Title
Sexual Selection, Flight Performance, and Signaling in Black-Chinned Hummingbirds (Archilochus alexandri)

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Publication Date
2020

UNIVERSITY OF CALIFORNIA
RIVERSIDE

Sexual Selection, Flight Performance, and Signaling in Black-Chinned Hummingbirds (*Archilochus alexandri*)

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Sean Charles Wilcox

September 2020

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Acknowledgements

Several people helped in the completion of this dissertation. In particular, many undergraduates and friends volunteered their time and energy to help conduct fieldwork during the hottest times of the year. I thank Lauren Conroy, Alexandra Cruz, Andrea Haller, Ralph Lacerda de Albuquerque, Jeffrey Liu, Misumi Ogawa, Ryan Oh, Victor Olivares-Moran, Molly Peters, Melani Ratenake, Par Singhaseni, and Paul Spradley for their help.

Thank you to my lab mates Ayala Berger, Jenny Hazelhurst, Elisa Henderson, Katie Johnson, Krista Le Piane, Emily Mistick, Bryan Myers, and David Rankin for your support. Also, thank you to Drs. Erin Rankin and Kevin Loope for help with a project that ultimately did not end up in this dissertation.

My graduate school experience would have been greatly diminished without the friends I made along the way. For those friends who are not also lab mates, I thank Clint Collins, Alex Cortez, Caitlin Eliason, Morgan Gray, Kevin Jagnandan, Ralph Lacerda de Albuquerque, Sarah Lillian, San McNamara, Keenan and Sarah Morrison, Emily Naylor, Aaron Olcerst, Matt O'Neill, Sonia Peterson, Kenji Quides, Parsa Saffarinia, Glenna Stomackin, and Jia Sun.

I thank Dr. Lucinda McDade and the staff of the California Botanic Garden, formerly the Rancho Santa Ana Botanic Garden, where much of the fieldwork for the third chapter of

this dissertation was conducted. Access to the population of black-chinned hummingbirds at the botanic garden was invaluable.

I was funded by the Pasadena Audubon Society in my first year as a graduate student which allowed me to purchase essential field equipment. I was also funded by a grant in aid of research from the Society for Integrative and Comparative Biology. Lastly, I was supported by the Department of Evolution, Ecology, and Organismal Biology in the form of Newell and Spieth Awards, and teaching assistantships. Without these generous funding sources, my dissertation would have been much more difficult to complete.

I thank my committee members, Drs. Ted Garland and Tim Higham for their help throughout my graduate experience. I especially thank Dr. Chris Clark for taking me on as a graduate student, guiding my through the program, and pushing me to finish.

My family encouraged and supported my love for animals since I was very young. Among elementary school friends, our house was known for the many different kinds of animals we kept as pets. I thank my mother, father, and sister for encouraging me in this endeavor.

I whole-heartedly thank my wife Lauren Conroy. We met as graduate students at UC Riverside. I am unsure if I would have completed this dissertation without her. Thank you for your support and encouragement.

Dedication

I dedicate this to my grandparents.

ABSTRACT OF THE DISSERTATION

Sexual Selection, Flight Performance, and Signaling in Black-Chinned Hummingbirds (*Archilochus alexandri*)

by

Sean Charles Wilcox

Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, September 2020
Dr. Christopher Clark, Chairperson

Locomotion is important for both naturally and sexually-selected behaviors. Maximal locomotor performance is physiologically challenging, and hence has been a focus of many studies that attempt to link morphology, performance and fitness. In particular, locomotor performance of males during courtship behaviors has become an interesting area of study because variation in performance can reveal individual differences in males which may be of interest to choosy females. Thus, sexual selection can result in extreme male locomotor performances which can lead to sexual dimorphism and sexual differences in locomotor performance. Hummingbirds are an ideal study organism to address these questions because males perform aerial courtship displays that seem to push the limits of their locomotor capabilities. Specifically, the focus of this dissertation is the shuttle display, a short-ranged low-speed side-to-side lateral flight display males perform for females. A hallmark of this display is a drastic increase in wingbeat frequency relative to hovering which must be challenging perform. This dissertation shows that flight performance is sexually selected in hummingbirds, most likely via female choice for male aerial displays. In black-chinned hummingbirds (*Archilochus alexandri*), sexual differences in flight performance exist, and variation in shuttle-display performance suggests the display is an index signal of male acceleration. Extreme male locomotor performance during courtship is seen in a wide diversity of species. These results further our understanding of how extreme male locomotor performances evolve, and the consequences of sexual selection on male locomotor performance.

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General Introduction:

Locomotion is crucial for behaviors with high fitness stakes, including predator-prey interactions (Webb, 1986; Jayne and Bennett, 1990; Watkins, 1996; Miles, 2004; Walker et al., 2005). Also, locomotor performance can be important during sexually-selected contexts (Byers et al., 2010; Barske et al., 2011). In particular, variation in male locomotor performance during courtship display behaviors can reveal differences among males which may be of interest to choosy females (Byers et al., 2010).

Hummingbirds perform two aerial displays that seem to exhibit extreme male locomotor performance: a high-speed dive and short-range low-speed shuttle display (Clark, 2009; Clark et al, 2018). During the shuttle display, a male flies laterally, side-to-side within ~1 meter of a female. Wingbeat frequency is drastically elevated (relative to hovering) during these displays. In some species, like black-chinned hummingbirds (*Archilochus alexandri*), accentuated wing sounds are produced during the shuttle display (Feo and Clark, 2010). Because the wing sounds are causally related to wingbeat frequency the shuttle display is hypothesized to function as an index signal (Maynard Smith and Harper, 2003) of male flight performance that females might use for mate choice decisions.

My dissertation examines the role of sexual selection for display behaviors on reversed size dimorphism in hummingbirds and attempts to understand how sexual selection for these displays has resulted in sexual differences in flight performances. Lastly, I document flight performance of the black-chinned hummingbird shuttle display and show that individual variation exists such that there is potential for females to select males based on variation in acceleration and display wingbeat frequency. Thus, in **Chapter 1**, I used a comparative approach to examine male and female allometry of flight-related morphology and hovering wingbeat frequency in hummingbirds. In **Chapter 2**, I used black-chinned hummingbirds (*A. alexandri*) to test for sexual differences in flight performance, and to test for a correlation between flight performances measured from different flight assays. In **Chapter 3**, I used male black-chinned hummingbirds (*A. alexandri*) to quantify shuttle-display flight performances to test for correlations among shuttle-display performance variables, and to test if shuttle-display performance variables were correlated with the performance of an asymptotic load lifting assay.

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Chapter 1: Sexual Selection for Flight Performance in Hummingbirds

Abstract:

Within animals exhibiting sexual size dimorphism, males are often larger than females. Certain clades, such as hummingbirds, tend towards the reverse: females are the larger sex (female-biased size dimorphism). What selects for small male size? Sexual selection for flight performance could drive the evolution of smaller, more agile males, either to improve performance in male-male combat or aerial courtship displays. Alternately, female body size could be under natural selection for fecundity (e.g. egg size influences female body size), or sex differences in foraging niche could favor body size differences. Sexual selection for flight performance predicts that males are dimorphic for other aspects of flight morphology (e.g. flight muscle size) whereas the natural selection hypotheses predict that male and female flight morphologies are isometric versions of each other. We tested these predictions through phylogenetic comparative analyses of allometric relationships of flight morphology and wingbeat frequency of 93 species of hummingbird, and courtship behaviors across 30 species within the "bee" hummingbird clade (tribe Mellisugini). Male “bees” tend to be smaller, have proportionately shorter wings, longer keels, larger flight muscles, and higher hovering wingbeat frequencies than females. Male “bees” also have higher hovering wingbeat frequencies and shorter wings compared to other male hummingbirds. Male wingbeat frequencies are greatly elevated during aerial displays, and the species with the greatest wingbeat frequencies have the greatest dimorphism, suggesting that female choice for male courtship displays has selected for aerial agility and small size in male hummingbirds.

Introduction:

Both natural and sexual selection can drive the evolution of sexual dimorphism, in which males and females exhibit dissimilar behaviors and morphologies (Andersson, 1994), such as body size. Among birds and mammals, males tend to be larger in size than females (Andersson, 1994; Fairbairn, 2013). The reverse pattern, female-biased size dimorphism (also called "reversed" size dimorphism) is less common, and is most prevalent in arthropods such as spiders (Moya-Laraño et al., 2002; Aisenberg et al., 2007), orthopterans (Hochkirch and Gröning, 2008) and beetles (Rudoy and Ribera, 2017).

Natural selection can favor larger female size in at least two ways (Blanckenhorn, 2005): 1) fecundity selection on females and 2) niche differentiation. Fecundity selection can select females to be larger, if this allows them to produce more or better (e.g. larger) offspring (Darwin, 1874; Shine, 1989; Pincheira-Donoso and Hunt, 2017). Under niche differentiation, natural selection favors different niches between the sexes (such as foraging niches), resulting in character displacement (Andersson and Norberg, 1981; Reviewed in Shine, 1989 and Fairbairn, 1997). For example, hawks, falcons, owls, or certain seabirds exhibit female-biased size dimorphism, which may result from natural selection on males and females to divide the foraging niche; small male size may allow males to specialize on smaller, more elusive prey (the "aerial agility" hypothesis, introduced below); whereas larger size may allow females to specialize on larger prey (Andersson and Norberg, 1981).

Alternately, sexual size dimorphism could arise via sexual selection through either male-male competition or female choice. Male-male competition may favor size dimorphism in species in which males fight for access to mates (Andersson, 1994; Blanckenhorn, 2005; Fairbairn, 2013). Larger male body size is selected for when it provides an advantage during fights and other contests (Andersson, 1994; Arnott and Elwood, 2009), when the probability of winning a fight is determined by brute force, rather than nimbleness. Male-male competition can instead favor small males if fights are won by the male that is more agile. For example, small midges are better able to maneuver in laboratory flight chambers which confers an advantage during aerial territorial behaviors between males (Crompton et al., 2003). Thus sexual selection, in theory, could select for small male size under male-male competition.

A final hypothesis of female-biased size dimorphism is sexual selection via female preferences for agile aerial display behaviors. Small males may be better able to maneuver during challenging flight displays performed for females during courtship. In birds, previous studies have suggested a correlation between aerial courtship-display behaviors directed at females and small male size in certain bird clades (Blomqvist et al., 1997; Székely et al., 2000; Székely et al., 2004). This pattern arises because male agility is negatively correlated with size (see aerial agility hypothesis, below). Hence, sexual selection resulting from female choice for dynamic male flight behaviors could select for small males, driving female-biased size dimorphism.

Thus there are four hypotheses of female-biased size dimorphism in hummingbirds: Natural selection may favor large female size under 1) fecundity selection or 2) niche differentiation. Sexual selection may favor small males when 3) this enables males to win fights; or, 4) small size enables superior performance of aerial displays. Under natural selection, we predict that body size alone (#1), or body size and bill morphology alone (#2) vary between the sexes, that is, we predict males and females, apart from body size differences, are otherwise isometric scaled versions of the other sex. By contrast, under the sexual selection hypotheses, we predict that sexual selection on flight performance would result in deviation from isometric relationships, such that males have a different flight phenotype (e.g. wing length, wing muscle size, and higher wingbeat frequencies) than females, which affects their flight performance.

The Aerial Agility Hypothesis

Flight performance, broadly defined, encompasses a suite of physiological and biomechanical variables, including the ability to turn, linearly accelerate, angularly accelerate, attain high speed, avoid obstacles (and other feats of coordination), and maintain the ability to fly at a high level over time (e.g., endurance, repeatability). Certain aspects of performance (particularly, accelerations) must scale negatively with increasing body size (Dial et al., 2008; Jackson and Dial, 2011). For instance, assuming isometry of limb proportions and muscle size, one model predicts that the ability to linearly accelerate scales as mass$^{-1/3}$ and rotational accelerations scale as mass$^{-2/3}$ (Norberg and Andersson, 1981; Altshuler and Dudley, 2002; Dudley, 2002). For our purposes, the aerial agility hypothesis states that smaller organisms are in some way more nimble or maneuverable than larger organisms, thus small body size may be selected for in organisms in which maneuvering flight plays an important role in their ecology (Dudley, 2002; Dial et al., 2008).

Focal Clade: Hummingbirds

Hummingbirds (Trochilidae) are a speciose clade of polygynous birds that follow Rensch's rule (Rensch, 1950). Specifically, male hummingbirds tend to be larger than females in large species, whereas small species instead tend to exhibit female-biased body size dimorphism (Colwell, 2000). Within hummingbirds, the "bee" hummingbird clade (tribe Mellisugini; hereafter: bees), on which many of our analyses are focused, is composed of 37 species of small to very small hummingbirds. All are dimorphic in size: the two largest species (*Calypte anna*, and *Thaumastura vesper*) are male-biased, and the remaining species are all female-biased in body size. Within the bees are two subclades, the North American bees and the woodstars (Clark et al., 2018).

Male hummingbirds do not provide help at the nest to females. Males of all species hold breeding territories which they defend against other males. In certain species these territories contain resources such as nectar that females utilize (Stiles and Wolf, 1970; Temeles and Kress 2010), whereas in others, male territories appear to contain nothing except the male himself (Stiles and Wolf, 1979), i.e. they lek.

Hummingbird flight is both a low-speed and high-speed affair: hummingbirds both hover for prolonged bouts and fly at high translational velocities (Clark and Dudley, 2009). This dynamic range of flight abilities are reflected in their impressive aerial courtship flights that males produce for females (Clark et al., 2018), as well as in male-male competition. Males of all species vigorously defend their courtship territories against other males by engaging in high-speed aerial chases and low-speed fights in which combatants maneuver in an attempt to stab each other with their sharpened bill-tips (Rico-Guevara and Araya-Salas, 2015). Whereas flight performance clearly plays a pivotal role in these contests, which aspects of performance enable one male to win a fight against another is unclear, and hence whether fights and fighting ability would select for large or small body size is not clear *a priori*.

Regarding courtship, males of nearly all bee species perform two types of acrobatic aerial displays: high-speed dives (Clark, 2009) and short-range “shuttle” displays. During both dive and shuttle displays, courtship-display wingbeat frequencies of some species are dramatically elevated relative to hovering wingbeat frequencies (Feo and Clark, 2010; Clark et al., 2013; Feo et al., 2015). During the shuttle, the male flies close (< 1 m) to the female (Figure 1.1E, 1.1F), gorget flared, and shuttles back and forth in a cyclical flight pattern. Most species fly rhythmically from side-to-side characterized by both a repetition rate (cycle frequency, *f*) and an amplitude (*a*), (Figure 1.1A) (Clark et al., 2013).

Since all animal locomotion is in some way acceleration-limited (Biewener and Patek, 2018), an individual cannot maximize both the frequency and amplitude of the overall flight pattern of this shuttle display. Behavioral traits that are subject to biomechanical constraints can appear as triangular distributions rather than linear tradeoffs (Podos, 1997; Cade et al., 1999; Wilson et al., 2014; Miles et al., 2018), where a negative relationship at higher quantiles of the distribution suggests a performance tradeoff (Miles et al., 2018). Small animals intrinsically have a greater capacity to accelerate (Altshuler et al., 2004; Altshuler, 2006; Dakin et al. 2018), thus the ability to perform shuttle displays may select for smaller body size.

Here, we present data for hovering wingbeat frequencies of males and females for 93 hummingbird species, and male courtship-display wingbeat frequencies for 30 bee hummingbird species. We demonstrate that, within and among hummingbird clades, flight morphology of males and females varies in ways that support the sexual selection hypotheses, and specifically that female choice for male aerial displays seems to be the selective pressure that has produced "reversed" sexual dimorphism in this clade.

Methods:

Data Collection

We compiled data from literature sources (see Suppl. Methods) in which hummingbird hovering wingbeat frequency was identified by sex. We included masses and wing lengths from literature sources (e.g. Greenewalt, 1962), or measurements from wild-caught birds in the course of field work in Argentina, the Bahamas, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, Guatemala, Jamaica, Mexico, and the United States (California, Connecticut, Texas). Masses from wild-caught birds were measured with a digital scale. Keel lengths (Zusi, 2013) were taken from literature sources (Wright and Steadman, 2012; Graves, 2015), measured on skeletal specimens from the Los Angeles County Natural History Museum (LACM), or on live wild-caught birds (Figure 1.1C). In many cases, hovering wingbeat frequency and morphological data were taken from different animals (for example, wingbeat frequency data were sometimes from high-speed videos of wild birds that were not captured), thus we were unable to examine within-species trait covariance. Rather, we analyzed average values for males and females within a species.

In addition to data from the literature, we measured wingbeat frequency from the species listed in Table S2 of Clark et al. (2018) by recording hovering and courtship-display wingbeat frequencies using a microphone (Sennheiser MKH 20) with a Sound Devices 24-bit recorder, to record wingbeat frequency acoustically; or from high-speed camera recordings (Miro EX4, Vision Research) to measure wingbeat frequency by counting frames. Acoustic measurements of wingbeat frequency were taken as the average of 10 or more wingbeats, to reduce quantizing error.

When wingbeat frequency data were available for both the dive and shuttle display, we included the higher value to represent maximal male courtship-display wingbeat frequency. The cycle frequencies of shuttle displays were measured from sound recordings (Figure 1.1A, 1.1B), and amplitudes were estimated by eye, as recorded in contemporaneous field notes, or from video (when available).

Allometric scaling can reveal the structural and functional consequences of changes in size (Gould, 1966; Schmidt-Nielsen, 1984). Hummingbird wing length, rather than scaling isometrically as mass$^{1/3}$, instead scales as mass$^{1/2}$ (Greenewalt, 1975; Clark, 2010; Skandalis, 2017). We generated a scaling hypothesis for hummingbird wingbeat frequency, based on wing length scaling as mass$^{1/2}$ (See Suppl. Methods). Our scaling hypothesis predicts that hummingbird hovering wingbeat frequency scales as mass$^{-1/2}$. Morphological and wingbeat frequency data were $\log_{10}$-transformed prior to analyses except where noted (see below).

Phylogenetic Statistics

We lacked data for many of the species in the McGuire et al. (2014) hummingbird phylogeny, and had data for a few missing species. In Mesquite (v 3.31) we substituted and added several species to the McGuire et al. (2014) phylogeny based on previous research (Hinkelmann and Schuchmann, 1997; Clark et al., 2018) or likely close relationships (Schuchmann, 1999). A full list of modifications to the McGuire phylogeny is in the Supplementary Methods. We pruned the modified phylogeny to include only species with data. Our sampling included data for all major clades of hummingbirds (Figures 1.2, 1.3, 1.4, 1.5). For analyses which estimated allometric slopes and intercepts, we transformed all branch lengths in Mesquite (v 3.31) using Pagel's (1992) arbitrary method. This transformation resulted in no significant correlations between independent contrasts and the square root of the sum of their branch lengths (Garland et al., 1992).

Some bee males had particularly high hovering wingbeat frequencies for their sizes (Figure 1.2A), chiefly in woodstars, and within woodstars, especially in a clade comprising most species in the genus *Chaetocercus* (Figures 2A). Thus we were predominantly interested in the evolutionary rates of male and female hovering wingbeat frequencies. However, there is no *a priori* way to know the number of rate shifts within any combination of data and phylogeny (Baker et al., 2016). Furthermore, once branch lengths were transformed (Pagel, 1992), standardized independent contrasts did not reveal substantial outliers. But, standardized independent contrasts are based on a Brownian motion model which can have an "averaging" effect (Elliot and Mooers, 2014) on the estimation of evolutionary rates such that small evolutionary rates are overestimated and large evolutionary rates are underestimated (pers. comm. Joanna Baker).

Therefore, we analyzed evolutionary rates of male and female body mass, wing length, keel length and hovering wingbeat frequency with the BayesTraitsV3 program (Baker et al., 2016) using our adjusted McGuire et al. (2014) phylogeny. BayesTraitsV3 uses Markov chain Monte Carlo (MCMC) reverse-jump simulations to estimate the background rate of phenotypic change (ΔB), and a second set of parameters that identify branch-specific rate shifts: the rate of phenotypic change (ΔV) which is not due to the background rate, and a rate scalar (r) which can be simplified as the ratio of the two rates of phenotypic change ($r = \Delta \mathrm{V}/\Delta B$) (Baker et al., 2016).

For all analyses of evolutionary rates using BayesTraitsV3, first we compared a null model where traits were assumed to evolve under a single-rate Brownian motion regime (i.e., equal-rate model), to a model in which the rates on all branches were allowed to vary (i.e., variable-rate model). We ran our MCMC chains for 10^9 iterations, with a burn-in period of 10^8 iterations. In a Bayesian framework, evidence for variable rates of evolution comprises Bayes factors (BF) values greater than 2 (Raftery, 1996). The Bayes factor is defined as $\mathrm{BF} = -2\log_e[m_1/m_0]$, where m_1 is the marginal likelihood of a variable-rates model and m_0 is that of an equal-rate Brownian motion model. We estimated m_1 and m_0 using stepping-stone sampling (Xie *et al.*, 2011) over 1000 stones at 10^5 iterations per stone implemented in BayesTraitsV3. Post-processing of the variable-rates models was conducted using an online post-processor (www.evolution.reading.ac.uk/VarRatesWebPP).

Within the BayesTraitsV3 program, the variable-rate regression model (Venditti et al., 2011) can identify heterogeneity in the rates of phylogenetically structured residual errors. Thus, the method can detect shifts in rate that apply to the residuals of a focal trait on another character (e.g., the residuals of hovering wingbeat frequency and body mass). Clades and/or branches that are outliers to the regression line are identified as having experienced a rate shift (Baker et al., 2016). We therefore used the variable-rate regression model with body mass as the independent variable to estimate evolutionary rates of wing length, keel length and hovering wingbeat frequency for male and female hummingbirds. From the variable-rate model output for body mass, and variable-rate regression model outputs for wing length, keel length, and hovering wingbeat frequency, we concluded that the estimated evolutionary rates were noteworthy if 1) the mean $r > 2$, and 2) the branch in question had mean $r > 2$ in more than 95% of the posterior sample (Baker et al., 2016).

We used the RegressionV2.m module (Lavin et al., 2008) in Matlab to perform phylogenetic generalized least squares regressions (PGLS) to calculate allometric relationships for all male and all female hummingbirds (i.e., including the bee hummingbirds), and for all male and all female bee hummingbirds. We treated bee hummingbirds separately from all hummingbirds, because the BayesTraitsV3 results suggested that an evolutionary rate shifts had occurred at the base of the bee hummingbirds. We estimated confidence intervals via bootstrapping with 10,000 iterations. This allowed for estimation of upper and lower 95% confidence intervals of PGLS allometric slopes which were then compared to predicted allometric slopes.

Allometric slopes among groups were not different (see results), whereas visual inspection of the data implied differences in intercepts. Thus, pANCOVA allowed us to test for differences in allometric intercepts of flight-related morphology and hovering wingbeat frequency between the bees and all other hummingbirds. We used mass as a covariate and group (clade) membership was assigned with categorical (0/1) dummy variables. We coded bees as our focal group (i.e., coded as 1) because branches within the bee clade exhibited higher evolutionary rates of male hovering wingbeat frequency (see results) compared to branches outside the bee hummingbird clade (including branches basal to the bee clade). Sexes were analyzed separately.

The sexual selection hypotheses predicted allometric differences between bee males and all other male hummingbirds, but not between bee females and all other female hummingbirds. Hence, we repeated the above analyses on female data to see if female hummingbirds also showed clade-specific differences in the evolutionary rates of body mass, hovering wingbeat frequency, wing length, and keel length, and allometric differences in flight morphology and hovering wingbeat frequency. We depict results as phylogenetically-corrected slopes overlaid onto plots of original species-level data per Garland and Ives (2000).

Phylogenetic comparative methods require species-level data, so there is no way to perform pANCOVAs with sex as a factor. Therefore, to assess whether clade-specific allometric patterns varied between the sexes, we compared female and male PGLS slopes and intercepts using post-hoc analyses. We wanted to test whether male bees had a different flight phenotype, as represented by a different intercept. To test this, we overlaid

PGLS lines onto the species-level data for female and male bees and calculated predicted values ($\hat{Y}$) for each. We then tested for allometric differences between the sexes in the bees by performing paired-*t* tests on the predicted values. Shapiro-Wilk tests showed the paired differences in predicted values were normally distributed.

Display Correlations and Flight-Performance Tradeoff

The sexual selection hypotheses predicted correlations between courtship-display performance and underlying flight morphology and hovering wingbeat frequency, all of which are already highly correlated with size (i.e., body mass). To search for effects after accounting for the effect of body mass, we calculated phylogenetic size-corrected residuals using the phytools package in R (Revell, 2009). We used these residuals to perform additional regressions to test for relationships between flight morphology and courtship-display wingbeat frequency.

To test whether there was a relationship between shuttle-display acceleration performance and body mass, we modeled the shuttle display as a sinusoidal oscillator to estimate maximal shuttle-display acceleration:

$$A_{max} = (2\pi f)^2 \times D \qquad (1)$$

We estimated maximum accelerations (A_{max}) in the display from our estimates of *D* (Displacement; Figure 1A) and *f* (Shuttle-display cycle frequency; Figure 1B), and regressed A_{max} against mass to test if shuttle display accelerations fit the scaling prediction that maximal linear acceleration scales as mass$^{-1/3}$ (Andersson and Norgberg, 1981; Dudley, 2002). Shuttle-display cycle frequency and amplitude were predicted to trade off. To test this, we used the R package quantreg (Koenker, 2013) to perform quantile regressions on shuttle-display cycle frequency versus amplitude for quantiles $\tau = 0.10$ to $\tau = 0.90$ at increments of $\tau = 0.01$. We ran multiple models and therefore we controlled for the false-discovery rate on all p-values (Benjamini and Hochberg, 1995). The Benjamini and Hochberg (1995) procedure ranks *p*-values in order from smallest to largest. Each individual *p*-value is compared to its Benjamini-Hochberg critical value, $(i/m)Q$, where i is the rank, m is the number of tests performed, and Q is the false-discovery rate (0.05). The largest *p*-value that is less than $(i/m)\underline{Q}$ is significant, and *all p*-values smaller than it are also significant.

Results:

We obtained hovering wingbeat frequency, body mass, and wing length data for 93 species and male courtship display wingbeat frequency for 30 species of bee hummingbirds. Of the 93 species for which we have data, hovering wingbeat frequency data were missing for males of 8 species (n = 85 males) and 38 females (n = 55 females). We obtained male keel length data for 45 species, and female keel length data for 34 species. Pagel's (1992) branch length transformation standardized independent contrasts (Supplementary Figures 1.1, 1.2, 1.3, 1.4, and 1.5) which allowed us to estimate allometric relationships.

Allometry of Hovering Wingbeat Frequency

Allometric slopes of hovering wingbeat frequencies were not different from our allometric predication (See Suppl. Methods) of -0.50, where this predicted value accounts for the known positive allometry of hummingbird wing length (Table 1.1; female slope = -0.50, male slope = -0.38). Similarly, courtship-display wingbeat frequency (slope = -

0.75; Lower CI = -0.99, Upper CI = -0.49) was not different from our prediction (Table 1.1; predicted slope = -0.50). Allometric slopes for bee male hovering (slope = -0.57) and courtship-display wingbeat frequency (slope = -0.75) were not different from each other (p = 0.37). Hence, neither hovering wingbeat frequency nor courtship-display wingbeat frequency allometric slopes differ from our hummingbird-specific scaling prediction of -0.5.

Within the bees, males of certain species had hovering wingbeat frequencies that were high for their body sizes (Figure 1.2A). For example, smallest bird in the world is the male Cuban bee hummingbird (*Mellisuga helenae*). Males of this species weighed an average of 1.73 ± 0.06 (n = 8) grams and have a hovering wingbeat frequency of 66.8 Hz (n = 8 males), whereas in the genus *Chaetocercus* males weigh 1.8 - 3.6 grams and males had hovering wingbeat frequencies ranging from 70.5 Hz - 99.1 Hz; within this group, the white-bellied woodstar (*C. mulstant*) weighs nearly twice as much (3.6 grams) as the remaining species (1.8 - 2.2 grams).

Allometry of Flight Morphology

For all hummingbirds, allometric slopes for flight morphology differed from isometry (0.33) for female and male wing lengths (female slope = 0.53, male slope = 0.51) and keel lengths (Table 1.1; female slope = 0.43, male slope = 0.41). Allometric slopes were not different between bees and other hummingbirds (all p > 0.12). Regarding allometric intercepts, male bees had shorter wings relative to all other male hummingbirds (Figure 1.3A; Table 1.2; t = -2.98, df = 81, p = 0.0038). Allometric intercepts for keel length did not differ between bee males and all other male hummingbirds (Figure 1.4A; Table 1.2; $p = 0.093$). In summary, there is an allometric shortening of wing length compared to the rest of the male hummingbirds, but keel length allometry was similar for males across the entire clade (Figure 1.3A, and 1.4A).

In females, comparing bees and all other female hummingbirds, wing length, keel length and hovering wingbeat frequency of was tnot different in allometric slopes or intercepts (Table 1.2) (all $p > 0.15$, pANCOVA). (Figures 1.2B, 1.3B, and 1.4B).

Allometric Sex Differences

In comparison to females, male bees had higher hovering wingbeat frequencies (i.e., a higher allometric intercept) (Figure 1.2A and B; $t = 43.87$, $df = 25$, $p < 0.0001$), relatively shorter wings (Figure 1.3A and B; $t = -12.28$, $df = 25$, $p < 0.0001$), and relatively longer keels (Figure 1.4A and B; $t = 8.33$, $df = 25$, $p < 0.0001$). Thus, bee males are not simply scaled versions of females.

Flight Morphology and Courtship Displays

If female choice for male flight performances has driven female-biased size dimorphism, we predicted significant correlation between flight phenotype (morphology, hovering wingbeat frequency) and the highest wingbeat frequencies attained during courtship-display (Figure 1.2C). After calculating phylogenetic residual values, residual courtship-display wingbeat frequency was negatively correlated with residual wing length (Figure 1.5A; $F_{1,28} = 26.44$, $p < 0.0001$), positively correlated with residual keel length (Figure 1.5B; $F_{1,19} = 6.86$, $p = 0.017$) and positively correlated with residual hovering wingbeat frequency (Figure 1.5C; $F_{1,28} = 45.14$, $p < 0.0001$). Hence, bee species with the greatest increases in wingbeat frequencies during their courtship displays also had the longest keels, shortest wings, and highest hovering wingbeat frequencies.

Among males, wingbeat frequency allometric slopes were not different between the bees and all other male hummingbirds ($p > 0.096$; all slopes approximately -0.5). Instead it is the intercept that changed. Male bees exhibited a higher allometric intercept for hovering wingbeat frequency relative to all other male hummingbirds (Figure 1.2A; Table 1.2; $t = 3.95$, $df = 81$, $p = 0.00017$). This elevation of the allometric intercept is what has produced species (particularly some of the woodstars such as members of the genus *Chaetocercus*) with greatly elevated hovering wingbeat frequencies relative to "typical" hummingbirds.

Evolutionary Rates

Variable rate (VR) models were supported over equal-rate (ER) models in the analyses of evolutionary rates of body mass for males (BF = 5.50) and females (BF = 7.26). In males, rate shifts for the evolution of body mass occurred predominately in the bee hummingbirds (Figure 1.6); only 2 branches exhibited rates > 2 (indicating a rate shift that is greater than twice the background rate of evolution) outside the bee hummingbird clade (Figure 1.6). Thus, branches within the bee clade exhibited higher evolutionary rates of male body mass (average $r = 4.09 \pm 2.31$) than branches outside the bee clade (average $r = 1.35 \pm 0.20$; $t = 11.66$, $df = 161$, $p < 0.0001$). In females, rate shifts in the evolution of body mass also occurred at a higher rate in the bee hummingbird clade (Figure 1.7) (average $r = 4.13 \pm 4.07$) than branches in other hummingbird clades (average $r = 1.43 \pm 0.24$; $t = 4.63$, $df = 100$, $p < 0.0001$).

We found strong support for variation in rates of male hovering wingbeat frequency evolution (Bayes Factor = 27.78). The VR regression model (which included body mass as a dependent variable) identified several evolutionary rate shifts of male hovering wingbeat frequency evolution (Figure 1.8). Branches within the bee clade had an average *r* = 16.02 ± 10.72, whereas branches outside the bee clade average *r* = 2.52 ± 0.95, a significant difference (t = 12.34, df = 161, $p < 0.0001$).

We also found strong support for variable rates of female hovering wingbeat frequency (BF = 70.79). Most of the rate shifts identified by the VR regression model occurred within the bee hummingbird clade (Figure 1.9). The evolutionary rates of female hovering wingbeat frequency for branches within the bee clade (average *r* = 23.04 ± 48.30) were significantly higher than those outside the bee clade (average *r* = 2.01 ± 1.32; t = 3.05, df = 100, p = 0.0030).

Wing length also exhibited variable rates for males (BF = 46.98) and females (BF = 16.99). The VR regression model for male wing length evolution showed rate shifts occurring along several branches, the highest of which were observed in the bee hummingbirds (Figure 1.10). Branches within the bee clade exhibited higher evolutionary rates (average *r* = 32.51 ± 26.04) for male wing length than branches outside the bee clade (average *r* = 3.40 ± 3.99; t = 10.84, df = 161, $p < 0.0001$). Similarly, female wing length also exhibited rate shifts mostly in the bee clade (Figure 1.11), and branches within the bee clade had higher evolutionary rates (average *r* = 16.64 ± 10.86) for female wing length compared to branches outside the bee clade (average *r* = 2.35 ± 1.82; t = 9.09, df = 100, $p < 0.0001$).

The VR regression model for male keel length evolution was supported over an ER model (BF = 5.15), however the VR model was not supported for female keel length (Figure 1.13; BF = 1.73). The VR regression analysis showed evolutionary rate shifts for male keel length the highest of which were found in the bee hummingbirds (Figure 1.12). Branches the bee clade exhibited higher rates for male keel length evolution (average r = 7.59 ± 3.36) compared to branches outside the bee clade (average r = 1.73 ± 0.73; t = 10.66, df = 82, p < 0.0001).

Altogether, analyses of evolutionary rates produced two consistent patterns: 1) variable rates of evolution for body mass, hovering wingbeat frequency, wing length, and male keel length. Only female keel length did not have better support in a variable rates model, versus the null equal rate model. 2) High evolutionary rates in the bee hummingbird clade. In particular, male body mass, hovering wingbeat frequency, wing length, and keel length had the highest evolutionary rates within the bee hummingbird clade (Figures 1.6, 1.8, 1.10, and 1.12). Likewise, evolutionary rates for female body mass, hovering wingbeat frequency, and wing length were also highest in the bee clade (Figures 1.7, 1.9, and 1.11).

Shuttle Display Performance Tradeoff

Shuttle display cycle frequency and amplitude potentially trade off, since organisms are acceleration-limited. Quantile regression showed a significant negative relationship between cycle frequency and shuttle-display amplitude for the 84th-87th quantiles (Figure 1.14A; p = 0.002) suggesting that species that perform in the upper distribution of cycle-frequency and amplitude are influenced by this tradeoff. However, maximal acceleration during the display was not significantly correlated with mass ($p = 0.56$). Our estimations of maximal acceleration based on simple harmonic motion yielded values for a few taxa that are unrealistically high (> 5Gs) (Figure 1.14B). This was likely caused by error in the measurement of shuttle-display amplitude.

Discussion:

Sexual Selection Produces Reversed Size Dimorphism

Hummingbirds are one of the few clades outside of arthropods in which reversed sexual size dimorphism is widespread. What has caused this pattern? Our data allow us to reject the hypotheses that natural selection is the cause of female-biased size dimorphism in bee hummingbirds. If size dimorphism was the product of natural selection for female fecundity or niche differentiation, males and females are predicted to have an isometric flight phenotype. This is not what the data show. Compared to females, male bees have a different suite of inter-related flight morphology and behaviors: they have higher hovering wingbeat frequencies (Figure 1.3B), shorter wings (Figure 1.4B), and longer keels (Figure 1.4D) for their size. These data suggest that sexual selection for male flight performance has driven evolution of female-biased size dimorphism, because they imply that males are under selection for flight demands that differ from those encountered by females. The two likeliest flight demands that could impose different selection on males than females are male-male competition for breeding territories, or female choice for aerial displays.

Regarding male-male competition, might small size confer advantages during aerial defense of breeding territories and other forms of male-male competition? Fights and other agonistic interactions are an important component of hummingbird territorial behaviors. Male territorial behavior includes frequent high-speed chases as well as actual physical fights, in which birds contact and hit each other with the bill (which is weaponized: Rico-Guevara and Araya-Salas, 2015) and wings, and sometimes grab and grapple with the feet. Whether winning a fight is a function of the amount of force one hummingbird can hit with the wings or peck with the beak (in which case large size should be favored); or whether fights are also mediated by the ability to dodge and turn (in which case small size could be favored) remains unknown.

The role of body size in hummingbird agonistic interactions is complex. Larger species tend to be favored in interspecific dominance hierarchies at patches of flowers or feeders (Colwell, 1989; López-Segoviano et al., 2018), suggesting that body size is positively correlated with fighting ability. But this is not a hard rule: under some ecological circumstances, smaller species outcompete larger species. For example, the smaller rufous hummingbird (*Selasphorus rufus*) is dominant over broad-tailed hummingbird (*S. platycercus*) at low elevations (Altshuler, 2006). The likely reason is broad-tailed hummingbird is a high-elevation specialist (e.g. it has relatively long wings). If interspecific differences in body size correlate with fighting ability, it is possible that intraspecific differences in body size do as well. Even if male-male competition could in some circumstances select for small males, there is no evidence that male-male competition is tied to a phenotype built for an increased wingbeat frequency. During territorial chases, there is no evidence that male hummingbirds increase their wingbeat frequencies to the same extreme extent observed during courtship displays as we show here. For instance, wingbeat frequency remains nearly unchanged as a function of flight speed in a wind tunnel (Clark and Mistick 2018; Tobalske, 2007). Thus, there is no direct evidence that production of ultra-high wingbeat frequencies that we document here (Figure 2C) is tied to male-male competition. So, although we do not have data that allow us to firmly reject the hypothesis that small male size is the product of selection for maneuverability during agonistic interactions, the available data suggest this explanation is less likely than female choice.

Selection for courtship-display performance is the more likely explanation for small male size within the bee hummingbirds. Males of nearly all species in this clade, when courting a female, greatly increase their wingbeat frequency (Figure 1.2C). Shorter wings and longer keels are correlated with elevated wingbeat frequency after accounting for body size, as well as with courtship-display performance (Figure 1.5A, 1.5B). That is, the species with males that have especially short wings and especially long keels (with big flight muscles attached) also elevate their wingbeats to especially high frequencies during courtship (Figure 1.5A, 1.5B). This strong residual correlation between morphology and courtship behavior (Figure 1.5A, 1.5B) implies that female choice for high wingbeat-frequency courtship-display performances has driven the evolution of male flight morphology, and along with it, small male size.

Male bees in general, and within bee hummingbirds a subclade called the woodstars recapitulates this pattern: male bees and, in particular, woodstars have evolved shorter wings (Figure 1.3A, 1.10) and higher wingbeat frequencies for their sizes (Figures 1.2A, 1.8) than other hummingbirds. For example, Greenewalt (1962) reported that the maximum wingbeat frequency of a hummingbird was 80Hz for the (male) amethyst-throated woodstar (*Calliphlox amethystina*). This value has stood, until now, as the highest published hovering wingbeat frequency for a hummingbird (Greenewalt, 1960; Warrick et al., 2012). This value, 80 Hz, is substantially greater than the wingbeat frequency of the very smallest hummingbird, the male bee hummingbird *Mellisuga helenae* (Figure 1.2C; 66 Hz). Here we present data for nearly every species of woodstar. Three woodstar species have hovering wingbeat frequencies that substantially exceed 80 Hz: males of *Chaetocercus burmeisteri* (96.6 Hz); *C. heliodor* (93.9 Hz); and *C. bombus* (99.1 Hz) have hovering wingbeat frequencies of nearly 100 Hz (Figure 1.2A).

100 Hz is a remarkably high rate of appendage oscillation for a vertebrate: these ~2-gram hummingbirds have hovering wingbeat frequencies that exceed that of most insects (Greenewalt, 1962)! Whereas *Drosophila* or honeybee (*Apis mellifera*) have greater wingbeat frequencies in absolute terms (both are ~200 Hz), these insects are a small fraction of the body mass of these hummingbirds, and they have such high wingbeat frequencies because they power their flight with indirect flight muscles (Pringle, 1949).

Evolution of Elevated Wingbeat Frequency

Previous studies of hummingbird flight have tended to treat hummingbird wingbeat frequency in simple allometric terms, as if all species and the two sexes are simply scaled versions of each other (Greenewalt, 1962; Altshuler 2003; Skandalis et al., 2017). For instance, lab studies of hummingbird flight performance often study only males, implicitly considering male flight morphology as the naturally-selected optimum for efficient flight. Authors sometimes averaged male and female wingbeat frequencies to present species averages (Greenewalt, 1962; Altshuler, 2000). Our data, including extensive new sampling of previously unmeasured species within the woodstars, suggest a more nuanced view is warranted. Bee males, woodstars in particular, and within woodstars, especially *Chaetocercus,* are not simply scaled versions of other hummingbirds. Their wingbeat frequencies are substantially higher than expected for their body sizes. Bee hummingbird species show elevated evolutionary rates for male hovering wingbeat frequency compared to most other male hummingbirds (Figure 1.8). Simply put, sexual selection has pushed several bee hummingbirds, and especially *Chaetocercus* woodstar males, into a new allometric space for hovering wingbeat frequency (Figure 1.2A, 1.8; Table 1.1). Lab studies of hummingbird flight performance would do well to expressly measure members of both sexes.

Other flying animals also perform challenging displays that can include substantial increases of wingbeat frequency. For example, male flappet larks (*Mirafra rufocinnamomea*) double their wingbeat frequencies (up to 24 Hz) during flight displays which may demand high muscle power output (Norberg, 1991). Male greater sac-winged bats (*Saccopteryx bilineatus*) perform hovering displays to females (Voigt and von Helverson, 1999), and small males tend to produce more offspring than larger individuals (Voigt et al., 2005). Male mosquitoes modulate their wingbeat frequency to match harmonics with female wingbeats (Cator et al., 2009), which can include increases in wingbeat frequency by ~175 Hz (a ~41% increase) above normal wingbeat frequency (~425 Hz; Gibson and Russell, 2006). Sexual selection for aerial display behaviors has been suggested to result in female-biased size dimorphism in shorebirds (Jehl and Murray, 1986; Figuerola, 1999; Blomqvist et al., 1997; Székely, 2000), and bustards (Raihani et al., 2006). Shorebird displays have not been described in any detail, thus what aspects of flight performance may be under selection are not entirely clear. Bustards are famous for dimorphism: most species are large, with male-biased dimorphism, and male size is positively correlated with ability to win male-male fights for lek territories (Alonso et al., 2009; Fairbairn, 2013). Only the smallest species of bustard has female-biased dimorphism: the lesser florican (*Sypheotides indicus*), males of which perform a remarkable leap display in which they repeatedly jump 2 meters in the air and flutter their wings to produce a sonation to advertise their territories to females (Dharmakumarsinhji, 1950).

Maneuverability and Flight Performance

Why courtship-display wingbeat frequency is elevated so dramatically during courtship remains unclear. Our results might appear to be at odds with recent research on hummingbird maneuverability. Hummingbird flight biomechanics has been extensively examined in laboratory studies. One general finding of a range of studies on different aspects of performance is how little hummingbird wingbeat frequency changes under fairly different flight conditions. In assays of top speed (Chai and Dudley, 1999; Clark and Dudley 2010), asymptotic load lifting (Chai et al., 1997; Chai and Millard, 1997; Altshuler, 2006; Groom et al., 2017) and flight in hypodense air (Chai and Dudley, 1995; Chai et al., 1996; Chai and Dudley, 1996; Altshuler and Dudley, 2003), wingbeat frequency changes little. The greatest increases in wingbeat frequency are reported from studies of maneuvering flight (Cheng et al., 2016a, 2016b) during which *Eugenes fulgens* and *Archilochus alexandri*, increased wingbeat frequency relative to hovering by 47% and 16%, respectively. In contrast to this lab result, displaying male *A. alexandri* increase wingbeat frequency by 90% relative to hovering (Feo and Clark, 2010). As shown here, this type of dramatic increase in wingbeat frequency is observed in most species in this clade (Figure 1.2C), most species elevate wingbeat frequency by at least 50%. No laboratory study of flight performance has documented anywhere near a 90% increase in wingbeat frequency. The lack of wingbeat frequency increase reported in laboratory studies of flight performance is not reflective of what happens in nature during courtship.

Moreover, previous authors have found that longer wing length is positively correlated with a hummingbird's willingness to perform voluntary (i.e., non-maximal) low-speed "pitch/roll" and "arcing turn" maneuvers (Segre et al., 2015; Dakin et al., 2018). Wing length is also positively correlated with the ability to produce downwardly directed thrust to lift a load (Altshuler et al., 2006). Are these lab results general, are longer wings always better for flight performance? Here we show that, in the context of high wingbeat frequency courtship displays, short wings seem to be favored (Figure 1.5A), likely because shorter wings are a part of the flight phenotype that allows for elevated wingbeat frequency. There are two plausible explanations for this apparent discrepancy between lab experiments on flight performance, and our field data on courtship: either the shuttle display does not maximize some aspect of flight performance per se; or lab experimental paradigms developed to study hummingbird flight performance may not paint a complete picture of what constitutes hummingbird flight performance. That is, some important region of flight performance parameter space has not been explored by these previous experiments.

The first alternative, that the shuttle display does not showcase flight performance, is plausible. Rephrased, this hypothesis states that perhaps reversed sexual size dimorphism has not evolved for agility, maneuverability, or nimbleness *per se*. Perhaps small male size is just about increasing wingbeat frequency: The shuttle display might entail a behavior that is intrinsically difficult to perform (i.e., dramatically elevated wingbeat frequency) but in ways that are unconnected to the animal's ability to accelerate or turn. For instance, elevating wingbeat frequency produces changes in wing-generated sounds and about half the bees have evolved to produce extra sound with modified wing feathers during the shuttle display (Clark, 2011; Clark et al., 2018). Production of these sounds could be uncoupled from flight performance (Clark and Mistick, 2018). Likewise, the shuttle display is also a visual display, including iridescent throat feathers (Simpson and McGraw, 2018a, 2018b), and strikingly colored tail feathers, which in certain species are held spread or flicked from side to side (Figure 1.1). Since assays of female preference have not been developed for hummingbirds (Hogan and Stoddard, 2018), it is unknown to which aspects of display females attend. It remains plausible that the male's performance of this aerial display does not showcase how “good” he is at flying.

The alternative view is that these flight courtship displays push against some aspect of flight performance limits, and thus do have the potential to allow females to assess variation in male flying ability (Clark 2009; Byers et al., 2010; Barske et al., 2011). A solid test of this hypothesis would be to examine whether flight performance differences between male courtship displays (e.g. maximum linear acceleration during the shuttle) is correlated with another aspect of flight performance (Chapter 3). Until then, the best current evidence that hummingbird courtship displays showcase extreme flight performance are our estimates of the translational accelerations during the shuttle display, which are very high in certain species (Figure 1.14). (Note: the highest accelerations reported in Fig. 1.14A we regard to be overestimates, the result of measurement error). The general picture offered by these data are that many shuttle displays entail linear accelerations on the order of 30 m s^{-2}, three times the force of gravity. This is high for a hummingbird engaged in a low speed, horizontal maneuver; maximum linear accelerations during take-off reported in Tobalske et al. (2004) were 37.4 ± 10.1 m s^{-2}; Clark (2010) reported maximal accelerations of approximately 25 m s^{-2} in a horizontal maneuver in response to a startle stimulus. Sholtis et al. (2015) reported average accelerations of 14.6 ± 5.7 m s^{-2} for hummingbirds chased during territorial disputes. Thus, shuttle display linear accelerations are roughly the same as reported in other studies on maximal hummingbird flight performance. But if this is the case, that hummingbird courtship displays exhibit relatively extreme flight performance, then we have not resolved the discrepancy: lab assays suggesting long wings enhance performance stand in sharp contrast against the data presented here that show males of some species evolve short wings to enhance wingbeat frequency.

We suggest the explanation for this apparent discrepancy is that lab tests of flight performance do not assay all aspects of flight performance that have ecological relevance. The maneuvers studied by Segre et al. (2015) and Dakin et al. (2018) primarily constituted voluntary “pitch/roll” and “arcing” turns. Neither of these maneuver types is all that similar to shuttle display maneuvers. Specifically, neither of these maneuver types entail all that much sideslip (sideways or lateral motion). In the shuttle display, by contrast, in every single species, the male remains facing towards the female while flying side to side (Figure 1.1E, 1.1F). Hence, the shuttle display tends to include substantial sideslip (lateral motion). Precisely how much is not clear (and there is substantial interspecific variation), since the detailed wing and body kinematics of a shuttle display have not yet been described for any species. A general theoretical model of flight performance predicts that elevated wingbeat frequency aids in turning (Hedrick et al., 2009), such as during sideslip. Thus, our results imply short wings (and increased muscle size) are beneficial during courtship behaviors of some species, and the maneuvers flown in courtship displays may have little overlap in performance-space with the types of maneuvers that have been the subject of previous biomechanical lab studies.

The dimorphism of keel length in the bee clade (Figure 1.4A, 1.4B) implies that males are selected to have larger flight muscles to achieve high flight performance during courtship-display behaviors. However, keel length scales similarly among males across hummingbirds of all sizes (Figure 1.4A), that is, large species outside of the bee clade (for which we have no data on their courtship displays) also have dimorphic keel lengths. Keel length dimorphism may be the result of sexual selection on male flight performance in general. Male hummingbirds tend to be more territorial than females during the breeding season (Pitelka, 1942; Altshuler et al., 2004). Thus, male keel length is sexually selected, but the relative roles of female choice for aerial displays and male-male competition remain unclear for the evolution of keel length dimorphism in hummingbirds in general. Large-bodied males may be selected to maintain high flight performance (and therefore longer keels) to better defend territories and win fights via forceful aerial combat, whereas the small bee species are under higher sexual selection pressure via female choice to perform aerial courtship display behaviors that require high accelerations.

Our estimates of shuttle display acceleration were not significantly correlated with male mass (Figure 1.14A), contrary to our hypothesis that display accelerations would decline with larger body size. Our estimates of shuttle-display amplitudes were crude for some species, but we do not think this measurement error is what accounts for the lack of a relationship between display acceleration and mass (Figure 1.14A). Rather, males of some species clearly do not perform shuttle displays in ways that push against a frequency-amplitude performance limit. For example, Calliope hummingbird (*Selasphorus calliope*) remain essentially stationary during the shuttle display, so this small species has a shuttle amplitude of zero, and thus display accelerations of zero. The relationship between shuttle-display cycle frequency and amplitude (Figure 1.14B) was negative at higher distributions (i.e., upper quantiles) of the data, suggesting a tradeoff. Hence, of the species for which we present cycle frequency-amplitude data, only a few may be subject to a tradeoff, whereas other species seem to have evolved away (i.e., away from the upper quantiles of the distribution) from this tradeoff (Figure 1.14B). These species may reflect situations in which sexual selection has favored other components of the display (e.g. a visual components of display), over translational and rotational accelerations of the shuttle-display flight path, since courtship-display wingbeat frequency is consistently elevated during display behaviors across all species (Figure 1.2C).

Implications for Muscle Physiology

The greatly elevated courtship-display wingbeat frequencies we report here raise interesting questions about hummingbird muscle physiology. The flight muscles (i.e., the pectoralis and supracoracoideus) are ~25% of body mass (Hartman, 1954) and move the wings. The maximal contraction rates of hummingbird flight muscles have not been measured directly, but are presumably the same as the wingbeat frequency. Previous research on manakins (Pipridae), which snap their wings during aerial courtship displays, report muscle contraction rates of up to 63 Hz (Fuxjager et al., 2016; Bostwick and Prum, 2003). The courtship-display wingbeat frequencies we describe here, particularly for certain species of woodstar, are far higher. The very highest wingbeat frequency in our sample is for *Chaetocercus berlepschi*, which very briefly attains 132 Hz at the bottom of its dive. This value likely represents the absolute maximum wing muscle contraction velocity; it occurs at a moment in the dive in which the wings are likely not supporting body weight (i.e., under low force conditions). Another record for sustained motor output is set by *Calliphlox amethystina*, which in displays can flap its wings at 112 Hz while hovering for > 30 seconds, i.e. with muscle contractions that *are* producing enough force to support body weight, and are sustained. How the muscles can flap this fast without going into tetanus, or indeed, while producing any force at all, remains unknown. The general tradeoffs among force production, muscle size, and contraction velocity are inherent to performance limits of muscle tissue (Rome et al., 1996), and must apply here in some way. In a comprehensive report on hummingbird anatomy, Zusi (2013) reports that woodstars alone have evolved a specialized (autapomorphic) "Type IV" wing muscle (M. tensor propatagialis, par brevis). Hummingbird males are certain to approach some sort of intrinsic muscle-contraction-rate limit during their courtship display, although we do not know what exactly this limit may be. For instance, the drastic increases in wingbeat frequencies during courtship-display behaviors may approach a fundamental limit set by the time available for muscle relaxation such that antagonistic flight muscles do not interfere with one another (Tobalske et al., 2010). Further research is needed to investigate the force-velocity tradeoff in hummingbird flight muscles and limits to shuttle-display performance.

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Figures:

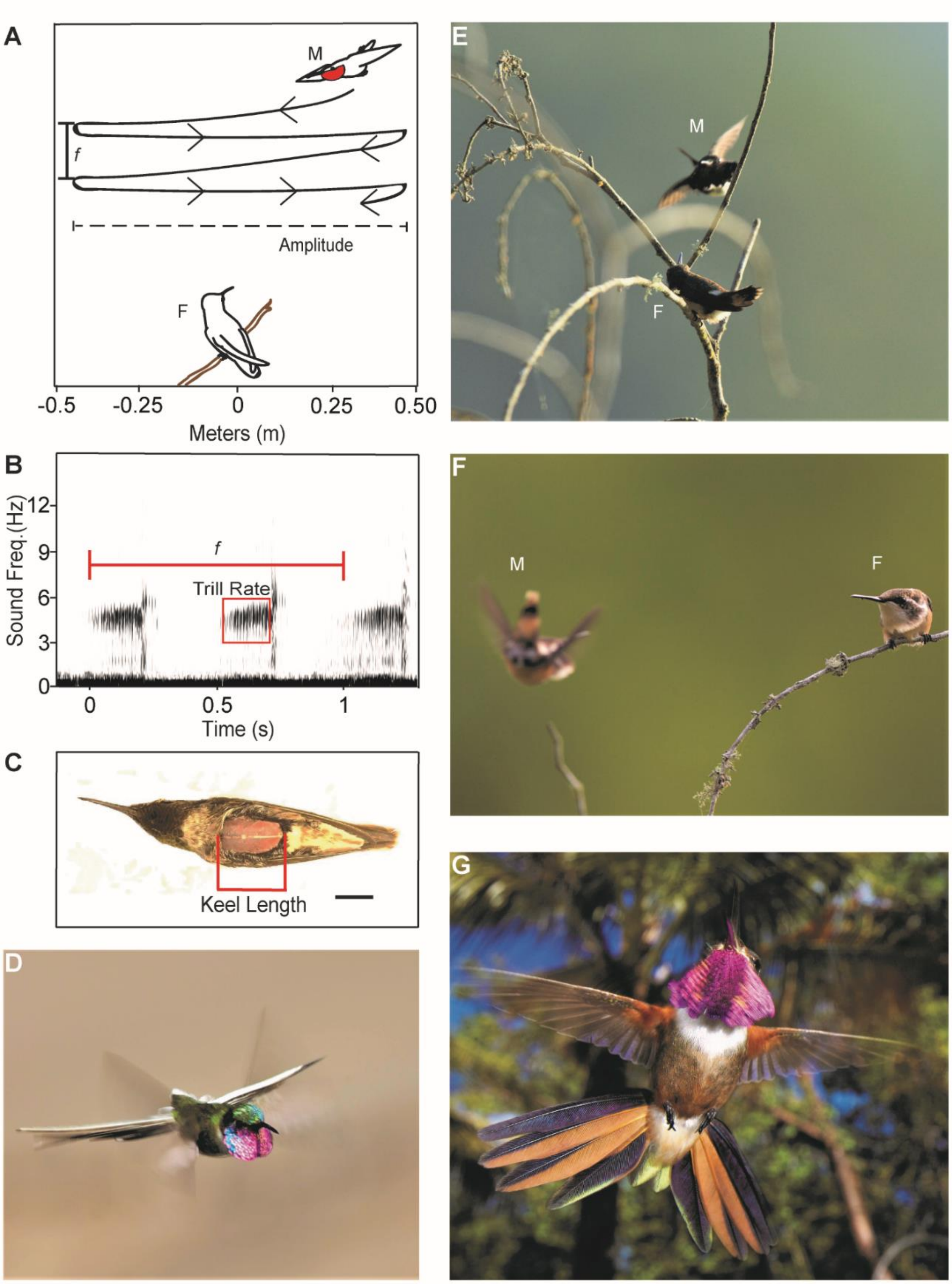

A
M
f
Amplitude
F
-0.5
-0.25
0
0.25
0.50
Meters (m)
B
Sound Freq.(Hz)
12
9
6
3
0
f
Trill Rate
0
0.5
1
Time (s)
C
Keel Length
D
E
M
F
F
M
F
G

Figure 1.1. Shuttle display characteristics and keel length in bee hummingbirds. **(A)** Typical trajectory of shuttle display performed by a male hummingbird (M) to a female hummingbird (F). Arrows along the solid black line represent the direction of the flight path. Shuttle-display cycle frequency is labeled *f*. Amplitude (dashed black line) is the translational distance (in meters) between two turns at either end of the oscillatory flight path. **(B)** A spectrogram of a black-chinned hummingbird (*Archilochus alexandri*) shuttle display. Shuttle-display cycle frequency (red bracket) is easy to measure from display sounds. Red box: Trill rate of wing sounds corresponds to the wingbeat frequency. **(C)** Keel length (red bracket) Scale bar: 10 mm. **(D-G)** Male hummingbirds performing shuttle displays to females. **D**: *Thaumastura cora*, **E, F**: *Chaetocercus berlepschi*, **G**: *Calliphlox evelynae;* Photographs courtesy Anand Varma.

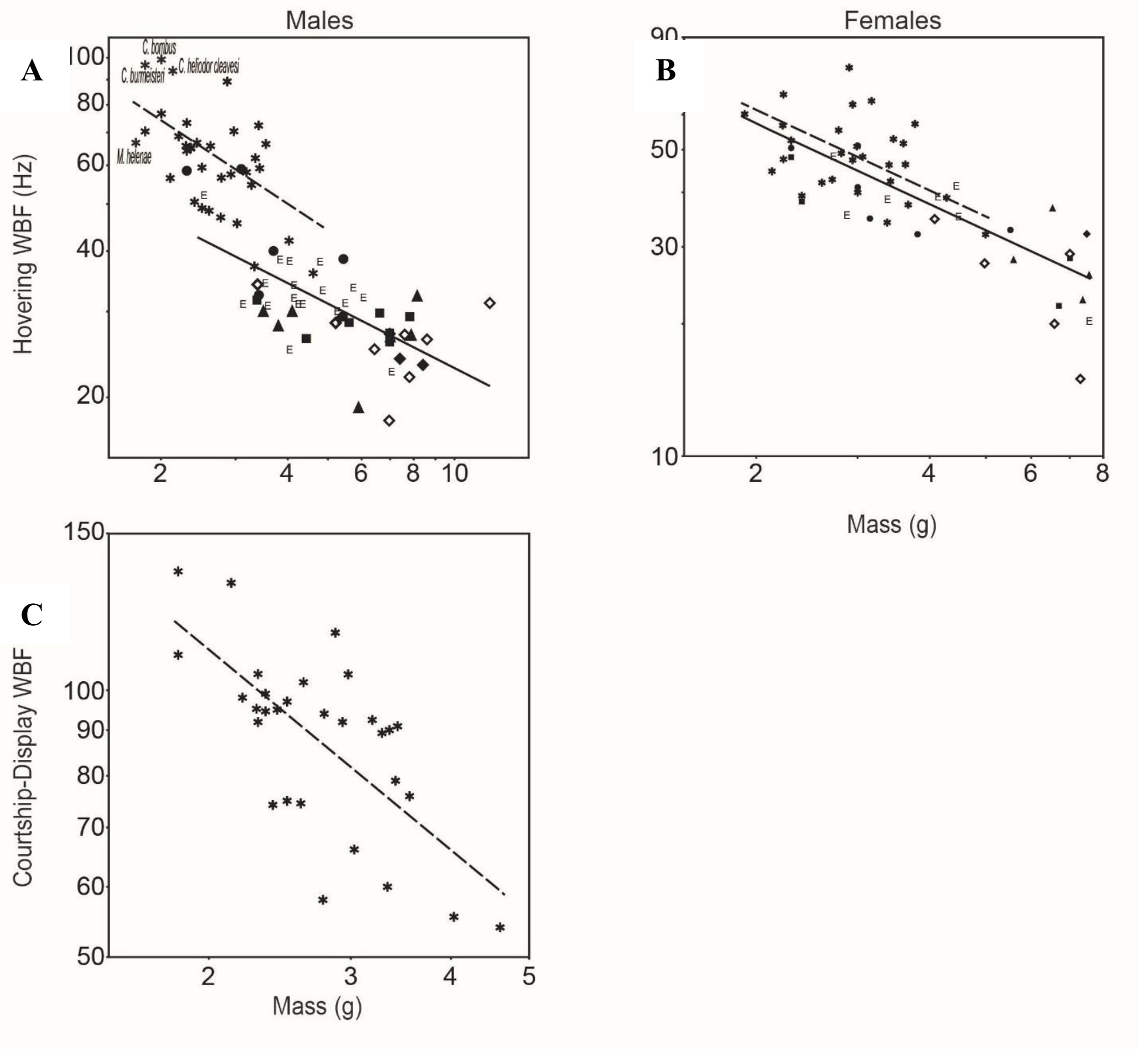

A
Males
Hovering WBF (Hz)
C. bombus
C. burmeisteri
C. heliodor cleavesi
M. helenae
100
80
60
40
20
2
4
6
8
10
B
Females
90
50
30
10
2
4
6
8
Mass (g)
C
Courtship-Display WBF
150
100
90
80
70
60
50
2
3
4
5
Mass (g)

Figure 1.2. Hummingbird wingbeat frequency regressed against body mass from PGLS regressions. **(A)** Male hovering wingbeat frequency against mass. Bee males (dashed line: $y = -0.57x + 110.2$) have significantly higher hovering wingbeat frequencies ($t = 3.95$, $df = 81$, $p = 0.00017$) compared to all other male hummingbirds (bee males excluded, dotted line: $y = -0.44x + 63.2$; Solid line: all male hummingbirds: $y = -0.38x + 63.0$). **(B)** Female hovering wingbeat frequency against mass (All females: $y = -0.50x + 71.6$). Bee males (**A** dashed line) have significantly higher hovering wingbeat frequencies ($t = 43.35$, $df = 25$, $p < 0.0001$) for their sizes compared to their female counterparts (**B** dashed line: $y = -0.54x + 78.4$). **(C)** Courtship-display wingbeat frequency ($y = -0.75x + 189.1$) scaled negatively with mass but not more negatively than our scaling prediction of -0.50 (Table 1). Data points are coded based on major hummingbird clades: squares are Topazes and Hermits, triangles are Mangoes, open diamonds are Brilliants, closed circles are Coquettes, closed diamonds are Mountain Gems, and stars are Bees. *Mellisuga helenae, Chaetocercus burmeisteri, C. bombus,* and *C. heliodor cleavesi* are labeled because they mentioned in text (see Discussion). Note $\log_{10}$ scales.

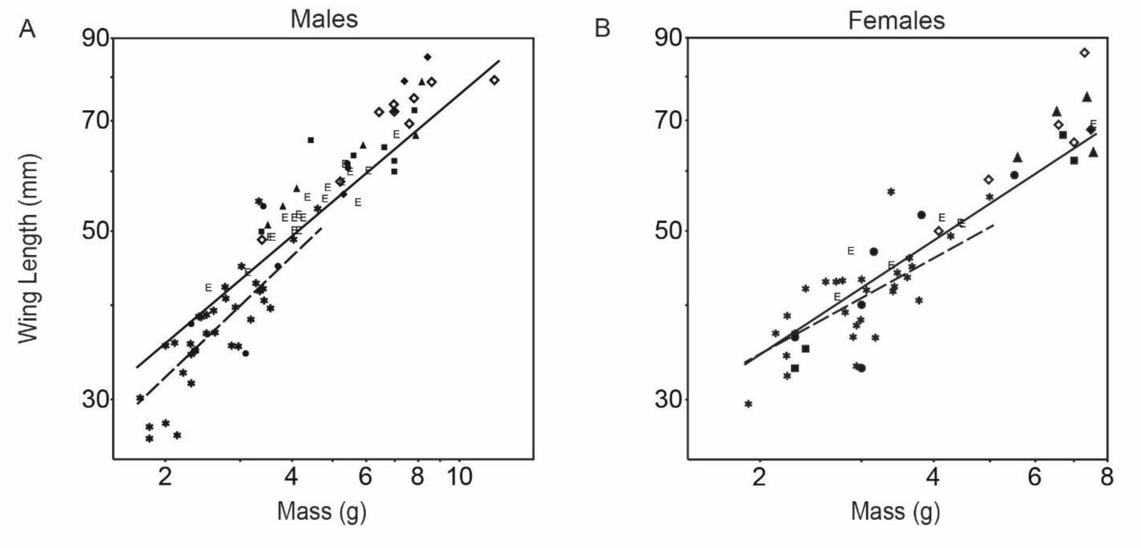


Figure 1.3. Wing length allometry for male hummingbirds (Left), and females (Right), from PGLS regressions. **(A)** bee males (dashed line: y = 0.53x + 22.32) have shorter wings (t = -2.98, df = 81, p = 0.0038) than other male hummingbirds (solid line: y = 0.47x + 26.42). **(B)** Female wing length against mass (All females solid line: y = 0.50x + 24.55; bee females dashed line: y = 0.42x + 25.56). Bee males (**A** dashed line) have shorter wings (t = -12.27, df = 25, p < 0.0001) for their sizes compared to bee females (**B** dashed line). Data points are coded based on major hummingbird clades: squares are Topazes and Hermits, triangles are Mangoes, open diamonds are Brilliants, closed circles are Coquettes, closed diamonds are Mountain Gems, and stars are Bees. Note $\log_{10}$ scales.

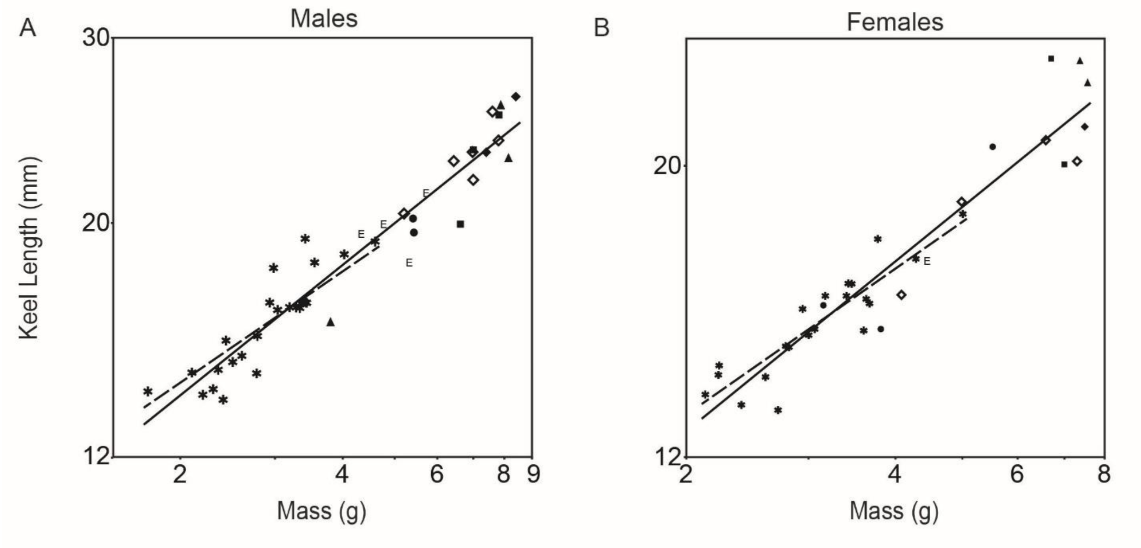


Figure 1.4. Keel length allometry of male (Left) and female (Right) hummingbirds, from PGLS regressions. **(A)** Males (**A** solid line: $y = 0.41x + 10.38$) tend to have longer keels than **(B)** females (**B** solid line: $y = 0.43x + 9.38$) in general ($t = 10.28$, $df = 46$, $p < 0.0001$), and in the bee clade (**A** dashed line and **B** dashed line: $t = 8.33$, $df = 25$, $p < 0.0001$). Data points are coded based on major hummingbird clades: squares are Topazes and Hermits, triangles are Mangoes, open diamonds are Brilliants, closed circles are Coquettes, closed diamonds are Mountain Gems, and stars are Bees. Note, plots are in $\log_{10}$ scales.

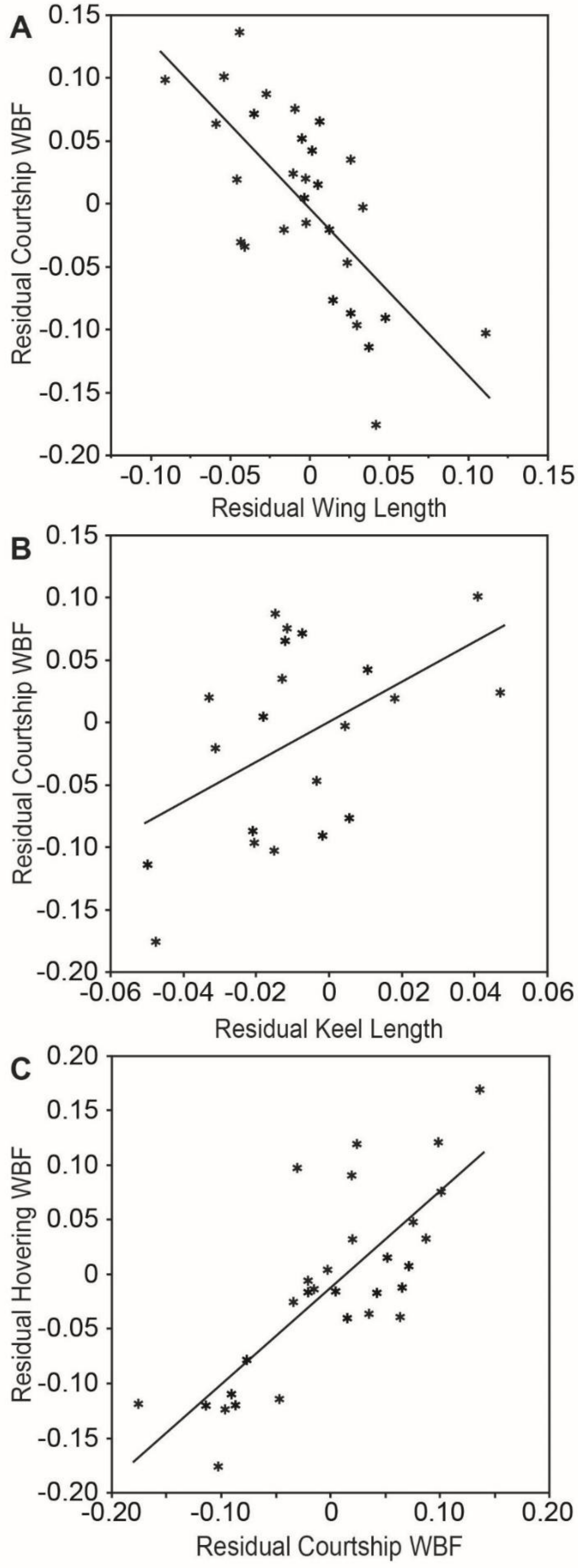

A
0.15
0.10
0.05
0
-0.05
-0.10
-0.15
-0.20
Residual Courtship WBF
-0.10
-0.05
0
0.05
0.10
0.15
Residual Wing Length
B
0.15
0.10
0.05
0
-0.05
-0.10
-0.15
-0.20
Residual Courtship WBF
-0.06
-0.04
-0.02
0
0.02
0.04
0.06
Residual Keel Length
C
0.20
0.15
0.10
0.05
0
-0.05
-0.10
-0.15
-0.20
Residual Hovering WBF
-0.20
-0.10
0
0.10
0.20
Residual Courtship WBF

Figure 1.5. Flight morphology and hovering wingbeat frequency correlate with courtship-display wingbeat frequency. Residual courtship-display wingbeat frequency is **(A)** negatively correlated ($F_{1, 28} = 26.44$, $R^2 = 0.49$, $p < 0.0001$) with residual wing length, and **(B)** positively correlated ($F_{1, 19} = 6.86$, $R^2 = 0.27$, $p = 0.017$) with residual keel length. **(C)** Residual courtship-display wingbeat frequency is positively correlated ($F_{1, 28} = 45.14$, $R^2 = 0.62$, $p < 0.0001$) with residual hovering wingbeat frequency.

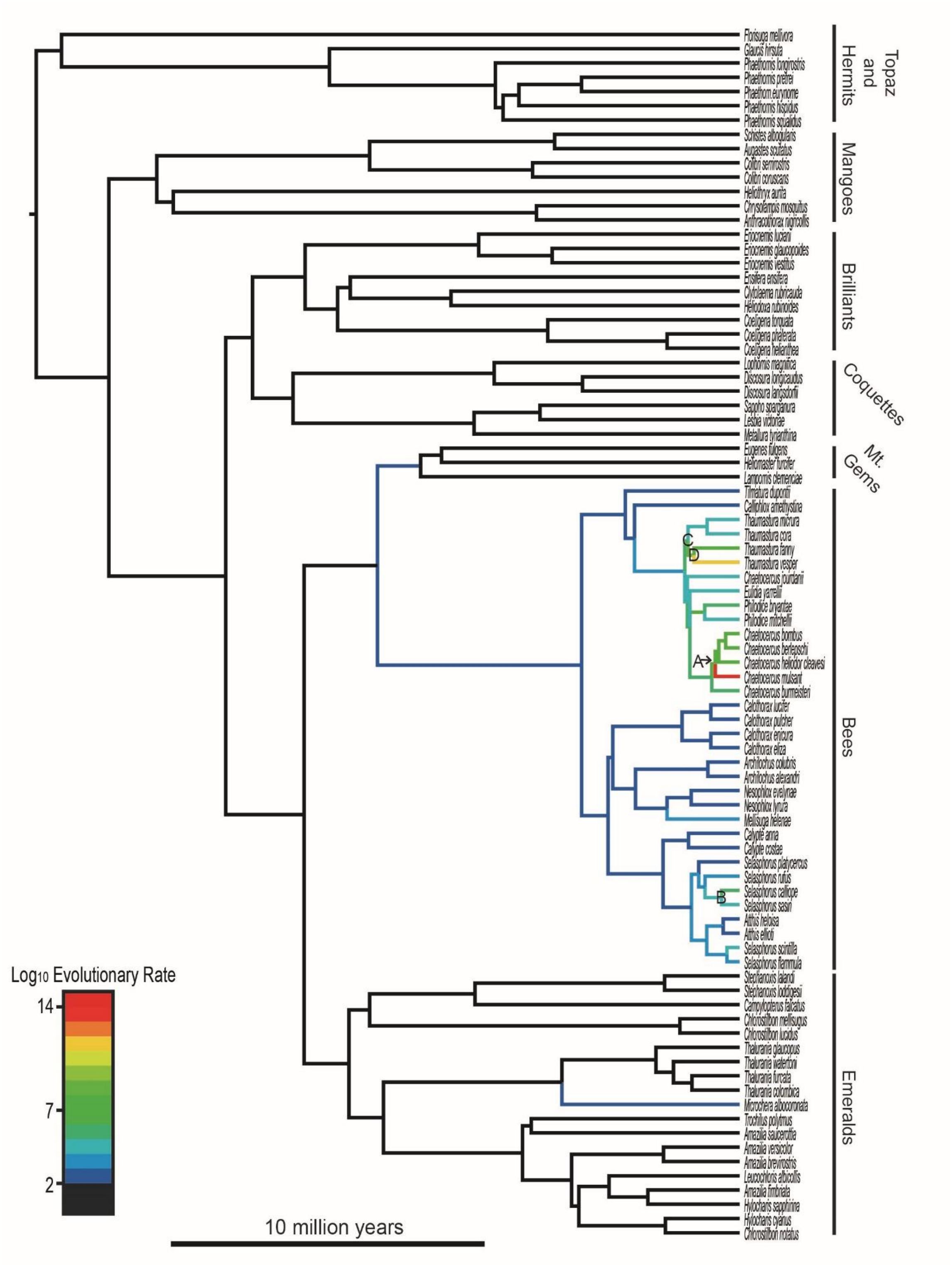
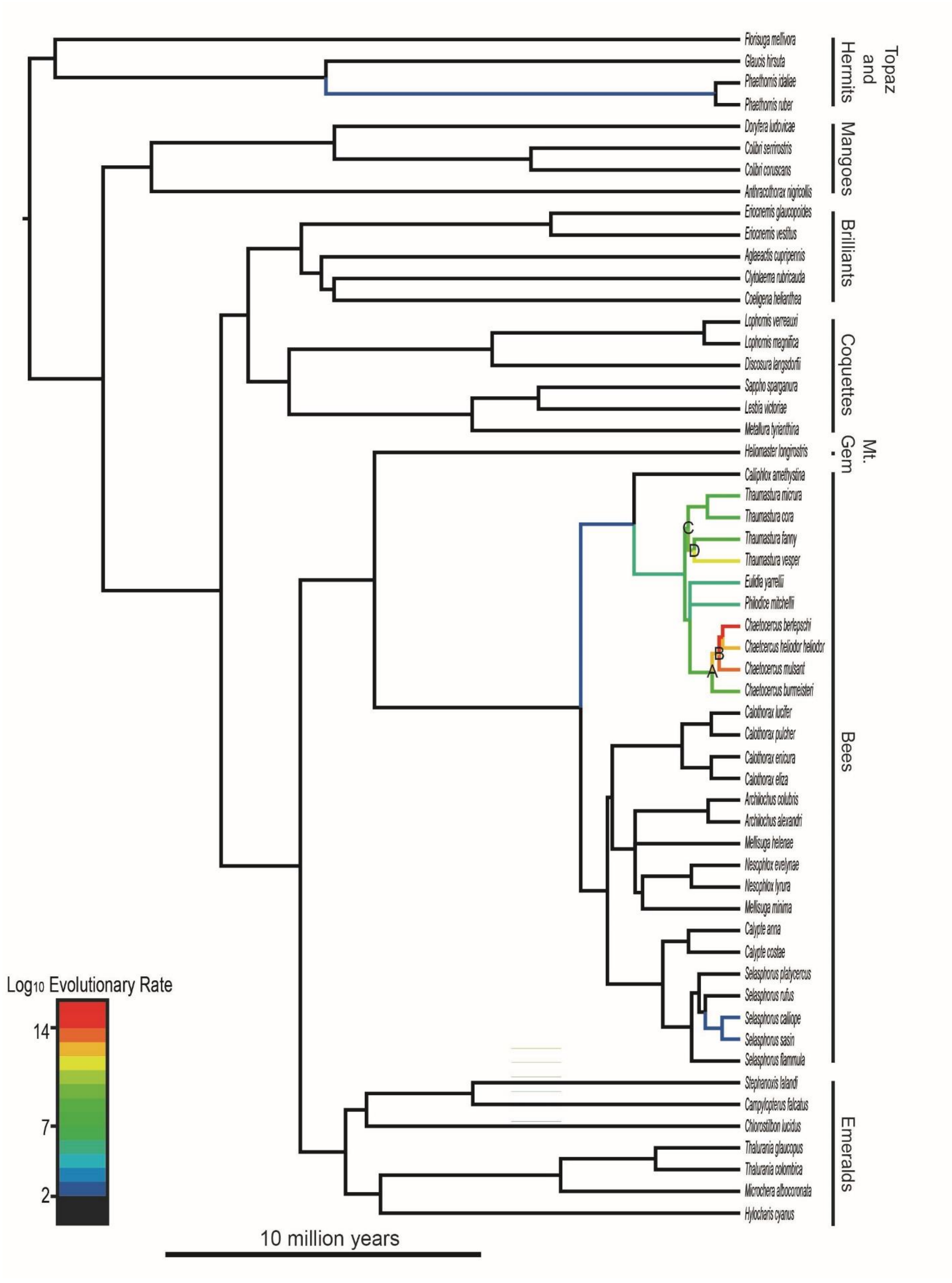

Florisuga mellivora
Glaucis hirsuta
Phaethornis longirostris
Phaethornis pretrei
Phaethorn.eurynome
Phaethornis hispidus
Phaethornis squalidus
Schistes albogularis
Augastes scutatus
Colibri serrirostris
Colibri coruscans
Heliothryx aurita
Chrysolampis mosquitus
Anthracothorax nigricollis
Eriocnemis luciani
Eriocnemis glaucopoides
Eriocnemis vestitus
Ensifera ensifera
Clytolaema rubricauda
Heliodoxa rubinoides
Coeligena torquata
Coeligena phalerata
Coeligena helianthea
Lophornis magnifica
Discosura longicaudus
Discosura langsdorfii
Sappho sparganura
Lesbia victoriae
Metallura tyrianthina
Eugenes fulgens
Heliomaster furcifer
Lampornis clemenciae
Tilmatura dupontii
Calliphlox amethystina
Thaumastura micrura
Thaumastura cora
Thaumastura fanny
Thaumastura vesper
Chaetocercus jourdanii
Eulidia yarrellii
Philodice bryantae
Philodice mitchellii
Chaetocercus bombus
Chaetocercus berlepschi
Chaetocercus heliodor cleavesi
Chaetocercus mulsant
Chaetocercus burmeisteri
Calothorax lucifer
Calothorax pulcher
Calothorax enicura
Calothorax eliza
Archilochus colubris
Archilochus alexandri
Nesophlox evelynae
Nesophlox lyrura
Mellisuga helenae
Calypte anna
Calypte costae
Selasphorus platycercus
Selasphorus rufus
Selasphorus calliope
Selasphorus sasin
Atthis heloisa
Atthis ellioti
Selasphorus scintilla
Selasphorus flammula
Stephanoxis lalandi
Stephanoxis loddigesii
Campylopterus falcatus
Chlorostilbon mellisugus
Chlorostilbon lucidus
Thalurania glaucopus
Thalurania watertonii
Thalurania furcata
Thalurania colombica
Microchera albocoronata
Trochilus polytmus
Amazilia saucerottia
Amazilia versicolor
Amazilia brevirostris
Leucochloris albicollis
Amazilia fimbriata
Hylocharis sapphirina
Hylocharis cyanus
Chlorostilbon notatus
C
D
A
B
Topaz and Hermits
Mangoes
Brilliants
Coquettes
Mt. Gems
Bees
Emeralds
Log10 Evolutionary Rate
14
7
2
10 million years

Figure 1.6. The evolution of mass in male hummingbirds. Results of analysis of evolutionary rates for male mass indicate several rate shifts, the highest of which are within the bee hummingbird clade. Branches are colored based on evolutionary rates of male mass which ranged from 1.10 – 14.61. Letters A, B, C, and D correspond to points in supplementary figures **S1.1 A** and **C**.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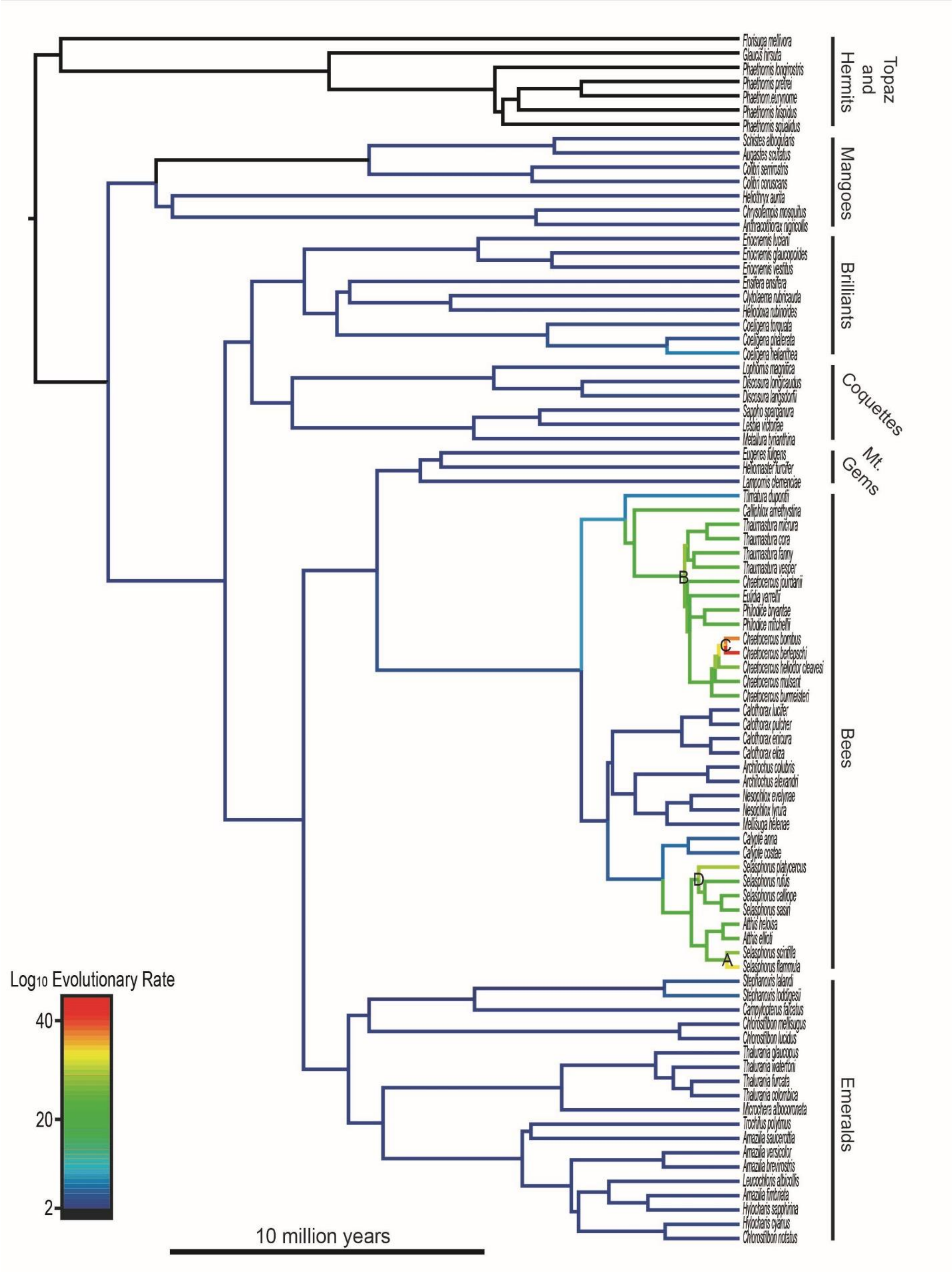
Glaucis hirsuta
Phaethornis idaliae
Phaethornis ruber
Doryfera ludovicae
Colibri serrirostris
Colibri coruscans
Anthracothorax nigricollis
Eriocnemis glaucopoides
Eriocnemis vestitus
Aglaeactis cupripennis
Clytolaema rubricauda
Coeligena helianthea
Lophornis verreauxi
Lophornis magnifica
Discosura langsdorfii
Sappho sparganura
Lesbia victoriae
Metallura tyrianthina
Heliomaster longirostris
Calliphlox amethystina
Thaumastura micrura
Thaumastura cora
Thaumastura fanny
Thaumastura vesper
Eulidia yarrellii
Philodice mitchellii
Chaetocercus berlepschi
Chaetcercus heliodor heliodor
Chaetocercus mulsant
Chaetocercus burmeisteri
Calothorax lucifer
Calothorax pulcher
Calothorax enicura
Calothorax eliza
Archilochus colubris
Archilochus alexandri
Mellisuga helenae
Nesophlox evelynae
Nesophlox lyrura
Mellisuga minima
Calypte anna
Calypte costae
Selasphorus platycercus
Selasphorus rufus
Selasphorus calliope
Selasphorus sasin
Selasphorus flammula
Stephanoxis lalandi
Campylopterus falcatus
Chlorostilbon lucidus
Thalurania glaucopus
Thalurania colombica
Microchera albocoronata
Hylocharis cyanus
C
D
B
A
Topaz and Hermits
Mangoes
Brilliants
Coquettes
Mt. Gem
Bees
Emeralds
Log10 Evolutionary Rate
14
7
2
10 million years

Figure 1.7. The evolution of mass in female hummingbirds. Results of analyses of evolutionary rates for female mass indicate several rate shifts, the highest of which are within the bee hummingbird clade. Branches are colored based on evolutionary rates of female mass which ranged from 1.11 – 15.48. Letters A, B, C, and D correspond to points in supplementary figure **S1.1 B** and **D**.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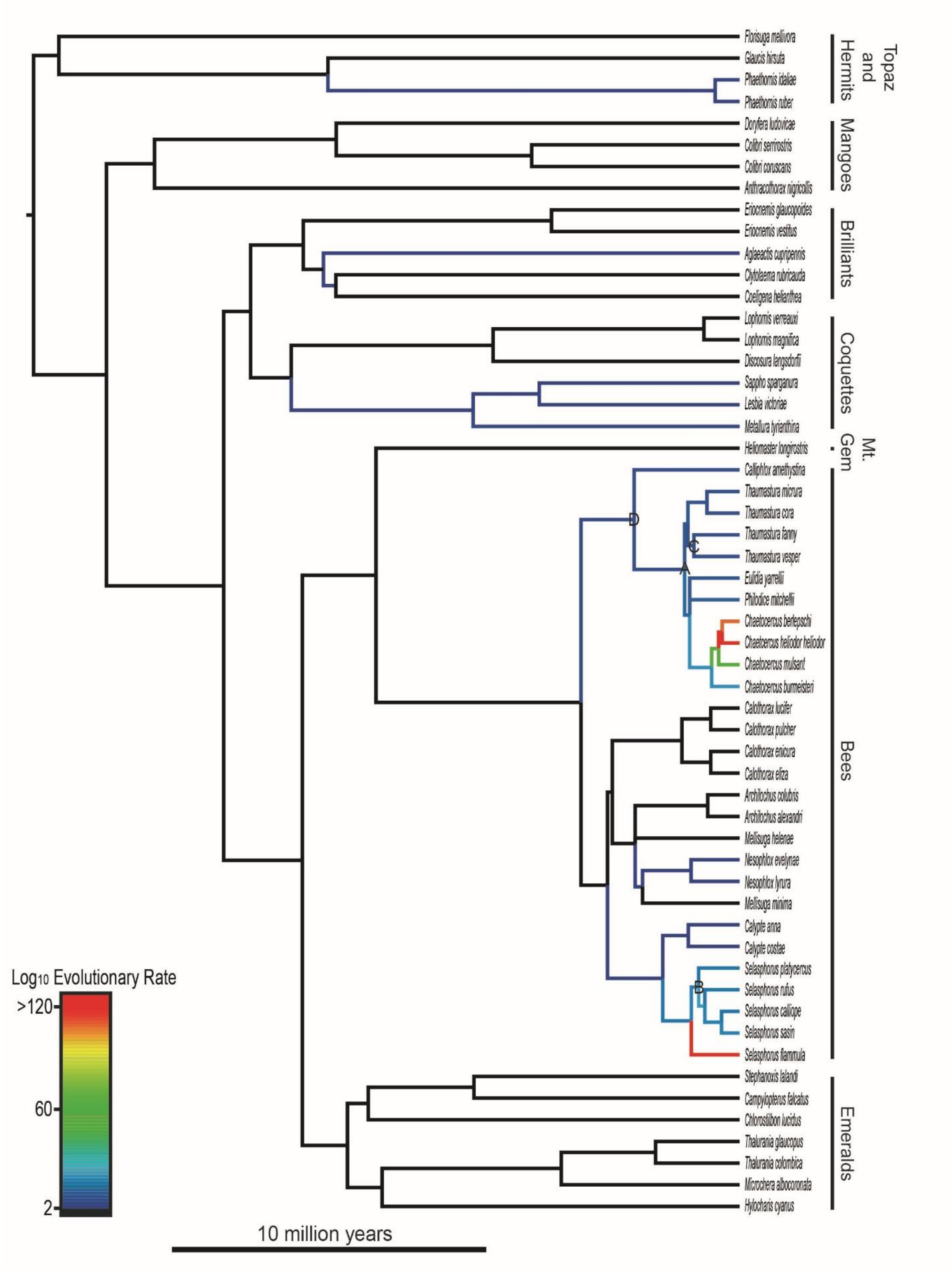
Phaethornis longirostris
Phaethornis pretrei
Phaethorn.eurynome
Phaethornis hispidus
Phaethornis squalidus
Schistes albogularis
Augastes scutatus
Colibri serrirostris
Colibri coruscans
Heliothryx aurita
Chrysolampis mosquitus
Anthracothorax nigricollis
Eriocnemis luciani
Eriocnemis glaucopoides
Eriocnemis vestitus
Ensifera ensifera
Clytolaema rubricauda
Heliodoxa rubinoides
Coeligena torquata
Coeligena phalerata
Coeligena helianthea
Lophornis magnifica
Discosura longicaudus
Discosura langsdorfii
Sappho sparganura
Lesbia victoriae
Metallura tyrianthina
Eugenes fulgens
Heliomaster furcifer
Lampornis clemenciae
Tilmatura dupontii
Calliphlox amethystina
Thaumastura micrura
Thaumastura cora
Thaumastura fanny
Thaumastura vesper
Chaetocercus jourdanii
Eulidia yarrellii
Philodice bryantae
Philodice mitchellii
Chaetocercus bombus
Chaetocercus berlepschi
Chaetocercus heliodor cleavesi
Chaetocercus mulsant
Chaetocercus burmeisteri
Calothorax lucifer
Calothorax pulcher
Calothorax enicura
Calothorax eliza
Archilochus colubris
Archilochus alexandri
Nesophlox evelynae
Nesophlox lyrura
Mellisuga helenae
Calypte anna
Calypte costae
Selasphorus platycercus
Selasphorus rufus
Selasphorus calliope
Selasphorus sasin
Atthis heloisa
Atthis ellioti
Selasphorus scintilla
Selasphorus flammula
Stephanoxis lalandi
Stephanoxis loddigesii
Campylopterus falcatus
Chlorostilbon mellisugus
Chlorostilbon lucidus
Thalurania glaucopus
Thalurania watertoni
Thalurania furcata
Thalurania colombica
Microchera albocoronata
Trochilus polytmus
Amazilia saucerottia
Amazilia versicolor
Amazilia brevirostris
Leucochloris albicollis
Amazilia fimbriata
Hylocharis sapphirina
Hylocharis cyanus
Chlorostilbon notatus
Topaz and Hermits
Mangoes
Brilliants
Coquettes
Mt. Gems
Bees
Emeralds
B
C
D
A
Log10 Evolutionary Rate
40
20
2
10 million years

Figure 1.8. The evolution of elevated hovering wingbeat frequency in male hummingbirds. Results of variable-rate regression analyses of evolutionary rates for hovering wingbeat frequency indicate several rate shifts. Branches are colored based on evolutionary rates of male hovering wingbeat frequency which ranged from 1.10 – 44.84. Letters A, B, C, and D correspond to points in supplementary figures **S1.2 A** and **B**, and **S1.5 A** and **B**.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Eriocnemis glaucopoides
Eriocnemis vestitus
Aglaeactis cupripennis
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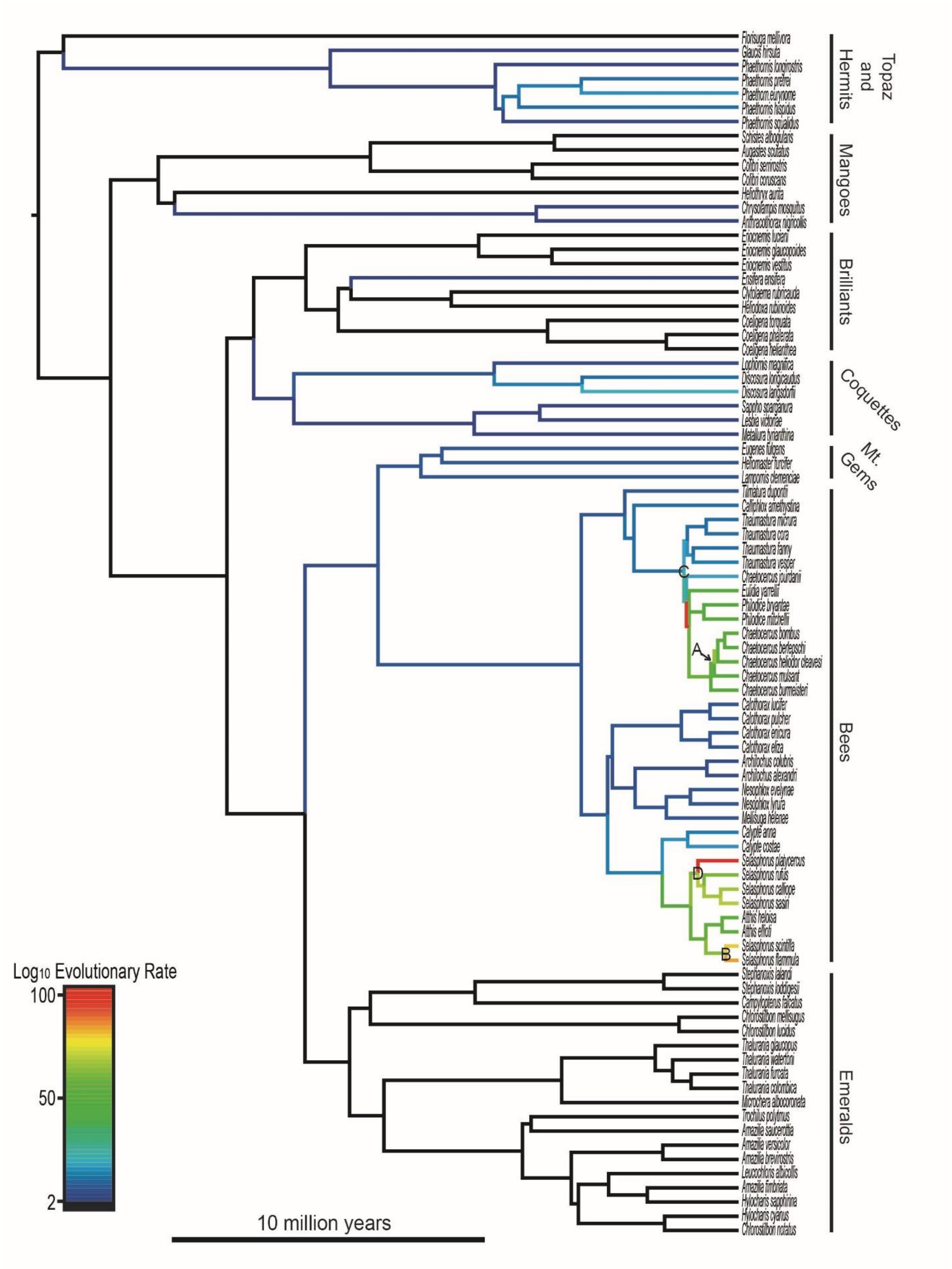
Thaumastura cora
Thaumastura fanny
Thaumastura vesper
Eulidia yarrellii
Philodice mitchellii
Chaetocercus berlepschi
Chaetcercus heliodor heliodor
Chaetocercus mulsant
Chaetocercus burmeisteri
Calothorax lucifer
Calothorax pulcher
Calothorax enicura
Calothorax eliza
Archilochus colubris
Archilochus alexandri
Mellisuga helenae
Nesophlox evelynae
Nesophlox lyrura
Mellisuga minima
Calypte anna
Calypte costae
Selasphorus platycercus
Selasphorus rufus
Selasphorus calliope
Selasphorus sasin
Selasphorus flammula
Stephanoxis lalandi
Campylopterus falcatus
Chlorostilbon lucidus
Thalurania glaucopus
Thalurania colombica
Microchera albocoronata
Hylocharis cyanus
D
C
A
B
Topaz and Hermits
Mangoes
Brilliants
Coquettes
Mt. Gem
Bees
Emeralds
Log10 Evolutionary Rate
>120
60
2
10 million years

Figure 1.9. The evolution of hovering wingbeat frequency in female hummingbirds. Results of variable-rate regression analyses of evolutionary rates for hovering wingbeat frequency indicate fewer rate shifts compared to males. Branches are colored based on evolutionary rates of female hovering wingbeat frequency which ranged from 1.15 – 293.41. Letters A, B, C, and D correspond to points in supplementary figures **S1.2 C** and **D**, and **S1.5 C** and **D**.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Schistes albogularis
Augastes scutatus
Colibri serrirostris
Colibri coruscans
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Chrysolampis mosquitus
Anthracothorax nigricollis
Eriocnemis luciani
Eriocnemis glaucopoides
Eriocnemis vestitus
Ensifera ensifera
Clytolaema rubricauda
Heliodoxa rubinoides
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Coeligena phalerata
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Discosura langsdorfii
Sappho sparganura
Lesbia victoriae
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Eugenes fulgens
Heliomaster furcifer
Lampornis clemenciae
Tilmatura dupontii
Calliphlox amethystina
Thaumastura micrura
Thaumastura cora
Thaumastura fanny
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Philodice bryantae
Philodice mitchellii
Chaetocercus bombus
Chaetocercus berlepschi
Chaetocercus heliodor cleavesi
Chaetocercus mulsant
Chaetocercus burmeisteri
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Calothorax pulcher
Calothorax enicura
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Calypte anna
Calypte costae
Selasphorus platycercus
Selasphorus rufus
Selasphorus calliope
Selasphorus sasin
Atthis heloisa
Atthis ellioti
Selasphorus scintilla
Selasphorus flammula
Stephanoxis lalandi
Stephanoxis loddigesii
Campylopterus falcatus
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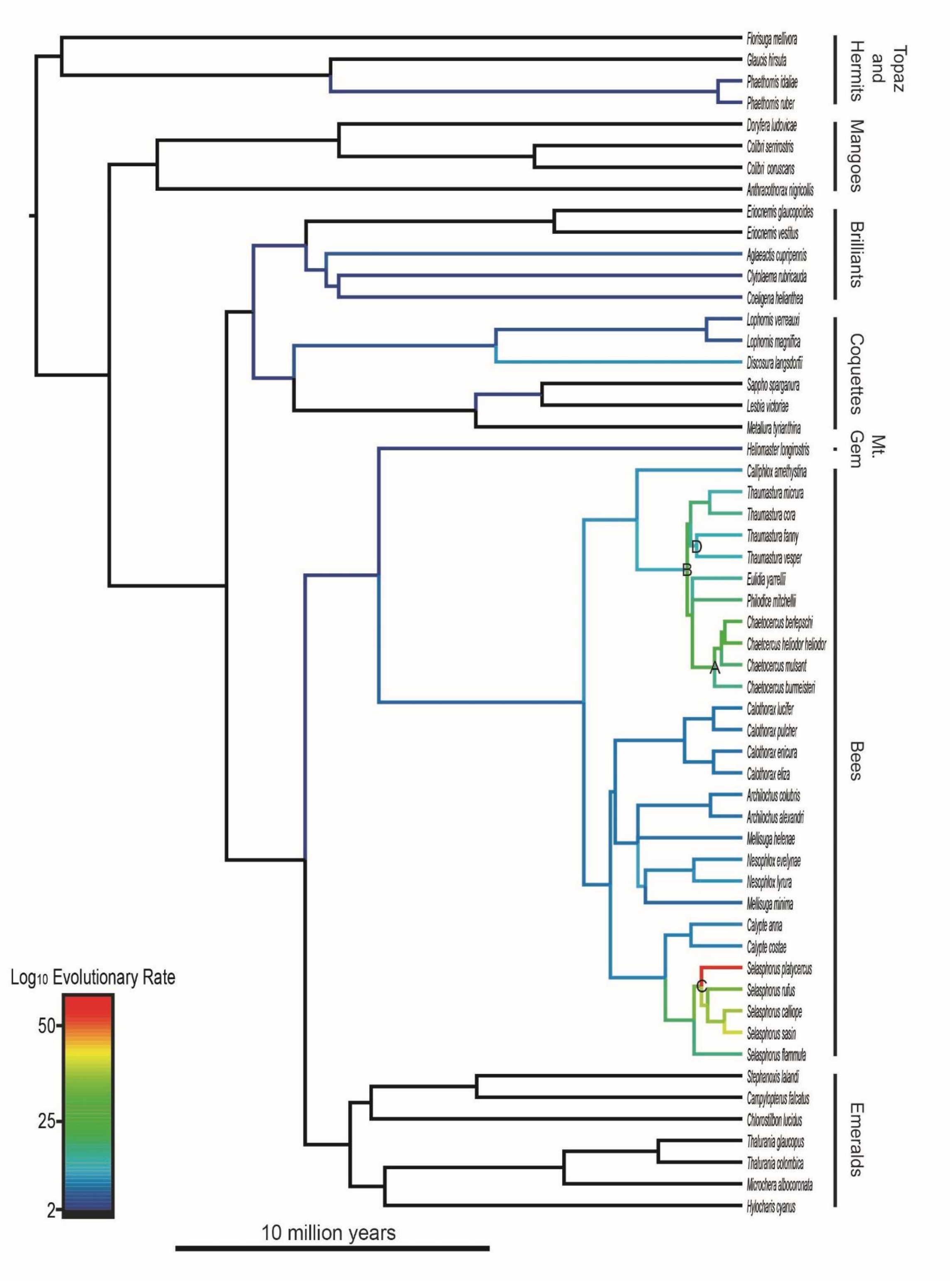
Chlorostilbon lucidus
Thalurania glaucopus
Thalurania watertoni
Thalurania furcata
Thalurania colombica
Microchera albocoronata
Trochilus polytmus
Amazilia saucerottia
Amazilia versicolor
Amazilia brevirostris
Leucochloris albicollis
Amazilia fimbriata
Hylocharis sapphirina
Hylocharis cyanus
Chlorostilbon notatus
C
A
D
B
Topaz and Hermits
Mangoes
Brilliants
Coquettes
Mt. Gems
Bees
Emeralds
Log10 Evolutionary Rate
100
50
2
10 million years

Figure 1.10. The evolution of wing length in male hummingbirds. Results of variable-rate regression analyses of evolutionary rates for wing length indicate several rate shifts, mostly occurring in the bee hummingbirds. Branches are colored based on evolutionary rates of male wing length which ranged from 1.20 – 102.21. Letters A, B, C, and D correspond to points in supplementary figure **S1.3 A** and **B**, and **S1.6 A** and **B**.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Anthracothorax nigricollis
Eriocnemis glaucopoides
Eriocnemis vestitus
Aglaeactis cupripennis
Clytolaema rubricauda
Coeligena helianthea
Lophornis verreauxi
Lophornis magnifica
Discosura langsdorfii
Sappho sparganura
Lesbia victoriae
Metallura tyrianthina
Heliomaster longirostris
Calliphlox amethystina
Thaumastura micrura
Thaumastura cora
Thaumastura fanny
Thaumastura vesper
Eulidia yarrellii
Philodice mitchellii
Chaetocercus berlepschi
Chaetcercus heliodor heliodor
Chaetocercus mulsant
Chaetocercus burmeisteri
Calothorax lucifer
Calothorax pulcher
Calothorax enicura
Calothorax eliza
Archilochus colubris
Archilochus alexandri
Mellisuga helenae
Nesophlox evelynae
Nesophlox lyrura
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Calypte costae
Selasphorus platycercus
Selasphorus rufus
Selasphorus calliope
Selasphorus sasin
Selasphorus flammula
Stephanoxis lalandi
Campylopterus falcatus
Chlorostilbon lucidus
Thalurania glaucopus
Thalurania colombica
Microchera albocoronata
Hylocharis cyanus
Topaz and Hermits
Mangoes
Brilliants
Coquettes
Mt. Gem
Bees
Emeralds
A
B
C
D
Log10 Evolutionary Rate
50
25
2
10 million years

Figure 1.11. The evolution of wing length in female hummingbirds. Results of variable-rate regression analyses of evolutionary rates for wing length indicate several rate shifts, mostly occurring in the bee hummingbirds. Branches are colored based on evolutionary rates of female wing length which ranged from 1.16 – 57.67. Letters A, B, C, and D correspond to points in supplementary figure **S1.3 C** and **D**, and **S1.6 A** and **B**.

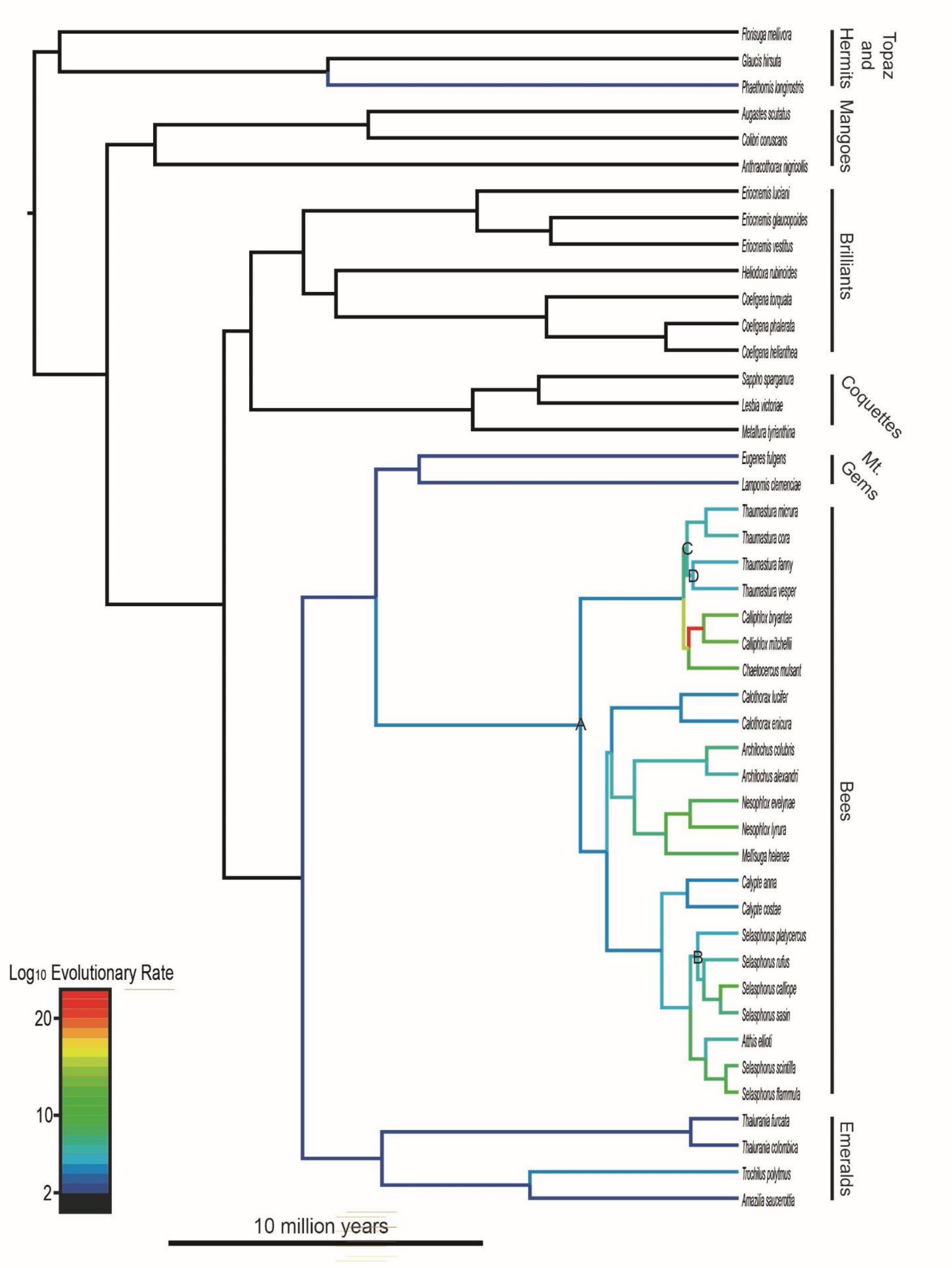

Florisuga mellivora
Glaucis hirsuta
Phaethornis longirostris
Topaz and Hermits
Augastes scutatus
Colibri coruscans
Anthracothorax nigricollis
Mangoes
Eriocnemis luciani
Eriocnemis glaucopoides
Eriocnemis vestitus
Heliodoxa rubinoides
Coeligena torquata
Coeligena phalerata
Coeligena helianthea
Brilliants
Sappho sparganura
Lesbia victoriae
Metallura tyrianthina
Coquettes
Eugenes fulgens
Lampornis clemenciae
Mt. Gems
Thaumastura micrura
Thaumastura cora
C
Thaumastura fanny
D
Thaumastura vesper
Calliphlox bryantae
Calliphlox mitchellii
Chaetocercus mulsant
Calothorax lucifer
A
Calothorax enicura
Archilochus colubris
Archilochus alexandri
Nesophlox evelynae
Nesophlox lyrura
Mellisuga helenae
Calypte anna
Calypte costae
Selasphorus platycercus
B
Selasphorus rufus
Selasphorus calliope
Selasphorus sasin
Atthis ellioti
Selasphorus scintilla
Selasphorus flammula
Bees
Thalurania furcata
Thalurania colombica
Trochilus polytmus
Amazilia saucerottia
Emeralds
Log10 Evolutionary Rate
20
10
2
10 million years

Figure 1.12. The evolution of keel length in male hummingbirds. Results of variable-rate regression analyses of evolutionary rates for keel length indicate several rate shifts, mostly occurring in the bee hummingbirds. Branches are colored based on evolutionary rates of male keel length which ranged from 1.15 – 22.26. Letters A, B, C, and D correspond to points in supplementary figure **S1.4 A** and **B**, and **S1.7 A** and **B**.

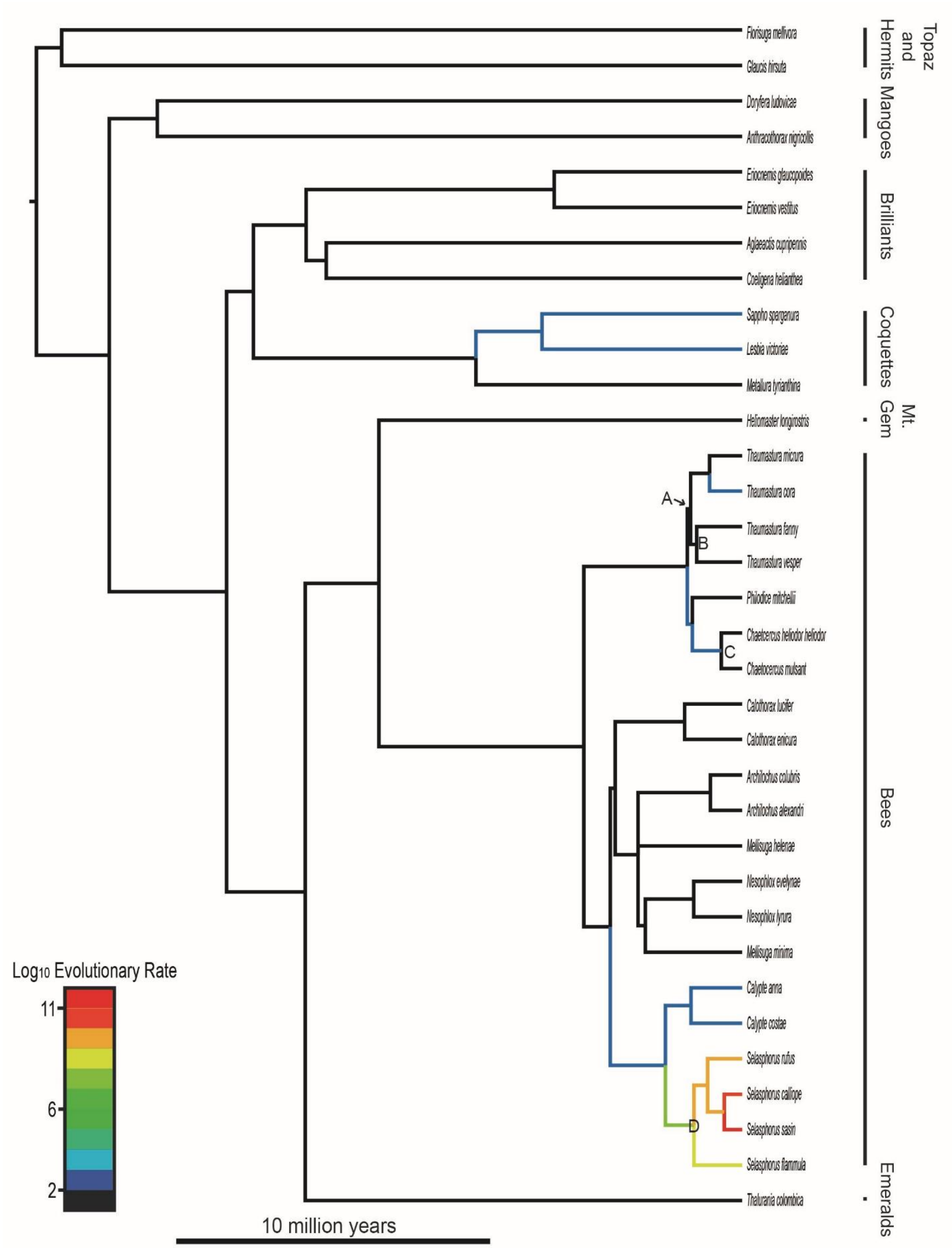


Figure 1.13. A variable rate model was not supported (Bayes Factor = 1.73) over an equal rate model for the evolution of keel length in female hummingbirds. Results of

variable-rate regression analyses of evolutionary rates for keel length indicate several rate shifts, mostly occurring in the bee hummingbirds. Branches are colored based on evolutionary rates of female keel length which ranged from 1.15 – 11.89. Letters A, B, C, and D correspond to points in supplementary figure **S1.4 A** and **B**, and **S1.7 A** and **B**.

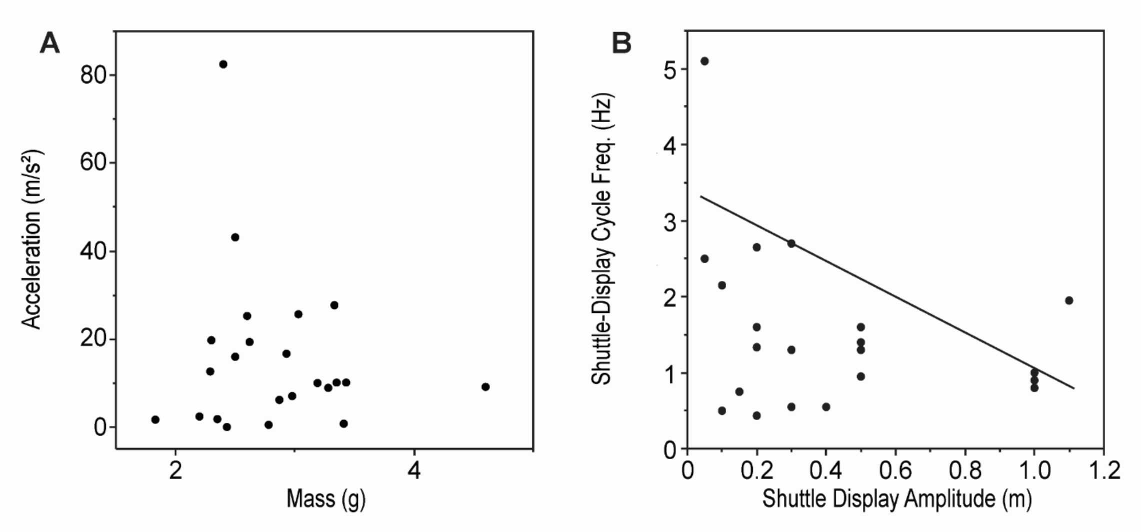


Figure 1.14. Shuttle display acceleration, cycle-frequency and amplitude. **(A)** Maximal acceleration was estimated by modeling the shuttle display as a simple-harmonic motion $A_{max} = (2\pi f)^2 \times D$. Acceleration was not correlated with mass ($F_{1, 21} = 0.64$, $p = 0.53$). **(B)** Quantile regression indicated significantly negative correlations between cycle-frequency and amplitude at upper quantiles (84th-87th, $p = 0.002$).

Tables:

Table 1.1. Allometry of female and male wingbeat frequencies and keel lengths.

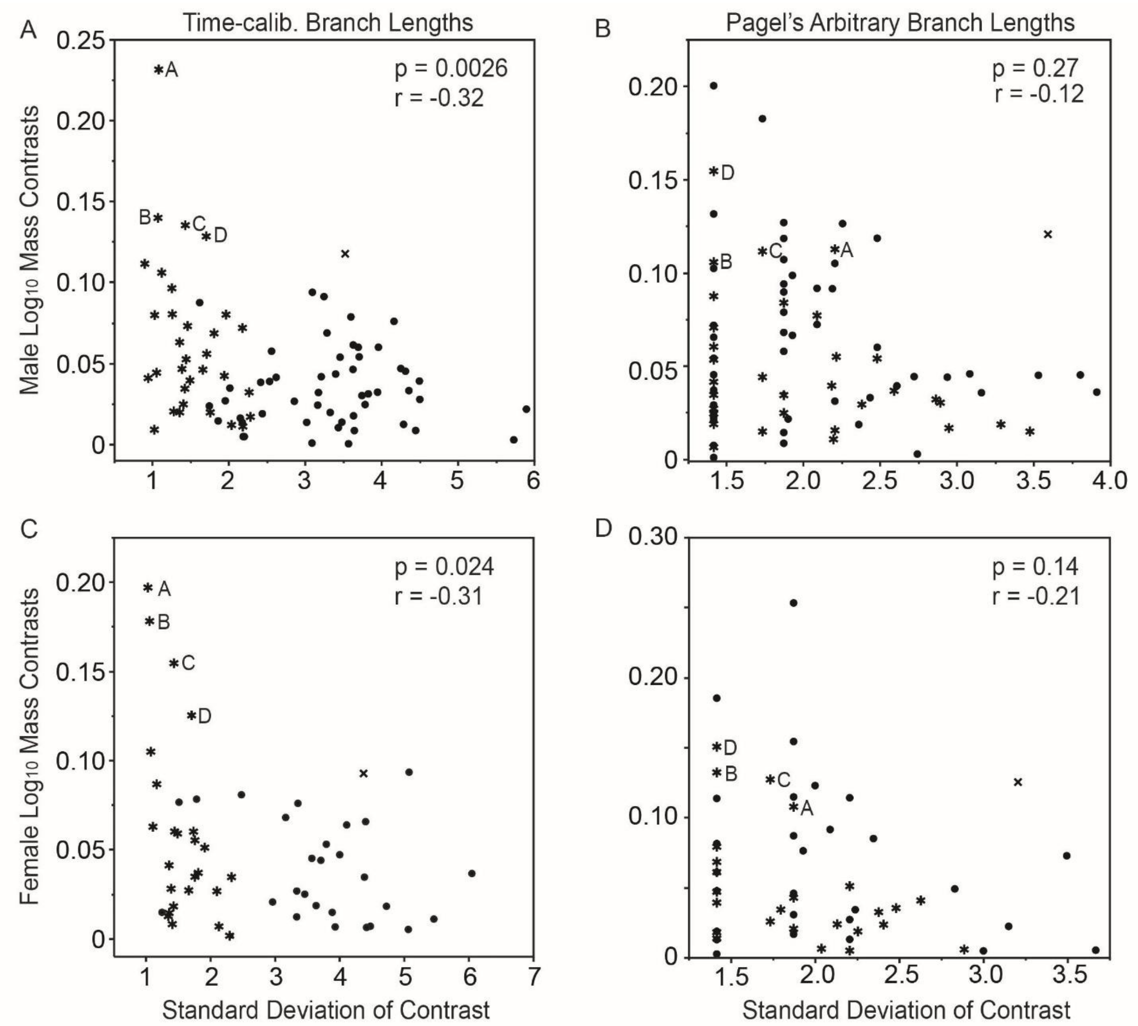
Trait	Group	Observed Allometry	SE	Lower CI	Upper CI	Predicted Allometry	N
Courtship-display WBF							
	bee males	-0.75	0.14	-0.99	-0.49	-0.50	30
Hovering WBF							
	all males	-0.38	0.070	-0.52	-0.25	-0.50	85
	bee males	-0.57	0.12	-0.81	-0.33	-0.50	34
	bee males excluded	-0.44	0.075	-0.57	-0.29	-0.50	51
	all females	-0.50	0.081	-0.63	-0.33	-0.50	55
Keel Length							
	all males	0.41*	0.028	0.37	0.47	0.33	45
	all females	0.43*	0.041	0.35	0.51	0.33	34

*Indicates 95% confidence interval does not overlap predicted allometry.

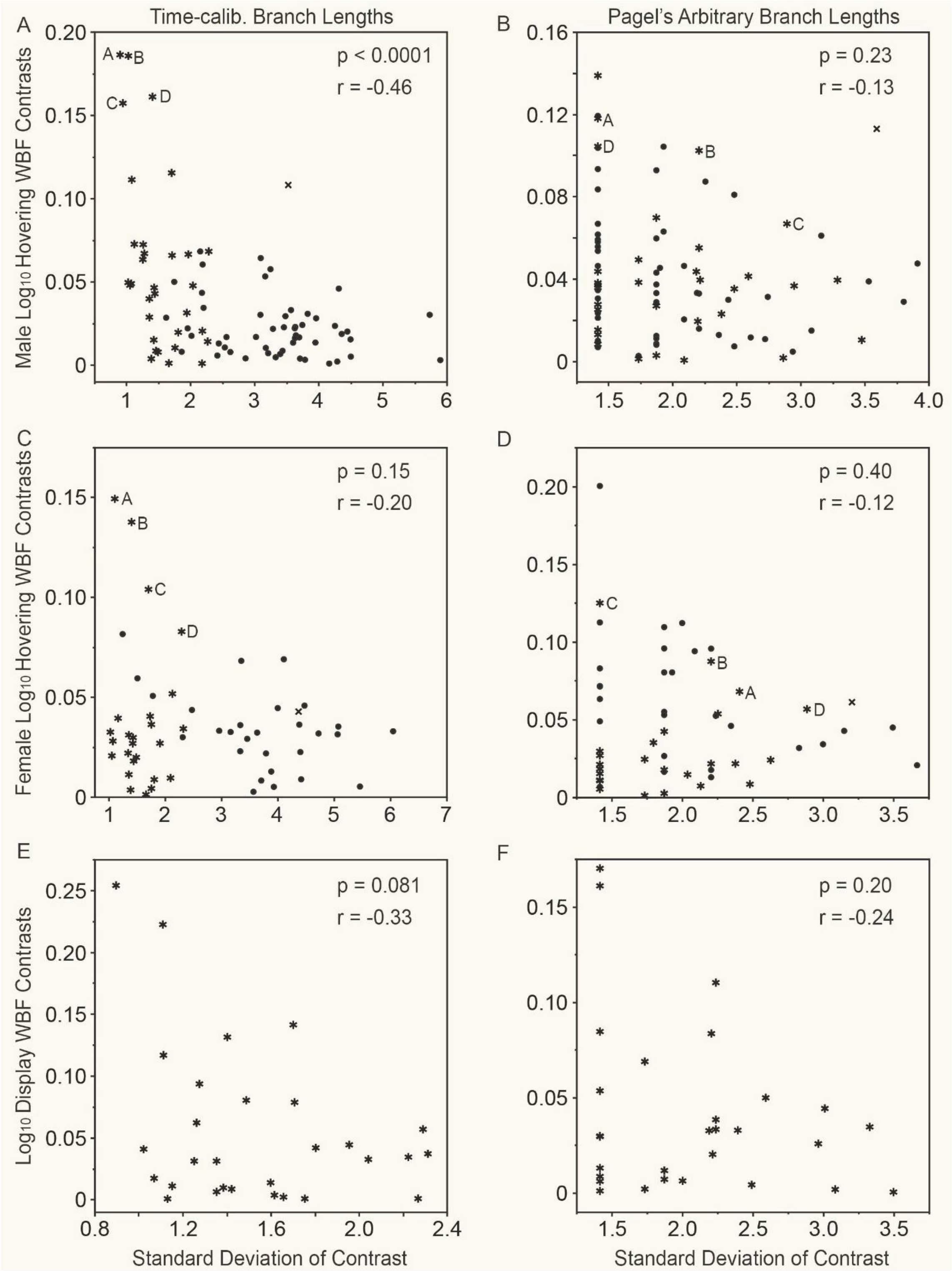
Table 1.2. Allometric differences of intercepts between focal groups of females and males for hovering wingbeat frequency, wing length and keel length from pANCOVA (RegressionV2).

Comparison	Trait	T	df	p
Male Bees vs. All Other Males	Hovering Wingbeat Frequency	3.95	81	0.00017
	Wing Length	-2.98	81	0.0038
	Keel Length	1.72	41	0.093
Female Bees vs. All Other Females	Hovering Wingbeat Frequency	0.14	51	0.89
	Wing Length	0.63	51	0.53
	Keel Length	1.47	30	0.15

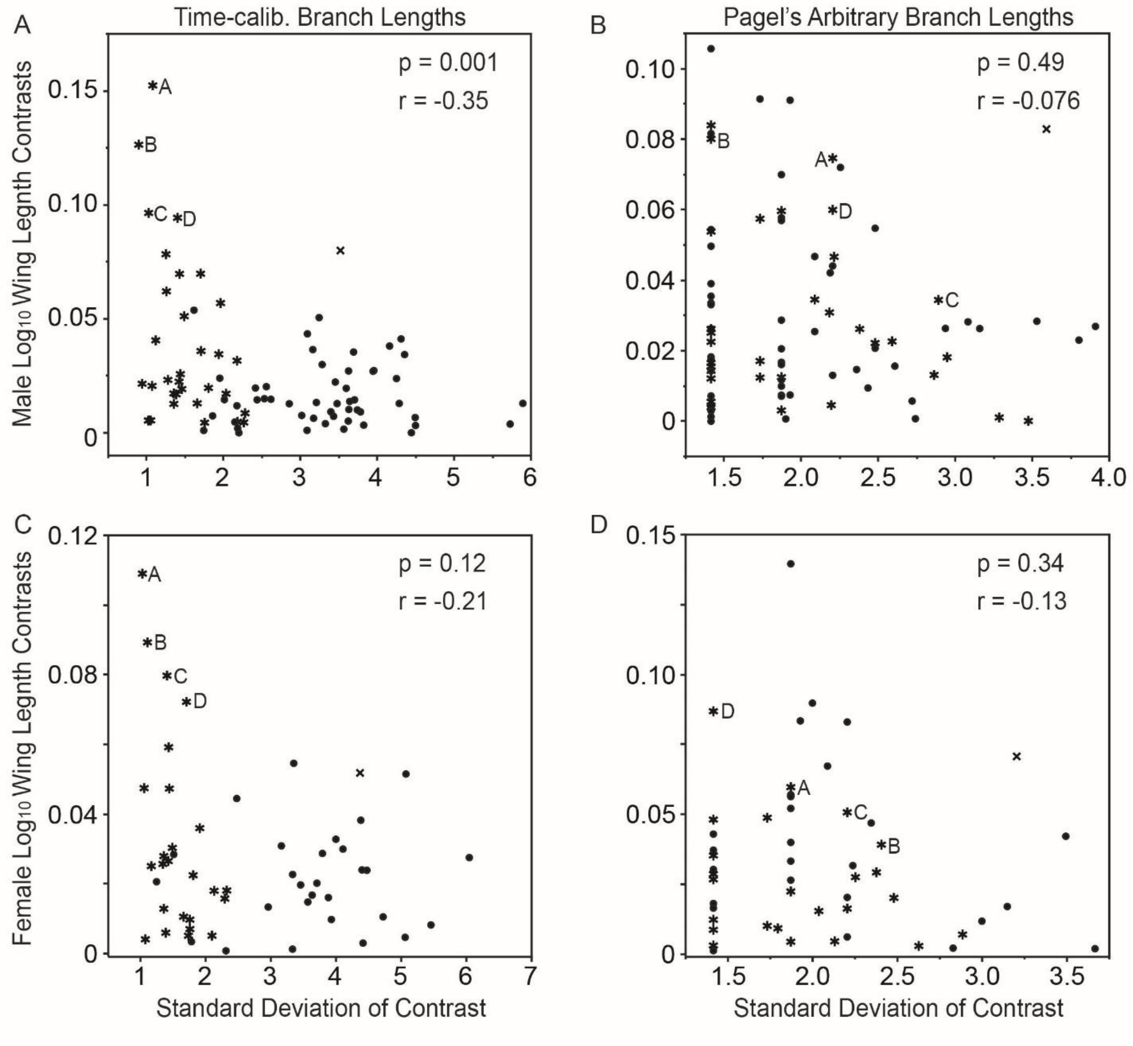
Supplementary Figures:



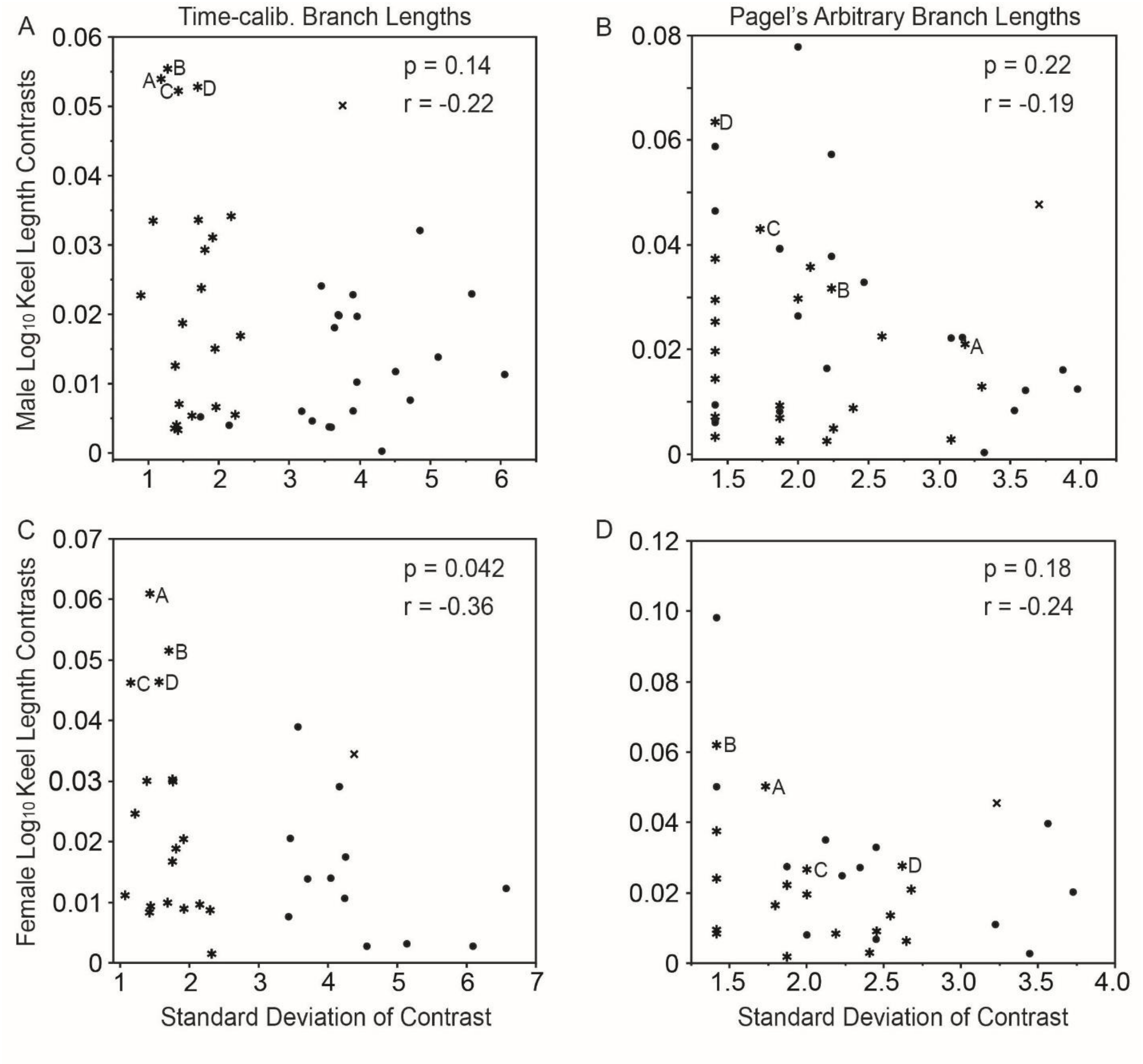
S1.1. Male and female $\log_{10}$ body mass contrasts against their standard deviations. Panels **(A)** and **(C)** are contrasts from the time-calibrated McGuire et al. (2014) phylogeny. Panels **(B)** and **(D)** are standardized contrasts from the Pagel (1992) branch length transformation. Within panels, letters A, B, C, and D represent focal branches from the BayesTraitsV3 variable rate analysis for body mass evolution (Figures 1.6 and 1.7).


Time-calib. Branch Lengths
Pagel's Arbitrary Branch Lengths
A
B
C
D
E
F
p < 0.0001
r = -0.46
p = 0.23
r = -0.13
p = 0.15
r = -0.20
p = 0.40
r = -0.12
p = 0.081
r = -0.33
p = 0.20
r = -0.24
Male Log10 Hovering WBF Contrasts
Female Log10 Hovering WBF Contrasts
Log10 Display WBF Contrasts
Standard Deviation of Contrast
Standard Deviation of Contrast
0.20
0.15
0.10
0.05
0
0.16
0.12
0.08
0.04
0
0.25
1
2
3
4
5
6
7
1.5
2.0
2.5
3.0
3.5
4.0
0.8
1.2
1.6
2.0
2.4

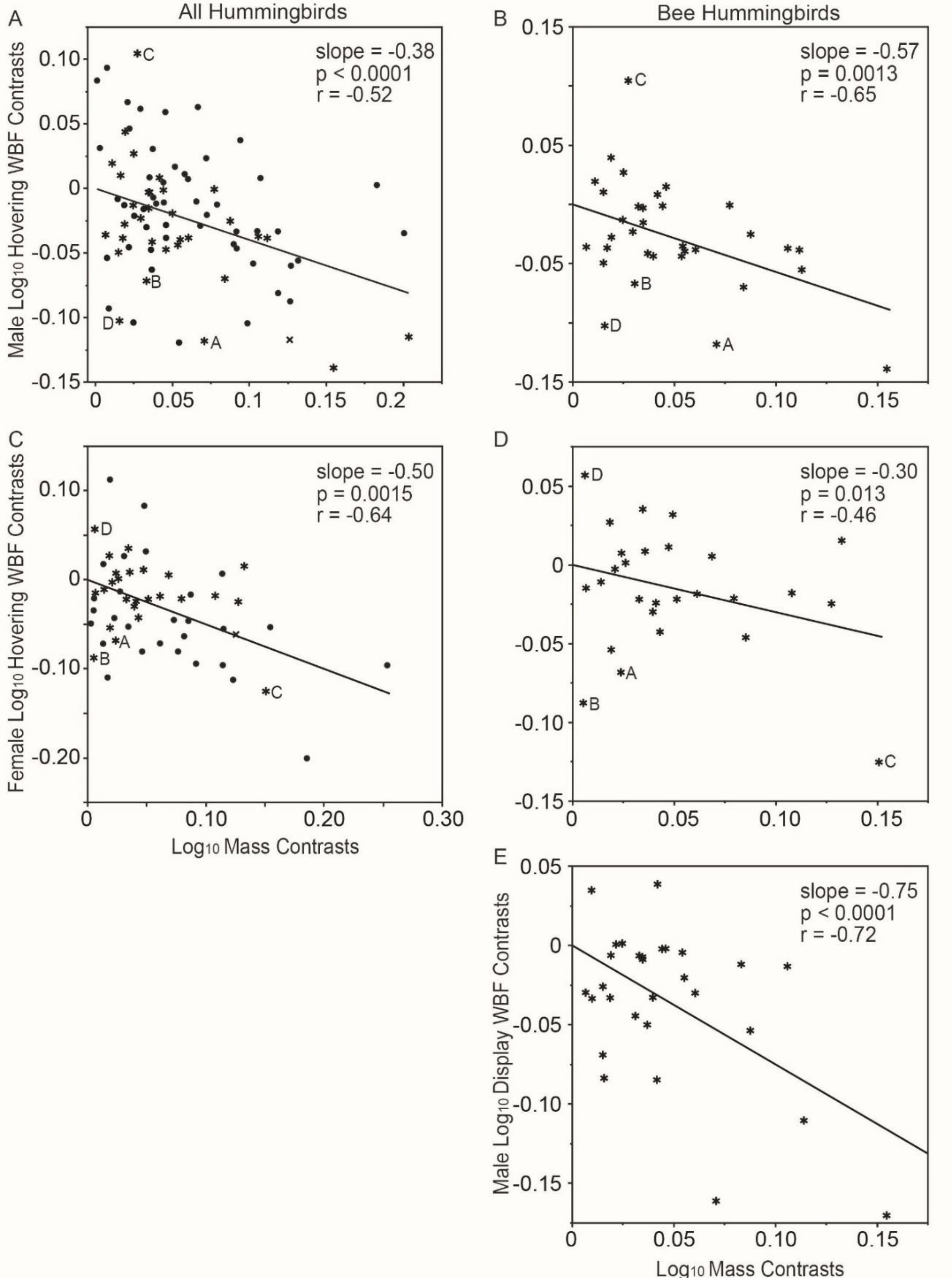
S1.2. Male and female $\log_{10}$ hovering and display wingbeat frequency contrasts against their standard deviations. Panels **(A)**, **(C)** and **(E)** are contrasts from the time-calibrated McGuire et al. (2014) phylogeny. Panels **(B)**, **(D)** and **(E)** are standardized contrasts from the Pagel (1992) branch length transformation. Within panels, letters A, B, C, and D represent focal branches from the BayesTraitsV3 variable rate regression analysis for hovering wingbeat evolution (Figures 1.8 and 1.9).



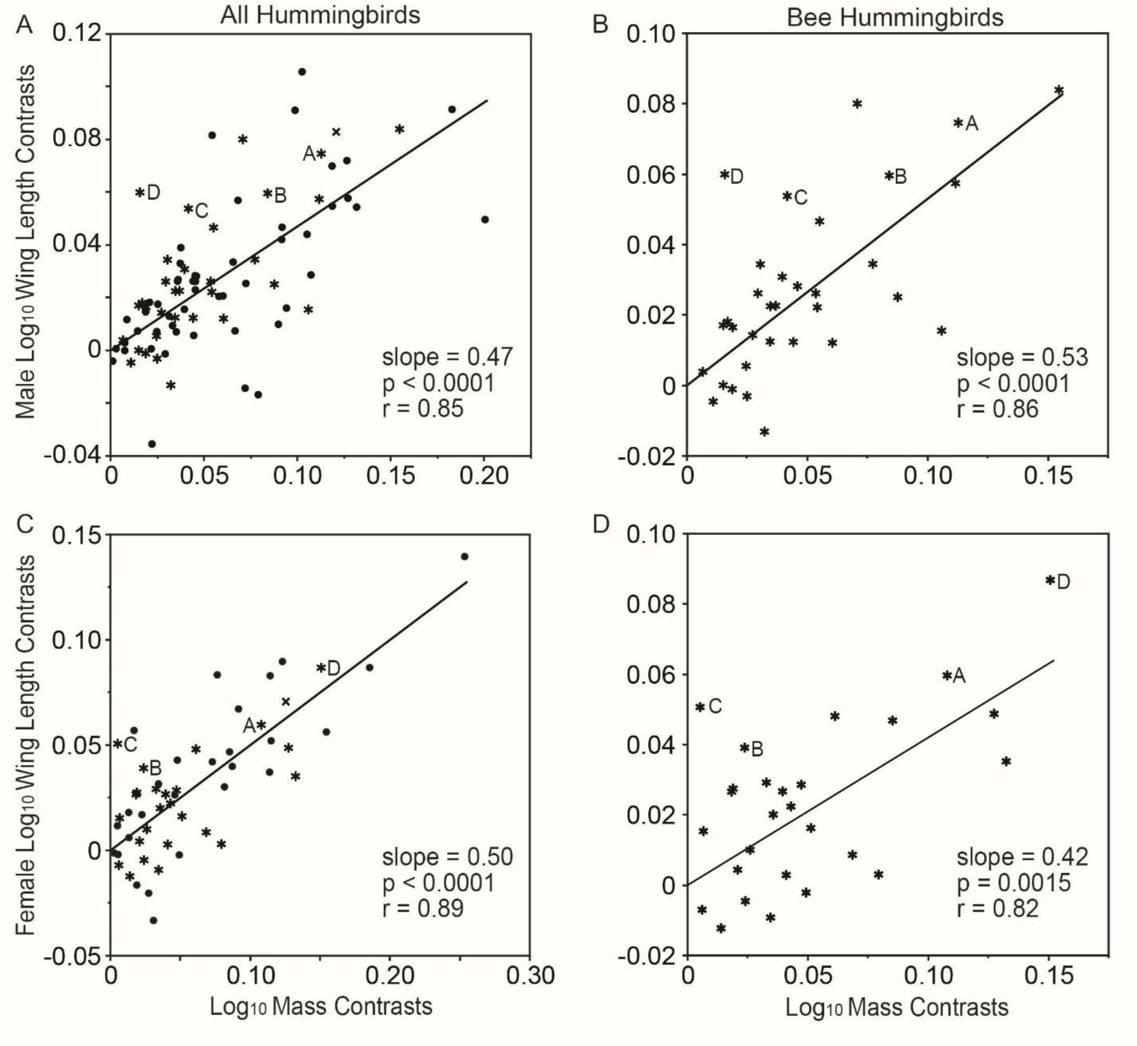
S1.3. Male and female $\log_{10}$ wing length contrasts against their standard deviations. Panels **(A)** and **(C)** are contrasts from the time-calibrated McGuire et al. (2014) phylogeny. Panels **(B)** and **(D)** are standardized contrasts from the Pagel (1992) branch length transformation. Within panels, letters A, B, C, and D represent focal branches from the BayesTraitsV3 variable rate regression analysis for wing length evolution (Figures 1.10 and 1.11).



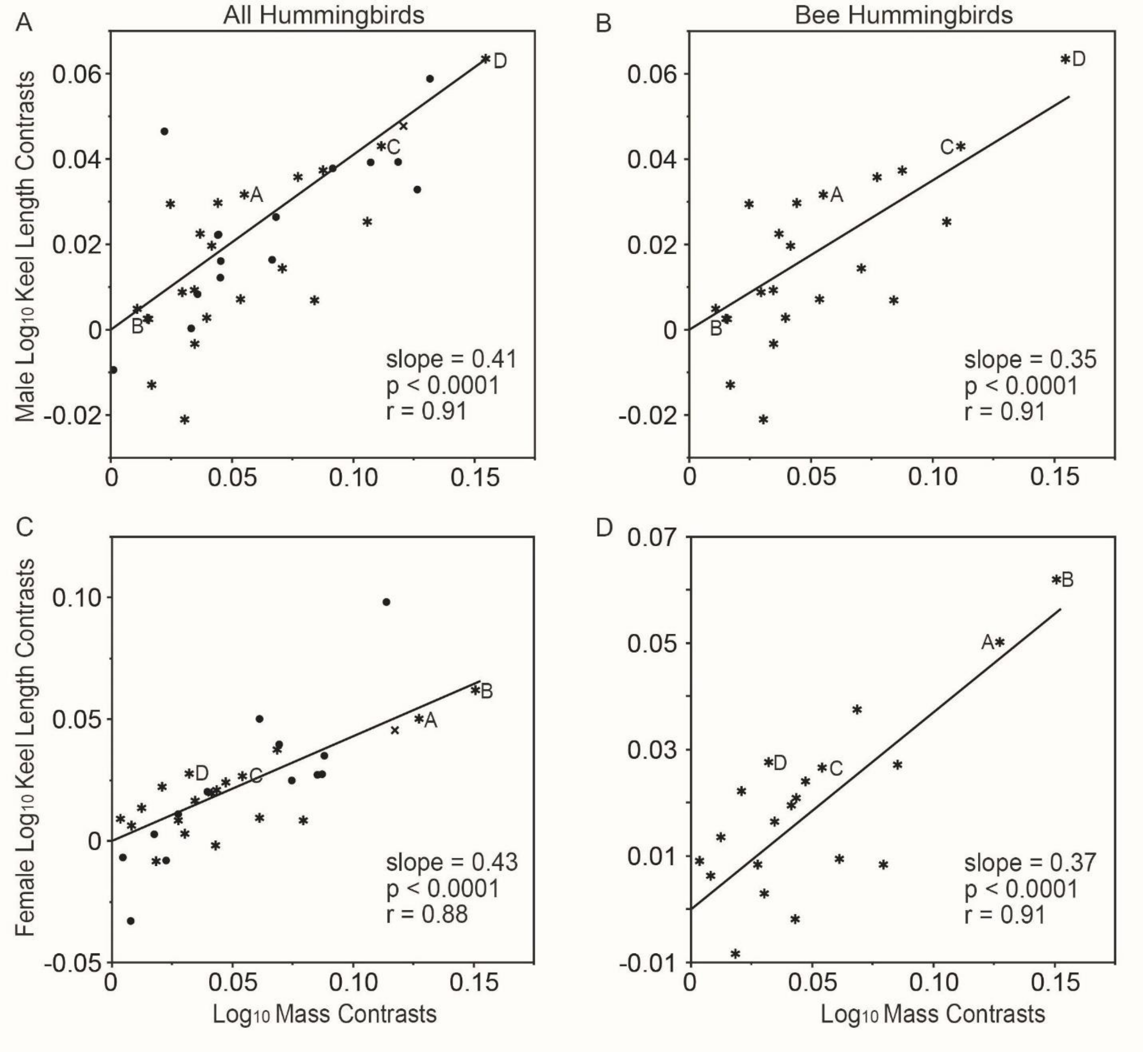
S1.4. Male and female $\log_{10}$ keel length contrasts against their standard deviations. Panels **(A)** and **(C)** are contrasts from the time-calibrated McGuire et al. (2014) phylogeny. Panels **(B)** and **(D)** are standardized contrasts from the Pagel (1992) branch length transformation. Within panels, letters A, B, C, and D represent focal branches from the BayesTraitsV3 variable rate analysis (Figures 1.12 and 1.13).


A
All Hummingbirds
B
Bee Hummingbirds
slope = -0.38
p < 0.0001
r = -0.52
slope = -0.57
p = 0.0013
r = -0.65
C
slope = -0.50
p = 0.0015
r = -0.64
D
slope = -0.30
p = 0.013
r = -0.46
E
slope = -0.75
p < 0.0001
r = -0.72
Male Log10 Hovering WBF Contrasts
Female Log10 Hovering WBF Contrasts
Male Log10 Display WBF Contrasts
Log10 Mass Contrasts
Log10 Mass Contrasts
0.15
0.10
0.05
0
-0.05
-0.10
-0.15
-0.20
0.2
0.1
0.20
0.30
A
B
C
D

S1.5. Wingbeat frequency allometry for all hummingbirds and the bee hummingbirds only. Regressions of standardized contrasts show the scaling slopes of hovering wingbeat frequency for **(A)** all male hummingbirds, and **(B)** male “bee” hummingbirds. **(C)** and **(D)** show the scaling of slopes of hovering wingbeat frequency for all female hummingbirds and female “bee” hummingbirds, respectively. The scaling slope of courtship display wingbeat frequency is shown in **(E)**. Within panels, letters A, B, C, and D correspond to branches highlighted on the phylogeny depicting the results of BayesTraitsV3 variable-rate regression analyses for evolutionary rates of hovering wingbeat frequency (Figures 1.8 and 1.9).



S1.6. Wing length allometry for all hummingbirds and the bee hummingbirds only. Regressions of standardized contrasts show the scaling slopes of wing length for **(A)** all male hummingbirds, and **(B)** male "bee" hummingbirds. **(C)** and **(D)** show the scaling of slopes of wing length for all female hummingbirds and female "bee" hummingbirds, respectively. Within panels, letters A, B, C, and D correspond to branches highlighted on the phylogeny depicting the results of BayesTraitsV3 variable-rate regression analyses for evolutionary rates of hovering wingbeat frequency (Figures 1.10 and 1.11).



S1.7. Keel length allometry for all hummingbirds and the bee hummingbirds only. Regressions of standardized contrasts show the scaling slopes of keel length for **(A)** all male hummingbirds, and **(B)** male “bee” hummingbirds. **(C)** and **(D)** show the scaling of slopes of keel length for all female hummingbirds and female “bee” hummingbirds, respectively. Within panels, letters A, B, C, and D correspond to branches highlighted on the phylogeny depicting the results of BayesTraitsV3 variable-rate regression analyses for evolutionary rates of hovering wingbeat frequency (Figures 1.12 and 1.13).

Supplementary Methods:

Much previous research on hummingbird hovering wingbeat frequency provides averaged data for species rather than for males and females separately. We only included data from sources that provided sex information for hovering wingbeat frequency. In cases where hovering wingbeat frequency was known from one source (e.g., Greenewalt, 1962) but mass, wing length, and/or keel length were not, we used data collected from other sources (e.g., Clark, 2010) to fill in missing morphometrics. Our literature sources for body mass, wing length, keel length, hovering and display wingbeat frequencies can be found in the Supplementary Literature Cited. Data are available from the authors upon request.

Phylogeny

In Mesquite (v 3.31) we added 8 species from the Clark et al. (2018) phylogeny to the McGuire et al. (2014) phylogeny. We substituted *Lophornis verreauxii* for *L. chalybeus*; added *Phaethornis idaliae* sister to *P. ruber* (Hinkelmann and Schuchmann, 1997); placed *Augastes scutatus* sister to *Schistes albogularis*, *Lophornis magnifica* sister to *L. verreauxii*, and *Stephanoxis loddigesii* sister to *S. lalandi* based on likely close relationships (Schuchmann, 1999).

Predicted Allometry of Hovering Wingbeat Frequency

Hovering wingbeat frequency is a ubiquitous and easily measured variable across hummingbirds, and is therefore a useful metric of flight performance for allometric studies. The scaling of wingbeat frequency is of interest because wingbeat frequency is a crucial aspect of hummingbird flight since increases in wingbeat frequency should promote enhanced maneuverability (Hedrick et al., 2009) within an individual, and prolonged hovering flight is a hallmark of all hummingbirds.

What is the expected allometry of hummingbird hovering wingbeat frequency (f)? According to the aerodynamic force (lift, drag) equation, wing forces (F) scale as

$$F \propto SA\, V^2 \qquad (1)$$

where SA is wing surface area and V is a characteristic wing velocity. Assuming the wing is flapped back and forth under simple harmonic motion gives:

$$V \propto f * L \qquad (2)$$

where f is wingbeat frequency and L is wing length. From geometry, assuming all hummingbird wings are approximately the same shape:

$$SA \propto L^2 \qquad (3)$$

inserting 2 and 3 into 1 yields

$$F \propto L^4 f^2 \qquad (4)$$

L is known to not scale isometrically: rather than scaling as $L \propto m^{1/3}$ (as expected for isometry) empirically, $L \propto m^{1/2}$ (Greenewalt, 1962; Altshuler, 2001; Clark, 2010). Inserting this empirical relationship into equation 4 produces:

$$F \propto m^2 f^2 \qquad (5)$$

Since a hovering hummingbird must produce $F = ma$ to remain aloft, $F \propto m$, and rearranging yields our predicted allometric relationship for hovering wingbeat frequency:

$$f \propto m^{-1/2} \qquad (6)$$

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Chapter 2: Sexual Dimorphism and Sex Differences in Flight Performance of Black-Chinned Hummingbirds (*Archilochus alexandri*)

Abstract:

Locomotion is important for both naturally and sexually selected behaviors. Maximal locomotor performance is physiologically challenging, and hence has been a focus of many studies that attempt to link morphology, performance and fitness. Hummingbirds are adept at both low and high speed flight. Many hummingbird species are sexually dimorphic in body size and flight morphology, suggesting there are sexual differences in flight performance. We measured performance of female and male black-chinned hummingbirds (*Archilochus alexandri*) in a top speed flight assay in a wind tunnel; and in a low-speed load-lifting assay. We hypothesized that top speed and the load an individual could lift would be correlated. We predicted that flight performances vary between males and females, and morphology is correlated with performance in female and male hummingbirds. We found top-speed flight and asymptotic load lifting were positively correlated, but only in females. Males are smaller than females and had higher top speeds, while females outperformed males at asymptotic load lifting. We did not find significant relationships between flight morphology and performances of either assay. Our results suggest the two flight assays are correlated due to shared muscle activity despite difference in flight kinematics in the performance of these assays. We suggest studies of flight performance may benefit from the use of more than one assay and by including both sexes to provide a broader comprehension of flight performance and performance consequences of sexual size dimorphism.

Introduction:

Locomotion is critical for behaviors with high fitness stakes, including predator-prey interactions (Webb, 1986; Jayne and Bennett, 1990; Watkins, 1996; Miles, 2004; Walker et al., 2005) and sexually-selected courtship behaviors (Barske et al., 2010; Clark, 2009). When escaping a predator, catching elusive prey, or performing 'athletic' displays to appeal to female preferences, success or failure of the behavior depends on out-performing other animals, and thus, individuals may be selected to perform a behavior at the maximal locomotor level they can produce. Maximal locomotor performance is physiologically challenging at the level of the whole organism. Hence, individual variation in maximal locomotor performance should be linked to variation in morphology and fitness (Arnold, 1983; Irschick et al., 2008). In contrast, if locomotor performance is submaximal or a behavior is not physiologically challenging, links between morphology, performance and fitness may be difficult to detect or non-existent (Irschick et al., 2008).

Here, we consider hummingbird flight performance. Hummingbirds routinely fly at many different speeds, from hovering at a flower to feed, to fast forward flight, when chasing other hummingbirds away from that flower (Greenewalt, 1960) (Chai et al., 1999). Therefore, their locomotor performance encompasses a wide 'speed spectrum' of flight. Whereas hovering and low-speed maneuvers can be elicited within small cages and so has been characterized in lab experiments (Greenewalt, 1960; Altshuler et al., 2004; Cheng et al., 2016a; Cheng et al., 2016b; Dakin et al., 2018), less is known about limits to high-speed flight in hummingbirds, because testing it in a lab requires a wind tunnel (Chai and Dudley, 1999; Clark and Dudley, 2010).

Low and high-speed flight potentially favor different wing morphologies. As airspeed increases, parasite drag increases, increasing the thrust that an individual must produce to overcome drag. Thus, Norberg (Norberg, 1990; Norberg, 1995) hypothesized that relatively short wings, and relatively large flight muscles to produce thrust are favored for high-speed flight. At the lowest flight speeds such as hovering, performance is a function of the ability to generate vertically-oriented thrust and overcome induced drag. Long, tapered wings may be favored because they can produce greater force with reduced induced drag (Chai and Dudley, 1999). Thus, we hypothesized that the two extremes of flight performances (i.e., low-speed and fast-forward flight) favor different flight morphology: short wings are favored at high speed but long wings are favored at low speed (Chai and Dudley, 1999).

Many hummingbird species are sexually dimorphic. In the "bee" hummingbird clade (tribe Mellisugini), males tend to be smaller than females, and after accounting for body size, males have proportionally shorter wings, longer keels, and higher hovering wingbeat frequencies (Chapter 1), than females. Because the keel is the site of flight muscle attachment (Zusi, 2013), males likely have proportionally larger flight muscles. Also, males perform acrobatic aerial displays during courtship (Clark, 2009; Clark et al., 2018), implying that they are sexually selected for certain aspects of flight performance (Chapter 1). Females do not perform aerial displays, and are presumably only under natural selection with regard to flight. The morphology-performance-fitness paradigm (Arnold, 1983) suggests that flight morphology is linked to flight performances. Thus, sexual dimorphism in flight morphology may be the product of sex differences in flight performance. Given sex differences in flight morphology, we expect to find sexual differences in flight performance in standardized lab assays.

Here we investigate this hypothesis in black-chinned hummingbirds (*Archilochus alexandri*), a species in the "bee" hummingbird clade (Mellisugini). Black-chinned hummingbirds migrate to the United States and Canada from Mexico in the late spring to breed during the summer months (Baltosser and Russell, 2000). Males perform two aerial display behaviors as part of their courtship repertoire: a high-speed dive display and a short-range shuttle display (Feo and Clark, 2010; Chapter 3).

Flight Performance Assays

One widely used assay of hummingbird flight performance is a low-speed asymptotic load lifting assay, during which a bird that is attempting to escape by flying straight up lifts a necklace of small beads (Chai and Millard, 1997; Chai et al., 1997). As the bird flies upward it generates vertically-oriented aerodynamic thrust to do so. As it rises, incrementally more mass is lifted until the bird can no longer ascend. Thus, the vertical aerodynamic force required to perform this assay must be at or near maximal at relatively low flight speeds (low speed: the bird's translational velocity is < 3 m s^{-1}, and associated parasite drag on the bird's body is negligible).

High-speed flight (i.e., flight at translational velocities of > 10 m s^{-1}, in which parasite drag is high) is ecologically relevant to hummingbirds, since hummingbirds routinely engage in high-speed flights both during territorial interactions (i.e., chases) as well as in high-speed aerial dive displays (Clark, 2009). Although the exact role of top speed in agonistic interactions has not been investigated, hummingbirds occasionally hit or collide with each other during high speed chases (CJC pers obvs), suggesting that the maximal speed they can fly is important. The top speed a bird can fly can be measured using a variable-speed wind tunnel, in which the airspeed of the tunnel is gradually increased until the bird is no longer able to hold position within the working section of the tunnel (Chai and Dudley, 1999; Clark and Dudley, 2009). Here we assayed flight performance in female and male black-chinned hummingbirds in two ways: high-speed flight in a wind tunnel, and asymptotic load lifting.

Hypotheses

Primary flight muscles (i.e., the pectoralis and supracoracoideus) power the wing kinematics responsible for generating lift and thrust. Under the hypothesis that both low and high speed flight are limited by flight muscle activity (e.g. muscle force or power), performance of high-speed and low-speed flight assays may be positively correlated because they rely on the same flight muscle (i.e., the pectoralis), which likely varies in size among individuals. Alternatively, top-speed flight and asymptotic load lifting may be uncorrelated or negatively correlated if there is a performance tradeoff. For instance, wing length might be beneficial for low speed load lifting but reduce the maximum speed a bird can fly.

In the bee hummingbirds, males tend to have lower masses, smaller wings and longer keels than their female counterparts (Chapter 1). Sexual differences in flight-related morphology suggest sexual differences in flight performance. Within a sex, the morphology-performance relationship may be the same or different than between the sexes. That is, we predicted that within a sex, longer-winged individuals would outperform shorter-winged individuals at load lifting, while individuals with shorter wings were hypothesized to reach higher top speeds in the wind tunnel. Also, within sex we expect individuals with relatively large keels to outperform individuals with relatively smaller keels at both asymptotic load lifting and top-speed flight assays. Here, we tested whether 1) high-speed flight is positively correlated with asymptotic load lifting, 2) flight performances vary between males and females, and 3) morphological variation, especially as it relates to sexual dimorphism in black-chinned hummingbirds, drives differences in flight performances between female and male hummingbirds.

Methods:

Wild black-chinned hummingbirds were captured using feeder traps in Riverside, CA from June to late August of 2015 and 2016. Late August is late in their breeding season, but before they began gaining fat in preparation for migrating south. The birds were transported in small cloth bags; transport time from the point of capture to the location where we tested them in a wind tunnel (below) was never more than 25 minutes. Since the birds were captured while attempting to visit a feeder, we infer that they were hungry when they were captured. Upon visiting the feeder-trap, birds were caught before they fed from the feeder. Five minutes before commencement of flight-performance trials each individual was fed a meal of 30% (w/w) sugar water for 3 seconds in order to diminish any effect of hypoglycemia caused by the transport time.

The protocols in 2015 and 2016 had one difference: in 2015 birds experienced 3 flight performance assays, while in 2016, we only subjected them to 2 assays. The three flight performance assays in 2015 were: asymptotic load lifting, high-speed flight in a wind tunnel, and flight through a string maze, which was an attempt to quantify maneuverability. Repeatability (measured as intraclass correlation) of performances was lower for the string-maze assay (0.61 for time taken to traverse the maze, and 0.17 for number of strings hit within the maze) than the other two assays (see Results), and the string maze assay was also more laborious to complete relative to the wind tunnel and asymptotic load lifting assays. To allow for more time to capture birds, and thereby increase sample size, we did not test birds in the string maze assay in 2016.

The flight performance assays were presented in a randomized order, with five minutes of rest between each flight-performance assay to avoid any carry-over effect of fatigue between assay performances. Each individual performed 4 consecutive trials per assay, the worst of which was discarded (i.e., 3 trials per individual were used for statistical analyses). We allowed 1 minute of rest between trials of high-speed flight in the wind tunnel, the same time for rest was given between trials of asymptotic load lifting. The string-maze trials took place within an outdoor aviary in which the individual escaped into the aviary, thus individuals had to be recaptured after each trial. Because we discontinued the use of the string-maze assay in 2016, for the statistics, we analyzed order effects with regard to which came first: asymptotic load lifting or the high-speed wind tunnel assay. All assays were performed on the same day an individual was captured. All individuals were banded and released at the end of performance trials.

Folded wing and keel lengths were measured using digital calipers. The keel is the site of flight muscle attachment (Zusi, 2013), and its length is a proxy for flight muscle size (Wright and Steadman, 2012; Wilcox and Clark, *in prep*). Body mass was measured to the nearest 0.01g with a digital scale (MyWeighGemPro 250) prior to each assay.

Asymptotic Load Lifting

Asymptotic load lifting followed the standard protocol for this assay (Chai and Millard, 1997; Chai et al., 1997) in which we placed a string of colored beads attached to a small rubber band around each bird's neck. We then placed the bird on the floor of a flight chamber (35cm x 35cm x 62cm) that had darkened sides and an open, light top, which induces the bird to fly upward. We recorded video (Canon Vixia HF R500; 30Hz) of the bird flying upward. As the bird flies upward, it lifts incrementally more beads until it can no longer ascend. After reaching the zenith, the bird either descends to the floor of the flight chamber or lands along the side of its walls. Video recordings allow for a frame-by-frame assessment of the maximum number of beads lifted in each trial. The mass of the bird was added to the mass of the beads to produce a total mass lifted variable. We divided total mass lifted by body mass to create a variable that represents the proportion of body mass an individual was able to lift (hereafter: proportional mass lifted).

Top Speed in a Wind Tunnel

Hummingbirds can fly in wind tunnels with minimal training (Clark and Dudley, 2010). Top speed was measured by placing a bird in the working section (94cm x 36cm x 36cm) of the variable speed wind tunnel described in (Clark and Mistick, 2018). Wind speed was calibrated using a pitot tube. The speed at which all birds started the trials was 9 m s^{-1}. We accelerated the wind speed at approximately 0.05 m s^{-2}, while the investigator stood near the back of the working section (downwind) to encourage the bird to fly at the front of the working section. When the bird was no longer able to maintain position and drifted downwind past a predetermined set point representing approximately the back ⅓ of the working section, airspeed was switched off and recorded. On rare occasions, birds hit the back mesh of the working section. When this occurred, the bird was allowed to preen its feathers for 1 additional minute before commencing the next trial.

Statistical Analyses

All statistical analyses were performed using JMP Pro 14.2.0. To estimate repeatabilities for each type of flight-performance assay we calculated intraclass correlation coefficients (ICC [Sokal and Rolf, 1995; Wolak et al., 2012]) from the variance components partitioned for the random effect of individual in our GLM models (see below). To examine sexual dimorphism in *A. alexandri*, we tested for morphological differences between females and males using *t*-tests or the non-parametric Welch's *t*-test when the assumptions of normality and even variances were violated (as determined by Levene's test).

We used Generalized Linear Mixed Models (GLMM) to test for a relationship between high-speed flight and asymptotic load lifting assays, and to test our hypotheses regarding between-sex differences of flight-assay performances and within-sex relationships with flight-assay performances and morphology. We wanted to know 1) if the amount of mass an individual lifted during asymptotic load lifting was positively or negatively correlated with top-speed flight in the wind tunnel, 2) if there were sex differences in flight performance in the two assays, and 3) if morphological variables within sex showed significant correlations with asymptotic load lifting and top-speed flight performances.

To test which variables affected top speed, we included in a GLMM: individual as a random effect, mass lifted, sex, the order in which the two assays were performed, the year, the trial number, and calendar day the assays were performed as fixed effects. Order, year, trial number, and day were included as nuisance variables, to test for effects such as of tiring, learning, or a seasonal decline in performance (i.e., if individuals performed better earlier in the summer). We dropped order, trial number and day because these terms were not significant. There were three possible ways that the weight of the string (i.e., asymptotic load lifting performance) could be included: string mass lifted, total mass lifted (string mass + bird mass) or proportional mass lifted [(string mass + bird mass)/bird mass]. Of these three, string mass lifted was uncorrelated with body mass, whereas the other two metrics were correlated with body mass (see below). Therefore, we included mass lifted in the model.

Including both sex and morphological variables into the GLMM posed a multicollinearity problem, because there were pronounced sex differences for all three morphological variables. So, to further examine the effects of morphology, we re-ran the GLMM analysis for females and males separately. This allowed us to test for sex-specific relationships between morphology and top-speed flight. For these models, top speed was the dependent variable, individual was a random effect, mass lifted, body mass, keel length, and wing length were included as fixed effects.

Regarding mass lifted, we used GLMM with individual as a random effect. Sex, top speed flight, the order in which the two assays were performed, the year, the trial number, the day the assays were performed (with order, year, trial, and day all included as nuisance variables), and body mass were initially included as fixed effects. Following the same procedure as above, we dropped order, trial number, and the day the assays were performed as they were not significant. As before, to examine morphological effects, we repeated the GLMM analysis for females and males separately to test for sex-specific relationships between mass lifted, and wing length, keel length, and body mass.

This research was approved by the UC Riverside animal care and use committee (IACUC; protocol #20130018 and 20160039). All individuals were captured in accordance with California state (SC-6598) and federal permits (Bird Banding Permit #23516), and banded and released at the end of trials.

Results:

Sexual Size Dimorphism in Black-Chinned Hummingbirds

We included 22 female and 31 male black-chinned hummingbirds (2015: $n = 12$ females, $n = 24$ males; 2016: $n = 10$ females, $n = 7$ males). Black-chinned hummingbirds are sexually dimorphic; females have significantly greater body masses (Figure 2.1A; Welch's $t = 4.48$, $df = 50.72$, $p < 0.0001$) and longer wing lengths (Figure 2.1B; Welch's $t = 13.01$, $df = 31.33$, $p < 0.0001$). However, males have significantly longer keels than females (Figure 2.1C; $t = -4.54$, $df = 51$, $p < 0.0001$).

Top-Speed Flight

Regarding top speed, year had a significant effect (final GLMM, t = 2.38, df = 54.13, p = 0.021), but the other nuisance variables had no effect (all p > 0.16). Mass lifted was positively correlated with top-speed flight (Figure 2.2A; GLMM, t = 2.41, df = 148.23, p = 0.017). Males exhibited higher top speeds (mean ± sd: males = 16.0 m s^{-1} ± 1.0, n = 31; female = 15.1 m s^{-1} ±1.1, n = 22) in the wind tunnel (Figure 2.2B; GLMM, t = -2.89, df = 53.29, p = 0.0056).

Re-running the GLMM on the sexes separately, females showed a nearly significant positive correlation between top-speed flight and mass lifted (Figure 2.2A, t = 1.81, df = 56.64, p = 0.0749), but no significant relationships were detected between top-speed flight and morphology (keel length: p = 0.18; wing length: p = 0.11, body mass: p = 0.15), and there was a significant effect of year (Figure 2.2C; t = 3.71, df = 18.42, p = 0.0016). For males, the relationship between top-speed flight and mass lifted was not significant (Figure 2.2A, p = 0.11). Also, males did not show significant relationships between top-speed flight and morphology (keel length: p = 0.82; wing length: p = 0.33 or body mass: p = 0.28). For males, year was not statistically significant (Figure 2.2C; p = 0.47). Repeatability was high for top-speed flight in the wind tunnel assay (all ICC = 0.83; females ICC = 0.84; males ICC = 0.82).

Load Lifting Ability

Because they were not significant, order in which the flight assays were performed, trial number and the day in which the assays were performed were not included in the final model (all p > 0.25). Sex (t = 3.73, df = 51.50, p = 0.0005), year (t = 2.64, df = 52.95, p = 0.011), and top speed (t = 2.37. df = 146.14. p = 0.019) had significant effects on mass lifted, while body mass was not a significant predictor (p = 0.22).

In the GLMM split by sex, for females, year was not significant (p = 0.36), none of the morphological variables were significant (body mass, wing length and keel length p > 0.26). Top speed was marginally nonsignificant (t = 1.74, df = 55.24, p = 0.088). Likewise, for males, none of the morphological variables were significant (body mass, wing length and keel length p > 0.26), top speed was not significant (p = 0.11), but year was marginally nonsignificant (Figure 2.2C; t = 1.89, df = 26.30, p = 0.069). Repeatabilities were high for this assay (all ICC = 0.75; females ICC = 0.73; males ICC = 0.80).

Discussion:

Sexual Dimorphism of Black-Chinned Hummingbirds

We found significant sexual differences in morphology and flight performance between female and male black-chinned hummingbirds. Females had greater masses (Figure 2.1A), and longer wings (Figure 2.1B) than males. Males had longer keels (Figure 2.1C), the site of primary flight muscle attachment (Zusi, 2013) suggesting they are sexually selected for flight performance (Chapter 1).

Flight Performance and Limits to Top-Speed Flight

Asymptotic load lifting has been used as a flight-performance metric in several studies of hummingbird flight performance (Chai and Millard, 1997; Chai et al., 1997; Segre et al., 2016; Dakin et al., 2018). However, comparatively little is known regarding the limits to high-speed flight performance of hummingbirds. Stroke amplitude increases dramatically and approaches a physical limit of 180° during asymptotic load lifting (Chai and Millard, 1997; Chai et al., 1997; Altshuler and Dudley, 2003; Altshuler et al., 2010). By contrast, stroke amplitude declines at the highest speeds in a wind tunnel (Tobalske et al., 2007; Clark and Dudley, 2009; Clark and Mistick, 2018). Thus, differences in flight kinematics might limit flight performances and decouple correlations between performances of different flight assays. Or, muscle activity which controls flight kinematics might be more important in which case different flight performance metrics that rely on the same muscle(s) will be positively correlated.

We found that top-speed flight was positively correlated with mass lifted in females, but not males (Figure 2.2A). This finding suggests that some individuals are generally better flyers (at low and high speeds) than others, and that kinematic differences observed between top-speed and asymptotic load lifting assays are less important than variation in flight muscle capabilities. Most studies of hummingbird flight performance focus only on males. Therefore, this pattern would have been missed if we studied only males, indicating that subsequent studies of hummingbird flight might benefit by including female flight-performance data in their samples.

Although males were smaller than females, they were able to reach higher top speeds in the wind tunnel (Figure 2.2B). Flying at high speed appears to be ecologically relevant. During the breeding season, males frequently engage in high-speed agonistic chases with other males on and around their courtship territories. Males also engage.in high-speed aerial courtship dives (Clark, 2009). Females were better at asymptotic load lifting (i.e., they lifted more mass; Figure 2.2C; GLMM $t = 3.73$, $df = 51.50$, $p = 0.0005$), an assay that exploits the quick-escape response of hummingbirds, which may be ecologically important for birds that need to avoid predators and competitors. Whereas, the relatively shorter wings of males (Figure 2.1B) might be suboptimal for this assay, and lead to decreased performances, or be offset by their larger flight muscles (i.e., keel length; Figure 2.1C).

We hypothesized that short wings, and relatively large flight muscles to produce thrust are favored for top-speed flight (Norberg 1990, 1995). Performance of asymptotic load lifting requires the production of vertically-oriented thrust to overcome induced drag. We hypothesized long, tapered wings are favored for this assay of flight performance because they can produce greater force. However, we did not find significant relationships between morphology and performance in females or males for either assay. This could have resulted from low variation in our morphological variables. In which case, expanding the range of morphological variation by sampling males and females of several species that range in size could potentially alleviate this issue and reveal the relationships between morphology and both top-speed and asymptotic load lifting flight performances for male and female hummingbirds.

Submaximal performance is a reason for why morphology-performance relationships might not be detected (Irschick et al., 2008). With regard to asymptotic load lifting, the values we report for female and male black-chinned hummingbirds (females: $\bar{x}$ = 204% body mass lifted, n = 22; males: $\bar{x}$ = 208% body mass, n = 31) are similar to those reported by Chai and Millard (1997) for males of the same species (204% body mass lifted ± 16, N = 5 males). Top speeds we report (males $\bar{x}$ = 16.0 m s^{-1}, n = 31;

females $\overline{x} = 15.1$ m s^{-1}, n = 22) are higher than those reported by Chai and Dudley (1999) for ruby-throated hummingbirds (*Archilochus colubris*; male max = 14.4 m/s, female max = 12.1 m/s), the sister taxa to black-chinned hummingbirds. However the design of the wind tunnel used by Chai and Dudley (1999) was different than the one used here. Repeatabilities (measured as intraclass correlations) of asymptotic load lifting (all ICC = 0.75; females ICC = 0.73; males ICC = 0.80) and top-speed flight (all ICC = 0.83; females ICC = 0.84; males ICC = 0.82) were high, suggesting that birds were performing consistently at or near maximal. Hence, we do not think submaximal performance is the reason for the lack of significant morphology-performance relationships in females and males.

Another possibility for why we did not detect significant morphology-performance relationships is that body mass might not be a good index of body size. Feeding increases body mass in hummingbirds, and previous research on male broad-tailed hummingbirds (*Selasphorus platycercus*) showed maintenance of low body mass during the breeding season (Calder et al., 1990). Thus, a large meal before the measurement of body mass (particularly in males which might be behaviorally maintaining low body mass) will introduce error, and affect the relationship between body size and performance. However, we do not think this was a major factor in our experiments. Birds were caught at feeder traps before feeding from them, thus birds were caught hungry. Also, prior to the start of assay trials, only a small meal of concentrated sugar water was fed.

The Year Effect

We found that the year in which birds were measured affected their flight performances. For females, this was the case for top speed flight (Figure 2.2C; t = 3.71, df = 18.42, p = 0.0016), but not asymptotic load lifting (p = 0.36). For males, the year effect was not significant for top speed flight (p = 0.47), but the effect was marginally nonsignificant for asymptotic load lifting (Figure 2.2C; t = 1.89, df = 26.30, p = 0.069. The likeliest explanation for this is that the birds had different average body condition between years. We measured birds that had finished breeding, but were pre-migratory (none of our birds had begun to accumulate body fat, as this species does shortly before departing on migration). Black-chinned hummingbirds, unlike the other hummingbirds that breed at low elevation in California, breed during the hottest and driest times of the year. Breeding birds are often thought to lose condition over the course of the breeding season, however we did not detect this: the day in which the assays were performed was not significant (GLMM, top speed: p > 0.16; mass lifted: p > 0.25). If one year was harder on the birds than another year, and thus the birds were in worse condition when we caught them, this could explain the difference. During summers 2015 and 2016, California was in the last years of an extreme drought (Prugh et al., 2018). The drought may have affected food resources, or changed the phenology of cessation of breeding and the onset of preparation for migration. Therefore birds captured in 2016 may have been in worse physical condition compared those captured in 2015, thus resulting in lower flight performances of females and males.

Conclusion

Most research on hummingbird flight has focused on one performance assay, and one sex (males). Studies of hummingbird flight performance may benefit from the use of more than one assay to better understand overall flight performance. In our study commonly used flight-performance assays were correlated in support of the idea that ecologically important flight performances are primarily driven by variation in flight musculature rather than flight kinematics. Given that most studies focus on only males, including both females and males provides for a broader comprehension of morphology-flight performance relationships, and a means to better understand the performance consequences of sexual size dimorphism.

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Figures:

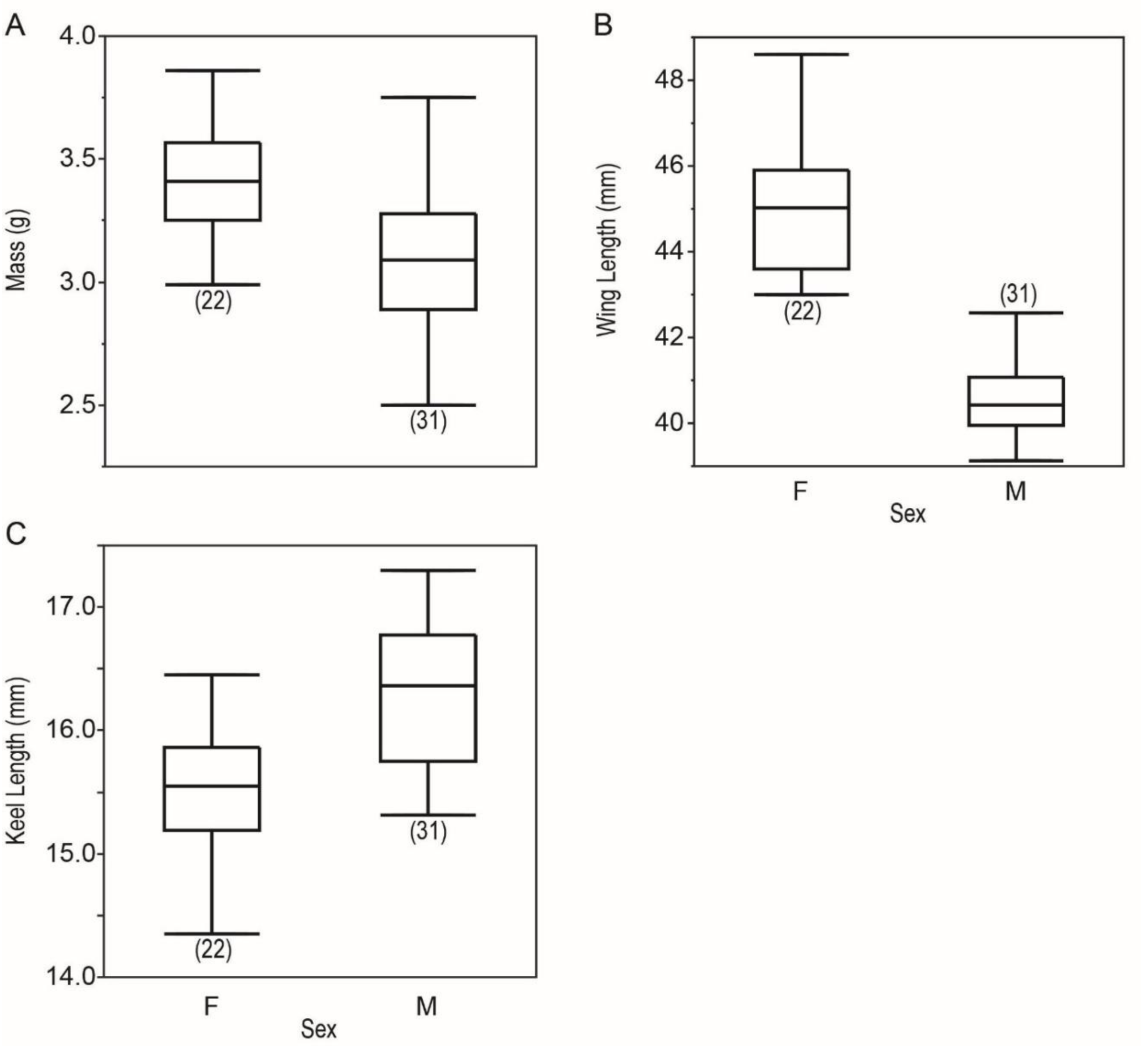
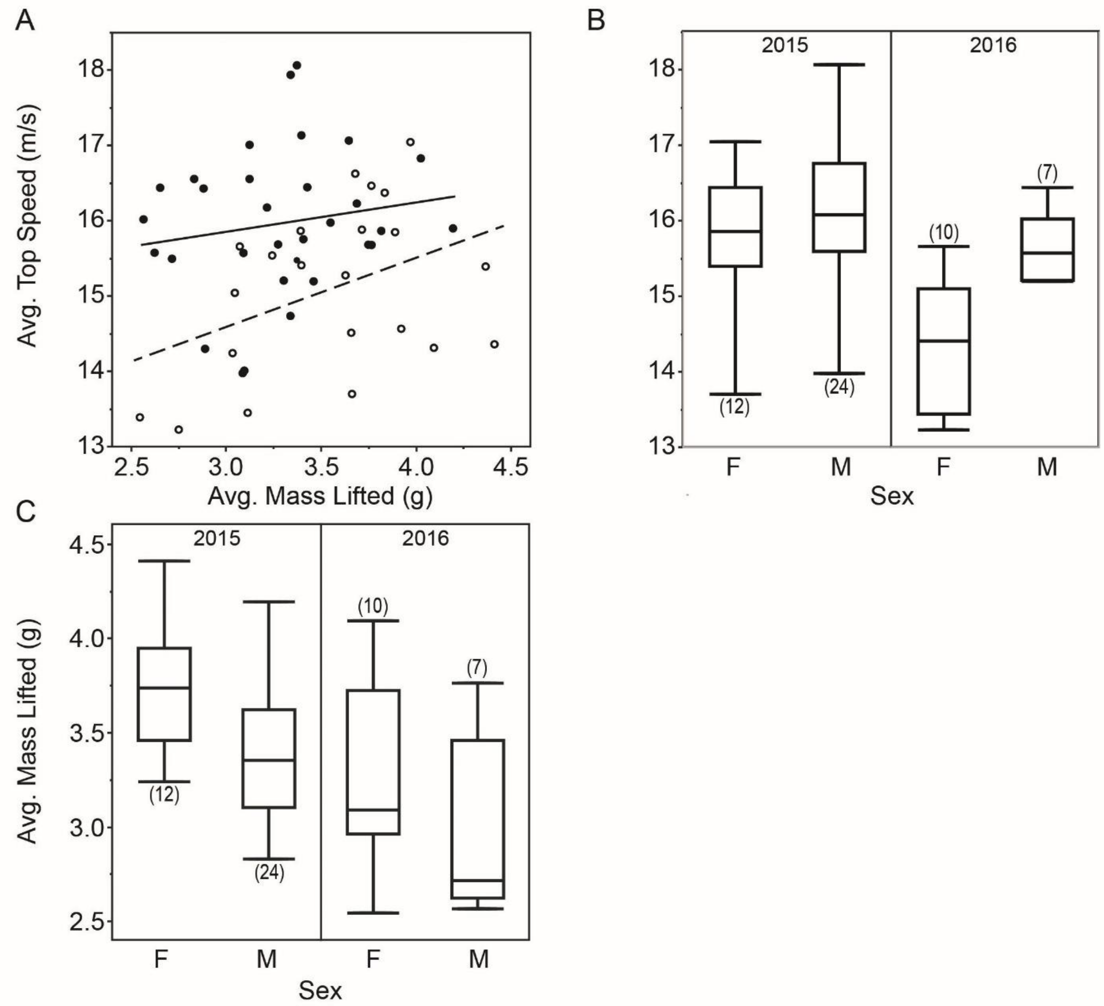


Figure 2.1. Female and male morphology of black-chinned hummingbirds. Box are the median, upper and lower quartiles, whiskers are the maximal and minimal values. **(A)** Females have greater body masses (Welch's $t = 4.48$, $df = 50.72$, $p < 0.0001$), and **(B)** longer wing lengths (Welch's $t = 13.01$, $df = 31.33$, $p < 0.0001$). **(C)** Males have longer keel lengths ($t = -4.54$, $df = 51$, $p < 0.0001$) compared to females.


A
Avg. Top Speed (m/s)
18
17
16
15
14
13
2.5
3.0
3.5
4.0
4.5
Avg. Mass Lifted (g)
B
2015
2016
(12)
(24)
(10)
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Avg. Mass Lifted (g)
4.5
4.0
3.5
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2015
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(12)
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(10)
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F
M
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M
Sex

Figure 2.2. Flight performance of female and male black-chinned hummingbirds. **(A)** Shows the positive relationship between top-speed flight and mass lifted (GLMM, t = 2.41, df = 148.23, p = 0.017), which is driven primarily by females (Open circles; GLMM, t = 1.81, df = 56.64, p = 0.075) since the relationship was not significant for males (Closed circles; p = 0.11). **(B)** The difference in top-speed flight performance between females and males by year; males had higher top speeds in the wind tunnel (GLMM, t = -2.89, df = 53.29, p = 0.0056). There was a significant year effect such that females (t = 3.71, df = 18.42, p = 0.0016), but not males (p = 0.47), performed better in 2015 relative to 2016. **(C)** Females lifted more mass compared to males (t = 3.73, df = 51.50, p = 0.0005). Birds performed the asymptotic load lifting assay better in 2015 than in 2016 (t = 2.64, df = 52.95, p = 0.011). This effect was not evident in females (p = 0.36), but was marginally nonsignificant in males (t = 1.89, df = 26.30, p = 0.069). For box and whisker plots (**B** and **C)**, the horizontal line within the box represents the median; upper and lower borders of the box are the upper and lower quantiles, respectively. Whiskers are the maximal and minimal values, respectively.

Chapter 3: Flight Performance During Courtship in Male Black-Chinned Hummingbirds (*Archilochus alexandri*)

Abstract:

Elaborate male display behaviors that require high locomotor performance have been hypothesized to honestly signal male quality to choosy females. Alternatively, challenging displays of locomotor performance may result from arbitrary female preferences. In several species of hummingbirds, males perform elaborate aerial displays during courtship. Black-chinned hummingbirds (*Archilochus alexandri*) perform a 'high performance' courtship display for females: a low-speed shuttle display during which wingbeat frequency is substantially elevated relative to hovering. We recorded males performing shuttle displays to live caged females to examine which display kinematic variables (such as wingbeat frequency) are correlated, and assess whether there is evidence that the shuttle display is constrained by a flight-performance tradeoff. Also, males were subjected to an asymptotic load lifting assay to test if shuttle-display flight performance was correlated with this general flight performance assay. We show that elevated shuttle display wingbeat frequency is positively correlated with acceleration and velocity during the display, and that the display is constrained by a tradeoff between cycle frequency and amplitude of the flight path. We found no relationship between asymptotic load lifting performance and any shuttle-display performance variable. We suggest this elaborate display behavior may serve as an index signal of male flight performance that females could use to judge individual males during mate choice decisions.

Introduction:

Adaptive mate-choice hypotheses suggest that females select mates on the basis of direct benefits (via nuptial gifts or access to high-quality territories, etc.). Alternately, male display traits may showcase indirect benefits such that their offspring will receive “good genes” (Fisher 1915; Williams, 1966; Zahavi, 1975). According to either of these adaptive hypotheses, male courtship behaviors serve as advertisements of quality that females can use during mate choice decisions. Alternatively, male courtship traits may be arbitrary with regard to male quality. Under this category of hypothesis, male courtship traits evolve in ways that are neutral with respect to female fecundity. Several mechanisms have been proposed, such as the Fisherian runaway mechanism (Fisher, 1930; Lande, 1981; Kirkpatrick, 1982), sensory biases, sensory exploitation, and aesthetic evolution (Prum, 2017). Under either adaptive or neutral paradigms, female preferences coevolve with male courtship traits leading to the exaggeration of male traits.

Locomotion is an important component of male courtship behaviors (Byers et al., 2010) and is particularly interesting because it is also intrinsically a part of other critical, naturally-selected behaviors, such as predator avoidance and prey capture (Jayne and Bennett, 1990; Garland and Losos, 1994; Watkins, 1996; Dickinson et al., 2000; Miles, 2004; Husak, 2006). Locomotion is also important for sexually-selected behaviors like male-male agonistic interactions (fights) (Garland et al., 1990; Robson and Miles, 2000; Brandt, 2003; Perry et al., 2004). Since locomotor performance is physically challenging, it may provide an index of individual variation in males which females could use during mate choice decisions (Byers et al., 2010). Hence, locomotor performance may play an important role in sexual selection via female choice for male courtship displays.

Animal signals are defined as behaviors (i.e., displays) and/or morphological structures that signalers have evolved to alter the behavior of intended receivers, and entail an evolved response from receivers (Maynard Smith and Harper, 1995; Maynard Smith and Harper, 2003). In particular, an index signal is a type of signal that is reliable because it is constrained in some way (e.g., anatomically, physiologically, biomechanically, etc.), and forms a causal link between the signal and the underlying advertised trait (Maynard Smith and Harper, 1995; Maynard Smith and Harper, 2003; Biernaske et al., 2014). Commonly cited examples of indices are those that signal morphology (i.e., body size; Davies and Halliday, 1978; Clutton-Brock and Albon, 1979; Thapar, 1986). However, behavioral, performance-based traits may provide a fruitful area of investigation because these traits require integration of the whole organism (Irschick et al., 2007; Vanhooydonck et al., 2007), and many are rate-based (e.g., accelerations, sound frequencies, etc.). Organisms are intrinsically physically constrained (Alexander, 1985), thus tradeoffs occur when the enhancement of one aspect of performance decreases another type of performance (Garland, 2014). Rate-based performances may be causally linked to biologically important underlying traits, and constrained by tradeoffs in their production. Thus, analysis of whole-organism performance may help to better understand the constraints involved in the index signal mechanism (Vanhooydonck et al., 2007).

Hummingbird males perform elaborate, extreme aerial courtship displays which are hypothesized to push against the limits of their locomotor abilities (Clark, 2009) and therefore potentially allow females to assess variation in male flying ability (Clark, 2009; Byers et al., 2010; Barske et al., 2011). Thus the adaptive hypothesis for male hummingbird courtship behaviors is that females select males based on their flight performances and indirectly benefit because their offspring inherit genes that will result in high flight performance. Alternatively, male flight performance is unconnected to aerial courtship performance, and the extreme displays are a result of female preferences only. Under this hypothesis, extreme locomotor performance during courtship might be intrinsically difficult to perform but in ways that are uncorrelated to the animal's ability to accelerate or turn. For example, the elevated wingbeat frequencies of male bee hummingbirds (Chapter 1) create wing-generated sounds, including black-chinned hummingbird (Feo and Clark, 2010). For instance, in Allen's hummingbird, the wing trill was produced specifically during the wing supination portion of the wingbeat cycle, such that production of the trill was relatively uncoupled from flight performance (Clark and Mistick, 2018). Likewise, the shuttle display is also a visual display, including iridescent throat feathers (Simpson and McGraw, 2018a, 2018b). Therefore, it is plausible that a male's performance of the shuttle display does not showcase how "good" he is at flying.

Black-chinned hummingbird (*Archilochus alexandri*) males perform two types of flight displays: courtship dives, and close-range aerial "shuttle" displays (Baltosser and Russell, 2000; Feo and Clark, 2010). During the shuttle display the male flies laterally side-to-side within ~0.5 meter of a female (Figure 3.1A; Baltosser and Russell, 2000) while also producing specialized sounds (the ‘wing trill’) with their wings (Figure 3.1B; Feo and Clark, 2010). The wing trill of the shuttle display consists of two sounds, one of which is produced in ordinary flight (Figure 3.1B, Element A; Feo and Clark, 2010), while the other element is produced only during this display (Figure 3.1B, Element B; Feo and Clark, 2010). Males perform this display ardently for females during the breeding season, suggesting that this display could function as a signal of male quality via flight performance.

A noticeable feature of the shuttle display is a drastic increase in wingbeat frequency relative to hovering: the wingbeat frequency is elevated from 51.2 Hz during hovering to 89.4 Hz during display flight (Clark and Feo, 2010; Chapter 1). This increase in frequency is far higher than observed in studies of hummingbird maneuvers or other types of flight. Whereas some kinds of flight (such as over a range of flight speeds in a wind tunnel) result in virtually no change in wingbeat frequency (Clark and Mistick, 2018; Clark and Dudley, 2010; Chai et al., 1997), the increases in wingbeat frequency of up to 30% were reported during turning maneuvers (Cheng et al., 2016a, 2016b). Thus, we hypothesized that shuttle-display performance is a reflection of some aspect of male flight performance. We tested this hypothesis by examining 1) whether shuttle-display wingbeat frequency positively correlated with other flight performance variables during the shuttle display, and 2) whether shuttle-display performance variables positively correlate with a load-lifting assay that has been frequently used to assess low-speed hummingbird flight performance (Chai et al., 1997; Chai and Millard, 1997; Altshuler, 2006; Segre et al., 2015; Groom et al., 2017; Dakin et al., 2018). Significant correlations between shuttle-display wingbeat frequency and shuttle-display flight performance variables (e.g., between acceleration and wingbeat frequency, etc.) and/or between shuttle-display performance variables and flight performance elicited via a load-lifting assay would provide support for the hypothesis that the display could signal flight performance ability. Null results would imply either 1) that females may be choosing males based on signals not associated with the shuttle display parameters we measured, and/or 2) the shuttle display is a result of aesthetic or runaway selection and therefore is not a signal of male flight ability.

The flight pattern of the shuttle display is approximately sinusoidal, characterized by both an amplitude and cycle frequency. An individual cannot maximize both the cycle frequency and amplitude of the overall flight pattern of the shuttle display because of the acceleration-limited nature of locomotion (Biewener and Patek, 2018; Chapter 1). Thus, the cyclical nature of the shuttle display suggests a tradeoff between the cycle frequency and amplitude that may constrain the overall display. We analyzed the relationship between the cycle frequency and amplitude of the shuttle display flight pattern to see if a negative relationship exists which would indicate a constraint to display performance.

Methods:

Shuttle Display Recordings and Digitization

Shuttle displays were recorded from two populations: a small number of males that held courtship territories on and around the UC Riverside campus (33.9737° N, 117.3281° W), and a larger number of birds breeding at the Rancho Santa Ana Botanic Garden in Claremont, California (34.1106° N, 117.7151° W). Individual males were captured and banded ensuring that they were different individuals. Shuttle displays were elicited by placing a live captive female in a wire mesh cage in an open space on a male's territory. We attempted to elicit multiple displays per male, but only one shuttle display was elicited per bird because males rapidly acclimated to the stimulus of a live caged female. Shuttle displays were recorded May-August of 2015, 2016 and 2018.

The shuttle displays were recorded with a microphone (Sennheiser MKH 20) attached to a Sound Devices 702 24-bit recorder, to record wingbeat frequency acoustically. Two video cameras (Canon Vixia HF R500; recording at 30 fps) also were used to record shuttle displays by placing the cameras at a 90° horizontal angle from each other. The cameras were slightly below the cage, pointed up at a 45° angle so that the sky was in the background to facilitate digitization of displaying males. The video recordings were temporally synchronized to within 1 frame using hand claps equidistant from the cameras (i.e., near the caged female), and spatially calibrated using a meter stick moved through the filmed volume (Theriault et al., 2014). Each male's head was manually digitized using DLTdv7 Matlab software (Hedrick, 2008). The resulting 3-dimensional digitizations of shuttle-display flight trajectories were smoothed with a lowpass (3.5 Hz) 4th order Butterworth filter in Matlab using a custom program (Crenshaw et al., 2000). The lowpass filter (3.5 Hz) was chosen based on the digitization of a dropped ball. 3.5 Hz gave the closest approximation of acceleration due to gravity on the dropped ball. All males were captured within 48 hours of shuttle display elicitation using hummingbird-feeder traps. Once captured, body mass, folded wing length, and keel length (i.e., the length of the sternum from the charinal apex to the caudal end of the sternal body; Fig. 13

in Zusi, 2013) were measured with digital calipers. The primary flight muscles (pectoralis and supracoracoideus) attach to the keel, thus this linear dimension served as a proxy for muscle size. After performance of the load-lifting assay (see below), all males were banded and released near their site of capture.

The Crenshaw et al. (2000) program produced estimates of the instantaneous velocities, accelerations and flight-path curvatures (a measure of turning performance) of shuttle displays. Curvature is the turning rate of velocity with respect to the arc length of the flight trajectory (Crenshaw et al., 2000; Barber et al., 2015). High curvature values correspond to sharp turns whereas low curvature values correspond to relatively straight trajectories. Since the male flies back and forth repetitively during the shuttle display, values for all of these kinematic parameters also oscillate. The peak values for velocity, acceleration, deceleration, flight-path curvature (Figure 3.1C, D, E) during a shuttle display were taken to represent male courtship-flight performance. We measured shuttle-display wingbeat frequency during the portion of the display in which wingbeat frequency was highest by counting wingbeats from sound recordings displayed by the program Raven Lite 2 (Figure 3.1B). To create a time series, we used sound recordings and recorded the time in seconds of the point at which Element A met Element B (Figure 3.1B). In video, this corresponded to the onset of display flight behavior in which the male began flying laterally while facing the caged female to the point at which the male ceased the behavior and began to fly away from the caged female.

3-dimensional flight trajectories were analyzed to quantify the amplitude (a) and cycle frequency (f) of the displays (Figure 3.1A).

Asymptotic Load-lifting

We assessed flight performance ability by attaching a string of small beads tied to a tiny black rubber band to each bird, following a previously established approach (Chai and Millard, 1997; Chai et al., 1997). For each male, the rubber band was placed around the neck and the bird was positioned on the floor of a 30.5 cm x 30.5 cm x 61.0 cm mesh field cage. A brief touch stimulated the male to fly upward. As the bird flew higher, incrementally more mass was lifted and therefore required more vertical force production. Each male was video recorded performing the load-lifting assay 4 times with a 60 second break between each trial. Video recordings allow for a frame-by-frame assessment of the maximum number of beads lifted in each trial. Out of the four trials, the maximal value for total mass lifted (i.e., the mass of the bird plus the mass of the beads lifted) was divided by body mass to create a variable that represents the maximal proportion of body mass lifted. Total mass lifted was correlated with body mass ($\rho = 0.79$, $N = 18$, $p < 0.0001$), whereas the proportion of body mass lifted was not ($\rho = 0.33$, $N = 18$, $p = 0.17$). Thus the proportion of body mass lifted was used in subsequent statistical analyses.

Statistical Analyses

All statistical analyses were performed using JMP Pro 14.2.0. We used General Linear Mixed Models to test the hypotheses that 1) shuttle-display wingbeat frequency is correlated with other aspects of shuttle-display flight performance (i.e., acceleration, velocity, and flight-path curvature), and 2) that shuttle-display performance variables are positively correlated with asymptotic load lifting. For each model, we included body mass, folded wing length, keel length, display wingbeat frequency, the time series of the display, the maximum proportion of body mass lifted during asymptotic load lifting, and the year the display was recorded as fixed effects, individual as a random effect, and a shuttle-display flight performance variable (i.e., acceleration, deceleration, velocity, or flight-path curvature) as the dependent variable. We also created a model in which shuttle-display wingbeat frequency was the dependent variable, and included body mass, folded wing length, keel length, the proportion of body mass lifted during asymptotic load lifting, the time series of the display, and the year the display was recorded as fixed effects, and individual as a random effect. Since the shuttle display is hypothesized to be physically challenging to perform, we included the time series of the display in our models because we predicted display performance variables would decline with time. For all models, the year was included as a nuisance variable, but was never significant (all $p > 0.073$) so was not included in any final GLM model. Likewise, the morphological variables body mass, folded wing length, and keel length were never significant (all $p > 0.12$) thus were not included in final GLM models. We tested interaction effects between individual and the time series variable (i.e., individual * time) to see if individuals varied in the decline of their performances over time, and the interaction between individual and shuttle-display wingbeat frequency (i.e., individual*wingbeat frequency) to see if individuals varied in their relationship between shuttle-display wingbeat frequency and other flight-performance variables. These interaction effects were only included in final models if they were significant.

To test for a negative relationship between shuttle-display cycle frequency and amplitude (which would indicate a performance tradeoff due to the acceleration-limited nature of locomotor behaviors), we used a GLMM with individual as a random effect, amplitude, acceleration, and individual*acceleration and individual*amplitude interactions as fixed effects, and cycle frequency as the dependent variable.

Results:

A total of one display from each of 18 male black-chinned hummingbirds were recorded. Males seemed to be enticed to display by the movement/flight of the caged live female stimulus, rather than a still, perched live female. Upon noticing her, a typical focal male performed a shuttle display within ~0.5 m of the cage. The side-to-side lateral flight (Table 3.1; mean ± standard deviation: cycle frequency $\bar{x} = 1.02$ Hz ± 0.11; amplitude $\bar{x} = 0.78$ m ± 0.13) of the shuttle display was repetitive and accompanied by specialized buzz-like sounds produced by the wings. The repetitive turning maneuvers were cyclical and therefore permitted repeated measurement of the peak accelerations as the birds maneuvered out of turns (Fig. 3.1A, 3.1C), the peak decelerations that preceded turn maneuvers (Fig. 3.1A, 3.1C), the peak velocities (Fig. 3.1A, 3.1D), and the peak curvatures of the turn maneuvers (Fig. 3.1C, 3.1E) of the shuttle display. The displays ended once the male had flown away from the female, or landed on the cage.

Averages of peak accelerations and decelerations were on the order of 1-1.5G (Table 3.1). However the maximum values for peak acceleration and deceleration were ~3G (Table 3.1). Shuttle displays were relatively low velocity, the greatest translational velocity reached was 4.33 m s^{-1} (Table 3.1). Flight-path curvature reached the highest values (Table 3.1) as the bird reached the ends of the shuttle segments (Figure 3.1A, 3.1E), as birds performed turn maneuvers during the display. Buzz-like sounds during the display, recorded by the microphone indicated that shuttle-display wingbeat frequency was elevated relative to hovering (Feo and Clark, 2010; Chapter 1). One male reached 95.24 Hz (Table 3.1). Shuttle displays lasted between 4.19 s and 40.72 s (Table 3.1).

Results of General Linear Mixed Model analyses showed significant positive relationships between shuttle-display wingbeat frequency and acceleration (Figure 3.2; GLMM: $F_{1,582.8} = 6.56$, $p = 0.011$. Although marginally nonsignificant, shuttle-display wingbeat frequency was negatively correlated with deceleration (GLMM: $F_{1,553.7} = 3.28$, $p = 0.071$). Shuttle-display wingbeat frequency was not related to velocity ($p = 0.21$) or flight-path curvature ($p = 0.28$). Also, asymptotic load lifting was not significantly correlated with any shuttle-display flight performance variable (all $p > 0.079$).

As males performed the display, their velocities (Figure 3.3B; GLMM: $F_{1,18.2} = 29.85$, $p < 0.0001$) and wingbeat frequencies (Figure 3.3C; GLMM: $F_{1,512.2} = 77.25$, $p < 0.0001$) declined with time. For velocity, the interaction between individual and time was significant (Figure 3.3B; GLMM individual*time: $F_{17,46.9} = 6.51$, $p < 0.0001$). For shuttle-display wingbeat frequency, this also was true (Figure 3.3C; GLMM individual*time: $F_{1,663.7} = 15.82$, $p < 0.0001$). Flight-path curvature showed a marginally nonsignificant increase (i.e., turns became sharper with time (Figure 3.3D; GLMM: $F_{1,8.6} = 3.80$, $p = 0.084$) and the interaction between individual and time was significant (Figure 3.3D; GLMM individual*time: $F_{17,14.2} = 4.02$, $p = 0.0056$). Acceleration ($p = 0.18$) and deceleration ($p = 0.25$) were not significantly affected by time. However, for acceleration, the interaction between individual and time was significant (Figure 3.3A: GLMM, individual*time: $F_{17, 39.8} = 4.20$, $p < 0.0001$).

We found a significant negative effect of amplitude on cycle frequency (Figure 3.4; GLMM: $F_{1, 384.7} = 122.39$, $p < 0.0001$), and a significant positive effect of acceleration on cycle frequency (Figure 3.4; GLMM: $F_{1, 565} = 6.14$, $p = 0.014$). Thus, a tradeoff exists between cycle frequency and amplitude that constrains the flight-path of the shuttle display. We also found significant interaction effects between individual and amplitude (Figure 3.4; GLMM individual*amplitude: $F_{17, 327.8} = 9.10$, $p < 0.0001$), and between individual and acceleration (Figure 3.4; GLMM individual*acceleration: $F_{17, 558.7} = 3.59$, $p < 0.0001$)

Discussion:

Flight Performance of the Shuttle Display

The shuttle display of male black-chinned hummingbirds entails dramatically elevated wingbeat frequencies, high accelerations and decelerations, and repetitive lateral turn maneuvers. We found support for the adaptive hypothesis that the shuttle display is a signal of male flight performance. Buzz-like sounds (Figure 3.1B) produced during the display correspond to wingbeat frequency which is significantly positively correlated with acceleration (Figure 3.2). Since the wing sounds are causally linked to wingbeat frequency, they might function as an auditory index signal (Maynard Smith and Harper, 1995; Maynard Smith and Harper, 2003) of flight performance that females may judge during courtship and mate selection. Although certain aspects of the shuttle display might be due to aesthetic evolution (e.g., flaring of the colorful gorget feathers), our results support the hypothesis that this display is a signal of male flight performance, and reliably reflects variation in male flight acceleration via the production of specialized buzz-like sounds created by the wings.

However, we did not find a significant relationship between asymptotic load lifting and shuttle-display performance variables. Asymptotic load lifting is a common assay of hummingbird flight performance (Chai and Millard, 1997; Chai et al., 1997; Altshuler and Dudley, 2003; Altshuler et al., 2010; Segre et al., 2016; Groom et al., 2017; Dakin et al., 2018). Because we did not find significant effects of asymptotic load lifting performance on any shuttle-display flight variable, a reasonable interpretation is that the shuttle display is not a signal of male flight performance. Asymptotic load lifting assays the vertical force output of a hummingbird as it flies a short distance relatively slowly (Chai and Millard, 1997; Chai et al., 1997). Perhaps, this assay of flight performance does not assay all aspects of flight performance that have ecological relevance. During the shuttle display the male remains facing towards the female while flying side to side (Figure 3.1A). Hence, the shuttle display tends to include substantial lateral motion (i.e., sideslip) rather than vertical motion. A theoretical model of flight performance predicts that elevated wingbeat frequency aids in turning (Hedrick et al., 2009), such as during sideslip. However, we did not detect a significant effect of shuttle-display wingbeat frequency on flight-path curvature ($p = 0.28$). It seems wingbeat frequency is instead a reflection of male acceleration.

The shuttle display is likely a challenging behavior for males to perform. Velocity (Figure 3.3B) and shuttle-display wingbeat frequency (Figure 3.3C) declined as the birds performed the display over time, suggesting that the behavior becomes more difficult to perform at high levels the longer an individual continues to display. We also found significant individual*time interactions for acceleration (Figure 3.3A), velocity (Figure 3.3B), shuttle-display wingbeat frequency (Figure 3.3C), and flight-path curvature (Figure 3.3D). This indicates there is variation in how birds alter their shuttle-display flight performances over time. That is, the performances of some birds declined with time more than others. Thus, there is potential for females to judge males based on individual variation of shuttle-display performances.

We also detected a tradeoff between cycle frequency and amplitude of the shuttle display (Figure 3.4) which seems to be related to individual variation in acceleration; higher accelerations were found near the edge of the cycle frequency-amplitude distribution (Figure 3.3). The tradeoff between cycle frequency and amplitude is important because it illustrates a flight performance constraint of the shuttle display. An index signal is a reliable signal because it is constrained in some way (e.g., anatomically, physiologically, biomechanically, etc.), and forms a causal link between the signal and the underlying advertised trait (Maynard Smith and Harper, 1995; Maynard Smith and Harper, 2003; Biernaske et al., 2014). The black-chinned hummingbird shuttle display is constrained by the cycle frequency-amplitude tradeoff. Furthermore, we found significant individual*amplitude and individual*acceleration interaction effects (Figure 3.4). This suggests individuals vary in how they deal with the cycle frequency-amplitude tradeoff. Thus, females could potentially judge males based on visual information regarding the cycle frequency-amplitude tradeoff.

However, signals must have an evolved response from receivers (Maynard Smith and Harper, 1995; Maynard Smith and Harper, 2003). Whether black-chinned hummingbird females actually select males based on shuttle-display performance traits is an unanswered question. There is currently no assay for female preference in hummingbirds (Hogan and Stoddard, 2018). Thus, further research is needed on hummingbird mating systems, particularly with regard to paternity analyses, to answer this question.

Morphology and Performance

We also did not detect significant relationships between morphology and shuttle display flight performance traits. Morphology-performance relationships can break down if the measured performance is submaximal (Irschick et al., 2008). Based on the cycle frequency-amplitude relationship (Figure 3.4) and the fact that we removed one underperforming outlier bird from Figures 3.2 and 3.3 (but not from analyses), it may be the case that submaximal performance is affecting the relationship between morphology and performance. However, performances seemed maximal for other traits. For instance, wingbeat frequencies ($\overline{x}$ = 83.28 Hz ± 2.72) we recorded were similar to those from a previous study on black-chinned hummingbird courtship displays ($\overline{x}$ = 89.4 Hz ± 3.36; Feo and Clark, 2010) and are substantially higher than hovering wingbeat frequency (Chapter 1). Also, the accelerations we report are similar to those reported for hummingbirds performing a horizontal maneuver in response to a startle stimulus (~25 m s^{-2}; Clark, 2010), and similar to those reported for hummingbirds during takeoff (37.4 ± 10.1 m s^{-2}; Tobalske et al., 2004).

Another reason for a lack of correlation between morphology and performance is low variation in morphology. We have argued that male hummingbirds in the bee clade (including black-chinned hummingbirds) are under sexual selection for flight performance (Chapter 1). Strong directional selection on males in the form of female choice sexual selection is predicted to decrease genetic variation (Fisher, 1930), and presumably phenotypic variation. Possibly this is the case in male black-chinned hummingbirds.

Conclusion

In summary, the black-chinned hummingbird shuttle display may serve as a signal since the specialized buzz-like sounds produced by the wings (Figure 3.1B) are causally linked to shuttle display wingbeat frequency which reflects variation in the underlying male trait: acceleration (Figure 3.2). Also, the shuttle display is constrained by a tradeoff between cycle frequency and amplitude which limits the distribution over which males are capable of performing the display (Figure 3.3). The significant interaction effects suggest that there is variation in how males perform the display over time. However, we did not find a significant correlation between asymptotic load lifting and shuttle-display performance which suggests the display is not a flight-performance signal. Furthermore, there are two parts to the definition of an animal signal: 1) the signal must evolve to alter the behavior of a receiver, and 2) the signal entails an evolved response from the receiver (Maynard Smith and Harper, 1995; Maynard Smith and Harper, 2003). Subsequent research is required to confirm that females attend to the shuttle display during courtship and mating decisions.

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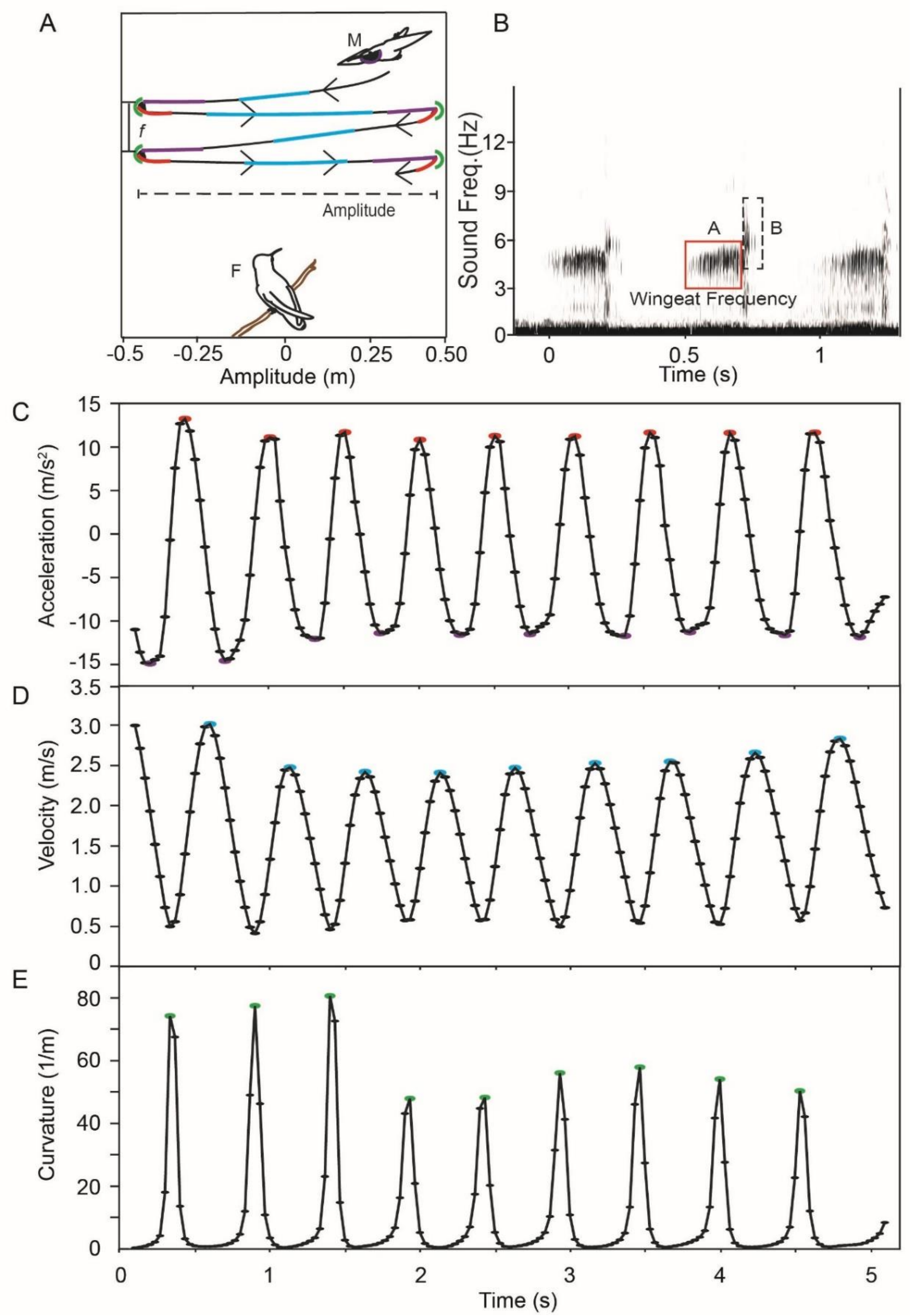
Figures:


A
M
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Amplitude
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Amplitude (m)
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Sound Freq.(Hz)
12
9
6
3
0
A
B
Wingeat Frequency
0
0.5
1
Time (s)
C
Acceleration (m/s^2)
15
10
5
0
-5
-10
-15
D
Velocity (m/s)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
E
Curvature (1/m)
80
60
40
20
0
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1
2
3
4
5
Time (s)

Figure 3.1. Five seconds of black-chinned hummingbird shuttle display flight kinematics. **(A)** In the shuttle display, the male (M) flies laterally back and forth in front of the female (F), in *segments*, where each segment has an amplitude of approximately 0.5 to 1.0 meter and cycle frequency (*f*). Peak decelerations (Purple; C) and peak accelerations (red; C) occur near the end of the segments. Velocity peaks midway through the segment (Blue; D). Flight-path curvatures (Green; E) also peaks at the end of the shuttle segments. **(B)** A spectrogram of specialized wings sounds produced after each turn maneuver. These specialized sounds consist of two elements: Element A outlined by the red box which also occurs in regular flight, and Element B outlined by the dashed black box which occurs only during displays. **(C)** Peak accelerations and decelerations are red and purple, respectively. **(D)** Velocity over time; peak velocities indicated with blue dots. **(E)** Peak flight-path curvature values are indicated in green.

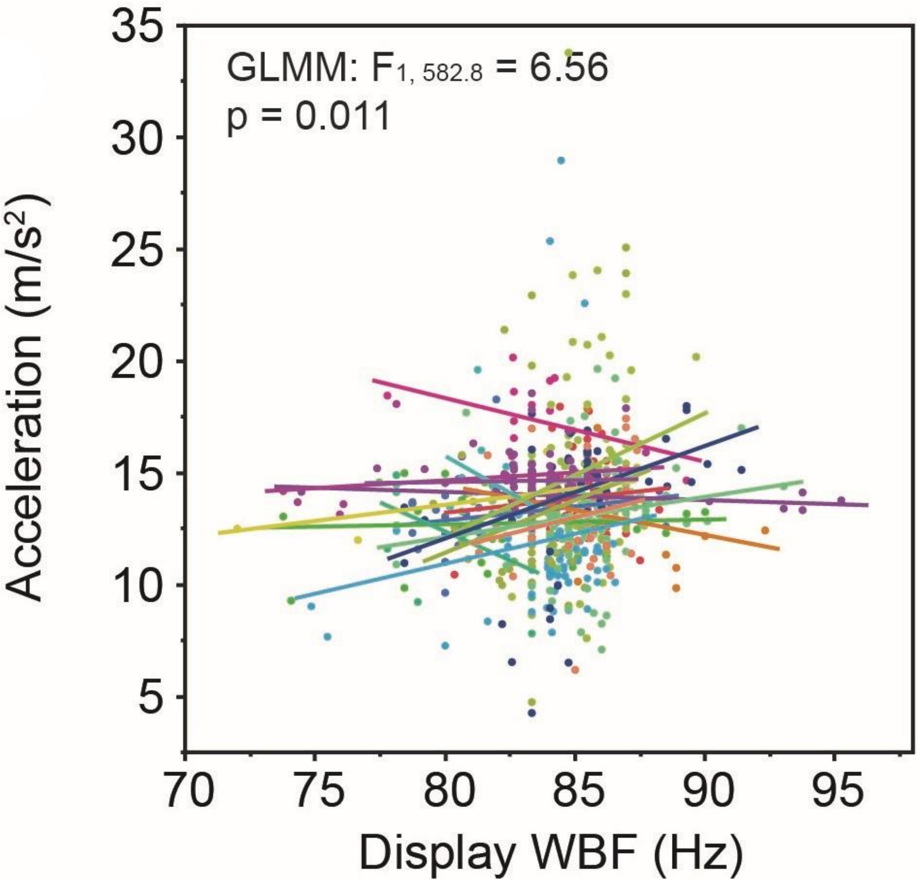

Figure 3.2. Elevated shuttle-display wingbeat frequency affects acceleration. Data points and lines are color coded by individual (N = 17). One bird was a clear underperforming outlier and therefore removed from the figure. Statistical analyses with and without the outlier individual did not change the results. Results presented here are from analyses with the outlier individual included. Shuttle display wingbeat frequency showed a general positive effect on acceleration ($t = 2.62$, $df = 596.8$, $p = 0.0090$).

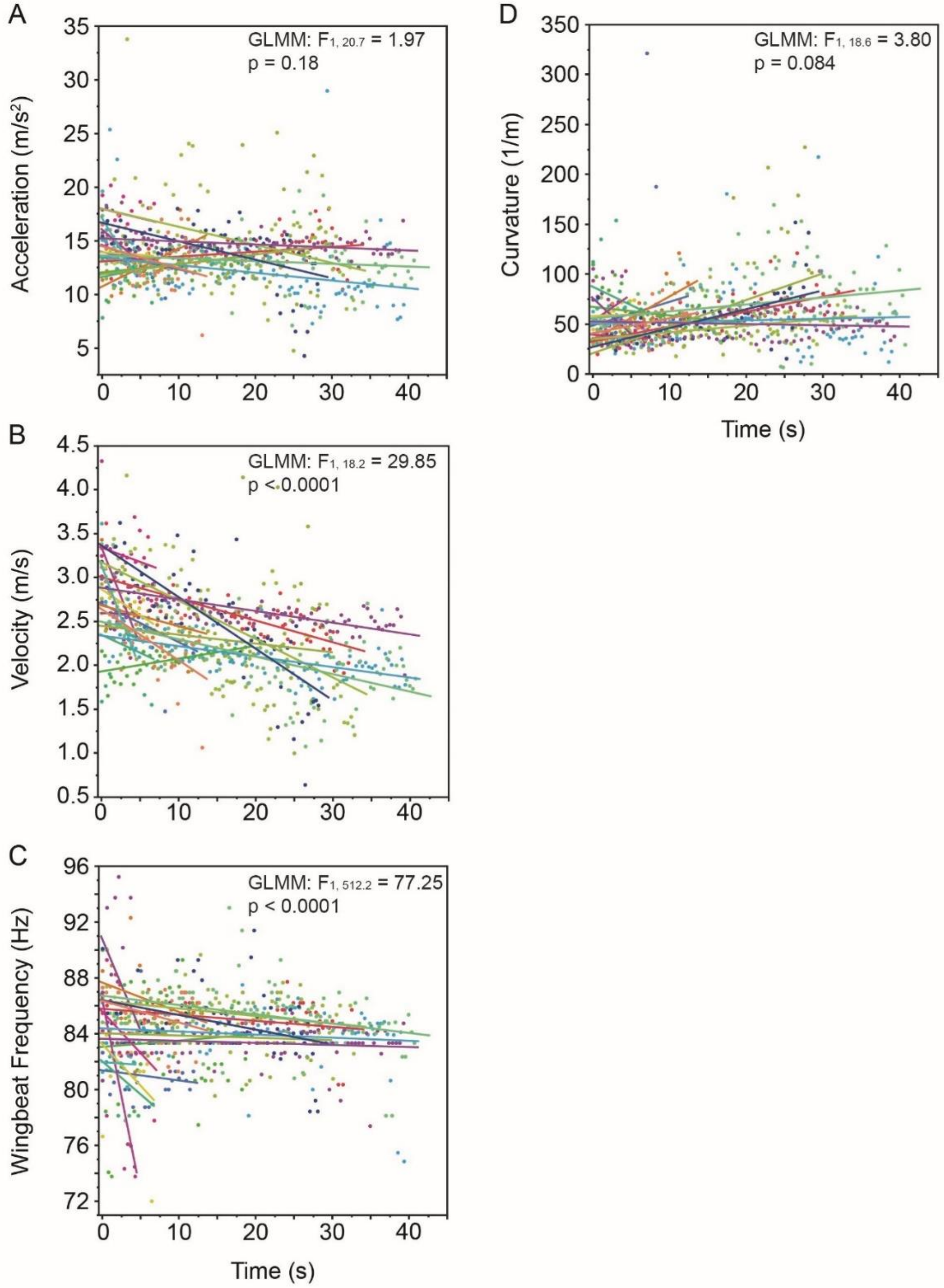

Figure 3.3. Shuttle-display flight performance parameters change with time. Data points and lines are color coded by individual. One bird was a clear underperforming outlier and

therefore was removed from the figure. Statistical analyses with and without the outlier individual did not change the results. Results presented here are from analyses with the outlier individual included. A) Acceleration did not decline with time ($F_{1, 20.7} = 1.97$, $p = 0.18$), but the interaction between individual and time was significant (individual*time: $F_{17, 39.8} = 4.20$, $p < 0.0001$). B) Velocity declines with time ($F_{1, 18.2} = 29.85$, $p < 0.0001$), and the slopes vary by individual (individual*time: $F_{17, 46.9} = 6.51$, $p < 0.0001$). C) Shuttle-display wingbeat frequency declines with time ($F_{1, 512.2} = 77.25$, $p < 0.0001$), and the slopes vary by individual (individual*time: $F_{17, 663.7} = 15.82$, $p < 0.0001$). D) Flight-path curvature showed a marginally nonsignificant increase with time ($t = 6.20$, $df = 658.8$, $p < 0.0001$), and the slopes vary by individual (individual*time: $F_{17, 14.2} = 4.02$, $p = 0.0056$). Some males decrease the amplitude and increase the cycle frequency of the shuttle display as the display flight progresses which seems to coincide with the maneuvering of sharper turns later in the display (pers. obs. SCW). It is possible, birds can maintain acceleration while decreasing amplitude and increasing cycle frequency.

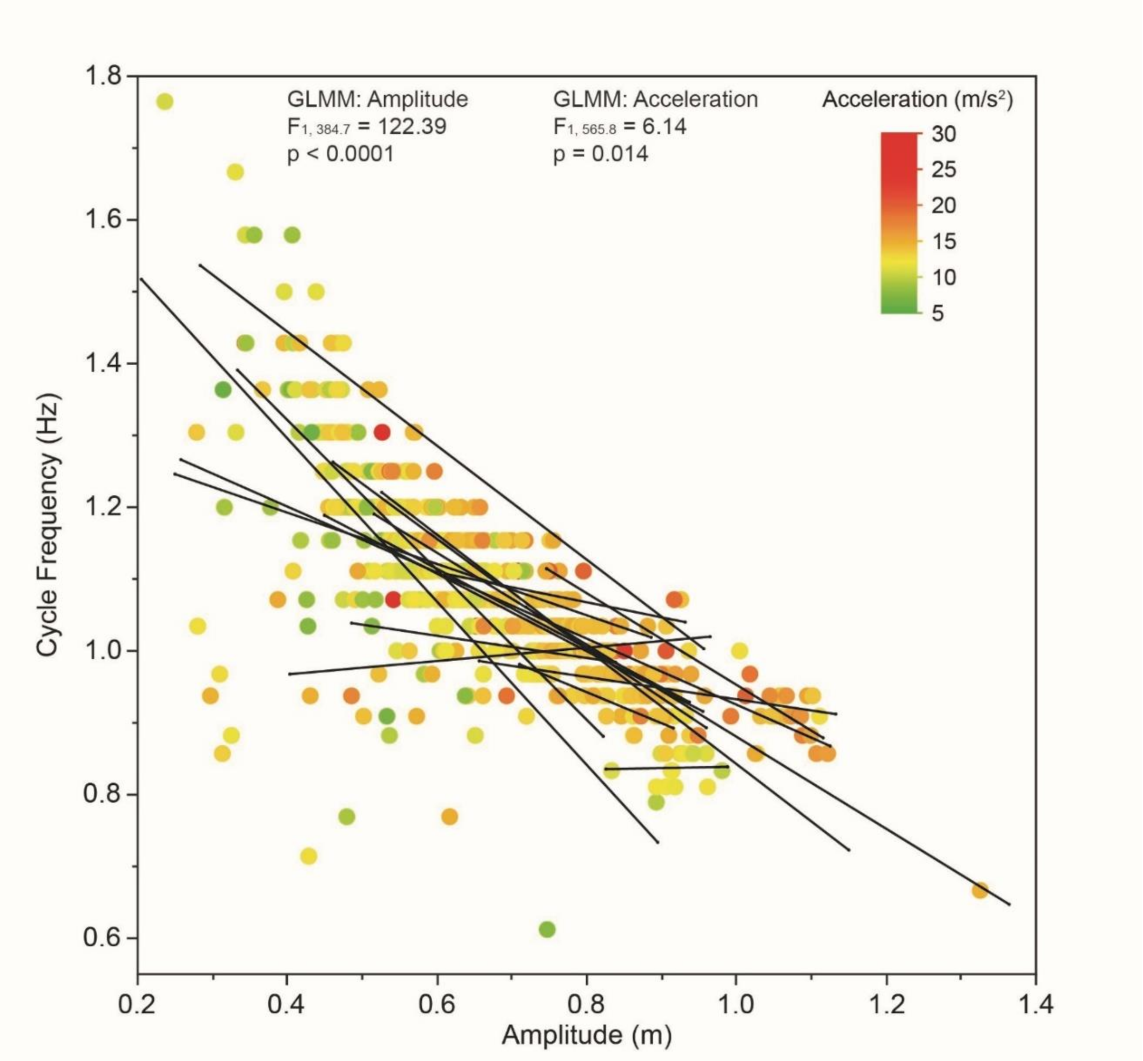

Figure 3.4. Shuttle-display cycle frequency and amplitude trade off within and among individual males (N = 18 displays, 1 display per male). Each point represents a different segment within the display (Figure 3.1A). The negative relationship between cycle frequency and amplitude is indicative of a flight-performance tradeoff (amplitude: $F_{1, 384.7}$ = 122.39, $p < 0.0001$). Data are color coded based on acceleration (acceleration: $F_{1, 565}$ = 6.14, $p = 0.014$). Individual slopes vary significantly for amplitude

(individual*amplitude; $F_{17, 327.8} = 9.10$, $p < 0.0001$), and for acceleration (individual*acceleration: $F_{17, 558.7} = 3.59$, $p < 0.0001$).

Tables:

Table 3.1. Shuttle-display flight performance means, standard deviations, minimum and maximum values. Means and standard deviations are grand means of individual performances (N = 17). Minimum and maximum values are from the entire pool of male performances (N = 708). One low-performing outlier was dropped from these calculations.

Flight performance variable	Mean	St. Deviation	Minimum	Maximum
Acceleration (m s^{-2})	13.76	1.28	4.25	33.79
Amplitude (m)	0.78	0.13	0.24	1.33
Curvature (m^{-1})	57.54	9.10	6.49	321.28
Cycle frequency (Hz)	1.02	0.11	0.61	1.76
Deceleration (m s^{-2})	-10.67	1.20	-27.2	-3.35
Duration (s)	19.46	13.85	4.19	40.72
Velocity (m s^{-1})	2.48	0.30	0.64	4.33
Wingbeat Frequency (Hz)	83.71	2.10	60.81	95.24

General Conclusion:

To summarize, this dissertation concludes that flight performance is sexually selected in hummingbirds. Explicitly, female choice for male aerial courtship behaviors is the most likely cause for small male size and reversed sexual size dimorphism in the bee hummingbird clade. We present data on the extraordinarily high courtship-display wingbeat frequencies of males in the bee hummingbird clade and suggest that more research is necessary on the flight muscle properties of these birds.

Sexual differences in flight performance is apparent in black-chinned hummingbirds (*Archilochus alexandri*), and the performances of two common flight assays are correlated suggesting that some birds are better fliers than others. Here, we suggest subsequent studies of flight performance in birds and other animals would do well to include both sexes to better encompass the variation in flight performance within a species. This might be particularly important if the species exhibits sexual size dimorphism or dimorphism of flight-related traits.

Lastly we characterize the flight performance of the black-chinned hummingbird (*A. alexandri*) shuttle display. We suggest this display functions as an index signal (Maynard Smith and Harper, 2003) of flight performance because the display is physically constrained by the cycle frequency-amplitude tradeoff, and the specialized sounds produced by the wings are causally related to wingbeat frequency. We show that shuttle display wingbeat frequency is correlated with acceleration, potentially advertising individual variation in this male locomotor performance trait which females may attend (Byers et al., 2010). However, more research is necessary to uncover if females prefer to mate with males based on shuttle-display performance traits like wingbeat frequency and acceleration.

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