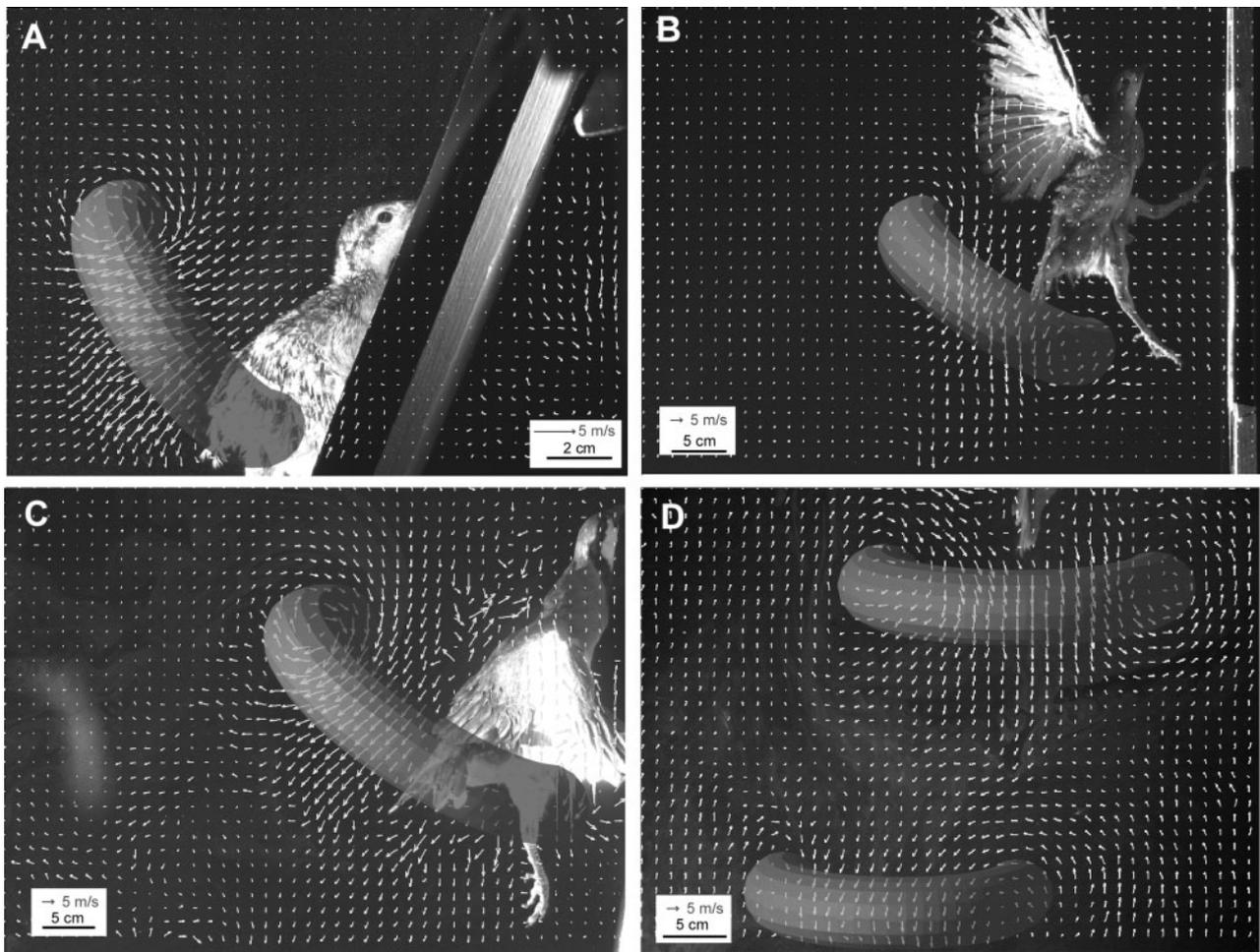


Fig. 4 Velocity fields in the wake of chukar during wing-assisted incline running (WAIR, **A–C**) and flight (**D**) as revealed using particle image velocimetry (Tobalske and Dial 2007). Backgrounds illustrate the bird and incline in **A–C** and the tip of the tail of the bird in **D**. Transparent loops represent an assumed 3D shape of vortex rings as inferred from concentrations of vorticity. (**A**) Days 6–8 dph at 65°, (**B**) 25–28 dph at 90°, (**C**) (45+ dph) at 80°, and (**D**) 45+ dph engaged in ascending flight at 80°, featuring wake patterns for two successive downstrokes.

and between 63 and 86% of body weight in older juveniles and adults engaged in WAIR. Forces during ascending flight are oriented vertically, indicating that lift is providing weight support and an increase in potential energy; in adults, lift is estimated to be 67% greater during ascending flight compared with WAIR (Tobalske and Dial 2007; Fig. 4).

Coefficients of vertical and horizontal force from spinning wings provide insight into the forces produced per unit wing area and velocity, thus control for absolute differences in wing size and angular velocity (Heers et al. 2011; Dial et al. 2012; Fig. 5). Polar diagrams represent vertical coefficient (C_V) as a function of horizontal coefficient (C_H) and obtained over a range of angles of attack (Fig. 5A). The ratio of vertical to horizontal coefficient ($C_V:C_H$) is generally interpreted as a measure of aerodynamic efficiency, which also varies as a function of angle of attack (Fig. 5B). From 8 dph through

to adults, peak C_V , peak $C_V:C_H$ and the resultant coefficient for any given angle of attack increase systematically with age. Several changes in feathers including increasing flexural stiffness, feather unfurling, feather asymmetry, and barbule overlap all may contribute to these improvements in performance. A cardboard cut-out with the same planform as an 8 dph bird wing exhibits peak C_V that is greater than the actual 8 dph wing, and it exhibits a greater $C_V:C_H$ ratio compared with the actual wings from birds of all age classes including adults. Similar trends are reported for mallard wings during ontogeny, but the onset of measurable forces begins at 4 dph in chukar compared with Day 30 in mallards (Dial et al. 2012).

Contractile behavior in the pectoralis

We undertook a new empirical study to test hypotheses about the development of pectoralis function in

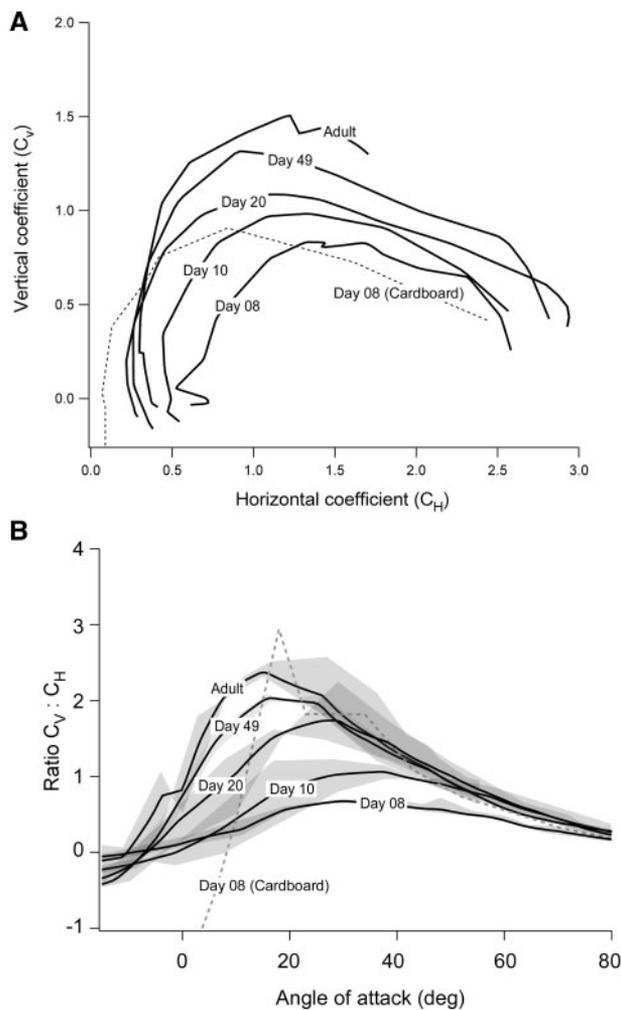


Fig. 5 Aerodynamic measurements from the dried, spread wings of an ontogenetic series of chukar obtained by spinning the wings as a propeller to emulate mid-downstroke during slow flight (Heers et al. 2011; Dial et al. 2012). (A) Polar traces of vertical force coefficient (C_v) and horizontal force coefficient (C_H). C_v and C_H were measured at angles of attack (α) from -15 to 80° . Gray dashed lines are from flat cardboard models of the youngest chukar. (B) Lift-to-drag ($C_v:C_H$) ratio.

chukar that emerged from the array of morphological, behavioral, kinematic and aerodynamic data summarized above.

Flapping flight, particularly at slow speeds such as immediately after takeoff or during explosive, escape flight that is typical of Galliform birds (Tobalske and Dial 2000), requires high output of work and power from the pectoralis muscle (Tobalske et al. 2003; Jackson et al. 2011). This high output is accomplished with relatively high muscle stress (e.g., ~ 80 kPa) and particularly large strains which scale in a positive manner with increasing body size among adults of various species (Tobalske et al. 2010). Pectoralis strain is $\geq 35\%$ during ascending flight in medium-sized birds including adult chukar

(Tobalske and Dial 2000), pigeons (*Columba livia*, Jackson et al. 2011), and corvids (Jackson and Dial 2011).

During flapping flight the pectoralis muscle exhibits precise timing without antagonist co-activation in the supracoracoideus, although it is estimated that some residual force from both muscles is present at each wing turnaround perhaps to promote joint stability (Tobalske and Biewener 2008). A variety of developing mammals including humans appear to lack precise timing, with antagonist co-activation for joint stability resulting in highly variable activation times (Forssberg 1999, Westerga and Gramsbergen 1994; Muir 2000, Chang et al. 2006). Insight into muscle strain during terrestrial locomotion is potentially problematic for predicting contractile behavior during flight because distal muscles of the hindlimb are generally used for elastic energy storage and recovery during running or galloping, which is associated with near-isometric contraction, strains $< 6\%$, during force development (Biewener et al. 1998). The proximal muscles of the limbs in terrestrial locomotion may exhibit comparable strains (12–27%) as in the pectoralis during flapping flight, but these muscles in terrestrial locomotion also exhibit pronounced co-activation of antagonists in adults (Gillis and Biewener 2001). Overall, the physical differences between terrestrial and fluid environments are understood to require animals to modulate neuromuscular recruitment patterns and contractile behavior (Gillis and Biewener 2000).

As flapping flight appears to require precise neuromuscular control and high power, and previously-studied juvenile vertebrates lack these characteristics (Westerga and Gramsbergen 1994; Forssberg 1999; Muir 2000, Chang et al. 2006), we hypothesized the youngest chukar would exhibit typical vertebrate patterns: (1) lack precise motor control of muscle activation and (2) exhibit variable contractile dynamics prior to the convergence of their external wing kinematics that occurs at 8–10 dph at the transition from Stage I to Stage II of development (Jackson et al. 2009; Fig. 3). We sought to explore how the development of adult-like neuromuscular function coincides with feather and skeletal development (Dial et al. 2006; Heers et al. 2016; Fig. 1), locomotor performance and kinematics (Jackson et al. 2009; Figs. 2 and 3) and the production of useful locomotor forces (Tobalske and Dial 2007; Heers et al. 2011; Dial et al. 2012; Figs. 4 and 5).

To test our hypothesis that variation in timing of neuromuscular activity and contractile dynamics would decrease with increasing age, we used

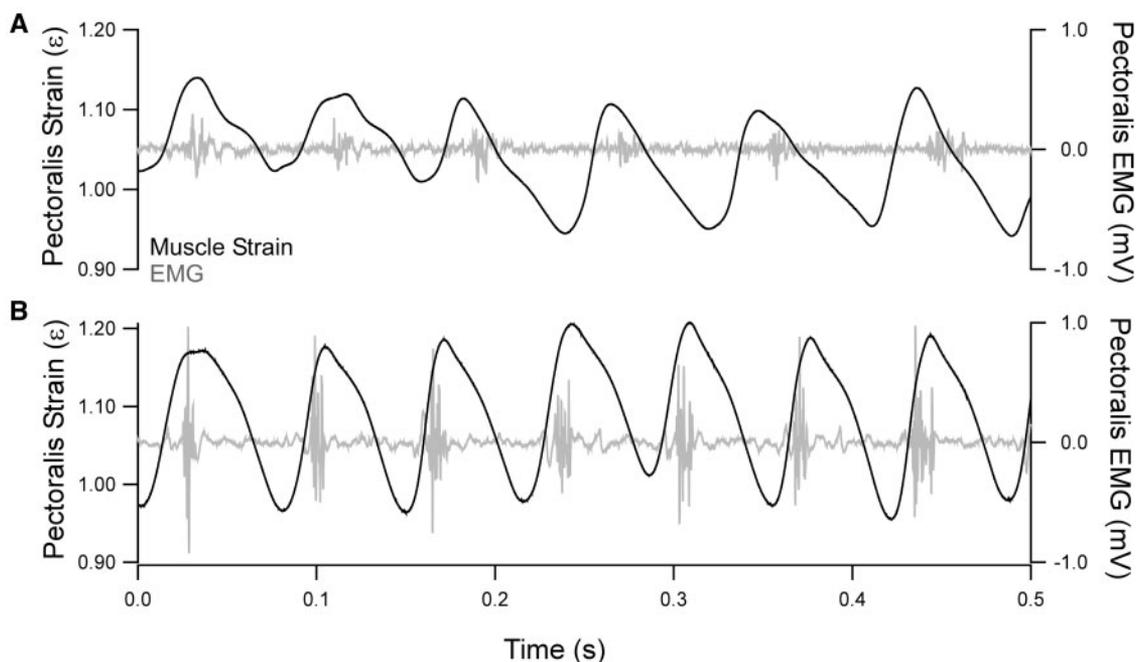


Fig. 6 Contractile activity in the pectoralis of a **(A)** juvenile (8–10 dph) and **(B)** adult (300+ dph) chukar engaged in wing-assisted incline running (WAIR) at 60–70°. Muscle length (strain, ϵ , $\Delta\text{Length}/L_{\text{rest}}$) was measured using sonomicrometry and neuromuscular activity was measured using electromyography (EMG, mV). The axes are set to the same scale to facilitate comparison of relative magnitude and variance of the signals between the two ages.

surgically-implanted electromyography electrodes and sonomicrometry transducers in the pectoralis to measure activation patterns and muscle shortening, in an ontogenetic series of chukar. If the kinematic-based hypothesis is true, we predicted that Stage I birds would show (1) high intra-individual variability in muscle activation timing; (2) relatively long duration of activation, including activation during times when the antagonist muscle (supracoracoideus m.) is expected to be activated; and (3) reduced muscle shortening strain and contractile velocity, possibly indicative of reduced shortening capacity, reduced force production, or both. The last prediction emerges from external kinematics (Jackson et al. 2009) whereas skeletal kinematics indicate that younger birds may exhibit greater strain associated with greater depression and retraction of their humeri during flapping (Heers et al. 2016).

Methods

Animals and experimental design

Our experiments involved seven age classes of chukar partridge (*Alectoris chukar*). Our sample size was $N=3$ individuals for each age class except 61 dph ($N=2$) and 300+ dph ($N=4$). Body masses (mean \pm SD) were: 5 dph, 18 ± 1 ; 9 dph, 26 ± 6 ; 14 dph, 48 ± 8 ; 25 dph, 87 ± 16 ; 39 dph 173 ± 12 ; 61 dph,

299 ± 13 ; 300+ dph, 549 ± 70 . The birds were reared from eggs purchased from a commercial supplier; the birds were housed at the Field Research Station at Fort Missoula; all housing and experimental procedures were approved by the University of Montana Institutional Animal Care and Use Committee).

To elicit maximal locomotor performance from the birds, we used the methods of Dial et al. (2006) and Jackson et al. (2009), for which the specific protocol varied according to age class. As chukar develop, they are able to ascend steeper inclines using wing assisted incline running (WAIR). The birds were motivated to use WAIR to join conspecifics in a darkened refuge (enclosure) at the top of the incline. We tested all birds using WAIR at 60–70°, birds 14 dph and older using 80° WAIR, birds 25 dph and older using 90° WAIR. We tested birds 9 dph and older in controlled flapping descent (CFD; Dial et al. 2006; Jackson et al. 2009). Birds 25 dph and older engaged in level ($\pm 20^\circ$) flight. Lastly, birds 300+ dph engaged in ascending flight at 80°.

We surgically implanted electromyography (EMG) electrodes and sonomicrometry transducers into the pectoralis using standard methods (Tobalske and Dial 2000; Jackson et al. 2011). All surgical implantation was performed with the birds anesthetized using isoflurane inhalant (HME109, Highland Medical Equipment, Temecula, CA, USA; 5% to induce,

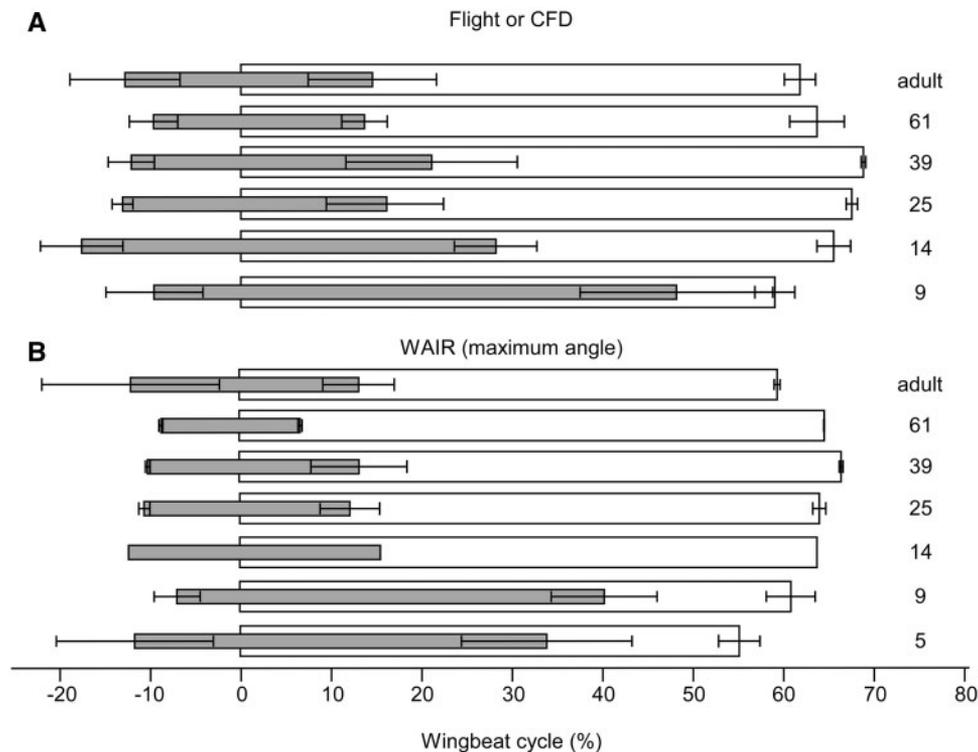


Fig. 7 Relative timing of length change and activation in the pectoralis of an ontogenetic series of chukar engaged either in **(A)** flight or controlled flapping descent and **(B)** wing-assisted incline running (WAIR) at maximum angle for which a given age class would perform. White, shortening phase of contraction; gray, electromyographic (EMG) activity. Mean \pm SD, $n = 2\text{--}4$ birds per age class.

2–3% to maintain), and the birds were allowed to fully recover for 12–15 h prior to the experiments. For birds 5–25 dph, we used 1-mm sonomicrometry crystals, and we used 2-mm crystals for older birds (Sonometrics, Inc., London, ON, Canada). Our electromyography electrodes were bipolar (twisted pair, 100 μ m diameter silver wire, 0.5 mm bared tips with 2 mm spacing; California Fine Wire, Inc., Grover Beach, CA, USA). One pair of sonomicrometry crystals and an EMG electrode were implanted parallel to the fascicle axis of the mid-anterior region of the sternobranchial portion of the pectoralis. The sonomicrometry crystals were implanted at a depth of about 4 mm beneath the superficial fascia of the muscle and a distance of 8–12 mm apart. The EMG electrode was inserted into the mid-belly of the muscle immediately adjacent to the sonomicrometry crystals.

Recordings were made by connecting the transducers and electrode to a shielded cable with leads connected to a sonomicrometry amplifier (Triton 120.1, Triton Technology, Inc., San Diego, CA, USA) and EMG amplifier (AM Systems Model 1700, Carlsborg, WA, USA). For EMG recording, we used $\times 1000$ amplification, 60 Hz notch and 100–5000 Hz band pass filters. We recorded the analog data to a computer using a Power Lab 8SP

analog to digital converter and Chart v. 4.5 software sampling at 10,000 Hz (ADInstruments, Inc., Colorado Springs, CO, USA). We recorded synchronized video (500 Hz, 1/5000 s shutter speed 1024 \times 1024 resolution, Photron SA-3, Photron, Inc., San Diego, CA, USA) to verify locomotor performance.

Subsequent analysis was performed using IGOR Pro v6.12 (Wavemetrics Inc., Portland, OR, USA). We measured 942 wingbeats, including at least three wingbeats per bird and locomotor style. The measured distance between sonomicrometry crystals was corrected for the conduction velocity of sound through muscle, the offset due to the epoxy lens and a 5-ms phase delay (Tobalske and Biewener 2008). Resting length (L_{rest}) was measured during standing at the top of the incline, with the wings folded and the pectoralis inactive. Muscle strain was calculated as $\Delta L/L_{\text{rest}}$, where ΔL is the difference between instantaneous length (L) and L_{rest} . For each contractile cycle (=wingbeat cycle), we measured the duration of EMG activity in the pectoralis (in ms) from onset to offset, average absolute amplitude of signal (mV), and 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 (%)

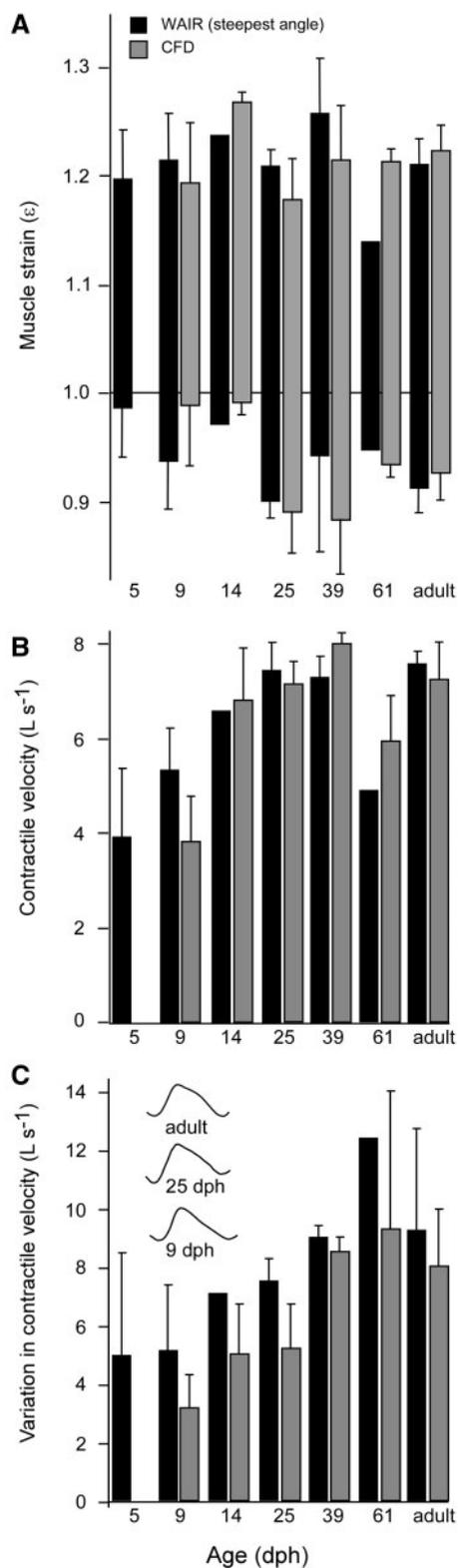


Fig. 8 Muscle contractile behavior in an ontogenetic series of chukar engaged in wing-assisted incline running (WAIR; black bars) and either controlled flapping descent or flight (gray). Mean \pm SD, $n=2-4$ birds per age class. (A) Muscle strain (ϵ , $\Delta\text{Length}/L_{\text{rest}}$), (B) contractile velocity (strain rate, L s^{-1}), and (C) variation in contractile velocity (L s^{-1}) during shortening phase of cycle.

were the proportions of pectoralis strain where 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 measured variation in strain rate during shortening by dividing the shortening phase into three equal intervals, calculating strain rate within each third, and computing a standard deviation among the three measures. We also measured duty factor (%), the time interval from maximum to minimum muscle length divided by total cycle time.

We used non-parametric statistical analysis: Spearman rank correlations to test for effects of age upon aspects of muscle function and paired Wilcoxon signed-rank tests to evaluate the significance of differences in muscle function between WAIR and flight. We adopted these tests following Heers et al. (2016) who had a similar experimental design with small sample sizes within age classes and non-repetitive sampling of individuals. We performed our statistical analysis using mean values within each individual ($n=21$ for WAIR, $n=17$ for CFD and flight) using R version 3.2.3 (R Foundation for Statistical Computing) within RStudio v. 1.0143, RStudio, Inc. Herein, we report means \pm SD.

Results

Wingbeat frequency (WBF) was slightly lower in all ages of implanted birds in our present study compared with non-implanted birds in which it averaged 18 Hz (Jackson et al. 2009; Fig. 4). WBF in implanted birds varied from 10.3 ± 0.78 Hz in Day 5 dph birds engaged in WAIR at $60-70^\circ$ to 16.6 ± 0.6 Hz in Day 25 dph engaged in CFD. There was a significant effect of age upon WBF during WAIR (Spearman's Rank Correlation, $S=681$, $P=0.0009$). Very young birds (5 and 9 dph) exhibited lower frequencies (10–11 Hz), frequencies peaked at near 17 Hz at 14 dph, and became similar to adult frequencies (~ 14 Hz) between 14 and 25 dph. After 25 dph, there was a trend of increasing wingbeat frequencies with effort at any given age. For example, frequency in adults (300+ dph) increased from 13.8 ± 0.5 Hz to 15.0 ± 0.7 Hz comparing WAIR at $60-70$ and 90° , respectively, and it was 15.9 ± 0.3 Hz during ascending flight. Differences in WBF between WAIR and flight were not significant (Wilcoxon Signed-Rank, $\nu=73$, $P=0.75$).

Neuromuscular activity (EMG) patterns and contractile behavior of the pectoralis as measured using

sonomicrometry varied with age class in a manner that generally tracked the developmental stages previously identified using wingbeat kinematics (Jackson et al. 2009; Fig. 4). Differences between juvenile and adult patterns were more apparent during Stage I, age ≤ 9 dph, and converged upon adult patterns during Stage II, age between 9 and 20 dph.

The amplitude of EMG signals from the pectoralis was less in Stage I juveniles and increased with age (Fig. 6). The effect of age upon mean EMG amplitude was statistically significant for WAIR ($S=415$, $P=0.0002$) and CFD or flight ($S=219$, $P=0.0008$). Comparing activity during WAIR at 60–70°, EMG amplitude in juveniles 5 and 9 dph was 0.04 ± 0.01 and 0.05 ± 0.02 mV, respectively, while means for other age classes were up to six times greater, varying from 0.16 to 0.20 mV, with one exception being at 39 dph where EMG amplitude was 0.09 ± 0.01 mV. During CFD, EMG similarly increased with age. It was 0.04 ± 0.02 mV and 0.09 ± 0.05 mV in ages 9 and 14 dph, respectively, and varied from 0.17 ± 0.04 mV to 0.29 ± 0.12 mV in older ages. For age classes ≥ 14 dph, capable of steeper incline angles during WAIR and either CFD or level flight, EMG amplitude increased with increasing angle of WAIR and reached greatest values during level or ascending flight. For example, in adults (300+ dph), EMG amplitude was 0.16 ± 0.06 mV during WAIR at 60–70°, increased to 0.23 ± 0.07 mV and 0.23 ± 0.04 mV during WAIR at 80 and 90°, and reached a maximum of 0.31 ± 0.08 mV during ascending flight. However, differences in EMG amplitude were not statistically different between WAIR and flight ($\nu=147$, $P=0.89$).

The relative shortening duration of a wingbeat cycle was slightly less in birds 5 dph engaged in WAIR at 60–70° (0.55 ± 0.2) compared with all other age classes and activities for which duty factor varied from 0.6 to 0.69 (mean 0.63 ± 0.03 ; Fig. 6). The effect of age upon duty factor was significant during WAIR ($S=736$, $P=0.02$) but marginally non-significant during flight ($S=459$, $P=0.08$). The relative duration of EMG activity within a wingbeat cycle declined with increasing age ($S>1450$, $P<0.00001$ for WAIR and flight). During WAIR it was 0.46 ± 0.01 and 0.47 ± 0.06 in juveniles 5 and 9 dph, and declined to a minimum of 0.15 ± 0.02 in birds 61 dph. In CFD and flight, relative duration of EMG during CFD was 0.58 ± 0.07 in birds 9 dph and declined to a minimum of 0.23 ± 0.09 in birds 61 dph. Within any age class, EMG durations were generally greater during CFD and flight (Fig. 7A) compared with WAIR ($\nu=145$, $P=0.0002$; Fig. 7B). EMG duration declined with age ($S>1450$,

$P<0.0001$). During WAIR, it averaged 45 ± 2 ms during in birds 5 dph and varied between 13 and 18 ms in birds 14 dph and older. Similarly, during CFD in birds 9 dph, duration of EMG activity was 54 ± 5 ms decreased to 28 ± 1.5 in birds 14 dph and varied between 12 and 20 ms in birds 25 dph and older during CFD and flight.

Pectoralis strain increased with age during WAIR, CFD, and flight (Fig. 8A); although this trend was not statistically significant for WAIR ($S=1163$, $P=0.29$), it was significant during CFD and flight ($S=215$, $P=0.0008$). Minimums were measured at 0.21 ± 0.08 in birds 5 dph engaged in WAIR and 0.21 ± 0.03 in birds 9 dph engaged in CFD. Among age classes in level flight, strain increased from 0.26 ± 0.01 in birds 25 dph to between 0.29 and 0.31 in older birds, and the overall maximum strain was exhibited during ascending flight in birds 300+ dph (0.31 ± 0.03). However differences between strain during WAIR and flight were not statistically significant ($\nu=50$, $P=0.82$). Fractional lengthening did not vary systematically with age class or activity ($P>0.05$), and the overall average was 0.22 ± 0.04 . In contrast, fractional shortening tended to increase with age within a given locomotor activity. For example, in CFD, fractional shortening was -0.01 ± 0.06 and -0.01 ± 0.01 in birds 5 and 9 dph, respectively, and it was between -0.08 and -0.12 in birds 25 dph and older. Within an age class, fractional shortening generally was less during WAIR at 60–70 and 80° than during WAIR at 90°, CFD and flight. The effect of age upon mean fractional shortening was statistically significant for CFD and flight ($S=1417$, $P=0.0008$) but not WAIR ($S=1828$, $P=0.42$). Average fractional shortening was significantly less during WAIR than CFD and flight ($\nu=58$, $P=0.04$). For birds 39 dph, fractional shortening $+0.01 \pm 0.06$ (i.e., pectoralis length was always greater than resting length in a cycle) during WAIR at 60–70°, and -0.01 ± 0.05 during WAIR at 80°. In these birds, it became more negative (increased in absolute amount of fractional shortening) to -0.06 ± 0.10 during WAIR at 80°, -0.12 ± 0.05 during CFD, and -0.07 ± 0.04 during level flight.

Contractile velocity in the pectoralis generally increased with age (Fig. 8B) from a minimum of $3.87 + 1.46 L s^{-1}$ in birds 5 dph engaged in WAIR at 60–70° to velocities of $5.39 L s^{-1}$ or greater for birds older than 14 dph during WAIR at 80°. This pattern was statistically significant during CFD and flight ($S=399$, $P=0.04$) but not WAIR ($S=954$, $P=0.09$). One exception to the overall trend with age was that contractile velocity in birds 61 dph was consistently less than for birds 39 dph or

300+ dph. For example, during CFD, contractile velocity was $5.99 \pm 0.96 \text{ L s}^{-1}$ in birds 61 dph, while it was $8.06 \pm 0.51 \text{ L s}^{-1}$ and $7.24 \pm 0.75 \text{ L s}^{-1}$ in birds 39 and 300+ dph, respectively. Similarly, during WAIR at 90° , contractile velocity in birds 61 dph was $4.80 \pm 0.0 \text{ L s}^{-1}$, and it varied between 7.25 L s^{-1} and 7.56 L s^{-1} among other age classes 25 dph and older. Maximum overall contractile velocity was exhibited by birds 300+ dph during WAIR at 90° ($7.56 + 0.27 \text{ L s}^{-1}$), which was slightly greater than during ascending flight ($7.4 \pm 1.21 \text{ L s}^{-1}$).

Strain trajectories during pectoralis shortening changed as a function of age, and this was reflected in the variation we observed in contractile velocity within the shortening phase of the cycles of contraction (Fig. 8C). Younger birds tended to have straighter shortening trajectories (Figs. 4 and 8C) whereas older birds exhibited a curved trajectory with less contractile velocity during the first third of shortening. For specific examples, birds 9 dph exhibited contractile velocities during WAIR at $60\text{--}70^\circ$ of $5.15 + 0.03 \text{ L s}^{-1}$, $5.29 + 0.99 \text{ L s}^{-1}$, and $5.56 + 1.69 \text{ L s}^{-1}$ during the first, second, and third sections of shortening, whereas birds 300+ dph engaged in WAIR at the same incline angle exhibited velocities of $5.03 \pm 1.77 \text{ L s}^{-1}$, $7.47 \pm 1.64 \text{ L s}^{-1}$, and $6.59 \pm 0.96 \text{ L s}^{-1}$. During WAIR, variance in strain rate was least in birds 5 dph climbing at $60\text{--}70^\circ$ ($4.99 \pm 3.50 \text{ L s}^{-1}$) and greatest in birds 61 dph climbing at 90° ($12.36 \pm 0.0 \text{ L s}^{-1}$). The effect of age upon variance in contractile velocity within the shortening phase of the contractile cycle was marginally non-significant during WAIR ($S = 925$, $P = 0.07$) but significant during CFD and flight ($S = 137$, $P = 0.00003$). During CFD, variance in contractile velocity increased from $3.19 \pm 1.3 \text{ L s}^{-1}$ in birds 9 dph to a maximum of $9.51 \pm 4.5 \text{ L s}^{-1}$ in birds 61 dph. Variance in contractile velocity was greater in birds 61 dph during level flight ($11.19 \pm 4.87 \text{ L s}^{-1}$) than in other age classes 25 dph and older (≤ 8.67) or in birds 300+ dph during ascending flight ($9.96 \pm 4.59 \text{ L s}^{-1}$).

Discussion

New recordings of EMG activity and contractile behavior in the pectoralis of developing chukar are consistent with our hypothesis that muscle function would converge upon adult patterns approximately at Day 8–10, the transition between Stages I and II of development (Jackson et al. 2009; Fig. 3). In general, most of the variables we measured in the pectoralis exhibited differences at 5 and 9 dph that converged upon adult (300+ dph) values with increasing age.

An important caution is that our study was not designed to test for discrete differences among age classes, so it is only appropriate to interpret the patterns as continuously-varying trends rather than as discrete changes in function.

The youngest chukar exhibited EMG bursts that were lower in amplitude (Fig. 6), with nearly-continuous low-level activation of the muscle and activation that occurred through most of the wingbeat cycle (Fig. 7). This is consistent with variable duration and significant coactivation of antagonists observed during development of locomotion in mammals engaged in terrestrial locomotion (Westerga and Gramsbergen 1994; Forsberg 1999; Muir 2000, Chang et al. 2006). We did not measure supracoracoideus activity, but data from strain-gauge sonomicrometry experiments suggests it is likely that EMG duty factors in birds 5 and 9 dph of over 50% of the entire wingbeat cycle (Fig. 2) would cause significant antagonistic forces between the pectoralis and supracoracoideus (Tobalske and Biewener 2008). An additional caution is necessary in interpreting amplitude of EMG (Fig. 6), as it is widely recognized that the amplitude of EMG recordings is sensitive to the exposed tips of the electrodes, the amount of uninsulated wire and tip separation (Loeb and Gans 1986). Usually, functional morphologists report relative amplitude or intensity of EMG recognizing that individual placement and tip preparation induces significant unwanted error (e.g., Tobalske et al. 2010, Jackson et al. 2011), but ethical considerations led to our experimental design precluding repeated sampling of individuals at different ages. Thus, we strove to use uniform tip preparation, but it is certain that some unwanted variation due to electrode preparation and tip separation contributed to observed variation we report for EMG amplitude (Fig. 6). Nonetheless, the overall trend for EMG amplitude to increase with age suggests that older birds are recruiting proportionally more motor units in the SB region of their pectoralis during maximal effort.

Juveniles exhibited less pectoralis strain, fractional shortening and contractile velocity compared with older birds (Fig. 8), and these differences are consistent with external wing kinematics where wingbeat amplitude and frequency are both least in Stage I birds engaged in WAIR (Jackson et al. 2009; Fig. 3). However, measures of skeletal kinematics suggest that juvenile birds use greater depression and retraction of their humeri (Heers et al. 2016), which would, in turn, suggest greater pectoralis strain and fractional shortening. While resolving this paradox will require further study, it is likely that the

structural complexity of the pectoralis can account for some of the discrepancy between the contractile behavior we report and the excursion of the humerus in separate 3D X-ray videography study (Heers et al. 2016). The pectoralis is anatomically complex, and strain, fractional lengthening, fractional shortening and strain rate all vary significantly among regions of the muscle during flight in adult birds (Biewener et al. 1998, Soman et al. 2005). Such regional variation may explain a lack of correspondence between humeral excursion reported for different age classes in Heers et al. (2016) and our measures of contractile behavior (Fig. 8). Also, it is feasible that the process of implanting electrodes and transducers affects kinematics (Tobalske et al. 2005). We observed lower wingbeat frequencies within any age class in our present study compared with non-implanted birds studied in Jackson et al. (2009).

We hypothesize that increasing variation in contractile velocity during the shortening phase as birds increase in age (Fig. 8C) was due to increasing external load (aerodynamic force) feeding back upon the contractile dynamics of the muscle (Marsh 1999). The intrinsic force-velocity relationship in muscle (Hill 1950) dictates that increasing external load slows contractile velocity. *In vivo* measures of muscle force using strain gauges implanted on the deltopectoral crest of pigeons and several other species reveal that pectoralis force in flight and WAIR is at its peak at the middle of the shortening phase of contraction (e.g., Jackson et al. 2011) which is when wingspan, angular velocity, and circulation about the wings indicates peak aerodynamic forces are being produced (Tobalske and Dial 2007). Unfortunately, chukar and other galliform birds do not have a deltopectoral crest that is amenable to *in vivo* strain-gauge measurements of pectoralis force (Tobalske and Dial 2000), but this hypothesis could be further tested using synchronized sonomicrometry and aerodynamic measures with high temporal resolution that can resolve instantaneous forces on the wings (Hubel et al. 2009; Henningson and Bomphrey 2013).

A fundamental wing stroke has been identified as an ancestral condition among flying birds based on the lack of variation in stroke-plane angles and the general trajectory of external wing excursion in WAIR, CFD, and level flight in chukar (Dial et al. 2008). It was previously revealed that certain external-wing and skeletal kinematics vary among age classes of chukar regardless of the overall similarity in wing trajectories (Jackson et al. 2009; Heers et al. 2016). Our EMG and sonomicrometry measures provide additional insight into variance within what is otherwise considered a fundamental stroke.

The duration of muscle activation tends to be less during WAIR than in CFD or flight (Fig. 7), while variation in contractile velocity during shortening is greater during WAIR (Fig. 8C). This variation is likely due to the increased power demands in flight compared with WAIR (Jackson et al. 2009). Such variation in muscle activity underscores the need for caution when extrapolating from performance in extant species to estimate the performance capacities of extinct forms (Heers and Dial 2012; Heers et al. 2014).

Birds 61 dph exhibited several patterns that departed from the general trends that EMG and sonomicrometry variables exhibited with increasing age. Compared with adults (300+ dph), birds 61 dph exhibited reduced EMG duty factor (Fig. 7), and, particularly during WAIR, reduced muscle strain and strain rate and increased variation in contractile velocity (Fig. 8). Chukar begin moulting the flight feathers of their wings 45–55 dph, and birds 61 dph possessed new distal primaries (P10 and P9). Perhaps these feathers were particularly useful at producing aerodynamic force compared with the worn feathers they replaced, and, at 61 dph, the birds were still learning to use their pectoralis in relation to increased functionality of their wings.

The available evidence from chukar indicates the components of their flight apparatus develop approximately in synchrony, consistent with the idea of symmorphosis (Weibel et al. 1991) during their ontogeny. Performance increases gradually: feathers emerge and the skeleton ossifies (Fig. 1), aerodynamic forces increase in a manner that suggests circulation is proportional to external wing area (Figs. 2, 4, and 5), and kinematics, neural activation and contractile behavior converge upon adult patterns (Figs. 3, 6–8). New comparative research is of course necessary for testing whether symmorphosis is applicable as a model for flight ontogeny in birds. One useful test would be to measure muscle function in precocial Anseriforms such as the mallard (Dial et al. 2012; Dial and Carrier 2012) where forelimb development occurs much later than in the chukar, between 30 and 45 dph. Caution would be required in such a two-species study (Garland and Adolph 1994) as many aspects of the ecology, anatomy and evolutionary history of ducks are different from chukars. Working instead within the Phasianidae clade to control for such variation, it would be useful to compare rates of development in flight ability in taxa such as the chukar that use flight primarily for escape with taxa such as ptarmigan and sharp-tailed grouse that exhibit short distance migration.

The latter species reportedly have wings of higher aspect ratio and pectoralis muscles with more myoglobin (Drovetski 1996).

As reviewed herein, and with the addition of our new measures of muscle function (Figs. 6–8), an understanding is emerging of the development of flight capacity in precocial birds, especially the chukar. Selective pressures during the juvenile period in development are thought to have profound effects upon life-history (Martin 2015). The next step in improving understanding of the ontogeny of flight and placing it in a broader ecological and evolutionary context will be to pursue similar studies in species that develop altricially or semi-altricially. It will be useful to select species that vary in nest environment and parental care (Dial 2003a). Exciting new research on an altricial passerine species, zebra finch, indicates that brood size has sustained, negative effects on flight performance during development that persist into adulthood and adversely impact reproductive success (Crino et al. 2017). Also in zebra finch, physiological traits affect flight performance at fledging; in particular, hemoglobin concentration and hematocrit affect energy gain and takeoff angle (Cornell et al. 2017). Within the clade of passerines, predation pressure during the nestling phase causes rapid development and early departure from the nest, with fledglings possessing wings that are underdeveloped relative to adults (Cheng and Martin 2012; Dial et al. 2016) and, presumably, lacking flight capacity. Given the importance of flight to the biology of extant birds, and persistent human fascination with the evolutionary origins of flight (Dial 2003a, 2003b; Dial et al. 2008; Heers and Dial 2012), novel and worthwhile insight remains to be gained by studying flight ontogeny.

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