

RESEARCH ARTICLE

Specialized primary feathers produce tonal sounds during flight in rock pigeons (*Columba livia*)

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ABSTRACT

For centuries, naturalists have suggested that the tonal elements of pigeon wing sounds may be sonations (non-vocal acoustic signals) of alarm. However, spurious tonal sounds may be produced passively as a result of aeroelastic flutter in the flight feathers of almost all birds. Using mechanistic criteria emerging from recent work on sonations, we sought to: (1) identify characteristics of rock pigeon flight feathers that might be adapted for sound production rather than flight, and (2) provide evidence that this morphology is necessary for *in vivo* sound production and is sufficient to replicate *in vivo* sounds. Pigeons produce tonal sounds (700±50 Hz) during the latter two-thirds of each downstroke during take-off. These tones are produced when a small region of long, curved barbs on the inner vane of the outermost primary feather (P10) aeroelastically flutters. Tones were silenced in live birds when we experimentally increased the stiffness of this region to prevent flutter. Isolated P10 feathers were sufficient to reproduce *in vivo* sounds when spun at the peak angular velocity of downstroke (53.9–60.3 rad s⁻¹), but did not produce tones at average downstroke velocity (31.8 rad s⁻¹), whereas P9 and P1 feathers never produced tones. P10 feathers had significantly lower coefficients of resultant aerodynamic force (C_R) when spun at peak angular velocity than at average angular velocity, revealing that production of tonal sounds incurs an aerodynamic cost. P9 and P1 feathers did not show this difference in C_R . These mechanistic results suggest that the tonal sounds produced by P10 feathers are not incidental and may function in communication.

KEY WORDS: Aerodynamic, Barb, Feather, Flutter, Frequency, Propeller, Sonation, Sound

INTRODUCTION

Sound is an intrinsic byproduct of all motion in the natural world. Perceptible motion-induced sound plays a crucial role in the lives of all multicellular organisms including plants (Appel and Cocroft, 2014), and perhaps even single-celled eukaryotes (Kolle-Kralik and Ruff, 1967). Motion-induced sound can act as a record of any biotic or abiotic movement, thus making it inherently informative (i.e. a cue). Nearly all animals possess the ability to detect this sound (Budelmann, 1989, 1992; Fay, 2009; Horch, 1971), and many may go to great lengths to mask their own locomotion-induced sounds (Conner, 2014; Graham, 1934; Roche et al., 1999). Conversely,

however, many species have also developed remarkable ways to amplify and otherwise modulate these motion-induced sounds for communication (Bostwick, 2006; Clark and Prum, 2015; Darwin, 1871).

Arguably, all aural communication – from the relatively simple sounds produced by stridulating insects (Huber, 1962) to the vast complexity of human language (Larsson, 2014) – can trace its origins to incidental, motion-induced sounds. This evolutionary link between incidental sounds and communicative signals is perhaps most easily explored in birds, in which motion-induced sounds associated with courtship behaviors can become the subject of novel female preferences, exaggerating them into complex signals (e.g. strut displays in greater sage grouse, wing-snapping displays in manakins; Clark and Prum, 2015; Prum, 1998). These non-vocal acoustic signals, or sonations, are common among birds, perhaps because of the inherently noisy nature of feathers, wings and flight (Clark and Prum, 2015; Fournier et al., 2013; Wei et al., 2013).

Incidental sounds produced during flight are ubiquitous among volant birds (even owls; Clark and Prum, 2015; Sarradj et al., 2011) and are, in part, due to the natural propensity of all stiff, light airfoils (e.g. feathers) to aeroelastically flutter under certain flow conditions (Clark and Prum, 2015; Clark et al., 2011, 2013a,b). Aerodynamic energy input from flow over an airfoil excites one or more resonance frequencies within the airfoil. Above a certain threshold (critical velocity, U^*), energy input from the airflow exceeds the structural damping of the airfoil (inertial and elastic forces), causing it to enter stable oscillations (Argentina and Mahadevan, 2005; Clark et al., 2011, 2013a,b; Mandre and Mahadevan, 2009). Feathers that enter these aeroelastic oscillations produce tones whose pitch, amplitude and harmonic content are dependent on complex interactions between flow conditions and the structural, resonant properties of the feather (Clark et al., 2013a,b), but are not dependent on the vortices generated by this flutter (Argentina and Mahadevan, 2005; Clark et al., 2013b). In addition to aeroelastic, tonal sounds, feathers and wings can also produce a variety of atonal sounds as turbulence is shed in their wake ('wooshes'; Blake, 1986; Clark and Prum, 2015; Wei et al., 2013), as feathers reposition and move during each wingbeat ('rustles'; Clark and Prum, 2015) or as flutter causes collisions between adjacent feathers (Clark, 2011; Clark and Prum, 2015). Percussive, broadband snaps and claps can also be produced as air is forced by the collision of wings, feathers and other parts of the body (Clark and Prum, 2015). Together, these passive mechanisms of tonal and atonal sound production make flight an inherently noisy mode of locomotion, suggesting that feather sonations could evolve easily and repeatedly among birds (Clark and Prum, 2015). The ways in which aeroelastically fluttering feathers have been evolutionarily co-opted for communication have been thoroughly described in two taxa – manakins (Bostwick and Prum, 2003; Bostwick et al., 2010; Prum, 1994, 1998) and hummingbirds

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(Clark, 2008; Clark and Feo, 2010; Clark et al., 2011; Hunter, 2008) – and are hypothesized to have evolved at least 27 times in at least nine orders of birds (Clark and Prum, 2015).

In many of these sonating species, selection for specific behaviors and sounds may have led to the evolution of feather morphologies specialized for sound production, particularly in sexually selected displays (Clark and Feo, 2010; Clark and Prum, 2015). Although the link between particular morphologies and their sound-producing abilities is tenuous (Clark and Prum, 2015), ornithologists have nevertheless hypothesized a direct connection between unique shape and sound in many species (Bahr, 1907; Craig, 1984; Hingee and Magrath, 2009; Johnston, 1960; Wetmore, 1926), whereas in many others, sounds are produced in the complete absence of obvious feather morphologies (Clark, 2008; Clark and Prum, 2015; Coleman, 2008; Lebreton, 1958).

Historically, no group has received as much attention in this regard as the pigeons and doves, whose wing sounds and unique feather morphologies have intrigued naturalists for nearly three centuries (Audubon, 1831; Craig, 1911; Cuvier, 1817; Darwin, 1871; Edwards, 1743, 1760; Selby, 1850; Wallace, 1865; Wilson, 1808). In spite of this impressive record of observations, the wing sounds and feather morphologies of these species have never been experimentally linked (but see Clark and Prum, 2015). Others (Barrera et al., 2011; Coleman, 2008; Hingee and Magrath, 2009) have attempted to link wing sounds to anti-predator behaviors, concluding that sounds produced by the wings during alarmed take-off (specifically tonal elements of wing sounds, i.e. ‘whistles’) are signals of alarm (Barrera et al., 2011; Hingee and Magrath, 2009). But the sounds of wings flapping are inherently informative (i.e. cues) because they are intrinsically linked to locomotion, and one would expect all listeners to have evolved some response to them regardless of their tonal content (Maynard Smith and Harper, 2003). The fact that these wing sounds contain tonal elements is not sufficient evidence to conclude that they are an evolved signal because all flight feathers possess the ability to produce tonal sounds through aeroelastic flutter. It is premature, therefore, to consider tonal wing sounds as signals, unless we can experimentally arrive at two conclusions: first, that these elements are critical for conveying alarm-related information (e.g. Hingee and Magrath, 2009) and, second, that these elements are produced by feathers that are specialized (i.e. co-opted) specifically for this purpose. Although this first line of evidence must be purely behavioral in origin, the second, which we have attempted to elucidate in this study, will largely rely on morphological and mechanistic investigations of feather structure and function.

To better understand the role that tonal wing sounds could play in communication, we investigated the link between unique feather morphologies and tonal sound production in the primary feathers of both male and female rock pigeons (*Columba livia* Gmelin 1789). First, we quantified variation in gross feather shape between male and female rock pigeons to determine whether a link between sexually selected displays and sound production could exist, as is common in other species (Clark and Prum, 2015; Prum, 1998). We then examined several characteristics of feather barbs, which are known to influence feather shape and stiffness (Ennos et al., 1995; Feo et al., 2015), two characteristics associated with aeroelastic flutter (Clark et al., 2013a,b). Finally, we experimentally tested the necessity of particular feather morphologies for natural, *in vivo* sound production, and the sufficiency of individual feathers to reproduce *in vivo* sounds in laboratory simulations of flapping wings.

MATERIALS AND METHODS

Feather morphology

The five outermost primary feathers (P10–P6; Fig. 1A) in 10 adult male and 10 adult female study skins from the Burke and Slater Museums of Natural History were photographed and digitally measured (Bachmann, et al., 2007) using tpsDig software (v.2.17, Rohlf, 2013). For each feather, the depth of the outer and inner vanes was measured every tenth percent of the vane length (Fig. 1B). Measurements of vane depth were normalized by the length of the vane for each feather (as per Bachmann et al., 2007).

In five additional birds (see Testing sufficiency, below), two primary feathers (P10 and P9) were removed from the wing and photographed. For each of these feathers, the barb length, distal barb angle and proximal barb angle on the inner vane were digitally measured (as above) at six points along the rachis (Fig. 1C). Measurements of barb length were also normalized by the length of the feather vane (as per Bachmann et al., 2007).

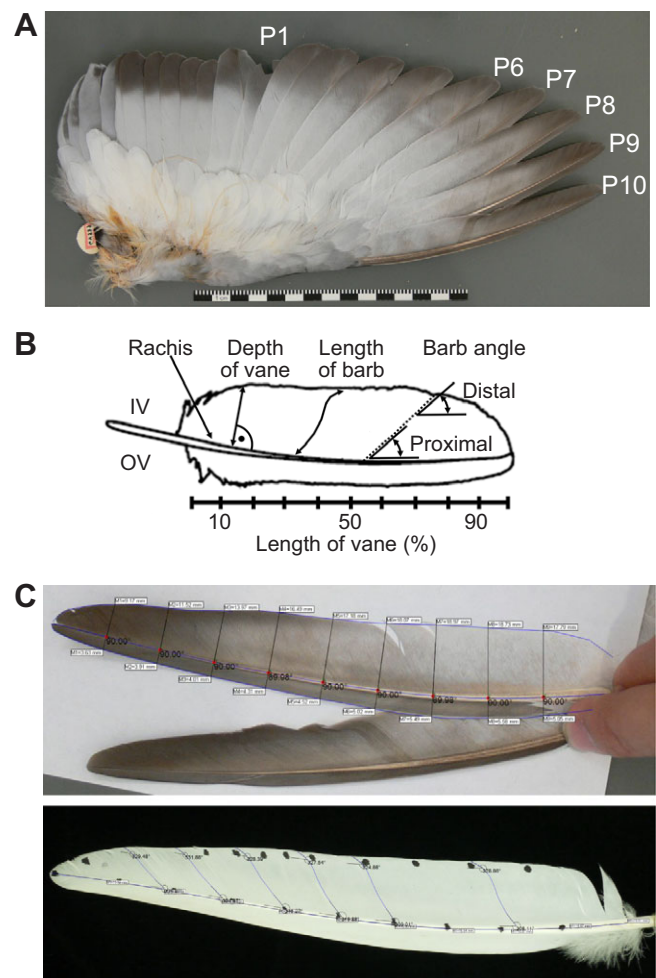


Fig. 1. Feather location on the wing and measurements taken on each feather. (A) A typical wing of *Columba livia*. The six sampled feathers are indicated. (B) Summary of the types of measurements taken in feathers. OV, outer vane; IV, inner vane. Vane depths were measured on both the OV and the IV, while barb measurements were only taken from the IV. Distal barb angles are measured relative to the rachis, as in proximal barb angles. (C) Example digital measurements of P9 feathers. Top, vane depths; bottom, barb measurements.

Testing necessity: feather manipulations on live birds

Five birds were tested in outdoor aviaries for their sound-producing abilities (IACUC, AUP 035-15). Birds were video and audio recorded during a single release flight as they returned to flockmates 10 m away at the opposing end of an aviary with dimensions 6×6×15 m. High-speed video was collected using a Photron FASTCAM SA-3 camera (Photron USA, San Diego, CA, USA; using PFV v.3282 Software) recording at 1000 frames s⁻¹ with a 1/6000 s shutter speed. Audio was recorded to desktop computer at 24 bits and sampling at 96 kHz through an audio interface (Raven Pro, v.1.4, Cornell Lab of Ornithology Bioacoustics Research Program, Ithaca, NY, USA) and preamplifier (Roland QUAD-CAPTURE UA-55, Roland Corporation, Hamamatsu, Japan) using a Sennheiser ME67 shotgun microphone (Sennheiser Electronic, Wedemark, Germany). Video and audio were trigger-synchronized using an ART AVDirect converter box (Applied Research and Technology, Rochester, NY, USA) to convert a TTL (transistor-transistor logic) trigger pulse input into an audio signal that was recorded on a second audio channel. This trigger-synchronization process created a repeatable 3.9 ms delay in the audio signal, which, along with a minor sound lag owing to distance (Clark et al., 2016), was accounted for in our analyses. Birds were recaptured and released individually into a separate aviary (of the same dimensions as above) where they were audio recorded during six to 18 consecutive escape flights motivated by a pursuing researcher. Audio recordings of these escape flights were collected continuously using the abovementioned microphone and a portable 24 bit Marantz PMD661 field recorder (Marantz America, Mahwah, NJ, USA) sampling at 96 kHz and were analyzed using Raven Pro software.

Birds were again recaptured and an aerosol plastic polymer fixative (i.e. hairspray; TRESemmé TRES Two® Freeze Hold Hair Spray, Godefroy Manufacturing Company, St Louis, MO, USA) was applied to a 5-cm-long region on P10 (identified in feather morphology analyses mentioned above) on both wings. The fixative was allowed to dry for approximately 5 min. Each treated bird was then video and audio recorded during a single flight (as above). Treated birds were then recaptured and released into a solitary aviary and audio recorded (as above) during nine to 14 consecutive escape flights motivated by a pursuing researcher.

Recordings of flights before and after the feather treatment were analyzed for their tonal content in Raven Pro using the ‘average entropy’ function. Entropy, or spectral flatness, is a dimensionless number representing the distribution of energy within a sound, where a tonally pure sound has an entropy approaching 0 and a tonally flat sound (i.e. white noise) has an entropy approaching 1. Raven Pro transforms these values, treated as probabilities, and averages them across each time slice, such that lower average entropy values correspond with more pure tones (Charif et al., 2010; Clark et al., 2016). Thus, the average entropy, as calculated using Raven Pro, describes the average amount of disorder in each frequency bin within the sound sample (Charif et al., 2010). Calculations were restricted to sound selections of equal duration (time) and bandwidth (frequency) across all recordings (Hann window size=1300 samples; 2048-sample FFT frequency grid) and were targeted at regions in which tonal sounds are known to occur (during downstroke, between 0.5 and 1 kHz; see Results).

Testing sufficiency: laboratory experiments on isolated feathers

Three primary feathers (P10, P9 and P1) were removed from five deceased rock pigeons (donated salvage from Montana, Oregon and

Washington, USA) and spun using a brushless DC motor (BL3056 Series Motor, BPMC Technology, Tsuen Wan, Hong Kong) controlled using a Luminary Micro Stellaris BLDC Motor Control Module (MDL-BLDC, Luminary Micro/Texas Instruments, Austin, TX, USA). High-speed video of spinning feathers was collected using a Photron FASTCAM SA-3 camera recording at 3000 frames s⁻¹ with a 1/6000 s shutter speed. Synchronized audio was recorded from a distance of 2 m using the previously mentioned microphone to a desktop computer and pre-amplifier, as above, at 24 bits and sampling at 96 kHz.

Feathers were spun at 0 deg angle of attack (α) at two biologically relevant velocities that were calculated from high-speed video of a single bird in a controlled, horizontal flight from perch to perch as motivated by a researcher (Crandell and Tobalske, 2011). The α at which feathers were spun was referenced to the orientation of the proximal 1/3 of the inner vane prior to spinning. This α was estimated to vary by ± 5 deg. Note that feathers exhibited spanwise twist when loaded and their α potentially decreased with increasing distance from the rotational axis; furthermore, feathers and whole wings are understood to deform under aerodynamic loading (Heers et al., 2011). Preliminary tests with feathers set with α ranging from -15 to $+15$ deg suggested that tonal sound production occurs primarily at 0 ± 5 deg. A more systematic assessment of all the feathers in the wing at all relevant angles of attack would provide more data, but our ability to interpret these data is hindered by the complex nature of these low-advance-ratio flights. The pigeon wingbeat is highly time-variant and dominated by unsteady aerodynamics at take-off. Any estimates of the function of angle of attack inferred from our relatively simple model are beyond the scope of this study.

Average wing-tip velocity (11.4 m s⁻¹) and peak wing-tip velocity (16.2 m s⁻¹) were calculated from the downstroke of the third wingbeat after take-off as per methods detailed in Crandell and Tobalske (2011). Wing-tip velocities were converted into average and peak angular velocities (38.1 and 53.9 rad s⁻¹, respectively), and rotational frequencies (rpm) were calculated for each feather given its length. At these velocities, feathers were operating at Reynolds numbers between 6000 and 11,000. Our flapping wing model assumes that each feather is placed at the tip and leading edge of the wing during downstroke, but it is important to caution that P10 and occasionally P9 (when P10 molts) are the only feathers that occur in this position *in vivo*. Feathers were tested at low rotational frequencies first (i.e. average wing-tip velocity before peak wing-tip velocity) to avoid potential hysteresis complications from feather flutter at higher frequencies. P10 feathers that did not flutter (i.e. activate) at peak wing-tip velocities were then spun faster until activation occurred. These activation velocities (U^*) were within 10–12% of the peak wing-tip velocities – a difference that can be accounted for by biologically relevant variation in flight motivation (e.g. high-powered escape flight), angle of ascent, wing span and/or body mass (Berg and Biewener, 2010; Kullberg et al., 1998).

Spinning feathers and the propeller assembly were mounted on a custom-built force plate (15×15 cm, Bertec Corp., Columbus, OH, USA) to measure vertical force along the y -axis and torque about the z -axis as a result of drag (Crandell and Tobalske, 2011; Usherwood, 2009). The voltage output from the force plate was amplified (10× digital gain, Bertec amplifier, model M6810) before being converted with an ADInstruments PowerLab 8SP A/D converter sampling at 1000 Hz and imported to a desktop computer using LabChart v5.2 software with a 1 Hz low-pass digital filter (ADInstruments, Colorado Springs, CO, USA) as per methods

detailed in Crandell and Tobalske (2011). Data from the force plate were converted into dimensionless coefficients of vertical (C_v) and horizontal (C_h) forces following Crandell and Tobalske (2011) and Usherwood and Ellington (2002), with average air density of 1.07 kg m^{-3} (for Missoula, MT, USA), and were expressed together as the resultant vector-sum coefficient of force (C_R ; $C_v + C_h = C_R$). The vertical coefficient was calculated using measured lift on the plate (N), angular velocity (ω ; rad s^{-1}) and second moment of area (S_2 ; m^4). The horizontal coefficient was calculated using torque (N m), ω and third moment of area (S_3 ; m^5). For details regarding these computations, see Usherwood and Ellington (2002), their eqns 8 and 10.

Statistical analyses

Statistical analyses were performed using SPSS (v.17.0, IBM, Armonk, NY, USA) and Excel (v.14.0, Microsoft Corporation, Redmond, WA, USA) software. To test for differences in vane depths between male and female rock pigeons, we used two-way ANOVAs to determine the effects of sex at a given position along the rachis for each vane on each feather. To test for differences between barb angles (distal and proximal angles separately) between feathers, we used two-way ANOVAs to determine the effects between feathers and positions along the rachis. Independent samples *t*-tests were used to analyze specific differences between feathers at a given position along the rachis. Average entropy of downstroke sounds for each bird across all flights was compared before and after feather manipulation using a paired samples *t*-test. To test for differences between coefficients of force production between angular velocities in different feathers, we used paired samples *t*-tests. Herein we report means \pm s.d.

RESULTS

Feather morphology

Across the five outermost primary feathers (P10–P6; Fig. 1A) in 10 adult male and 10 adult female rock pigeons, there were no significant differences in vane depths (Fig. 1B) at any point along any feather between the sexes (all $P > 0.5$; Fig. 2). The inner vane (IV) of all P10 feathers showed slight attenuation around 60% of the vane length (Fig. 2). Barb measurements (Fig. 1B,C) from the IV of P10 showed that this area of attenuation possessed barbs that were between 14 and 30% longer than barbs in adjacent regions of the same feather, in spite of the distinct narrowing in this region (Fig. 3). This increase in barb length concurrent with a narrowing of vane depth was due to significant changes in the distal barb angle but not in the proximal barb angle (Fig. 4) within the attenuated region. P10 possessed barbs with a distal angle that was significantly more acute (i.e. more parallel to the rachis) at 40, 50 and 60% (all $P < 0.05$) and

significantly more obtuse (i.e. more perpendicular to the rachis) at 70% ($P = 0.01$) than barbs in the same region on P9. In contrast, P10 and P9 feathers had similar distal barb angles at other points along the rachis (20 and 80%; both $P > 0.5$).

Testing the necessity of P10 for sound production in live birds

Three of five birds (60%) produced tonal sounds in 100% of flights ($N = 18$, 14 and 12 flights per bird) prior to feather manipulations. Two other birds produced tonal sounds in 83 and 31% of flights ($N = 6$, 13 flights per bird, respectively) prior to manipulations. All tones had an average fundamental peak frequency of $700 \pm 50 \text{ Hz}$ with up to 20 harmonics. The average entropy (a measure of the distribution of energy, where 0 is a pure tone with all energy distributed in a single frequency) of each downstroke sound was 2.6 ± 0.2 . Following the application of an aerosol plastic polymer fixative (i.e. hairspray, see Materials and methods) to the small target region of P10 (see Feather morphology, above), the tonal aspect of wing sounds was eliminated in 100% of flights (e.g. Fig. 5) in four birds ($N = 14$, 13, 10 and 9 flights per bird; Audio 1). The average entropy of these downstroke sounds was 3.1 ± 0.1 , which was significantly less tonal than feather sounds before manipulation ($t = -13.4$, d.f. = 4, $P < 0.001$). One individual produced fewer wing sounds with tonal elements (83%; $N = 12$ flights) (but see Discussion).

Qualitative comparisons of high-speed video of test flights pre- and post-manipulation suggested that the application of fixative to the small region of the IV on P10 did not significantly alter the birds' flight kinematics. On average ($N = 20$ wingbeats across five take-off events), the peak fundamental frequency of the tonal aspect of unmanipulated flight sounds occurred at $69 \pm 16\%$ of downstroke ($40 \pm 10\%$ of the wingbeat cycle), and did not occur during the upstroke (Fig. 6). A broadband 'clap' aspect of pre- and post-manipulation flight sounds occurred at the end of the upstroke and was caused by dorsal wing-to-wing contact (Fig. 6) (Crandell and Tobalske, 2015).

Testing the sufficiency of individual feathers to replicate *in vivo* tones

P10 feathers that were spun at the average angular velocity of downstroke (38.1 rad s^{-1}) never produced tonal sounds and never fluttered. Three of the five P10 feathers we tested produced tonal sounds and fluttered at peak angular velocities (53.9 rad s^{-1}). The remaining two P10 feathers produced tonal sounds and fluttered when spun at a biologically plausible speed 10–12% faster than peak angular velocity (up to 60.3 rad s^{-1}). All tones produced by P10 feathers had a fundamental frequency $\sim 500 \text{ Hz}$ ($480 \pm 20 \text{ Hz}$;

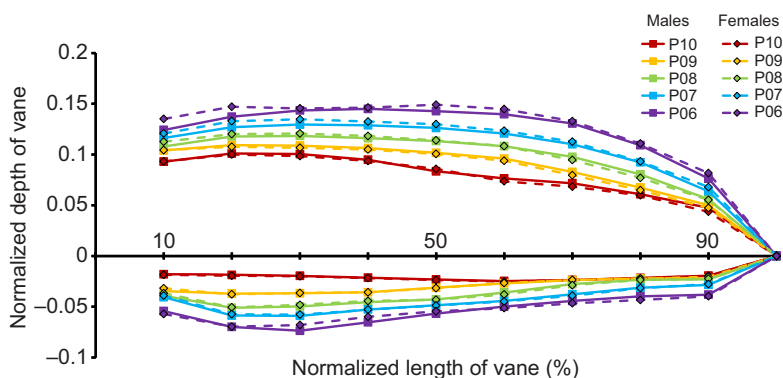


Fig. 2. Mean normalized vane depth at every 10% of vane length for five feathers (P10–P6) of male and female rock pigeons. The x-axis represents the rachis (calamus towards the origin; feather tip at 100%) with measurements every 10% of the vane. Positive values are inner vane depths; negative values are outer vane depths. Dashed lines and diamonds represent females; solid lines and squares represent males. $N = 10$ for each point. Error bars removed for clarity. Vane depths are not significantly different between males and females at any point in any feather (all $P > 0.5$).

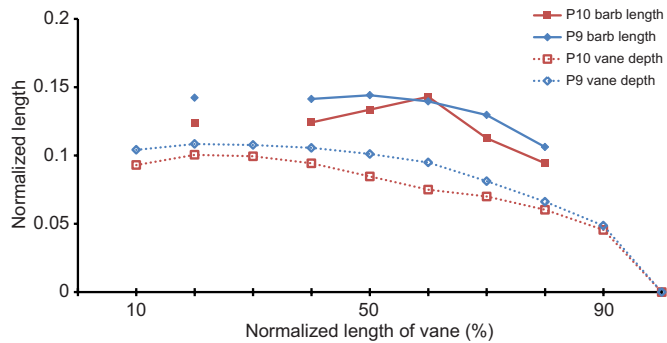


Fig. 3. Vane depths (dashed lines, open markers) in P10 and P9 as compared with their barb lengths (solid lines, closed markers). Red squares are P10 feathers; blue diamonds are P9 feathers. For vane depths, $N=20$ for each point; for barb lengths, $N=5$ for each point. All measurements are normalized by the length of the vane. At the point of attenuation in P10 (at 60% of the vane length), barbs are 15% longer than more proximal barbs (at 40% of the vane length) and 30% longer than more distal barbs (at 70% of the vane length). In the same region on P9, barbs are approximately the same length as more proximal barbs, and 8% longer than more distal barbs.

$N=5$) with three to seven harmonics (4.4 ± 1.5), whereas *in vivo* wing sounds had frequencies ~ 700 Hz (700 ± 50 Hz; $N=24$) with one to 20 harmonics. Flutter in all P10 feathers occurred in the region of the IV identified in our morphological investigations, but also occurred, at comparatively smaller amplitudes, more proximally on the IV (Movie 1). Flutter in the target region of the IV appeared to occur at 450 ± 15 Hz, but the precision of these measurements is somewhat constrained by the relatively low frame rate of our camera ($3000 \text{ frames s}^{-1}$).

P9 feathers never produced tones and never fluttered when spun at average or peak angular velocities. Additionally, P1 feathers never produced pure tonal sounds, but consistently fluttered at peak angular velocities and sporadically at average velocities. Flutter in P1 feathers was always irregular (i.e. non-limit cycle; Clark et al., 2013b) and often resulted in barb separation at one or multiple points in both the IV and outer vane.

Activation velocity (U^*) for three of five P10 feathers was at or slightly below the calculated peak angular velocity. Two P10 feathers required higher-than-peak angular velocities to activate (within 12% of peak; up to 60.3 rad s^{-1}). Activated P10 feathers had significantly lower coefficients of resultant force (C_R) than inactive (i.e. not fluttering; at average angular velocity) P10 feathers ($t=9.12$, d.f.=4, $P=0.001$; Fig. 7). This reduction in C_R was due to significant decreases in force production in horizontal ($t=5.68$, d.f.=4, $P=0.005$) and vertical components ($t=4.68$, d.f.=4, $P=0.009$), with a slightly larger decrease in C_h than in C_v . However, overall change in the $C_v:C_h$ ratio was insignificant ($t=-0.97$, d.f.=4, $P=0.39$). Coefficients of variance were higher for C_v (ranging from 0.32 to 0.80) than for C_h (ranging from 0.03 to 0.30). No differences in coefficients of force production were observed between average and peak velocities in P9 and P1 feathers (all $P>0.05$; Fig. 7).

DISCUSSION

Morphological investigations of the outer primaries on rock pigeons revealed one region of the outermost feather (P10) that may be specialized for sound production in both sexes (Figs 2–4). This morphology was previously noted by Bachmann et al. (2007), but the authors did not speculate at its function. This small, 5-cm-long region of P10 (between 50 and 70% of IV length) has barb characteristics that decrease its stiffness (Ennos et al., 1995; Feo

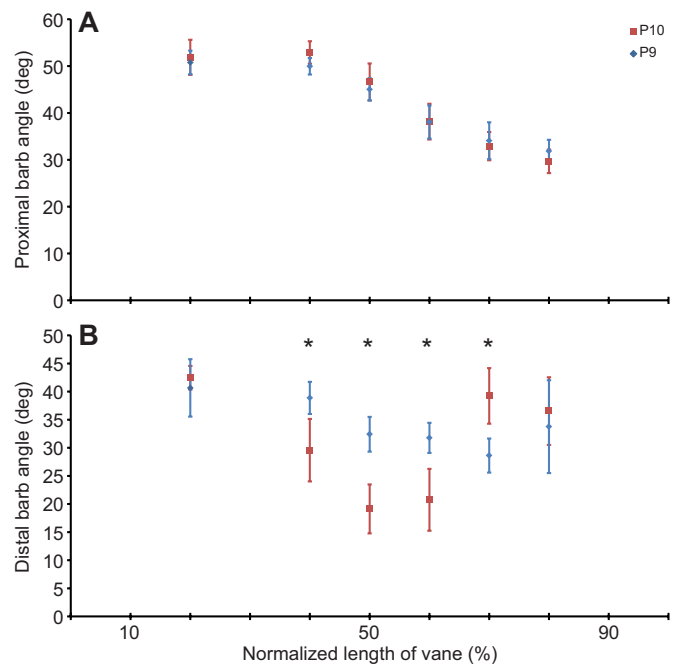


Fig. 4. Barb angles measured proximally and distally to the rachis. Red squares are P10 feathers; blue diamonds are P9 feathers. $N=5$ for each point; data are means \pm s.d. (A) Proximal barb angles vary significantly at different points along any given feather ($F=66.9$, d.f.=5,47, $P<0.001$), but between P10 and P9 feathers, proximal angles are statistically similar ($F=0.19$, d.f.=1,47, $P=0.66$) and vary along the rachis in similar ways ($F=0.73$, d.f.=5,47, $P=0.60$). (B) The way that distal barb angles vary at different points along the rachis is different between P10 and P9 ($F=7.44$, d.f.=5,47, $P<0.001$). Distal barb angles are significantly more acute (more parallel to the rachis) in P10 than in P9 at 40, 50 and 60% of the vane length (indicated by asterisks; all $P<0.05$). Distal barb angles are significantly more obtuse (more perpendicular to the rachis) in P10 than in P9 at 70% of the vane length (indicated by an asterisk; $t=3.32$, d.f.=7, $P=0.01$). Distal barb angles at 20 and 80% of the vane length are not significantly different between P10 and P9 (all $P>0.5$).

et al., 2015) and promote aeroelastic flutter, which is necessary for the production of tonal sounds during flight. P10 feathers stiffened with a temporary fixative (hairspray) were effectively silenced (i.e. produced atonal sounds only; Fig. 5) in all the birds we tested except one, which had accidentally crimped its feather during the drying process. This crimped feather produced tones, but with fewer or no harmonics and at a different frequency than all the other individuals we tested. Preliminary tests of hairsprayed and unmanipulated feathers in an aeroacoustic wind tunnel revealed that the fixative successfully prevents flutter in the target region of P10, providing further support for our conclusion that this region has been co-opted to promote flutter (Movie 2).

Flutter in this specialized region of P10 is sufficient on its own to nearly replicate the tonal sounds produced *in vivo*. We determined that the velocity at which flutter activates (U^*) in P10 occurs within 12% of the peak velocity of wing-tips during downstroke in take-off in live birds. This was corroborated using synchronized kinematic and audio data that indicate that tonal wing sounds only occur during downstroke (specifically the latter two-thirds of downstroke; Fig. 6) and are most easily detected during take-off when wing-tip velocity is highest (Berg and Biewener, 2010), and not during steady, level flight when wing-tip velocity is lower. In level flight at 4 m s^{-1} , we estimate that average wing-tip velocity is $\sim 15.1 \text{ m s}^{-1}$ (Berg and Biewener, 2010), which is below the threshold we observed for sound production. The wing-tip velocities we modeled

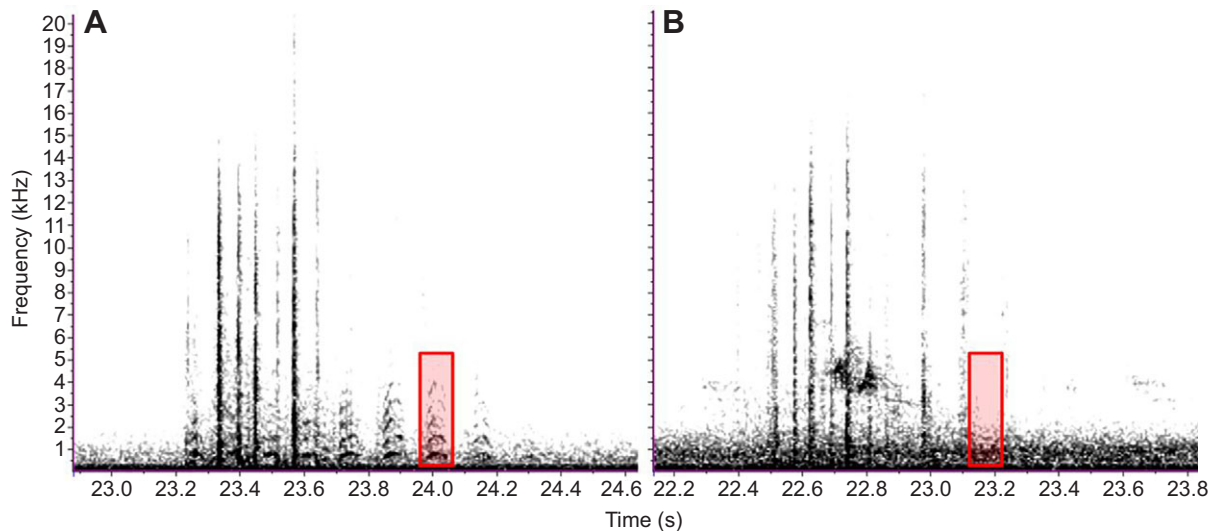


Fig. 5. Take-off flights of a captive rock pigeon (*Columba livia*) in response to a pursuing researcher. Data shown are (A) before and (B) after a fixative was applied to the outermost primary feather (P10). Red highlighted region notes the presence of tonal elements in wing sounds prior to manipulation (A) and the absence of tonal elements after manipulation (B). Tonal elements in A have a fundamental frequency around 0.65 kHz, up to five harmonics, and an average entropy of 2.5 ± 0.1 (see Materials and methods, Testing necessity). Conversely, wing sounds in the post-manipulation flight have no apparent tonal elements and have an average entropy of 3.1 ± 0.1 . These two recordings are statistically representative of the average of our dataset ($t=1.29$, d.f.=38, $P=0.20$; $t=-1.03$, d.f.=28, $P=0.31$). Vertical, broadband sounds are wing claps in both A and B. In B, the 4.5 kHz tonal sound between 22.7 and 22.9 s is a house sparrow (*Passer domesticus*) call, not a tonal element of rock pigeon wing sounds. Hann window size=1300 samples; 2048-sample FFT frequency grid. The spectrograms here correspond to Audio 1.

in these experiments are somewhat higher than those reported in other kinematic investigations of rock pigeon take-off (e.g. Crandell and Tobalske, 2011), which is reasonable considering that take-off speed is greater under perceived threat of predation (Hingee and Magrath, 2009; Kullberg et al., 1998). The two P10 feathers that required slightly higher than peak angular velocities to activate (up to 60.3 rad s^{-1} ; wing-tip velocities up to 18.1 m s^{-1}) were still moving well within biologically relevant boundaries for rock pigeons (Berg and Biewener, 2010). These same peak and average angular velocities were insufficient to produce tones in the other feathers we tested, revealing that P10 was more susceptible to aeroelastic flutter – a trait that we attribute to the barb characteristics of its IV (Ennos et al., 1995). Because of this intrinsic link to high-speed downstroke (i.e. wing-tip velocities above U^*), tonal wing sounds can act as a cue for discerning the speed at which a bird takes off (e.g. alarmed or non-alarmed), as has been shown for crested

pigeons (*Ocyphaps lophotes*) in Australia (Hingee and Magrath, 2009).

Tones produced by individual P10 feathers under laboratory conditions had a peak fundamental frequency around 500 Hz with three to seven harmonics, while *in vivo* tonal sounds had peak fundamental frequencies around 700 Hz. Differences between our propeller model and *in vivo* flight could explain the discrepancy in this tonal content. Specifically, our propeller model spun feathers at constant angular velocity and at a single angle of attack, whereas *in vivo* flights at low advance ratio are characterized by continuous variation in wing-tip velocity, body velocity, angle of attack and associated aerodynamics (Crandell and Tobalske, 2015; Dickson and Dickinson, 2004), all of which have the potential to influence aeroacoustic ability. Alternatively, the adjacent P9 may either aerodynamically or structurally couple to P10 to modify its sound production (Clark, 2014). Differences in tonal content could also be

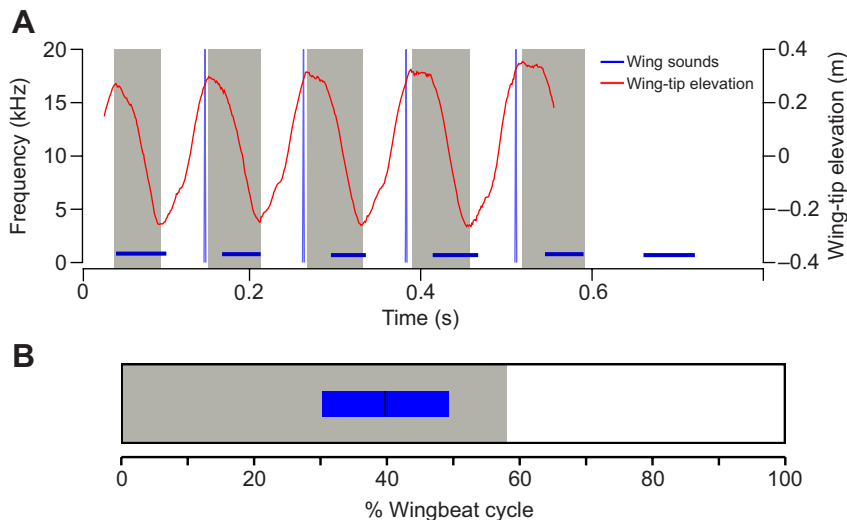


Fig. 6. Synchronized audio and high-speed video recordings of take-off flights reveal that tonal components of wing sounds occur late in downstroke. (A) A single take-off event of synchronized kinematic and acoustic data shows that broadband, atonal claps (vertical blue lines) occur at the top of upstroke, when the wing-tip is highest above the body (red line), and occurs at the end of the first four wingbeats only. Tonal elements (horizontal blue lines) appear to occur late in downstroke and have a peak fundamental frequency of around 700 Hz with up to four harmonics (removed for clarity). Tonal elements occur during the first six downstrokes during this take-off event. Wing-tip elevation data end shortly after the fourth cycle, when the bird flies out of the video frame. (B) Across all video-recorded wingbeats in all birds ($N=20$), the peak fundamental frequency of the tonal component of wing sounds occurs at $30 \pm 10\%$ of the wingbeat cycle (or $69 \pm 16\%$ of each downstroke) during take-off (blue bar). The gray shaded regions in A and B represent the downstroke.

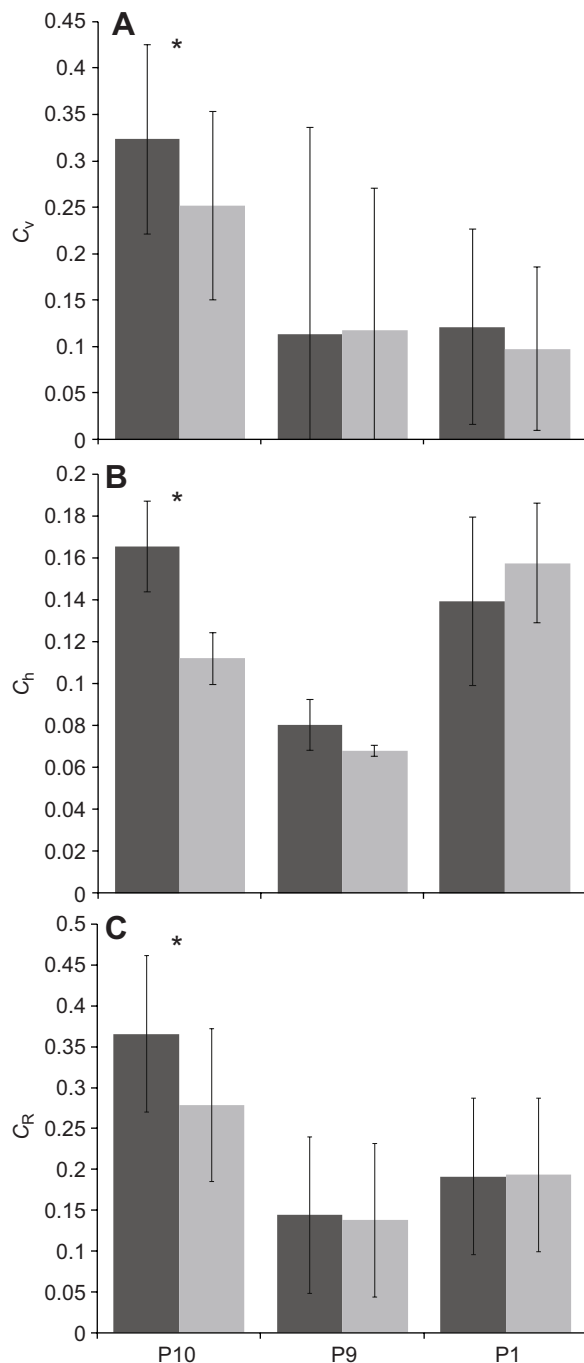


Fig. 7. Mean coefficients of aerodynamic force production of spinning rock pigeon (*Columba livia*) feathers at two velocities. Dark bars represent coefficients of force for feathers spun at average angular velocity (38.1 rad s⁻¹) and light bars represent coefficients of force for feathers spun at the angular velocity at which P10 begins to flutter (i.e. U^* ; between 53.9 and 60.3 rad s⁻¹). (A) Vertical force coefficient (C_v), (B) horizontal force coefficient (C_h) and (C) resultant coefficient (C_R), which is a vector-sum of $C_v + C_h$. P10 displayed a significant decrease in all coefficients of force when spun at higher angular velocities. All other feathers showed no significant change. Asterisks indicate statistically significant differences among means ($P < 0.05$). $N = 5$ for each bar; data are means \pm s.d.

partially explained by differences in spectrogram window size between time-invariant laboratory recordings (larger sample window, maximized for frequency resolution) and temporally variable *in vivo* flights (smaller sample window, intermediate

frequency resolution), which has been identified as a source of error in similar experiments (Clark et al., 2013b).

There has been some debate regarding the mechanisms responsible for aeroacoustic tonal sound production (e.g. Clark and Prum, 2015; Clark et al., 2013b; Reddig, 1978; van Casteren et al., 2010). The feathers we tested in these experiments are operating within a range of Reynolds numbers (6000 to 11,000) that is known to coincide with the production of vortices in a Von Kármán vortex street (Blake, 1986; Clark et al., 2013b). Some have argued that these vortex streets, which are known to produce tones (e.g. ‘singing’ telephone wires), are responsible for the whistling sounds of many aerial displays in birds. This vortex-whistle hypothesis has yet to find any empirical support, however, and several key lines of evidence in the present study continue this trend. In particular, according to the vortex-whistle hypothesis, whistling feathers should produce tones with a fundamental frequency (f) that is directly proportional to airspeed (U_{air}) and contains no more than one harmonic ($2f$; Blake, 1986). Our data revealed that f does not scale proportionally with U_{air} , but instead requires some critical velocity to activate (U^*). Furthermore, recordings of tonal sounds produced by unmanipulated birds *in vivo* contained up to 20 harmonics, and laboratory tests of P10 produced up to seven harmonics. Lastly, the characteristic frequency of vortex shedding (f) is expected to relate to chord length (L) and velocity (U) according to the dimensionless Strouhal number of 0.2 ($St = fL/U$; Vogel, 1994), while St for P10 feather tones is 0.7. An St of ~ 0.7 is within the range of St for fluttering hummingbird rectrices (Clark et al., 2013b). Together, these data lend additional, strong support to the aeroelastic flutter hypothesis for tonal feather sound production.

Our laboratory experiments on individual feathers revealed that flutter in P10 significantly reduces the coefficient of resultant forces (C_R) of the feather in both the horizontal (C_h) and vertical directions (C_v ; Fig. 7). At comparable velocities, P9 and P1 feathers showed no significant changes in coefficients of force. This suggests that, although P10 may more easily enter stable oscillations, those oscillations reduce the overall aerodynamic activity of the feather. This reduction in aerodynamic activity could indicate that P10 feathers have been co-opted for sound production at the cost of aerodynamic performance. However, we estimate this as a minor cost relative to the aerodynamic demands for weight support and thrust. If C_v had remained constant at higher velocities in P10, we estimate that the force gained would be approximately 12.3 mN, approximately 1% of the peak vertical force obtained from a whole wing (Crandell and Tobalske, 2011) spinning under similar conditions. Further research is needed to fully understand how flutter influences aerodynamic performance and whether this cost to take-off efficiency is evolutionarily viable in the context of alarm signals that are produced by fleeing.

Conclusions

Our morphological and functional investigations of primary feathers in rock pigeons revealed a subtle, yet critically important, morphology for the production of tonal wing sounds during escape take-off. These types of wing sounds have been implicated as signals of alarm in the past (Barrera et al., 2011; Coleman, 2008; Hingee and Magrath, 2009; Townsend, 1915), but discerning them from incidentally produced sounds of locomotion has proven difficult and consequently has impeded any conclusions about their function as signals. Providing a link between a unique feather morphology and sound production, especially one that appears specialized at the expense of aerodynamic performance, allows us to conclude that tonal wing sounds in this species are non-incidentally.

The behavioral significance of these tones has yet to be experimentally investigated in this species, but considering the information they contain (i.e. P10 only produces tones above a critical velocity, U^*), we encourage future experiments to study these tones in the context of predator avoidance and flock cohesion.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Both authors were involved in the research design, interpretation of findings and preparation of the manuscript. R.L.N. additionally analyzed the data.

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Data availability

Digital images of the spread wings used in this study can be found at <http://digitalcollections.pugetsound.edu/cdm/landingpage/collection/slaterwing>.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.131649.supplemental>

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