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Complex coevolution of wing, tail, and vocal sounds of courting male bee hummingbirds

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Phenotypic characters with a complex physical basis may have a correspondingly complex evolutionary history. Males in the "bee" hummingbird clade court females with sound from tail-feathers, which flutter during display dives. On a phylogeny of 35 species, flutter sound frequency evolves as a gradual, continuous character on most branches. But on at least six internal branches fall two types of major, saltational changes: mode of flutter changes, or the feather that is the sound source changes, causing frequency to jump from one discrete value to another. In addition to their tail "instruments," males also court females with sound from their syrinx and wing feathers, and may transfer or switch instruments over evolutionary time. In support of this, we found a negative phylogenetic correlation between presence of wing trills and singing. We hypothesize this transference occurs because wing trills and vocal songs serve similar functions and are thus redundant. There are also three independent origins of self-convergence of multiple signals, in which the same species produces both a vocal (sung) frequency sweep, and a highly similar nonvocal sound. Moreover, production of vocal, learned song has been lost repeatedly. Male bee hummingbirds court females with a diverse, coevolving array of acoustic traits.

KEY WORDS: **Biomechanics, dynamical system, flight, locomotion induced sound, rectrix, remix, sonation, Trochilidae, wind tunnel.**

Multiple clades, including orthopterans, spiders, anurans, mammals, birds, and fish, have evolved to use sound and vibration to communicate. The physical acoustic mechanisms that animals employ to produce sound include many internal and external anatomical structures (Ewing 1989; Fletcher 1992; Patek and Oakley 2003). For instance, birds are famous for their capacity to vocalize with an internal syrinx, but many also produce sounds nonvocally such as with the wings or tail (Prum 1998; Clark and Prum 2015). The physical and neural mechanisms underlying sound production both promote and constrain acoustic diversity (Nowicki et al. 1992). For example, entire clades of birds share certain vocal features, and hence sound similar (Farnsworth and Lovette 2008; Miller and Baker 2009). Thus, the mechanistic basis of sound production both shapes and

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constrains evolution of the acoustic structure of communication sounds.

Darwin (1871) used the term *instrumental music* to describe the nonvocal sounds produced by birds with their feathers during displays. This metaphor of a musical instrument is apt when considering how these nonvocal sounds may evolve. Just as a musical instrument's sound is a product of both its structure and the style (and skill) with which it is played, animal sounds are likewise the result of an interaction between the animal's morphology and behavior (Nowicki et al. 1992; Clark 2016). Acoustic structure of the sounds they produce may thus evolve through either modification of the morphology of the instrument itself, or by changes in the behavior by which it is played (Prum 1998). For example, interspecific diversity in jumping spider vibratory songs arises through both changes in morphology and complex behavioral sequences (Elias et al. 2012), while kangaroo rats (*Dipodomys* spp.) produce species-specific patterns of foot drumming (Randall 2001). Here, we study the evolution of hummingbird tail-feathers and the sounds they make during courtship displays in the "bee" hummingbirds (Mellisugini).

We first examine how these tail sounds evolve in response to changes in morphology. Recent experiments have revealed several properties of the mechanism that produces these sounds: aeroelastic flutter of feathers (described below). As the phenotype is integrated and hierarchical (West-Eberhard 2003), physical processes at lower levels of integration (e.g., mechanics of feathers flutter) enable and constrain emergent phenotypic characters at higher levels of integration (i.e., the sounds they produce). Since organisms obey the laws of physics at every evolutionary step (Alexander 1985), we develop a model of character evolution that is fully consistent with the physical principles underlying flutter, which has a complex dynamical basis.

Flutter is also influenced by kinematics (behavior) of an animal's display, which may also evolve in response to mate choice, producing effects on sound production. A parameter of flutter is U^*, the minimum airspeed required for a feather to flutter and produce sounds (Clark and Feo 2008). In Anna's Hummingbird, U^* of its outer tail feather is greater than the top speed the birds can fly in level flight (Clark and Dudley 2009) but below the speeds attained during the display dive (Pearson 1960; Clark 2009). This means that the behavior of diving is essential to produce the tail sound in this species (Clark and Feo 2008). Therefore, as a test of how behavior could drive display evolution, we hypothesized that dive height (a proxy for dive speed) correlates with U^* across the bee clade.

We then examine two related evolutionary patterns: how hummingbirds "switch instruments" between vocal, wing, and tail sound sources. In this regard, the transfer hypothesis posits that elaborate ornamentation may transfer between different ornament types, either among traits of the same sensory modality (within-mode transference) or between different sensory modalities (between-mode transference). Evidence for the transfer hypothesis constitutes finding a negative phylogenetic correlation between characters of the two types. Most tests for transference are of between-mode transference between visual and acoustic characters. However, empirical support for this idea is mixed. For example, while Badyaev et al. (2002) found a negative correlation between song and plumage traits in cardueline finches, by contrast, Mason et al. (2014) found no evidence of concerted evolution of acoustic and plumage traits in tanagers. Within-mode transference has been reported for elaborate visual signals, which may transfer from the plumage to the bower decorations of bowerbirds (Gilliard 1956; Endler et al. 2005), although this interpretation of the bowerbird data is disputed (Borgia et al. 2007; Endler 2007). Here, we test whether transference occurs between acoustic characters of different types. While previous accounts have suggested that transference between vocal and nonvocal acoustic characters has occurred in a variety of birds (Prum 1998)—species such as *Smithornis* broadbills or Ruffed Grouse (*Bonasa umbellus*) have highly developed sonations and produce few vocalizations (Prum 1998; Garcia et al. 2012; Clark et al. 2016)—here we present the first tests of transference between acoustic characters in an explicitly phylogenetic context.

Another pattern of interest is whether acoustic similarity between vocal and nonvocal sounds has evolved nonrandomly across the entire "bee" hummingbird clade, as a form of within-species convergent evolution between two independent components of the phenotype (Clark and Feo 2010). This pattern was previously demonstrated in two sister species within the bee hummingbirds, Costa's and Anna's hummingbird (*Calypte costae* and *C. anna*). In these two species, sounds generated by the tail are highly similar in acoustic structure to sounds generated vocally, despite the fact that they are physically produced by two completely different morphological structures. The theoretical basis for why this might occur is not entirely clear: Clark and Feo (2010) suggested that this pattern might be caused by sexual sensory bias, in which preexisting female preferences for one sexual character produced a similar acoustic structure in an unrelated character. Here, we examine whether this pattern is repeated across the entire bee hummingbird clade.

BACKGROUND: FOCAL CLADE

The "bee" hummingbirds are a monophyletic clade of approximately 37 species that are found in North, Central, and South America, and the Caribbean (McGuire et al. 2009; McGuire et al. 2014). All species in this clade are polygynous and most lek. Males spend the breeding season occupying courtship territories, waiting for females to visit. When a female does visit, a male performs multiple flight displays, one of which is a dive, in which he rises up, then descends at high speed, swooping past the

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female while abruptly spreading his tail one or more times (Clark 2009). During this dive, when the tail is spread, males produce sound with one or more of their five tail-feathers, called rectrices, numbered R1, the innermost, through R5, the outermost (Clark et al. 2011a). The principle physical mechanism by which these feathers produce sound is aeroelastic flutter, or flutter for short (Clark et al. 2011a, 2013b). Flutter is an aerodynamically driven stable vibration of a feather at an audible frequency. Lab experiments on flutter have revealed several physical properties, which we next synthesize into inferences that inform and constrain how sounds generated by flutter may evolve.

PROPERTIES OF FLUTTER

The properties of flutter influence how tail sounds evolve. First we consider how single feathers flutter. For flutter to occur, airflow over a feather must exceed a critical airspeed, U^*, which for tail-feathers is set by the speed the bird reaches during the dive. Wind tunnel experiments reveal that feathers are complex dynamical systems that may flutter by more than one possible mode, that is, a given feather may vibrate stably in more than one discrete way. Clark et al. (2011a) classified modes of hummingbird tail feathers into four types. Two prevalent modes of vibration are of the feather's tip, or the trailing vane, while two rarer mode types include a torsional (twisting) mode observed in *Selasphorus calliope* feathers, and *Chaetocercus mulsant* tail-feathers exhibit a whole-feather bending "mode" (Clark 2011; Clark et al. 2011a; Clark and Prum 2015). Physically, whether and how one of these modes of flutter is expressed is set by state (independent) variables that include the speed of the airflow, the feather's geometric and structural properties (size, shape, stiffness, orientation, and static bending), and proximity to other feathers (Clark 2011; Clark et al. 2011a; 2013a; 2013b; Clark 2014). A change in any of these independent variables can have either a linear (continuous) or a nonlinear (discontinuous) effect in how the feather flutters (Clark 2014). As an example of a linear response, a slight change in feather orientation or airspeed often causes a commensurate slight change in pitch of sound, or even, no change in pitch at all. But these domains of feather flutter performance space are bounded by thresholds. If a threshold is crossed, a nonlinear response ensues. For instance, in wind tunnel experiments, rotating a feather slightly in certain cases causes the feather to transition, or "jump" from one mode of vibration to a completely different one (Clark et al. 2013a), such as from tip mode of flutter to a trailing vane mode of flutter, in which an entirely different part of the feather flutters, and at an entirely different frequency. Cast in the language of dynamical systems, there are multiple stable states (modes of flutter) within the feather flutter performance space, and abrupt transitions from one stable state to another occurs when a state variable (such as orientation, or feather shape) crosses some threshold value—much as water remains a liquid across a wide range of temperatures, but abruptly transitions to a distinct, solid state when the state variable temperature crosses a threshold, such as 0°C when under standard pressure etc. In the case of feather morphology, although we know empirically that these thresholds exist, we do not know exactly where in parameter space they lie, particularly for ancestral feather morphologies, which do not have shapes that correspond exactly to the extant feathers available for lab experiments.

A fluttering feather also potentially interacts either aerodynamically or structurally with neighboring feathers. When a source feather flutters, it moves the air around it, and this may cause neighboring feathers to vibrate in unison, aerodynamically driven by the motion of the source feather. The effects of these feather–feather interactions include substantial increases in acoustic amplitude, the production of sideband frequencies, or flutter-induced collisions between neighboring feathers (Clark 2011; Clark et al. 2011a; Clark 2014). Neighboring feathers are thus not independent evolutionary characters. Hummingbird species studied thus far use a subset of their tail feathers as the sound source, often just a single feather (Clark 2014). Different species have different feather(s) as the source, thus which feather(s) act as the source must have changed from one feather to another over evolutionary time (Clark 2014). One result of the present study is that individual feathers can be gained (e.g., R5 → R5 + R4) or lost (e.g., R5 + R4 → R5) as sound sources, which we term source addition and source loss, respectively. Entirely changing which feather produces sound (e.g. R5 → R2) we term a *source switch*. Species also vary in mode of flutter, and so we name the inferred change that causes this a *mode switch*. We specifically use the word "switch" to refer to phenomena inferred to occur over evolutionary time, whereas "transition" refers to nonlinear change in sound production observed in lab experiments. But this distinction is subtle, as we will argue in the discussion that the two are tightly coupled, mode switches must and source switches likely are accompanied by nonlinear transitions in frequency.

Put together, the physical properties of flutter of individual feathers, along with the feather–feather interactions, suggest the hypothesis that over evolutionary time, sounds produced by flutter may evolve in multiple, distinct ways. Gradual evolution in feather morphology (size, shape, stiffness) may produce slight, gradual (continuous) evolution of sound frequency; or when continuous evolutionary change of feather morphology crosses a threshold value of a critical variable, then a discrete, discontinuous, saltational change (*sensu* Landis et al. 2013) in frequency can occur.

Methods

SONGS AND COURTSHIP DISPLAYS

We obtained sound recordings, video, or other descriptions of courtship behavior from 35 bee hummingbird taxa, as well as

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data from the literature for six outgroups from different major hummingbird lineages: *Phaethornis longirostris*, *Eulampis jugularis*, *Popelaria spp*., *Ocreatus underwoodii*, *Trochilus polytmus*, and *Eugenes fulgens* (references in Table S1). Data from 33 bee hummingbird species come from 10 years of fieldwork by CJC and collaborators, 16 of which include data from previous publications (Table S1) and another 17 of which are previously unpublished. Whenever possible, we sampled >10 males per species, but in a few cases we only succeeded in recording displays of one or two males. Our own data were supplemented with data from the literature and other sources (Table S1). Behavioral homology was established following Wenzel's (1992) criteria.

We recorded song and displays from males that we found on their courtship territories. Some displays were unelicited, but when possible we elicited displays, such as with a live female in a cage, or stuffed mount, because eliciting displays enabled better recordings. Nearly all displays described here were highly stereotypic; the species evidencing the least stereotypy in display was *Thaumastura cora*, as described in detail in Clark et al. (2013c). Sound recordings and videos were both used to reconstruct display kinematics, following a previously established approach (Clark 2009, 2016).

EVOLUTION OF TAIL SOUNDS

All flight feathers can produce sounds when tested in a wind tunnel, but many of these sounds are spurious and unrelated to the behavioral repertoire of the animal (Clark et al. 2013b; Clark and Prum 2015). So, we first compared the dive sounds the birds produced to the sounds feathers produced in a wind tunnel, to establish which feathers could produce the dive sound (Clark et al. 2011a). The relevant wind tunnel data come from previous articles on the mechanics of flutter (Clark et al. 2011a, 2013a, b), and tests of a few additional feathers from species not sampled previously. We scored each tail-feather as producing sound, or not, according to the four criteria described in Clark and Prum (2015). We then categorized these feathers according to their mode of flutter (as in Clark et al. 2011a, 2013a). Finally, we examined the frequency-velocity profile, which describes how sound pitch varies with airspeed; and U^*. For taxa for which we were unable to obtain feather samples for wind tunnel tests (particularly Woodstars), we made the conservative assumption that similarly shaped feathers in closely related species produce sound via the same feather and mode of flutter.

PHYLOGENY

We constructed a molecular phylogeny of bee hummingbird species and subspecies known to vary in male tail morphology, except missing *Chaetocercus heliodor heliodor*, *C. astreans*, and *Selasphorus ardens*, including outgroup species from six of the major hummingbird clades. The tree included DNA sequence data from previously published hummingbird phylogenies (McGuire et al. 2007, 2014), supplemented with DNA for eight additional bee species. For most specimens, we obtained the same six genes included in McGuire et al. (2014), which includes the mitochondrial ND2 and ND4 genes, and the nuclear loci *beta-fibrinogen intron 7* (FGB), *adenylate kinase intron 5* (AK1), a segment of *ornithine decarboxylase* extending from the end of exon 6 to the beginning of exon 8 (ODC), and a segment of the Z-linked *muscle skeletal receptor tyrosine* gene including parts of exons 4 and 5 and the intervening intron (MUSK). We obtained sequences from nonstandard specimens for three species. For *Mellisuga helenae*, we obtained a nearly complete mitochondrial ND2 gene sequence from a museum specimen toepad (MCZ 80780). For one individual *Chaetocercus berlepschi* and four individuals of *Calothorax pulcher* we sequenced our six target genes from feather samples. The new DNA sequence data were deposited in GenBank (Accession numbers MG754240-MG754347).

DNA was isolated using Qiagen DNeasy extraction kits following standard protocols. Amplification of target sequences and cycle sequencing was performed using the polymerase chain reaction with the primer sets identified in McGuire et al. (2014). We performed partitioned Bayesian phylogenetic analyses using the program MrBayes version 3.2 under the same models and partitioning scheme used by McGuire et al. (2014). Briefly, this included 11 separate partitions, including a partition for the mitochondrial tRNAs flanking the ND2 and ND4 genes, separate partitions for the first, second, and third codon positions of both ND2 and ND4, and separate partitions for the FGB, AK1, ODC, and MUSK genes. Similar analyses produced with RA × ML (Stamatakis 2014) generated the same phylogenetic hypothesis.

Using the resulting phylogenetic estimate, we then generated an ultrametric tree by applying the penalized likelihood approach of Sanderson (2002), implemented in ape (Paradis 2012) to our MrBayes tree, with lambda = 0.1. We reconstructed character states on this tree with a mixture of parsimony and maximum likelihood (ML), using the program Mesquite 3.03 (Maddison and Maddison 2015). We assumed gains and losses to be equally likely, since we had no theoretical basis for considering any of the phenotypic characters to be more or less likely to be gained than lost. Parsimony is used to present ancestral character state reconstructions in the figures, and to present the minimum number of character state changes within the tree. We considered reconstructions with accelerated and delayed character transformations (Acctran vs Deltran) to be equivalent (Agnarsson and Miller 2008), and therefore we present gains and losses as ranges. We calculated a phylogenetic generalized least squares (PGLS) regression using the *ape* library in R version 3.4.1. Using the function corPagel, we estimated Pagel's λ for individual characters, with 999 randomizations. Species with a character absent (such

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Table 1. Summary of OUwie model rankings (sorted by AICc) of evolution of dive sound frequency.

Model	Log likelihood	AIC	AICc	Model	Parameters	Weights	Branches/clades with alternate rate
BMS.a4	–63.8	133.6	134.6	BMS	3	0.72	Clade 1 (including V_1)
BMS.a3	–63.4	134.8	136.6	BMS	4	0.28	V_1, Clade 1 (separate)
BMS.a2	–72.8	151.6	152.6	BMS	3	0.0001	V_1
BM1.null	–74.3	152.6	153.1	BM1	2	0.0001	None
BMS.a1	–74.3	154.6	155.6	BMS	3	0.0000	V_1–V_7 (1 rate)
BMS.a5	–67.3	152.5	162.5	BMS	9	0.0000	V_1–V_7 (each separate rate)

as tail sound frequency) were coded as NA rather than 0, and thus dropped from the PGLS. Pagel's λ is an index of the phylogenetic correlation of variation a continuous character state with $\lambda = 1$ is complete phylogenetic correlation.

Outgroups for which we had phenotypic data were added to the tree per the phylogenetic position reported in McGuire et al. (2014). Correlations between discrete characters were tested with Pagel's (1994) test, with a *P*-value calculated estimated from 1000 simulations, and in Bayestraits version 2 (Pagel and Meade 2006), using the discrete function, MCMC with 1,010,000 iterations. Bayes factors were calculated from the harmonic mean of the log likelihood. Taxa missing data were dropped from the relevant statistical test, but for graphical consistency, these taxa were not dropped from the figures. All of the tests with statistical significance had "replicated codistribution," and so single influential evolutionary events are not of concern here (Maddison and FitzJohn 2015).

Finally, our results indicated that certain branches contained source and mode switches (labeled V_1–V_7 in Fig. 6), indicating that these branches have a different rate of evolution (since a discrete change in value can be modeled as an infinitely high rate of change). We tested whether differences in rate of evolution were detectable, using an information theoretic approach implemented in OUwie (Beaulieu et al. 2012), an R package that allows one to model and rank hypothesis regarding how multirate Brownian motion regimes may evolve on a phylogeny. For these tests we first pruned taxa from the tree that did not produce a dive-sound or were missing data. Next a 3-way polytomy within the woodstars was resolved by placing *Eulidia* sister to *Chaetocercus*. This produced a tree topology with one fewer inferred mode switches than the two other possible topologies, and is thus conservative for this analysis.

For analyses with OUwie, we used the same ultrametric phylogeny to compare alternative Brownian motion models with different rates of trait evolution across the tree (models listed in Table 1). We compared a null model where all branches were assumed to evolve under a single-rate Brownian motion regime (model = BM1), to four multirate Brownian alternatives (model = BMS), selected on account of patterns suggested by results of a McPeek (1995) test (data not shown). Alternative 1 was a 2-rate model in which branches V_1–V_7 (Fig. 6) evolve under one rate, while all others have another (BMS.a1). Alternative 2 is a 2-rate model in which branch V_1 (representing the *Eulidia-Chaetocercus* (hereafter "Clade 1") stem lineage) has one rate and all other branches have another rate (BMS.a2). Alternative 3 (BMS.a3) is a 3-rate model that gives V_1 a unique rate, the entire clade 1 a unique rate (corresponding to dynamic-bending modes of flutter in this clade), and all other branches another unique rate. Alternative 4 (BMS.a4) is a 2-rate model that gives V_1 and clade 1 a unique rate, and all other braches another rate. The root.station parameter was set to FALSE as per the OUwie documentation recommendation for BMS models, and diagnostics were turned on to check that all eigenvalues were positive, indicating that parameter estimates were reliable for each ML search. We ran the models with both log-transformed data and nontransformed data. The log-transformed data are not presented, because both versions generated similar model rankings and thus does not affect any of the conclusions we present.

Results

Phylogenetic analysis with MrBayes and RAxML returned a newly detailed, well-resolved and well-supported phylogenetic hypotheses of the bee hummingbirds, including two large subclades as indicated in the figures: the Woodstars, a clade found mostly in South America (two are in Central America), and the North American Bees, found in North and Central America, and the Caribbean. We have phenotypic data for 15 of the woodstars and 20 of the North American Bee Hummingbirds. The full molecular phylogeny is presented in the supplemental material (Fig. S1).

EVOLUTION OF DIVING, SINGING, AND WING TRILLS

Dive displays are high-speed flights past the female, in which the male ascends and then descends with the aid of gravity, swooping past the female. The flight trajectory and discrete behavioral components of dives are highly variable among species (Fig. 1). Most hummingbirds outside the bee clade, including five of six

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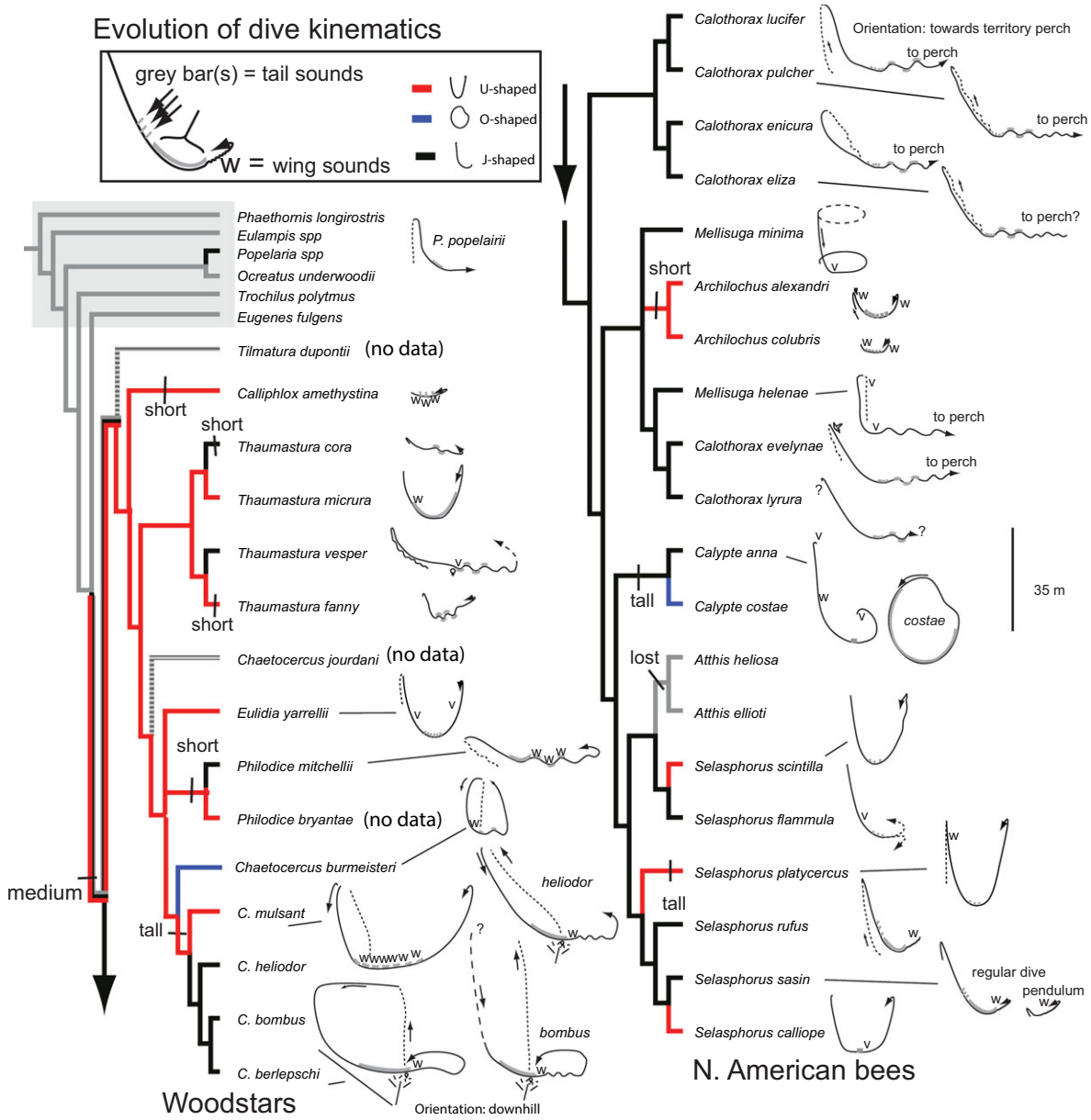

Figure 1. Evolution of display dive trajectory in bee hummingbirds. Gray box indicates six outgroups representing most of the major hummingbird lineages, for which data on displays are available. Outside of bees, only coquettes (*Popelaria* spp. and close relatives) are known to have display behaviors that closely resemble dives of bee hummingbirds. Line drawings of dive trajectories are to scale; birds dive from left to right. Black box: w = wing sound, gray bands indicate approximate location, and duration of tail-sound; v = vocalization. Question marks indicate incompletely observed kinematics. Dive height evolution: 0–12 m = short, 15–25 m = medium, >25 m = tall. "Medium" is the ancestral character state.

outgroups sampled, lack dives or other clearly homologous behaviors. Thus, diving has likely evolved in the most recent common ancestor of the bee clade. Dives are present in 31 species, are absent in the two *Atthis* spp. (one phylogenetic loss), and unknown (but likely present) in two species. Twenty-nine also produce sounds with the tail during the dive (Fig. 2). Producing sound with the tail also likely evolved in the common ancestor of the bee hummingbirds, and has been lost once or twice in *Mellisuga* spp., which do dive, and was also lost in the nondiving *Atthis* spp. In addition to producing sounds with the tail during the dive, many species also either vocalize, or produce sounds with the wings, during the dive (Fig. 2). In some but not all cases these vocalizations are song, and in some but not all cases these wing sounds are wing trills (significance of this nuance is explained below). A behavioral component of many bee hummingbird dives called *undulating*, in which birds repeatedly maneuver while spreading

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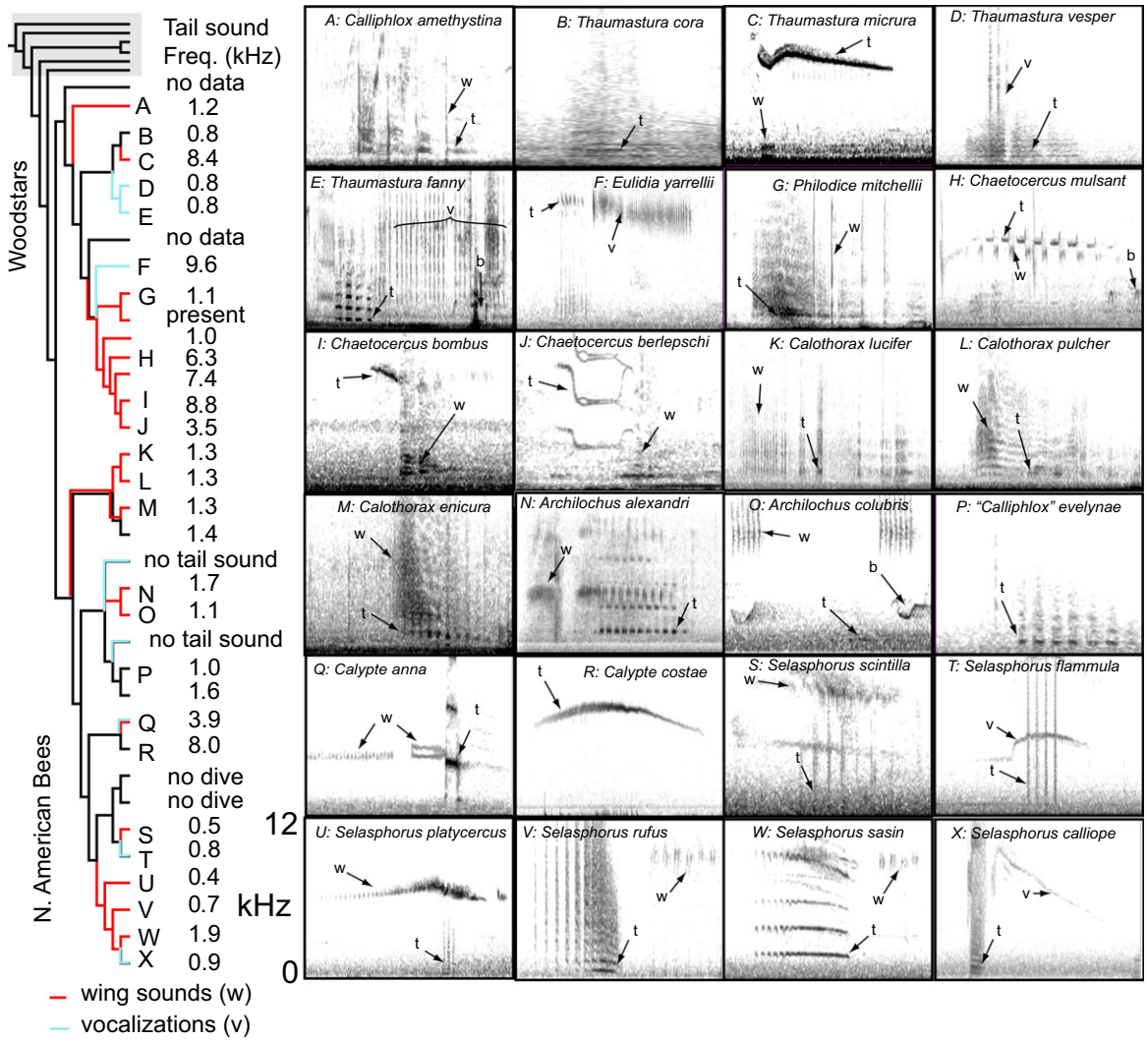

Figure 2. Evolution of dive-sounds within the bee hummingbirds. Left: Wing sounds (red branches), and vocalizations (blue branches) present within the dive. Tree topology as in Figure 1. There is a negative correlation between presence of wing sounds and presence of vocalizations (Pagel test, $P < 0.001$, $n = 33$); only one species, Anna's Hummingbird (Q), does both. Right: Dive sounds of most bees that produce tail-sounds (*t*) during the dive. Many species also produce wing sounds (*w*) or vocalizations (*v*) in conjunction with dives; *b* = background sound (e.g., nontarget bird) and some vocalizations produced between dives are not shown in spectrograms (e.g., Anna's Hummingbird, Q). Spectrograms produced with a 512 (48 kHz) or 1024 sample window (96 kHz); Y axis: 0–12 kHz. Duration (seconds) in lower right. Tail sound frequency on phylogeny refers to the fundamental frequency of sound produced by the tail, which is difficult to see in some spectrograms, either because an integer harmonic is dominant (louder), for example in some *Selasphorus* spp. (S-U), or because the sound is faint (e.g., O, *Archilochus colubris*).

the tail once per maneuver (Fig. 3; see description in Clark et al. 2013c). This behavior is also present in Emeralds, an outgroup to the bees, thus presuming this specific behavior is homologous, this display behavior is ancestral to bees and subsequently lost in some lineages.

Singing was defined as spontaneous, undirected vocalizations uttered by males at least once an hour from their territory; vocalizations given by both sexes, or only directed toward another hummingbird (such as during agonistic interactions between males) were instead calls. Species that sang undirected song also produced directed song toward a target individual (female), and some species sang during flight displays. Singing is widespread in hummingbirds and is ancestral to the bee clade, but only about half the bee species sing. Singing has been lost 6–7 times and re-evolved 1–2 times within the bee clade. Songs are often highly divergent in form among sister taxa, and several unrelated species' songs contain trills or frequency modulated (FM) sweeps. Vocal trills were defined as pulses of sound produced at a rate of between 20 and 137 Hz (corresponding to the definition of wing trill), and frequency modulated sweeps were defined as a tone or

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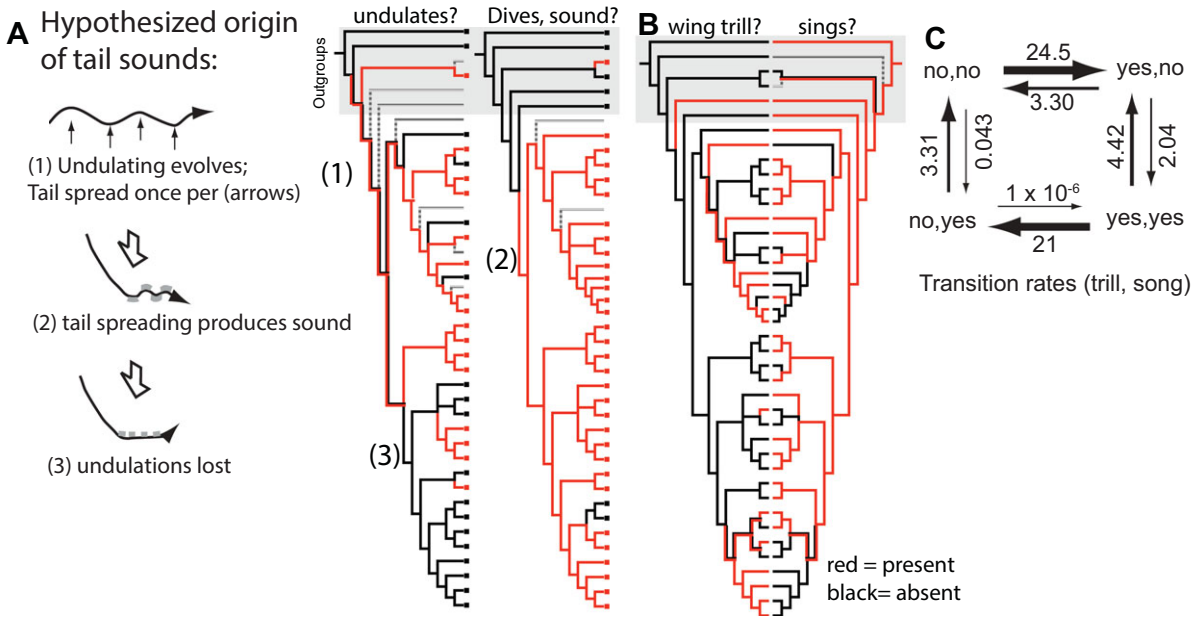

Figure 3. Hypothesized origin of the dive sound (A), and correlated evolution of vocal song and wing trill (B). Characters plotted with parsimony (ML analyses are similar), assuming gains and losses are equally likely; tree topology as in Figure 1. (A) The behavior undulating is widespread in bee dives and is also present in at least one outgroup, implying it precedes evolution of the dive (1). We propose producing sound with the tail evolved out of undulating (2); and then some taxa have lost undulating but have retained spreading the tail repeatedly (3). (B) There is a negative correlation between presence of vocal song and production of a wing trill during ordinary flight (Pagel test, $P = 0.001$). (C) Transition rates for the 8-parameter model, from the Pagel (1994) test; see also Table S3.

a trill that rose or fell in frequency by more than 15% (of any type of sound). The value of 15% fell in a gap in the distribution of the data.

Wing trills are widespread within the bee clade. The definition of wing trill required distinguishing these communication sounds from wing hum, which is produced by all hummingbird species as an inevitable product of flapping wings. Most sound energy of wing hums is in frequencies below 500 Hz. We therefore defined wing trills as pulses of tonal sound produced by the wings during ordinary flight at frequencies >500 Hz, similar to Hunter's definition (Hunter and Picman 2005; Hunter 2008). A few species (e.g., *Eulidia yarrellii*, *Calypte anna*) do not produce these sounds in ordinary flight but do produce them during displays (particularly during the shuttle display, a display not considered here; see Clark et al. 2013c). These species were coded as not producing a trill under the definition presented here. This does not matter: rerunning these analyses under various reasonable permutations of the definition of wing trill yielded similar statistical results, so for brevity these equivalent alternatives are not presented. We also separately scored wing sounds from dive displays as any sound produced by the wings. This category included wing trills, but also snapping (percussive, atonal) sounds that a couple species (*Philodice* spp., *Calliphlox amethystina*) produce instead. Wing trills produced during ordinary flight evolve 6–10 times (with 1–5 losses) in the phylogeny (Fig. 3B). Irrespective of which definition is employed, production of wing trills is a dynamic trait with more than nine state changes on the phylogeny. Out of 35 bee species, two have both a wing trill and song, five have neither, nine taxa have a wing trill and no song, and 18 have a song but lack a wing trill.

COEVOLUTION OF SONGS AND MECHANICAL SOUNDS

Across the bee clade, presence of a wing trill is negatively correlated with presence of singing (Fig. 3B and C; Pagel–Meade discrete test, $P < 0.001$, $n = 35$ bee taxa; Table S2). In a Pagel test (Pagel 1994) of the same traits, the largest parameter value was for the term representing gain of a trill in the presence of song (Table S2). However, a 6-parameter model testing whether wing trill evolution depends on song was not significantly different from the full 8-parameter model ($P > 0.2$; Table S2).

Within dives, presence of dive-vocalizations is negatively correlated with presence of wing sounds (Fig. 2, Pagel test, $P < 0.001$, $n = 33$). These two patterns (wing trill vs song; vocalizations vs wing sounds during dives) are related but not identical, as not all vocalizations produced during the dive are song (e.g., *Selasphorus calliope*, *S. flammula*; Fig. 2T, 2X) and not all wing sounds produced during the dive are wing trill (Fig. 3). The point of examining these overlapping alternatives is to establish that a negative correlation between wing sounds and vocalizations is

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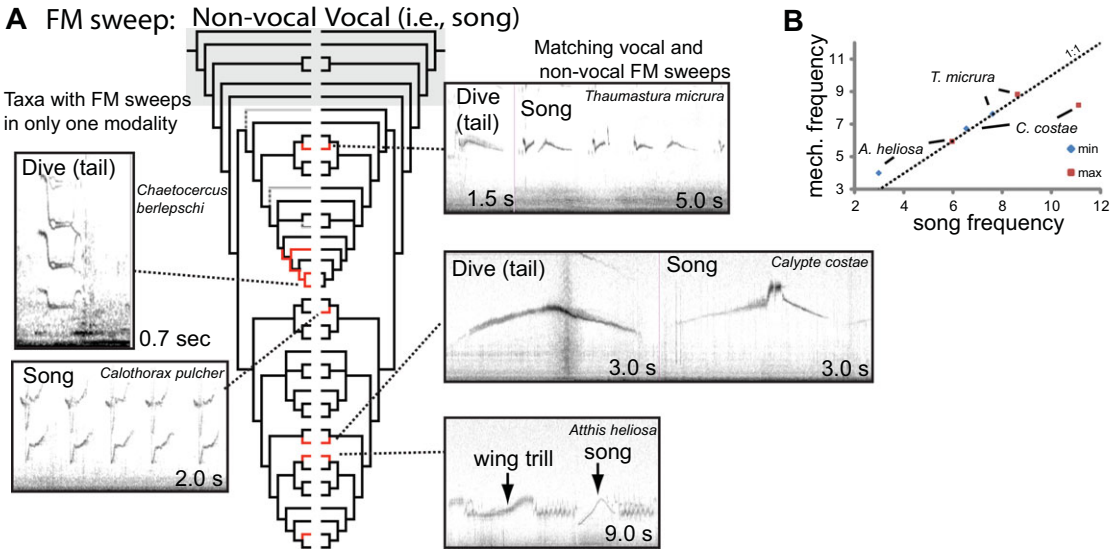

Figure 4. Coevolution of vocal song and nonvocal displays sounds. There is a positive correlation between FM modulated sweeps in song and in nonvocal (displays) (Pagel test, $P = 0.01$). Spectrograms are all 0–16 kHz and FFT windows of 512 samples (48 kHz) or 1024 samples (96 kHz). Duration in seconds indicated in lower right. (B) Minimum and maximum frequency of song and mechanical sounds among the three species with matching mechanical sounds and song. Dashed line represents 1:1 fit. Topology of phylogeny as in Figure 1.

general and does not hinge on one exact definition of wing sound or song.

Acoustic similarity between mechanical sounds and songs has evolved nonrandomly. Presence of frequency modulation (FM) in song is positively correlated with presence of an FM sweep in either dive-sound or shuttle display (Pagel–Meade discrete test, $P = 0.006$; $n = 32$, Fig. 4A). Moreover, in the three species that have both vocal and mechanical FM sweeps, there is also a striking correspondence in frequency range and temporal pattern between the vocalization and mechanical sound (Fig. 4B). By contrast, with respect to trills, contrary to our hypothesis, there is no significant acoustic convergence between vocal trills and wing trills. Vocal trills that generally resemble wing trills in structure have evolved in the songs of three species independently (*Eulidia yarrellii*, *Mellisuga helenae*, *Calypte anna*) but only *C. anna* produces a similar mechanical wing sound, and as a result there is no significant phylogenetic correlation between these two types of sounds (Pagel–Meade discrete test, $n = 28$, $P = 0.3$). Finally, we also detected no correlations between tail-generated dive sounds and other song or wing trill-related characters (null results not shown).

COMPLEX EVOLUTION OF TAIL SOUNDS

Every species in the bee hummingbird clade has both unique tail morphology (Fig. 5) and a unique dive sound, with fundamental frequencies of the tail-sound from 0.35–10.4 kHz (Fig. 2). Pagel's λ of tail sound frequency is 0.24 ($n = 28$ species, species with no tail-sound omitted), implying a low correlation with phylogenetic relatedness. No two species have tails that are completely alike in shape, implying tail morphology has evolved on every branch of the phylogeny. To investigate how sound production by the tail has evolved (Fig. 5), we first mapped on the tree whether each rectrix separately was a sound source. Parsimony implies that in the ancestral bee, R5 alone was the sound source (Fig. 5). R5 as a source has been lost 3–4 times, while the remaining tail-feathers were all added as sources at least once, for a total of 14 character state changes among the five tail feathers (9–13 gains, 1–5 losses). Four of them are concentrated on the branches leading to *Selasphorus sasin* and *S. calliope* (Fig. 5C). Six branches on the phylogeny have either only a gain or only a loss of a feather source and are thus source additions and source losses (Fig. 5B), while another three branches contain both a gain and a loss, that is they are source switches, a complete change in which feather(s) are the source of sound.

This reconstruction shows that when feathers are evolutionarily added as a sound source (source addition), the new feather is usually adjacent to a neighboring feather that also flutters (Fig. 5C). Wind tunnel experiments demonstrated that adjacent feathers can be aerodynamically coupled (Clark et al. 2011a) and tend to flutter at the same frequency. Therefore, during the evolution of source addition, the simplest hypothesis is that frequency evolves gradually—the new feather flutters at the same frequency as its neighbor, as it evolves to become a sound source (Fig. 5). The hypotheses to explain the three source switches within the tree (Fig. 5A) are more complex, so we develop this topic in the Discussion.

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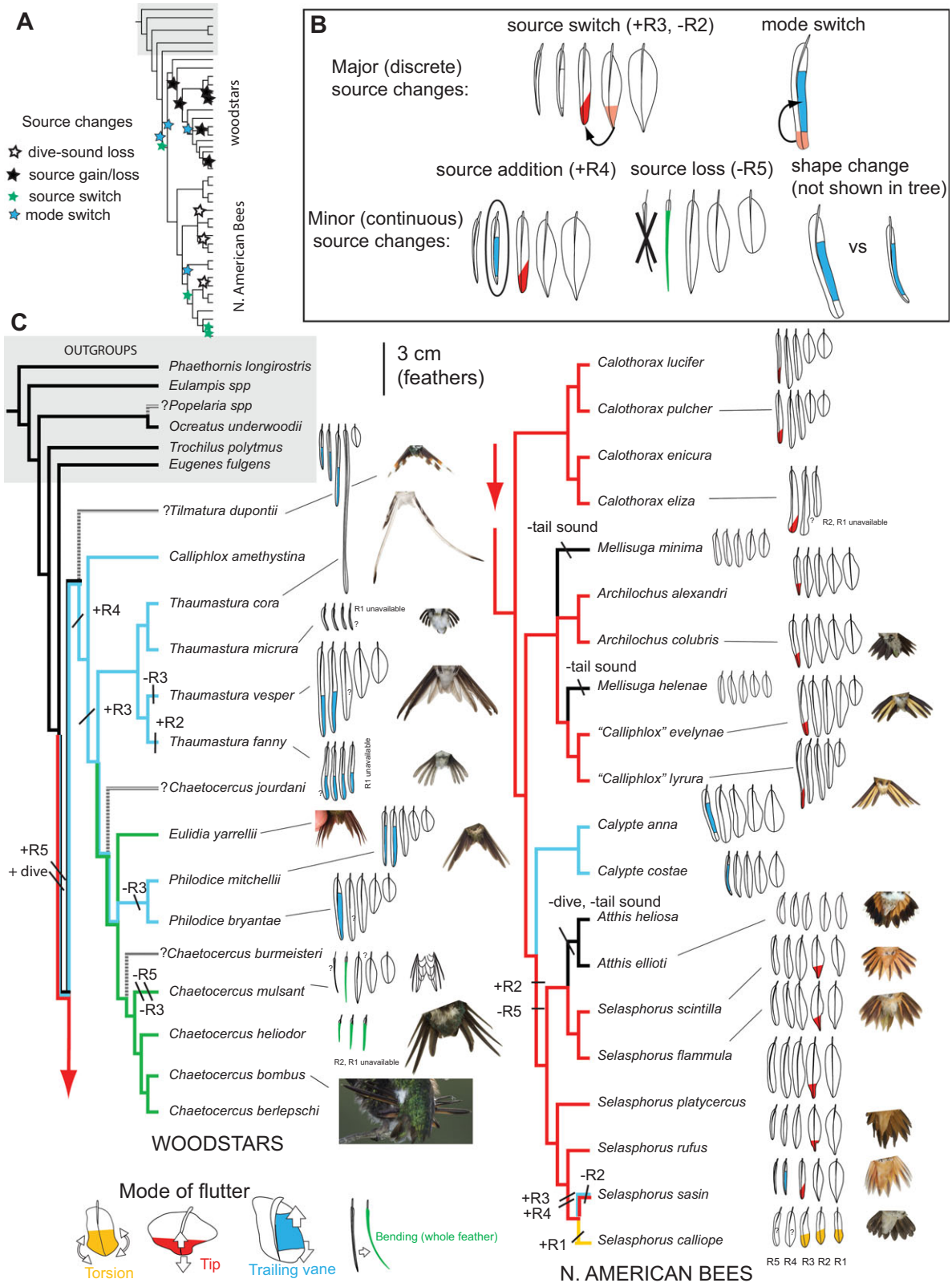

Figure 5. Evolution of tail feather mode of flutter and tail feather sound source in male bee hummingbirds. (A) Summary of the details presented in panels B and C. In this clade there are at least nine major (discrete) changes in sound source occur: four source switches (green stars), including the origin of dive-sounds at the base of the clade, and five mode switches (blue stars). As a result of these nonlinear changes in the source, frequency of sound produced evolves discretely on these branches, jumping from one value to another. There are an additional six source changes that are minor (black stars) and three branches in which tail-sounds are lost altogether (white.

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Mode of flutter also varies across the phylogeny. Physically speaking, flutter is a property of individual feathers, thus each species could in theory have several modes, one per tail-feather. But the available data indicate that in all but one case (*Selasphorus sasin*), neighboring feathers have the same mode of flutter during sound production. Thus in Figure 5C we plot mode as a property of the tail rather than of individual feathers, and *S. sasin* is polymorphic. Tip modes have evolved once and are lost multiple times, trailing vane (TV) modes have evolved 3–4 times, whole feather modes have evolved 1–2 times in the woodstar clade spanning *Eulidia-Chaetocercus*, and torsional flutter has evolved once, in *Selasphorus calliope*. The ancestral character state to the entire clade was either a tip mode, as expressed at the base of the North American Bees; or trailing vane mode per the base of the woodstars.

In total, there are at least six internal branches with source changes (i.e., branches with either a mode switch or source switch; Fig. 4A), along with the base of the entire clade, where, as we argue in the discussion, tail sound frequency evolves in a discontinuous, saltational manner. Isolating each of these branches results in seven subgroups (Fig. 6). Analyses with OUwie reject a null model in which a single rate Brownian motion model can best explain the extant diversity of character states (Table 1). Model a4, in which clade 1 (including the stem lineage; Fig. 6) evolves under a unique rate of trait evolution ($\sigma^2 = 151.89$) and all other branches evolve under another unique rate ($\sigma^2 = 9.69$), received ~70% of the model weight ($dAIC_{A4\text{-null (log)}} = 4.04$, $dAIC_{A4\text{-null (raw)}} = 18.46$). Model a3, a 3-rate model that gives branch V1 a unique rate ($\sigma^2 = 291.41$), the entire clade 1 a unique rate ($\sigma^2 = 5.77$), and all other branches another unique rate ($\sigma^2 = 9.69$), received ~25% of the model weight ($dAIC_{A3\text{-null (log)}} = 1.64$, $dAIC_{A3\text{-null (raw)}} = 16.52$). Other tested models received negligible model weight.

DIVE-SOUND EVOLUTION AND BEHAVIOR

Dive kinematics are highly variable in form (Fig. 1), but relatively little of this behavioral diversity was clearly linked to how sound was produced by the tail. Contrary to our hypothesis that producing the dive-sound drove the evolution of dive height, dive height is not significantly correlated with U^* (Fig. 7). Pagel's λ of dive height was estimated to be 0.84 (95% ce: 0.24–1.43). Our hypothesis was in retrospect based on an anomalous species, Anna's Hummingbird: the critical velocity for all tail-feathers except Anna's hummingbird R5 was below 14.8 m/s (Table S3), below the top speeds male hummingbirds can attain in level flight (Chai et al. 1999; Clark and Dudley 2009). Hence the behavior of diving is not strictly necessary to produce the tail sound–except in Anna's Hummingbird. We identified three clades in which pitch of the tail sound is modulated behaviorally: in two it is modulated by flight speed (*Calypte costae*, *Thaumastura* (*Myrmia*) *micrura*), and a third in which it is affected by feather orientation (*Chaetocercus* spp.). Behavior also affects the sound through the number of times the tail is spread during the dive. Number of tail-spreads during the dive is not particularly variable: most species spread their tail 3–5 times in association with the behavior undulating (as described below), with four reductions to one spread, and 1–2 increases to >5 spreads.

Discussion

Males of the 37 species in the bee hummingbird clade court prospective mates with three "musical instruments:" the wings, tail, and syrinx. Close relatives produce display sounds that differ dramatically in acoustic structure (Fig. 2). Acoustic sexual displays have thus rapidly diversified in this clade under the action of sexual selection by mate choice. The data presented here allow us to address three evolutionary topics. First, we further develop our

stars). (B) The mechanics of flutter reveal five ways the acoustic mechanism can change the sound produced. Two mechanisms of changes that produce discrete changes in sound source (top) are source switches, when flutter jumps from one feather to another (outline: *Selasphorus sasin*; Clark 2014); and mode switches (shown: *Calypte anna* R5; Clark et al. 2013b), when flutter jumps from one feather region to another within the same feather. Bottom: three types of changes produce continuous changes in sound source: an individual feather is gained (outline: *Selasphorus sasin*) or lost (outline: *Chaetocercus mulsant*) from the set of feathers that comprises the source, but flutter of one or more other source feathers remains the same. Finally, flutter frequency may change gradually with evolved gradual changes in feather shape, which occurs on all branches in the tree (outline: Anna's vs Costa's R5). Gradual changes in morphology occur on all branches but are not depicted in panels A or C. (C) Evolution of mode of flutter, indicated by branch color, and which feathers are the source of sound, indicated with hatches. Flutter mode, coded by branch color, has changed state a minimum of 10 times (includes 2–3 losses, white branches). Which tail-feathers are included in the source has a minimum of individual 16 character-state changes (± R1, R2, R3, R4, R5), and are reconstructed assuming accelerated transformations (ACCTRAN). Several branches contain multiple changes in mode and/or changes in source, such as the branch leading to *Selasphorus calliope*, and these are depicted as single changes in A, because they are single complex evolutionary change (Clark 2014). Feather outlines are to scale; color photographs are not. Outlines include species with at least one feather tested individually in a wind tunnel; colored regions show approximate region of flutter. Some species, particularly most woodstars, have poor data; small question marks indicate character states and feathers especially prone to reinterpretation upon further study. Taxa lacking feather outlines are those for which feathers were unavailable for wind tunnel tests. For these taxa, most plausible character states are presented based on morphology, dive-sound, and homology with sister taxa.

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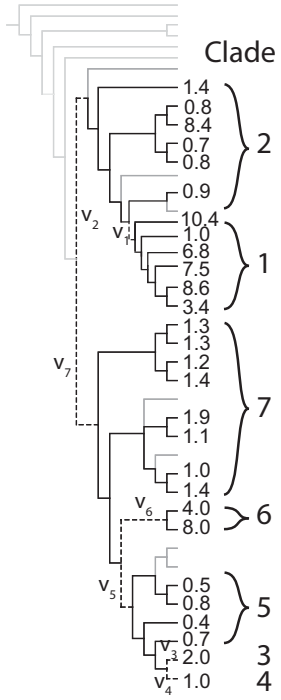

Figure 6. Branches and clades/grades investigated for alternate rates of evolution of dive sound frequency. Tip values: fundamental frequency of the dive-sound. Dashed branches (V_1–V_7) are those on which a major source change is inferred to have produced a discrete change in dive sound (Fig. 5). Gray branches = no data or dive-sound absent. Clade 1: *Chaetocercus-Eulidia*. 2: Other woodstars. 3: *Selasphorus sasin*. 4: *S. calliope*. 5: other *Selasphorus* (including *Atthis*). 6: *Calypte*. 7: a grade of other North American bees. Note: Unlike in other figures, a polytomy has been resolved by placing *Eulidia* sister to *Chaetocercus*. This reconstruction posits one fewer non Brownian change (on branch V1) than the other two possible topologies, and is thus conservative relative to the OUwie analyses.

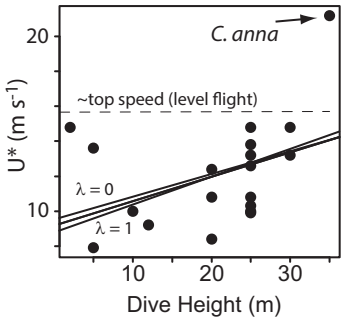

Figure 7. Dive height against U^* for 19 species of Bee Hummingbird. U^* is the critical velocity for feather flutter and produce sound. PGLS regressions with Pagel's λ set to 0 (top line), set to 1 (bottom line), and middle line is actual estimate of λ = 0.84 from the data. Slope is marginally significant (λ = 1, slope = 0.16, P = 0.099; λ = 0, slope = 0.13, P = 0.098), and this marginal significance is driven by data for *C. anna,* which is a clear outlier. Dashed line represents the approximate top flight speed of male hummingbirds tested in a wind tunnel; U^* values below this line indicate that diving is not essential for males to reach speeds sufficient to produce sound with their tail feathers (thus, below this line there is no clear reason to expect U^* and dive height to coevolve). See also Table S3.

hypothesis of how tail-sounds have evolved rooted in our physical understanding of how feathers flutter. We argue that while sound frequency likely evolves as a gradual, continuous character on most branches of the phylogeny, the dynamics of how feathers flutter precludes it from having done so on others. Instead, sound frequency also evolves as a genuinely saltational (discontinuous) character (Landis et al. 2013), physically jumping from one state to another without passing through intermediate frequencies. Second, we then discuss how changes in instrument (morphology) and playing style (kinematics and behavior) affect the evolution of the tail-sound. Finally, we consider how tail sounds evolve in the context of the two other instruments, the wings and the syrinx. The data presented here show that these three acoustic characters coevolve with each other in two ways: (1) vocalizations and wing sounds (including wing trills) are negatively correlated, supporting the transfer hypothesis, and (2) there are three independent instances of coevolutionary convergence, or concerted evolution, in which a species produces both vocal and nonvocal frequency-modulated sweeps that are a striking match in acoustic structure, despite being produced by two completely different mechanisms.

COMPLEX EVOLUTION OF TAIL SOUNDS, CONTINUED

Our reconstruction of the evolution of sounds produced by the tail of bee hummingbirds (Fig. 5) indicates that the evolution of this acoustic organ is complex. No two species are exactly alike in the shape of their tail-feathers, thus shape of each of the rectrices has evolved on every branch of the bee hummingbird tree. Most branches of the tree show no evidence of source or mode switches (Fig. 5). For two feathers expressing the same mode of flutter, differences in frequency are produced by commensurate differences in feather size and stiffness, just as a slight change of the stiffness of a guitar string shifts its frequency slightly (Clark et al. 2011a). On these branches, the simplest explanation is that acoustic frequency has evolved gradually and continuously as a function of changes in feather shape. By contrast, on six internal branches on which source switches and mode switches have evolved, we next argue that, owing to the underlying physics, fundamental frequency likely instead evolved as a discontinuous character, "jumping" from one frequency to another.

There are three source switches in the tree, all of which are associated with the *Selasphorus* clade, and two of which were described by Clark (2014). Mode and source switches are always associated with large jumps in frequency. In *S. calliope* this is

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because the tail-feathers of this species collide to produce sound, rather than fluttering on their own to generate sound (Clark 2011). Regarding the source switch R2 → R3 on the branch leading to *S. sasin*, Clark (2014) showed that the ancestor of *S. sasin* likely underwent "harmonic hopping" (Kingston and Rossiter 2004), in which R3 was initially a filter of the even harmonics of R2, before becoming a source, vibrating at twice the frequency (i.e., the even harmonics) of R2, such that fundamental frequency of sound hopped from ∼1.0 kHz → ∼2.0 kHz without passing through intermediate frequencies (Clark 2014). The third source switch within bee hummingbirds, uncovered here, is R5 → R2 at the base of *Selasphorus*. There are at least two hypotheses that can explain how this switch evolved. For sound frequency to evolve as a gradual character, the source would creep down the tail from R5 to R2 through a series of six source additions and losses, for example R5 → R5+R4 → R4 → R4+R3 → R3 → R3+R2 → R2 (or similar, gains, and losses need not alternate as just implied). Under this "source creep" hypothesis, frequency could evolve gradually, because each feather that joins the source may do so by initially vibrating in forced response to the vibration of its immediate neighbor, and hence at the same frequency. But as this hypothesis requires six individual evolutionary steps, we suggest it is unlikely. Instead we propose that R2 began to flutter independently of R5 during the dive, when the tail is spread as the bird is flying at high speed, and so became a sound source; R5 was then lost as a source. This hypothesis is more parsimonious as it posits only two changes on the same branch, +R2 and –R5. As R2 begins vibrating independently of R5, whatever R2's original resonance frequency was would set its initial sound frequency, unrelated to the frequency of sound produced by R5. Thus, we propose, in this source switch, frequency jumped discontinuously from one value to another.

Mode switches also occurred at least four times within the bee hummingbird phylogeny (Fig. 5). During a mode switch, frequency also evolves as a saltational character. This is easy to justify because intermediate frequencies are often not physically possible. In wind tunnel experiments, feathers may transition between flutter modes over a few milliseconds in response to a change in a state variable (such as when rotated), jumping from one frequency of sound to another (Clark et al. 2013a). In certain orientations, two modes of flutter may even be elicited from the same feather simultaneously, meaning it is physically possible for this character to be truly polymorphic at an intermediate step (Clark et al. 2013a). Alternatively, individuals could be temporally polymorphic, producing sound via one mode of flutter at one point in time, and via the other mode at other times, depending on the exact angle to which the tail is spread, or dive speed, or another geometric variable. Either way, the fact that frequency varies discontinuously in wind tunnel experiments, via transitions between one stable mode of vibration to another, provides a physical analogy that strongly implies the same thing happens over evolutionary time during mode switches. As feather shape evolves, if a small change in feather shape crosses a threshold in state space, it causes the feather to transition to an entirely different mode of flutter.

It is common in evolutionary biology to assume that phenotypic characters evolve in either a continuous or discrete manner in the absence of information about the mechanistic basis of that character, because the physical basis for many characters is poorly known. Here, we have some understanding of the complex physical basis for feather sound frequency. We have shown that out of 67 internal branches within the bee hummingbird phylogeny, the tail sound evolves as a continuous character on as many as 61 branches, but as a discontinuous, saltational trait on at least six branches. That is, frequency likely evolves as under a Gaussian process on most branches of the phylogeny, but under a Levy process on at least six branches (Landis et al. 2013). The underlying cause is that flutter and the ensuing sound it produces is an emergent phenotypic character with a complex dynamical basis.

We are unaware of other phenotypic characters that have been shown to evolve under both genuinely continuous and saltational regimes. Similar phenomena seem likely to be common in phenotypic traits that are rooted in a complex dynamical systems, or are emergent properties. There are many other phenotypic characters that arise out of a complex physical basis, such as structural colors (coloration mechanism (Prum 2006) or plumage pigments (Prum and Torres 2013)), locomotor gaits, jaw mechanics such as of fishes (Westneat 2004), or the forces produced by appendages (e.g., mantis shrimp; Blanco and Patek 2014).

The analyses in OUwie recovered an elevated rate of evolution of frequency on one of the individual branches that we reconstructed as having saltational evolution, but not the others (Fig. 6, Table 1). We attribute this to two factors: the number of and location of branches within the phylogeny inferred to have saltational evolution; and the elevated rate of evolution of frequency present in the woodstars (estimated to be 30 times higher than the rate estimated for other branches (e.g., BMS.a3), or up to eight times the background null rate (e.g., BM1.null), Table 1). For example, branch V_1 (Fig. 6), was not a statistical outlier, with respect to the rest of clade 1. This coincides with the evolution of the strange whole feather bending "mode" of vibration in *Chaetocercus* (Fig. 5). Moreover, this pattern occurred in the clade for which we have more limited data: several of the *Chaetocercus* woodstars are rare or of conservation concern, and we did not obtain feathers to test in the wind tunnel. Therefore, we assumed that they all produce sound via the same feather and mode. Additional instances of saltational change could have occurred within the woodstars that we did not detect (Fig. 5), due to our paucity of data on how the tail makes sounds.

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RELATIVE ROLES OF MORPHOLOGY AND BEHAVIOR

Our hypothesis for the origin of the dive-sound is similar to the model proposed for the origin of arthropod communication sounds (Ewing 1989) or for manakins (Prum 1998): the display behavior evolves first, the sound is initially a byproduct of the behavior, then subsequent selection for the sound causes the morphology to evolve in response. The undulating behavior widespread in bee dives appears to precede the dive-sounds (Fig. 3A). We suggest that spreading the tail repeatedly while flying at high speed during this undulating display evolved first. Flutter is especially prone to occur at high airspeeds, thus it is during high speed maneuvering flight that flutter of tail-feathers is most likely to arise as an incidental byproduct of flight, as hummingbirds spread their tails during maneuvers (Clark 2010). Thus, we argue incidental tail-sounds began to accompany undulating. Once the tail then began to produce sound, it converted from an incidental cue to a coevolving sexual communication signal, and sexual selection began to modify the tail and associated behaviors for sound production. Finally, some members of Mellisugini subsequently lost the original behavior of undulating during the dive, but maintained the associated behavior of spreading the tail repeatedly, which now serves the function of producing sounds (Fig. 3A). This evolutionarily novel behavior that has been innovated into a dive was a necessary precursor for hummingbird tails to become a source of "instrumental music."

After the evolution of the dive, it appears that changes in the instrument, tail morphology, generated greater diversity in the pitch of the sounds produced. Across the species we studied, there are three instances in which pitch varies because of behavior (Fig. 2), whereas essentially every species produces a unique pitch due to changes in morphology. This is not to imply display behaviors are static; most species have unique kinematics (Fig. 1) that potentially modulates effects such as the Doppler shift of the dive (Clark and Feo 2010), and in some species, sound frequency is correlated with dive-speed. But overall, behavior does not appear to drive the interspecific differences in pitch. Unquantified aspects of the sounds, especially amplitude, might be modulated behaviorally. For instance, we failed to find a correspondence between dive height and the U^* of the tail-feathers, but as many hummingbird tail feathers increase in amplitude at higher speeds in a wind tunnel tests (Clark et al. 2011a, Clark et al. 2013b), it remains possible that dive height relates to this acoustic variable, which is difficult to measure in the field.

In other taxa with complex displays, the interplay of morphology and behavior in signal design is variable. In a comparison of 11 species of jumping spiders, Elias et al. (2012) found a positive correlation between number of morphological display traits and behavioral complexity of displays. In Manakins, "whirr" mechanical sounds may diversify behaviorally, through variation in pulse number (Prum 1998; Bostwick and Prum 2003), as these sounds are broadband and atonal, providing less capacity for acoustic structure to change with morphology. Similarly to manakins, Kangaroo rats (*Dipodomys* spp.) produce patterns of drumming with their feet that are complex and species-specific (Randall 2001, 2013), yet every species has essentially the same "instrument": there seem to be no identified morphological structures specialized for sound production in Kangaroo rat feet (Randall pers. comm.).

TRANSFERENCE AND CONVERGENCE BETWEEN FEATHER SOUNDS AND VOCALIZATIONS

Our results support the within-mode transfer hypothesis for two types of sounds: those produced by the wings, and vocalizations. The hypothesis is supported in two related ways: males when alone on their territories tend to produce either vocal undirected song, or a wing trill, but usually not both (Fig. 3B), the only exceptions being *Atthis heloisa* and *Chaetocercus jourdani*. Second, during dives directed toward females, in addition to tail-sounds, hummingbirds produce either wing sounds or vocalizations but usually not both (Fig. 2). Why does transference occur between wing sounds and vocalizations? We propose that they are functionally equivalent. Vocal songs and wing trills have common features: both are sounds broadcast into the environment in an undirected fashion. Hummingbird songs are actively sung spontaneously from territorial perches, similar to passerine birds. Wing trills produced in ordinary flight are automatically also broadcast into the environment, indicating the presence of a male. Experiments on *Selasphorus platycercus* have demonstrated that loss of the wing trill causes males to lose their territories (Miller and Inouye 1983). Hunter (Hunter and Picman 2005; Hunter 2008) has shown in a nonterritorial context that *Archilochus alexandri*, *Selasphorus calliope*, and *S. rufus* hummingbirds use wing trills to identify the sex-species class of nearby individuals. Both features imply wing trills have functional similarity to vocal song; a male that produces a wing trill will be audible to other birds (both rival males and prospective mates) as he flies around his territory, thus proclaiming presence and ownership. He may not need to sing as well. We propose that, as wing trills can serve the same functions as vocal song, species that evolve wing trills tend to lose vocal song as these two traits are functionally equivalent.

Gilliard (1956) proposed the transfer hypothesis in the context of within-mode transference between plumage and bower decorations of bowerbirds (Endler et al. 2005). The proposed cause of intramodal transference is differences in costliness of the traits in question (Gilliard 1956; Endler et al. 2005). While it is possible that either songs or wing trills could be costlier than the other, we are unaware of any actual cost specific to one of these characters. A reasonable null is that this pattern could arise through an essentially neutral process: if these two traits are functionally redundant, one could replace the other neutrally.

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Indeed, Pagel tests of a 6-parameter models to test whether either trills or songs is an independent variable that drives the other were not statistically significant relative to the full 8-parameter model, implying mutual codependence between these two types of characters (Table S2).

We also uncovered a potentially related pattern: repeated coevolutionary convergence, or concerted evolution, between vocal song in an acoustic repertoire with highly similar form has occurred multiple times within the bee clade (Fig. 4). In two, *Calypte costae* and *Thaumastura* (*Myrmia*) *micrura*, the match is between tail-sound and vocal song, while in the third, *Atthis heloisa*, the match is between wing trill and vocal song. In each case, both vocal song and mechanical sounds are frequency sweeps that rise and then fall in frequency, and do not resemble their respective outgroups. There are no data available that would allow an evaluation of the proximate causes (such as female preferences) behind this convergence. The concerted evolution between vocal and feather sounds in hummingbirds is particularly interesting because the songs are socially learned. Learning of vocalizations and potentially of female acoustic preferences may have facilitated this process. We do not know of any comparable examples in other taxa. As a hypothetical example: an organism has evolved two color patches with the same appearance (hue, chroma, etc.) but one is produced by pigments and the other produced by a structural color.

EVOLUTIONARY LOSS OF LEARNED SONG

A final noteworthy pattern documented here is the repeated evolutionary loss of singing. Hummingbirds in at least two distantly related clades, hermits and bee hummingbirds (*Calypte* spp.), learn their songs socially (Baptista and Schuchmann 1990; Gahr 2000; Araya-Salas and Wright 2013) while swifts and other outgroups are not known to socially learn their songs. Hummingbirds have thus independently evolved song learning relative to songbirds and parrots (Jarvis et al. 2000). Here, we document multiple evolutionary losses of singing in one small clade. The repeated loss of vocal advertisement behavior in an ancestrally song learning clade provides a unique opportunity for future research on the evolution of song learning brain circuitry in the absence of selection to maintain their vocal learning function.

AUTHOR CONTRIBUTIONS

C.J.C. conceived of study, collected field data, and analyzed feather data. J.M. and J.S.B. constructed molecular phylogenies. E.B. and J.S.B. performed sequencing and contributed to field work. J.S.B. and C.J.C. conducted comparative analyses. R.P. conceived of the study, contributed to data collection and funding. C.J.C. wrote the manuscript with input from all authors.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

DATA ARCHIVING

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Supporting Information

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Figure S1. Phylogenetic estimate of the bee hummingbird clade (MrBayes).
Table S1. Data sources for courtship displays of species in the bee hummingbird clade.
Table S2. Parameter matrix from Pagel's (1994) test for correlated evolution of the evolution of wing trill (X) and vocal song (Y).
Table S3. Parameter estimate from Pagel and Mead's (2006) test for correlated evolution of the evolution of wing trill (X) and vocal song (Y).
Table S4. Feather critical velocity and dive height of bee hummingbirds (N).

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