

Research



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Phylogenetic and kinematic constraints on avian flight signals

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Many birds vocalize in flight. Because wingbeat and respiratory cycles are often linked in flying vertebrates, birds in these cases must satisfy the respiratory demands of vocal production within the physiological limits imposed by flight. Using acoustic triangulation and high-speed video, we found that avian vocal production in flight exhibits a largely phasic and kinematic relationship with the power stroke. However, the sample of species showed considerable flexibility, especially those from lineages known for vocal plasticity (songbirds, parrots and hummingbirds), prompting a broader phylogenetic analysis. We thus collected data from 150 species across 12 avian orders and examined the links between wingbeat period, flight call duration and body mass. Overall, shorter wingbeat periods, controlling for ancestry and body mass, were correlated with shorter flight call durations. However, species from vocal learner lineages produced flight signals that, on average, exceeded multiple phases of their wingbeat cycle, while vocal non-learners had signal periods that were, on average, closer to the duration of their power stroke. These results raise an interesting question: is partial emancipation from respiratory constraints a necessary step in the evolution of vocal learning or an epiphenomenon? Our current study cannot provide the answer, but it does suggest several avenues for future research.

1. Introduction

Vocal signals are commonly given in flight in a wide assortment of bird species and social contexts [1–4], suggesting many potential benefits. Airborne vocal production can increase acoustic broadcast space of song, and presumably other functional vocal types, with height above the substrate [5]. Contact calls are more commonly given in flight and can help coordinate movements at multiple spatial, temporal and life-history scales, including: early parent–offspring interactions [6]; daily movements of small numbers of mates and close kin [7–10]; larger, population level foraging and roosting congregations [11–15]; and transcontinental migrations involving multiple populations [16]. In select taxa, airborne vocal production can also be used in echolocation to navigate dark environments [1,14]. In these and other cases vibrating structures must be coupled with systems capable of producing sufficient expiratory pressure in order to propagate acoustic energy in ways that are adaptive. Bat echolocation involves a fortuitous and synergistic coupling of wing, respiratory and vocal tract movements, allowing for energetically efficient amplification of high-frequency signals synched with the power stroke [17,18]. However, the biomechanics of avian flight signals, while different in many ways, has received little attention [2], and it is unclear how birds coordinate vocal production amidst the physiological rigours of flapping flight.

Unlike respiration during mammalian flight, avian flight involves a rigid, fixed-volume lung and up to 14 interconnected air-sacs that work in synergistic and phasic coordination with the kinematics of wingbeat and respiratory cycles [19,20]. During the down stroke, the sternum moves dorsally compressing the thoracic cavity, while abdominal muscle movements create positive expiratory pressure in the intraclavicular air-sac (IAS). During the recovery stroke, the

sternum moves ventrally allowing the IAS to expand, coinciding with negative pressure for inhalation. One exception is backwards flight in hummingbirds, where the axis of the wingstroke is rotated so that the upstroke causes thoracic compression and expiratory pressure [21]. Superimposed on the general theme above, birds often produce several wingbeat cycles during each respiratory cycle, though wing kinematics continue to influence net-airflow directionality [19,22]. In perching birds, independent contrasts in respiratory frequency show a strong, inverse, allometric relationship with body mass across species ($\text{mass}^{-0.35}$) [23]. Wingbeat frequency, which also scales inversely with body mass ($\text{mass}^{-0.35}$) [24,25], would presumably constrain the duration of expiratory periods in smaller birds, but has been measured in fewer species [19]. Interestingly, wind-tunnel experiments show wingbeat frequency is largely impervious to different flight speeds, while respiratory frequency shows a U-shaped functional response to flight speed [26,27]. Wingbeat frequency is a function of species-specific body mass, wing surface area and stroke amplitude, presumably under strong genetic control, and atmospheric pressure [25,28], while plasticity of the respiratory system allows birds to tailor metabolic processes to specific environmental contexts, providing a highly variable substrate upon which selection could shape vocal production strategies [29–31].

The IAS also houses the avian sound-producing organ, the syrinx. Avian vocal production requires a build-up of expiratory pressure at the sub-syringeal aperture [32,33], however, it is unknown whether the expiratory phase, or related wing kinematics, limit the period during which airborne vocal signal production would be energetically most efficient; or how changes in flight styles may have influenced the evolution of mobile communication signals. If respiratory flexibility tailors to species-specific demands of vocal production independently of wingbeat cycling, there need be no direct, temporal synchronization between vocal production and power stroke phases. On the other hand, if selection has favoured amplification of acoustic signals to avoid adverse biomechanical or energetic effects of respiratory cycling, then vocal production should, on average, coincide with the powerstroke [19,26]. This should be the case in all birds that flap and vocalize simultaneously. Songbirds, parrots and hummingbirds, known for their vocal plasticity and heightened respiratory control, may be special cases [29,33]. Regardless, it is not clear how the above plays out across groups with varying ancestries, aerodynamic designs and levels of vocal flexibility.

Why should we care whether flight dynamics limit call durations? There is a limit to how rapidly any avian syrinx can modulate a call. The shorter the call, the fewer the number of modulations and thus the fewer possible call variants. Where all individuals in a species emit the same simple call, this is not a problem. But for birds like parrots, where each individual may have its own unique contact call [4], constrained call durations would limit the number of individuals that could be identified. Species in which males emit a variety of aerial vocal displays would be similarly constrained [5,34,35]. Thus, limits on signal duration imposed by flight dynamics could easily limit the options for mating systems and social organization and may favour flexible behavioural strategies.

With this question in mind we quantified flight signal duration and wingbeat kinematics in free-ranging birds, incorporating mechanistic and phylogenetic comparative approaches. We restricted the analysis to contact calls, which were the most commonly given flight signals in both vocal

learner lineages (oscine passerines, parrots and hummingbirds) and vocal non-learner lineages, while acknowledging that the precise function and motivational scenarios, and extent of learning of flight signals probably varies. From high-speed audio-video and acoustic triangulation, we demonstrate how acoustic energy of the contact call is distributed among the phases of the wingbeat cycle in a small sample of free-ranging avian species spanning a spectrum of flight styles, body sizes and ancestries. In a broader analysis of 150 species recorded vocalizing during flapping flight, we compared one-half wingbeat period (a proxy for the power stroke period), contact call duration and body mass, theorizing that if the power stroke and vocal production are on average phase-locked to avoid adverse biomechanical or energetic constraints of the recovery phase, one-half wingbeat period should be approximately equal to vocal signal period and both traits positively correlated with body mass across the species. Both mechanistic and phylogenetic approaches indicate some surprising differences in vocal learning and vocal non-learning taxa.

2. Methods

(a) Animal care

All experiments were reviewed and approved by the Institutional Animal Care and Use Committee (2014-003), University of Texas Rio Grande Valley and the Ministerio del Poder Popular para el Ambiente in Venezuela (no. 1430).

(b) Avian power stroke and vocal signal production

We determined relationships between wingbeat phases and vocal production during free-ranging, flapping flight in 12 species at various localities in the United States (US) (Texas, California & New York), Venezuela (Guárico) and Ecuador (Pichincha, Guayas). When birds were a considerable distance away from the microphone and, or wingbeat frequency was high, audio delay becomes a factor in determining phase relationships with vocal recordings extracted from video. We circumvented this by: (i) attaching dynamic microphones to feeders (SM11, SM 57, Shure Microphone Corp., Niles, Illinois, USA); (ii) or by capturing and releasing individuals near a video camera (XF105 or EOS 2ti, Canon, Tokyo, Japan). When birds were farther away we estimated distance by using a range-finder (CRF2400-R, Leica, Wetzlar, Germany), or a video-microphone array, described as follows. Four omnidirectional microphones (SM63, Shure) were mounted on poles in the ground at the vertices of a 7 m × 7 m square. Three microphones were mounted at 1.5 m above the ground and the fourth was mounted 4.5 m above the ground to provide a third altitudinal dimension to location software. The four microphones were fed into separate channels of a digital audio recorder (788t, Sound Devices, Reedsburg, Wisconsin, USA) and audio saved as wave files with a 44.1 kHz, 16-bit sample rate. The audio recorder was synchronized via SMPTE time code to the video frames of the camcorder, which was set to record at 60 frames per second (FPS). Acoustic triangulation software (ISHMAEL V2.4) was used to quantify signal arrival times to the different microphones to determine the location of the individual when vocalizing in flight and distance to the video camera. Distance between the sound source and video camera were used to correct for audio delay using AUDITION (CCS v. 6, Adobe Inc., San Jose, California, USA). For birds with wingbeat frequencies above the Nyquist frequency of the camera (i.e. greater than 30 Hz) we incorporated into the above design a higher speed camera (EX-FH20, Casio Inc., Tokyo, Japan), which filmed at 210 FPS and was synched with the audio–video set-up above via a video time

slate. In other cases, we used audio–video recordings made simultaneously on the same camcorder at 120 FPS (FDR-AX53, Sony Corp., Tokyo, Japan). Only high quality recordings where we were able to capture the specific wingbeat cycle associated with a clear spectrogram of call production was used in spectrographic analysis of phase relationships. Wingbeat periods were measured to the nearest millisecond (± 3 milliseconds) in Adobe Audition and audio saved as WAVE files with 16-bit, 44.1 k sample rate. Audio files were analysed in RAVEN (v1.5, Cornell Lab of Ornithology, Ithaca, NY, USA), where we measured the duration of signals on the waveform, while simultaneously viewing the signals as a continuous trace on a spectrogram.

(c) Wingbeat and flight signal period

We complimented the dataset above with audio–video recordings where one-half wingbeat period (a proxy for the power phase) and flight call production could be measured, but distance between the bird and the recorder was unknown. While this negated any analysis of direct phase relationships, vocal signal duration and one-half wingbeat period estimates are relevant for assessing evolutionary constraints on signal duration. The combined datasets included measurements for 150 species, 79 of which belonged to lineages known for vocal learning and 71 came from lineages where vocal learning does not occur. We excluded songs and alarm calls and to the best of our knowledge restricted analysis to contact calls. Estimates were obtained from audio–video recordings of free-flying individuals made at various localities in the USA, Venezuela and Ecuador between 2008–2017. Birds were recorded with a professional camcorder (Canon XF105) with a directional shotgun microphone (MKH70, Sennheiser, Wedemark, Germany) fed into one of the stereo channels. For some species we used wingbeat frequencies and audio recordings from published sources [25,36,37] and audio specimens from digital repositories (Macaulay Library, Lab of Ornithology, Ithaca, NY, USA), where authors indicated birds were in flight. In hummingbirds this invariably meant flapping flight, however, we were careful to include only material where gliding or bounded flight seemed improbable (e.g. flapping noises). Body mass measurements were taken directly with a Pesola balance (± 0.1 g) and birds released near the microphone array. In other cases body mass was taken from the literature [36–41] or from online museum collection records (Cornell Museum of Vertebrates, Ithaca, New York; Museum of Vertebrate Zoology, Berkeley, California, USA). Measurements taken from the literature were highly predictable from our own measurements for 16 species common to both datasets (adjusted $r^2 = 0.99$, $f_{1,14} = 1399$, $p < 0.0001$). In addition to these three attributes, we calculated the ratio of one-half wingbeat period to call duration.

(d) Phylogenetic and statistical analysis

We used a Bayesian statistical analysis to model trait evolution [42]. One hundred phylogenetic trees of varying topologies and branch lengths were obtained for the 150 species using birdtree.org (Hackett *et al.* 6670 operational taxonomic units; electronic supplementary material, figure S1) [43]. Pagel's λ was used to estimate phylogenetic signal in each of the traits, given the tree topologies. Because results indicated strong phylogenetic signal, λ was estimated simultaneously for each of the correlations. Phylogenetic generalized least-squares regression (PGLS) was used to compare ($\log_{10}$ -transformed) call duration, one-half wingbeat period, body mass and the ratio of one-half wingbeat period to call duration, while simultaneously estimating λ given the tree topologies. Markov chain Monte Carlo (MCMC) was used to create posterior distributions of parameter estimates. Each run consisted of 5 million iterations with a 1 million burn-in period. Chain acceptance rates were typically 38% (± 0.04 s.d.). Two methods were used to assess statistical significance. First, we assessed the consistency of phylogenetically-corrected regression coefficients by

analysing posterior distributions from MCMC iterations; to avoid autocorrelation, every thousandth-iteration was used to create posterior distributions. Distributions indicating a high likelihood of beta coefficients (slopes) being different from zero were considered significantly correlated. Second, each run was repeated with λ set to zero and marginal likelihood ratios were used to calculate log Bayes factors (IBF)(IBF = $2 \times (\text{marginal likelihood accounting for } \lambda - \text{marginal likelihood, where } \lambda = 0)$). Marginal likelihoods estimates were calculated with the stepping stone method (250 stones, 10 000 samples). An average IBF of less than 2 was considered uncorrelated and 10 or above was considered to be strongly correlated [42]. We also tested whether a PGLS multiple regression model comparing one-half wingbeat period to call duration differed between vocal learners and vocal non-learners, by including call duration as a dependent variable, one-half wingbeat period as an independent variable, a dummy variable (vocal learner = 1; non-vocal learner = 0) and a dummy interaction, where one-half wingbeat period was repeated, but with values for non-vocal learners set to zero [44]. The complex model was thus: $y = x_1(\text{one-half wingbeat period}) + x_2(\text{dummy variable}) + x_3(\text{dummy interaction}) + b$, where y = call duration. Analyses were conducted separately for each of the models and replicated six times using LONESTAR5 and LINUX operating system at the Texas Advanced Computing Center at the University of Texas (Austin, Texas USA). Replicate runs were conducted simultaneously and results pooled to calculate IBF and create posterior distributions of parameters and r^2 coefficients. To choose among models, we calculated maximum-likelihood estimates using a random walk model and used these and the number of free parameters in Akaike information criteria (AIC), ranking models according to their AIC weights. Phylogenetically correct means (PCMs) and variances of the four traits and the two behavioural groups were calculated using a continuous random walk model while simultaneously estimating lambda.

3. Results

(a) Vocal and wingbeat phase relationships

As expected, the acoustic energy of contact calls emitted during forward flapping flight was often strongly biased towards, or completely contained within, the down stroke (i.e. power stroke) of the wingbeat cycle. The sample included free-ranging avian species spanning a spectrum of body sizes, flight styles and taxonomic groups (body mass range: 0.003–1.24, $\bar{x} = 0.3 \pm 0.43$ kg; figure 1*a–i*) [45]. As a positive control, we show a biomechanical reversal during backwards flight in hummingbirds (figure 1*h,i*; electronic supplementary material, movies M1 and M2) [45]. Though there were exceptions, vocal non-learners often produced flight signals in a nearly 1 : 1 ratio with the power stroke (figure 1*a–c*; electronic supplementary material, movies M3–M6). Interestingly, vocal-learners, across a spectrum of body sizes and ancestries, commonly, though not universally, produced flight signals that exceeded multiple phases of their respective wingbeat cycles (figure 1*d–g*; electronic supplementary material, movies M7–M9) [45]. However, testing for group differences requires controlling for body mass and ancestry in a representative set of species and lineages [46].

(b) Phylogenetic comparisons

Across 150 species from 12 avian orders, PGLS regression showed body mass was positively related to one-half wingbeat period ($(\log)y = 0.35 (\pm 0.03 \text{ s.e.}) x - 0.89 (\pm 0.07 \text{ s.e.})$ $r^2 = 0.49$; IBF = 52, $p < 0.001$; figure 2*a*), and to call duration ($(\log)y = 0.27 (\pm 0.05 \text{ s.e.}) x(\text{body mass}) - 0.78 (\pm 0.11 \text{ s.e.})$; $r^2 = 0.15$;

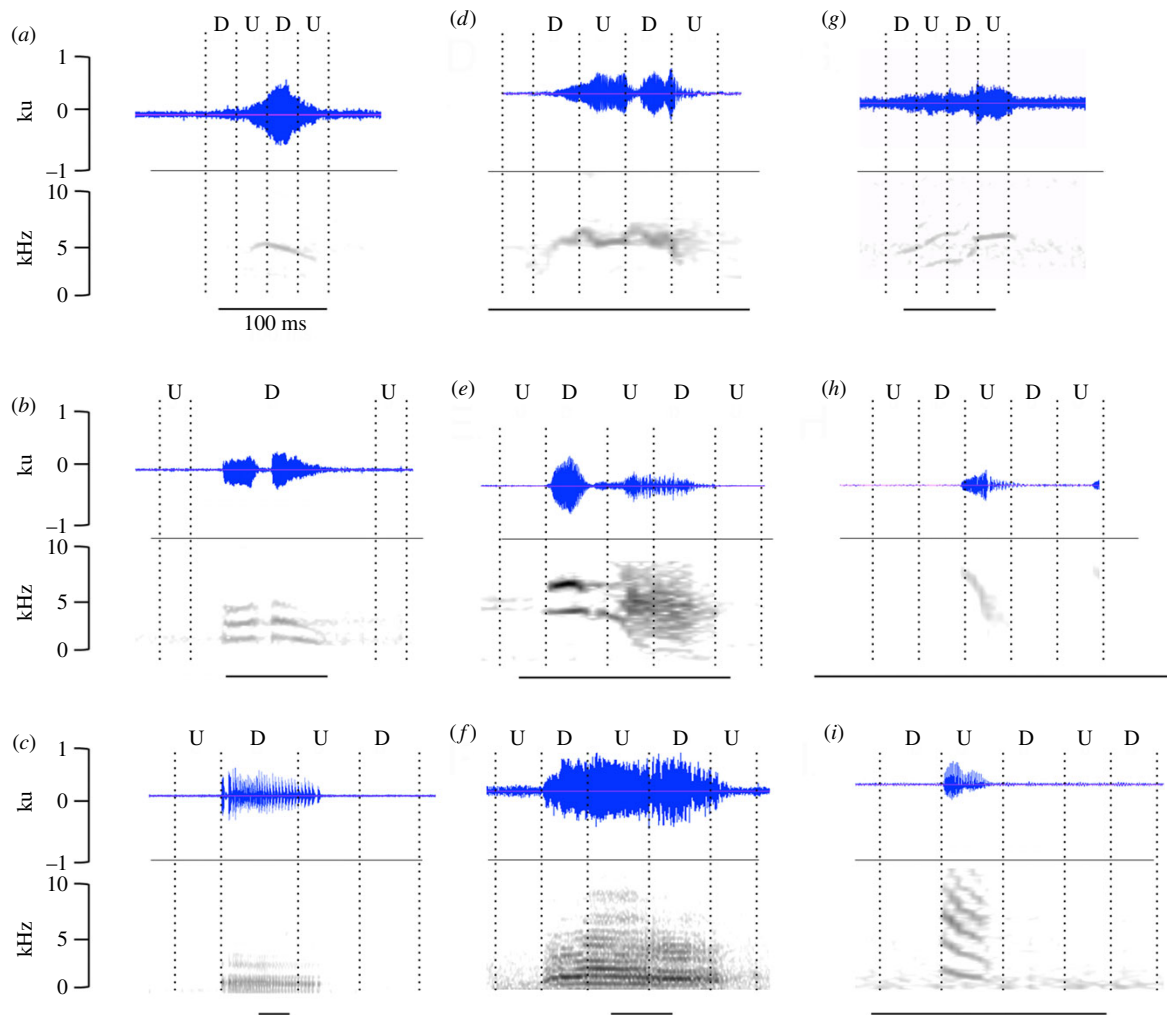


Figure 1. Avian power stroke is synchronized with vocal signal production in vocal non-learners, but more variable in vocal learners. Wingbeat phases superimposed on wave forms and spectrograms of calls given during forward flapping flight in three species of vocal non-learners: (a) black Phoebe (*Sayornis nigricans*), (b) limpkin (*Aramus guarana*), (c) great egret (*Ardea alba*); and four species from vocal learner lineages: (d) green-rumped parrotlet (*Forpus passerinus*), (e) black-crested titmouse (*Baeolophus atricristatus*), (f) Lear's macaw (*Anodorhynchus leari*) and (g) saffron finch (*Sicalis flaveola*). Vocal production synchronized with upstroke during backwards flight in: (h) buff-bellied hummingbird (*Amazilia yucatanensis*) and (i) ruby-throated hummingbird (*Archilochus colubris*). Timing of stroke phases was corrected for audio-delay (see methods). Spectrograms were produced with a 256 fast Fourier transform (FFT) sample size; spectrogram in (i) was made with 113 FFT. (Online version in colour.)

IBF = 10; $p < 0.001$; figure 2c). Moreover, one-half wingbeat period was positively related to call duration ($(\log)y = 0.57 (\pm 0.11 \text{ s.e.})x(\text{one-half wingbeat period}) - 0.32 (\pm 0.16 \text{ s.e.})$, $r^2 = 0.16$; IBF = 12, $p < 0.001$; figure 2b). Controlling for body mass in multiple PGLS regression, one-half wingbeat period was a significant predictor of call duration ($(\log)y = 0.16 (\pm 0.08 \text{ s.e.})x(\text{body mass}) + 0.32 (\pm 0.02 \text{ s.e.})x(\text{one-half wingbeat period}) - 0.49 (\pm 0.18 \text{ s.e.})$; $p < 0.001$, $p < 0.01$, respectively, $r^2 = 0.18$; IBF = 9), indicating that transitions to shorter wingbeat periods for a given body size were accompanied by transitions to shorter call durations and not the result of a spurious correlation with body mass. The ratio of one-half wingbeat period-to-call duration was not significantly related to body mass ($p < 0.10$), and had a lower phylogenetic signal ($\lambda = 0.38$) compared to the three main traits ($\lambda_{\text{mass}} = 0.96$, $\lambda_{\text{wingbeat}} = 0.92$, $\lambda_{\text{call}} = 0.66$).

(c) Vocal learners versus vocal non-learners

We grouped species into vocal learner and vocal non-learner groups and used this as a covariate in PGLS ANCOVAs. AIC

criteria indicated the model including the vocal learner covariate; body mass and one-half wingbeat period had the highest weight among models tested (0.70) and the lowest phylogenetic signal ($\lambda = 0.31$; table 1). Species from vocal learner lineages had longer call durations than expected for either body mass or one-half wingbeat period (table 1, figure 3a). Including an interaction effect did not markedly improve models and was not significant, indicating that while allometric slopes were not significantly different, y -intercepts were shifted upwards in the vocal learner group. Consistent with these findings, vocal learners had on average smaller body masses ($\text{PCM} = 0.032 \text{ kg} \pm \text{s.d. } 0.01$; IBF = 176; $p < 0.000$) and shorter one-half wingbeat periods ($\text{PCM} = 0.033 \text{ s} \pm \text{s.d. } 0.007$) compared to non-learners ($\text{PCM}_{\text{body}} = 0.32 \text{ kg}$, $\pm \text{s.d. } 0.057$; $\text{PCM}_{\text{wingbeat}} = 0.087 \text{ s} \pm \text{s.d. } 0.01$; IBF = 132, $p < 0.05$; figure 3a). Despite these differences, the two groups did not differ in their call durations ($\text{PCM}_{\text{vocal learners}} = 0.081 \text{ s} \pm \text{s.d. } 0.022$; $\text{PCM}_{\text{non-vocal learners}} = 0.090 \text{ s} \pm \text{s.d. } 0.022$; $p < 0.55$; figure 3a). A higher one-half wingbeat per call ratio might be

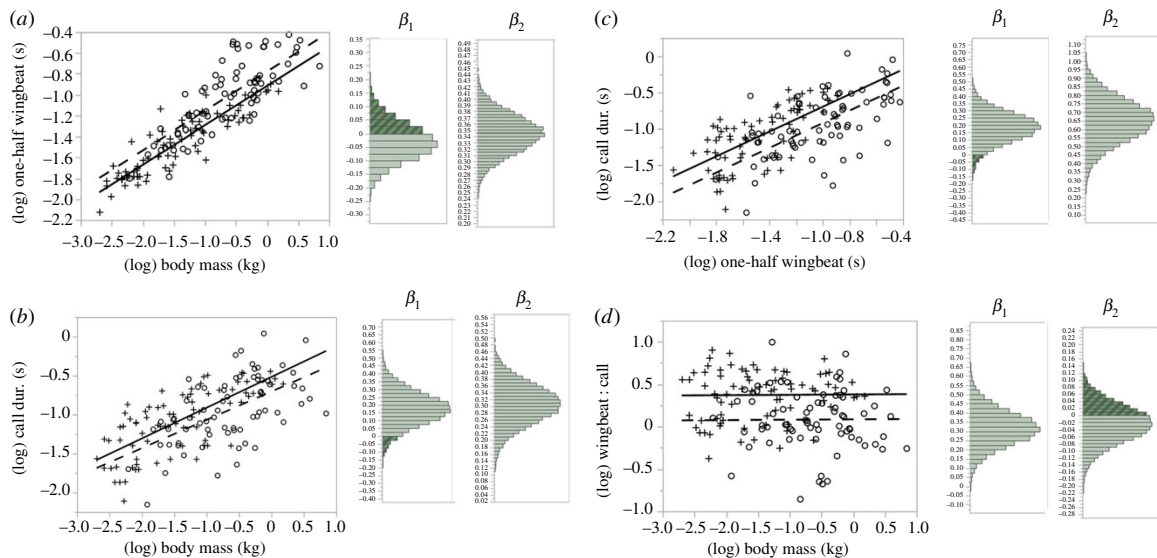


Figure 2. Allometric relationships in vocal-flight integration differ between vocal learner and vocal non-learner lineages. (a–d) Log-log standard least-squares regression models including vocal learning as a covariate: (a) one-half wingbeat period as a function of body mass; (b) call duration as a function of body mass; (c) call duration as a function of one-half wingbeat period. (d) Ratio of one-half wingbeat period : call duration as a function of body mass. Species from vocal learner lineages are indicated by plus symbols; vocal non-learners are shown as open circles. Regression lines on graphs were fit assuming no phylogenetic signal ($\lambda = 0$), for visual representation and comparison to Bayesian posterior distributions: β_1 (slope of covariate), β_2 (slope of continuous regressor) from phylogenetic generalized least-squares regression models. Only every thousandth estimate is displayed from 24 million estimates ($n = 24\,000$). Large, dark-coloured portions of distributions of β_1 in (a) and β_2 in (d) illustrate a high probability of sign-reversal in slope coefficients, suggesting they are not significantly correlated (not different from zero). (Online version in colour.)

Table 1. Model selection predicting flight call duration using Akaike information criteria (AIC). Models were ranked according to their AIC weights and compared to parameters from phylogenetic generalized least-squares regression (PGLS). Vocal learners (79 species) and vocal non-learners (71 species) were used as a covariate. LH, maximum-likelihood estimates; λ = phylogenetic signal; AIC, $\Delta AIC = \exp(-\Delta AIC/2)$; w_i = AIC weights; b = beta coefficient; $P(bc > 0)$ = percentage of beta coefficients greater than 0; IBF = log Bayes factor; r^2 = PGLS and standard least squares (SLS) adjusted regression coefficients. p = p -value of model terms (SLS): *0.01, **0.001, ***0.0001.)

model	parameters	LH	λ	AIC	w_i	b	$P(bc > 0)$	IBF	r^2		p
									PGLS	SLS	
I	voc. learn	−36.3	0.31	82.7	0.70	0.21	98%	1.8	0.23	0.47	***
	mass					0.18	98%				**
	wingbeat					0.39	98%				*
II	mass	−39.4	0.40	86.7	0.09	0.16	99%	8.6	0.18	0.42	**
	wingbeat					0.32	98%				
III	voc. learn	−39.5	0.34	86.9	0.08	0.19	95%	5.1	0.20	0.42	**
	wingbeat					0.65	100%				***
IV	voc. learn	−39.9	0.41	87.8	0.05	0.18	95%	7.9	0.17	0.44	*
	mass					0.31	100%				***
V	mass	−41.4	0.45	88.9	0.03	0.28	100%	9.0	0.15	0.41	***
VI	wingbeat	−41.4	0.41	88.8	0.03	0.57	100%	9.0	0.16	0.37	***

expected to vary with body mass, however, controlling for body mass, vocal learners still had a significantly higher ratio of one-half wingbeat phases per call compared to non-vocal learners; the term for body mass was again not significant (figure 2d). Taken together, flight signal duration appears to have become more liberated from potential biomechanical or energetic constraints of the power stroke early in the lineages of vocal learners.

4. Discussion

(a) Synting of vocal production with power stroke

Birds appear to have exploited vocal-locomotor synergisms that are similar to those used by bats, even though the avian power stroke is synchronized with vocal production used primarily for social communication rather than for

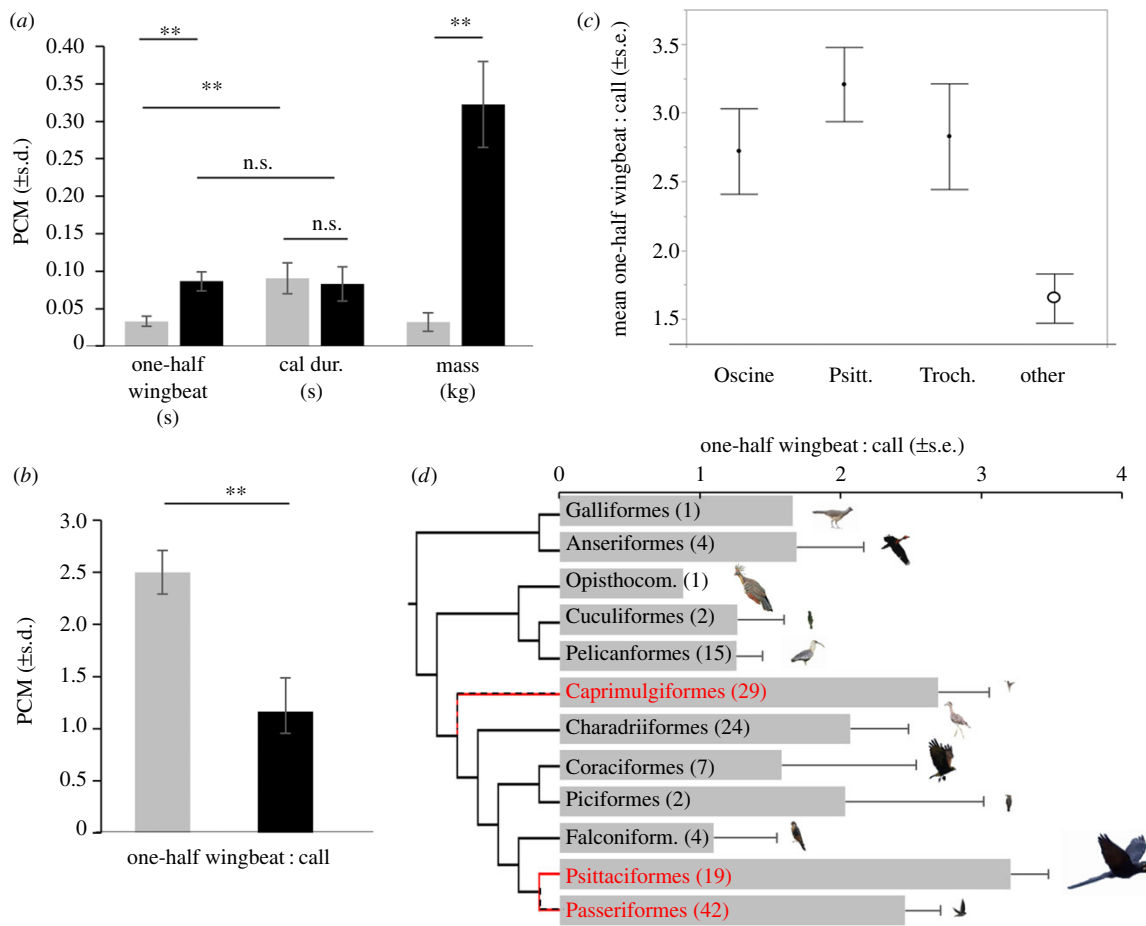


Figure 3. Vocal non-leaners have, on average, flight signal durations equal to their one-half wingbeat periods, while vocal learners have signal durations lasting multiple phases of the wingbeat period. (a) Phylogenetic correct mean (PCM) (±s.d.) one-half wingbeat period (seconds), call duration (seconds), body mass (kg) and ratio of one-half wingbeat period : call duration for vocal learner and vocal non-learner groups. (b) PCM (±s.d.) ratio of one-half wingbeat period : call duration for vocal learners and vocal non-learners. Grey and black bars represent vocal learner and vocal non-learner groups, respectively. (c) Uncorrected mean (±s.e.) ratio of one-half wingbeat period : call duration for each of the three vocal learning groups (Oscine, Oscine Passerines; Psitt. Psittacidae; Troch. Trochilidae) and vocal non-learners. (d) Uncorrected mean (±s.e.) ratio of one-half wingbeat period : call duration for each of 12 orders with simplified tree showing hierarchy of relatedness. Lineages with vocal learning are shown in red; hashedlines indicate groups with and without vocal learning. (Online version in colour.)

echolocation. An exception to this is found in the echolocating signals of swiftlets and oilbirds [14,47]. Unlike the echolocating bats, we have shown that several groups of birds appear to have relaxed the coupling between wing power strokes and vocal call emission. The need to facilitate varied social functions of avian flight signals; exploitation of multiple wingbeat cycles per respiratory cycle, which is common in birds, and the unidirectional airflow of the avian respiratory system [48] might have facilitated the observed departure from vocal-locomotor coupling. However, experimental mass manipulations and measurement of energetics [17] and sound pressure levels of vocalizations of birds in different contexts [49] would be required before we can understand the mechanisms and consequences of this relaxed coupling. Might wingbeat phases superimpose complex amplitude envelopes on the acoustic structure of flight signals, thereby providing information about the sender (e.g. flight speed, motivation, gender, fitness, etc.)? Additionally, might major differences in syringeal morphology (e.g. tracheal, bronchial or tracheobronchial syringes) [30,50,51] induce different kinematic challenges to vocalizing during flapping flight? Clearly, much more work remains.

(b) Wingbeat and flight signal allometry

Our results indicate a well-known trade-off between wingbeat frequency and body mass owing primarily to aerodynamic effects on wingloading [24,26,38] and mass-specific power requirements [52]. In our study this yielded a slope coefficient of 0.35 (figure 2a), controlling for phylogeny. This was identical to the value estimated by a previous study on a smaller assemblage of bird species ($n = 32$ species), ignoring phylogeny (-0.35) [24], and identical to the slope of avian body mass on respiratory frequency (during perching), controlling for phylogeny (-0.35 ; $n = 50$ species) [23], indicating that traits were estimated adequately in this study, and that scaling relationships of physiological systems are often highly conserved [46]. This value is also similar to the slope coefficient from body mass on call duration (0.32; figure 2b), suggesting that wing, respiratory and vocal tract movements follow similar scaling relationships. Interestingly, the slope coefficient of the third regression, one-half wingbeat period on call duration, was closer to 1 (0.68), suggesting that call duration and wingbeat period may be more directly related. While many life-history and physiological variables scale allometrically with body mass, in this case a more interesting question is whether

wingbeat period is related to call duration *after* removing the effects of body mass. Controlling for body mass in multiple PGLS regression showed that changes to shorter wingbeat periods for a given body mass were significantly correlated with changes to shorter call durations. While it is widely appreciated that vocal production and flapping flight each coopted underlying respiratory physiology [2,29,53], our results represent phylogenetic comparative evidence linking a motor attribute of flight with a vocal trait. Auditory-guided motor entrainment of limb movements was found to be more prevalent in mammals and bird species known for vocal mimicry [54], however, neither natural limb movements nor vocal signals were measured. Evolutionary changes in motor performance influence the spectral attributes of songs in Emberizine sparrows [55], via bill movement-size trade-offs, however, the focus was on song given while perched and did not address locomotor movements. While an analysis of bill movements was beyond the scope of our study, conducted entirely on free-ranging individuals, we did find that vocal signal duration was inversely related to several frequency measurements (figure 1; K. S. Berg 2019, unpublished data). Signal duration was synchronized with bill movements in many cases and is implied or explicit in much of our supporting audio–video material (electronic supplementary material, movies M4, M6 and M9) [45]. Given the role of bill movements in vocal production, our results may also implicate changes in motor systems controlling bill movements, in addition to motor systems controlling limb, respiratory and syringeal movements, however, this will require further work.

(c) Vocal learners versus vocal non-learners

The model including a vocal learner covariate, body mass and wingbeat period yielded the highest AIC weight among models predicting flight call duration (0.70; table 1). This suggests that, after accounting for phylogeny and removing the effects of body mass, birds from vocal learner lineages, on average, integrated contact call production with wingbeat cycles in ways that differ significantly from vocal non-learners. Regression slopes of body mass on wingbeat period did not differ significantly between the two groups (figure 2a), suggesting that general principles of physiology and aerodynamics apply equally to both groups. However, with or without controlling for phylogeny, y -intercepts in wingbeat-call duration regressions were shifted upwards in vocal learners across a large spectrum of body sizes (table 1; figures 2c and 3), indicating an increased tendency to propagate vocal signals independently of power-stroke phases. Because we included the vocal learning covariate and body mass in PGLS multiple regression models and wingbeat period was still significantly correlated with call duration (table 1), our results represent phylogenetic comparative evidence showing significant differences in vocal-locomotor integration between species from vocal learning and vocal non-learning lineages.

Avian vocal learning has been hypothesized to have evolved as a specialization of an ancestral motor system controlling limb movements [56], but it is not clear how alterations to locomotor systems led to increased vocal plasticity. One possibility is that an uncoupling of central pattern generators controlling bill, limb and respiratory rhythms, often phase-locked in more ancestral lineages, allowed each system to evolve independently, opening the door to increased plasticity and eventually vocal

learning [29,57]. However, at this point we cannot conclude that vocal learning *per se* is responsible for the results above, because we know little about the biomechanics of flight signals in any avian taxa, or how vocal production learning may have factored into our sample of contact calls analysed. Contact call learning is purportedly widespread in parrots [4], but has been less often studied in songbirds [2,9,10,58] and even less in hummingbirds, a group containing the smallest of avian taxa.

Body size figures prominently in many comparative studies of morphology, physiology and behaviour [46,59,60] and avian flight signals integrate all three. Species from vocal learner lineages had, on average, much smaller body masses compared to the vocal non-learners (figure 3a). Thus our finding of differences between the two groups could be an artefact of smaller-bodied birds and not related to vocal learning *per se*. We attempted to control for potential non-linearity associated with these differences by including body mass in phylogenetically-controlled multiple regression models (table 1), and by standardizing vocal signal periods to wingbeat durations, and controlling for body mass (table 1, figure 2d), while estimating phylogenetic signal. Both approaches showed the number of wingbeat phases per call differed between behavioural groupings, but was not directly related to body mass. Furthermore, parrots, which had body masses more comparable to vocal non-learners (parrots: $\bar{x} = 0.43$ kg, s.e. ± 0.10 kg; vocal non-learners: $\bar{x} = 0.65$ kg, s.e. ± 0.13 kg), had the highest mean number of wingbeat phases per call (figure 3c,d). They also showed the strongest within-clade relationship between wingbeat period and call duration, and the highest slope coefficient (0.78; K. S. Berg 2019, unpublished data; electronic supplementary material, Appendix I). If our results are an epiphenomenon of small body masses of vocal learners, and not related to vocal plasticity itself, there would be no need to think that larger-bodied parrots would also have longer calls than expected for their wingbeat periods and body masses. Yet, they do. Parrots are highly social; noted for their contact call learning; and are often vocally ubiquitous in flight as they coordinate flock movements to far-ranging foraging and roosting sites [4]. They show auditory entrainment of limb movements [54] and cognitive, neural and developmental peculiarities related to flight [56,61–63]. Thus, at the very least, parrots may require longer call durations to the extent that these aid individual, group or population recognition [4].

5. Conclusion

A well-known example of vocal-locomotor coupling in mammals has a potential analogue in avian flight signals. However, the varied social functions of avian flight signals; greater morphological, physiological and behavioural diversity; different sound production and respiratory mechanisms; dependence on lower frequency sounds; and longer evolutionary history may thus explain the larger variability exhibited by birds. While we cannot yet say to what extent an uncoupling of underlying systems precipitated vocal learning or was instead a consequence, our study demonstrates that avian vocal and locomotor systems share a long and intimate history, and highlights the importance of multiple oscillating mechanisms and physiological processes in the evolution of avian vocal learning.

Ethics. All experiments were reviewed and approved by the Institutional Animal Care and Use Committee (2014-003), University of Texas Rio Grande Valley and the Ministerio del Poder Popular para el Ambiente in Venezuela (no. 1430).

Data accessibility. Electronic supplementary material: Berg_etal_FigI_ESM; Berg_etal_Appendix I, II ESM; movies, M1–9; Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.7g7ff0s> [45].

Authors' contributions. K.S.B. designed the study, collected and analysed data and wrote the manuscript. S.D. helped design the study, and collect and analyse data. A.M.-B. helped design the study and interpret results.

Competing interests. We declare we have no competing interests.

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