Title

A 'Tail' of Two Signals: Acoustic Mating Displays in Anna's and Costa's Hummingbirds (Calypte anna; Calypte costae)

Author

Berger, Ayala Noga

Publication Date

2024

UNIVERSITY OF CALIFORNIA
RIVERSIDE

A 'Tail' of Two Signals: Acoustic Mating Displays in Anna's and Costa's Hummingbirds (*Calypte anna; Calypte costae*)

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

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Ayala Noga Berger

September 2024

Dissertation Committee:
Dr. Polly Campbell, Co-Chairperson
Dr. Christopher Clark, Co-Chairperson
Dr. Bret Pasch

The Dissertation of Ayala Noga Berger is approved:

Committee Co-Chairperson

Committee Co-Chairperson

University of California, Riverside

Acknowledgements

I thank Dr. Polly Campbell for her support and guidance. She taught me how to become a better scientist, mentor, teacher, and human being. I will greatly miss walking down the hall to talk about science, dogs, and everything in between (puffin chicks feeding log and bat echolocation evolution to name a few). I thank Dr. Chris Clark for introducing me to the coolest system and for the great talks about ornithology. I thank Dr. Bret Pasch for his input and edits on my chapters. I thank the UC natural reserve system and reserve managers especially Dr. Chris Tracy at Boyd Deep Canyon who worked hard to ensure access to the reserve after flashfloods took down the access road.

I deeply thank my family for their unconditional love and support. I thank Elon for lending me Happi and Ori for lending my Hank and I thank both for visiting me and for the many great adventures we have had. I thank Tamar for listening to me and for the many niece photos and video calls. I especially thank my parents for their unwavering, constant, and all-encompassing support and guidance.

I express my gratitude to my lab mates, Nadje Najar, Sean Wilcox, Katie Johnson, Krista Le Piane, Sarah Gardner, Jon Hughes, Bongnikosi Gumbi, Louise Heitzmann, David Rankin. I thank Sarah Gardner who finished her dissertation and started her new position on the same timeline as me–so much sour candy was involved. I thank David Rankin for his help in the field and Nadje Najar for the many discussions about my research. I thank multiple undergraduate researchers notably Wendy Yeh, Trinh Kondo, and Ashley Padollina–their help analyzing the many song-recordings was instrumental in the completion of the project.

I thank my friends Shaina Hammer and Ilana Kornfeld for accompanying me to random and remote places and for their constant championship and friendship. I would like to thank Tamar Danielle Wenocur and Hannah Katznelson for the writing sessions together. To all my friends at UCR including April Arquilla, Kristen Edwards, Sam Kubica, Catherine Nguyen, and Sandhya Sriram, thank you for talking science and for all the fun times we have had. I thank Sam Kubica and Kristen Edwards for reading many versions of my work, listening to multiple practice presentations, and for teaching me to like cats.

I thank Tulip for her magical diving board chats and slow walks. I thank Happi for being my shadow.

I would like to thank my dissertation and oral exam committee members, Drs. Polly Campbell, Kim Hammond, Khaleel Razak, Wendy Saltzman, and Lawrence Rosenblum and Blue for their advice, encouragement, and mentorship. Kim and Wendy especially have been constant cheerleaders and extremely supportive of my current and future endeavors.

I thank the Animal Behavior Society, the Society of Integrative and Comparative Biology, Western Ornithology Society, Graduate Assistance in Areas of National Needs (GANN), Shoemaker foundation UCR, Spieth family UCR, and Newell fund, and UCR Graduate Division for funding my research and studies.

Dedication

To my parents for championing my passions and interests and supporting me in every way of my life. My curiosity and humanist view of the world stems from the examples they set.

ABSTRACT OF THE DISSERTATION

A 'Tail' of Two Signals: Acoustic Mating Displays in Anna's and Costa's Hummingbirds (*Calypte anna; Calypte costae*)

by

Ayala Noga Berger

Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, September 2024
Dr. Polly Campbell and Dr. Chris Clark, Co-Chairpersons

The evolution of complex courtship displays is of central interest in animal behavior. Anna's and Costa's hummingbirds (*Calypte anna* and *C. costae*) produce complex acoustic courtship signals mechanically with wing and tail feathers during dives, and vocally with their syrinx. Both the song and the dive-sounds of Anna's are complex and multisyllabic whereas in Costa's both signals are one phrase and monosyllabic. I first (chapter 1) test how similar the dive-sounds and songs are to one another within individual, then (chapter 2) characterize macrogeographic variation in Anna's and Costa's song, and lastly (chapter 3) characterize microgeographic variation in Anna's song.

First, I analyzed dive-sound and song covariation within individual. I audio-recorded dive-sounds and songs from 13 Anna's and 14 Costa's and measured spectral and temporal components of both courtship signals. I found between-individual variation in all components but little within-individual covariation between dive-sound and song, suggesting that the correspondence between song and dive-sounds is not maintained within individual.

Second, I studied macrogeographic variation in Anna’s and Costa’s songs. Cultural transmission of song components can lead to the accumulation of variants that differ among populations. I recorded 5-24 males in 6 populations of each species across their ranges in the Western US and tested for geographic variation in song. I found few differences in frequency measures of Costa’s song and invariant song form across populations. Anna’s song was contrastingly variable, with population differences in both syllable use and multiple spectral and temporal measures. The most strongly differentiated Anna’s population in my study, Seattle (WA), is the product of a recent northward range expansion. The loss and modification of syllables in this population suggests a founder effect.

Third, I analyzed Anna’s song within one population, Golden Gate Park, San Francisco, CA, and tested inter-individual distance effects on song. I recorded 29 birds and analyzed spectral and temporal components of their songs. I found an effect of inter-individual distance on song, evidence of syllable sharing, 11 song types, and 3 song neighborhoods.

This dissertation provides insight into song evolution in non-passerines and contributes to understanding of how complex signals evolve.

Table of Contents

Chapter 3: Song Variation Across Space and Time in Anna's Hummingbird (*Calypte anna*)

List of Figures

Chapter 1

Chapter 2

Chapter 3

List of Tables

Chapter 1

Chapter 2

Chapter 3

Introduction

The field of acoustic ecology has its roots in antiquity. In the first book of *Historia Animalia* (written in 350 BC), Aristotle described the nature of song learning in birds. Four hundred years later, Pliny the Elder similarly documented the process of song learning noting, in the tenth book of his *Natural History* (c. 50 AD), variations between individual nightingale songs. The elaborate nature of birdsong similarly captured Darwin's attention, partially stumping him, and pushing him to develop the theory of sexual selection (Darwin, 1871). With the advent of technology such as the sound spectrograph in the 1940's, it became possible to quantify structural variation in song. The discovery of dialects in birds such as white-crowned sparrows (*Zonotrichia leucophrys*) (Marler and Tamura, 1962) and work on the neural mechanisms of song-learning (e.g., Nottenbohm, 1972) further pushed the field forward. Since then, the field of acoustic ecology has flourished, with particular emphasis/focus on the evolution and mechanistic basis of song in oscine passerines (Podos and Warren, 2007). However, vocal learning is present in three distinct avian lineages: songbirds (Passeriformes), parrots (Psittaciformes), and hummingbirds (Trochilidae) (Nottenbohm, 1972; Jarvis et al., 2014; Tyack, 2019; Johnson and Clark, 2020). This dissertation is focused on the latter.

Hummingbirds are speciose (>280 species; McGuire et al., 2014) and exhibit diverse singing behavior and song form (Monte et al., 2023). Despite this diversity, research on song hummingbirds is limited and centered on few, primarily neotropical, species (Snow, 1968; Wiley, 1971; Baptista and Schuchmann, 1990; Gaunt et al., 1994; González and Ornelas, 2009; Araya-Salas and Wright, 2013; Lara et al., 2015). Consequently, knowledge of hummingbird song lags far behind that for songbirds. To help fill this gap, this dissertation focuses on a pair of closely related species from North America, Anna's and Costa's hummingbirds (*Calypte anna*, *C. costae*, respectively), hereafter Anna's and Costa's.

Anna's and Costa's males hold display territories and produce two mechanistically different acoustic signals during courtship: a song produced with their syrinx, and a dive-sound produced mechanically during dynamic displays in which feathers flutter (Clark et al., 2018; Clark & Prum, 2015). The two acoustic signals are produced in bouts, often one bout after the other, but not simultaneously. In both species, there is an unusual correspondence between male song and dive-sounds. Moreover, despite their close evolutionary relationship, Anna's and Costa's have very different song and dive-sound structures, with a multi-syllable song and dive-sound in Anna's and a simpler one-phrase song and dive-sound in Costa's.

The aim of this dissertation is to examine whether intraspecific correspondence in song and dive-sound is maintained at an individual level, and to investigate variation in song at multiple spatial scales. In Chapter one, I focused on an individual level within one population per species and tested for evidence that males match their learned song to their dynamic mechanically produced dive-sound. In Chapter two, I tested for patterns of geographic variation in song across a large portion of each species' range. In Chapter three, I analyzed spatial structure in song within a single Anna’s population. Together these chapters provide new insight into the causes and spatial scale of variation in acoustic courtship signals in an understudied lineage of song-learning birds.

References

Araya-Salas, M., Smith-Vidaurre, G., Mennill, D. J., González-Gómez, P. L., Cahill, J.,& Wright, T. F. (2019). Social group signatures in hummingbird displays provide evidence of co-occurrence of vocal and visual learning. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, *286*, 20190666.

Aristotle. (1965). Historia Animalia, Volume 1. Translated by A. L. Loeb Classical Library, Cambridge, MA: Harvard University Press.

Baptista, L. F., & Schuchmann, K. L. (1990). Song learning in the Anna hummingbird (*Calypte anna*). *Ethology*, *84*(1), 15–26.

Clark, C. J., McGuire, J. A., Bonaccorso, E., Berv, J. S., & Prum, R. O. (2018). Complex coevolution of wing, tail, and vocal sounds of courting male bee hummingbirds. *Evolution; International Journal of Organic Evolution*, *72*(3), 630–646.

Clark, C. J., & Prum, R. (2015). Aeroelastic flutter of feathers, flight and the evolution of non-vocal communication in birds. *The Journal of Experimental Biology*, *218*(21), 3520–3527.

Darwin, C. (1871). *The Descent of Man and Selection in Relation to Sex Vol I.* John Murray, London, UK.Angell, C. S., Janacek, R., & Rundle, H. D. (2022). Maternal and Paternal Age Effects on Male Antler Flies: A Field Experiment. *American Naturalist*, *199*(3), 436–442. https://doi.org/10.1086/718236.

Gaunt, S. L. L., Baptista, L. F., Sánchez, J. E., & Hernandez, D. (1994). Song learning as evidenced from song sharing in two hummingbird species (*Colibri coruscans* and *C. thalassinus*). *The Auk*, *111*(1), 87–103.

González, C., & Ornelas, J. F. (2009). Song variation and persistence of song neighborhoods in a lekking hummingbird. *The Condor*, *111*(4), 633–640.

Jarvis, E. D., Mirarab, S., Aberer, A. J., Li, B., Houde, P., Braun, E. L., Warnow, T., Jun, W., Gilbert, M. T., & Zhang, G. (2014). Whole-genome analyses resolve early branches in the tree of life of modern birds. *Science*, *346*(6215), 1320–1331.

Johnson, K. E., & Clark, C. J. (2020). Ontogeny of vocal learning in a hummingbird. *Animal Behaviour*, *167*, 139–150.

Lara, C., Martínez-García, V., & Ornelas, J. F. (2015). Microgeographical variation in song repertoire and structure between the leks of Green Violetears *Colibri thalassinus* in Central Mexico. *Acta Ornithologica*, *50*(1), 23–32.

Marler, P., & Tamura, M. (1962). Song “dialects” in three populations of white-crowned sparrows. *The Condor*, *64*(5), 368–377.

McGuire, J. A., Witt, C. C., Altshuler, D. L., & Remsen, J. V. (2007). Phylogenetic systematics and biogeography of hummingbirds: Bayesian and maximum likelihood analyses of partitioned data and selection of an appropriate partitioning strategy. *Systematic Biology*, *56*(5), 837–856.

Monte, A., Da Silva, M., & Gahr, M. (2023). Absence of song suggests heterogeneity of vocal-production learning in hummingbirds. *Journal of Ornithology*, 164, 721-727.

Nottebohm, F. (1972). The origins of vocal learning. *The American Naturalist*, *106*(947), 116–140.

Pliny the Elder (50 A.D) Natural History. Accessed John Bostock, M.D., F.R.S. H.T. Riley, Esq., B.A. London. Taylor and Francis, Red Lion Court, Fleet Street.

Snow, D., W. (1968) The singing assemblies of Little Hermits. *The Living Birds* 7(1), 47-55.

Tyack, P. L. (2019). A taxonomy for vocal learning. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *375*(1789), 20180406.

Wiley, R. H. (1971). Song groups in a singing assembly of Little Hermits. *The Condor*, *73*(1), 28–35. What is parental care. *The Evolution of Parental Care*, 1–14.

Chapter 1

A 'Tail' of Two Signals: Acoustic Mating Displays in the Anna's and Costa's Hummingbirds (*Calypte anna* and *C. costae*)

Abstract

The diversity of animal signals are of central interest in animal behavior. Lacking however, is research into the potential covariation of complex signals that are temporally disjunct. Anna's and Costa's hummingbirds (*Calypte anna, C. costae*) produce complex acoustic courtship signals mechanically with wing and tail feathers during a dive (dive-sound), and vocally with their syrinx (song). Despite being sister taxa, Anna's and Costa's have remarkably different songs and dive-sounds. Interestingly, in both species there is a correspondence in the mechanistically distinct signal forms–the songs acoustically resemble sounds of the dive displays. The correspondence between the two signals demand explanation. I investigate to what degree there is a match between an individual's dive-sounds and songs by evaluating correlations between corresponding spectral and temporal elements of the song and dive-sounds. I tested for unequal variances in the corresponding elements at a population level. I elicited and audio-recorded dive-sounds and songs from 13 male Anna's and 14 male Costa's and recorded twenty iterations of each signal type per individual. Both dive-sounds and songs were elicited and the bird's position in relation to the microphone was standardized. I measured acoustic features of elements of both signals, including peak frequency, trill-rate, and duration. I found that in both species, generally song and dive-sound elements

did not covary with one another and that largely the songs and dive-sound variances at the population level were significantly different from one another. Results from this study will provide insight into signal evolution and the development of complex displays.

1. Introduction

Complex courtship displays abound in the natural world and have a long history of study in the fields of evolutionary biology and animal behavior (Darwin, 1871; Johnstone 1996; Hebets et al., 2016). Complex signaling is a broad term that encompasses signals that contain multiple components, multiple messages, and signals that cross sensory modalities (Hebets and Papaj, 2005). Courtship displays, whether simple or complex, evolve through the stochastic processes of genetic and cultural drift, and in response to both biotic and abiotic selection pressures, and receiver preferences and sensory biases (Marler and Tamura, 1962; Morton, 1975; Endler and Basolo, 1998). Given that both the energetic costs of signal production and the probability of predator detection typically increase with signal complexity (Cady et al. 2011; Halfwerk et al. 2014; Tobiansky et al. 2020), the fact that complexity is a widespread feature of sexually selected signals demands explanation.

Proposed adaptive benefits to complexity include enhanced signal transmission (Endler, 1992; Hebets et al., 2008) and increased information transfer (Blanco and De La Puente, 2002; Doucet and Montgomerie, 2003). Discriminating between these and other potential explanations for the evolution of signal complexity is a major challenge. Because the components of complex signals often interact (Hughes 1996; Borgia and

Presgraves 1998; Thornhill and Møller, 1998; Hebets and Uetz 1999; Hölldobler 1999; Partan and Marler 1999; Uetz and Roberts 2002), a better understanding of the processes underlying complex signal evolution can be gained from measuring the covariation between individual components.

Simultaneously produced components of complex signals are often mechanically linked (James et al., 2021; Moody et al., 2022) and are therefore inherently covariant. For example, the tap-dancing cordon-bleu bird's movements create a vibrational signal that cannot be produced in isolation from the visual signal of the bird's foot movements (Ota, 2020; Ota and Soma, 2022). Similarly, in male túngara frogs, call amplitude, vocal sac inflation size, and water ripple diameter are all correlated, but none of these signal components can be produced in isolation of the others (James et al., 2021). Covariation between independently produced components of complex signals has garnered little attention. In this study, I evaluate how pairs of acoustic display components interact with one another, and seek to understand the selection pressures that maintain similarity between them. Specifically, I test for evidence of covariation between two acoustic components of hummingbird courtship displays that are produced in independent bouts and are mechanistically distinct.

Hummingbird males are promiscuous and do not contribute parental care (Höglund & Alatalo, 1995; Martínez-García and Ornelas, 2013). Instead, males court females that visit their breeding territory. Courtship displays vary in form but often include a dynamic display that comprises a series of aerial flights and dives. Although the shape of the dives and the type of displays varies by hummingbird species most produce acoustic signals or "dive-sounds".

Hummingbirds have three instruments in which they produce sounds - two mechanical instruments (wings and tail feathers) and their syrinx, which produce vocalizations (Clark et al., 2018; Prum, 1998; Clark & Prum, 2015; Darwin, 1871). In both species of the hummingbird genus *Calypte*, *C. anna* and *C. costae* (hereafter, Anna's and Costa's, respectively), males produce two acoustic mating displays: a song produced with their syrinx and a dive-sound produced mechanically through feather fluttering during dive displays (Prum, 1998; Clark and Prum, 2015; Clark et al., 2018). The songs and dive-sounds are produced in bouts, often one bout after the other but never simultaneously. In both species there is a striking similarity between the songs and their respective dive-sounds. In fact, the resemblance is so close that ornithologists originally argued that both sounds were vocally produced (Wells et al., 1978; Baptista and Matsui, 1979; Clark and Feo, 2008).

Despite the species' close evolutionary relationship, Anna's and Costa's songs and dive-sound forms are highly distinct. Costa's males sing a simple one phrase song that is a high-pitched rapidly modulated whistle rising and falling from 6 to 9 kHz, and produce a dive-sound sonation with wings and tail feathers that has the same structure and frequency range as the song. In contrast, Anna's males sing a complex song with three phrases (A-C) and produce multi-syllabic dive-sounds with their wings and tail feathers. The third phrase of the song, phrase C, resembles the dive-sound whereas phrases A-B do not. In this paper, I focus on Phrase C, the dive-like portion of the song.

In both species all courtship displays incorporate visual signals that include flaring gorget feathers, in Costa's purple, and Anna's pink.

Both Anna's and Costa's learn their songs (Baptista and Schuchmann, 1990; Johnson and Clark, 2020). Therefore, if the acoustic components of courtship displays were evolving independently, I would expect the song to diverge in form from the dive-sound due to a faster rate of change in a culturally transmitted signal relative to one that is tightly morphologically constrained. The fact that the two signals remain highly similar suggests that the correspondence between them is maintained by selection. However, strong support for this hypothesis requires that the two signals vary among individuals and covary within individual, predictions that to this point are untested. To fill this gap, I recorded multiple song and dive-sound iterations for Costa's and Anna's males, evaluated correlations between corresponding elements of song and dive-sound, and tested for unequal variances in corresponding song and dive-sound elements at the population level. If sexual selection promotes matching between different acoustic components of male courtship signals, then corresponding elements of song and dive-sound should co-vary within-individual. If variation in learned song is constrained by selection to match the dive-sound, then corresponding elements should have statistically similar variances. If, alternatively, selection for match between dive-sound and song is weak or absent variance in song, the more plastic learned signal, should be greater that variance in dive-sound.

2. Methods

2.1. Birds and Locations -

The dive-sounds and songs of Anna's and Costa's hummingbirds were recorded in the field over the course of two breeding seasons (December – May, 2018 – 2020). Anna's hummingbirds (*n* = 13) were recorded in coastal sage-scrub habitats on the University of California, Riverside campus (33.96379, -117.31073) and in Sycamore Canyon Wilderness Park (33.94347, -117.31406) in Riverside, CA. Costa's hummingbirds (*n* = 14) were recorded at Boyd Deep Canyon Desert Research Center, Indian Wells, CA (33.65022, -116.36990; Reserve doi:10.21973/N3V66D). All work was done in accordance with UCR IACUC protocols 20160039 and 20190019.

2.2. Elicitation of Behavior and Recording –

Anna's and Costa's males both hold breeding territories on which they sing and produce athletic dive displays. The songs and dive-sounds are produced independently from one another in sequential bouts. Most recordings from individual territorial males were collected consecutively over the course of one day, with a maximum of two consecutive days taken to record a given male. Because territories are usually stable from day to day (Stiles, 1973; Clark and Russell, 2020), a male was assumed to be the same individual if he occupied the same perches and exhibited consistent territorial behavior. Displays were elicited using either a female mount or a caged live female placed on the male's breeding territory. The focal birds' position in relation to the microphone was standardized–

microphones were placed within 30 cm of the mount or female (Clark and Feo, 2010; Simpson and McGraw, 2018). I recorded 20 iterations of each signal type per individual.

Recordings were made using a Zoom F8 multitrack field recorder (Zoom Corporation, Tokyo, Japan) with a sample rate of 48kHz with a 24-bit depth. A Sennheiser MK8 omnidirectional microphone was used (Sennheiser electronic GmbH & Co. KG, Wedemark, Germany; frequency range 30Hz -20 kHz, ± 1dB frequency handling) and was positioned within a meter of the stimulus female to record sounds from the position of the female receiver.

2.3. Sound Analysis -

Spectrograms were generated in Raven Pro v1.6 (Cornell University, Ithaca, NY) using a 512 FFT smooth Hamming window. All variables were measured using visual selection. A single observer measured all the spectral variables.

Anna's song consists of three phrases: A, B, and C (Figure 1). Each phrase is complex and multisyllabic. Phrase C visually resembles the dive-sound and is the focus of this study (Figure 1). The dive-sound comprises one complex, multisyllabic, phrase. Song and dive-sound iterations were divided into four discrete syllables that were used as units of comparison (Table 4, Figure 1). For each syllable, I measured peak, minimum and maximum frequency, and duration. For syllable 1, number of pulses and pulse rate was measured. Pulse rate was calculated as the number of pulses-1/element 1 duration. I also measured the duration of silence between syllables 1 and 2 (Table 4, Figure 1).

Costa’s song and dive-sound are simpler in form than Anna’s. Both the song and dive-sound comprise one phrase with a tonal up and down-sweep (Figure 1). For each song and dive-sound, I measured total duration, low frequency at the beginning and end of the signal, high frequency, and peak frequency (Table 3, Figure 1). I divided the signals into three elements, up-sweep, apex, and down-sweep, and measured the same variables for each (Figure 1). Pulse rate was measured for the up-sweep. In the dive-sound I chose a 100ms segment of the upsweep and counted the number of pulses for that duration (Figure 1). Pulse rate was measured by taking pulse number -1/100ms. In the song, the pulses occur in delineated segments throughout the upsweep. I calculated pulse-rate as the number of pulses in a segment-1/segment duration.

2.4. Statistical Analysis -

Differences in variance between corresponding song and dive-sound variables were tested with Levene’s test. One Costa’s male was a clear outlier for song frequency, so this bird was removed from the comparison of song and dive-sound variances. Because songs and dive-sounds are produced in sequential bouts, I could not make pairwise comparisons between any one song and dive-sound. Instead, I evaluated within-male covariation between the signals by testing for correlations between corresponding song and dive-sound elements using within-male mean values. All birds were retained in this analysis.

3. Results

In Costa’s, 17 of the 23 elements measured had unequal variances between dive-sound and song; differences were split as to whether variance in dive-sound or song was higher with higher variance in dive-sound for 10 variables and higher variance in song for 7 (Table 1). None of the 23 variables were significantly correlated and only one showed evidence of a positive trend. Males with higher average dive-sound pulse rates tended to have higher song pulse-rates ($n = 14$, $R^2 = 0.227$, $p = 0.085$) (Table 3, Fig 2).

In Anna’s, 9 of 12 dive-sound/song variables had unequal variances and, in the majority of these cases (7/9; Table 2), the dive-sound had higher variance than song. Three variables were significantly positively correlated (Table 4, Fig. 1). Males who on average produced faster dive-sound pulse rates also produced faster song pulse rates ($n = 13$, $R^2 = 0.457$, $p = 0.011$). Similarly, males with higher peak frequency and larger delta frequencies in their lower whistle dive-sounds also produced higher frequency ($n = 13$, $R^2 = 0.379$, $p = 0.025$) and larger delta frequency songs ($n = 6$, $R^2 = 0.974$, $p = 0.0003$) (Table 4, Fig. 2).

4. Discussion

Complex, multi-component signals are common features of sexually selected courtship displays. Displays in which two or more independently produced components are matched to one another provide a unique opportunity to test for evidence of selection to maintain this match. I studied this phenomenon in two species of hummingbirds in which song and mechanical feather sonations are strikingly similar in spectral and temporal structure. Specifically, I predicted that, if sexual selection favors the match between these components of male courtship displays, corresponding song and dive-sound elements should be significantly positively correlated across males in the same population. In Costa's, none of the corresponding dive-sound/song elements were significantly correlated. In Anna's, I found some evidence of covariation: 25% of corresponding dive-sound/song elements were significantly correlated. In both species, most dive-sound and song variances were significantly different. Opposite to our prediction, variance in dive-sound was generally higher than variance in song.

4.1. Little covariation between courtship display components -

The species-level match between song and dive-sound in Anna's and Costa's is reported in several other hummingbird species with equally distinct courtship displays (Clark et al., 2018). Thus, it seems likely that these replicated cases of matched signal components have evolved in response to selection. Three elements in Anna's covaried within-male, as predicted if selection favors the match between song and dive-sound. Interestingly, the dive-sound component of all three elements is generated by wing feathers rather than tail feathers (Clark, 2009; Clark et al., 2018). Whereas tail feather sounds are velocity limited (Clark, 2009), the mechanisms behind wing sound production is largely unknown and might be more plastic and therefore more mechanistically amenable to acoustic matching with song. Alternatively, though very unlikely, the production of the signals may be phenotypically integrated such that developmentally the sonation-inducing feathers and syrinx are linked (Wilkinson et al., 1990; Clark and Feo, 2010; Penna et al., 2017). The covariation in these elements may indicate female preference for those signal components to match (Rosenthal and Evans, 1998; Candolin, 2003). Determining whether covariation between these particular elements is favored by Anna's females will require measuring female response to song and dive-sound elements with the degree of match manipulated.

Given that 9/12 elements measured in Anna's did not covary and 0/23 elements covaried in Costa's, I treat this positive result with caution and consider potential explanations for the general lack of covariation between song and dive-sound in either species. First, Anna's and Costa's males provide no parental care or other direct benefits to females. Therefore, female mating decisions are based entirely on assessment of male displays. If high correspondence between song and dive-sound is difficult to achieve, it is possible that the correspondence between the two signals serves as an honest signal of male quality. In this case, I would not expect to observe strong correlations between song and dive-sounds when comparing a random, and relatively small, sample of males in a population. Second, the similarity between the two signals may be a secondary consequence of female auditory tuning to sounds with a particular spectral and temporal structure, rather than a direct consequence of female preference for signal match (Arak and Enquist, 1993, Fuller et al., 2005). If females attend to acoustic signals within the range of songs and dive-sounds performed by adult males in their population there would be no selection for within-male matching. Rather, females may prefer a display form that is exploited by males (Basolo, 1990; Ryan and Rand, 1990; Rosenthal and Evans, 1998; Ryan and Cummings, 2013; Cummings and Endler, 2018) in this case, the two signals may be eliciting responses independently, rather in relation to one-another. Third, it is possible that the apparent match between song and dive-sounds observed in Anna’s and Costa’s represents and evolutionarily transitional state in which one acoustic component will ultimately replace the other. The notion that one component of a complex courtship display can be replaced by another on evolutionary time scales was proposed to explain the fact that some related species in the bee hummingbird clade produce wing trills rather than song (Clark et al. 2018). Extending this hypothesis to song and dive-sounds in general, similarity between display components may reflect evolutionarily temporary redundancy.

Our study had several limitations that may make patterns of covariation hard to detect. As noted above, sample sizes for individual males were relatively small. Moreover, because males sing and dive in sequential bouts rather than alternating between the two, I could not make pairwise comparisons between song and dive-sound and therefore analyzed male means for each signal. This approach eliminates information provided by within- relative to between-male variation.

Whereas larger sample sizes and analyses that incorporate within-individual variance could validate the results of the present study, analysis of song and dive-sound ontogeny and variation among populations could provide additional evidence for or against selection for match between the two display components. Complex courtship displays often require practice and sensorimotor learning (Madden, 2008; Clark et al., 2015; Makino et al., 2016; Janisch et al., 2020; reviewed in Spezie et al., 2022) and social experience (Patricelli et al., 2004; Smith and Martins, 2006; HoI et al., 2008; Balsby and Dabelsteen, 2002), resulting in displays that change over time. In Anna’s and Costa’s, both song and dive are practiced and follow offset ontogenetic timelines; song likely crystalizes in adult form prior to dive-sounds (Baptista and Schummann, 1990, Johnson. and Clark, 2020, Baltosser and Scott, 2020). Future research should sample dive-sounds and songs as they develop and stabilize. Convergence of song and dive-sounds during display ontogeny would provide independent evidence of active matching.

Both learned songs and dynamic visual signals can evolve culturally and vary geographically (Marler and Tamura, 1964; Marler and Peters, 1987; Uy and Borgia, 2000; Frith and Frith, 2004; Madden et al., 2004; Podos et al., 1992; reviewed in Podos and Warren, 2007). Some hummingbirds such as the long-billed hermit (*Phaethornis longirostris*) learn displays in both visual and auditory modalities, resulting in micro-geographic variation in dynamic visual and song displays (Araya-Salas et al., 2019). If there is geographic variation in one of the display types, geographic covariation in the other display-type would provide evidence for concordant selection on both display-types. Anna's song varies geographically and birds from Seattle sing a unique syllable (C1) in the dive-like portion of the song (Berger et al., 2023). Covariation between the variant C1 syllable in song and corresponding dive-sound elements would support selection for song-dive-sound match.

4.2. Song and dive-sounds differ in variance -

I predicted that variances would either be similar between dive-sounds and songs or would be higher in songs. Neither prediction was supported. In Anna's, the dive-sounds had higher variance than songs, whereas in Costa's there was a near split between dive-sound and songs driving the trend. For covariation to occur, there needs to be variation in signals–studying the magnitude of variance is essential to confirm that there is sufficient variance on which covariation can occur. The higher-than-expected variance in dive-sound indicates that there might be either more variation in feather morphology of the sound-inducing feathers than originally thought and/or that males have more control over the dive-sounds than predicted. Studying the degree of variance and the sources of variance will help inform the covariation, and lack there-of, patterns I found. Furthermore, examining the causes and consequences of signal variance on the covariance of the signals will help inform the selection pressures shaping complex signals.

5. Conclusion

In conclusion, complex courtship has fascinated researchers for millennia. The diversity and ubiquity of complex courtship displays demands explanation and exploration. Much progress has been made in studying components of multimodal and multicomponent signals in isolation from one another, and the recent movement towards a systems-wide approach to studying complex signaling has further advanced the field (Hebets, 2011, Hebets, et al., 2015; Patricelli and Hebets, 2016). In this paper I studied two cases in which acoustic components of complex signals correspond to one another through different production mechanisms. The phenomenon of matching display components is understudied and provides a new dimension to the study of the evolution and maintenance of complex signals.

References

Höglund, J. & Alatalo, R. V. (1995). *Leks*. Princeton University Press, New Jersey, USA.

Arak, A., & Enquist, M. (1993). Hidden preferences and the evolution of signals. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *340*(1292), 207–213.

Araya-Salas, M., Smith-Vidaurre, G., Mennill, D. J., González-Gómez, P. L., Cahill, J., & Wright, T. F. (2019). Social group signatures in hummingbird displays provide evidence of co-occurrence of vocal and visual learning. *Proceedings of the Royal Society Biological Sciences, 286*(1903), 20190666.

Balsby, T. J. S., & Dabelsteen, T. (2002). Female behaviour affects male courtship in whitethroats, Sylvia communis: an interactive experiment using visual and acoustic cues. *Animal Behaviour*, *63*(2), 251–257.

Baltosser, W. H., & Scott, P. E. (2020). Costa's Hummingbird (Calypte costae). *Birds of the World*. https://doi.org/10.2173/bow.coshum.01

Baptista, L. F., & Matsui, M. (1979). The source of the dive-noise of the anna's hummingbird. *The Condor*, *81*(1), 87.

Baptista, L. F., & Schuchmann, K.-L. (1990). Song learning in the Anna hummingbird (*Calypte anna*). *Ethology: Formerly Zeitschrift Fur Tierpsychologie*, *84*(1), 15–26.

Basolo, A. L. (1990). Female preference for male sword length in the green swordtail, Xiphophorus helleri (Pisces: Poeciliidae). *Animal Behaviour*, *40*(2), 332–338.

Berger, A., Ye, W., Padilla, A., Gumbi, B., Clark, C., & Campbell, P. (2023). Geographic variation in the songs of two closely related song-learning species, Anna's and Costa's hummingbird (Calypte anna, C. costae). In *bioRxiv* (p. 2023.11.26.568754). https://doi.org/10.1101/2023.11.26.568754

Blanco, G., & de la Puente, J. (2002). Multiple elements of the black-billed magpie's tail correlate with variable honest information on quality in different age/sex classes. *Animal Behaviour*, *63*(2), 217–225.

Borgia, G., & Presgraves, D. C. (1998). Coevolution of elaborated male display traits in the spotted bowerbird: an experimental test of the threat reduction hypothesis. *Animal Behaviour*, *56*(5), 1121–1128.

Smith, B., & Martins, E. P. (2006). Display plasticity in response to a robotic lizard: Signal matching or song sharing in lizards? *Ethology: Formerly Zeitschrift Für Tierpsychologie*, *112*(10), 955–962.

Cady, A. B., Delaney, K. J., & Uetz, G. W. (2011). Contrasting energetic costs of courtship signaling in two wolf spiders having divergent courtship behaviors. *Arachnologische Mitteilungen*, *39*(1), 161–165.

Candolin, U. (2003). The use of multiple cues in mate choice. *Biological Reviews of the Cambridge Philosophical Society*, *78*(4), 575–595.

Clark, C. J. (2009). Courtship dives of Anna's hummingbird offer insights into flight performance limits. *Proc. Biol. Sci.* 276, 3027-3052.

Clark, C. J., & Russell, S. M. (2020). Anna's Hummingbird (*Calypte anna*). Version 1.0. In, *Birds of the World* (A. F. Poole, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.annhum.01.

Clark, C. J., & Prum, R. (2015). Aeroelastic flutter of feathers, flight and the evolution of non-vocal communication in birds. *The Journal of Experimental Biology*, *218*(21), 3520–3527.

Clark, C. J., & Feo, T. J. (2010). Why do Calypte hummingbirds "sing" with both their tail and their syrinx? An apparent example of sexual sensory bias. *The American Naturalist*, *175*(1), 27–37.

Clark, C. J., McGuire, J. A., Bonaccorso, E., Berv, J. S., & Prum, R. O. (2018). Complex coevolution of wing, tail, and vocal sounds of courting male bee hummingbirds. *Evolution; International Journal of Organic Evolution*, *72*(3), 630–646.

Clark, C. J, & Feo, T. J. (2008). The Anna's hummingbird chirps with its tail: a new mechanism of sonation in birds. *Proceedings. Biological Sciences*, *275*(1637), 955–962.

Cummings, M. E., Endler, J. A.(2018). 25 Years of sensory drive: the evidence and its watery bias. *Current Zoology*, *64*(4), 471–484.

Darwin, C. (1871). *The Descent of Man and Selection in Relation to Sex Vol I*. John Murray, London, UK.

Doucet, S. M., & Montgomerie, R. (2003). Multiple sexual ornaments in satin bowerbirds: ultraviolet plumage and bowers signal different aspects of male quality. *Behavioral Ecology: Official Journal of the International Society for Behavioral Ecology*, *14*(4), 503–509.

Endler, J. A., & Basolo, A. L. (1998). Sensory ecology, receiver biases and sexual selection. *Trends in Ecology & Evolution*, *13*(10), 415–420.

Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *The American Naturalist*, *139*, S125–S153.

Frith, C. B., & Frith, D. W. (2004). Family Ptilonorhynchidae: the bowerbirds. In *Bird Families of the World* (pp. 225–438). Oxford University PressOxford.

Fuller, R. C., Houle, D., & Travis, J. (2005). Sensory bias as an explanation for the evolution of mate preferences. *The American Naturalist*, *166*(4), 437–446.

Halfwerk, W., Dixon, M. M., Ottens, K. J., Taylor, R. C., Ryan, M. J., Page, R. A., & Jones, P. L. (2014). Risks of multimodal signaling: bat predators attend to dynamic motion in frog sexual displays. *The Journal of Experimental Biology*, *217*(Pt 17), 3038–3044.

Hebets, E. (2008). Seismic signal dominance in the multimodal courtship display of the wolf spider Schizocosa stridulans Stratton 1991. *Behavioral Ecology: Official Journal of the International Society for Behavioral Ecology*, *19*(6), 1250–1257.

Hebets, E. A., & Uetz, G. W. (1999). Female responses to isolated signals from multimodal male courtship displays in the wolf spider genus Schizocosa (Araneae: Lycosidae). *Animal Behaviour*, *57*(4), 865–872.

Hebets, E. A. (2011). Current status and future directions of research in complex signaling. *Current Zoology*, *57*(2), i–v.

Hebets, E. A., Barron, A. B., Balakrishnan, C. N., Hauber, M. E., Mason, P. H., & Hoke, K. L. (2016). A systems approach to animal communication. *Proceedings. Biological Sciences / The Royal Society*, *283*(1826), 20152889.

Hebets, E. A., Hansen, M., Jones, T. C., & Wilgers, D. J. (2015). Octopamine levels relate to male mating tactic expression in the wolf spider Rabidosa punctulata. *Animal Behaviour*, *100*, 136–142.

Hebets, E. A., & Papaj, D. R. (2005). Complex signal function: developing a framework of testable hypotheses. *Behavioral Ecology and Sociobiology*, *57*(3), 197–214.

Hölldobler, B. (1999). Multimodal signals in ant communication. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology*, *184*(2), 129–141.

Howe, M. J., Hemmi, J. M., Zeil, J., & Peters, R. (2008). Claw waving display changes with receiver distance in fiddler crabs, Uca perplexa. *Animal Behaviour*, *75*(3), 1015–1022.

Hughes, M. (1996). The function of concurrent signals: visual and chemical communication in snapping shrimp. *Animal Behaviour*, *52*(2), 247–257.

James, L. S., Halfwerk, W., Hunter, K. L., Page, R. A., Taylor, R. C., Wilson, P. S., & Ryan, M. J. (2021). Covariation among multimodal components in the túngara frog's courtship display. *The Journal of Experimental Biology*, *224*(12), jeb241661.

Janisch, J., Perinot, E., & Fusani, L. (2020). Behavioural flexibility in the courtship dance of golden-collared manakins, *Manacus vitellinus*. *Animal Behaviour*, *166*, 61–71.

Johnson, K. E., & Clark, C. J. (2020). Ontogeny of vocal learning in a hummingbird. *Animal Behaviour*, *167*, 139–150.

Johnstone, R. A. (1997). The evolution of animal signals. *Behavioural Ecology: An Evolutionary Approach*, 155–178.

Madden, J. R. (2008). Do bowerbirds exhibit cultures? *Animal Cognition*, *11*(1), 1–12.

Madden, J. R., Lowe, T. J., Fuller, H. V., Dasmahapatra, K. K., & Coe, R. L. (2004). Local traditions of bower decoration by spotted bowerbirds in a single population. *Animal Behaviour*, *68*(4), 759–765.

Makino, H., Hwang, E. J., Hedrick, N. G., & Komiyama, T. (2016). Circuit mechanisms of sensorimotor learning. *Neuron*, *92*(4), 705–721.

Marler, P., & Peters, S. (1987). A sensitive period for song acquisition in the song sparrow, *Melospiza melodia*: A case of age-limited learning. *Ethology, 76*(2), 89–100.

Marler, P., & Tamura, M. (1962). Song "dialects" in three populations of white-crowned sparrows. *The Condor*, *64*(5), 368–377.

Marler, P., & Tamura, M. (1964). Culturally transmitted patterns of vocal behavior in sparrows. *Science*, *146*(3650), 1483–1486.

Martínez-García, V., Lara, L., & Ornelas, J. F. (2013). Lek characteristics and the static male song of the green violet-ear (*Colibri thalassinus*) during a 3-year study in a temperate forest of Central Mexico. *Ornitologia Neotropical*, *24*, 183–200.

Moody, N. M., Vivlamore, E. K., & Fuxjager, M. J. (2022). Woodpecker drum evolution: An analysis of covariation in elements of a multicomponent acoustic display among and within species. *Evolution; International Journal of Organic Evolution*, *76*(7), 1469–1480.

Morton, E. S. (1975). Ecological Sources of Selection on Avian Sounds. *The American Naturalist*, *109*(965), 17–34.

Ota, N. (2020). Tap dancers in the wild: field observations of multimodal courtship displays in socially monogamous songbirds. *The Science of Nature*, *107*(4), 30.

Ota, N., & Soma, M. (2022). Vibrational signals in multimodal courtship displays of birds. In *Biotremology: Physiology, Ecology, and Evolution* (pp. 237–259). Springer International Publishing.

Partan, S., & Marler, P. (1999). Communication goes multimodal. *Science (New York, N.Y.)*, *283*(5406), 1272–1273.

Patricelli, G. L., & Hebets, E. A. (2016). New dimensions in animal communication: the case for complexity. *Current Opinion in Behavioral Sciences*, *12*, 80–89.

Patricelli, G. L., Uy, J. A. C., & Borgia, G. (2004). *Female signals enhance the efficiency of mate assessment in satin bowerbirds (Ptilonorhynchus violaceus).* https://doi.org/10.1093/beheco/arh013

Penna, A., Melo, D., Bernardi, S., Oyarzabal, M. I., & Marroig, G. (2017). The evolution of phenotypic integration: How directional selection reshapes covariation in mice. *Evolution; International Journal of Organic Evolution*, *71*(10), 2370–2380.

Podos, J., Huber, S. K., & Taft, B. (2004). Bird song: The interface of evolution and mechanism. *Annual Review of Ecology, Evolution, and Systematics*, *35*(1), 55–87.

Podos, J., Peters, S., Rudnicky, T., Marler, P., & Nowicki, S. (1992). The organization of song repertoires in song sparrows: Themes and variations. *Ethology*, *90*(2), 89–106.

Podos, J., & Warren, P. S. (2007). The Evolution of Geographic Variation in Birdsong. In *Advances in the Study of Behavior* (Vol. 37, pp. 403–458). Academic Press.

Prum, R. O. (1998). Sexual selection and the evolution of mechanical sound production in manakins (Aves: Pipridae). *Animal Behaviour*, *55*(4), 977–994.

Rosenthal, G. G., & Evans, C. S. (1998). Female preference for swords in Xiphophorus helleri reflects a bias for large apparent size. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(8), 4431–4436.

Ryan, M. J., & Rand, A. S. (1993). Sexual selection and signal evolution: the ghost of biases past. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *340*(1292), 187–195.

Ryan, M. J., & Cummings, M. E. (2013). Perceptual biases and mate choice. *Annual Review of Ecology, Evolution, and Systematics*, *44*(1), 437–459.

Ryan, Michael J., & Rand, A. S. (1990). The sensory basis of sexual selection for complex calls in the túngara frog,Physalaemus pustulosus(sexual selection for sensory exploitation). *Evolution; International Journal of Organic Evolution*, *44*(2), 305–314.

Simpson, R. K., & McGraw, K. J. (2018). It's not just what you have, but how you use it: solar-positional and behavioural effects on hummingbird colour appearance during courtship. *Ecology Letters*, *21*(9), 1413–1422.

Spezie, G., Quigley, C., & Fusani, L. (2002). *Learned components of courtship: A focus on postural displays, choreographies and construction abilities*. https://doi.org/10.1016/bs.asb.2022.01.00.

Stiles, F. G., & Wolf, L. L. (1973). Techniques for color-marking hummingbirds. *The Condor*, *75*(2), 244–245.

Thornhill, R., & Møller, A. (1998). The relative importance of size and asymmetry in sexual selection. *Behavioral Ecology: Official Journal of the International Society for Behavioral Ecology*, *9*, 546–551.

Tobiansky, D. J., Miles, M. C., Goller, F., & Fuxjager, M. J. (2020). Androgenic modulation of extraordinary muscle speed creates a performance trade-off with endurance. *The Journal of Experimental Biology*, *223*(11), jeb222984.

Uetz, G. W., & Roberts, J. A. (2002). Multisensory cues and multimodal communication in spiders: insights from video/audio playback studies. *Brain, Behavior and Evolution*, *59*(4), 222–230.

Uy, J. A., & Borgia, G. (2000). Sexual selection drives rapid divergence in bowerbird display traits. *Evolution; International Journal of Organic Evolution*, *54*(1), 273–278.

Wells, S., Bradley, R. A., & Baptista, L. F. (1978). Hybridization in Calypte Hummingbirds. *The Auk*, *95*(3), 537–549.

Wilkinson, G. S., Fowler, K., & Partridge, L. (1990). Resistance of genetic correlation structure to directional selection in Drosophila melanogaster. *Evolution; International Journal of Organic Evolution*, *44*(8), 1990–2003.

Table 1.1: Levene's test for homogeneity of variances of Costa's hummingbird song and dive-sounds spectral and temporal measurements.

	Measurement	$F_{(1,11)}$	*p*
Entire	**Duration (seconds)**	**6.48**	**0.011_d**
	Peak frequency (kHz)	**9.09**	**0.0027_s**
	High frequency (kHz)	0.54	0.461
	Low frequency (kHz)	**4.27**	**0.002_s**
	Delta frequency (kHz)	1.49	0.223
	Start frequency (kHz)	2.560	0.108
	End frequency (kHz)	**6.08**	**0.014_s**
Up-sweep	**Duration (seconds)**	**16.07**	**$<0.0001_d$**
	Peak frequency (kHz)	0.41	0.521
	High frequency (kHz)	**13.01**	**0.0003_d**
	Low frequency (kHz)	**4.13**	**0.043_s**
	Delta frequency (kHz)	0.0078	0.929
	Pulse rate (#pulses/duration pulses)	**27.18**	**$<0.0001_s$**
Apex	**Duration (seconds)**	**90.70**	**$<0.0001_d$**
	Peak frequency (kHz)	**26.40**	**$<0.0001_d$**
	High frequency (kHz)	**4.76**	**0.0297_d**
	Low frequency (kHz)	**36.04**	**$<0.0001_d$**
	Delta frequency (kHz)	**21.41**	**$<0.0001_s$**
Down-sweep	Duration (seconds)	**17.94**	**$<0.0001_d$**
	Peak frequency (kHz)	**12.01**	**0.0006_s**
	High frequency (kHz)	**20.66**	**$<0.0001_d$**
	Low frequency (kHz)	**18.40**	**$<0.0001_d$**
	Delta frequency (kHz)	0.22	0.636

s, variance significantly higher in song; **d**, variance significantly higher in dive-sound

Table 1.2: Levene's test for homogeneity of variances of Anna's hummingbird song and dive-sounds spectral and temporal measurements.

	Measurement	$F_{(1,11)}$	p
Element 1	**Peak frequency pulses (kHz)**	**17.37**	**<0.0001**$_d$
	Duration pulses (seconds)	**93.27**	**<0.0001**$_d$
	Pulse rate (# pulses/duration pulses)	**4.43**	**<0.0001**$_d$
Element 2	**Duration between elements 1 and 2 (seconds)**	**33.91**	**<0.0001**$_d$
	Peak frequency lower whistle (kHz)	**26.05**	**<0.0001**$_d$
	Duration lower whistle (seconds)	**54.65**	**<0.0001**$_d$
	Delta frequency lower whistle (kHz)	**9.32**	**0.0024**$_d$
Element 3	**Peak frequency (kHz)**	**465.30**	**<0.0001**$_s$
	Duration (seconds)	0.021	0.886
	Delta frequency (kHz)	**331.58**	**<0.0001**$_s$
Element 4	Duration (seconds)	0.022	0.885
	Peak frequency (kHz)	0.019	0.893

d, variance significantly higher in dive-sound; **s**, variance significantly higher in song

Table 1.3: Linear regression results for mean spectral and temporal measures of Costa's

	Measurement	***n***	**$F_{(1,12)}$**	***p***	**R^2**
Entire	Duration (seconds)	14	0.131	0.724	0.0107
	Peak frequency (kHz)	14	0.117	0.739	0.0096
	High frequency (kHz)	14	0.010	0.923	0.0008
	Low frequency (kHz)	14	0.343	0.569	0.0278
	Delta frequency (kHz)	14	0.820	0.383	0.0639
	Start frequency (kHz)	14	0.945	0.350	0.073
	End frequency (kHz)	14	0.024	0.880	0.002
Up-sweep	Duration (seconds)	14	0.869	0.370	0.067
	Peak frequency (kHz)	14	0.204	0.659	0.017
	High frequency (kHz)	14	0.005	0.947	0.0004
	Low frequency (kHz)	14	0.722	0.412	0.057
	Delta frequency (kHz)	14	0.530	0.480	0.0423
	Pulse rate (#pulses/duration pulses)	14	3.522	0.085	0.227
Apex	Duration (seconds)	14	0.026	0.874	0.0022
	Peak frequency (kHz)	14	0.0061	0.939	0.0005
	High frequency (kHz)	14	0.0278	0.870	0.0023
	Low frequency (kHz)	14	0.314	0.585	0.0255
	Delta frequency (kHz)	14	0.413	0.533	0.0333
Down-sweep	Duration (seconds)	14	0.039	0.847	0.0032
	Peak frequency (kHz)	14	0.081	0.781	0.0067
	High frequency (kHz)	14	0.076	0.788	0.0063
	Low frequency (kHz)	14	0.017	0.898	0.0014
	Delta frequency (kHz)	14	0.600	0.453	0.0477

hummingbird song and dive-sounds. 20 songs and dive-sounds measured per individual.

Table 1.4: Linear regression results for mean spectral and temporal measures of Anna's hummingbird song and dive-sounds. 20 songs and dive-sounds measured per individual.

	Measurement	***n***	$F_{(1, 11)}$	p	R^2
Element 1	Peak frequency pulses (kHz)	13	0.404	0.538	0.035
	Duration pulses (seconds)				
	Number of pulses (#)	13	1.005	0.338	0.084
	Pulse rate (#	13	1.995	0.185	0.154
	pulses/duration pulses)	**13**	**9.274**	**0.011**	**0.457**
Element 2	Duration between elements 1 and 2 (seconds)	13	0.051	0.825	0.005
	Peak frequency lower whistle (kHz)	13	**6.712**	**0.025**	**0.379**
	Duration lower whistle (seconds)	13	1.195	0.298	0.098
	Delta frequency lower whistle (kHz)	**6**	**147.47**	**0.0003**	**0.974**
Element 3	Peak frequency (kHz)	13	0.141	0.714	0.013
	Duration (seconds)	13	0.032	0.863	0.003
	Delta frequency (kHz)	13	1.233	0.291	0.101
Element 4	Duration (seconds)	13	0.219	0.885	0.002
	Peak frequency (kHz)	13	0.161	0.696	0.060

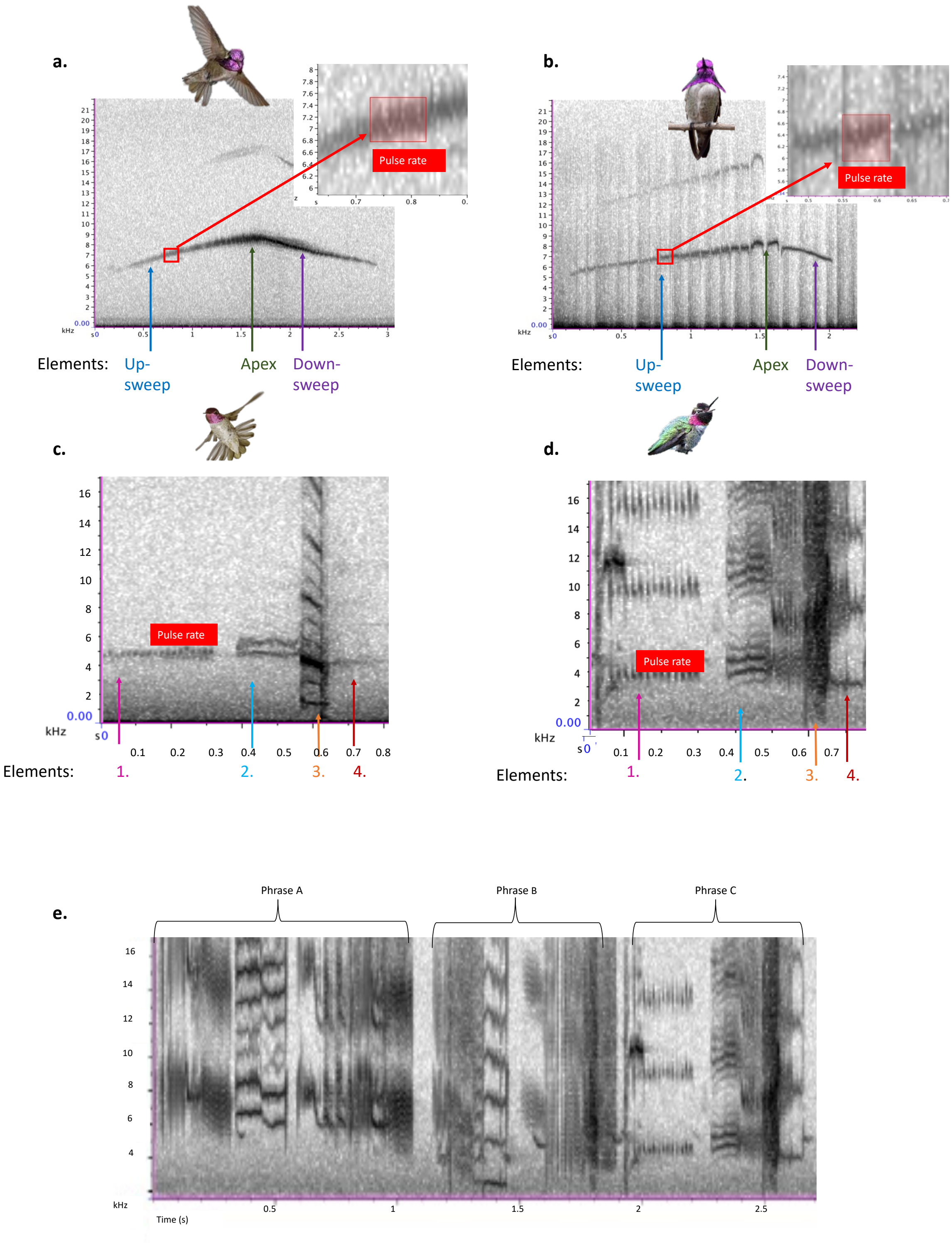

a.
Pulse rate
kHz
s
0.00
Elements:
Up-sweep
Apex
Down-sweep
b.
Pulse rate
kHz
s
0.00
Elements:
Up-sweep
Apex
Down-sweep
c.
Pulse rate
kHz
s
0.00
Elements:
1.
2.
3.
4.
d.
Pulse rate
kHz
s
0.00
Elements:
1.
2.
3.
4.
e.
Phrase A
Phrase B
Phrase C
kHz
Time (s)

Figure 1.1: Song and dive-sound resemble each other in form. Spectrograms of an individual Costa's dive-sound **a)** and song **b)** and Anna's dive-sound **c)** and song **d)**. Anna's song is comprised of 3 phrase (A-C) and the dive-like song is Phrase C **e**). Song and dive-sound were divided into Up-sweep, Apex, and Down-sweep in Costa's and four elements labeled 1-4 in Anna's. Labels correspond to matching elements in the songs and dive-sounds of each species. Spectrograms produced in Raven Pro 1.6, FFT 512, Hamming smooth window. Recordings taken with Zoom F8 recorder, Sennheiser Mk8.

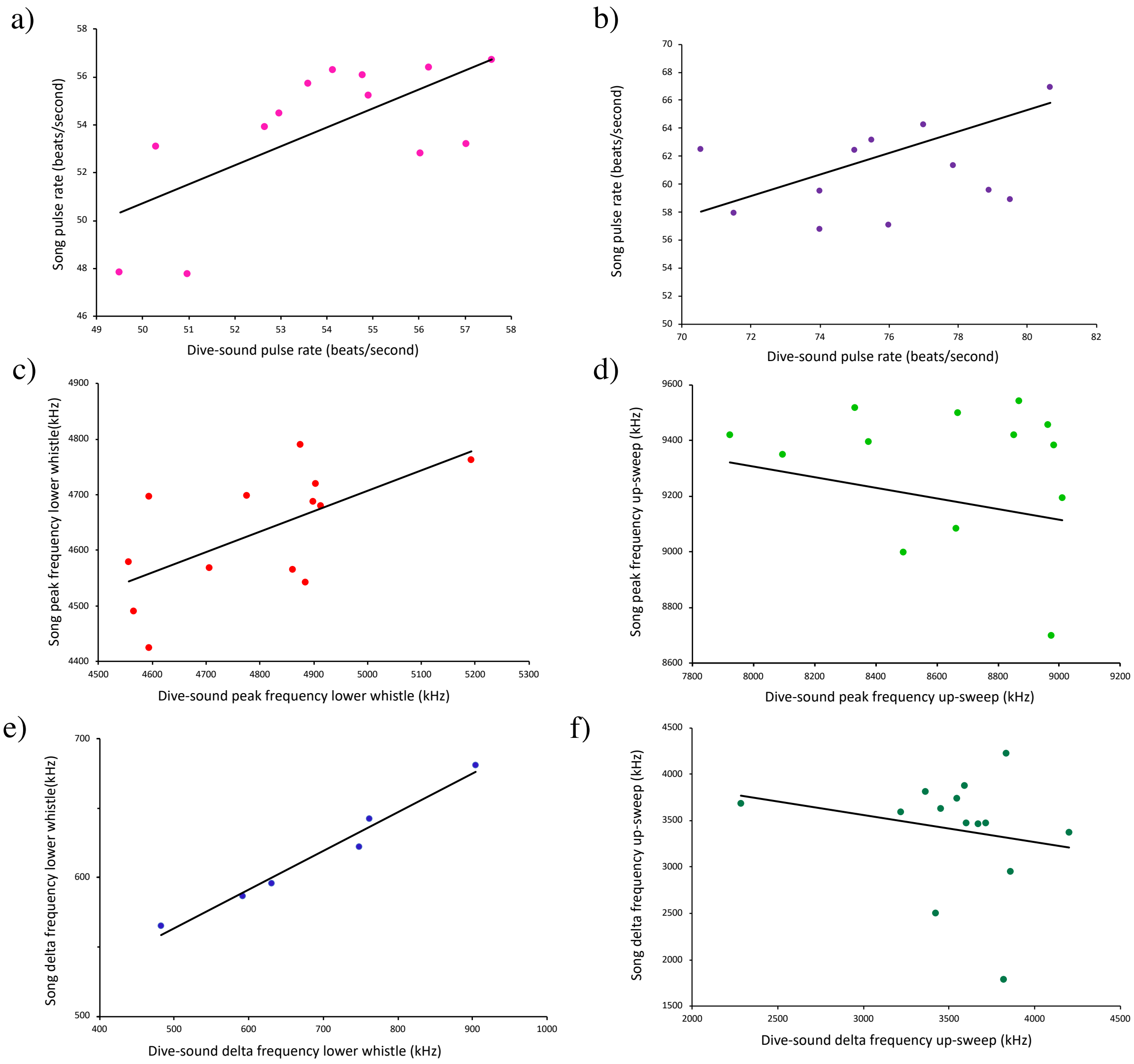


Figure 1.2: Covariation between song and dive-sounds in Anna's (**a, c, e**) and Costa's (**b, d, f**) hummingbirds. Song and dive-sound pulse rates are significantly correlated in **a)** Anna's, with a non-significant trend in the same direction in **b)** Costa's. **c)** In Anna's, song and dive-sound peak frequency of lower whistle and **e)** delta frequency of lower whistle are significantly correlated. Comparable measurements of song and dive-sound do not covary in Costa's: **d)** up-sweep peak frequency; **f)** Up-sweep delta frequency. In both species, 20 songs and 20 dive-sounds per individual were measured and averaged.

Chapter 2

Geographic Variation in the Songs of Two Closely Related Song-learning Species, Anna's and Costa's Hummingbird (*Calypte anna, C.* costae)

Abstract

In species that learn their vocalizations, cultural transmission of song components can lead to the accumulation of variants that differ among populations, resulting in geographic variation in song. Three avian clades have evolved song learning – parrots, oscine passerines, and hummingbirds. Dialects have mainly been studied in passerines. I extend the study of geographic variation in learned song to the bee hummingbird clade, focusing on Anna's and Costa's hummingbirds (*Calypte anna* and *C. costae*). Anna's produces complex, three phrase, multi-syllable songs whereas Costa's produces simple, one phrase songs. I recorded 5-20 males per population (6 Costa's and 6 Anna's populations) across the species' ranges in the Western United States and tested for evidence of geographic variation in song. I found minor population differences in temporal and frequency measures of Costa's song, but song form was invariant across populations. Anna's song was contrastingly variable with population differences in both syllable use and multiple spectral and temporal measures. The most strongly differentiated Anna's population in our study, Seattle (Washington State), is the product of a recent northward range expansion. The loss of a syllable in this population is suggestive of a founder effect on song. This study provides insight into song evolution in

non-passerine vocal learners and contributes to understanding how both complex and simple songs evolve and are maintained.

1. Introduction

Acoustic communication has intrigued scientists for millennia (Pliny the Elder, 50 A.D.; Darwin, 1871). Of particular interest are the evolution and mechanistic basis of vocal learning – a defining feature of humans that evolved independently in a subset of other mammalian taxa (pinnipeds, cetaceans, elephants, bats), and three bird lineages (oscine songbirds, parrots, hummingbirds) (Nottenbohm, 1972; Janik and Slater, 1997; Tyack, 2019; Janik and Knörnschild, 2021). Vocal learning is a complex and imitative social process that requires the capacity to memorize acoustic input and to match acoustic output to this internal template. At every step of the vocal learning and production process there is a potential for errors that generate vocal novelties. Like genetic mutations, these vocal novelties can rise to high frequency and ultimately become fixed in a population, resulting in among population acoustic differences. (Marler and Tamura, 1964; Lemon, 1975; Baptista, 1977; Marler and Peters, 1987; Slater, 1989).

The study of geographic variation in oscine birdsong has a long history. Marler and Tamura set the stage with their foundational study on the white-crowned sparrow (*Zonotrichia leucophrys*), in which they documented stereotyped local dialects in three populations in California (Marler and Tamura, 1962). This work catalyzed an explosion of studies looking at incidences of dialects (Lemon, 1966; Nottebohm, 1969; Baptista and King, 1980; Wang et al., 2022), the adaptive function of dialects (Baker, 1975; Mundinger, 1982; Baker and Cunningham, 1985), and the ecological predictors and mechanisms of dialect formation (Lemon, 1975; Morton, 1975; Slater, 1989; Kroodsma and Miller, 1996; Podos and Warren, 2007; Derryberry, 2009). In parallel, research on dialects has expanded to include the two non-oscine lineages of vocal learners, parrots (Bond and Diamond, 2005; Wright et al., 2005; reviewed in Wright and Dahlin, 2018) and hummingbirds (Snow, 1968; Wiley, 1971; Baptista and Schuchmann, 1990; Gaunt et al., 1994; González and Ornelas, 2009; Araya-Salas and Wright, 2013; Lara et al., 2015). Our focus is on the latter group.

Lek mating systems are common in hummingbirds (reviewed in Martínez-García et al., 2013) and most prior research on hummingbird dialects has focused on vocal variation within and between leks in neotropical species that have well defined lek boundaries (Gaunt et al., 1994; González and Ornelas, 2005; González and Ornelas, 2009; González et al. 2011; Lara et al., 2015; Araya-Salas et al., 2019). Whereas microgeographic variation in song is documented in several species (e.g., González and Ornelas, 2005; Lara et al., 2015; Araya-Salas et al., 2019), analyses of song differences among multiple geographically distinct populations are, to our knowledge, published for just one species (wedge-tailed saberwings, *Campylopterus curvipennis*; González et al. 2011; González and Ornelas, 2014). The songs of wedge-tailed saberwings, sampled along a 500 km transect in eastern Mexico, exhibited strong within-lek cohesion and contrastingly high between-lek differentiation, with a significant positive relationship between geographic and song distance based on syllable sharing (González and Ornelas, 2014). These results suggest that song differentiation on a macrogeographic scale is a byproduct of social selection for song sharing within leks, coupled with rapid turnover in syllable usage (González and Ornelas, 2014). Whether the songs of hummingbird species that lack spatially defined leks exhibit equivalently strong patterns of geographic differentiation is an open question. More generally, the independent origin of song learning in hummingbirds relative to oscine songbirds, and the paucity of studies on hummingbird song dialects compared to the large body of work on dialects in songbirds, provide strong motivation to extend the study of macrogeographic variation in hummingbird song to multiple species.

In this study, I test for evidence of geographic variation in the songs of two closely related hummingbird species whose ranges overlap in the southwestern United States, Anna's and Costa's hummingbirds (*Calypte anna* and *C. costae*, respectively; hereafter, Anna's and Costa's). Anna's and Costa's, the sole members of the *Calypte* genus within the bee (*Mellisuginii*) hummingbird clade (McGuire et al., 2007), exhibit multiple extravagant multimodal displays including aerobatic dives and shuttle and perched songs (Stiles, 1982; Clark and Feo, 2008; Clark and Feo, 2010). Both species learn their songs (Baptista and Schuchmann, 1990; Johnson and Clark, 2020) and sing during territorial and courtship displays (Wells et al., 1978; Stiles, 1982, Clark and Feo, 2008; Clark and Feo, 2010). Despite being sister taxa, Anna's and Costa's have remarkably different song structures. Anna's song is spectrally complex and multi-syllabic with multiple phrases whereas Costa's song is pure tone, monosyllabic, and comprises a single phrase (Fig. 1).

As for other hummingbird species, there is evidence for within-population variation in both Anna's and Costa's songs (Williams and Houtman, 2008; Yang et al., 2007). The only prior study in the *Calypte* clade conducted at a larger spatial scale found acoustic differences between the songs of an isolated Anna's population from Guadalupe Island (240 km from the nearest mainland) and birds from a mainland population (Mirsky, 1976). Whether song differences exist between mainland populations of Anna's that are not geographically disjunct from one another is unknown.

I recorded Anna's and Costa's across their ranges in the Western United States (Fig. 2), and collected spectral, temporal, and qualitative measurements of the songs to test for acoustic differences between populations. Since vocal learning provides ample opportunity for improvisation and error, I expected to find evidence of geographic variation in both species' songs. However, given the considerable structural differences between Anna's and Costa's songs', I were particularly interested in how population differences would manifest in a complex, multi-phrase song as compared to a simple, single phrase song.

2. Methods

*2.*1. Recording –

I sampled Anna's and Costa's song from geographically distinct populations in the Western United States during the January to June breeding season in 2020 and 2021 (Anna's, *n*=6 populations, 10-20 birds/population; Costa's, *n*=6 populations, 5-20 birds/population; Fig. 2). The ranges of straight line distances between sampled populations were 171-1,571 km for Anna's and 116-848 km for Costa's. I recorded songs from males singing on their breeding territories (5-30 songs/male). Breeding males of both species are territorial and can be identified by perch fidelity (Stiles, 1982). Territories are stable from day to day (Stiles, 1973; Clark and Russell, 2020). Recordings from a given male were collected consecutively over the course of one to two days, with most recordings taken in a single day. Recordings were captured using a Zoom F8 multitrack field recorder (Zoom Corporation, Tokyo, Japan), at a sample rate of 48kHz with a 24-bit depth and a Sennheiser K6 microphone (Sennheiser electronic GmbH & Co. KG, Wedemark, Germany; frequency range 30Hz -20 kHz, ± 1dB frequency handling) with a parabola shell (Wildtronics, LLC Mono Parabolic microphone, Newton Falls, OH, USA) at a distance of ≤15 meters from the focal bird. In one population of Costa's hummingbirds, Deep Canyon (DC, Fig. 2b), shuttle display song was elicited using a mount or live female and was recorded at <1 meter from the focal bird using a Sennheiser MK8 omnidirectional microphone (Sennheiser electronic GmbH & Co. KG, Wedemark, Germany). In all other populations, songs were recorded opportunistically from males singing from perches. I divided the songs into phrases, syllables, and elements by visual inspection of the spectrograms generated in Raven Pro v1.6 (Cornell Lab of Ornithology, Cornell University, Ithaca, NY, USA) using a 512 FFT smooth Hamming window.

2.2. Anna's Hummingbird Song Analysis

Anna's song consists of three multi-syllabic phrases (A-C; Fig. 1a). Phrase A consists of 2-4 syllables, phrase B consists of 2-3 syllables, and phrase C consists of 1-2 syllables. The song is complex with high entropy and a frequency range of 1.5 – 20 kHz, with most of the energy between 1.5 and 4 kHz. I defined syllables as repeatable components separated by less than 0.08 seconds and defined phrases as repeated groupings of syllables separated by more than 0.08 seconds. I used the same designation and nomenclature for phrases as Yang et al. (2007) but developed our own naming scheme for syllables. Song syntax is stereotyped: the phrases are always sung in the same order, starting with phrase A, followed by phrase B, and then phrase C (Yang et al., 2007). The number of times phrase A is repeated before singing the full song (A-C) is variable within individual and likely dependent on context. However, the syntax of the full song is conserved between all individuals irrespective of singing context (Stiles, 1982; Yang et al., 2007; ANB personal observation). I visually evaluated 10 songs per male to confirm that the syllable types where invariant within individual and selected the song with the highest signal to noise ratio and the lowest background noise to represent each male. For each syllable I collected one or more measures of duration and frequency in Raven (Table 1, Fig. 1a).

To define distinct syllables within and among songs I visually cataloged the syllables through inspection of the spectrograms with the bird IDs hidden (Searcy et al., 1985; Podos et al., 1992; González and Ornelas, 2009). To check the repeatability of the syllable designations, this process was repeated three times by the same observer (ANB) for each song. The process was repeatable and the same 55 syllable types were identified in all three iterations (Fig. 3). However, multiple syllables in this initial catalog were sung by only one bird, suggesting either that I were over-splitting syllable types or that these were rare variants that would require larger sample sizes to detect in multiple individuals. I dealt with this issue in two ways. First, I removed syllables that were only sung by one individual; this reduced our number of syllable types to 42. Second, I binned syllables together that were produced in analogous syntax positions and had clear similarities in spectral shape, prominent frequency, harmonic structure, and duration (Fig. 3). To group these similar syllables together, I isolated syllables by splicing recordings into syllable units in Audacity 3.3.1 (https://audacityteam.org), such that each recording only consisted of one syllable and individual identity was hidden. To confirm that the visual binning process accurately captured acoustic similarity, I grouped syllables auditorily by listening to each representative syllable recording at 0.25 speed and then at full speed. The visual and auditory groupings matched one another, resulting in 11 syllable groups that were scored as present or absent for each bird. Subsequent analyses were done on both this binned dataset and the full dataset with single incidence syllables removed (hereafter, full dataset).

To test for an effect of geographic distance on syllable use, I used the proportional abundance of each syllable by population to calculate Jaccard dissimilarity indices (hereafter, syllable use distances) with the R package, vegan (Oksanen et al. 2018). Correlation between geographic distance and syllable use distance was tested with a Mantel test in XLSTAT (Lumivero, Burlington, MA, USA). To test for evidence of geographic structure in patterns of syllable use without a priori assignment of birds to populations, I converted individual syllable use to a binary matrix (1 = presence, 0 = absence) and ran a population structure analysis using the *snmf* function in the R package, LEA v3.11.6 (Frichot and François, 2015). LEA is typically used in population genetics to estimate individual ancestry proportions from *K* populations or clusters, where the *K* is the number of populations that provides the best fit to the data. I follow González and Ornelas (2014) in extending this approach to the analysis of bird song. I ran ten replicates for each *K* value from 1 to 10, with a regularization parameter (α) value of 100 as recommended (Frichot et al., 2014). I chose the value of *K* with the lowest cross-entropy criterion value (Frichot et al., 2014) and evaluated the relationship between geographic origin and cluster membership.

I tested for an effect of population on each measure of duration and frequency (Table 1) with analysis of variance (ANOVA), with Bonferroni-corrected $\alpha = 0.0024$. To better summarize and visualize spectral and temporal song differences among populations, I entered all measurements for phrases B-C into a principle component analysis (PCA). I excluded measurements for phrase A from this analysis because high inter-individual variation in the form and structure of syllables in phrase A reduced the explanatory power of the first two principle components by 47% relative to the analysis with phrases B and C only. I used population averages for the first three principal component (PC) scores to calculate Euclidian distance among populations and tested for a correlation between geographic distance and song distance with a Mantel test. ANOVAs and PCA were run in JMP (SAS Institute Inc., Cary, NC, USA).

2.3. Costa's Hummingbird Song Analysis

Costa's hummingbirds sing a one-phrase song consisting of a rapidly modulated tonal up and down sweep. I divided the songs into three elements: up-sweep, apex (notes

1 and 2), and down-sweep (Fig. 1b). The up-sweep and down-sweep span an approximately 6 kHz range from 6 kHz to 12 kHz, with the apex at about 12 kHz. I visually evaluated 3-10 songs for each male to confirm that the structural form of the phrase and elements was invariant within individual. As for Anna's, I chose one representative song per male that had the highest signal to noise ratio and the lowest background noise to analyze temporal and frequency components of the elements of the song (Table 2). For the apex I measured, low, high and delta frequencies, and duration. I divided the apex into two notes (1 and 2) and for each note measured peak frequency and duration. Due to its high frequency nature, the song degraded quickly and was affected by the birds' head movements; the up- and down-sweeps were most sensitive to recording conditions. Because the beginning of the up-sweep and the end of the down-sweep were particularly difficult to define accurately, I did not take temporal and frequency measurements for the entirety of each element. Since the apex was clear in all recordings, I instead standardized measurements of the up- and down-sweeps by selecting the areas that flanked the apex. I selected two durations, 0.25s and 0.5s (Fig. 1b), and measured the delta frequency, minimum and maximum frequency using the center frequency at the start and end of the selected windows. Duration and frequency measurements were taken in Raven and I ran the same set of analyses described in Section ii. for quantitative measures of Anna's song, with Bonferroni-corrected $\alpha = 0.0028$ for the ANOVAs. For the Deep Canyon population, I tested for an effect of the context in which songs were produced (perched vs. shuttle display), using songs from six males that were recorded in both contexts (Table 3).

3. Results

3.1. Evidence for Geographic Variation in Anna's Song

There was a significant positive correlation between syllable use distance and geographic distance for both the full (42 syllables; Mantel, $r = 0.76$, $r^2 = 0.58$, $P < 0.0001$; Fig. 4a) and the binned datasets (11 syllable groups; Mantel, $r = 0.81$, $r^2 = 0.65$, $P < 0.0001$; Fig. 4b). For the spectral and temporal components of Anna's song, I found a weak positive correlation between song distance and geographic distance (Mantel, $r = 0.48$, $r^2 = 0.24$, $P = 0.076$; Fig. 4c).

Patterns of syllable use were spatially structured. For both full and binned datasets, K=4 clusters had the lowest cross-entropy criterion value, with similar proportional assignments of individual birds to clusters (Fig. 5). In the full dataset, 12 of 13 Seattle (SE) birds were assigned to a Seattle-limited cluster (Cluster 1) and all Mendocino (ME) and Santa Ynez (SY) birds were assigned to a single cluster (Cluster 3). Henderson (HE), San Francisco (SF), and Riverside (RI) birds were less differentiated in syllable use; individuals from all three populations, together with one bird from Seattle, were represented in Cluster 2. Riverside was distinct from all other populations in lacking representation in Cluster 3, and Cluster 4 was unique to Riverside and San Francisco (Fig. 5). Seattle birds notably lacked syllable B1 and had a unique C1 syllable type.

There was a significant effect of population on 13 of the 21 spectral and temporal measures of Anna's song; seven of these survived Bonferroni correction ($\alpha = 0.0024$; Table 1; Fig. 6; Supplemental Material, Fig. S1). I ran post hoc Tukey HSD tests on these seven variables to determine which populations were driving each result (Fig. 6; p-values for significant post hoc tests provided in Table 4). Although the number of significant pairwise population contrasts differed between variables, some general patterns were evident. As for the syllable use clustering analysis, Seattle birds were most consistently differentiated for significant temporal measures (shorter duration for Phrase B elements B2 and B3 with fewer B3 pulses; longer duration for Phrase C element C1; Fig. 6a-e), and Mendocino and Santa Ynez birds were consistently similar to one another (Fig. 6a-g). Population differences were more variable for significant frequency measures, with higher B3 frequency in Henderson, Riverside, and Seattle relative to Mendocino and Santa Ynez (Fig. 6g), and higher C1 frequency in Riverside only (Fig. 6f).

Principle component analysis of all phrase B and C variables similarly separated Seattle from the other five populations. The first two components explained 45% of the total variance with duration and frequency positively loaded on PC1 and PC2, respectively (Fig. 7a). Mean scores for Seattle were negative on both components, reflecting shorter duration and lower frequency relative to all other populations. PC1 also distinguished San Francisco and Riverside from Santa Ynez and Henderson, with more positive mean scores indicative of longer durations in the latter two populations (Fig. 7a).

3.2. Little Geographic Variation in Costa's Song

There was no association between song distance and geographic distance among Costa's populations (Mantel, $r = -0.17$, $r^2 = 0.028$, $P = 0.532$) (Fig. 4d). Visual categorization of songs did not detect any discrete differences in song form (see Fig. 2b).

There was a significant effect of population on five of the eighteen spectral and temporal measures of Costa's song, but only minimum frequency of the up-sweep at 0.25s survived Bonferroni correction ($\alpha = 0.0028$; Table 2; Fig. 8). A post hoc Tukey HSD test indicated that this result was mainly driven by higher minimum frequency in Deep Canyon relative to Henderson, Lompoc, and Rancho Cucamonga (Fig. 8; p-values for significant post hoc tests provided in Table 5). Two of the other measures that were significant at $\alpha = 0.05$ were similarly differentiated with higher apex note 1 peak frequency in Deep Canyon relative to Henderson and Rancho Cucamonga, and higher minimum frequency of the up-sweep at 0.5s in Deep Canyon relative to Henderson (Fig. 8; Table 2). Finally, duration of apex note 1 was shorter in Deep Canyon relative to Superior (Fig. 8; Table 2). All other duration and frequency measures were more variable within than between populations (Supplemental Material, Fig. S2). Within Deep Canyon, of the five measures highlighted above, only duration of apex note 1 was significantly different between shuttle and perched songs recorded from the same males in the Deep Canyon sample ($n = 6$, ANOVA, $F = 44.42$, $P < 0.0001$; Table 3). Notably, this measure was longer in shuttle relative to perched song but moderately shorter in Deep Canyon relative to Superior. I are therefore confident that the spectral and temporal differences between Deep Canyon and other populations are not an artifact of the different context in which songs from this population were recorded.

Principle component analysis of all eighteen variables recovered a similar pattern of low differentiation between most populations (Fig. 7b). The first two components explained 39% of the total variance and all populations clustered together. All populations clustered together on PC1, on which frequency was positively loaded. Duration was positively loaded on PC2, which separated Deep Canyon from other populations (Fig. 7b).

4. Discussion

Song learning evolved convergently in hummingbirds relative to songbirds and parrots (Jarvis et al., 2014). The effect of population origin on song has been studied for more than sixty years in oscine songbirds (Marler and Tamura, 1962; Lemon, 1966; Nottebohm, 1969; Baptista and King, 1980; Wang et al., 2022; reviewed in Podos and Warren, 2007). Contrastingly few studies have tested for among population differences in hummingbird song (González et al., 2011; González and Ornelas, 2014). I tested for geographic variation in the songs in a pair of closely related species with overlapping distributions in the Southwestern United States, Anna's and Costa's hummingbirds. Interestingly, despite the fact that both species learn their songs and sing in similar contexts, I found robust geographic variation in Anna's song and contrastingly little in Costa's. I discuss the patterns of geographic variation in both species' songs and consider the factors that might drive song differentiation in Anna's and constrain variation in Costa's.

Consistent with numerous studies of geographic variation in birdsong (e.g., Mundinger, 1982; Roach and Phillmore, 2017), Anna's song varied between populations in both syllable usage and spectral and temporal components. The largest differences for both types of data were between Seattle and all other populations. Whereas most populations differed in relative usage of shared syllables, Seattle birds sing a unique C1 syllable and appear to have lost the B1 syllable sung in all other populations. With all quantitative measures of Phrases B and C combined in a PCA, Seattle songs were distinct based on both shorter syllable durations and lower frequencies. In contrast, Mendocino and Santa Ynez shared syllable use patterns and were not significantly different from each other in spectral and temporal measurements indicating that, despite being geographically separated, the two populations share song-types. Henderson, San Francisco, and Riverside birds were more variable in syllable use and clustered together. San Francisco and Riverside birds were tightly clustered in the PCA based on temporal measures (PC1). Thus, with the exception of Seattle (discussed below), I did not find evidence for the population-defining song features, or dialects, that characterize many songbird species (reviewed in Podos and Warren, 2007; Williams, 2021).

Anna's hummingbird has undergone a recent and rapid northward range expansion. Count data from hummingbird feeders suggest that the species' northern-most winter occurrence at coastal sites expanded from northern California in the late 1990's to southern British Columbia by 2013 (Grieg et al., 2017). The timeframe of the expansion is strongly associated with increases in human activities that provide additional food sources for hummingbirds (e.g. hummingbird feeders, plantings that attract hummingbirds; Grieg et al., 2017). In our sampling, Mendocino is close to the presumed historic northern limits for year-round Anna's populations on the West Coast whereas Seattle is in the northern part of the recent range expansion.

Rapid range expansions are predicted to reduce song diversity or complexity in recently established relative to historically stable populations (Xing et al., 2013). Processes that can produce this pattern include founder effects (founding birds sing a subset of songs from the source population) and cultural drift (increased random loss of song diversity), both of which may be compounded by subsequent isolation of newly founded populations (Lack and Southern, 1949; Podos and Warren, 2007). Consistent with these predictions, numerous studies of the effect of range expansion on oscine birdsong found fewer or less complex song types in recently established populations (e.g., Mundinger, 1975; Baker, 1996; Newman et al., 2008; Parker et al., 2012; White, 2012; Lachlan et al., 2013; Xing et al., 2013; Malykh et al., 2018). In some cases, however, song diversity is higher in young populations due to post-expansion addition of novel variants (e.g., Lynch and Baker, 1994; Baker et al., 2003; Baker et al., 2006).

In Anna's songs from Seattle, I find evidence for both reduction in song complexity (loss of syllable B1), and the appearance of new variants (novel syllable C1). Whereas syllable loss is consistent with a founder effect, both the presence of novel variation in syllable form, and the overall reduction in syllable duration and frequency in Seattle songs, highlight the rapid rate of cultural evolution in this recent range expansion. Determining whether song differences between recently founded and historic populations are disjunct or clinal awaits sampling of birds from intervening populations in southern Washington State and Oregon. Since song differences among other Anna's populations in both syllable usage and temporal and spectral features are not strongly associated with geographic distance between them, it will be important to evaluate biotic and abiotic features of the acoustic environment to test for evidence of locally adapted song features.

In Costa's, I found just one population-level difference in song: songs from Deep Canyon tended to have higher frequency range relative to other populations. Although Deep Canyon songs were recorded in a different context than songs from other Costa's populations (elicited dynamic shuttle displays vs. perched song), none of the frequency measures that differentiate Deep Canyon songs were significantly different in comparisons of shuttle display and perched songs from the same individuals. This suggests that the moderately higher frequency range in Deep Canyon songs reflects a true population-level difference in song.

Other among-population differences in temporal and spectral measures of song were minimal and there was no relationship between song distance and geographic distance, and no qualitative differences in song structure among populations. Given that I did not sample at the southern limits of Costa's range in Baja California, and that Costa's was sampled over a smaller geographic area than Anna's, it is possible that an effect of geographic distance on song might emerge with additional sampling. However, the tendency for song distance to decrease with spatial distance suggests that the addition of more distant populations would not uncover a pattern of isolation-by-distance in Costa's song.

Why is there so little geographic variation in Costa's song when variation in Anna's song is substantial? This question is specifically motivated by the close evolutionary relationship between these two song-learning species and by the fact that both species sing in territorial defense and mate attraction, and incorporate song into elaborate female-directed flight displays. I consider three non-mutually exclusive factors that might explain low variation in Costa's song as compared to Anna's: differences in song complexity, differences in sexually selected constraints on song, and differences in seasonal movement patterns.

The relative complexity of Anna's and Costa's songs is the most obvious difference between them. Whereas Anna's song is multi-syllabic and multi-phrase with both pure tones and high-entropy portions, Costa's song is mono-syllabic and mostly pure tone with prominent harmonic stacks. The simpler structure of Costa's song provides less opportunity for song-learning error and improvisation. However, geographic variation in song is documented in multiple passerine species with low complexity songs, including the brown-headed cowbird (*Molothrus ater*, Rothstein and Fleischer, 1987), black-capped chickadee (*Poecile atricapillus*, Kroodsma et al., 1999), yellowhammer, (*Emberiza citrinella*, Petusková et al., 2015), and river warbler (*Locustella fluviatilis*, Czocherová et al., 2022). Thus, song simplicity alone may be an insufficient explanation for low variation in Costa's song.

Another possibility is that Costa's song is constrained by its relationship to other features of the species' courtship display. Both Anna's and Costa's males produce two mechanistically distinct acoustic signals during courtship: song produced with their syrinx and a dive-sound produced mechanically through feather fluttering during aerial displays (Stiles, 1982; Clark and Feo, 2008; Clark and Feo, 2010; Clark et al., 2011, Clark and Mistick, 2018). In both species, there is a striking acoustic similarity between song and dive-sound (Clark and Feo, 2010). However, whereas Anna's dive-sound matches only the terminal phrase of the song (C1), Costa's dive-sound matches the entire song. Indeed, Costa's song and dive-sounds are so similar that ornithologists originally argued that both were produced vocally (Baptista and Matsui, 1979). If the two signals were evolving independently, I would expect the song to diverge in form from the dive-sound due to a faster rate of change in a culturally transmitted signal relative to one that is more morphologically constrained. The fact that the two signals remain highly similar suggests that the match between them is sexually selected and maintained by female preference. Thus, selection for a song that is dive-like may constrain variation in Costa's song both within and among populations. A direct test this hypothesis would require measuring female response to matched vs. mismatched song and dive-sounds.

Finally, population connectivity and individual movement patterns (migration and dispersal) can impact the extent of geographic variation in song (Kroodsma, 1974; Podos and Warren, 2007). For example, both migration and high rates of dispersal can erode population differences in song in open-ended learners (Podos and Warren, 2007). Although the annual movement patterns of Anna's and Costa's are not well-defined, at least some populations of both species migrate seasonally, likely in response to food availability (reviewed in Baltosser and Scott, 2020; Clark and Russell, 2020). For example, some populations of Anna's breeding at lower elevations in California migrate up elevation by the summer, whereas others fly south (Clark and Russell, 2020). Likewise, Costa's that breed in Sonoran desert habitat in Arizona and California move to Pacific coastal scrub in the summer, whereas birds breeding in Mojave desert habitat are thought to move to higher altitudes by mid-summer (Baltosser and Scott, 2020). In both species, the onset and duration of breeding differs across habitats and latitudes (Baltosser, 1989, Baltosser and Scott, 2020, Clark and Russell, 2020).

Importantly, in both Anna's and Costa's, females provide all parental care and do not nest on male breeding territories (Stiles, 1973; Vleck, 1981) and, in populations that undergo seasonal migration, males leave breeding habitats before females and young of the year (Baltosser and Scott, 2020; Clark and Russell, 2020). Thus, unlike many oscine songbirds in which juveniles preferentially learn their father's song, the social context in which young Anna's and Costa's males first learn their song may depend on hatch date. Determining when, where, and from whom males of both species learn their songs will be critical to understanding the effects of seasonal movements on song cohesion and differentiation.

5. Conclusion

In summary, the results of this study identify contrasting effects of geography on the songs of two closely related hummingbird species. A critical next step in this line of research is to determine whether the statistically significant population differences in Anna's song are biologically significant, using field playbacks of population-matched vs. mismatched songs. Conversely, the absence of statistical differences among most populations does not guarantee that Costa's songs from different populations are equivalent from the birds' perspectives. Therefore, playback experiments are similarly well motivated in Costa's. Given the independent acquisition of song-learning in hummingbirds and songbirds, hummingbirds represent an important and currently underutilized resource for comparative studies of the evolution of song variants in natural populations. As one of very few analyses of macrogeographic patterns of variation in hummingbird song, our study helps to fill this gap. Our comparison of two song-learning species whose songs differ in complexity and variability contributes to understanding of the evolution of complexity and the maintenance of simplicity in animal signals.

References

Araya-Salas, M., Smith-Vidaurre, G., Mennill, D. J., González-Gómez, P. L., Cahill, J., & Wright, T. F. (2019). Social group signatures in hummingbird displays provide evidence of co-occurrence of vocal and visual learning. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, *286*, 20190666.

Araya-Salas, M., & Wright, T. (2013). Open-ended song learning in a hummingbird. *Biology Letters*, *9*(5), 20130625.

Baker, M. C. (1975). Song dialects and genetic differences in White-Crowned Sparrows (*Zonotrichia leucophrys*). *Evolution*, *29*(2), 226–241.

Baker, M. C., & Cunningham, M. A. (1985). The biology of bird-song dialects. *Behavioral and Brain Sciences, 8*(1), 85-100.

Baker, M.C. (1996). Depauperate meme pool of vocal signals in an island population of singing honeyeaters. *Animal Behaviour*, *51*, 853–858.

Baker, M. C., Baker, M. S. A., & Baker, E. M. (2003). Rapid evolution of a novel song and an increase in repertoire size in an island population of an Australian songbird. *Ibis*, *145*, 465–471.

Baker, M. C., Baker, M. S. A., & Tilgman, L. M. (2006). Differing effects of isolation on evolution of bird songs: examples from an island-mainland comparison of three species, *Biological Journal of the Linnean Society*, *89*(2), 331–342.

Baltosser, W. 1989. Costa's Hummingbird: Its Distribution and Status. Western Birds, 20, 41-62.

Baltosser, W. H. & Scott, P. E. (2020). Costa's Hummingbird (*Calypte costae*), version 1.0. In *Birds of the World* (A. F. Poole and F. B. Gill, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA.

Baptista, L. F. (1977). Geographic variation in song and dialects of the Puget Sound White-Crowned Sparrow. *The Condor*, *79*(3), 356–370.

Baptista, L. F., & Matsui, M. (1979). The source of the dive-noise of the Anna's hummingbird. *The Condor*, 81, 87-89.

Baptista, L. F., & King, J. R. (1980). Geographical variation in song and song dialects of Montane White-Crowned Sparrows. *The Condor*, *82*(3), 267–284.

Baptista, L. F., & Schuchmann, K.-L. (1990). Song learning in the Anna hummingbird (*Calypte anna*). *Ethology*, *84*(1), 15–26.

Bond, A. B., & Diamond, J. (2005). Geographic and ontogenetic variation in the contact calls of the Kea (*Nestor notabilis*). *Behaviour*, *142*(1), 1–20.

Clark, C. J., & Feo, T. J. (2008). The Anna's hummingbird chirps with its tail: a new mechanism of sonation in birds. *Proceedings of the Royal Society B: Biological Sciences*, 275(1637), 955-962.

Clark, C. J., & Feo, T. J. (2010). Why do *Calypte* hummingbirds "sing" with both their tail and their syrinx? An apparent example of sexual sensory bias. *American Naturalist*, 175(1), 27-37.

Clark, C. J., Elias, D. O., & Prum, R. O. (2011). Aeroelastic flutter produces hummingbird feather songs. *Science*, 333, 1430-1433.

Clark, C. J., & Mistick, E. A. (2018). Strategic acoustic control of a hummingbird courtship dive. *Current Biology*, 28, 1-8.

Clark, C. J., & Russell, S. M. (2020). Anna's Hummingbird (*Calypte anna*). Version 1.0. In, *Birds of the World* (A. F. Poole, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.annhum.01.

Czocherová, I., Rubáčová, L., Petrusek, A., & Petrusková, T. (2022). Contrasting patterns of geographical song variation in two closely related passerine species with a simple song. *Journal of Ornithology*, *163*, 93–104.

Darwin, C. (1871). *The Descent of Man and Selection in Relation to Sex Vol I.* John Murray, London, UK.

Derryberry, E. P. (2009). Ecology shapes birdsong evolution: variation in morphology and habitat explains variation in white-crowned sparrow song. *The American Naturalist*, *174*(1), 24–33.

Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution, 6*, 925-929.

Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G., & François, O. (2014). Fast and efficient estimation of individual ancestry coefficients. *Genetics*, *196*(4), 973–983.

Gaunt, S. L. L., Baptista, L. F., Sánchez, J. E., & Hernandez, D. (1994). Song learning as evidenced from song sharing in two hummingbird species (*Colibri coruscans* and *C. thalassinus*). *The Auk*, *111*(1), 87–103.

González, C., & Ornelas, J. F. (2005). Song structure and microgeographic song variation in Wedge-Tailed Sabrewings (*Campylopterus curvipennis*) in Veracruz, Mexico. *The Auk*, *122*(2), 593–607.

González, C., & Ornelas, J. F. (2009). Song variation and persistence of song neighborhoods in a lekking hummingbird. *The Condor*, *111*(4), 633–640.

González, C., & Ornelas, J. F. (2014). Acoustic divergence with gene flow in a lekking hummingbird with complex songs. *PloS One*, *9*(10), e109241.

González, C., Ornelas, J. F., & Gutiérrez-Rodríguez, C. (2011). Selection and geographic isolation influence hummingbird speciation: genetic, acoustic and morphological divergence in the wedge-tailed sabrewing (*Campylopterus curvipennis*). *BMC Evolutionary Biology*, *11*:38.

Greig, E. I., Wood, E. M., & Bonter, D. N. (2017). Winter range expansion of a hummingbird is associated with urbanization and supplementary feeding. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, *284,* 20170256.

Janik, V. M. and Slater, P. J. B. (1997). Vocal learning in mammals. *Advances in the Study of Behaviour*, *26*, 59–100.

Janik, V. M., & Knörnschild, M. (2021). Vocal production learning in mammals revisited. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *376*(1836), 202-244.

Jarvis, E. D., Mirarab, S., Aberer, A. J., Li, B., Houde, P., ...Braun, E. L., Warnow, T., Jun, W., Gilbert, M. T., & Zhang, G. (2014). Whole-genome analyses resolve early branches in the tree of life of modern birds. *Science*, *346*(6215), 1320–1331.

Johnson, K. E., & Clark, C. J. (2020). Ontogeny of vocal learning in a hummingbird. *Animal Behaviour*, *167*, 139–150.

Kroodsma, D. E., & Miller, E. H. (1996). *Ecology and Evolution of Acoustic Communication in Birds*. Cornell University Press, Ithaca, NY, USA.

Kroodsma, D. E. (1974). Song learning, dialects, and dispersal in the Bewick's Wren. *Z. Tierpsych*. 35, 352–380.

Kroodsma, D. E., Byers, B. E., Halkin, S. L., Hill, C., Minis, D., Bolsinger, J. R., Dawson, J. A., Donelan, E., Farrington, J., Gill, F. B., Houlihan, P., Innes, D., Keller, G., Macaulay, L., Marantz, C. A., Ortiz, J., Stoddard, P. K., & Wilda, K. (1999). Geographic variation in black-capped chickadee songs and singing behavior. *Auk*, *116*, 387–402.

Lachlan, R. F., Verzijden, M. N., Bernard, C. S., Jonker, P.-P., Koese, B., Jaarsma, S., Spoor, W., Slater, P. J. B., & ten Cate, C. (2013). The progressive loss of syntactical structure in bird son along an island colonization chain. *Current Biology*, *23*(19), 1896–1901.

Lack, D., & Southern, H. N. (1949). Birds on Tenerife. *Ibis*, *91*, 607–626.

Lara, C., Martínez-García, V., & Ornelas, J. F. (2015). Microgeographical variation in song repertoire and structure between the leks of Green Violetears *Colibri thalassinus* in Central Mexico. *Acta Ornithologica*, *50*(1), 23–32.

Lemon, R. E. (1966). Geographic variation in the song of cardinals. *Canadian Journal of Zoology*, *44*(3), 413–428.

Lemon, R. E. (1975). How birds develop song dialects. *The Condor*, *77*(4), 385–406.

Lynch, A., & Baker, A. J. (1994). A population memetics approach to cultural evolution in chaffinch song: differentiation among populations. *Evolution*, *48*, 351–359.

Malykh, I. M., Red'kin, J. A., Ivanitskii, V. V., and Marova, I. M. (2018). Differences in the songs of birds from island and mainland populations. *Biology Bulletin*, *45*(8), 803–811.

Marler, P., & Peters, S. (1987). A sensitive period for song acquisition in the song sparrow, *Melospiza melodia*: A case of age-limited learning. *Ethology, 76*(2), 89–100.

Marler, P., & Tamura, M. (1962). Song "dialects" in three populations of white-crowned sparrows. *The Condor*, *64*(5), 368–377.

Marler, P., & Tamura, M. (1964). Culturally transmitted patterns of vocal behavior in sparrows. *Science*, *146*(3650), 1483–1486.

Martínez-García, V., Lara, L., & Ornelas, J. F. (2013). Lek characteristics and the static male song of the green violet-ear (*Colibri thalassinus*) during a 3-year study in a temperate forest of Central Mexico. *Ornitologia Neotropical*, *24*, 183–200.

McGuire, J. A., Witt, C. C., Altshuler, D. L., & Remsen, J. V. (2007). Phylogenetic systematics and biogeography of hummingbirds: Bayesian and maximum likelihood analyses of partitioned data and selection of an appropriate partitioning strategy. *Systematic Biology*, *56*(5), 837–856.

Mirsky, E. N. (1976). Song divergence in hummingbird and junco populations on Guadalupe Island. *The Condor*, *78*(2), 230–235.

Morton, E. S. (1975). Ecological sources of selection on avian sounds. *The American Naturalist*, *109*(965), 17-34.

Mundinger, P. (1975). Song dialects and colonization in the house finch, *Carpodacus mexicanus*, on the east coast. *Condor*, *77*, 407–422.

Mundinger, P. C. (1982). Microgeographic and macrogeographic variation in the acquired vocalizations of birds. In, *Acoustic Communication in Birds*. Academic Press, New York, NY, USA.

Newman, M. M., Yeh, P. J., & Price, T. D. (2008). Song variation in a recently founded population of the dark-eyed junco (*Junco hyemalis*). *Ethology*, *114*, 164–173.

Nottebohm, F. (1969). The Song of the Chingolo, *Zonotrichia capensis*, in Argentina: description and evaluation of a system of dialects. *The Condor*, *71*(3), 299–315.

Nottebohm, F. (1972). The origins of vocal learning. *The American Naturalist*, *106*(947), 116–140.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2018) vegan: Community Ecology Package. R package version 2.5-2.

Parker, K. A., Anderson, M. J., Jenkins, P. F., & Bruton, D. H. (2012). The effects of translocation-induced isolation and fragmentation on the cultural evolution of bird song. *Ecology Letters*, *15*, 778–785.

Petrusková, T., Diblíková, L., Pipek, P. Frauendorf, E., Procházka, & P., Petrusek, A. (2015). A review of the distribution of Yellowhammer (*Emberiza citrinella*) dialects in Europe reveals the lack of a clear macrogeographic pattern. *Journal of Ornitholo*gy, 156, 263–273.

Pliny the Elder (50 A.D) Natural History. Accessed John Bostock, M.D., F.R.S. H.T. Riley, Esq., B.A. London. Taylor and Francis, Red Lion Court, Fleet Street.

Podos, J., Peters, S., Rudnicky, T., Marler, P., & Nowicki, S. (1992). The organization of song repertoires in song sparrows: Themes and variations. *Ethology*, *90*(2), 89–106.

Podos, J., & Warren, P. S. (2007). The Evolution of Geographic Variation in Birdsong. In *Advances in the Study of Behavior* (Vol. 37, pp. 403–458). Academic Press.

Roach, S. P., Philmore, L. S. (2017). Geographic variation in song structure in the Hermit Thrush (*Catharus guttatus*). *The Auk*, 124(3), 612-626.

Rothstein, S. I., & R. C. Fleischer. (1987). Vocal dialects and their possible relation to honest status signaling in the brown-headed cowbird. *The Condor*, *89*, 1–23.

Searcy, W. A., Marler, P., & Peters, S. S. (1985). Songs of isolation-reared sparrows function in communication, but are significantly less effective than learned songs. *Behavioral Ecology and Sociobiology*, *17*(3), 223–229.

Slater, P. J. B. (1989). Bird song learning: causes and consequences. *Ethology Ecology & Evolution*, *1*(1), 19–46.

Snow, D., W. (1968) The singing assemblies of Little Hermits. *The Living Birds* 7(1), 47-55.

Stiles, F. G. (1973). Food supply and annual cycle of the Anna Hummingbird. *University of California Publications in Zoology, 97*, 1-109.
Stiles, F. G. (1982). Aggressive and courtship displays of the male Anna's Hummingbird. *The Condor*, *84*(2), 208–225.

Tyack, P. L. (2019). A taxonomy for vocal learning. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *375*(1789), 20180406.

Vleck, M.,C. (1981). Hummingbird Incubation: Female Attentiveness and Egg Temperature. *Oecologia*, 51. 199-205.

Wang, D., Forstmeier, W., Farine, D. R., Maldonado-Chaparro, A. A., Martin, K., Pei, Y., Alarcón-Nieto, G., Klarevas-Irby, J. A., Ma, S., Aplin, L. M., & Kempenaers, B. (2022). Machine learning reveals cryptic dialects that explain mate choice in a songbird. *Nature Communications*, *13*(1), 1630.

Wells, S., Bradley, R. A., & Baptista, L. F. (1978). Hybridization in *Calypte* Hummingbirds. *The Auk*, *95*(3), 537–549.

White, P. J. C. (2012). Song characteristics vary clinally across an active colonization zone in Eurasian nuthatches *Sitta europaea*.
Bird Study, *59*, 296–302.

Wiley, R. H. (1971). Song groups in a singing assembly of Little Hermits. *The Condor*, *73*(1), 28–35.

Williams, B. R., & Houtman, A. M. (2008). Song of Costa's Hummingbird (*Calypte costae*). *The Auk*, *125*(3), 663–669.

Williams, H. (2021). Mechanisms of cultural evolution in the songs of wild bird populations. *Frontiers in Psychology*, *12*, 643343.

Wright, T. F., & Dahlin, C. R. (2018). Vocal dialects in parrots: patterns and processes of cultural evolution. *The Emu*, *118*(1), 50–66.

Wright, T. F., Rodriguez, A. M., & Fleischer, R. C. (2005). Vocal dialects, sex-biased dispersal, and microsatellite population structure in the parrot *Amazona auropalliata*. *Molecular Ecology*, *14*(4), 1197–1205.

Xing, X. Y., Alström, P., Yang, X. J., Lei, F. M. (2013). Recent northward range expansion promotes song evolution in a passering bird, the Light-vented Bulbul. *Journal of Evolutionary Biology*, 26(4), 867-877.

Yang, X., Lei, F., Wang, G., & Jesse, A. J. (2007). Syllable sharing and inter-individual syllable variation in Anna's hummingbird *Calypte anna* songs, in San Francisco, California. *Folia Zoologica*, *56*(3), 307-318.

Tables and Figures

Table 2.1. Analysis of variance results for spectral and temporal measures of Anna's hummingbird song.

	Measurement	***n***	***F***	***p***
Phrase A				
Syllables	**A1 Duration (seconds)**	62	**3.91**	**0.004**
A1-A2	A1 Peak frequency (kHz)	62	1.54	0.190
	A2 Duration (seconds)	61	**2.43**	**0.047**
	A2 Peak frequency (kHz)	61	1.88	0.113
Phrase B				
B1	**B1 Duration (seconds)**	47	**2.94**	**0.030**
	B1 Peak frequency (kHz)	47	2.33	0.071
	Upper whistle (kHz)	47	0.62	0.653
B2	B2 Peak frequency (kHz)	63	2.15	0.072
	B2 Duration (seconds)	63	**5.23**	**0.0005***
B3	**B3 Peak frequency (kHz)**	63	**7.27**	**<0.0001***
	B3 Duration (seconds)	63	**22.63**	**<0.0001***
	Peak frequency pulses (kHz)	63	1.32	0.266
	Duration pulses (seconds)	63	**13.80**	**<0.0001***
	Number of pulses (#)	63	**11.21**	**<0.0001***
	Pulse rate (# pulses/duration pulses)	63	**3.15**	**0.014**
Phrase C	**Peak frequency (kHz)**	63	**6.20**	**0.0001***
C1	**Duration (seconds)**	63	**11.93**	**<0.0001***
	Peak frequency lower tone (kHz)	63	0.09	0.992
	Duration lower tone (seconds)	63	**2.93**	**0.020**
	Peak frequency upper tone (kHz)	61	0.17	0.974
	Duration upper tone (seconds)	61	**2.46**	**0.044**

*Significant at Bonferroni $\alpha = 0.0024$

Table 2.2. Analysis of variance results for spectral and temporal measures of Costa's hummingbird song.

	Measurement	***n***	***F***	***p***
Up-sweep	Delta frequency 0.25s (kHz)	46	1.06	0.394
	Max frequency 0.25s (kHz)	46	1.49	0.214
	Min frequency 0.25s (kHz)	46	**5.44**	**0.0006***
	Delta frequency 0.5s (kHz)	45	0.37	0.866
	Min frequency 0.5s (kHz)	45	**3.20**	**0.016**
Down-sweep	**Delta frequency 0.25s (kHz)**	45	**2.85**	**0.028**
	Max frequency 0.25s (kHz)	45	2.15	0.079
	Min frequency 0.25s (kHz)	45	1.82	0.131
	Delta frequency 0.5s (kHz)	45	1.14	0.354
	Min frequency 0.5s (kHz)	45	0.98	0.441
Apex	Duration (seconds)	49	1.82	0.130
	High frequency (kHz)	49	1.61	0.176
	Low frequency (kHz)	49	2.31	0.06
	Delta frequency (kHz)	49	1.07	0.390
Apex note 1	**Peak frequency (kHz)**	49	**3.91**	**0.0052**
	Duration (seconds)	49	**2.48**	**0.046**
Apex note 2	Peak frequency (kHz)	49	2.18	0.074
	Duration (seconds)	49	1.15	0.349

Table 2.3. Analysis of variance results for spectral and temporal measures of Costa's hummingbird song perched (N=6) vs. shuttle song (N=6), recorded at Deep Canyon, CA.

	Measurement	***F***	***p***
Up-sweep	Delta frequency 0.25s (kHz)	4.02	0.076
	Max frequency 0.25s (kHz)	3.43	0.097
	Min frequency 0.25s (kHz)	0.03	0.858
	Delta frequency 0.5s (kHz)	0.13	0.728
	Min frequency 0.5s (kHz)	0.04	0.854
Down-sweep	Delta frequency 0.25s (kHz)	4.19	0.071
	Max frequency 0.25s (kHz)	0.36	0.563
	Min frequency .25s (kHz)	0.25	0.628
	Delta frequency 0.5s (kHz)	4.36	0.067
	Min frequency 0.5s (kHz)	**7.36**	**0.024**
Apex	Duration (seconds)	2.45	0.149
	High frequency (kHz)	0.05	0.820
	Low frequency (kHz)	0.001	0.971
	Delta frequency (kHz)	0.05	0.835
Apex note 1	Peak frequency (kHz)	0.001	0.979
	Duration (seconds)	**44.42**	**<0.0001**
Apex note 2	Peak frequency (kHz)	0.07	0.800
	Duration (seconds)	0.10	0.756

Table 2.4. Significant population pairwise comparisons with Tukey's HSD correction for 13 spectral and temporal measures for which there was a main effect of population in Anna's hummingbird.

	Measurement	**Population comparisons**[a]	***p***
Phrase A Syllables A1-A2	A1 Duration (seconds)	SE vs. ME	0.0007
		SE vs. RI	0.020
		SE vs. SY	0.052
		SF vs. ME	0.037
	A2 Duration (seconds)	SF vs. RI	0.029
Phrase B Syllables B1-B3	B1 Duration (seconds)	HE vs. ME	0.062
		RI vs. ME	0.066
	B2 Duration (seconds)*	RI vs. HE	0.048
		RI vs. SE	0.006
		SY vs. SE	0.012
		SF vs. SE	0.010
	B3 Peak Frequency (kHz)*	RI vs. ME	0.0005
		RI vs. SY	0.004
		HE v. ME	0.015
		HE vs. SY	0.042
		SE vs. ME	0.002
		SE vs. SY	0.012
	B3 Duration (seconds)	HE vs. SE	<0.0001
		HE vs. SF	<0.0001
		SY vs. SE	<0.0001
		HE vs. RI	<0.0001
		ME vs. SE	<0.0001
		HE vs. ME	0.006
		RI vs. SE	0.0004
		HE vs. SY	0.024
		SY vs. SF	0.011
		SF vs. SE	0.009
		ME vs. SF	0.00

	Measurement	**Population comparisons**[a]	***p***
Phrase B B3			
	Duration pulses (seconds)*	HE vs. SE	<0.0001
		SY vs. SE	<0.0001
		HE vs. SF	0.0003
		ME vs. SE	<0.0001
		HE vs. RI	0.011
		SY vs. SF	0.013
		RI vs. SE	0.012
		ME vs. SF	0.008
	Number of pulses (#)	HE vs. SE	<0.0001
		ME vs. SE	<0.0001
		SY vs. SE	<0.0001
		HE vs. SF	0.020
		RI vs. SE	0.004
		ME vs. SF	0.023
	Pulse rate (# pulses/duration of pulses)	ME vs. SE	0.027
Phrase C C1			
	Peak frequency (kHz)	RI vs. SF	0.0001
		RI vs. SE	0.0002
		RI vs. HE	0.016
		RI vs. SY	0.004
		RI vs. ME	0.003
	Duration (seconds)	SE vs. SY	<0.0001
		SE vs. RI	<0.0001
		SE vs. HE	0.003
		SE vs. ME	<0.0001
		SF vs. SY	0.002
		SE vs. SF	0.050
	Duration lower tone (seconds)	RI vs. HE	0.036
		SE vs. HE	0.028
	Duration upper tone (seconds)	SE vs HE	0.020

---

[a] HE, Henderson; ME, Mendocino; RI, Riverside; SE, Seattle; SF, San Francisco; SY, Santa Ynez.

Table 2.5. Significant population pairwise comparisons with Tukey's HSD correction for four spectral and temporal measures for which there was a main effect of population in Costa's hummingbird.

	Measurement	**Population comparisons**[a]	***p***
Up-sweep	Min frequency 0.25 (kHz)		
		DC vs. HE	0.0014
		YU vs. HE	0.039
		DC vs. RC	0.030
		DC vs. LO	0.023
	Min frequency 0.5 (kHz)		
		DC vs. HE	0.043
Apex note 1	Peak frequency (kHz)		
		DC vs. HE	0.010
		DC vs. RC	0.045
	Duration (seconds)		
		SU vs. DC	0.016

[a] HE, Henderson; LO, Lompoc; DC, Deep Canyon; RC, Rancho Cucamonga; YU, Yuma; SU, Superior.

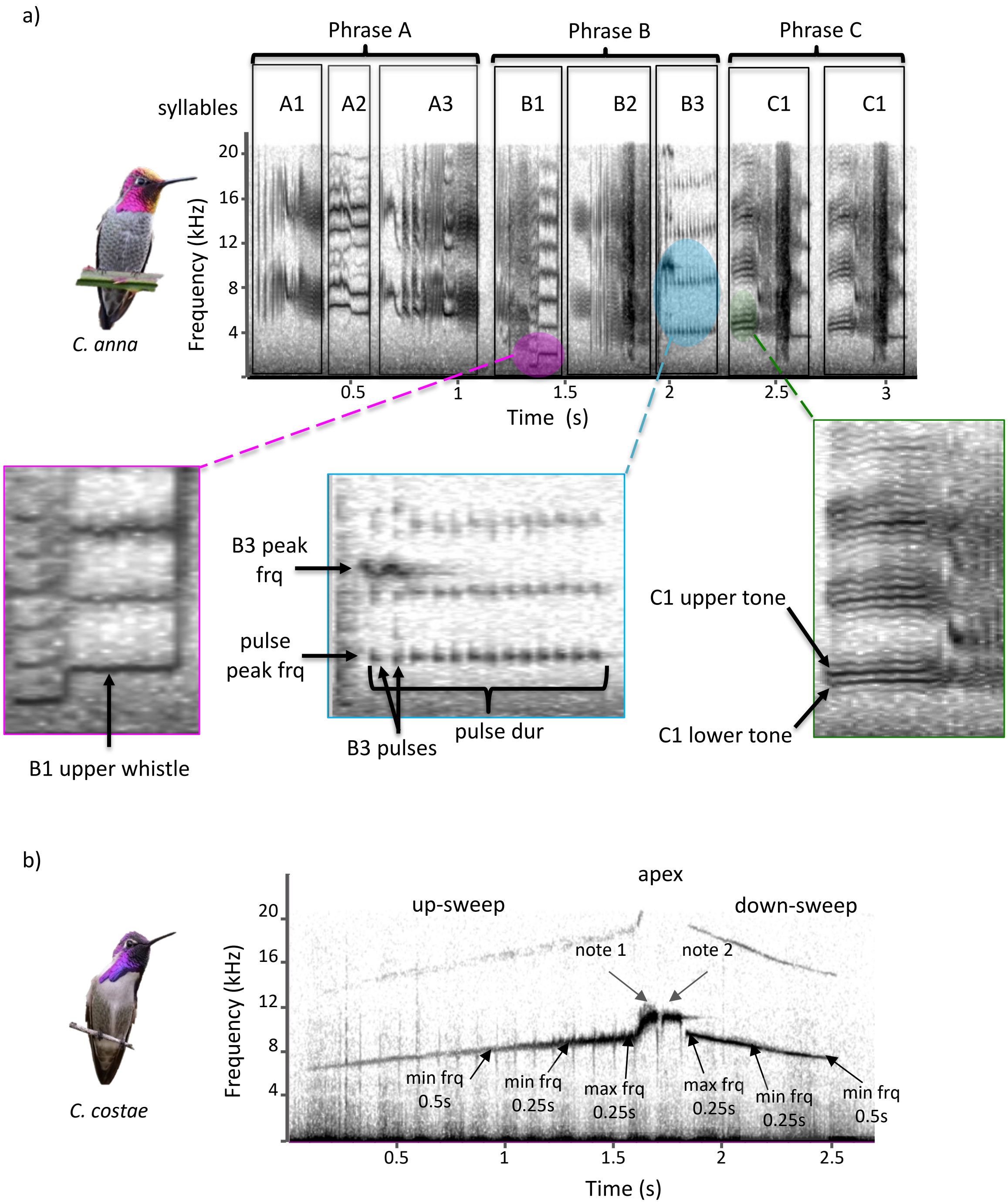

a)
Phrase A
Phrase B
Phrase C
syllables
A1
A2
A3
B1
B2
B3
C1
C1
C. anna
Frequency (kHz)
20
16
12
8
4
0.5
1
1.5
2
2.5
3
Time (s)
B1 upper whistle
B3 peak frq
pulse peak frq
B3 pulses
pulse dur
C1 upper tone
C1 lower tone
b)
C. costae
apex
up-sweep
down-sweep
note 1
note 2
Frequency (kHz)
20
16
12
8
4
min frq 0.5s
min frq 0.25s
max frq 0.25s
max frq 0.25s
min frq 0.25s
min frq 0.5s
0.5
1
1.5
2
2.5
Time (s)

Figure 2.1. Anna's and Costa's song and their spectral and temporal measurements. **a)** Anna's hummingbirds sing a complex three phrase (A-C) song with multiple syllables (A1-A3, B1-B3, C1). Peak frequency and duration was taken for full syllables (A1-C1), and for each of the highlighted elements (B1 upper whistle, B3 pulses, C1 upper tone, and C1 lower tone). B3 number of pulses and pulse rate were also measured. **b)** Costa's hummingbirds sing a simple one phrase song with three elements: up-sweep, two note apex, and down-sweep. For the up-sweep and down-sweep max and min center frequency and delta frequency was measure at 0.25 s and 0.5s from the apex. For the apex (note 1 and 2 combined) duration and high and low frequency was measured. For note 1 and 2 peak frequency and duration was measured. Images, Steven Mlodinow, Tom Friedel. Spectrograms and measurements generated in Raven Pro v1.6 (Cornell Lab of Ornithology, Cornell University, Ithaca, NY, USA) using a 512 FFT smooth Hamming window.

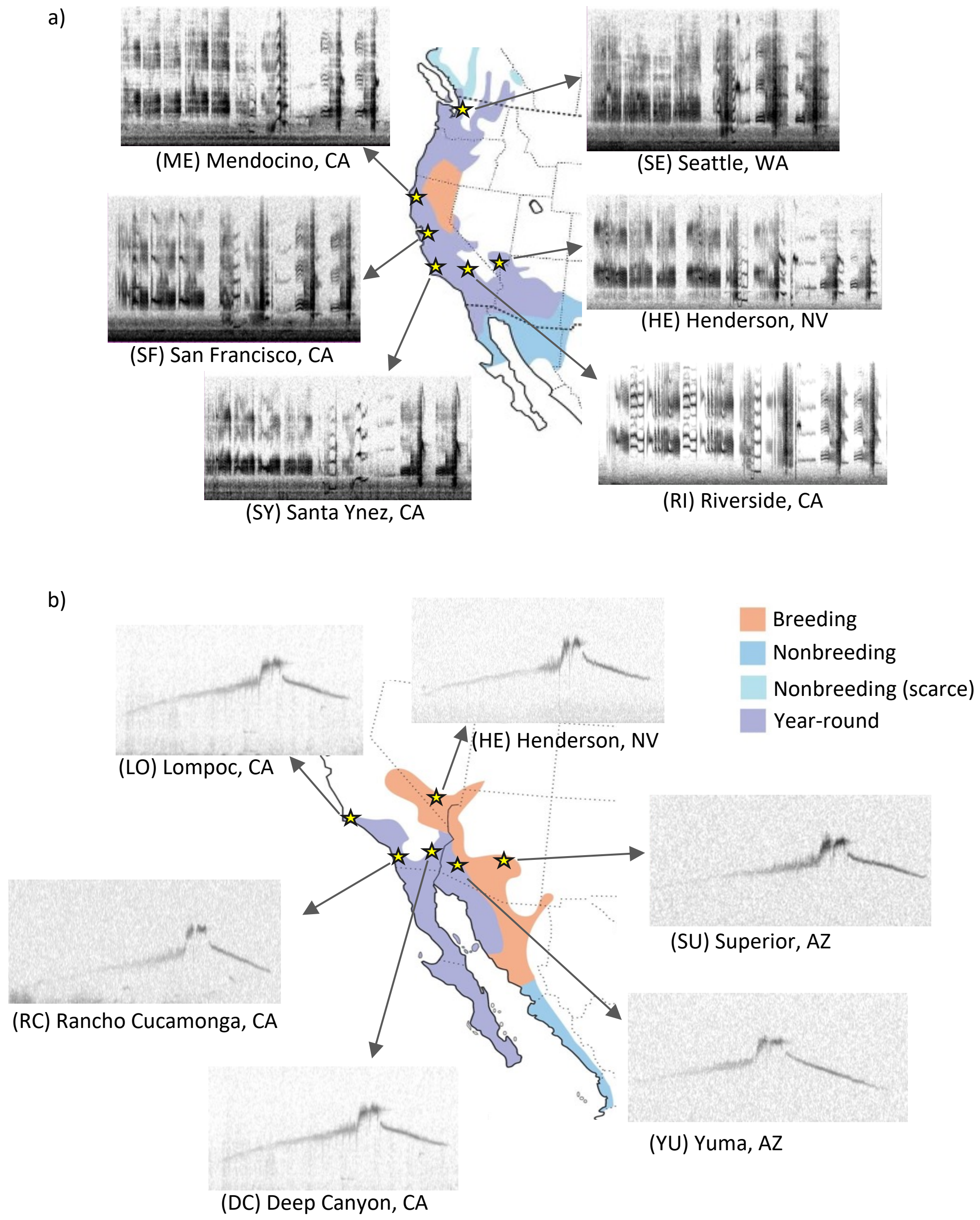
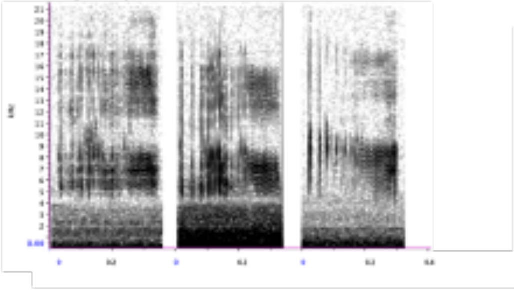


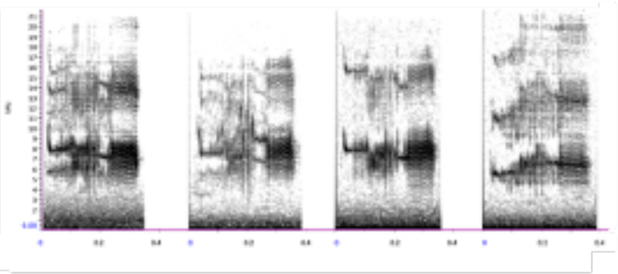
Figure 2.2 Anna's and Costa's geographic distributions and approximate locations of sampling sites with representative spectrograms. **a)** Anna's sample sizes: RI (Riverside, CA) *n*=15; SY (Santa Ynez, CA) *n*=10, SF (San Francisco, CA) *n*=25; ME (Mendocino, CA) *n*=10; SE (Seattle, WA) *n*=25; HE (Henderson, NV) *n*=5. **b)** Costa's sample sizes: DC (Deep Canyon, CA) *n*=15; RC (Rancho Cucamonga, CA), *n*=7; LO (Lompoc, CA) *n*=10; HE (Henderson, NV) *n*=6; SU (Superior, AZ) n=4. Range maps modified from All About Birds (allaboutbirds.org).

Phrase A

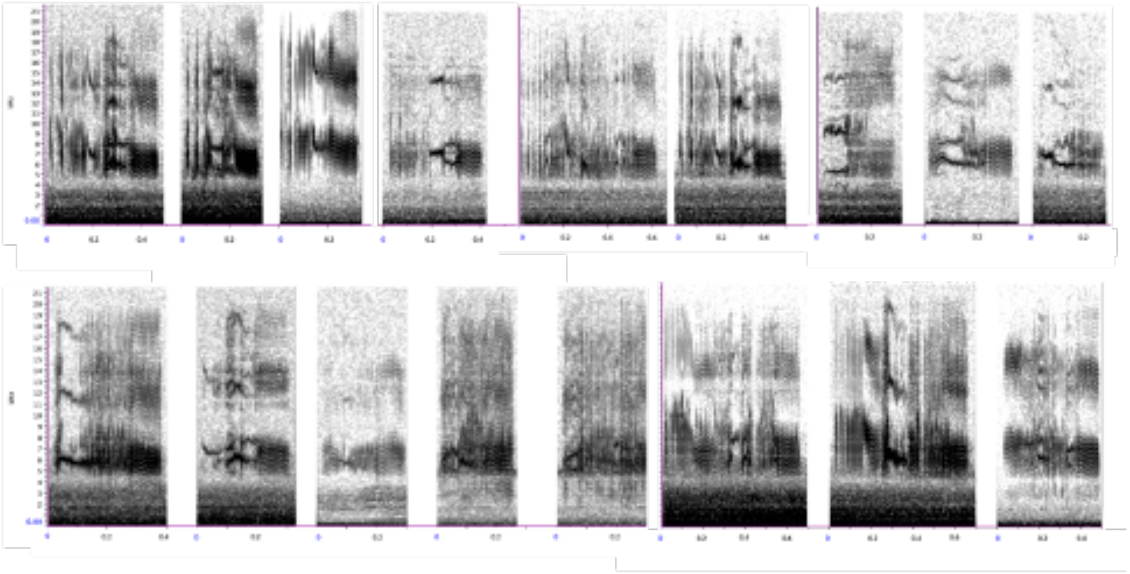
A group



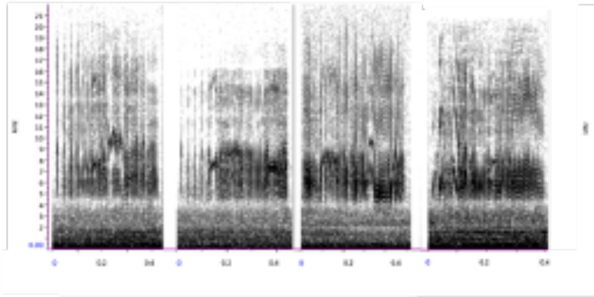
B group



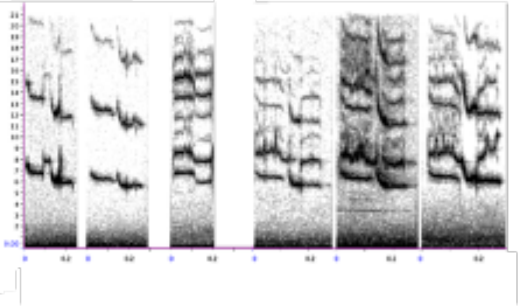
C group



D group



F group



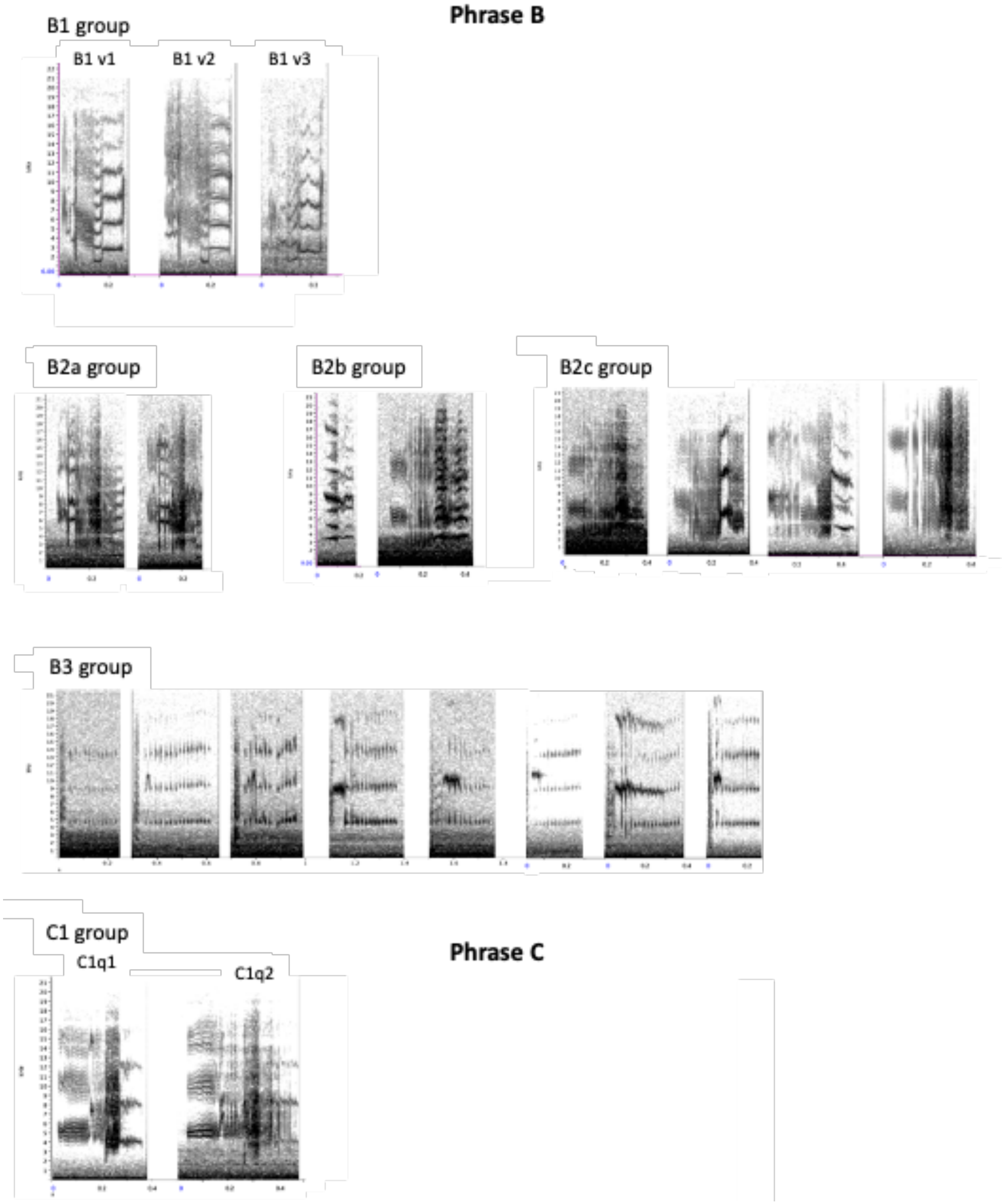


Figure 2.3 Syllable groupings in Anna's hummingbird song. Fifty-five syllables were identified and were binned into 11 groups.

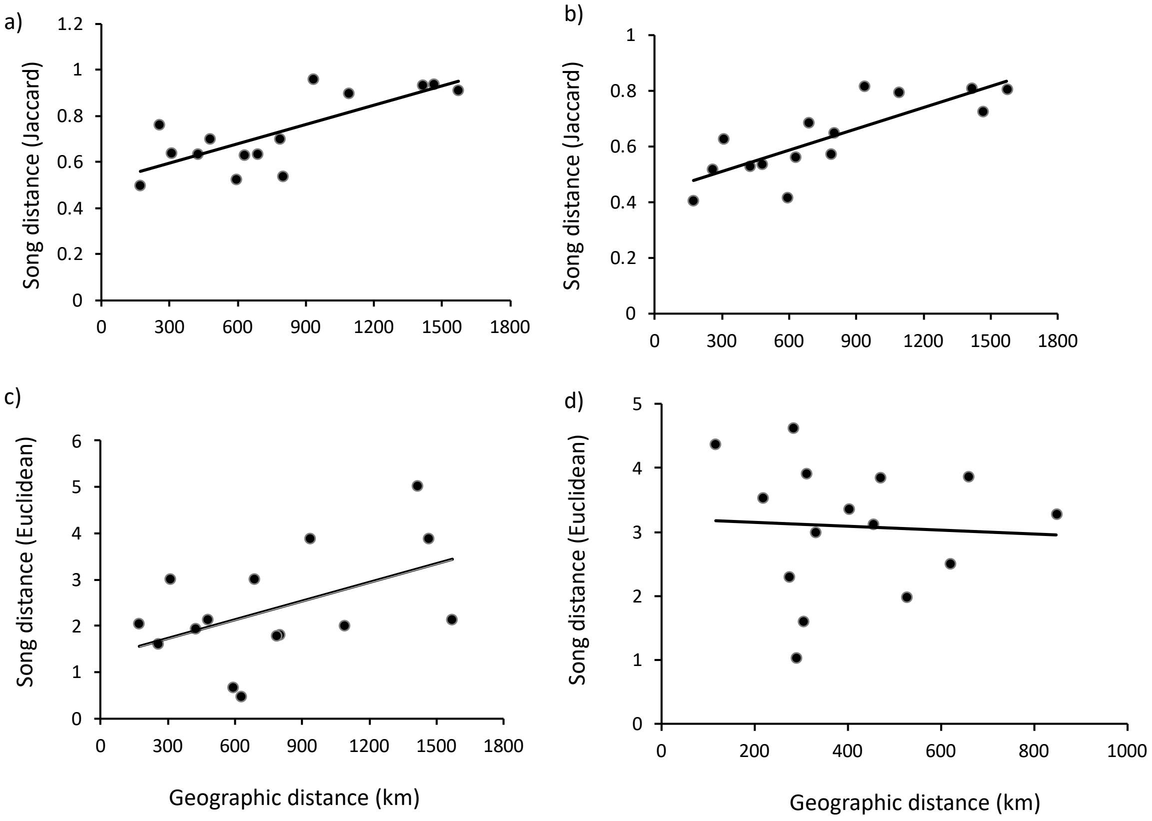


Figure 2.4. Effect of geographic distance on song distance in Anna's (**a-c**) and Costa's (**d**) hummingbirds. **a)** Geographic and song distance for syllable use in Anna's (full dataset; Mantel, $r = 0.76$, $r^2 = 0.58$, $P < 0.0001$). **b)** Geographic and song distance for syllable use in Anna's (binned dataset; Mantel, $r = 0.81$, $r^2 = 0.65$, $P < 0.0001$). **c)** Geographic and song distance for spectral and temporal components of Anna's song (Mantel, $r = 0.48$, $r^2 = 0.24$, $P = 0.076$). **d)** Geographic and song distance for spectral and temporal components of Costa's song (Mantel, $r = -0.17$, $r^2 = 0.028$, $P = 0.532$). Geographic distance estimated as linear distance in kilometers from center of each sampling locality; song distance calculated as Jaccard distance for syllable use and Euclidian distance for spectral and temporal measures.

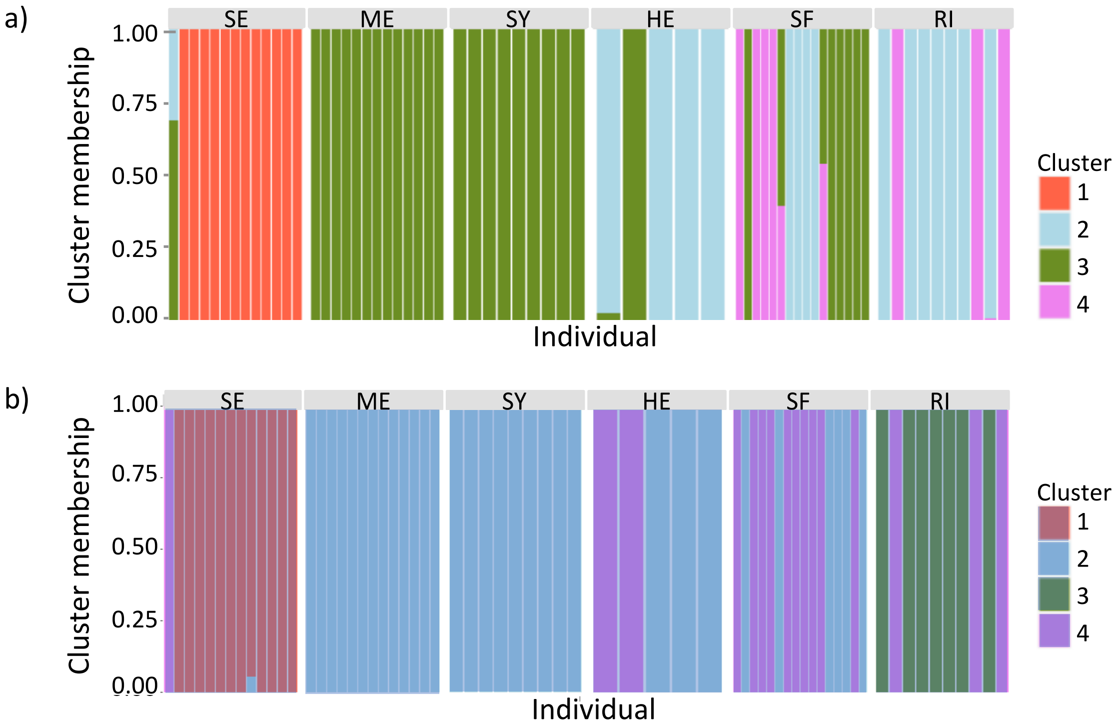


Figure 2.5. Geographically structured patterns of syllable use in Anna's hummingbird based on a) full dataset and b) binned dataset. Bars are individual birds; colors indicate proportional assignment to $K = 4$ clusters. SE, Seattle; ME, Mendocino; SY, Santa Ynez; HE, Henderson; SF, San Francisco; RI, Riverside.

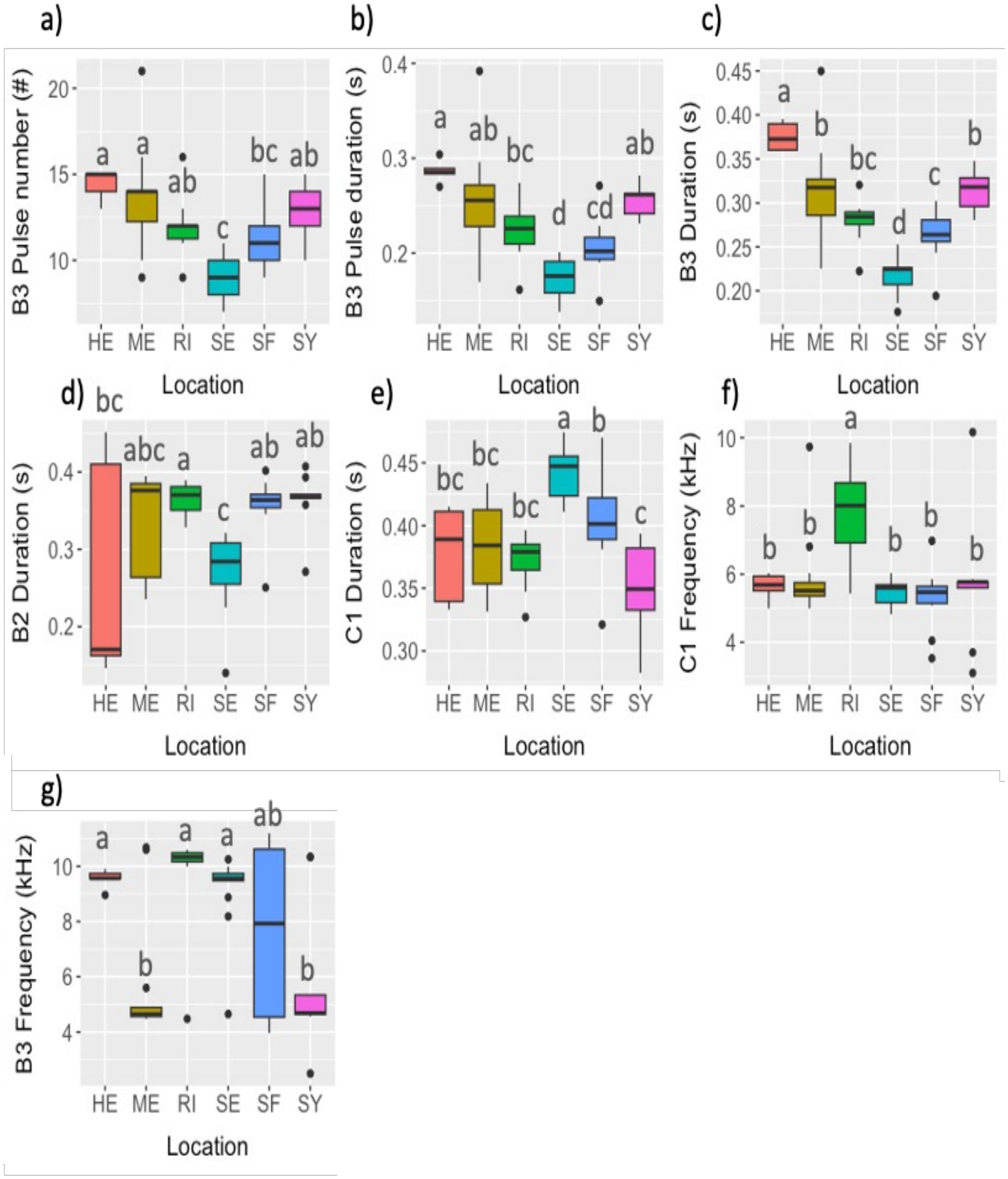


Figure 2.6. Geographic variation in spectral and temporal measures of Anna's hummingbird song. Populations that do not share letters are significantly different (Tukey HSD test). All ANOVA p-values ≤ 0.0005 (see Table 1). HE, Henderson; ME, Mendocino; RI, Riverside; SE, Seattle; SF, San Francisco; SY, Santa Ynez.

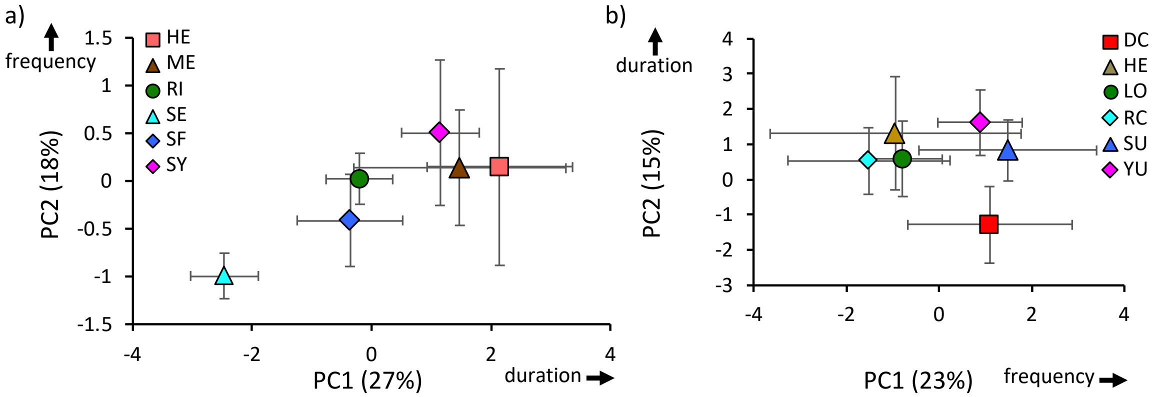


Figure 2.7. Principle component analysis of spectral and temporal measures of Anna's and Costa's hummingbird song. Plots of population mean scores for first (PC1) and second (PC2) principle components from, **a)** six Anna's populations with duration positively loaded on PC1 and frequency positively loaded on PC2 and **b)** six Costa's populations with frequency positively loaded on PC1 and duration positively loaded on PC2. Axis labels include percent variance explained by each PC; error bars are standard deviations. HE, Henderson; ME, Mendocino; RI, Riverside; SE, Seattle; SF, San Francisco; SY, Santa Ynez; LO, Lompoc; PD, Deep Canyon; DC, Rancho Cucamonga; YU, Yuma; SU, Superior.

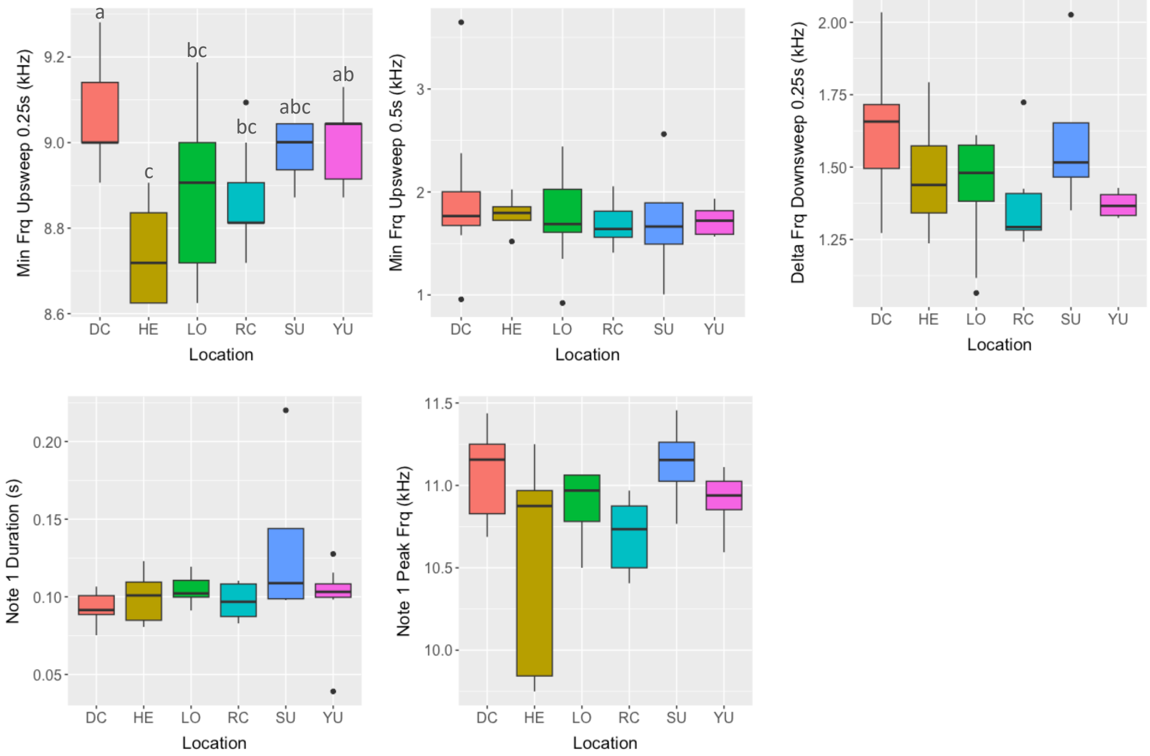


Figure 2.8. Geographic variation in spectral measures of Costa's hummingbird song. All ANOVA p-values > 0.0028 (see Table 2). DC, Deep Canyon; HE, Henderson; LO, Lompoc; RC, Rancho Cucamonga; SU, Superior; YU, Yuma.

Chapter 3

Song Variation Across Space and Time in Anna’s Hummingbird (*Calypte anna*)

Abstract

Birds use song to communicate in multiple contexts, including territoriality and courtship. Of particular interest is the phenomenon of song sharing, in which direct neighbors’ songs are more similar to one another than those of non-direct neighbors. Song sharing has been studied extensively in oscine songbirds. However, despite the high diversity of hummingbird vocalizations, less attention has been given to this lineage. Anna’s hummingbird (*Calypte anna*) has a spectrally complex, three-phrase multisyllabic song that varies on both macrogeographic and microgeographic scales. In this study I tested for song sharing between neighbors and for an effect of physical distance on song distance within a single population. In January - February of 2021 I audio recorded 29 Anna’s hummingbirds in Golden Gate Park, San Francisco, CA. I characterized syllable types, and collected spectral and temporal measures of songs in Raven Pro 1.6. I evaluated how Anna’s song form may change over time by comparing our results to those of a study conducted at the same site in 1999 and a pair of recordings from 1985. I identified 29 syllable types and 11 song types, and found strong evidence for both syllable and song type sharing between males with adjacent territories. I found a nearly complete turnover of syllable types between 1999 and 2021, demonstrating the potential for rapid cultural evolution in hummingbird song.

1. Introduction

How, why, and when birds learn their songs are enduring questions in animal behavior. Song learning has evolved in three avian clades, the oscine songbirds, parrots, and hummingbirds (Nottenbohm, 1972; Janik and Slater, 1997; Tyack, 2019; Janik and Knörnschild, 2021). Subject to errors and improvisation, learned song can facilitate song variation, and the formation of song types that can be stable or labile over time. Most research on avian song has focused on oscine songbirds (Slater, 1981; Baker and Cunningham, 1985; Tsipoura and Morton, 1988; Ellers and Slabekoorn, 2003; DeVoogd et al., 1993; Podos and Warren, 2007). Despite having complex and diverse songs, hummingbirds have received less attention in general and few studies have evaluated whether and how hummingbird songs may change over time.

Like the songs of oscines, hummingbird songs can vary on both micro- and macrogeographic scales (Snow, 1968; Wiley, 1971; Baptista and Schuchmann, 1990; Gaunt et al., 1994; González and Ornelas, 2009; González and Ornelas, 2014; Araya-Salas and Wright, 2013; Lara et al., 2015; Araya-Salas et al., 2019). One mechanism for the generation of microgeographic variation in song is song sharing between birds with neighboring territories (Payne et al., 1988; Hill et al., 1999). Song sharing, and the associated formation of song neighborhoods in which sub-sets of locally aggregated individuals share song-types, is widespread in oscines (Payne, 1985; McGregor and Thompson, 1988; Hughes et al., 1998) and is documented in several neotropical hummingbird species (e.g., wedge-tailed sabrewings [*Campylopterus curvipennis*]: González and Ornelas, 2009, hermit hummingbirds [*Phaethornis*]: Snow, 1968; Wiley, 1971; Snow, 1974; Stiles and Wolf, 1979; Araya-Salas and Wright, 2013). To our knowledge, only two such studies have evaluated whether shared song types change over time. In wedge-tailed sabrewings, song neighborhoods were stable over the course of a four-year study with a low rate of syllable type turnover within each neighborhood (González and Ornelas, 2009). In contrast, the monosyllabic song types of long-billed hermits (*Phaethornis longirostris*) had turnover rates of a few months in some males (Araya-Salas and Wright, 2013). These contrasting patterns of song stability and plasticity within single generations highlight the need for temporal studies in a wider range of hummingbird species, and raise the question of how hummingbird songs may change across time periods longer than individual lifetimes.

Here, I characterize the spatial distribution of song and syllable types in a population of Anna's hummingbirds (*Calypte anna*), and evaluate temporal change in syllable types across multiple generations. To do so, I re-sampled an Anna's population in Golden Gate Park (San Francisco, CA, USA) that was recorded at two earlier time points: March, 1985 (LF Baptista, archived in the Borror Laboratory of Bioacoustics) and April-May, 1999 (Yang et al., 2007).

Male Anna's hummingbirds learn their songs (Baptista and Schuchmann, 1990) and sing during territorial and courtship displays (Wells et al., 1978; Stiles, 1982, Clark and Feo, 2008; Clark and Feo, 2010). Anna's song is spectrally complex with three multi-syllabic phrases (A-C) that are integrated into both static and dynamic multimodal courtship displays. The full song (phrases A-C) is sung from perches and in flight during territorial chases. The first phrase of the song (phrase A), is sung repeatedly as part of two dynamic courtship displays, the dive-display, in which a male hovers while singing phrase A and subsequently dives towards females while flaring his gorget and sonating (Clark and Feo, 2008), and in a circling display, in which a male repeatedly flies around the female, flying low to the ground and then up to perch where he sings phrase A while flaring his gorget (Stiles, 1982). In this study I focused on static perched song.

Anna's hummingbird song varies between populations in both syllable usage and spectral and temporal components (Berger et al., 2023), and syllable usage can vary within population (Yang et al., 2007). The 1999 study of Anna's song in Golden Gate Park found a weak negative association between song similarity and between-bird distance, indicating that birds with adjacent territories tend to share syllables (Yang et al. 2007). Here, I re-sample the same population and 1) identify syllable and song types, 2) determine the spatial distribution of song sharing and test the effect of inter-individual distance on song distance, based on both syllable usage and temporal and spectral measures, and 3) evaluate the extent of syllable turnover across decades. Documenting spatial patterns of song sharing provides insight into the social mechanism and function of song-learning. Furthermore, documenting how songs in a single population may change over time helps to connect population level variation to the evolution of among-population differences in song.

2. Methods

2.1 Recording

I sampled Anna's hummingbird song from Golden Gate Park, (San Francisco, CA, USA), a 1,000 acre urban public park. Recordings were collected in January and February 2021 during the January to June breeding season (n= 29 birds, Fig. 1a). I recorded songs from males singing on their breeding territories (5-30 songs/male). Breeding males have stable territories and can be identified by perch fidelity (Stiles, 1973; Stiles, 1982; Clark and Russell, 2012). Recordings from a given male were collected consecutively over the course of one day. Recordings were captured using a Zoom F8 multitrack field recorder (Zoom Corporation, Tokyo, Japan), at a sample rate of 48kHz with a 24-bit depth and a Sennheiser K6 microphone (Sennheiser electronic GmbH & Co. KG, Wedemark, Germany; frequency range 30Hz -20 kHz, ± 1dB frequency handling) with a parabola shell (Wildtronics, LLC Mono Parabolic microphone, Newton Falls, OH, USA) at a distance of ≤15 meters from the focal bird. GPS coordinates were taken with Gaia GPS (Trailbehind Inc., Berkeley, CA, USA) and straight line distance between individuals was calculated using the Haversine formula and the geosphere package (Hijmans et al. 2022). I obtained recordings of two male Anna's made in March, 1985 by LF Baptista and archived in the Borror Laboratory of Bioacoustics (blb.osu.edu/database/; The Ohio State University). Recordings from both 1985 (*n* = 2 males) and 1999 (*n* = 44 males; Yang et al., 2007) were captured using a Nagra tape recorder and ME 20 Sennheiser microphone (Sennheiser electronic GmbH & Co. KG, Wedemark, Germany; frequency range 50Hz - 15 kHz, ± 3dB frequency handling).

2.2. Song Analysis -

Anna's song is complex with high entropy and a frequency range of 1.5 – 20 kHz, with most of the energy between 1.5 and 4 kHz. I divided the songs into phrases, syllables, and elements by visual inspection of the spectrograms generated in Raven Pro v1.6 (Cornell Lab of Ornithology, Cornell University, Ithaca, NY, USA) using a 512 FFT smooth Hamming window. Anna's song consists of three multi-syllabic phrases (A-C; Fig. 2). Phrase A consists of 2-4 syllables, phrase B consists of 2-3 syllables, and phrase C consists of 1-2 syllables. I defined syllables as repeatable components that have an inter-syllable interval less than 0.08 seconds and defined phrases as repeated groupings of syllables with between phrase intervals greater than 0.08 seconds. I used the same designation and nomenclature for phrases as Yang et al. (2007) with the exception of phrase B, which I divided into three syllables as opposed to two. I used different nomenclature for syllables. The number of times phrase A is repeated before singing the full song (A-C) is variable within individual but the syntax of the full song (phrases A-C) is stereotyped and conserved between all individuals (Stiles, 1982; Yang et al., 2007; ANB personal observation). I visually evaluated 10 songs per male to confirm that individuals had a single song type and selected the song with the highest signal to noise ratio and the lowest background noise to represent each male. A single observer (WWY) measured the duration and peak frequency of each syllable in Raven (Table 1; Fig. 2).

To define distinct syllables, a single observer (ANB) visually cataloged syllables through inspection of the spectrograms with the bird IDs hidden (Searcy et al., 1985; Podos et al., 1992; González and Ornelas, 2009). I did the same with recordings from

1985. To check the repeatability of the syllable designations, this process was repeated two times for each song. I compared these syllable types visually to those defined by Yang et al. (2007). To evaluate how well our sampling represented all syllable types in the population I plotted a cumulative curve of the number of syllables identified in the songs of the 29 males I recorded.

I defined song types as songs that share the same syllable types and syntax across the full song (Tsipoura and Morton, 1988) and identified them by visual evaluation of spectrograms. I used discriminant function analysis (DFA) to test the accuracy of our qualitative classification of song types based on syllable usage. DFA was run in JMP (SAS Institute Inc., Cary, NC, U SA).

To test for an effect of physical distance between males on syllable use, I used the presence/absence of each syllable type in each male's song to produce a Jaccard's dissimilarity matrix with the R package vegan (Oksanen et al. 2018; https://CRAN.R-project.org/package=vegan). To test for an effect of between male distances on spectral and temporal measurements I entered all measurements into a principle component analysis (PCA) and used PC1-3 scores for each individual to calculate Euclidian distance (song distance). Correlations between syllable use distance and physical distance, and between song distance and physical distance, were tested with Mantel tests (10,000 iterations) in XLSTAT (Lumivero, Burlington, MA, USA). PCA was run in JMP (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1 Reproductive Outcomes

I identified a total of 25 syllable types and 11 song types (Figs. 1 and 3). The most common song types, 1, 4, and 6, were sung by 55% (16/29) of males and were spatially clustered, a pattern suggestive of song neighborhoods (Fig. 1a). The majority of syllable type diversity was found in phrases A and B (Fig. 3). I found a significant positive relationship between physical distances between males and syllable use distances (Fig. 4a; Mantel, $r = 0.32$, $r^2 = 0.108$, $P = <0.0001$) but no relationship between physical distance and song distance derived from spectral and temporal measures (Fig. 4b; Mantel, $r = 0.056$, $r^2 = 0.0031$, $P = 0.328$). The first two components in the PCA explained 36% of the total variance in spectral and temporal measures, with duration and frequency negatively loaded on PC1 and PC2, respectively (Fig. 5). The PCA did not cluster individuals by song type (Fig. 5). In contrast, DFA based on syllable sharing classified 93% of individual songs (27/29) to the correct song type.

In the comparison with 1985 and 1999 recordings from the same population, A syllable types from the "bzz" category of Yang et al. (2007) were present in all earlier recordings and were analogous to the syllable types I identified as Ai, Ak, and Am (Fig. 3). These syllables were sung in 7/11 song types found in 2021 (types 4, 6, 7, 8, 9, 10, 11; Fig. 1b). However, because the rapid, broadband nature of these trills made them difficult to delineate into separate syllable types, temporal differences and similarities that I could not distinguish may exist. In 1985 and 1999 songs, phrase B consisted of two main syllables whereas in 2021 the second B syllable ("Zwee" in Yang et al., 2007) was split in two, with a gap between syllable B2 and B3 and a broadband click marking the start of syllable B3 in all songs (Fig. 3). Only one syllable type was fully conserved across all three time points; the syllable designated here as C1 resembled syllable “Db” of Yang et al., (2007). Overall, I identified fewer syllable types than did Yang and colleagues in 1999 (25 vs. 38). None-the-less, the fact that the cumulative curve for syllables approaches an asymptote suggests that I sampled most, if not all, current variants in the population (Fig. 6).

4. Discussion

Anna’s hummingbird males have remarkably complex learned songs that are sung from breeding territories during the species’ January-June breeding season. I sampled male songs from a single breeding population in Golden Gate Park with the goals of cataloguing acoustic diversity and evaluating spatial and temporal effects on acoustic variation. I identified 25 syllables and 11 song types, and found that both were affected by inter-male distances such that males with adjacent territories were more similar in syllable and song type usage than were males with more distant territories. The high turnover of syllable types between 1999 and 2021 in Golden Gate Park is indicative of rapid cultural evolution of song in a single population. I consider the potential function and social mechanism of song sharing in Anna’s hummingbird, and discuss evidence for contrasting patterns of rapid cultural evolution and stability of Anna’s song.

Why and how do male Anna's sing their neighbors' songs?

Song sharing has proposed adaptive functions in territorial defense (Krebs et al., 1981; Stoddard et al., 1991; Stoddard, 1996) and reproduction (Payne, 1982; Payne et al., 1988). Shared song types may, for example, facilitate neighbor recognition, allowing reduced aggression between neighbors holding established territories (the 'dear enemy' effect; Fisher, 1954; Temeles 1994; Jaska et al., 2015). In birds with repertoires of song types and syllables, counter-singing matched song types can also be used to indicate threat and escalate agonistic interactions (Bertram, 1970; Beecher et al., 2000a; Anderson et al., 2005, Beecher and Campbell, 2005).

Anna's hummingbirds are extremely aggressive and territorial and engage in frequent high-speed chases with conspecific males and other sympatric hummingbird species (Pitelka, 1951). Fights are costly, taking time away from displaying and feeding (Powers, 1987; Stiles, 1982). I hypothesize that sharing song types with neighbors decreases the number of agonistic interactions between males on neighboring breeding territories. Testing this hypothesis will require playback experiments to determine whether shared and non-shared song types elicit different levels of aggressive response.

Song sharing is facilitated by song learning – the social process in which a young bird hears and memorizes a song template from an adult conspecific, practices singing to match their memorized template, and then crystalizes a song form. Birds have diverse song learning timelines that are broadly categorized as closed- or open-ended. Closed-ended song learners are classified as birds whose song repertoire is fixed by the end of their first year whereas open-ended song learners can add new variants and embellishments, including the songs of their neighbors, to their repertoire later in life (Lemon, 1968; McGregor and Krebs, 1989; Beecher and Brenowitz, 2005). The conditions under which song sharing can occur in closed-ended learners are more restrictive. Young males must either memorize multiple song types pre-dispersal and retain those that match their neighbors' on breeding territories, or learn songs and hold territories in the same location (Beecher et al., 1997; Payne, 1997; MacGregor and Krebs, 1989; Nelson, 2000). In hummingbirds, open-ended learning is well supported in the long-billed hermit (*Phaethornis longirostris*; Araya-Salas and Wright, 2013) and probable in Costa's hummingbird (*Calypte costae*; Johnson and Clark, 2020, 2022), the sister species to Anna's. However, where Anna's hummingbird falls on the song-learning timeline is currently uncertain. When raised in captivity without conspecific acoustic stimuli, an Anna's male sang incomplete songs with varied syntax and unique syllable types whereas males exposed to conspecific song as juveniles later produced songs comparable to wild-recorded males (Baptista and Schuchmann, 1990). As an adult, the male reared in acoustic isolation was temporarily housed with another adult male but did not modify his incomplete song to match that of his tutor (Baptista and Schuchmann, 1990). This experiment clearly demonstrates that, like most other song-learning birds, male Anna's hummingbirds need to hear their species' song as fledglings in order to produce fully formed songs as adults (Marler, 1987; Baptista and Schuchmann, 1990; Beecher and Brenowitz, 2005). However, whether the lack of change in the acoustically isolated male's song following adult exposure to a conspecific male supports closed-ended learning in Anna's is uncertain. Because the period of exposure was brief (1 month), the potential for seasonal variation in male Anna's capacity to modify song was not taken into account (Baptista and Schuchmann, 1990). Moreover, acoustic isolation during the stage when song templates are initially formed might disable the capacity to modify song in later life (Chaiken and Bohner, 2007). Thus, a more complete understanding of the social mechanism of song sharing in Anna's awaits long term studies of the ontogeny and social context of song acquisition in this species.

4.1. Why and How do Male Anna's Sing Their Neighbor's Songs?

Song sharing has proposed adaptive functions in territorial defense (Krebs et al., 1981; Stoddard et al., 1991; Stoddard, 1996) and reproduction (Payne, 1982; Payne et al., 1988). Shared song types may, for example, facilitate neighbor recognition, allowing reduced aggression between neighbors holding established territories (the 'dear enemy' effect; Fisher, 1954; Temeles 1994; Jaska et al., 2015). In birds with repertoires of song types and syllables, counter-singing matched song types can also be used to indicate threat and escalate agonistic interactions (Bertram, 1970; Beecher et al., 2000a; Anderson et al., 2005, Beecher and Campbell, 2005).

Anna's hummingbirds are extremely aggressive and territorial and engage in frequent high-speed chases with conspecific males and other sympatric hummingbird species (Pitelka, 1951). Fights are costly, taking time away from displaying and feeding (Powers, 1987; Stiles, 1982). I hypothesize that sharing song types with neighbors decreases the number of agonistic interactions between males on neighboring breeding territories. Testing this hypothesis will require playback experiments to determine whether shared and non-shared song types elicit different levels of aggressive response.

Song sharing is facilitated by song learning – the social process in which a young bird hears and memorizes a song template from an adult conspecific, practices singing to match their memorized template, and then crystalizes a song form. Birds have diverse song learning timelines that are broadly categorized as closed- or open-ended. Closed-ended song learners are classified as birds whose song repertoire is fixed by the end of their first year whereas open-ended song learners can add new variants and embellishments, including the songs of their neighbors, to their repertoire later in life (Lemon, 1968; McGregor and Krebs, 1989; Beecher and Brenowitz, 2005). The conditions under which song sharing can occur in closed-ended learners are more restrictive. Young males must either memorize multiple song types pre-dispersal and retain those that match their neighbors' on breeding territories, or learn songs and hold territories in the same location (Beecher et al., 1997; Payne, 1997; MacGregor and Krebs, 1989; Nelson, 2000).

In hummingbirds, open-ended learning is well supported in the long-billed hermit (*Phaethornis longirostris*; Araya-Salas and Wright, 2013) and probable in Costa's hummingbird (*Calypte costae*; Johnson and Clark, 2020, 2022), the sister species to Anna's. However, where Anna's hummingbird falls on the song-learning timeline is currently uncertain. When raised in captivity without conspecific acoustic stimuli, an Anna's male sang incomplete songs with varied syntax and unique syllable types whereas males exposed to conspecific song as juveniles later produced songs comparable to wild-recorded males (Baptista and Schuchmann, 1990). As an adult, the male reared in acoustic isolation was temporarily housed with another adult male but did not modify his incomplete song to match that of his tutor (Baptista and Schuchmann, 1990). This experiment clearly demonstrates that, like most other song-learning birds, male Anna's hummingbirds need to hear their species' song as fledglings in order to produce fully formed songs as adults (Marler, 1987; Baptista and Schuchmann, 1990; Beecher and Brenowitz, 2005). However, whether the lack of change in the acoustically isolated male's song following adult exposure to a conspecific male supports closed-ended learning in Anna's is uncertain. Because the period of exposure was brief (1 month), the potential for seasonal variation in male Anna's capacity to modify song was not taken into account (Baptista and Schuchmann, 1990). Moreover, acoustic isolation during the stage when song templates are initially formed might disable the capacity to modify song in later life (Chaiken and Bohner, 2007). Thus, a more complete understanding of the social mechanism of song sharing in Anna's awaits long term studies of the ontogeny and social context of song acquisition in this species.

4.3. Contrasting Patterns of Stability and Lability in Anna's Song

I found differences in the rate of cultural evolution in Anna's song, both across time periods, and within the song itself. Specifically, A syllable types were largely conserved between 1985 and 1999 but underwent high turnover between 1999 and 2021. The very limited sample size from 1985 notwithstanding, the apparent complete overlap in syllable types between 1985 and 1999 demonstrates that multiple syllable types were stable across this time period. Neither syllable stability nor high rates of turnover are surprising; both patterns are common in longitudinal studies of oscine birdsong (e.g. Payne, 1985, Sorjonen, 1987; Trainer, 1983; Harbison et al., 1999). However, why the rate of cultural evolution of song should be so different across comparable time periods (14 and 22 years) is unclear. As a large protected green space in an urban area, I would expect the biotic and acoustic environment of Golden Gate Park to be stable over recent decades and thus not have significantly different sound transmission or social interaction characteristics.

I also found substantial change in the structure of phrase B. Whereas all males recorded in 1985 and 1999 had two syllables in phrase B, all birds recorded in 2021 had three. The third syllable in phrase B (B3) shares characteristics with the end of phrase B in 1985 and 1999 songs but begins with a broadband note and a clear inter-syllable gap. Interestingly, this change to phrase B in the Golden Gate Park Anna's population is ubiquitous in recordings collected during the 2020 and 2021 breeding seasons from five other populations across the Western United States (Berger et al., 2023). If the structural change to phrase B arose after 1999, such rapid spread of the new three-syllable variant would require strong positive selection, perhaps due to female preference. Alternatively, the two-syllable phrase B from earlier recordings at Golden Gate Park was a local variant that disappeared from that population some time after 1999. Discriminating between these possibilities will require evaluation of the structure of phrase B in earlier recordings from populations across the species' range.

Interestingly, birds from all three time points sang the same version of syllable C1. The stability of C1 relative to the rest of the song is indicative of a constraint on variation, possibly because this syllable signals species identity. In some species, songs are hypothesized to segregate into elements containing information of varying specificity, such as individual, population, or species identity (Marler, 1960; Brenowitz, 1982; Elfström, 1990; Nelson and Poesel, 2007). Individual identity signals have high intraspecific variation in both song form and fine temporal structure (Katharina and Nieder, 2020; Pruchová et al., 2017; Prior et al., 2018), whereas signals of species or population identity have less variation and are less labile (Marler, 1960). For example, in white-crowned sparrows (*Zonotrichia leucophrys*), introductory whistles did not vary geographically as compared to the other phrases in the song and were stable over a 30-year period (Harbison et al., 1999; Nelson and Poesel, 2007). In our study, the high diversity of A syllables and the structural change in B syllables suggest that the first part of the song encodes individual or song neighborhood identity information, whereas the C phrase may signal species identity.

5. Conclusion

I show that Anna's hummingbirds exhibit song sharing and that common song types are loosely organized into song neighborhoods. I find evidence for rapid syllable turnover in the first and most variable phrase of Anna's song, and contrasting stability in the terminal phrase. These results motivate future work on the function of song sharing in Anna's, and the information content of the species' multi-phrase song. As a species with a complex song that is invariant within individual, Anna's is just one of over 300 hummingbird species whose diverse vocalizations range from no song, to simple songs, to large individual repertoires of complex songs (Monte et al., 2023). Such diversity of learned song makes hummingbirds a fascinating clade for comparative analysis of the evolution of song structure and complexity.

References

Anderson, R. C., Searcy, W. A., & Nowicki, S. (2005). Partial song matching in a eastern population of song sparrows, *Melospiza melodia*. *Animal Behaviour*, *69*(1), 189-196.

Araya-Salas, M., Smith-Vidaurre, G., Mennill, D. J., González-Gómez, P. L., Cahill, J., & Wright, T. F. (2019). Social group signatures in hummingbird displays provide evidence of co-occurrence of vocal and visual learning. *Proceedings of the Royal Society B: Biological Sciences, 286*(1903), 20190666.

Araya-Salas, M., & Wright, T. (2013). Open-ended song learning in a hummingbird. *Biology Letters*, *9*(5), 20130625.

Baker, M. C., & Cunningham, M. A. (1985). The biology of bird-song dialects. *Behavioral and Brain Sciences, 8*(1), 85-100.

Baptista, L. F., & Schuchmann, K.-L. (1990). Song learning in the Anna hummingbird (*Calypte anna*). *Ethology*, *84*(1), 15–26.

Beecher, M. D., & Brenowitz, E. A. (2005). Functional aspects of song learning in songbirds. *Trends in Ecology and Evolution*. *20*(3), 143-149.

Beecher, M. D., Campbell, S. E., Burt, J. M., Hill, C. E., & Nordby, J. C. (2000). Song type matching between neighbouring song sparrows. *Animal Behaviour*, *59*(1), 21–27.

Beecher, M. D., & Campbell, S. E. (2005). The role of unshared songs in singing interactions between neighbouring song sparrows. *Animal Behaviour*, *70*(6), 1297–1304.

Beecher, M. D., Nordby, J. C., Campbell, S. E., Burt, J. M., Hill, C. E., & O'Loghlen, A. L. (1997). What is the function of song learing in songbirds? *Perspectives in Ethology*, *12*, 77-97.

Beecher, M. D., Stoddard, P. K., Campbell, E. S., & Horning, C. L. (1996). Repertoire matching between neighbouring song sparrows. *Animal Behaviour*, *51*(4), 917–923.

Berger, A., Ye, W., Padilla, A., Gumbi, B., Clark, C., & Campbell, P. (2023). Geographic variation in the songs of two closely related song-learning species, Anna's and Costa's hummingbird (*Calypte anna, C. costae*). *bioRxiv* (2023.11.26.568754).

Bertram, L. F. (1970). The vocal behaviour of the Indian Hill Mynah, *Gracula relifgiosa*. *Animal Behavior, 3,* 79-192.

Brenowitz, E. A. (1982). Long-range communication of species identity by song in the Red-winged Blackbird. *Behavioral Ecology and Sociobiology, 10*, 29-38.

Chaiken, L. M., & Böhner, J. (2007). Song learning after isolation in the open-ended learner the European Starling: Dissociation of immitation and syntactic development. *The Condor*, *4*, 968-976.

Clark, C. J., & Feo, T. J. (2008). The Anna's hummingbird chirps with its tail: a new mechanism of sonation in birds. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1637), 955-962.

Clark, C. J., & Feo, T. J. (2010). Why do *Calypte* hummingbirds "sing" with both their tail and their syrinx? An apparent example of sexual sensory bias. *The American Naturalist*, *175*(1), 27–37.

Clark, C. J., & Russell, S. M. (2012). Anna's Hummingbird (Calypte anna). *Birds of North America*. https://doi.org/10.2173/bna.226.

DeVoogd, T. J., Krebs, J. R., Healy, S. D., & Purvis, A. (1993). Relations between song repertoire size and the volume of brain nuclei related to song: comparative evolutionary analyses amongst oscine birds. *Proceedings of the Royal Society B: Biological Sciences*, *254*(1340), 75-82.

Elfstrom, S. T. (1990). Responses of territorial Meadow Pipits to strange and familiar song phrases in playback experiments. *Animal Behavior, 40*(4), 786-788.

Ellers, J., & Slabbekoorn, H. (2003). Song divergence and male dispersal among bird populations: a spatially explicit model testing the role of vocal learning. *Animal Behaviour*, *65*(4), 671-681.

Ewert, D. N., & Kroodsma, D. E. (1994). Song sharing and repertoires among migratory and resident Rufous-sided Towhees. *Condor*, *96*, 190-196.

Fisher, J. (1954). Evolution and bird sociality. In J. Huxley, A. C., Hardy, & E. B., Ford (Eds.), *Evolution as a process* (pp 71-83). London: Allen & Unwin.

Gaunt, S. L. L., Baptista, L. F., Sánchez, J. E., & Hernandez, D. (1994). Song learning as evidenced from song sharing in two hummingbird species (*Colibri coruscans* and *C. thalassinus*). *The Auk*, *111*(1), 87–103.

González, C., & Ornelas, J. F. (2009). Song variation and persistence of song neighborhoods in a lekking hummingbird. *The Condor*, *111*(4), 633–640.

González, C., & Ornelas, J. F. (2014). Acoustic divergence with gene flow in a lekking hummingbird with complex songs. *PloS One*, *9*(10), e109241.

Hansen, P. (1979). Vocal learning: its role in adapting sound structures to long-distance propagation, and a hypothesis on its evolution. *Animal Behaviour*, *27*, 1270–1271.

Harbison, H., Nelson, D. A., & Hahn, T. P. (1999). Long-Term Persistence of Song Dialects in the Mountain White-Crowned Sparrow. *The Condor*, *101*(1), 133–148.

Hijmans, R. J., Karney, C., Williams, E., & Vennes, C. (2022). geosphere 1.5-18 https://github.com/rspatial/geosphere.

Hill, C. E., Campbell, S. E., Nordby, J. C., Burt, J. M., & Beecher, M. D. (1999). Song sharing in two populations of song sparrows (Melospiza melodia). *Behavioral Ecology and Sociobiology*, *46*(5), 341–349.

Hughes, M., Nowicki, S., Searcy, W. A., & Peters, S. (1998). Song-type sharing in song sparrows: implications for repertoire function and song learning. *Behavioral Ecology and Sociobiology*, *42*, 437-446.

Ince, S. A., Slater, P. J. B., & Weismann, C. (1980). Changes with time in the songs of a population of chaffinches. *The Condor*, *82*(3), 285.

Janik, V. M., & Knörnschild, M. (2021). Vocal production learning in mammals revisited. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *376*(1836), 20200244.

Janik, V. M., & Slater, P. J. B. (1997). Vocal learning in mammals. *Advances in the Study of Behaviour*, *26*, 59–100.

Jaška, P., Linhart, P. ,& Fuchs, R. (2015). Neighbour recognition in two sister songbird species with a simple and complex repertoire – a playback study. *Journal of Avian Biology*, *46,* 151-158.

Johnson, K. E., & Clark, C. J. (2020). Ontogeny of vocal learning in a hummingbird. *Animal Behaviour*, *167*, 139–150.

Johnson, K. E., & Clark, C. J. (2022). Costa's hummingbird has an extended sensitive phase of vocal learning. *Animal Behaviour*, *188*, 75–84.

Krebs, J. R., Ashcroft, R., & Van Orsdol, K. (1981). Song matching in the great tit *Parus major* L. *Animal Behaviour*, *29*(3), 918–923.

Kroodsma, D. E., Sanchez, J., Stemple, D. W., Goodwin, D., Da Silva, M. L, & Vielliard, J. M. (1999). Sedentary life style of Neotropical sedge wrens promotes song imiataion. *Animal Behaviour*, *57,* 855-863.

Lara, C., Martínez-García, V., & Ornelas, J. F. (2015). Microgeographical Variation in Song Repertoire and Structure between the Leks of Green Violetears *Colibri thalassinus* in Central Mexico. *Acta Ornithologica*, *50*(1), 23–32.

Lemon, R. E. (1968). The relation between organization and function of song in cardinals. *Behaviour*, *32*(1–3), 158–177.

Lynch, A. (1996). The population memetics of birdsong. *Ecology and evolution of acoustic communication in birds* (D. E. Kroodsma, and E. H. Miller, Eds.) Cornell University Press, New York, 181-197.

Marler, P. (1960). Bird songs and mate selection. *In:* Lanyon, W. E., and Tavolga, W. N. eds. *Animal Sounds and Communication,* 7: 348-367. American Institute of Biological Sciences, Washington, DC.

Marler, P., & Peters, S. (1987). A sensitive period for song acquisition in the song sparrow, *Melospiza melodia*: A case of age-limited learning. *Ethology*, *76*(2), 89–100.

Marler, P., & Tamura, M. (1962). Song "dialects" in three populations of white-crowned sparrows. *The Condor*, *64*(5), 368–37.

McGregor, P. K., & Krebs, J. R. (1989). Song learning in adult great tits (*Parus major*): Effects of neighbours. *Behaviour*, *108*(1-2), 139–159.

McGregor, P. K., & Thompson, D. B. A. (1988). Constancy and Change in Local Dialects of the Corn Bunting. *Ornis Scandinavica*, *19*(2), 153–159.

Monte, A., da Silva, M. L., & Gahr, M. (2023). Absence of song suggests heterogeneity of vocal-production learning in hummingbirds. *Journal of Ornithology*, *164*(3), 721–727.

Nelson, D. A. (2000). Song overproduction, selective attrition and song dialects in the white-crowned sparrow. *Animal Behaviour*, *60*(6), 887-898.

Nelson, D. A., & Poesel, A. (2007). Segregation of information in a complex acoustic signal: individual and dialect identity in white-crowned sparrow song. *Animal Behaviour*, 74(4), 1073-1084.

Nordby, J. C., Campbell, S. E., & Beecher, M. D. (2002). Adult Song Sparrows do not Alter their Song Repertoires. *Ethology*, *108*(1), 39–50.

Nottebohm, F. (1972). The origins of vocal learning. *The American Naturalist*, *106*(947), 116–140.

Payne, R. B. (1981). Song learning and social interaction in indigo buntings. *Animal Behaviour*, *29*(3), 688–697.

Payne, R. B. (1982). Ecological consequences of song matching: Breeding success and intraspecific song mimicry in indigo buntings. *Ecology*, *63*(2), 401–411.

Payne, R. B. (1985). Behavioral continuity and change in local song populations of village indigobirds *Vidua chalybeata*. *Zeitschrift Für Tierpsychologie*, *70*(1), 1–44.

Payne, R. B., & Payne, L. L. (1997). Field observations, experimental design, and the time and place of learning bird songs. *Social influences on vocal development*. 57-84.

Payne, R. B., Payne, L. L., & Doehler, M. S. (1988). Biological and cultural success of song memes in Indigo Buntings. *Ecology*, *69*, 104-117.

Payne, R. B., & Westneat, D. F. (1988). A genetic and behavioral analysis of mate choice and song neighborhoods in Indigo Buntings. *Evolution*, *42*(5), 935–947.

Pitelka, F. A. (1951). Ecological overlap and interspecific strife in breeding populations of Anna and Allen hummingbirds. *Ecology*, *32*, 641-661.

Podos, J., Peters, S., Rudnicky, T., Marler, P., & Nowicki, S. (1992). The organization of song repertoires in song sparrows: Themes and variations. *Ethology*, *90*(2), 89–106.

Potvin, D. A., & Clegg, S. M. (2015). The relative roles of cultural drift and acoustic adaptation in shaping syllable repertoires of island bird populations change with time since colonization. *Evolution, 69(2), 368-380.*

Powers, D. R. (1987). Effects of variation in food quality on the breeding territoriality of the male Anna’s hummingbird. *The Condor*, *89*(1), 103.

Searcy, W. A., Marler, P., & Peters, S. S. (1985). Songs of isolation-reared sparrows function in communication, but are significantly less effective than learned songs. *Behavioral Ecology and Sociobiology*, *17*(3), 223–229.

Slater, P. J. B. (1989). Bird song learning: causes and consequences. *Ethology Ecology & Evolution*, *1*(1), 19–46.

Snow, D. W. (1968). The singing assemblies of Little Hermits. *The Living Bird,* 7(1), 47 55.

Snow, B. K. (1974). Lek Behaviour and Breeding of Guy's Hermit hummingbird *Phaethornis Guy*. *The Ibis*, *116*(3), 278-297.

Snow, B. K. (1973). The Behavior and Ecology of Hermit Hummingbirds in the Kanaku Mountains, Guyana. *The Wilson Bulletin*, *85*(2), 163–177.

Sorjonen, J. (1987). Temporal and spatial differences in traditions and repertoires in the song of the thrush nightingale (Luscinia Luscinia). *Behaviour*, *102*(3–4), 196–211.

Stiles, F. G. (1982). Aggressive and Courtship Displays of the Male Anna's Hummingbird. *The Condor*, *84*(2), 208–225.

Stiles, F. G., & Wolf, L. L. (1973). Techniques for color-marking hummingbirds. *The Condor*, *75*(2), 244–245.

Stiles, F. G., & Wolf, L. L. (1979). Ecology and Evolution of Lek Mating Behavior in the Long-Tailed Hermit Hummingbird. *Ornithological Monographs*, *27*, iii–78.

Stoddard, P. K., Beecher, M. D., Horning, C. L., & Campbell, S. E. (1991). Recognition of individual neighbors by song in the song sparrow, a species with song repertoires. *Behavioral Ecology and Sociobiology*, *29*(3), 211–215.

Stoddard, P. (1996). Vocal recognition of neighbors by territorial passerines. *Ecology and Evolution of Acoustic Communications in Birds,* 356-374.

Temeles, E. J. (1994). The role of neighbours in territorial systems: When are they "dear enemies"? *Animal Behaviour, 47*(2), 339–350.

Trainer, J. M. (1983). Changes in song dialect distributions and microgeographic variation in song of White-crowned Sparrows *Zonotrichia leucophrys nutatalli*, *Auk*, *100*, 190-204.

Trainer, J. M. (2010). Cultural evolution in song dialects of yellow-rumped caciques in panama. *Ethology*, *80*(1–4), 190–204.

Tsipoura, N., & Morton, E. S. (1988). Song-Type Distribution in a Population of Kentucky Warblers. *The Wilson Bulletin*, *100*(1), 9–16.

Tyack, P. (2019). Communication by Sound and by Visual, Tactile, and Chemical Sensing. In B. Würsig (Ed.), *Ethology and Behavioral Ecology of Odontocetes* (pp. 25 50). Springer International Publishing.

Wells, S., Bradley, R. A., & Baptista, L. F. (1978). Hybridization in *Calypte* Hummingbirds. *The Auk*, *95*(3), 537–549.

Wiley, R. H. (1971). Song Groups in a Singing Assembly of Little Hermits. *The Condor*, *73*(1), 28–35.

Yang, X., Lei, F., Wang, G., & Jesse, A. J. (2007). Syllable sharing and inter-individual syllable variation in Anna's hummingbird *Calypte anna* songs, in San Francisco, California. *Folia Zoologica*, *56*(3), 307-318.

Table 3.1. Spectral and temporal measures of Anna's hummingbird song.

	Measurements
Phrase A	
A1	A1 Duration (seconds)
	A1 Peak frequency (kHz)
A2	A2 Duration (seconds)
	A2 Peak frequency (kHz)
A3	A3 Duration (seconds)
	A3 Peak frequency (seconds)
Phrase B	
B1	B1 Peak frequency (kHz)
	B1 Duration (seconds)
	Upper whistle (kHz)
B2	B2 Peak frequency (kHz)
	B2 Duration (seconds)
B3	B3 Peak frequency (kHz)
	B3 Duration (seconds)
	Peak frequency pulses (kHz)
	Duration pulses (seconds)
	Number of pulses (#)
	Pulse rate (# pulses/seconds)
Phrase C	
C1	Peak frequency (kHz)
	Duration (seconds)
	Peak frequency lower tone (kHz)
	Duration lower tone (seconds)
	Peak frequency upper tone (kHz)
	Duration upper tone (seconds)

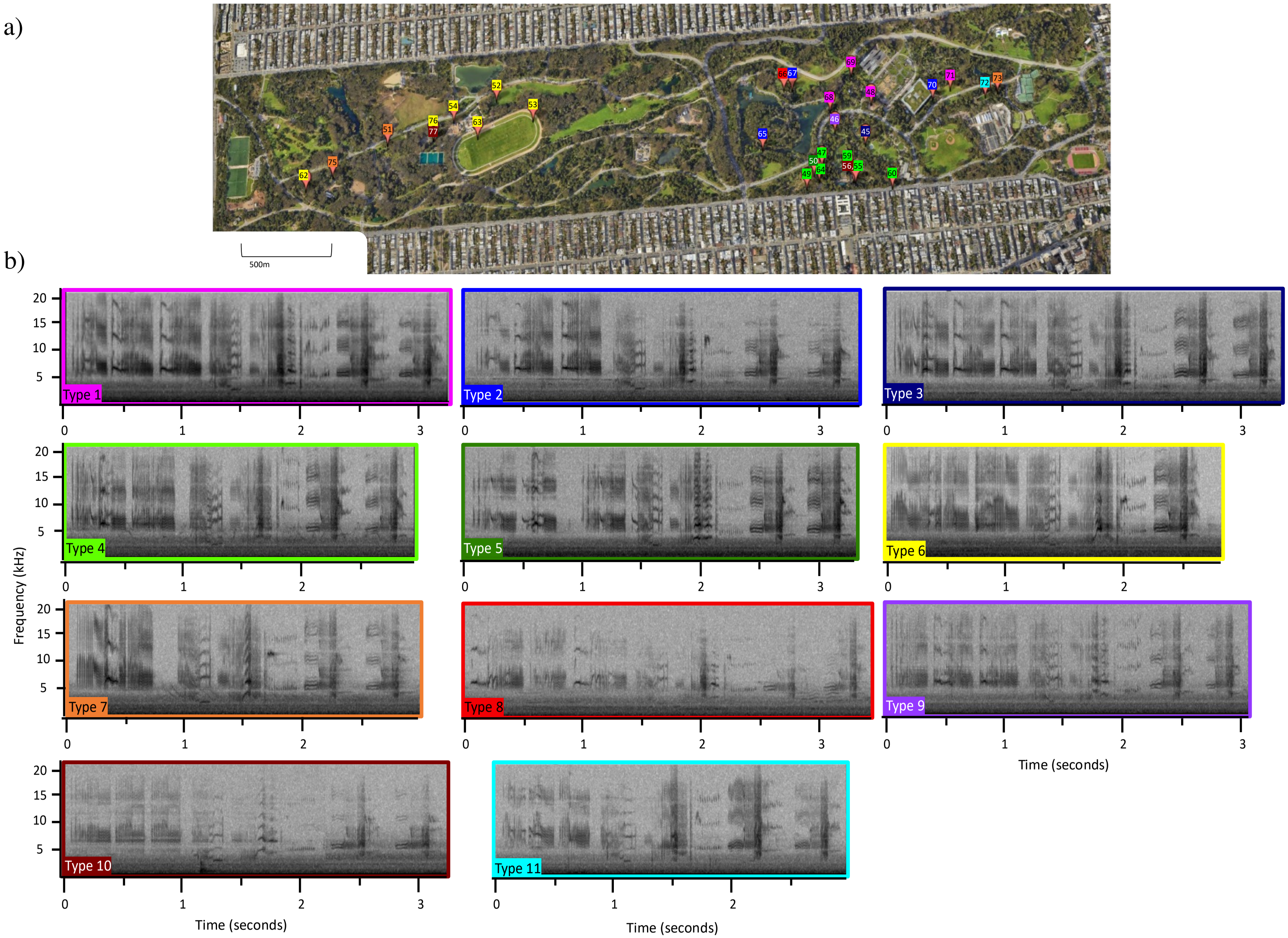

a)
62
75
51
76
77
54
63
52
53
65
66
67
69
68
48
46
45
47
50
49
64
59
56,
55
60
70
71
72
73
500m
b)
Type 1
Type 2
Type 3
Type 4
Type 5
Type 6
Type 7
Type 8
Type 9
Type 10
Type 11
20
15
10
5
0
1
2
3
Frequency (kHz)
Time (seconds)

Figure 3.1. Recording locations and song types of Anna's hummingbird males in Golden Gate Park. a) Locations of 29 males, colored by song type. Individual ID's are indicated by numbered square (45-77). b) Representative spectrograms for 11 song types. Map from Google Earth. Spectrograms generated in Audacity version 3.3. 1 using a 512 FFT smooth Hamming window.

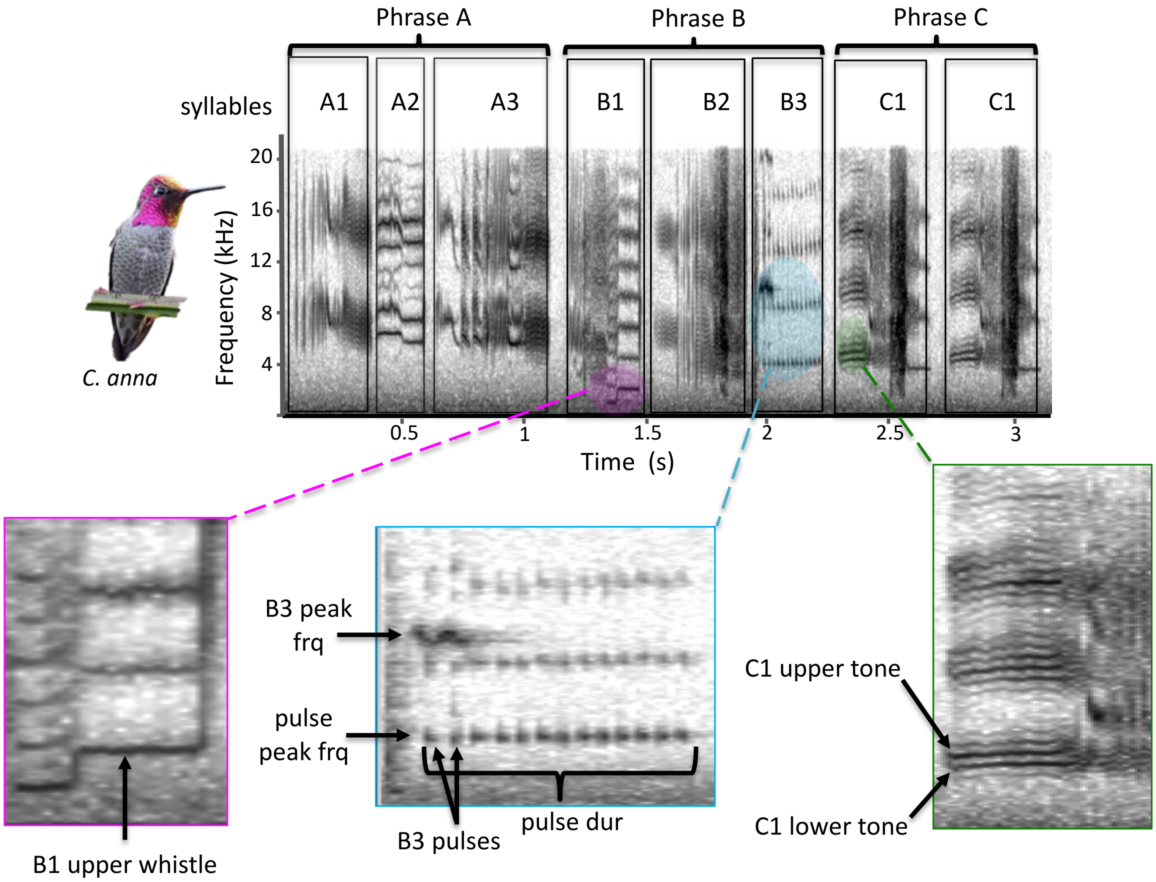


Figure 3.2. Anna's hummingbird song and spectral and temporal measurements. Anna's hummingbirds sing a complex three phrase (A-C) song with multiple syllables (A1-A3, B1-B3, C1). Peak frequency and duration was taken for full syllables (A1-C1), and for each of the highlighted elements (B1 upper whistle, B3 pulses, C1 upper tone, and C1 lower tone); pulse number and rate were measured for B3. Images, Steven Mlodinow. Spectrograms and measurements generated in Raven Pro v1.6 (Cornell Lab of Ornithology, Cornell University, Ithaca, NY, USA) using a 512 FFT smooth Hamming window.

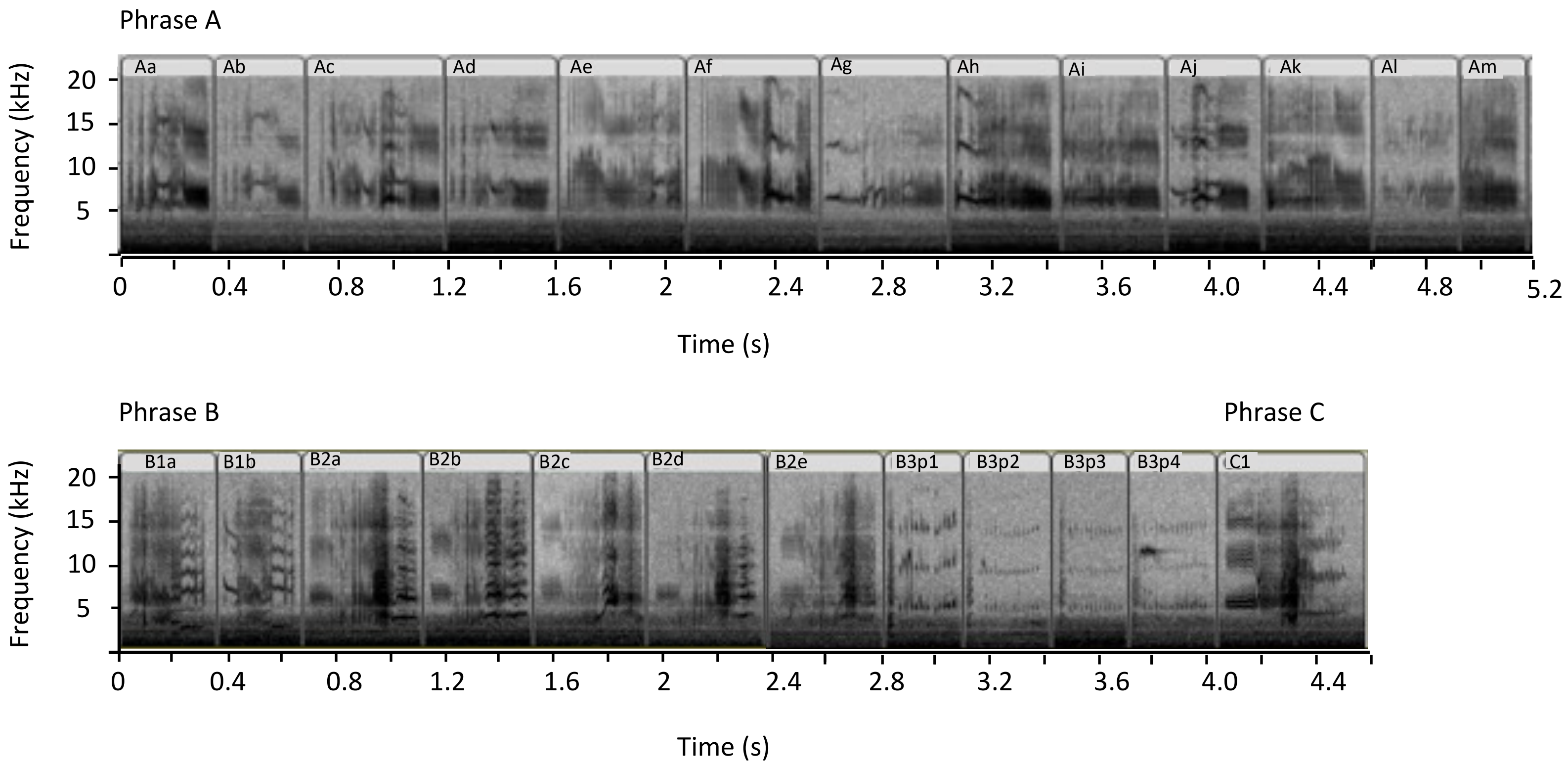


Figure 3.3. Anna's hummingbird syllable types. 25 syllable types were identified among 29 individuals sampled. Spectrograms generated in Audacity version 3.3.1 using a 512 FFT smooth Hamming window.

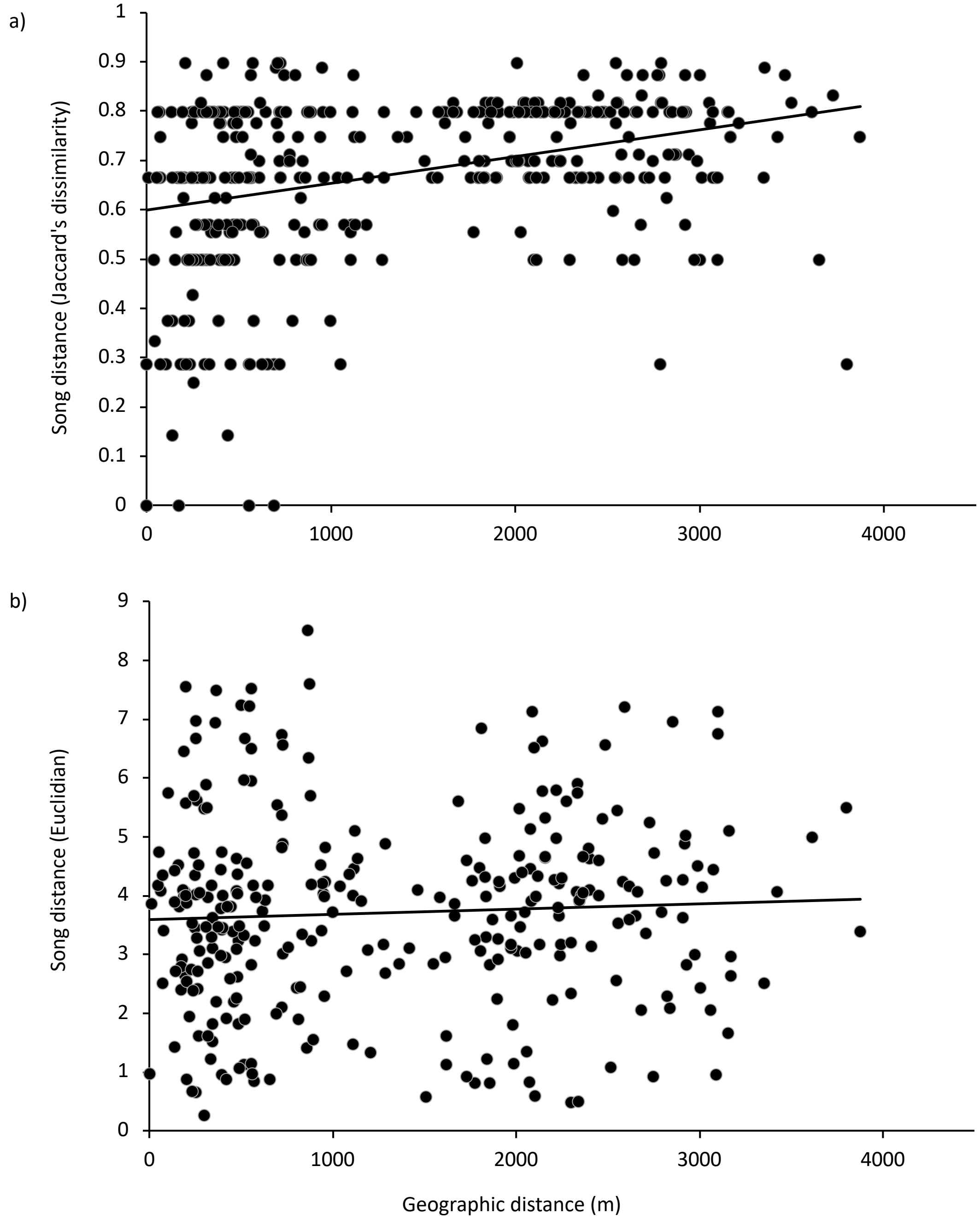


Figure 3.4. Effect of physical distance on song distance for a) syllable use (Mantel, $r = 0.32$, $r^2 = 0.108$, $P < 0.0001$) and b) spectral and temporal components (Mantel, $r = 0.056$, $r^2 = 0.0031$, $P = 0.328$). Physical distance calculated as linear distance in meters between each individual. Song distance of syllable use calculated as Jaccard distance; song distance of spectral and temporal components calculated as Euclidian distance.

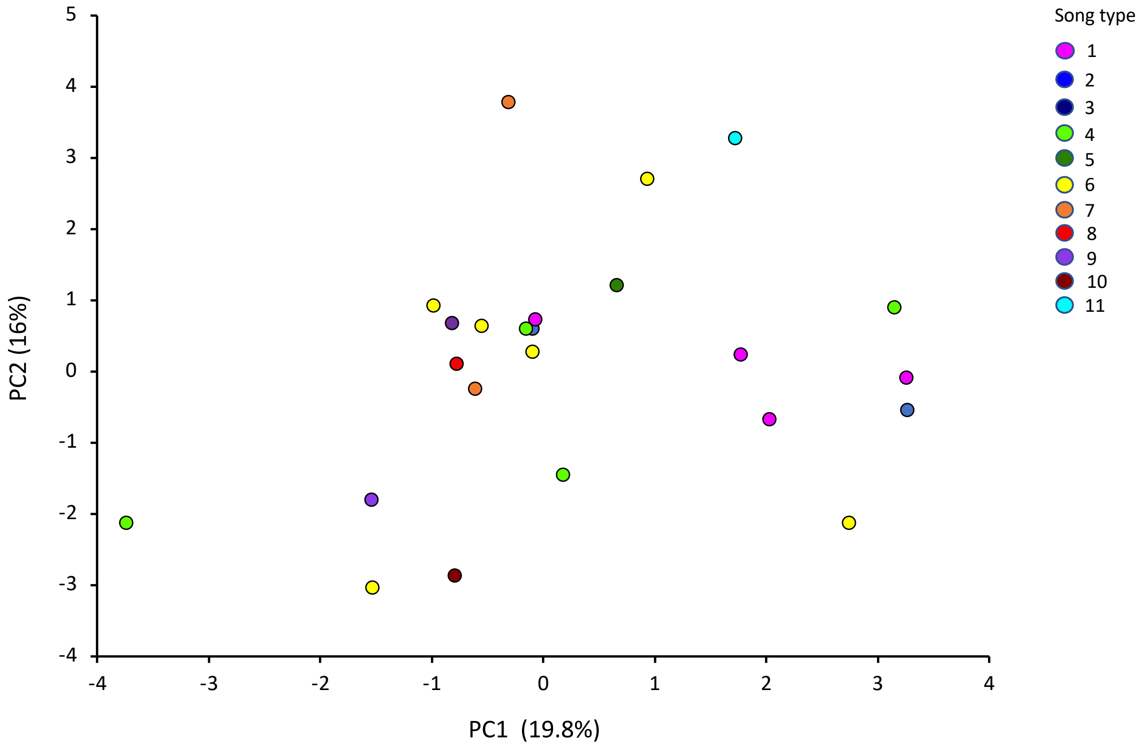


Figure 3.5. Principle component analysis of spectral and temporal measures of Anna's hummingbird song. Birds (*n* = 29) are colored by their assigned song type. Duration is negatively loaded on PC1, and frequency is negatively loaded on PC2.

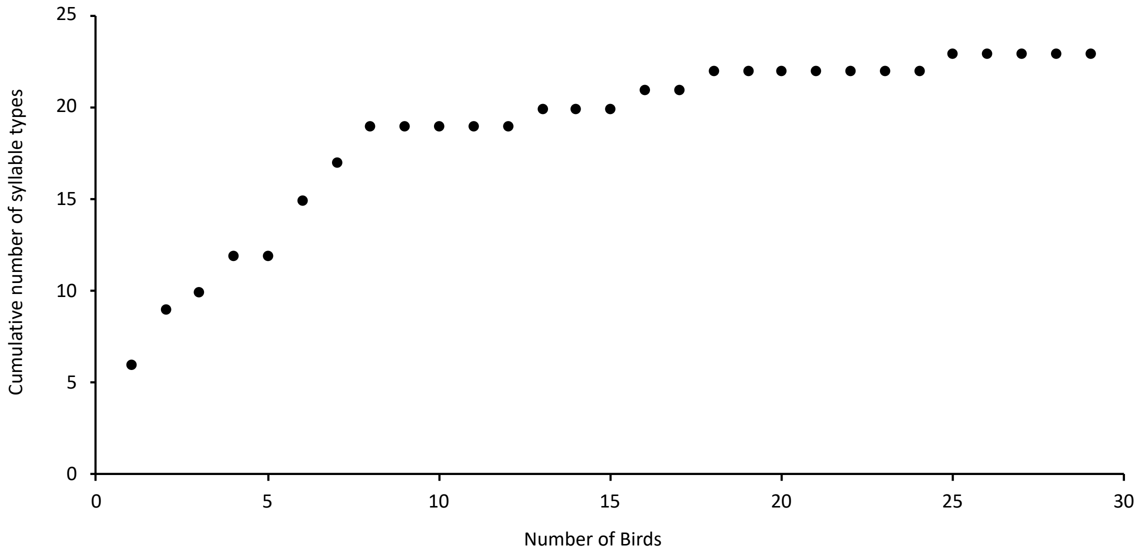


Figure 3.6. Cumulative number of syllable types for 29 Anna's hummingbirds sampled. Count of animals that behaved parentally, attacked, or behaved neutrally (e.g., neither parentally nor attacked) during the parental-behavior test.