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Supporting Online Material for

Aeroelastic Flutter Produces Hummingbird Feather Songs

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Other Supporting Online Material for this manuscript includes the following:
(available at www.sciencemag.org/cgi/content/full/333/6048/1430/DC1)

Movies S1 and S2

Supplemental online materials

Two supplemental movies accompany this manuscript.

Supplemental s1.avi

This narrated movie shows different ways feathers can flutter and produce sound, including the four types of modes of flutter depicted in Fig. 1G. Video clips of feathers in the wind tunnel are 30 frames per second (fps) clips with accompanying sound, and high-speed videos (23,121 fps) that show the flutter of the feather. Shot with a Sony Handicam HD and a Miro Ex4 high-speed camera; edited in Windows Movie Maker.

Supplemental s2.avi

This narrated movie is a demonstration of sympathetic vibrations in Anna's Hummingbird R4 that amplify the loudness of the sound produced by R5 (i.e., Fig. 3). Sound recordings of the displays of Anna's Hummingbird (*Calypte anna*) with and without R4 are played; then a demonstration of the amplification effect that R4 has when placed behind R5 is shown, both with conventional video (30 fps, including sound) and high-speed video (23,121 fps). Finally SLDV scans of Anna's R5 are shown, demonstrating that R4 vibrates at the same frequency as R5 when placed behind R5, whereas a flat plate does not. Shot with a Sony Handicam HD and a Miro Ex4 high-speed camera; edited in Windows Movie Maker.

Supplemental Methods

Evidence that the feathers make sounds during courtship displays

In this study, we measured a series of tail-feathers from males of species of hummingbird from the 'bee' clade (*5*). Twenty seven types of rectrices were tested as summarized in table S1. They were from adult males of 14 species; 1 to 4 rectrix types (R5, R4, etc) were tested per species. N = 1 feather per type except in 4 cases, N = 2 feathers per type (table S1), thus a total of 31 individual feathers were tested.

There are four different lines of evidence that indicate a given tail-feather produces courtship display sounds.

1. A strong correlation between the wing and tail kinematics and sound production during a flight display. All of the hummingbirds studied here for which high-speed videos are available spread and shut the tail one or more times during a courtship dive, and produce the sound only when the tail is spread (6-*9*).

2. Manipulative experiments on wild birds demonstrate which feathers are <u>necessary</u> to produce the dive sound. A male's capacity to produce a dive-sound is first recorded as a control (as not all unmanipulated individuals in a population are competent, see refs 7, *9*). Then he is captured and a manipulation is performed, typically removal of one or more pairs of tail-feathers. Next

the sounds the male produces in post-manipulation displays are recorded (*5-8*). This type of experiment shows which feathers are necessary to produce the dive-sound, but does not demonstrate which feathers are sufficient for sound production.

3. Sufficiency is demonstrated by putting individual feathers in a wind tunnel and getting them to produce sounds similar to the dive-sound (*7-9*), which is what was done in the present study.

4. After it has been established that one species produces display sounds with a given tail-feather, homology can be used to attribute dive sounds to similarly-shaped tail-feathers in a sister species.

The tail-feathers used in this study are listed in Table S1. Of the 27 types of feathers tested with the SLDV in this study, there is wind-tunnel evidence that at least 24 of them are sufficient for the production of sounds matching courtship display sounds of the species in question (although some are not necessary, see below). Thus far across our previous work (refs 6-9), all individual feathers found to be necessary (via field manipulations) to produce the dive-sound are also sufficient. But, in some cases it is known that a feather is sufficient but not necessary to produce the dive sound, as indicated by a match between the sounds it produces in the wind tunnel with the sounds the bird produces in the wild, but a bird missing this feather still makes the dive sound[1]. Specifically this is true of R4 of the Black-chinned Hummingbird, *Archilochus alexandri* (*8*), and R2 and R5 of Allen's Hummingbird, *Selasphorus sasin* (this study). In both of these cases, a neighboring feather has been demonstrated to be both necessary and sufficient for sound production (R5 in Black-chinned; R3 and R4 in Allen's). Therefore, while any of the four lines of evidence outlined above can be used to assign courtship display-sounds to a particular tail-feather, field manipulations are necessary to make this diagnosis definitive.

The specific evidence as to whether a bird uses a given feather for sound production during courtship displays is presented in table S1. Two examples of feathers that are sufficient to produce dive sounds are provided for the White-bellied Woodstar (*Chaetocercus mulsant*) and the Bahama Woodstar (*Calliphlox evelynae evelynae*) in Fig. S1. In many cases our diagnosis is a working hypothesis because time, expense, and other logistical difficulties have prevented us from performing field manipulations on all of the species studied here. Thus some of these diagnoses as to which tail-feather produces dive sounds that are presented in table S1 could be overturned with additional data involving manipulations of wild birds (see table S1).

The feather-feather interactions demonstrated for the Allen's and Anna's Hummingbirds (Figs 3, 4) seem likely in additional taxa, including all of those studied here. Table S1 only considers whether a given feather is primarily responsible for production of a given sound (i.e. its removal

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[1] In the field, quantitative measurements of loudness of a sound are challenging to make, due to variable distance from microphone to bird, variable gain settings on the recorder, etc. Therefore removal of a feather could result in a decrease in sound loudness that was not detected. The argument in this paragraph therefore applies to which feather is *primarily* necessary for sound production, and does not address whether these feathers amplify the sound (e.g. result shown for Anna's Hummingbird R4 in Fig. 3)

is predicted to produce a pronounced decrease in sound production), and does not consider whether a given feather could play a secondary role in sound production, such as amplifying the sound produced by a neighbor.

Materials and methods

The acoustic properties of feathers were tested in the working section of an Eiffel-style open wind tunnel in the Department of Mechanical Engineering at Yale University. The top and bottom surfaces of the working section of the tunnel were lined with 1” flat acoustic foam (Noise Reduction Coefficient of 0.72), reducing reverberation within the working section. Airspeed could be continuously varied between 0 and > 40 m s^{-1} and was calibrated (after the acoustic foam was installed) using a Pitot tube connected to a pressure transducer.

Sounds were recorded with a ½” free-field microphone (B&K 4190; Brüel & Kjær, Norcross, GA, USA) with a turbulence-reducing nose cone (B&K UA 0386), suspended approximately 10 cm downstream from (but not in the wake of) the test subject. The microphone’s frequency-response curve was flat between 3 Hz and 20kHz. Output from the microphone was routed through an amplifier to a 24 bit recorder sampling at 48 kHz (Sound Devices 702, Reedsburg, WI, USA). The microphone was calibrated with a B&K 4231 sound level calibrator, and SPL levels (ref 20 µPa) at 1 meter from the source were calculated based on the distance from the feather to the microphone (assuming uniform radiation and negligible reverberation).

To mount the feathers, an insect pin dipped into cyanoacrylate glue was inserted into the calamus. The opposite end of the pin was inserted into a pin vise at the end of a sting projecting down from the top of the tunnel. With the airflow turned on, the feather’s orientation could be finely adjusted outside of the tunnel by rotating the sting and by raising and lowering the feather. For the 2-feather experiments (see below), a second, identical sting was positioned in the tunnel, directly downstream from the first sting. The distance between the two stings could be adjusted using a custom-built stage. The supplemental online video shows part of this setup, including the types of movements possible.

Possible pinning effects

How an object is mounted (pinned in this case) is a boundary condition that could influence its mechanical resonant frequencies and therefore the vibratory modes expressed in airflow. Therefore, the insect pin and pin vise to which the feathers were attached may influence how the feathers fluttered, both by adding additional vibratory modes (due to the resonant frequencies of the pin and pin vise), and by affecting damping (i.e., damping provided by the pin and pin vise is likely dissimilar to the damping naturally experienced by a rectrix attached to the bird’s pygostyle, rectricial musculature, and in contact with neighboring feathers). The insect pin and pin vise tended to have a resonant frequency of < 1 kHz, which was occasionally present and recorded as an additional vibratory peak by the SDLV. In some feather orientations, vibratory modes (< 1 kHz) that included large amplitude vibrations (>0.5 mm) of the pins were observed;

these were clearly artifactual and were not measured. Beyond these feather/pin modes, there is no indication that the pinning geometry had a large influence on the sounds produced by the feathers. Individual feathers will produce approximately the same tone (frequency) when non-vibrating parts of the feather are trimmed away (*6,8*), or if the feather is mounted in different ways (e.g. various types of pins, or by gluing a pin to the rachis rather than the calamus), or even if it is aerodynamically activated by different flow regimes, e.g. placed in a jet or air or a wind tunnel (*6,8*). It is possible that the pin influenced the exact shape of the mode of vibration expressed in the feather, but this possible effect is not expected to affect any of the results presented here, as detailed analyses of the mode shape were precluded by the velocity limits inherent to the SLDV measurements (see below).

High-speed video

High speed video of feathers in the wind tunnel was obtained with a monochrome Phantom Miro EX4 (Vision Research, Wayne, NJ, USA) filming at 20,000 or 23,121 fps at a resolution of 160 × 120 pixels. The feather was backlit with a 300W incandescent light.

Scanning Doppler Laser Vibrometer

Vibration velocities were measured using a Scanning Laser Doppler Vibrometer (SLDV) (PSV-I-400 LR, Polytec Inc, Irvine, CA, USA) with an OFV-505 scan head fitted with a close-up attachment (PSV-A-410). This allowed the laser spot (~1 µm diameter) to be positioned with an accuracy of ~5 µm. A single scan consisted of approximately 100 points in a hexagonal array fitted to the shape of each individual feather. Measurements across the feather were taken without readjusting feather position. Each point was measured 3 times at a sampling frequency of 51.2 kHz and averaged. In order to reconstruct the topography of feather motion, a second laser Doppler vibrometer (PDV 100, Polytec Inc, Irvine, CA, USA) was used a reference. The reference point was positioned at the base of each feather. Both the SLDV and reference vibrometers were mounted on a tripod (Manfrotto 058B) and orientated so that the scan head was orientated perpendicular to the feather surface. Both laser points were focused through the acrylic wall of the tunnel on the feather surface. Sound decibel level was monitored through each scan to ensure that the acoustic behavior remained constant. Scans in which the feather behavior abruptly changed were removed from the analysis. Microphone recordings were recorded simultaneously with the vibrometer recordings.

The SLDV measures a maximum velocity of 3 m s^{-1}. At higher airspeeds all feathers had regions that vibrated well above this maximum velocity. Calculation of average vibration power spectra at these higher airspeeds only include parts of the feather that were below this maximum velocity, hence reported feather vibration amplitudes ($V_{feather}$) at these airspeeds are conservative and underestimated the average velocity amplitude of the feather.

This systematic bias was present in range III (see Fig. 1A) of all feathers, and affects the data we present in three ways. First, as mentioned above, it prevented a quantitative analysis of the mode shape of feather vibrations, which is why we qualitatively divided vibrations into four types (Fig. 1H). Second, it is likely the reason $V_{feather}$ does not increase with U_{air} in range III of the example presented in Fig. 1A. Likewise it may be the reason why the $V_{feather}$-dB sound relationship was negative in a few of the feathers measured (see faint lines with negative slope in Fig. 1C), as these feathers tended to exhibit high values of $V_{feather}$ that resulted in especially limited SLDV scans. Third, this limitation also means that in Fig. 1C, no points fall above 3 m s^{-1}, whereas high-speed video demonstrates that some of the feathers measured must well exceed this velocity. For example in the supplemental movie S1 (0:43 – 0:50), a Broad-tailed Hummingbird (*Selasphorus platycercus*) R2 exhibits tip flutter in which the tip has an amplitude of > 2 cm at a frequency of ~0.5 kHz, thus the tip of the feather has an average velocity of >20 m s^{-1}, nearly an order of magnitude higher than the SLDV can measure. $V_{feather}$ as presented in Fig. 1 cannot be quantified from video. This technological limitation of the SLDV prevented more refined analyses, but it did not affect any of the conclusions presented in this paper.

Experiments

The acoustic behavior and flutter of 31 feathers[2] was explored over a range of airspeeds at a single orientation. Prior to the experiment, a given feather was placed at a relatively high airflow and adjusted until an orientation in which the feather produced sound was found. SLDV scans were then taken over a range of speeds; airspeed was then changed with minimal adjustment of the feather's orientation. The exact orientation of a feather could not be held exactly constant, as aerodynamic loading of a feather caused it to bend (i.e. slightly changing orientation), and this effect varied with airspeed.

Anna's Hummingbird rectrix experiments in wind tunnel

For a demonstration of this experiment, see the supplemental online video. Eight sets (R5 plus R4) of male Anna's Hummingbird rectrices were tested at an airspeed of 24.4 or 26.8 m s^{-1}, (these speeds are similar to the speed that species flies at the bottom of its dive: Fig 1B, ref *21*). In all cases the feathers were matched pairs taken from the same side of the bird. First, R5 was oriented such that it produced the loudest sound possible (as determined by ear), followed by SLDV scan and microphone measurement. The reference laser was positioned at the base of R5. Second, without changing airspeed of the tunnel, an Anna's R4 (that had previously been in the wind tunnel but not near R5) was moved immediately behind R5 and its position adjusted until the largest possible increase in sound was attained. Once positioned a second SLDV scan and microphone measurement was taken. Next the wind tunnel was stopped and the R4 was replaced with a flat plate (0.1mm aluminum shim stock) cut in the shape of R4 (control); the orientation of R5 was not adjusted. Then tunnel was then re-started and R4 was positioned behind R5 and adjusted to find the position at which the sound was the loudest. A third SLDV scan and microphone measurement was taken. Finally, without changing the airflow or adjusting R5, the

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[2] Except the data shown in Figure 1B, which were collected from 3 additional Anna's Hummingbird R5s.

flat plate was removed and a SLDV scan and microphone measurement of R5 taken. No differences in loudness were observed between the first R5-alone measurement and the last R5-alone measurement ($P = 0.82$, paired t-test).

Field experiments on Allen's Hummingbirds

Male Allen's Hummingbirds (*Selasphorus sasin*) were studied on their breeding territories at the 'Albany Bulb' portion of the East Shore State Park, Albany, CA, in spring of 2005 - 2009. All procedures were approved by the UC Berkeley Animal Care and Use Committee, and performed under the relevant park and government permits to film, capture and band hummingbirds. The experiments performed were similar to previously reported field experiments presented for the Anna's, Costa's, Black-chinned, and Calliope Hummingbirds (*Calypte anna, C. costae, Archilochus alexandri,* and *Stellula calliope*) (6-*9*). Specifically, individual male Allen's Hummingbirds were sound-recorded as they performed courtship dives on their territories. They were then captured and underwent a manipulation of a specific pair of tail-feathers, and were marked and banded for re-identification. The manipulated bird was then sound-recorded again to determine the effects of manipulating those specific tail-feathers.

Allen's Hummingbird rectrix experiments in wind tunnel

Male Allen's Hummingbird (*Selasphorus sasin*) rectrices were tested at an airspeed of 22.8 m s^{-1}. R4 produced sounds ranging from ~7 to 9 kHz that scaled with airspeed, whereas R3 produced sound at ~2 kHz with less variation with airspeed. Therefore at some airspeeds the sound R4 produced were similar to R3's 4th harmonic of ~8kHz. At this speed it would be impossible to detect heterodyne interactions. So, the airspeed of 22.8 m s^{-1} was used for the experiment specifically because there was not harmonic matching of R3 by R4 at this speed. Eight sets of R4 and R3 were paired together, although they were often from different individual birds, as most of the feathers were collected from field experiments in which only one feather type was plucked. Both R4 and R3 were mounted in the wind tunnel in an orientation that produced sounds matching sounds made in the wild (R4:7 to 9 kHz; R3 2kHz plus harmonics). In the first treatment, both feathers positioned so that R3 was positioned immediately downstream from R4 in a realistic orientation. A SLDV scan and microphone measurement was taken in this configuration (R3+R4) with the reference laser positioned at the base of R4 Second, without adjusting R4's orientation, R3 was moved away from R4, and an R4-alone SLDV scan and microphone measurement was taken . Third, R3 was positioned back to its initial configuration and a second R3+R4 SLDV scan and microphone measurement taken. Fourth, without touching R3, R4 was moved away. The reference laser was positioned at the base of R3, and a SLDV scan and microphone measurement of R3 alone taken. Fifth, R4 was returned to its position upstream of R3, and a final R3+R4 SLDV scan and microphone measurement taken. Thus, the first, third and fifth treatments were similar, and treatment 2 was compared to treatment 1 and 3, while treatment 4 was compared to treatment 3 and 5.

In all 8 sets, R4 by itself produced tones of 7.5 ± 0.4 kHz whereas R3 alone produced tones of 1.8 ± 0.2 kHz and harmonics. No tones resembling the heterodyne frequencies were detected in the solo feather trials of any of the sets. When one feather (either R3 or R4) was producing sound and had the other placed in proximity, heterodyne frequencies were produced in all 8 sets. Both the sum and difference heterodyne frequencies (i.e., $f_1 \pm f_2$) were observed in the vibrations of the feathers (as measured with SLDV). In terms of sound recorded by the microphone, all 8 sets produced the sum heterodyne frequency ($f_1 + f_2$) of 9.27 ± 0.35 kHz, but the difference heterodyne frequency ($f_1 - f_2$) was only audible above the wind tunnel background sound in 4 of the 8 sets.

Table S1. Evidence whether the 31 feathers tested in this study produce sounds in the courtship display. All feathers are from adult males.

Species	Feather (n)	Uses feather to make sound in display?	Evidence
Calypte anna	R5 (2)	Yes	Experimental manipulations of wild birds
Calypte costae	R5 (1)	Yes	Experimental manipulations of wild birds
Calypte costae	R4 (1)	No[a]	Experimental manipulations of wild birds
Archilochus alexandri	R5 (2)	Yes	Experimental manipulations of wild birds
Archilochus alexandri	R4 (1)	No[a]	Experimental manipulations of wild birds
Archilochus colubris	R5 (1)	Yes	Match between wind tunnel sound and sound recordings of courtship displays; homology with *A. alexandri*
Selasphorus sasin	R5 (1)	No[a]	Experimental manipulations of wild birds
S. sasin	R4 (2)	Yes	Experimental manipulations of wild birds
S. sasin	R3 (1)	Yes	Experimental manipulations of wild birds
S. sasin	R2 (1)	No[a]	Experimental manipulations of wild birds
S. rufus	R5 (1)	No	Lack of match between sound produced in wind tunnel and sound during courtship display
S. rufus	R4 (1)	No	Lack of match between sound produced in wind tunnel and sound during courtship display
S. rufus	R3 (1)	Yes[b]	Match between sound produced in wind tunnel and sound during courtship display
S. rufus	R2 (1)	Yes[b]	Match between wind tunnel sound and sound recordings of courtship displays; homology with other *Selasphorus*
S. platycercus	R2 (1)	Yes	Match between wind tunnel sound and sound recordings of courtship displays; homology with other *Selasphorus*
S. scintilla	R2 (1)	Yes[b]	Match between wind tunnel sound and sound recordings of courtship displays; homology with other *Selasphorus*
S. flammula	R2 (1)	Yes[b]	Match between wind tunnel sound and sound recordings of courtship displays; homology with other *Selasphorus*
S. flammula	R3 (1)	Yes[b]	Match between sound produced in wind tunnel and sound during courtship display
Selasphorus [Stellula] calliope	R3 (1)	Yes	Experimental manipulations of wild birds
S. calliope	R2 (1)	Yes	Experimental manipulations of wild birds
S. calliope	R1 (1)	Yes	Experimental manipulations of wild birds
Chaetocercus mulsant	R4 (2)	Yes	Match between wind tunnel sound and sound recordings of courtship displays. See Fig. S1.
C. mulsant	R3 (1)	No[a]	Lack of match between wind tunnel sound and sound recordings of courtship displays
Calliphlox bryantae	R5 (1)	Yes[b]	Likely homology with *Calliphlox mitchellii* (no sound recordings of displays available)
C. mitchellii	R5 (1)	Yes[b]	Match between wind tunnel sound and sound recordings of courtship displays
C. mitchellii	R4 (1)	Yes[b]	Match between wind tunnel sound and sound recordings of courtship displays
C. e. evelynae	R5 (1)	Yes	Match between wind tunnel sound and sound recordings of courtship displays. See Fig. S1.

[a] This diagnosis disregards the possibility that this feather is involved in feather-feather interactions (e.g. Figs 3 - 4).

[b] diagnosis is not definitive because manipulations have not been performed on wild birds; a neighboring feather may instead be primarily responsible for sound production. See text above for further explanation.

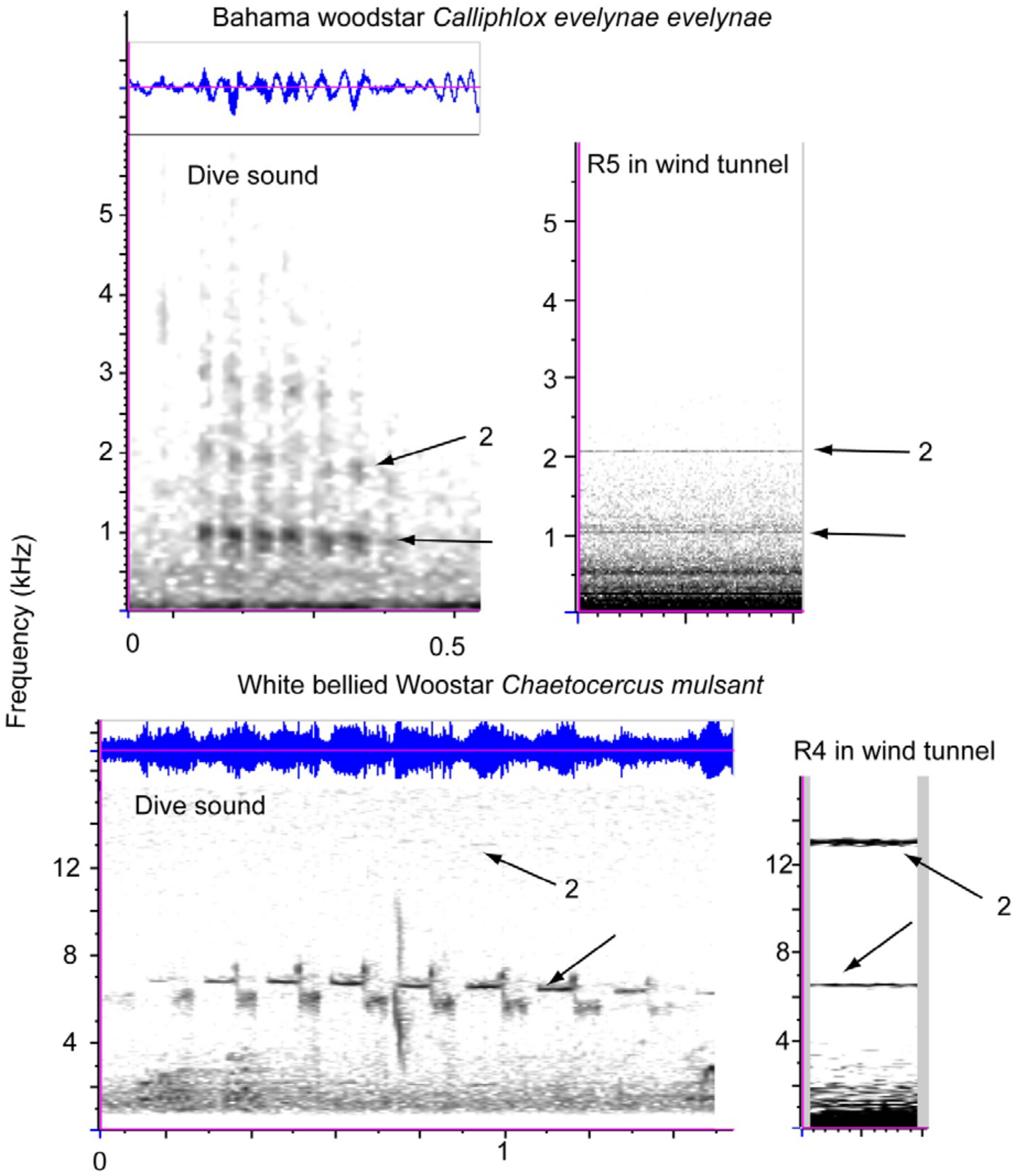

Figure S1. Examples of the dive-sounds of two hummingbird species, *Calliphlox evelynae evelynae* and *Chaetocercus mulsant* (left), and the sounds produced by their tail-feathers in the wind tunnel (right). Arrows points to fundamental frequency and 2nd harmonic in each. Harmonics are more apparent in the wind tunnel recordings than in the dive sounds. This is likely due to the distance between the microphone and the feather or bird in the two setups. The dive sounds were recorded meters away from the rapidly moving bird (with a shotgun microphone), thus greater attenuation of high frequencies is expected in comparison to the wind tunnel experiments, in which the microphone is ~10 cm from the feather.