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Vocal Learning in the Costa's Hummingbird

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May you all enjoy your retirements in San Diego and Florida!

ABSTRACT OF THE DISSERTATION

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Hummingbirds represent one of three avian groups that have the ability to vocal learn, and yet they have received very little attention. Here I examine vocal learning in the Costa's hummingbird. I used the standard isolation experiment technique utilized for decades to study vocal learning in songbirds. I raised young male Costa's hummingbirds in isolation, with playback of adult song, and with playback in the presence of an adult model. I recorded all of the songs produced by the birds to examine song ontogeny. I found the necessary conditions required for vocal learning of a tutor song to take place in a lab setting, which include the presence of an adult model during tutoring and playback of Costa's like tutor songs. I developed a timeline of song ontogeny events including information about the onset of singing, and the stages of song development. Another experiment examined the timing of the sensitive phase by tutoring Costa's for 20 hours in one of three different 30-day tutoring periods (Early, Mid, and Late). Results indicate that Costa's can learn some vocal information across all three tutoring periods but that the amount of tutoring during one test period was insufficient for production of normal Costa's song. Finally, I examined open-ended vocal learning by exposing previously isolated birds to other Costa's hummingbirds and found that songs change after exposure, even after one or two years of isolation.

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Introduction:

Vocal learning is observed in only a few groups of mammals including humans, and three groups of birds: songbirds, parrots, and hummingbirds (Nottebohm, 1972). Vocal learning is a type of social learning through which an organism can "modify vocal production as a result of auditory experience" (Marler, 1976). Non-vocal learners cannot change their own vocalizations based on what they hear; instead, they inherit their vocalizations (Kroodsma & Konishi, 1991; Marler & Slabbekoorn, 2004). In birds, the most common learned vocalization is song. Bird songs differ greatly both in length and complexity but are unified by function in that song is most commonly used for mate attraction and male-male interactions (Catchpole & Slater, 2003).

It has been proposed for decades that hummingbirds are vocal learners (Nottebohm, 1972), and yet our knowledge of the mechanism and intricacies of hummingbird vocal learning are almost non-existent. The purpose of this dissertation is to examine vocal learning in Costa's hummingbird, *Calypte costae*. Currently, there are fewer than a dozen papers showing indirect evidence of vocal learning in hummingbirds, usually through the presence of regional dialects. Additionally, there is an experimental study on vocal learning in the Anna's hummingbird with a sample size of four (Baptista & Schuchmann, 1990), another looking at syllable sharing and inter-individual song variation in Anna's hummingbirds (Yang, Lei, Wang, & Jesse, 2007), a study looking at variation in adult Costa's hummingbird song (Williams & Houtman, 2008), and a study reporting open-ended song learning in the long-billed hermit (Araya-Salas & Wright, 2013). Many aspects of vocal learning in hummingbirds have yet to be explored.

Moreover, in order to achieve a more complete understanding of vocal learning in the avian lineage, additional data on hummingbirds are necessary.

Broader Implications of Vocal Learning: Vocal learning is complex behavior that requires multiple brain regions devoted to memorizing vocalizations, comparing sensory input to motor output, and fine control of both vocal musculature and the breathing apparatus to name a few (Marler & Slabbekoorn, 2004). Behaviors like vocal learning are often studied as models of the evolution of complex traits. Vocal learning is of particular interest because it is central to language acquisition in humans. Infants learn language by hearing other humans talk. A baby starts to mimic the sounds it hears and eventually learns to produce the words and phrases a mature human can. This process takes close to three years in most children (Doupe & Kuhl, 1999). The question of how humans evolved such a complex language system is a mystery. Chimpanzees do not have the ability to vocalize in the complex way humans do. That is not say that they do not vocalize; there is evidence that chimpanzees and a few other non-human primates may vocally learn (Margoliash & Tchernichovski, 2015; Marshall, Wrangham, & Arcadi, 1999). However, the sheer complexity of human vocalizations and in turn the learning process is unmatched by any other group of save the vocal learning birds. As Darwin explained in *The Descent of Man, and Selection in the Relation to Sex*, “the sounds uttered by birds offer in several respects the nearest analogy to language” (Darwin, 1871).

Several important similarities in bird song and human language acquisition make birds a good model for studying vocal learning. For example, both humans and birds can vocalize from a very early age. Monitoring vocalizations from the very first utterances of a chick to the fully mature song of an adult allows for an in-depth examination of vocal learning ontogeny. Birds and humans also have similarities in the brain regions involved in vocal learning. Jarvis (2004) examined brain regions associated with vocal learning in humans, songbirds, parrots, and hummingbirds. In the three bird groups, Jarvis found "seven similar but not identical cerebral vocal nuclei distributed into two vocal pathways, one posterior and one anterior" (Jarvis, 2004). Jarvis also found brain similarities between the birds and humans. These specialized brain regions are not found in non-vocal learning birds and mammals (Jarvis, 2004). Humans, songbirds, parrots, and hummingbirds represent at least three and possibly four potentially independent acquisitions of vocal learning and yet the similarities among the brains are striking. Jarvis states that the similar brain structures across divergent groups represent strong "constraints of a pre-existing basic neural network of the vertebrate brain" (Jarvis, 2004). The brain regions are not homologous; nonetheless, they function in nearly the same way. Lastly, there appears to be convergent evolution of a gene, FoxP2, that is found in both humans and vocal learning birds and appears to have a direct role in vocal control, coordination, and learning (Fitch & Mietchen, 2013). Consequently, the evolution of vocal learning in these groups took similar yet independent paths. Therefore, this convergent evolution offers a strong line of evidence for the use of both songbirds and hummingbirds as model systems for studying vocal learning.

Songbirds have been the model organism of choice for studying vocal learning since the inception of the vocal learning field in the 1950s. Because hummingbirds evolved vocal learning independently of songbirds (Jarvis, 2004), they warrant examination. The last common ancestor of the hummingbird and the songbird/parrot lineage is believed to have lived during the Upper Cretaceous more than 60 million years ago, and no intermediate birds are known to be vocal learners, therefore, vocal learning is likely not the ancestral state (Prum et al., 2015). Therefore, the evolution of hummingbird vocal learning seems likely to be separate and distinct from that of the songbird/parrot linage.

Vocal Learning Terminology: Vocal learning is full of field-specific terms that need to be defined. For vocal learning birds, there are two major stages of learning, the **sensory period** and the **sensory-motor period** (Figure 1). The **sensory period** is the stage when vocal learning birds listen to and memorize their species-specific song. Associated with the **sensory period** is the **sensitive phase/period** of vocal learning (Figure 1). The **sensitive phase** is a period in the development of song, that if missed or postponed, can completely disrupt the vocal learning process and result in abnormal vocalizations (Kroodsma, 1978). During the **sensitive phase**, regions of the brain associated with vocal learning are altered, auditory and motor nuclei are organized, and neural connections that will enable the bird to memorize and learn to sing are being forged. The **sensitive phase** overlaps with the **sensory period** for all vocal learners, however the **sensitive phase** may or may not overlap with the next learning stage, the **sensory-motor period** (Eales, 1985; Marler & Peters, 1987).

The **sensory-motor period** is characterized by the commencement of singing (Figure 1). During this time, the baby bird learns to use its vocal apparatus. The bird learns to match the motor output i.e. the vocalizations, with the memory of the song from the **sensory period** (Marler, 1970). Throughout the **sensory-motor period** there are three major stages of development based on what the song resembles. The **subsong** is the first stage of the **sensory-motor period**(Marler & Slabbekoorn, 2004). The **subsong** is often very different from the adult song, containing wide frequency modulations and no apparent rhyme or reason to the sounds produced. During the next stage, the **plastic song**, the vocalizations are starting to shape into the adult song. There are hints of the adult song woven into the baby's vocalizations. As the bird practices its song, and matches its vocalizations to its memorized song, the adult song begins to emerge. The final song stage is the **crystalized song** (Marler & Slabbekoorn, 2004). The **crystalized song**, or **adult song**, can remain unchanged throughout the life of the bird, or can change from year to year. Birds that sing only the song they crystallize on in their first year of life are considered **closed-ended** (Marler & Slabbekoorn, 2004). Other birds can alter their song from year to year and are considered **open-ended learners**. Each year the bird will have an opening of the **sensitive phase**, during which time the bird can learn new syllables or songs to incorporate into its repertoire.

One unifying theme of songbird vocal learning is variation in almost every aspect of the learning process including, the timing of the sensory and sensory-motor periods, whether there is overlap between the sensory and the sensory-motor period, when learning commences and when it ends (sensitive phase) or if learning never ends (open-ended learning), the stages during the ontogeny of song, and if males and females sing or just males. The mechanisms of hummingbird vocal learning are almost completely unknown. Baptista and Schuchmann (1990), who reared four Anna's males in isolation, suggest that hummingbird vocal learning is similar to songbirds and that Anna's hummingbirds have an early sensitive period and are closed-ended learners (Baptista & Schuchmann, 1990b). Baptista and Schuchmann's results are the foundation of my work, but additional and more rigorous experiments are necessary to find where hummingbirds fall on the continuum of avian vocal learning. It is possible that hummingbird vocal learning is unique; however, it is also plausible that Costa's hummingbirds are similar to songbirds. My dissertation focuses on a few central topics of vocal learning: the presence or absence of vocal learning, song ontogeny, the sensitive phase of learning, and open-ended vs. closed-ended learning.

Evidence of Vocal Learning in Songbirds: Songbirds are arguably the most prolific singers of any animal clade and are the first group outside of humans to be used to examine vocal learning. Traditionally, the technique to test for vocal learning is the isolation experiment, which involves removing young birds from their natural environment, raising them in a controlled acoustic setting, and observing how (or if) song develops. Isolation experiments have been used for a variety of songbirds and their closest relatives the suboscines (Kroodsma & Konishi, 1991) to establish which species are vocal learners and which are not. If a bird, reared from a very young age in acoustic isolation, is able to produce a normal song, then the bird is thought to acquire its song innately (Marler, 1970). If, however, a bird raised in isolation develops an abnormal song it is possible that the bird is a vocal learner and additional experiments with playback and tutors can be used to confirm the hypothesis.

W.H. Thorpe's examination of chaffinch (*Fringilla coelebs*) vocal learning (Thorpe, 1958) and Peter Marler's studies of vocal learning in white-crowned sparrows (*Zonotrichia leucophrys*) gave the first direct evidence of vocal learning in birds. Marler and Tamura (1962) caught male white-crowned sparrows at different ages and examined song development. The birds were brought in at either three to fourteen or thirty-days post-hatching and then raised in captivity. Males caught between three and fourteen days developed abnormal songs while males taken in at thirty days produced normal songs. Marler and Tamura (1962) concluded that white-crowned sparrows are vocal learners and that there is a 'critical period' in song development. During the critical period, the young bird must hear the song of its species to develop a normal song. For white-crowned sparrows, this critical period is some time after fourteen days post-hatching but before thirty days old. Marler also examined vocal learning in the red-winged blackbird (*Agelaius phoeniceus*; Marler et al., 1972), the swamp sparrow (*Melospiza georgiana*; Marler & Peters, 1982), and the song sparrow (*Melospiza melodia*; Marler & Peters, 1988), finding vocal learning in these species as well.

In another important study, Chaiken, Böhner, and Marler (1993) examined vocal learning in the European starling (*Sturnus vulgaris*). In this experiment twenty-four male starlings were collected and put into different tutor regimes including live tutor (actual bird), tape tutor (song played on speaker), and no tutor. Both the live-tutored and tape-tutored starlings learned the normal song while the non-tutored males did not (Chaiken, Böhner, & Marler, 1993). This experiment shows the effectiveness of isolation experiments in their ability to provide information about the presence or absence of vocal learning as well as important information about the effectiveness of tutor regimes. Chapter 1 of my dissertation implements similar methods as Chaiken et al. Once it is established that a bird species is a vocal learner, more experiments can be done to reveal the different mechanisms of vocal learning.

Evidence of Vocal Learning in Hummingbirds: Some of the first evidence for vocal learning in hummingbirds came from studies describing song dialects in lekking species. Observations of dialects in singing assemblies were found in the green hermit (Phaethornis guy; Brewster & Chapman, 1895), reddish hermit (*Phaethornis ruber*; Davis, 1934), long-tailed hermit (*Phaethornis superciliosus*; Davis, 1934; Nicholson, 1931) and little hermit (*Phaethornis longuemareus*; Arp, 1957; Skutch, 1951). Snow (1968) and then Wiley (1971) further examined song groups in lekking little hermits of Trinidad. It was discovered that little hermits form song sharing groups, as "birds with neighboring perches tend to have similar songs" (Snow, 1968). Individuals within a group have much more similar songs to each other than birds from farther away (Wiley, 1971). While the studies never explicitly refer to vocal learning, they mention the idea of birds being able to imitate their neighbors.

Mirsky (1976) found indirect evidence of vocal learning in a population of Anna's hummingbirds (*Calypte anna*) on Guadalupe Island, which have a distinct song, unlike that of conspecifics from the mainland. Using a playback experiment in the wild, Mirsky elicited different responses from adult island Anna's and mainland Anna's males. Island males responded aggressively to playback of other island male songs, which is expected, but had no reaction to mainland Anna's song. A similar but reversed response occurred with mainland males. This result suggests that males from the two groups do not recognize the song from the other group as an intruder song in their territory. Mirsky believes that because of an initial rarity of tutors when the Guadalupe population was established, young males had no chance to learn normal song and therefore produced an aberrant song. Because of vocal learning, the aberrant song was then passed on to subsequent generations as a form of cultural transmission (Mirsky, 1976).

The presence of dialects has often been cited as an indicator of vocal learning in both the songbird and hummingbird literature. However, an alternative explanation is that there are genetic differences between populations that cause the dialects. González and Ornelas (2014) examined genetic differentiation in lekking wedge-tailed sabrewings (*Campylopterus pampa*) with different dialects. The authors found that “neutral genetic and song divergence were not correlated, and measures of genetic differentiation and migration estimates indicated gene flow across leks” (González & Ornelas, 2014). The authors concluded that the dialects are not the result of genetic differences but are caused by vocal learning.

In 1990, Baptista and Schuchmann performed the only experiment to explicitly test vocal learning in hummingbirds. They captured four nestling male Anna’s hummingbirds (all less than twenty days old) and then raised them in isolation. All developed abnormal song. The authors believe that because the young males were isolated from adult males, they did not have the chance to learn the correct song. They concluded that because the isolated males ultimately produced abnormal songs, Anna’s hummingbirds are vocal learners (Baptista & Schuchmann, 1990b). Since this experiment, there have been very few studies on hummingbird vocal learning.

The Sensitive Phase of Vocal Learning: The sensitive phase of vocal learning has been identified in a few species of songbirds. Marler and Peters (1987) were able to experimentally identify the sensitive phase in song sparrows. Song sparrow nestlings were brought into the lab and tutored for different periods of time between day ten and day three hundred and fifty. The authors found learning began around day twenty-two and for some birds continued until day two hundred (Marler & Peters, 1987). Marsh wrens (*Cistothorus palustris*) have a sensitive phase from around day fifteen to day sixty (Kroodsma, 1978) and the zebra finch (*Taeniopygia guttata*) has a sensitive phase from day twenty-five to day sixty (Eales, 1985). In the zebra finch, Eales (1985) found that the sensitive period was associated with certain life history traits. Zebra finches become independent from their parents around day thirty-five and become sexually mature at day sixty-five (Eales, 1985). The young males start to learn in the nest from their fathers but can adjust song to match their neighbors once they become independent. The cut-off point for the sensitive phase must occur prior to day sixty-five since young males are fully capable of singing an adult song at that point (Eales, 1985). The sensitive phase also appears to be linked to hormone levels during development (Schlinger, 1997).

In both Anna’s (Yang et al., 2007) and Costa’s (Williams & Houtman, 2008) hummingbirds there is considerable inter-individual variability of song. This variation may be important in male-male contact and neighbor identification. If so, individuals may benefit by modifying their song when entering a new territory, which has important implications about when vocal learning can take place. The timing of the sensitive phase can be restricted to early development, can re-open seasonally before the breeding season, or may never end (Doupe & Kuhl, 1999). The re-opening of the sensitive phase or having a sensitive phase that never ends may make it possible to modify song after the hatch-year to match song with neighbors.

The sensitive phase seems to vary from species to species in songbirds, but most sensitive periods fall within the first few months of life. The standard way to identify the sensitive phase is to vary the period of tutoring .(Marler & Peters, 1987) For instance, if a bird with a sensitive phase from day thirty to day fifty is kept in isolation until day fifty-five and then tutored, the bird will produce an abnormal song. Similar methods are utilized in Chapter 2 of my dissertation to try and identify the sensitive phase of learning in Costa's hummingbirds.

Open-ended vs. Closed-ended Vocal Learning: The ability to vocally learn is not only limited to certain groups of animals but it often seems to be limited to specific periods of development in the life of an animal. An ability to learn vocally past the first year of life has been reported in mockingbirds (*Mimus polyglottos*), canaries (*Serinus canaria*), saddlebacks (*Philesturnus carunculatus*), starlings, great tits (*Parus major*), and nightingales (*Luscinia megarhynchos*) (Chaiken, Böhner, & Marler, 1994). In contrast, species such as song sparrows, swamp sparrows, white-crowned sparrows, chaffinches, and zebra finches are unable to learn after their hatch-year (Marler & Slabbekoorn, 2004) and are therefore considered closed-ended learners.

Only one hummingbird has been examined in terms of open-ended learning. Araya-Salas and Wright (2013) recorded songs from long-billed hermit (*Phaethornis longirostris*) leks over four breeding seasons. After catching and marking all the males, the authors tracked songs over time. Nine out of forty-nine males showed song replacement. This is truly open-ended learning because replacement songs (when they occurred) were not present until after the first year of life (Araya-Salas & Wright, 2013).

One uncertainty in the open-ended song learning literature is whether new components added to the repertoire are newly learned. It is possible that a 'new' vocalization was in fact learned much earlier in development but never sung until considerably later in life. A way to circumvent this problem is to use hand-reared males and control (or at least keep track of) what is heard and when. In Chapter 3 of my dissertation I test whether Costa's hummingbirds are closed-ended or open-ended learners by exposing previously isolated male Costa's hummingbirds, housed in isolation from 21 dph until 1 year of age, to other male Costa's hummingbirds.

The primary aim of this dissertation is an in-depth examination of vocal learning in the Costa's hummingbird. I want to find where this species lies on the spectrum of avian vocal learning. I first confirm that Costa's hummingbirds are vocal learners (Chapter 1), and then examine two important components of vocal learning: the sensitive phase (Chapter 2) and open-ended learning (Chapter 3).

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Chapter 1: Ontogeny of Vocal Learning the Costa's Hummingbird

Abstract:

Hummingbirds have evolved to learn song from conspecifics independently of the other bird clades that have this behavior, parrots and oscine passerines. Little is known about how vocal learning occurs in hummingbirds. To determine the necessary and sufficient conditions for learning, I raised young Costa’s hummingbirds (*Calypte costae*) in isolation. The first experiment tested whether three pairs of males, raised either in silence or with only acoustic exposure to song, produce wild-type song. They did not: each pair instead produced stereotypical 'isolate' songs dissimilar to wild-type song. Each member of a pair produced song indistinguishable from his cage-mate's song but with subtle differences from other pairs. Thus, exposure to song alone is insufficient to trigger learning. The second experiment tested whether ten individually housed males learn songs when exposed to a live male model plus song (mainly playback but some adult model-produced song). Seven of eight birds that heard Costa's-like songs learned to sing their tutor song. Specifically, they faithfully matched idiosyncratic features of the individual songs played to them, demonstrating attention to and learning of the exact acoustic stimuli received. Two control birds exposed to acoustically dissimilar Anna's Hummingbird song developed isolate song. Thus, exposure to species-specific song with a conspecific male is sufficient to trigger learning. The sensory and sensory-motor phases of song learning overlap, and song crystallization occurs approximately 125 days post-hatch. Hummingbird song ontogeny is strikingly similar to song ontogeny in passerines, despite its convergent origin, in support of the 'deep homology' hypothesis of vocal learning in vertebrates.

Introduction:

Vocal learning is the social acquisition of vocalizations based on auditory experience (Marler, 1976). Species exhibiting this behavior include hummingbirds (Trochilidae), passerines (Passeriformes) and parrots (Psittaciformes), and certain mammals (e.g. humans) (Nottebohm, 1972). Vocal learning requires brain regions and neural connections devoted to memorizing vocalizations, comparing sensory input to motor output, and fine control of both vocal and breathing musculature (Marler & Slabbekoorn, 2004). This neural complexity is reflected in its complex ontogeny. Among songbirds (oscine passerines), vocal learning begins with a **sensory phase** followed by a **sensory motor phase** (Table 1). During the sensory-motor phase, the initial vocalizations produced, **subsongs,** do not resemble adult song, but these progress to **plastic songs** that change over time and eventually turn into **crystalized songs** that are the adult songs (Beecher & Brenowitz, 2005; Konishi, 1965).

Oscine passerines (hereafter referred to as “songbirds”) exhibit substantial variation in these stages. The onset of the sensory phase can begin in the egg, as a nestling, or after fledging (Marler & Slabbekoorn, 2004). Though many species, including the Zebra Finch (*Taeniopygia guttata*), overlap sensory and sensory-motor phases, others, such as swamp sparrows (*Melospiza georgiana*) delay the sensory-motor phase until long after the song has been memorized (Marler & Peters, 1977, 1981, 1982, 1987).

Parrots are the sister clade to passerine birds within the clade Psittacopasserae and also learn vocalizations (Suh et al., 2011). Parrots mimic heterospecific sounds, including other species in nature (Amsler, 1947; Cruickshank, Gautier, & Chappuis, 1993; Hensley, 1980; Pepperberg, 1998), as well as human language (Aristotle, 323AD). Though the literature on parrot vocal learning has used different terminology, much of their vocal ontogeny is similar to songbird song ontogeny. Like songbirds, parrots extract auditory information from the vocalizations they hear early in life (sensory phase), have a ‘babbling’ stage (subsong), and then use the memorized vocalizations to guide their vocal development (sensory-motor phase) (Pepperberg, 2010). These similarities may arise from the phylogenetic proximity of parrots and songbirds, since, plausibly, vocal learning could have a single origin at the base of Psittacopasserae.

Hummingbirds and Psittacopasserae are distantly related; their most recent common ancestor is at the base of the clade neoaves, about 60 MYA (Prum et al., 2015). Some fifteen outgroups, none of which are known to learn vocalizations (Brenowitz, 1997; Jarvis et al., 2014), lie in between the two. Therefore, hummingbird vocal learning has presumably evolved convergently relative to the origin(s) of this trait within Psittacopasserae. Hummingbirds also have certain life history differences: they invariably lay two eggs per nest, and become independent from their mother shortly after fledging, whereas most Psattacopasserae have larger clutches and longer post-fledging associations. Thus, a male hummingbird's song development is potentially less influenced by his siblings than is the case for most Psittacopasserae. Most species within Psittacopasserae are monogamous with biparental care, exposing nestlings to their father’s song at an early age (Marler & Slabbekoorn, 2004; Masello, Sramkova, Quillfeldt, Epplen, & Lubjuhn, 2002). Hummingbirds have lek-like mating systems and uniparental care by the mother, who does not sing (Ruschi, 1965), and she often nests far from male territories. Thus, young male hummingbirds are unlikely to encounter their own father or hear him sing, unlike in Psittacopasserae (Snowdon & Hausberger, 1997), and sexual selection is expected to act intensely on hummingbird display traits, such as male song. Finally, unlike most Psittacopasserae species, hummingbirds do not flock; most of their social interactions arise from competitive or aggressive encounters around food sources.

Apart from observations that many hummingbird songs exhibit regional dialects (Arp, 1957; Brewster & Chapman, 1895; Davis, 1934; González & Ornelas, 2005; González, Ornelas, & Gutiérrez-Rodríguez, 2011; Nicholson, 1931; Skutch, 1951; Snow, 1968; Wiley, 1971), little is known about hummingbird vocal learning ontogeny. Raising young birds in varying degrees of isolation can reveal how vocal learning occurs (Marler & Sherman, 1985; Mulligan, 1966). Baptista and Schuchmann (1990) performed the only such study in hummingbirds, raising 4 young Anna's Hummingbirds (*Calypte anna*) (Baptista & Schuchmann, 1990). Both a single isolate (n = 1) and group isolates (n = 3) failed to produce Anna's wild-type song, implying that Anna's hummingbirds are vocal learners (Baptista & Schuchmann, 1990). The group of three isolate birds sang songs similar to each other, hinting that they learned to match each other's songs. As these experiments lacked a positive control, they do not identify the conditions necessary and sufficient for young hummingbirds to learn song.

Here I describe two experiments on nestling Costa's Hummingbirds (*C. costae*), hereafter Costa's, raised in isolation chambers. Our first experiment recapitulates Baptista and Schuchmann's (Baptista & Schuchmann, 1990) result: pairs of Costa's hummingbirds raised in isolation chambers, exposed to either no song or to species-specific song, develop an atypical 'isolate' song that does not closely resemble wild-type Costa's song nor the tutor song. Our second experiment included a positive control: captive reared birds, exposed to a live male model paired with tutor song, later sing the tutor song. This experiment demonstrates the elements of a tutoring regime that are necessary and sufficient for song learning. Finally, I propose a preliminary timeline of song ontogeny in Costa's Hummingbirds, including the sensory phase, the sensory-motor phase, and the subsong, plastic songs, and crystalized song stages that apparently have evolved convergently with the song ontogeny of songbirds.

Methods summary:

Animal Husbandry

During January through June of 2015 (Experiment 1) and 2016 (Experiment 2), Costa's Hummingbird nests were located on the University of California, Riverside (UCR) campus and nearby localities. Nests fledged at approximately 22 days post-hatch, so I removed nestlings at age 21 days and brought them into the lab. Chicks found after hatching (unknown hatch date) were approximately aged based on size and development relative to birds of known age. For Experiment 1, six males and two females were collected (the latter inadvertently). For Experiment 2 the sample was 10 males. All birds were collected under U.S. Fish and Wildlife Service federal permit MB087454-0, California Department of Fish and Wildlife permit SC-006598, and UCR IACUC Animal Use Protocols 20130018 and 20160039.

Chicks were fed by hand until they were capable of independent feeding, usually within 3 days of intake. All chicks were fed Nectar 9 (Roudybush™, Woodland, California) until 49 days old. Thereafter they were fed adult hummingbird diet, Nektar plus (Nekton Produkte, Keltern, Germany), except during molt (approximately 100-200 days post hatch) when I supplemented Experiment 2 birds' diet with Nectar 9, for additional protein.

Equipment

All isolation chambers were in the same room. Each consisted of an outer wood box, a layer of acoustic insulation, an inner plastic box (acrylonitrile butadiene styrene), and a powder-coated zinc 91.4 × 50.8 × 55.8 cm cage (Quality Cage Crafters, Portland, Oregon, USA). Each chamber had a microphone (Pyle PDMIC45, 20 Hz - 16 kHz, Brooklyn, New York, USA), two speakers (Gear Head™ SP2500USB 180 Hz - 20 kHz), two puck LED lights (Utilitech™) and an air supply of 10 air changes/hour. Experiment 1 had a 12 l / 12 d photoperiod, Experiment 2 was set to local sunrise and sunset times. To reduce air pump noise inside each chamber, a 1 L surge tank was placed between each pump and the chambers it served. The microphones and speakers were connected to either an M-Audio Eight High-resolution USB 2.0 Audio Interface (8-channel, 24-bit, Cumberland, Rhode Island, USA) or Vocal Studio Pro II M-Track USB Audio interface (2-channel, 24-bit, Cumberland, Rhode Island, USA). On occasion, a web camera was placed into cages for direct observation.

Tutor Songs

Tutor songs were recorded in spring of 2015 at the Boyd Deep Canyon Natural History Reserve (33.6474°, -116.3778°W; doi:10.21973/N3V66D), with a Sennheiser MKH 20 microphone in a Telinga Pro parabola and a Sound Devices 702 24-bit recorder sampling at 44.1 kHz. I recorded a Costa's singing bout from one male and one Costa's diving bout. An Anna's song was recorded in 2011 at the Albany Bulb, East Shore State Park, (37.8911, -122.3157). Anna's Hummingbird song was a "non-Costa's like" song (Figure 1C). Anna's are the closest relatives of *C. costae*, are partially sympatric, and the two species sometimes hybridize (Wells, Bradley, & Baptista, 1978). Since they are sympatric, many young Costa's will encounter Anna's song during their ontogeny. Thus, it is reasonable to expect that there has been selection on Costa's to avoid learning Anna's song.

Playback

Sound Analysis Pro 2011 software (hereafter, SAP) (http://soundanalysispro.com/, (Tchernichovski, 2011)) was used for tutoring, and to constantly monitor vocalizations of a bird from intake until the end of the experiment, the following January. The chambers reduced but did not entirely eliminate sound transmission: playback songs, as well as the songs of the experimental subjects were faintly audible outside of the chambers. The results suggest that transmission of sound from one chamber to another was unlikely to have had an effect.

SAP allowed for automated playback of separate sounds into each isolation chamber. Each channel contained one tutor song file of a song bout, approximately 20 seconds long. With these settings, SAP played the file for varying lengths of time, mimicking the variation of a natural tutoring experience. During an hour of tutoring, approximately 200 song bouts were played. The SAP settings were passive playbacks, 2.0 s gap and 0.1/s odds.

Recording Parameters

The sound recording parameters used by the SAP software used for both Experiment 1 and 2 were as follows: 44.1 kHz 16-bit depth, trigger activated recordings, pre-threshold recording duration 0 ms, post-threshold recording duration 3000 ms, maximal recording duration limit 30 s, and save if threshold was crossed 2000 times. The sound level of the chambers was measured with a sound level meter (Class 1 Sound level meter, DSM403SD, General® Tools and Instruments, New York, New York, USA) with a range of 50-100 dB, A weighting, 200 ms time interval). The sound level within the closed chambers with air-pumps on and without a bird or playback was 42 dB ± 0.98 and during playback the sound level is 51 dB ± 3.4.

Protocol: Experiment 1

For Experiment 1, six males were housed in three pairs and placed into one of two possible treatments, silence or speaker playback. Playback was initiated at 35 days post hatch for three hours a day, every day, and continued until the birds were approximately 275 days old. The tutor songs used were Backwards Costa's song, and Dive sound, since these sounds resemble but do not exactly match actual Costa's song. The most prominent difference of a song played in reverse is the placement of a series of clicks that are normally early in the dive (Figure 1, A2). The dive sound, which is non-vocal, is also similar to normal Costa’s song (Figure 1, B), but is missing the highest frequency elements, #3 and #4 (Figure 1, A3 & A4). I used these Costa’s-like tutor songs to examine what the birds were memorizing and learning to produce. If these birds developed song that matched tutor songs that were similar to but not exactly like 'normal' Costa’s song, this would constitute strong evidence of vocal learning.

For Experiment 1, I use only speaker playback to tutor the birds, but the birds failed to learn. Therefore, during Experiment 2, I placed a live adult male in with the pupil bird while I tutored them with speaker playback, to test the hypothesis that a live tutor was necessary.

Protocol: Experiment 2

All of the birds from experiment 1 produced isolate songs, irrespective of treatment (Figure 2), indicating that the social stimulus during tutoring was insufficient to stimulate learning. Accordingly, Experiment 2 followed a different protocol: of ten fledgling males, nine were housed separately, and the tenth, Squirrel, was housed with his sister for the entire experiment. For Experiment 2 I added three additional tutor song treatments: Normal Costa’s song and Costa’s upsweep only (both within the category of ‘Costa’s-like’ tutor song) and Anna’s song (‘non-Costa’s like’ tutor song). Anna’s song was used as a negative control since it seemed unlikely a Costa’s hummingbird would learn its sister species’ song. Upsweep only was used to test whether the birds need to hear the whole song in order to learn their species-specific song or if they just need to hear a portion of it. Under the 'innate song storage' hypothesis, I predicted that birds that received the upsweep-only tutor song might produce a complete species typical song. By contrast, according to a 'true learning' hypothesis, males should instead faithfully reproduce the tutor song, i.e. sing just the Costa’s upsweep.

During this experiment, all birds received speaker playback in the presence of an adult male Costa’s hummingbird (the 'adult model'). There were five male Costa’s adult models, four of which were from Experiment 1 and sang isolate songs, and the last was a wild-caught adult male. The wild-caught male presumably had learned normal Costa’s song; however, he never sang in captivity. For each pupil, only one of the five adult models were used throughout the entire tutoring period. During tutoring the adult male model was placed inside the isolation chamber with the pupil male for one-hour intervals, three times per week. Thus, the pupil and adult model were in physical, visual, and acoustic contact. The adult models were housed at other times in an outdoor aviary, where they were in visual and acoustic contact with each other. Tutoring began when the birds reached 35 days post hatch and lasted for three months until the bird was approximately 125 days old. Each pupil was tutored mid-morning (between 10:00-12:00) 3 days a week, for a total of 75 days. One potential problem with using adult models is that they might sing when they are in an isolation chamber with a pupil. SAP captured any songs sung by the adult models, so I could assess the impact those songs had on the juvenile birds.

Analysis

For both Experiments 1 and 2, vocalizations were monitored continuously, 24 hours a day, from the time a bird was placed in an isolation chamber around day 21 post hatch. This permitted tracking of song ontogeny beginning with first vocalizations of each bird. Once the songs stabilized, and became consistent, or crystalized, they were compared with the tutor songs by running a cross-correlation test in the program Raven v.1.5 (http://www.birds.cornell.edu/brp/raven/) on the spectrograms (normalized, linear power). The spectral cross-correlation function is a quantitative measure of similarity in frequency over time and returns a score between 0 and 1. A score of 0 indicates the spectrograms are have no frequency overlap, and 1 indicates the spectrograms are identical. To perform the cross-correlation test, each song file was converted into a spectrogram of an individual song (2-3 seconds in length). Some birds incorporated scolding into their song repertoire, which I excluded from the cross-correlation test. All recordings made by SAP had a sampling rate of 44.1 kHz, FFT (Fast Fourier Transformation) window of 60. Each recording was band pass filtered from 4 to 13kHz to remove background sound, such as the sound of the air pumps.

The cross-correlation function used to quantify song matching was sensitive to minor stochasticity in the recordings, such as background sound or slight differences in characteristics such as the timing of song elements. Therefore, songs that sound (to human ears) and appear spectrally appear similar can nevertheless have low correlation scores. To benchmark what constitutes a high cross-correlation score with the experimental setup, I played the original tutor song recordings into an isolation chamber and re-recorded the playback with SAP. The original vs. re-recorded versions had cross-correlation scores that ranged from 0.364 – 0.805 with an average cross-correlation score of 0.44 ± 0.181 (n = 6 song-song comparisons, ± S.D.). This variation was affected by the particular song used; thus, cross-correlation scores between different specific treatments (e.g. backwards song vs upsweeps only) are not comparable quantitatively.

I computed a self-similarity score for single birds, in which I compared a series of individual songs that a bird produced against each other. The cross-correlation self-similarity ranged from 0.217 - 0.99 (n= 2, birds). Therefore, I interpreted a cross-correlation score of ≥ 0.2 as evidence that two sounds were "similar" to each other. As a negative control, comparing Costa's song with Anna's song produced cross-correlation scores ranged from 0.001-0.032 with an average of 0.0099 ± 0.003 (n = 6 song-song comparisons). Therefore, I interpreted a cross-correlation score of less than 0.2 as evidence of dissimilarity between two sounds (i.e., the bird did not learn to sing the tutor song).

To assess learning over time and differences between treatment types, i.e. Costa's-like and non-Costa's-like tutor song, a multilevel modeling approach was implemented with the lme4 and lmerTest packages in R (Bates, Mächler, Bolker, & Walker, 2015), with bird identity as a random effect. Variables included bird identity, bird age, average correlation score, and tutor song (Costa's-like or non-Costa's like).

Results:

Experiment 1

None of the six male Costa's hummingbirds removed from nests on day 21 (mean fledging date) produced a song resembling typical Costa's hummingbird song (Figure 2A-C). Cross-correlation scores of the birds' songs compared to the tutor Costa's song were low: 0.15-0.21 (Table 2). I term the resulting songs 'isolate song'. Isolate songs were similar among the three pairs (Figure 2). Individuals within a pair sounded so similar that it was not possible to identify which bird of a pair sang a given song. However, the organization of isolate song syllables differed among pairs (Figure 2).

The production of isolate song implied the following: (a) Costa’s Hummingbirds socially learn their song, (b) they did not undergo substantial learning in the nest before capture, and (c) the social context of the Experiment 1 playback regime was not sufficient to produce vocal learning. Since hummingbirds lay two eggs, I hypothesized that housing two "brothers" in a single chamber would mimic possible inter-sibling interactions such that the birds would learn song with their cage-mate acting as a model. Contrary to this hypothesis, each pair learned to match each other’s songs but without any apparent influence of the playback song. Since playback was presented for three hours per day, but the lights were on (and birds were active) for 12 hours per day, the two birds were exposed to each other much more often than they were exposed to the playback song. These findings, as well as data presented below, are consistent with the statistical learning hypothesis, in which pupils memorize what they hear most often.

Experiment 2

Experiment 2 differed from Experiment 1 in that young males were housed individually, and a live adult male Costa’s ‘tutor’ (henceforth, the adult model) was placed in the cage with the pupil during playback of tutor songs (three days per week, one h / day). After receiving tutoring from day 35-125 (post-hatch), seven of eight birds presented with Costa's-like songs successfully learned the songs played to them (Figure 3, Table 3). An eighth bird (Figure 3L), did not learn his tutor song, but his experimental conditions differed from the other 9 birds (see *Additional Results* below). Cross-correlation scores for the seven birds that learned their tutor song ranged from 0.22-0.58 (Table 3).

Two males, instead of receiving Costa's-like song, were presented with Anna's Hummingbird song (Figure 1C) as a negative control. Neither of these birds learned to sing Anna's hummingbird song. They instead sang isolate song similar to that produced by the birds in Experiment 1 (cross-correlation scores with Anna's song of 0.01 and 0.02).

Treatment (Costa's like versus non-Costa's like song) was highly significant (Multi-Level Model, t-value = 3.66, df = 7.97, p = 0.006) indicating that the type of song that a bird heard determined whether that song was learned (Figure 3, Figure 4). Across all 10 birds, age was positively associated with correlation score (MLM, t-value = 12.71, df = 3.743, p-value < 2^{-16}); that is, the match between the bird's song and playback song improved over time (Figure 5). The average cross-correlation score of the first vocalizations for birds presented with Costa's-like song (against the song they received) was 0.15 (95% CI: 0.0477, 0.261) (Figure 4).

Song Ontogeny

The ontogeny of song in Costa's hummingbird closely resembles songbird song ontogeny (Figure 7). Costa's first detectable subsongs started on day 54 ± 12 (n ± S.D; n = 16 males, Figure 4). These subsongs consisted of short undulating (ascending and descending) whistles with a frequency range from 4 – 10 kHz (Figure 5A), similar to that adult song. The subsongs of all the birds were similar, despite exposure to a variety of playback songs (Figure 5, A). As birds aged, their vocalizations became plastic, and for birds that received Costa’s-like songs,-correlation with playback song increased over time (Figure 4, Figure 5, B-D). The song continued to change until adult song stage, when the songs became consistent (variation in frequency and structure decreased) and the cross-correlation scores plateaued (Figure 4, Table 3), which occurred 125 ± 23 days (n = 8 birds tutored with Costa’s like tutor song; Figs 4, 5 E-G).

Isolate Costa’s Song

All birds from Experiment 1 (n = 3 pairs) sang isolate songs, as did the three birds from Experiment 2 that failed to learn playback songs. The subsongs of these birds were similar to those of the birds that heard Costa’s-like song. However, when they began to sing their plastic song, over time the ascending whistles slowly disappeared, and a pure 'whistled' high-frequency down-sweep, similar to the last part of natural Costa's song, remained. The down-sweeps became more stereotyped, and seven (of nine) birds produced two versions of different duration, long (L; approx. 0.5 sec), and short (S, approx. 0.25 sec) (Fig 2A-C). The isolate song crystalized (~96 days, n=3 birds experiment 2) with a stereotyped syntax: song typically began with a single long down-sweep (Figure 2, L) followed by a series of short down-sweeps (Figure 2, S) where the number of repeats of S varied among birds, from 2 or 3 short down-sweeps (i.e. LSSS, LSS, LSSS, etc., Figure 2B) to as high as 20 short down-sweeps in a row (Figure 2A). One pair from experiment 1 sang just the long down-sweeps in succession (i.e. L L L L L… Figure 2C).

Additional results

Three additional results are necessary to document: adult model song, scolding calls in song, and the case of Squirrel.

Adult model song:

One effect of using live adult Costa's males as models for the Experiment 2 birds was the possibility of the models themselves singing, thereby ‘contaminating’ the auditory environment experienced by the pupil birds. Since I primarily used the now-mature birds from Experiment 1 as the models, they sang isolate song (Figure 2) that was very different from any of the (playback) songs (Fig. 3). The recording software tracked all instances of singing by models. The maximum number of model songs a pupil heard was 172 bouts, compared to about 15,000 playback song bouts. Since 7 of 8 pupil birds learned playback songs (Figure 3), there is no evidence that song from models affected the song learned by the pupil birds. These data are consistent with statistical learning, in which pupils determine what to memorize based on what they hear the most often (Fehér, Ljubičić, Suzuki, Okanoya, & Tchernichovski, 2017). According to this hypothesis, the more times a certain stimulus is heard, the more likely the pupil is to match that stimulus.

Scolding calls in song:

Another effect of using live adult models during tutoring was the incorporation of scolding calls into the pupil’s songs (Figure 6). In addition to song, Costa’s also produce several other calls, all of which appear to be innate (since the experiment 1 birds would produce them without having heard them before, and to our ears these vocalizations sounded similar to the calls of wild birds). One of these is a “scolding” call used in agonistic interactions. Scolding calls are acoustically different from song, with scolding having a wide frequency bandwidth including much lower frequency ranges than present in the song (Figure 6). Introducing a live adult model into a cage with a pupil bird produces a social situation that likely does not reflect how songs are learned in the wild: the pupil bird considers the cage his territory, which he will aggressively defend, and the adult male model is an intruder that does not flee when chased. Some tutoring sessions were captured on video in order to examine behavior and prevent serious pupil-tutor fighting. All live adult models produced scolding calls during tutoring. The young males thus heard scolding only when the model was in the chamber, also coinciding with when the tutor song was played. These scolding calls were omitted from all analyses, and their presence does not affect our conclusions.

The case of Squirrel:

An Experiment 2 bird named Squirrel was tutored with Backwards Costa's song but instead learned Isolate song. I considered several hypotheses as to why he failed to learn the tutor-song. The adult model used to tutor Squirrel sang 47 bouts of isolate song over the entire tutoring period. Thus, I regard it as unlikely that this affected Squirrel's song. Because the isolation chambers were in the same room, there could have been 'leakage' (contamination) between boxes. The bird housed above Squirrel, named Cricket, was tutored with Anna's Hummingbird song, and so also sang Isolate Costa's song. Cricket was by far the loudest and most persistent singer (i.e., hundreds of times per day) of all the Experiment 2 subjects. Thus, a hypothesis for Squirrel's learning the 'wrong' song was that he could hear Cricket as well as his tutor song when the model was in his cage. However, this hypothesis is not supported: while both birds sang isolate Costa's song, the syllable structure is different and there was is no indication that Squirrel matched Cricket specifically (Figure 3, Squirrel (B3) & Cricket (E2)), as occurred within the pairs of cohabitating birds in Experiment 1.

The most plausible hypothesis as to why Squirrel sang isolate Costa's song is that, unlike the other nine birds in Experiment 2, his experimental conditions had greater similarities with the three pairs of birds in Experiment 1. Specifically, unlike the other birds in Experiment 2, but like the three pairs in Experiment 1, he shared his cage with another bird, his sister (who was brought into captivity by accident, since our method for sexing chicks was not 100% accurate) for the entirety of the experiment. I hypothesize that living with another bird affected his response to tutoring. For instance, the arousal of adding an adult model to the chamber of a bird housed alone may be greater than that of a bird housed with another bird. Therefore, the necessary stimulus to learn may have not been present for Squirrel and therefore he developed isolate song.

Discussion:

These results show that exposure to species-typical song in isolation from adult birds is insufficient to elicit song learning in Costa's Hummingbirds: despite exposure to Costa's-like songs in the presence of an age-matched conspecific "brother," the Experiment 1 birds produced isolate song (Figure 2). This is consistent with the findings of Baptista and Schuchmann (1990) for Anna's Hummingbird. In these two experiments, a total of nine birds produced isolate song, which consisted of similar frequency down-sweeps (Figures 2, 3). This similarity suggests that down-sweeps may be an innate 'species universal' portion of Costa’s song, similar to what is observed in passerines (Marler, 1997; Marler & Slabbekoorn, 2004). Possibly, this helps a vocal-learning species to direct attention to their own species’ song and not that of another species (Marler & Slabbekoorn, 2004).

I also established the conditions that permit captive Costa’s hummingbirds to learn typical adult song (Figure 3). Young males that interact with a live adult model during playback of an appropriate song learn to sing the playback song (Figure 3). However, they will not learn a sister-species’ song even with a live model present. Costa’s hummingbirds need auditory, visual, and/or tactile interaction with an adult male in order for song learning to occur. Thus, production of isolate song is not the result of raising hummingbirds in captivity *per se*. Moreover, our experimental subjects faithfully copied subtle features of slightly atypical tutor songs (e.g., a song played backwards). This demonstrates that they copied the specific sounds they heard (Figure 3) and rejects the hypothesis that hearing a Costa’s-like song is sufficient to elicit production of an innate species-specific song.

From which adult(s) does a young Costa's male learn? In passerines, this is largely a function of whether the sensory phase starts in the nest or after fledging. Because most songbirds have biparental care, it was initially thought that most learn from their fathers in the nest. Although true in some species (Millington & Price, 1985; Zann, 1990), other species learn their songs after dispersal from their natal territory (Payne & Payne, 1997). In songbirds, tutors can be fathers (Grant, 1984; Immelmann, 1969), unrelated males encountered after leaving natal territory (M. D. Beecher, Campbell, &

Stoddard, 1994; Kroodsma, 1974), a single dominant male of a breeding territory (Payne & Payne, 1977), or other males encountered after arriving in the breeding territory (Baptista & Morton, 1988; Kroodsma, 1974).

The life history of hummingbirds (no paternal care) largely eliminates the father as a possible tutor. I began our experiment when chicks were 21 days old and had been exposed to natural stimuli since hatching. Since none of the experimental birds produced typical adult song unless playback was accompanied by a live model, I conclude that Costa's do not memorize song while in the nest and instead memorize from males they encounter post-fledging. Adult male Costa's sing year-round (Williams & Houtman, 2008) and young males occupy the same habitats as adults (pers. obvs.). Therefore, a young male will almost certainly encounter singing adults after he fledges.

Our experiments provide a preliminary timeline of song ontogeny for Costa's hummingbird (Figure 7). Among songbirds, Costa's ontogeny most closely resembles that of the Zebra Finch. Like Zebra Finches, Costa's have a late (post-fledging) sensitive phase, overlap between the sensory and sensory motor phases, and production of adult song during their hatch year (Figure 7) (Eales, 1985; Slater, Eales, & Clayton, 1988). There is one apparent difference: Zebra finches have close-ended learning, whereas other work indicates that Costa's Hummingbirds have open-ended song learning (Chapter 3).

Finally, it is noteworthy that Costa's Hummingbirds seem to lack dialects, i.e. regional variation in song (Williams & Houtman, 2008). This is strange for two reasons. First, dialects arise easily in vocal learning species, because slight differences between what a bird hears and then sings are culturally transmitted to other birds in the same region, permitting rapid local cultural evolution. Second, in songbirds, dialects are more common in resident populations than in migrant populations (Handley & Nelson, 2005). Although local migration or other annual movements of Costa's Hummingbird remain unclear, the species does not have long-distance migration and is a year-round resident over much of its range, including our collection site in Riverside, CA (Baltosser & Scott, 1996). For both these reasons Costa's Hummingbird should be especially prone to develop dialects, yet no dialects are known. The lack of dialects could result from young males using multiple males as song tutors instead of learning from just one male. Learning from multiple males, instead of just one, would homogenize song as opposed to creating dialects. Additionally, the migration patterns of Costa's hummingbirds may have a similar homogenizing effect on song. It has been observed that Costa's sometimes migrate during the breeding season, starting in one location and then moving to another (Stiles, 1971). Moving during the breeding season and listening to and learning songs from multiple males from each location may erase any differences in song that arise.

I have shown that isolation chamber techniques used extensively in experiments on passerine birds can also be used with hummingbirds. The number of apparent similarities between hummingbird vocal learning and songbird vocal learning is interesting, given the evolutionary divergence of these lineages over 60 million years ago (Prum et al., 2015). Convergent evolution usually follows different 'paths' to the same destination, thus subtle details of mechanism often differ between convergent clades. While additional studies on hummingbird vocal learning may uncover differences relative to passerines, the surprising number of common features between hummingbirds and passerines suggests that vocal learning has deep origins in all birds.

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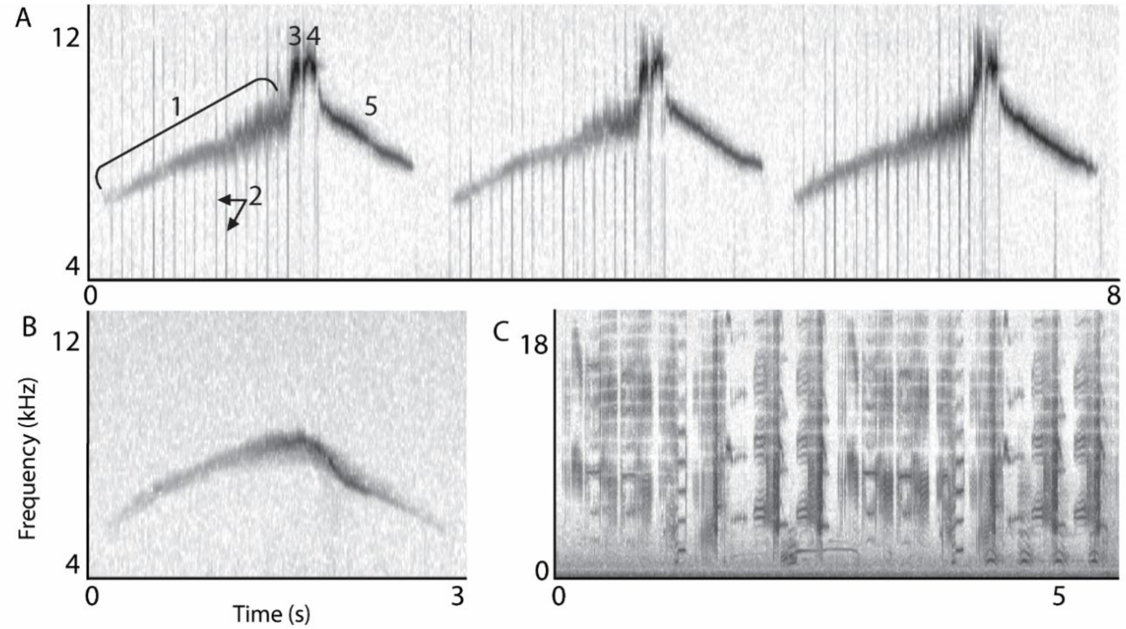

Figure 1.1: Costa's Hummingbird (*Calypte costae*) song, dive sound, and Anna's Hummingbird (*C. anna*) song. **(A)** Song bout of 3 songs. Individual songs are short (two seconds), comprising of a frequency upsweep (1) with a series of broadband clicks (2), two short high-frequency notes (3 & 4), then a frequency down-sweep (5). Songs bouts varying in length from single songs to over 20. **(B)**. Costa's dive sound resembles song, but is produced by the tail feathers during a courtship dive (Clark & Feo, 2010). **(C)**. Song of Anna's hummingbird. Spectrograms parameters (all figures): 44.1 kHz, FFT window size of 1024 samples.

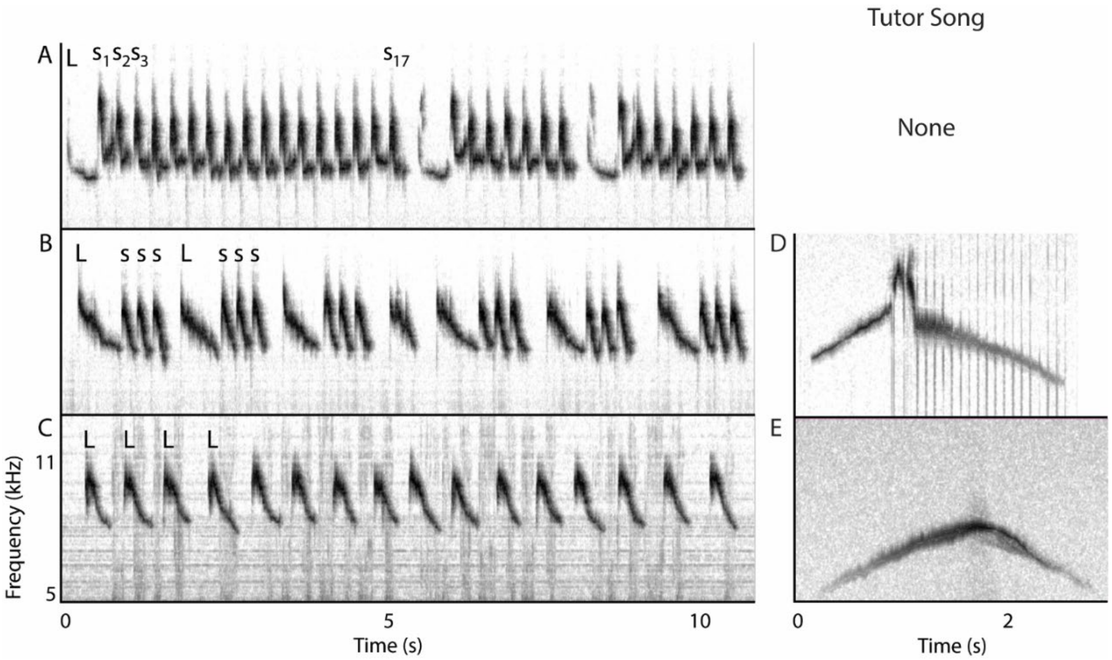

Figure 1.2: Isolate song of Costa's Hummingbirds that developed in the absence of an adequate tutoring regime. In experiment 1, three pairs of age-matched young male Costa's hummingbirds, (A) Pip and Pap, (B) Mist and Jack, and (C) Encelia and Farinosa, were housed in separate isolation chambers and exposed to tutor song for three hours/day, daily for 240 days. Birds produced a series of long (L) and short (s) duration frequency down-sweeps.

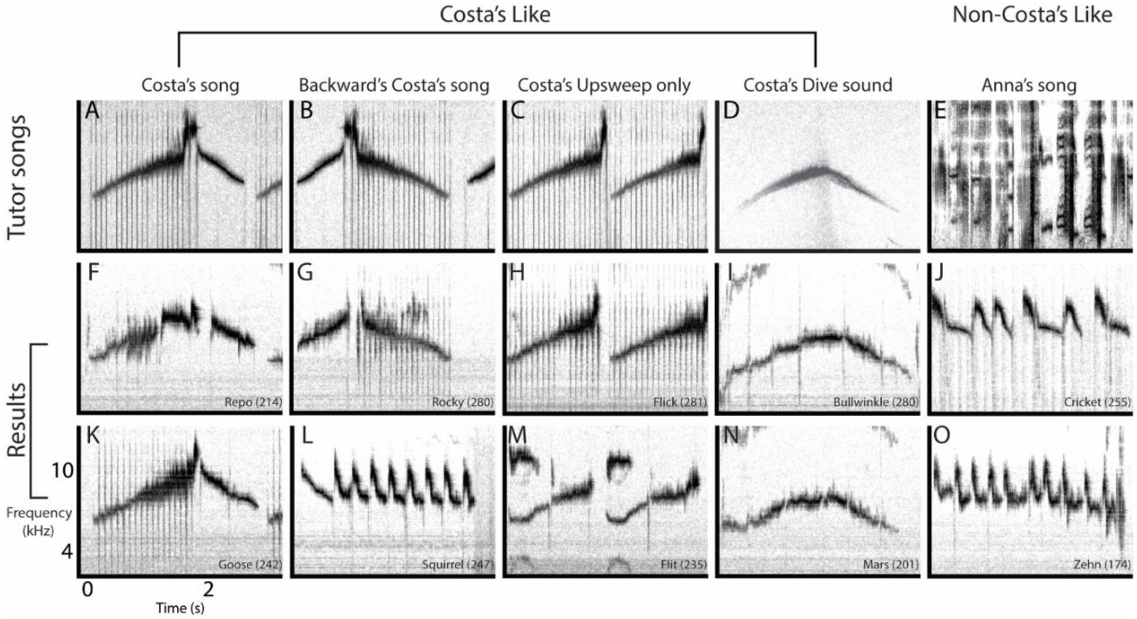

Figure 1.3: Songs developed by 10 individually isolated Costa's Hummingbirds simultaneously exposed to a live model (adult male Costa's hummingbirds) and tutor song (top row, A-E). Exposure regime: for 1 hr/day, 3 days/week. Top row (**A-D**): Tutor songs. **A**: species-typical song, **B-D**, sounds resembling species-typical song. **E**: Song of Anna's Hummingbird (see also Fig. 1**C**). Middle and bottom rows: songs developed by 10 pupil birds (two per tutor song type). Birds **F, G, H, I, K, M** and **N** produced songs with similarities to the tutor song, whereas birds **L** and **J** and **O** produced isolate song with no evidence of matching the tutor song. Lower corner: name of each bird, and age of recording. See the Case of Squirrel in additional results for a hypothesis as to why Squirrel (**L**) failed to learn backwards Costa's Song.

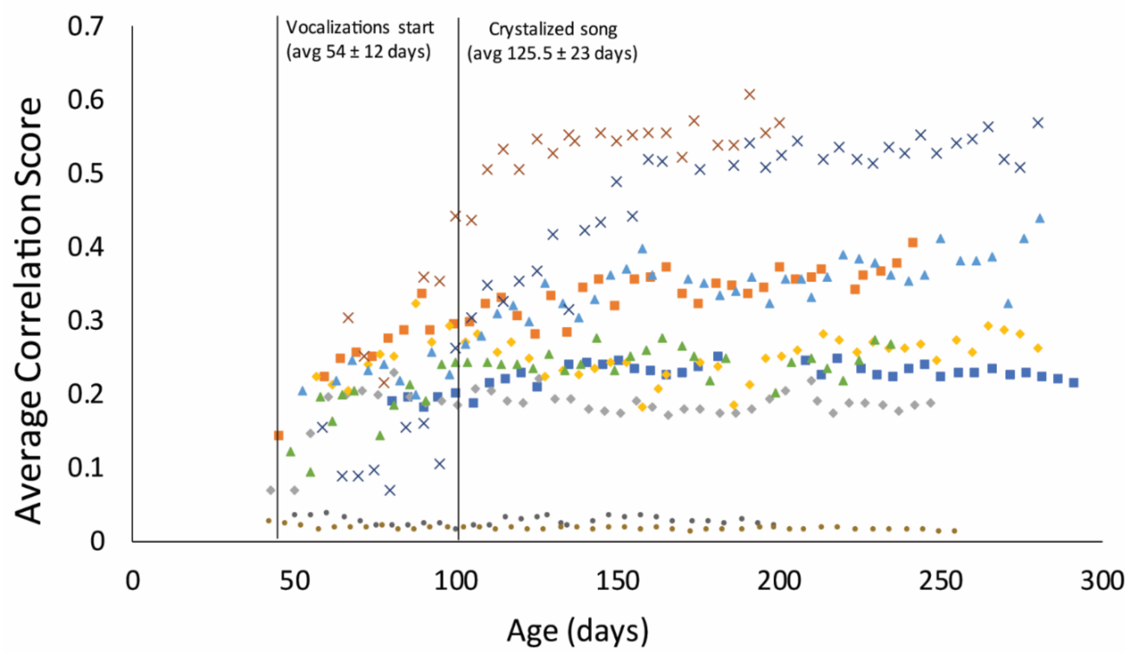

Figure 1.4: Learning curves for 10 Costa's Hummingbirds tutored individually with an adult model live tutor (experiment 2). Each point is an average score from n = 30 songs cross-correlated against tutor song (Top row in Fig. 3). Tutor song types: Normal Costa's song (squares; Fig. 3 F, K), Backwards Costa's song (diamond; Fig. 3 G, L), Upsweep only (triangle; Fig. 3 H, M), Dive sound (x; Fig. 3 I, N), and Anna's song (circle; Fig 3 J, O). Note: the asymptotes of curves for different tutor song types are not directly comparable, since the cross-correlation function intrinsically produced higher scores for some tutor song types than others. See also Figure 5.

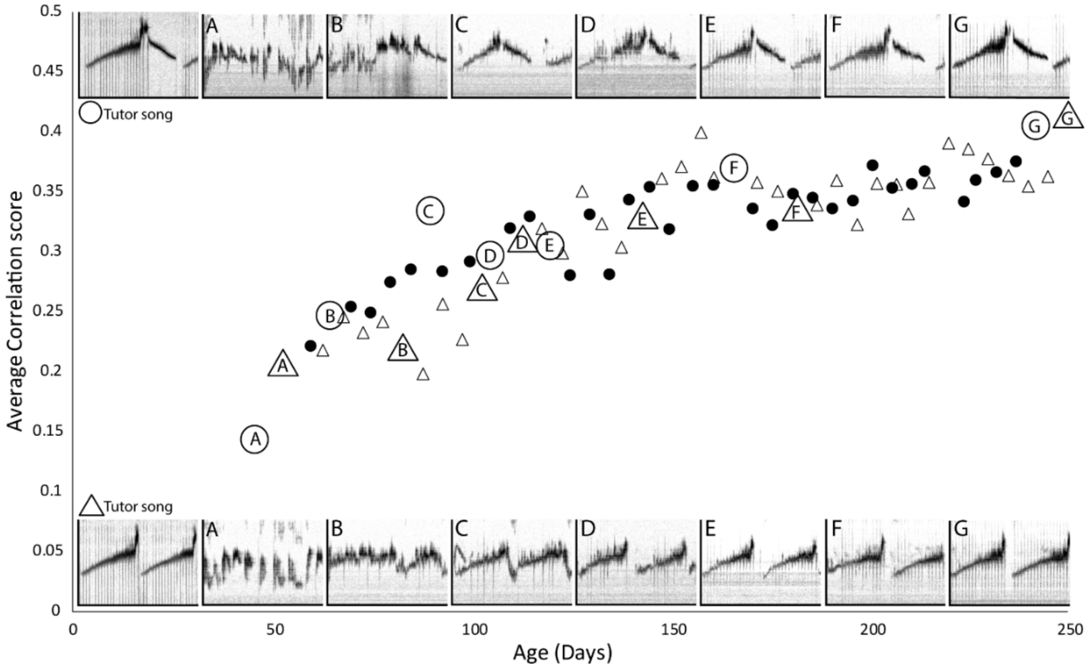

Figure 1.5: Song ontogeny of two Costa's Hummingbirds, Goose (circles; top row) and Flick (triangles; bottom row). Subsongs spectrograms A & B, Plastic songs C-F, and Crystalized songs G. All spectrograms are 3.3 seconds, 3 – 10 kHz.

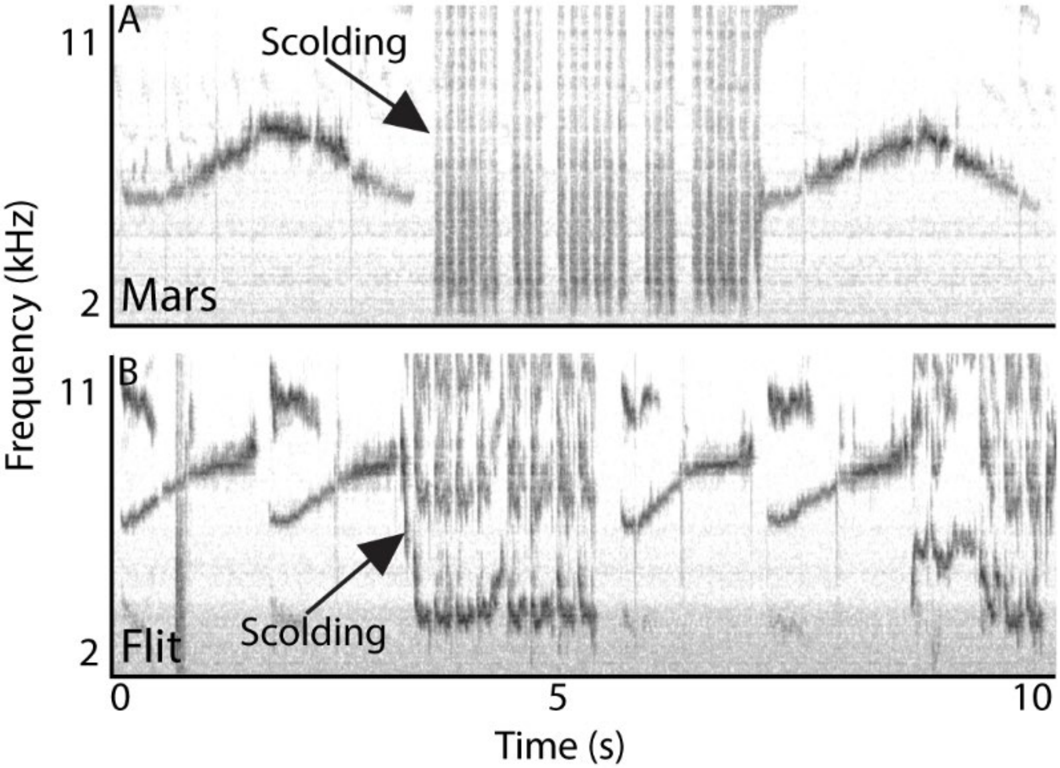

Figure 1.6: Costa's Hummingbirds incorporated "scolding" calls into their songs.

Scolding calls are typically produced by both sexes in agonistic (aggressive) interactions and are not observed in song of wild male Costa's (Williams & Houtman, 2008). I hypothesize that incorporation of scolding calls into song was a side-effect of introducing adult models into the juvenile bird's cage. The two panels show two birds **(A)** Mars and **(B)** Flit. Mars and Flit had different adult models and therefore heard different scolding types. Spectrograms were created from recordings with sampling rate 44.1 kHz and FFT window 1024.

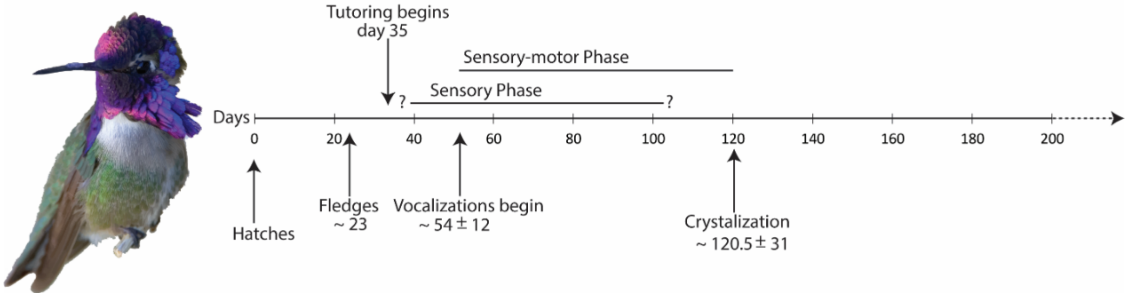

Figure 1.7: Preliminary Timeline of Song Ontogeny in the Costa's Hummingbird. I placed nestling Costa's males in isolation chambers on day 21 post-hatch. On day 35 tutoring began for both experiment 1 and experiment 2; see text for further details. Question marks indicate uncertainty. Numbers are means ± s.d., n = 8 birds.

Table 1.1: Vocal Learning Definitions

Term	Definition	Citation
Sensory phase	Subject listens to and memorizes species-specific song.	(Konishi, 1985; Marler & Peters, 1987)
Sensory-motor phase	Subject commences singing. Animal learns to use its vocal apparatus and to match the motor output i.e. the vocalizations, with the memory of the song from the sensory phase.	(Marler, 1970)
Subsong	The first vocalizations a vocal learning bird produces. The vocalizations are usually short, vary in frequency, and are acoustically and structurally dissimilar in sound and appearance to the adult song (Fig. 5A).	(Thorpe, 1958; Thorpe & Pilcher, 1958)
Plastic song	Stage of song development in which the bird practices its vocalizations and starts to match its vocalizations to its memorized song (Fig. 5B-F)	(Marler & Peters, 1981, 1982)
Crystalized song	Final stage of song development, when the song matches the memorized song, is stereotyped (i.e. has low variability) and ceases changing (Fig. 5G)	(Marler, Peters, Ball, Dufty, & Wingfield, 1988)
Open-ended learning	Learning that takes place after the first year of life, in which new song material is incorporated into the repertoire of the animal	(Laskey, 1944; Nottebohm, Nottebohm, & Crane, 1986)
Closed-ended learning	Vocal learning birds that do not learn new song material after their first year.	(Immelmann, 1969; Thorpe, 1958)
Isolate song	Song produced by a vocal learning bird raised in isolation, with insufficient stimuli to elicit learning (e.g. failure to hear its species-specific song)	(Marler & Sherman, 1985)

Table 1.2: Experiment 1 results: Correlation scores of pupil song vs Costa's song or tutor song

Bird pair	Treatment	Average Correlation score (n ± s.d.)	
		Songs vs. Normal Costa's	Songs vs. Tutor song
Mist & Jack (2B)	Silence	0.15 ± 0.07	NA
Pip & Pap (2A)	Backwards Costa's song	0.21 ± 0.08	0.21 ± 0.09
Encelia & Farinosa (2C)	Costa's Dive sound	0.17 ± 0.06	0.02 ± 0.01

Table 1.3: Average correlation scores for Experiment 2 Table

Bird (Fig. 4 ID)	Tutor Song	Costa's- like	Average Correlation score (mean ± s.d.)	Crystallization age (days)
Repo (A2)	Normal Costa's song	Yes	0.22 ± 0.003	111
Goose (A3)	Normal Costa's song	Yes	0.38 ± 0.018	140
Rocky (B2)	Backwards Costa's song	Yes	0.28 ± 0.01	128
Squirrel (B3)	Backwards Costa's song	Yes	0.18 ± 0.005	96
Flick (C2)	Costa's Upsweep only	Yes	0.39 ± 0.05	143
Flit (C3)	Costa's Upsweep only	Yes	0.26 ± 0.01	96
Bullwinkle (D2)	Costa's Dive sound	Yes	0.53 ± 0.03	160
Mars (D3)	Costa's Dive sound	Yes	0.58 ± 0.02	125
Cricket (E2)	Anna's song	No	0.01 ± 0.001	97
Zehn (E3)	Anna's song	No	0.02 ± 0.003	95

Chapter 2: The Sensitive Phase of Vocal Learning in Costa's Hummingbirds

Abstract:

Vocal learning is an ability known from only three groups of birds, songbirds (oscine passerines), parrots, and hummingbirds, as well as a few groups of mammals including humans. It is an experience-dependent behavior that requires exposure to appropriate vocalizations in order for learning to take place. The timing of the exposure is often restricted to early in life, when the brain is particularly plastic and can be altered by experience. If an organism receives no exposure to the necessary auditory experience during this sensitive phase, it will produce abnormal vocalizations. Here I examine the sensitive phase of vocal learning in the Costa's hummingbird (*Calypte costae*). I tutored young Costa's males ($n = 16$) with Costa's song in the presence of an adult model under three different tutoring periods: Early (35-65 days post-hatch (dph)), Mid (75-105 dph), or Late (115-145 dph). Birds only tutored during the sensitive phase should be able to learn to sing the tutor song, while those tutored too early or too late should not. Birds received 20 total hours of tutoring over the 30 days of their tutoring period. I tracked song development for each bird and assessed learning by examining similarity of each bird's adult song (average age 276 ± 29) to Costa's song. No birds produced isolate song, meaning some learning of the tutor song took place. Each bird produced a unique song that contained some aspect of Costa's song along with some abnormalities. A linear mixed model showed no significant difference in the similarity of the experimental birds' adult songs to wild-type Costa's song across all three treatments. This result suggests that the sensitive phase extends throughout all of the tutoring periods, but the amount of tutoring was insufficient. These results suggest that Costa's hummingbirds have a sensitive phase spanning from soon after fledging (starting before day 35) until the bird is more than 115 days old.

Introduction:

Vocal learning is the process of acquiring vocalizations through auditory experience, usually by listening to conspecifics, early in life (Marler, 1976; Saffran, Aslin, & Newport, 1996). The ability to acquire vocalizations from experience occurs in a few groups of mammals, including humans, and three groups of birds: songbirds, parrots, and hummingbirds (Nottebohm, 1972). Hummingbirds are thought to have evolved vocal learning independently of these other groups, but similarities in vocal learning brain regions and gene expression suggest that vocal learning has deep roots in vertebrate evolution, somehow restricting how vocal learning evolves (Jarvis, 2004).

Vocal learning, like some other experience-dependent behaviors, is often restricted to specific sensitive phases in an organism's life (Knudsen, 2004; Marler & Peters, 1987). Sensitive phases are discrete periods when the brain is plastic and easily altered by experience (Bottjer, 2004). During the sensitive phase, the brain undergoes changes as a result of auditory experience: axons and dendrites are altered, synapses form or disappear, and strengthen or weaken based on the experience (Knudsen, 2004; Nottebohm, 1992). In many vocal-learning species, the high neural plasticity of the sensitive phase occurs during a discrete sensitive period, after which learning is limited or completely halts (Knudsen, 2004).

There has been considerable research into vocal learning, in part because it serves as a model of how complex behaviors evolve. Examination of the sensitive phases of learning are of particular interest on account of human parallels: humans have multiple sensitive phases throughout early life that are extremely important for normal social, vocal, visual, auditory, and locomotor development (Lewis & Maurer, 2005; Ruben, 1997).

Much of what we know about sensitive phases comes from oscine passerines. The first stage of vocal learning is the sensory phase (Thorpe, 1958), when a vocal learning bird listens to and memorizes its species-specific song (Marler & Slabbekoorn, 2004). This early auditory experience modifies the song nuclei in the brain, establishing a neural representation of the song that the bird will use as a template to match its vocalizations to in the next stage of development (Konishi, 2010). If a vocal learner is prevented from hearing its species-specific vocalizations during its sensitive phase, abnormal vocalizations develop because a vocal learning bird has no model to match its own vocalizations against (Thorpe, 1958; Gobes, Jennings, & Maeda, 2017; Marler & Peters, 1987). The production of abnormal vocalizations also occurs in humans, usually the result of extreme neglect and abuse (Krashen, 1973). However, timing of the closure of a sensitive phase can be prolonged (Knudsen, 2004). In the absence of the correct stimulus, the brain regions involved in the sensitive phase remain plastic (Knudsen, 2004). This extension of the sensitive phase occurs in some oscines and is called the rescue effect (Knudsen, 2004). However, at some point, usually when a songbird reaches sexual maturity, the sensitive phase closes (Knudsen, 2004).

Little is known about the sensitive phase of vocal learning in hummingbirds. In previous experiments I showed that Costa’s hummingbirds are vocal learners and developed an approximate timeline of song ontogeny (Chapter 1, figure 7). Like oscines, Costa’s hummingbirds go through three developmental stages of song: subsong, plastic song, and finally the adult song (Adult song see Figure 1A). Costa’s hummingbirds have an overlap between their sensory and sensory-motor phase due to the early onset of singing, which is at roughly 50 days post-hatch. Twenty-one-day old males raised in isolation sing isolate song (Figure 1B), suggesting the sensory phase starts after fledging, where 'isolate song' is the song a vocal learning bird produces when raised without hearing the correct tutor song (Marler & Sherman, 1983). Isolate song consists of only down-sweeps (Figure 1B).

In this study of vocal learning in Costa’s hummingbird (*Calypte costae*), I tested the timing of the sensitive phase and its duration by controlling auditory experience during development. If tutoring occurs too early or too late, the resulting song should be abnormal. The experiment consisted of raising 16 young male Costa’s hummingbirds (captured at 21 days old) in one of three tutoring treatments: Early tutoring (35-65 days post-hatch (dph)), Mid tutoring (75-105 dph), and Late tutoring (115-145 dph). I chose the tutoring periods to correspond to song ontogeny events from previous experiments: The first song vocalizations, subsongs, begin around day 50, which I define as the beginning of the sensory-motor period (Marler & Slabbekoorn, 2004). Therefore, it is likely that the sensitive phase starts earlier, sometime after the young bird leaves the nest (Chapter 1), although it could conceivably start just prior to the plastic song stage (approximately 5 to 10 days after the first vocalizations). Accordingly, I timed the early tutoring period (day 35-65) to begin prior to the commencement of singing. The mid tutoring period (day 75-105) started after the first onset of plastic song and extended to the period when some birds start to crystalize their songs. The late tutoring period (115-

145) started after the first vocalizations and corresponded to the time when birds from a previous experiment started to crystallize their song at around 125 days post-hatch (Chapter 1).

Methods Summary:

Animal Husbandry:

Husbandry of Costa’s hummingbird chicks is briefly summarized here; other details (such as equipment used) are the same as described in Chapter 1.

Males were collected from their nests when they were 21 days post-hatch in 2017 (n = 12) and 2018 (n = 4). The 2017 birds were placed in the Early and Mid-tutoring treatments, while the 2018 birds were placed in the Late tutoring treatment. All birds were collected under the auspices of U.S. Fish and Wildlife Service permit MB087454-0, California Department of Fish and Wildlife permit SC-006598, and UCR IACUC Animal Use Protocol 20160039.

Playback and recording:

Sound Analysis Pro 2011 software (hereafter, SAP; http://soundanalysispro.com/) was used for tutoring, and to constantly monitor vocalizations of a bird from intake until the end of the experiment (Tchernichovski, 2000). SAP allowed for automated playback of Costa’s song into each of the 12 isolation chambers. Playback consisted of a series of six Costa’s songs from one wild adult male. Songs used for tutoring were recorded in spring 2015 at the Boyd Deep Canyon Natural History Reserve (33.6474°, -116.3778°W), with a Sennheiser MKH 20 microphone in a Telinga Pro parabola and a

Sound Devices 702 24-bit recorder sampling at 44.1 kHz. During an hour of tutoring, approximately 200 song bouts were played.

Experimental Set-up

Tutoring:

Speaker playback alone is not sufficient for normal song learning in this species (Chapter 1). Therefore, during tutoring sessions, an adult male Costa's hummingbirds, henceforth the adult model, was placed in the cage with the pupil for one hour while Costa's song was played through speakers. Pupil and model were in physical, visual, and acoustic contact, which is necessary for learning to take place (Chapter 1). Adult models were housed at other times in an outdoor aviary. The models were from a previous experiment, and all sang atypical songs. However, songs by models during tutoring sessions were rare and had no apparent impact on the pupil's song in a previous experiment (Chapter 1).

Tutoring for all of the tutoring treatments (Early 35-65 dph, Mid 75-105 dph, and Late 115-145 dph) took place between mid-morning and early afternoon (10:00 am-3:00 pm). The pupils were tutored four days a week for a total of 20 days over the 30 days of the treatment period.

Analysis:

Cross-correlation:

To measure learning, I compared each bird's adult song with Costa's song. This was done by running a cross-correlation test in the program Raven v.1.5 (http://www.birds.cornell.edu/brp/raven/) on the spectrograms. The spectral cross-correlation function is a quantitative measure of similarity in frequency over time and returns a score between 0 and 1. A score of 0 indicates the spectrograms are have no frequency overlap, and 1 indicates the spectrograms are identical. To perform the cross-correlation test, a song file was converted into a spectrogram of an individual song (2-3 seconds in length). For each day sampled I selected 20 individual songs, selected haphazardly from multiple recordings from the same day. The only requirement was that the song was complete and not a partial recording (e.g., due to the recording starting partway into a song or cutting off before a song ended). Some birds incorporated scolding into their song repertoire (Chapter 1); scolding was excluded from cross-correlation tests. I examined each birds' songs from their first vocalizations and then every two weeks until the bird was at least 250 days old.

Song Scoring:

In addition to the cross-correlation analysis a song scoring analysis was done. I also hand-scored a subsample of five songs from each day sampled. I chose 11 elements to score based on the major features of a typical Costa's song (Williams & Houtman, 2008): the upsweep, clicks, down-sweep, song duration, high frequency, low frequency, and frequency range (Figure2; Table 1). The songs of two wild Costa's hummingbirds (6 songs each) were examined in Raven to find averages for the different categories and what ranges I deemed acceptable (for averages and acceptable ranges see Table1). Each song was examined and scored based on the presence or absence of the 11 components. Given that virtually all wild adult male Costa's Hummingbirds sing highly similar songs

(Williams and Houtman 2008), virtually all wild adult male Costa's hummingbirds score an 11.

Statistical analysis:

To evaluate learning differences between treatment types in both of the analyses, a multilevel model was implemented with lmerModLmerTest package in R, with treatment as the factor with three levels (early, mid, and late) and bird identity as random effect. Because the birds learned to match a tutor song, the variance in their correlation scores changed over time, thus violating the assumption of equal variance for a repeated measures ANOVA.

Results:

None of the birds from any of the treatment groups produced Costa's isolate song (Figure 1B for isolate song, Figure 3). The expected adult song produced by a bird tutored outside the sensitive phase is the isolate song; therefore, all three tutoring periods fell within the sensitive phase. The final adult songs (Figure 3), across all three tutoring periods, varied in their similarity to Costa's song. Below I present the results from two similarity analyses, the cross-correlation score and the song scoring analysis. Most birds incorporated scolding into their song repertoire, but scolding was excluded from both analyses.

Cross-correlation analysis:

The first vocalizations of the birds had low cross-correlation scores, 0.03 ± .02 (n = 16) (Figure 4). The low cross-correlation scores were expected due to the differences between subsong and adult Costa's song (Figure 5, column A). The correlation scores of songs beyond the first vocalizations thereafter took differing trajectories (Figure 4, Figure 5, column B-H). A few birds (Figure 3: see A, B, H, I, J, L, M, O, and Q) had the general structure of a normal Costa's song, with an initial upsweep and then down-sweep. Some birds also incorporated the click element (Figure 3). However, the cross correlations scores were generally low, with the highest scores reaching 0.3 (Figure 4). For comparison, Figure 4 shows the learning trajectory of a bird from a previous experiment that received 75 days of tutoring over three months and eventually sang Costa's song with a correlation score of 0.4 (Figure 4, black line). One aspect of song that contributed to the low cross-correlation scores was song duration, because the analysis is sensitive to the timing of song elements. Typical Costa's song is around 2.4 seconds (n = 2 birds, for a total of 12 songs), but many birds from this experiment developed songs with average durations close to 4 seconds (Figure 3).

There was no significant difference in cross-correlation scores between the three treatment groups. Linear mixed models of average correlation as a function treatment with three levels (Early, Mid, or Late), age, and the treatment × age interaction had no significant differences between treatment groups: Multi-Level Model (MLM): Age t-value = 0.74, df = 13, p = 0.47; Early × Late t-value = -0.71, df = 13, p = 0.49; Early x Mid t-value = -0.54, df = 13, p = 0.6; Age x Treatment t-value = 0.97, df = 13, p = 0.348.

Song scoring analysis:

The first vocalizations of birds from all tutoring periods, scored very low, with an average score of 0.13 ± 0.3out of a possible 11 points (Figure 7). Subsequently, songs started to acquire aspects of tutor song, causing scores to increase (Figure 5, Figure 7).

The final song score (average score from last three days sampled for each bird) ranged from 0.93-9.5 (n = 16, average age 276 ± 29 days). The majority of birds consistently scored over 5, but there were a few birds that scored very low (Figure 8, Bolt and Lescoe). There was no significant difference in song scores between the Early, Mid, or Late treatment groups (MLM: Early x Late t-value = -0.37, df = 13, p = 0.72; Early x Mid t-value = 0.54, df = 13, p =0.6). There was an Age x Song Score interaction (MLM: t-value = 11.5, df = 275.8, $p < 2^{-16}$), but no Treatment x Age interaction (MLM: t-value = -1.1, df = 276, p = 0.27).

Discussion:

These results show that juvenile male Costa's hummingbirds that heard normal Costa's song, in the presence of an adult model during one of three tutoring periods, Early (35-65 dph), Mid (75-105 dph) or Late (115-145), learned equally well (Figure 3, Figure 5). Neither cross-correlation scores (Figure 4) nor song scoring analysis (Figure 7) revealed significant differences in how well birds in the different tutoring groups matched tutor song. Therefore, Costa's hummingbirds can memorize adult song sometime within the 35 – 145 dph period and there is no evidence that at any point in this window they learn better than at any other point in this window. The sensitive phase start at some point after the birds fledge, because males taken prior to fledging and raised in isolation produce isolate song (Chapter 1). Though our tutoring period extended to day 145 dph, it is possible that the sensitive phase extends beyond this period.

How well did they learn?

None of the birds from any of the tutoring periods produced imitations of wild-type Costa's song that contained all of the song elements, in the correct amount (i.e. more than one upsweep), and in the correct order. Most birds produced songs that contained some aspects of Costa's song but also abnormalities (Figure 3). Many birds produced songs also retained high variability song to song (Figure 6). Almost all birds produced songs longer than normal with a few producing songs shorter than normal (Figure 3, Longer: A-D, F-J, & L-Q; Shorter: E & K). During song ontogeny in Costa's hummingbirds, songs start out as long continuous vocalizations. As the song develops, during the plastic song stage, the vocalizations become more discrete with periods of silence between songs. For many birds in this experiment, the songs remain in the more continuous vocalization stage, with few birds producing songs with periods of silence before and after the song (Figure 5, Figure 6). This result hints that the timing of the song may be one of the final stages of the song learning process. The order and placement of certain elements, the up and down-sweeps as well as the clicks, were also atypical. Multiple birds included both the upsweeps and down-sweeps, but sometimes more than one of each and they were sometimes out of order (Figure 3, Early: A, C, & F, Late: P). Many birds also produced clicks, but no bird consistently placed them only in the upsweep, where they are in a typical Costa's song (Figure 3, Early B & C). As with the timing of the song, the ordering and placement of the song elements may be one of the last aspects of song development.

In addition to atypical song duration and song element placement, the songs of the birds retained a high level of variability song to song (Figure 5, Figure 6). In songbirds, at the end of the plastic song stage, the song starts to stabilize and becomes stereotyped, with low variance song to song (Marler & Peters, 1981). It is at this point the song is said to have crystallized (Marler, Peters, Ball, Dufty, & Wingfield, 1988). In a previous experiment in Costa's hummingbirds, towards the end of the plastic song stage the songs became stereotyped, with consistent song duration and song element placement (Chapter 1). This is not the result of this experiment (Figure 6). One of the biggest differences between the previous experiment, where song stabilized, and this experiment, where it did not, is that the birds received less tutoring. Therefore, it is possible that the abnormalities in song developed because the birds did not enough receive enough exposure to the tutor song.

Possible reasons for imperfect song acquisition:

Insufficient tutoring: The amount of tutoring the birds received—20 hours of playback over a 30-day period—may have been insufficient. In a previous experiment, birds received 75 hours of playback over 125 days and were able to produce songs that closely matched their tutor song, containing all elements in the correct order, and with the correct timing (Chapter 1). Based on these results, 20 hours over 30 days is insufficient, while 75 hours over 125 days is sufficient. Even with 20 hours of tutoring, some birds were able to imitate Costa's song better than others, possibly indicative of individual differences in learning abilities. In oscines, there is variation in how much exposure to a tutor song is necessary for a bird to produce a faithful copy. For most species the minimum necessary exposure is unknown. A few species need very little exposure to tutor songs. Nightingales (*Luscinia megarhynchos*) can learn a string of 12 songs if exposed to the 12 songs once per day for 15 days (Hultsch & Todt, 1989). Nightingales also have large repertoires, which may be related to their ability to learn songs based on very few exposures (Zeigler & Marler, 2008).

Imitation vs. Improvisation: Insufficient exposure to tutor song does not explain the variation between individuals within a tutoring group. In oscines there is variation in the fidelity of matching the tutor song (Beecher & Brenowitz, 2005), ranging from imitation (faithful matching), improvisation (some variation on tutor song), to invention (no similarity to tutor song) (Beecher & Brenowitz, 2005).

In a previous experiment, I showed that young Costa's hummingbirds can produce faithful copies of tutor songs that contain atypical features (e.g., backwards song or upsweep only; see Chapter 1). Therefore, Costa's hummingbirds do not seem to have an inflexible song template encoded within their brain that needs only be activated by hearing Costa's song. Instead, they apparently memorize tutor song, including any slight abnormalities, as long as it has some resemblance to typical Costa's song. However, in this previous experiment some birds produced songs that only were slightly different than the tutor song (see Chapter 1). For example, a bird tutored with Costa's Upsweep only, sang an upsweep but did not sing the clicks that accompany the upsweep. This was assumed to reflect individual differences inabilities to learn. An alternative explanation is variation in improvising on tutor song. One line of evidence consistent with this idea is incorporation of scolding into a song repertoire. In this experiment as well as in a previous study (Chapter 1) most birds included scolding in their song repertoire. This is likely the result of juvenile birds hearing scolding from the adult model when they also received playback. Therefore, they memorized scolding as well as tutor song, and developed a song repertoire more complex than typical of wild birds. An ability to improvise could explain the diversity of song observed in this experiment. However, this result is somewhat paradoxical given the lack of variation in wild Costa's song. To my knowledge, wild Costa's hummingbirds never include scolding in their song and typically produce one stereotyped song (Williams & Houtman, 2008).

Do Hummingbird Sensitive Phases Close?

The traditional view of song acquisition in Oscine passerines is that they are either open-ended or closed-ended vocal learners(Nottebohm, Nottebohm, & Crane, 1986). To be categorized as an open-ended vocal learner, a species must be able to learn acquire (and produce) new vocal patterns beyond its first year of life. Open-ended learning can be either a seasonal re-opening of the sensitive phase, or a sensitive phase that never closes (Beecher & Brenowitz, 2005; Doupe & Kuhl, 1999). Conversely, in closed-ended vocal learners, once the sensitive phase closes, it does not re-open, and the bird is unable to learn new song material (Brenowitz & Beecher, 2005). My data do not reveal whether (or when) the sensitive phase closes in Costa's hummingbird. It appears that the concluding phase of our tests in the late tutoring period (day 115-145) was still within the sensitive phase (Knudsen, 2004). But it is also possible that the normal sensitive phase of vocal learning in the Costa's hummingbird is very long, extending late into development and possibly into adulthood. At this time, I have no evidence that

Costa's hummingbirds undergo a closing of their sensitive phase, as seen in some Oscine passerines (e.g., Zebra Finch, *Taeniopygia guttata*; Eales, 1985). Future studies should focus on sequential hierarchical learning of different song elements (e.g., Ziegler & Marler, 2008), as well as establishing the existence of open-ended learning (i.e., can adult birds acquire new songs).

It is not completely clear how these experimental results model the song acquisition process in nature, in large part because the movements and intraspecific interactions of young male Costa's hummingbirds' post fledgling are poorly understood. Adult males do not provide parental care so there is no obvious male parent to learn from. Anecdotally, I have observed hatch year males near adult male territories, and the young birds are occasionally courted in the breeding season by adult males (personal observation). These behaviors could provide song learning opportunities.

Here I present the first experiments done to examine the sensitive phase of vocal learning in any hummingbird. Combined with previous experiments, the results show considerable similarities between hummingbird and songbird vocal learning despite the independent evolution of this ability in these two highly divergent lineages.

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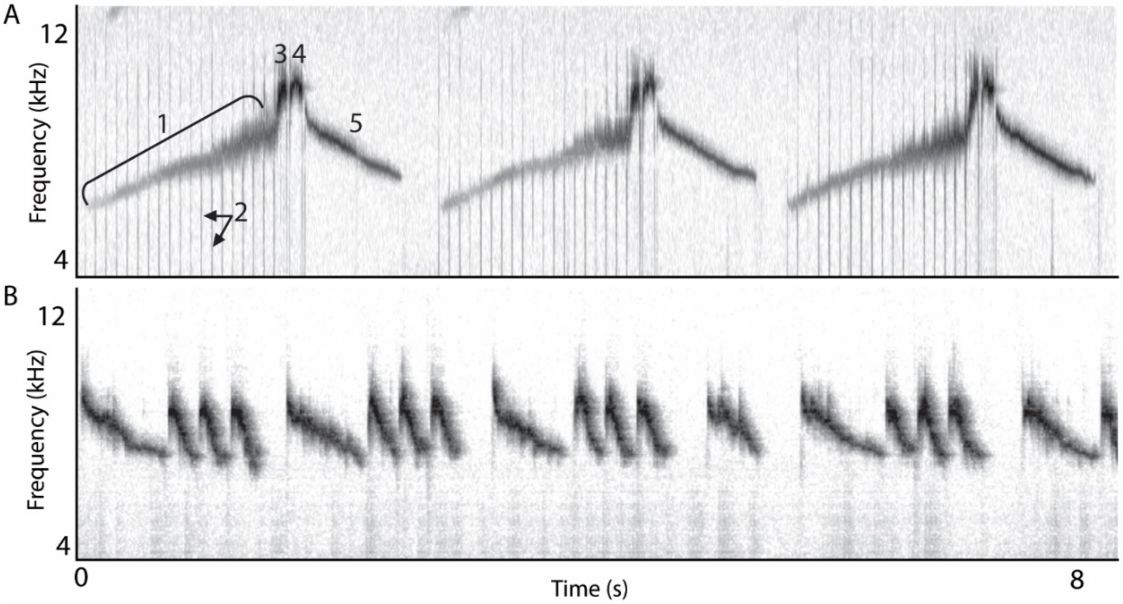

Figure 2.1: Normal Costa's song and Isolate Costa's song. (A) Costa's song is composed of a frequency upsweep (1) with a series of clicks (2), two short notes at the highest frequency (3 & 4), and then a frequency down-sweep (5). Songs are usually sung in bouts of several songs sung in succession. Bouts contain varying numbers of songs, from single songs to over 20. (B) Costa's Isolate song is composed of a series of frequency down-sweeps. Isolate song is produced when a Costa's hummingbird is raised without the correct tutoring regime. Spectrograms parameters (all figures): 44.1 kHz, FFT window size of 1024 samples.

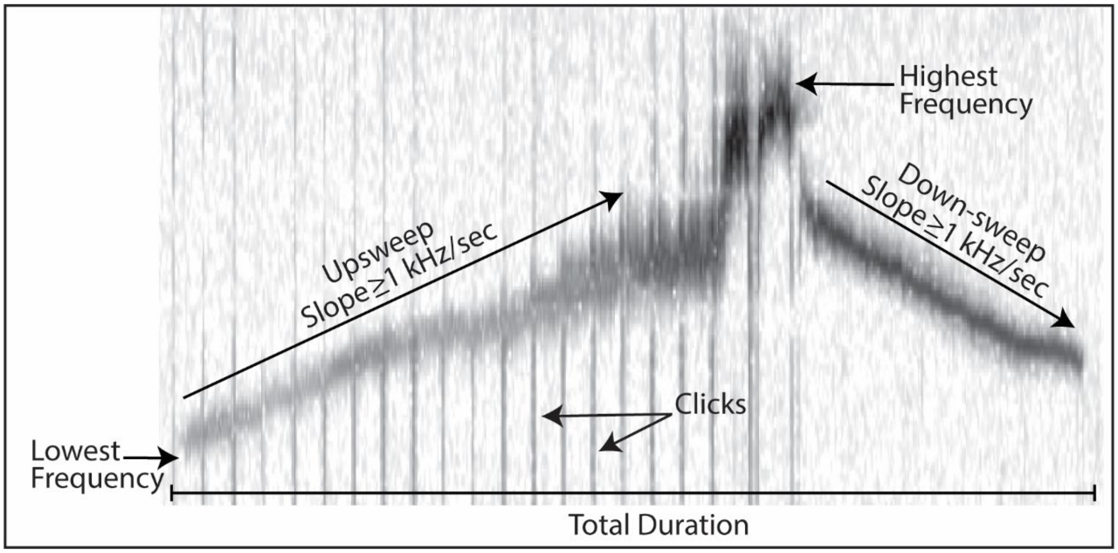

Figure 2.2: Song features used in song scoring analysis. We chose 11 elements song elements to score for each song including: upsweep, clicks, down-sweep, song duration, high frequency, low frequency, and frequency range. For both the upsweep and down-sweep in addition to being present we determined that the slope of these two song components needed to be greater or equal to 1kHz/sec. We examined both the presence of the Click element as well as if the Clicks were in the appropriate part of the song, just the upsweep. Many of the birds produced both as upsweep and down-sweep, but they were not in the correct order, therefore we added an additional category of upsweep and down-sweep in correct order. We examined each song’s high and low frequency, as well as the total frequency range. Some birds produced songs that fell into the high frequency range but failed to fall into the low frequency range, and vice versa. The total frequency range category allowed us to see if the song fell over the entire frequency range that a normal Costa’s song encompasses.

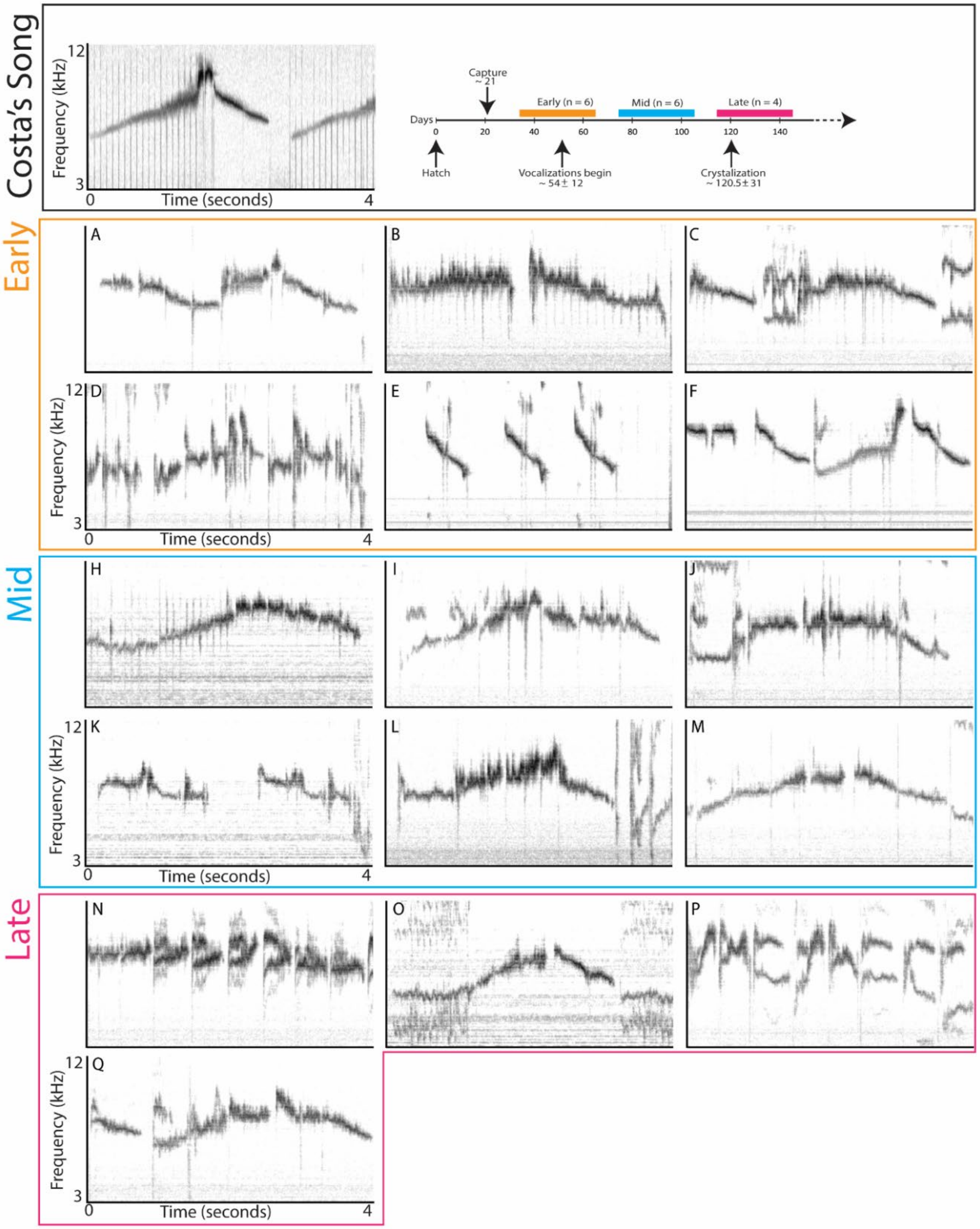

Figure 2.3: Songs developed by 16 individually isolated Costa's Hummingbirds tutored with Costa’s song in the presence of live model (adult male Costa's

hummingbirds) in one of three possible treatment periods. Orange panel: Early tutoring treatment 35-65 days post-hatch (dph). Blue panel: Mid tutoring treatment 75-105 dph. Pink panel: Late tutoring treatment 115-145 dph. All spectrograms are 4 seconds and 3-12 kHz.

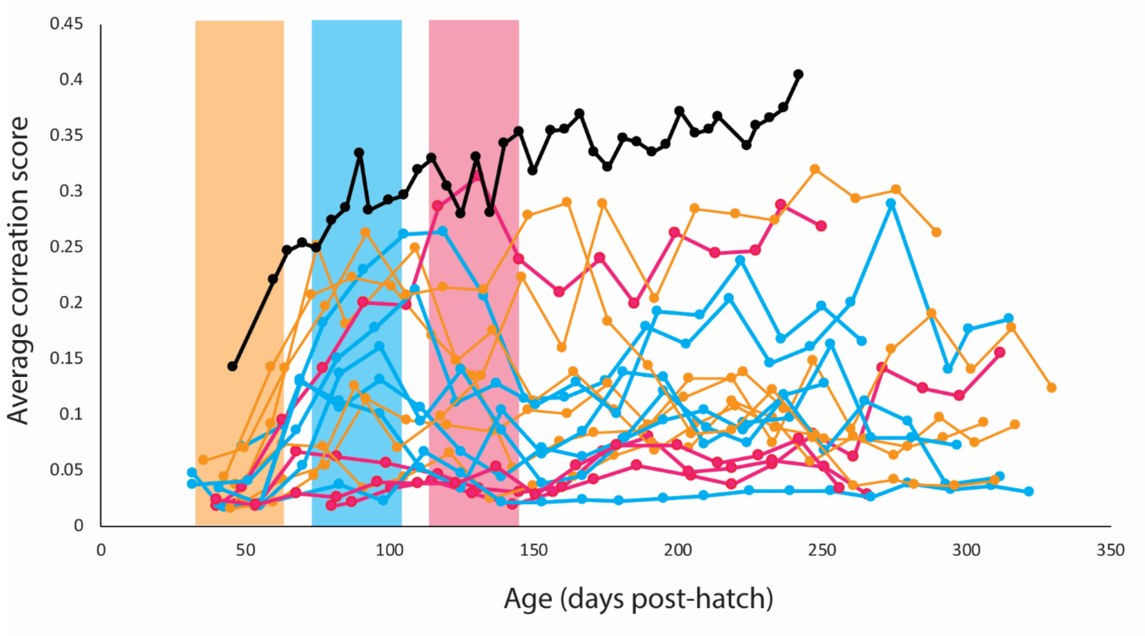

Figure 2.4: Learning curves of average cross-correlation scores over song ontogeny for 16 Costa's hummingbirds tutored with Costa's song in the presence of live model (adult male Costa's hummingbirds) in either Early (35-65 dph, orange), Mid (75-105dph, blue), or Late (115-145dph, pink) tutoring period. Each time point represents the average of cross-correlation scores of 20 songs compared to the Costa's tutor song. Cross-correlation scores were calculated in Raven with the settings: normalized, linear power. All recordings made by SAP had a sampling rate of 44.1 kHz, FFT window of 60 band pass filtered from 4 to 13kHz to remove background. The black line represents the learning curve of Goose, a bird from a previous experiment (Chapter 1), that learned to sing Costa's song. No significant difference was found between the song score and the three tutoring treatments (MLM: Early × Late t-value = -0.71, df = 13, p = 0.49; Early x Mid t-value = -0.54, df = 13, p = 0.6).

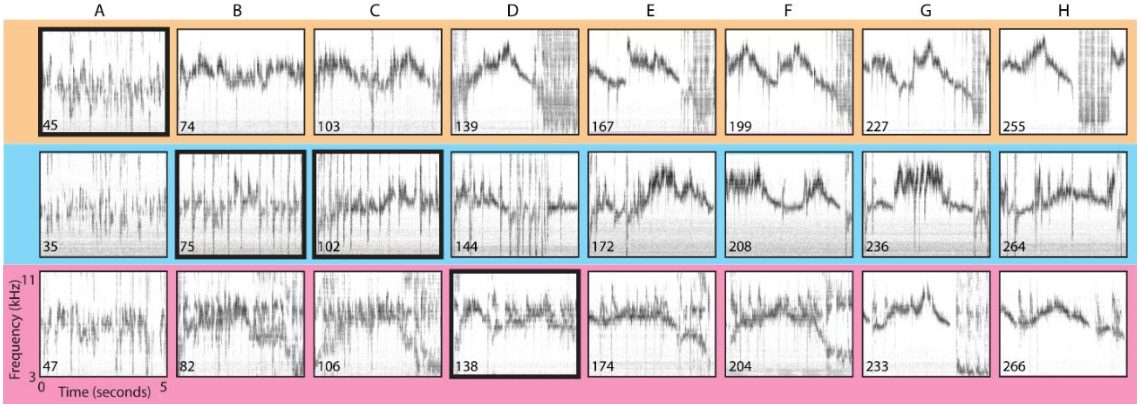

Figure 2.5: Song ontogeny of three birds, one from each of the tutoring treatment Early (35-65 dph, orange), Mid (75-105 dph, blue), or Late (115-145 dph, pink). Boxes with bolded outline represent songs sung while tutoring was taking place and the age of a bird at time of recording is located in the lower left corner of each spectrogram. All spectrograms are 5 seconds, 3 – 12 kHz.

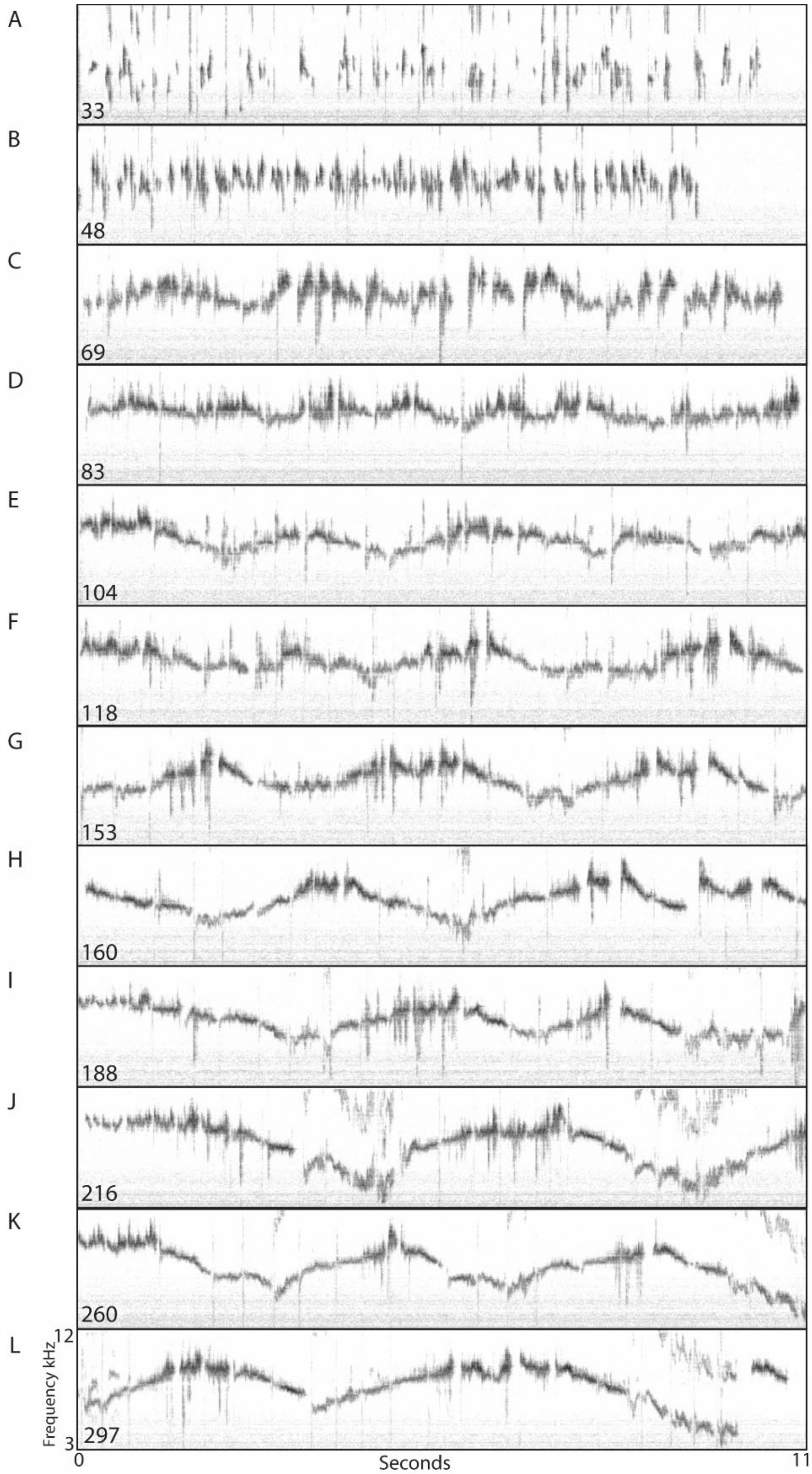
A
33
B
48
C
69
D
83
E
104
F
118
G
153
H
160
I
188
J
216
K
260
L
297
12
Frequency kHz
3
0
Seconds
11

Figure 2.6: Song ontogeny of bird tutored Mid (75-105). Age at recording is in the lower left corner. The first two rows (ages 33 and 48 dph) represent subsong stage. Row C (age 69) the first signs of plastic song emerge, with a slight upsweep and down-sweep formation. Rows D-L, songs continue to incorporate upsweeps and down sweeps, in the correct order, and clicks start to appear. Songs are much longer than the 2.4 seconds of normal Costa's song, and songs are sung in a continuous sting of upsweeps and down-sweeps. Spectrograms parameters (all figures): 44.1 kHz, FFT window size of 1024 samples. All spectrograms are 11 seconds, 3 – 12 kHz.

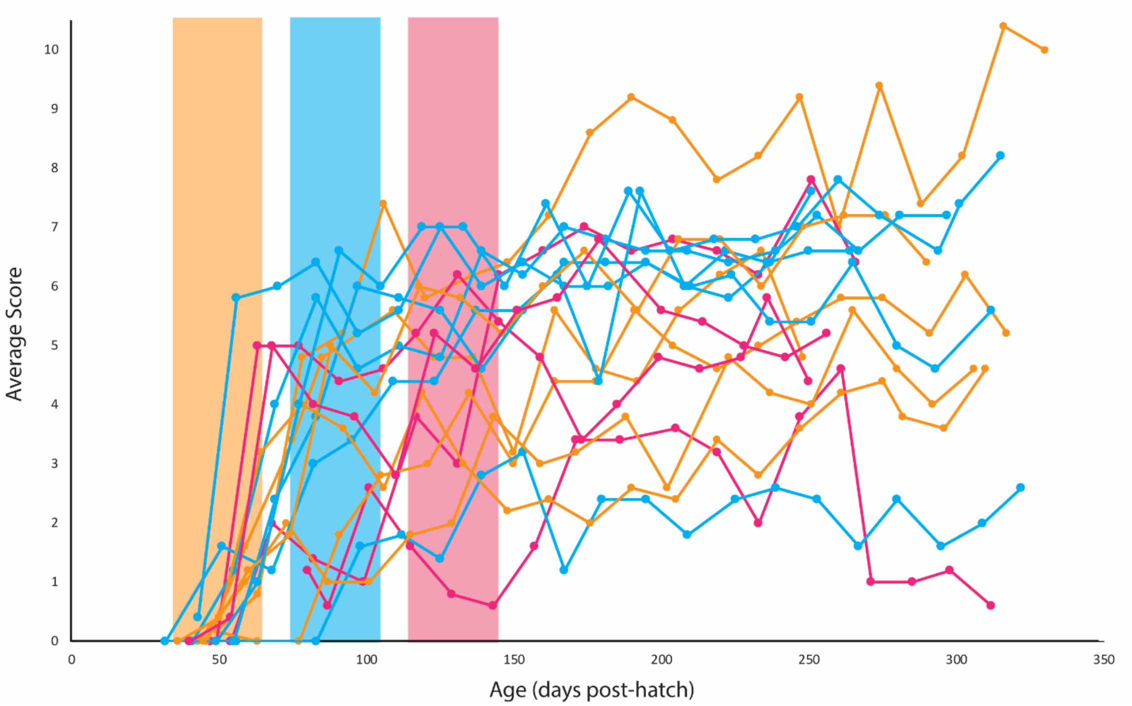

Figure 2.7: Song Scoring Analysis for 16 Costa's hummingbirds tutored with Costa's song in the presence of live model (adult male Costa's hummingbirds) in either Early (35-65 dph, orange), Mid (75-105, blue), or Late (115-145, pink) tutoring period. Each point represents the average score of 5 songs. Each song was assessed for 11 different elements. There was no significant difference in song score between the three tutoring treatments (MLM: Early x Late t-value = -0.37, df = 13, p = 0.72; Early x Mid t-value = 0.54, df = 13, p =0.6).

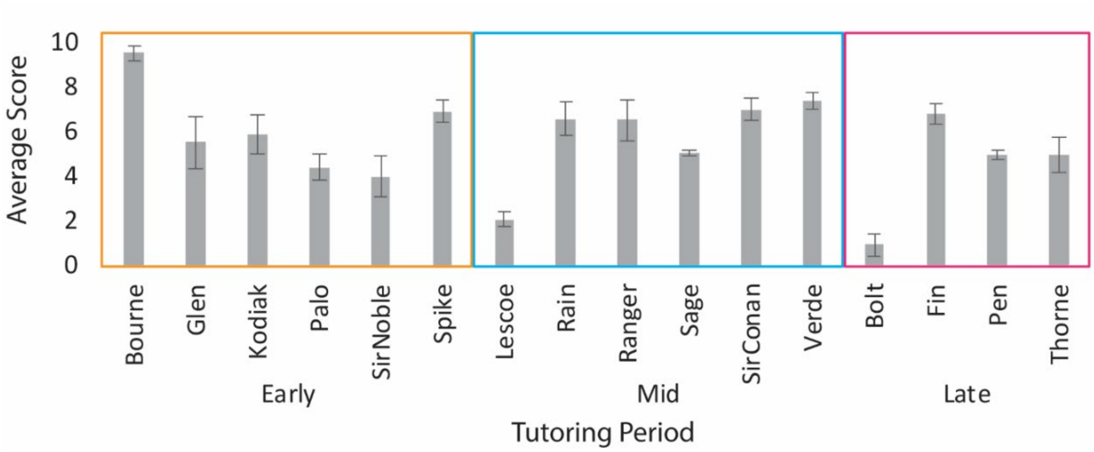

Figure 2.8: The average song score (the last three days sampled; average age 276 ± 29) for birds in each of the three tutoring treatments, treatment Early (35-65 dph, orange), Mid (75-105 dph, blue), or Late (115-145 dph, pink. Each bar represents the average song score, with standard deviation, of the last three days sampled (15 songs). No significant difference was found between the song score and the three tutoring treatments (MLM: Early x Late t-value = -0.37, df = 13, p = 0.72; Early x Mid t-value = 0.54, df = 13, p =0.6).

Table 2.1: Categories for Song Scoring Analysis

Category	Score Yes	 No
Upsweep present	+1	0
Upsweep slope ≥ 1 kHz/sec	+1	0
Clicks present	+1	0
Clicks present only in upsweep	+1	0
Down-sweep present	+1	0
Down-sweep slope ≥ 1 kHz/sec	+1	0
Upsweep and down-sweep in correct order	+1	0
Song Duration Range 2.081-2.681 sec	+1	0
Highest frequency between 11.45-12.45 kHz	+1	0
Lowest frequency between 6.075-8.875 kHz	+1	0
Frequency range: song falls within the both the highest and lowest frequency ranges	+1	0

Chapter 3: Open-ended vocal learning in Costa's Hummingbird

Abstract:

Here I examine open-ended vocal learning in the Costa's hummingbird (*Calypte costae*). Open-ended learning is the ability of a vocal learning animal to incorporate new material into their vocal repertoire after the first year of life. Open-ended vocal learners have either a sensitive phase that never closes or a seasonal re-opening of the sensitive phase, where they are able to memorize and learn to produce new song material. In a prior experiment, I raised 18 individually housed Costa's males in isolation chambers from day 21 post-hatch until they were approximately 1 year old. During that time, they were tutored, learned song, and developed individual-specific stable song. In this experiment I moved 14 of these ~1-year-old birds to an outdoor aviary, placing them in physical, visual, and acoustic contact with other adult Costa's hummingbirds that sang different songs, for eight months. A second group of four 1-year-old birds was kept in isolation for their second year as a control. I then moved these birds to the outdoor aviary, so that their first exposure to other hummingbirds came at 2 years of age. All of the birds in the initial treatment group changed their songs to match the other birds they were living with within two months of exposure, while the control birds that remained in isolation for a second year did not. These second-year birds then changed their songs when moved to the aviaries and exposed to conspecifics for the first time at 2 years of age. This result suggests that Costa's hummingbirds are capable of open-ended vocal learning, and it is possible that learning to match territorial neighbors may be important in Costa's Hummingbird ecology.

Introduction:

The ability to learn vocalizations based on auditory experience is found in three lineages of birds: passerines, parrots, and hummingbirds, as well as a few groups of mammals, including humans (Nottebohm, 1972). In many of these animals the ability to learn vocalizations is restricted to early, pre-adult life (Marler & Slabbekoorn, 2004). In many birds, vocal learning is often restricted to the period between fledging until arrival and settlement on the breeding grounds (Marler & Slabbekoorn, 2004). Vocal learning birds restricted to learning vocalizations early in life are closed-ended learners (Thorpe, 1958). However, some vocal learning species, including humans, some songbirds, parrots, and at least one hummingbird retain the ability to learn new vocalizations as adults (Araya-Salas & Wright, 2013; Chaiken, Böhner, & Marler, 1994; Marler & Slabbekoorn, 2004). This is considered open-ended learning (Nottebohm, Nottebohm, & Crane, 1986).

Vocal learning birds go through two main phases of learning. The first is the sensory phase, during which species-specific vocalizations are listen to and memorized (Marler & Peters, 1987). The next stage is the sensory-motor phase, during which the bird makes vocalizations and tries to match them to the song memorized during the sensory phase (Marler, 1970). During the sensory-motor phase the vocalizations go through three stages: subsong, plastic song, and adult song (Marler & Slabbekoorn, 2004). In closed-ended vocal learners, once the song reaches adult song it is said to crystallize and does not change later in life (Thorpe, 1958). In open-ended learners, two possible mechanisms could allow new song material to be added: (1) the sensitive phase never closes, and the bird can continuously learn new song material (Doupe & Kuhl, 1999); (2) each year the sensitive phase re-opens and neural plasticity in the song nuclei increases, permitting learning of new material (Doupe & Kuhl, 1999).

Evidence for open-ended vocal learning arises from experiments that track songs over multiple years to see if new song material is added after the first breeding season. A challenge in many open-ended song learning experiments is ascertaining whether new acoustic components added late in life are newly learned, as opposed to being learned early in development but not sung until later (Chaiken et al., 1994; Marler, 1997). A method to control acoustic exposure is to rear birds in an isolated acoustic setting from the nestling stage (or earlier).

Hummingbirds remain a largely unexplored group of vocal learners and evolved this ability independently of the evolution of vocal learning in the parrot-passerine lineage (Jarvis et al., 2014). To date only one experiment has suggested open-ended vocal learning in a hummingbird. Araya-Salas and Wright (2013) recorded songs from long-billed hermit (*Phaethornis longirostris*) leks over four breeding seasons. Nine out of forty-nine males showed song replacement, and the replacement songs were not produced until after the first year of life (Araya-Salas & Wright, 2013). The ability to learn past the first year has not been examined in any other hummingbird. Previous experiments on Costa's hummingbirds *(Calypte costae)* revealed that their vocal ontogeny is similar to songbird vocal learning (Chapter 1), with a sensory phase, sensory motor phase, subsong, plastic song, and adult song.

In this study I performed three experiments examining open-ended learning in the Costa’s hummingbird. The subjects (n = 18) were males that had previously undergone vocal learning experiments over their first year of life. For each individual I had a complete record of all the songs, sounds, and other *C. costae* they were exposed to since they were 21 days old, as well as a complete record of all of the vocalizations each individual produced (Figure 1). I moved these year-old birds into a large communal aviary where they were exposed to songs from each other, from two male Anna’s x Costa’s hybrids that had learned isolate song from previous occupants of the aviaries, and from free-living Anna's and Costa's hummingbirds. If *C. costae* are open-ended vocal learners, individuals previously kept in acoustic isolation may change their songs after exposure to other individuals with different songs. Closed-ended learning would be indicated if songs do not change after exposure to novel song.

Methods:

Animal Husbandry

Husbandry procedures are summarized here; further details are as in Chapter 1. Adults are fed adult hummingbird diet, Nektar plus (Nekton Produkte, Keltern, Germany). The hummingbirds used for these open-ended vocal learning experiments were housed in one of two possible locations, an isolation room or an outdoor aviary.

Isolation Chambers:

The isolation room was the location where all of the song recordings took place, as well as where the isolation birds for experiment 2 were housed. The isolation room contained 12 individual isolation chambers, each consisting of an outer wood box, a layer of cotton batting insulation, an inner plastic box (acrylonitrile butadiene styrene), and a powder-coated zinc 91.4 × 50.8 × 55.8 cm cage (Quality Cage Company, Portland Oregon). Each chamber had a condenser microphone (Pyle PDMIC45 range 20 Hz-16 kHz, 2 puck LED lights, and an air supply of 10 air changes/hour. The microphones and speakers were connected to an M-Audio Eight High-resolution USB 2.0 Audio Interface and the computer software program Sound Analysis Pro (Tchernichoviski et al. 2000). I used Sound Analysis Pro (hence forth SAP), to acquire all song recordings.

I took periodic recordings of songs produced in isolation chambers. Aviary-housed birds were temporarily placed in an isolation chamber for several hours and sometimes overnight, to record their individual songs. After recording at least five song bouts that contained at least 10 individual songs, I returned birds to the aviary. Similar periodic recordings were not feasible in the outdoor aviary because there was too much background noise and often multiple birds singing simultaneously.

Outdoor Aviary:

The outdoor aviary consisted of seven individual enclosures that keep birds physically separated but in visual and acoustic contact with occupants of other enclosures.

Open-ended Experimental Set-up:

Subjects (n = 18) were male Costa's hummingbirds that had previously undergone vocal learning experiments over their first year of life (Figure 1).

Experiment 1:

Eight Costa's males captured in February through June 2016 (~21 days post-hatch) were housed individually, then tutored from day 35-125 with either a Costa's-like song or non-Costa's-like tutor song in the presence of an adult male Costa's. Five out of six birds that heard Costa's-like song learned to produce good matches of their tutor song. The two birds tutored with Anna's song did not learn the tutor song, and instead produced isolate song (the song that develops when a male *C. costae* is raised in acoustic isolation).

On 25 November 2016, when the birds were on average 242 ± 31 days post hatch, I took the covers off of the isolation chambers, thus permitting acoustic contact. I subsequently took one more recording from each bird and on 25 January 2017 (average 303 ± 31 days post hatch), I randomly placed them in two enclosures (Aviary 1 and Aviary 2, 4 birds each) in the outdoor aviary. The birds remained in these groups until the end of the experiment the following November. Both groups were in acoustic and visual contact with the other group, but the two enclosures were separated by 1.5 m. All of these individuals served as adult models for another experiment examining the sensitive phase of learning. This is relevant because while being used as an adult model, they are exposed to the pupils they were models for and to Costa's playback. However, there is no evidence that acting as an adult model had any effect on the bird's songs. I took recordings of all of the birds' songs every 2 months.

Experiment 2:

These eight birds had been captured at 21 dph and given speaker playback of Costa's song in the presence of an adult male over one of two tutoring time periods: 35-

65 dph or 75-105 dph. Birds received one hour of tutoring on 20 days over their 30-day treatment period. Even though all birds were received the same tutor song (wild-type Costa's song), each individual acquired a unique abnormal Costa's song (see Chapter 2).

Experiment 1 did not have a control group that stayed in isolation for a second year. Therefore, for experiment 2, I had two treatment groups, an exposure group and an isolation group (average bird age at beginning of experiment 373 ± 35 dph). The isolation group consisted of four birds that stayed in their isolation chambers ('isolation' birds) and continued to not be exposed to other individuals over their second year of life. The second group of four 'exposure' birds were moved to the outdoor aviary and were exposed to each other, over their second year of life. The four exposure birds acted as adult models for another experiment, and thus were exposed to Costa's song playback.

Individuals were assigned to a group based on the song they developed. As mentioned above, each bird produced a unique song, and individuals differed in the similarity of their songs to normal Costa's song. Therefore, I separated the birds into two groups, so each group contained a mixture of song types ranging from high to low similarity to Costa's songs. Unlike experiment 1, exposure males were not housed with the same birds throughout the experiment. Hummingbirds are aggressive and form dominance hierarchies when housed communally (Mobbs, 1982). In experiment 2 this created husbandry problems: dominant birds sometimes excluded subordinates from feeders, which would cause them to starve if we did not intervene. The solution was to disrupt the hierarchy by occasionally exchanging males between aviaries. Therefore, experiment 2 males were housed with different aviary-mates over the course of the experiment. I took recordings of all individuals once a month for experiment 2, instead of every two months as in experiment 1.

Experiment 3:

Four of the six birds used for experiment 3 were the birds kept in isolation in their second year of life from experiment 2. The two other birds were captured at 21 dph and given speaker playback of a Costa's song in the presence of an adult male from day 115-145 dph. Birds received one hour of tutoring on 20 days over the 30-day treatment. Each bird developed their own unique abnormal Costa's song (see Chapter 2). I moved the four isolation birds (average age 706 ± 37 dph), and the two additional birds (average age 315 ± 21 dph) to the outdoor aviary. I initially housed birds is two randomly assigned groups of three, but after aggression developed (as in experiment 2), the birds were split into a group of three, one pair, and one bird was housed alone. None of these birds were used as adult models. Due to time constraints experiment 3 had only one post exposure recording which took place one month after moving to outdoor aviary.

Analysis of pre and post exposure song:

I compared each individual's post-exposure songs with its pre-exposure songs with a cross-correlation test on the spectrograms (44.1 kHz , FFT window of 256, band pass filtered from 13kHz to 4kHz to remove background sound) in the program Raven v.1.5 (http://www.birds.cornell.edu/brp/raven/).The spectral cross-correlation function is a quantitative measure of similarity and returns a score between 0 and 1, with a score of 0 indicating the spectrograms are have no frequency overlap, and 1 indicates the spectrograms are identical (Raven 1.5 settings: normalized, linear power). All recordings made by SAP had a sampling rate of 44.1 kHz 16-bit depth.

For each bird-day sampled, I selected 10 individual songs, selected randomly from multiple recordings from the same day. The only requirement was that the song was complete and not a partial recording (e.g., due to the recording starting partway into a song or cutting off before a song ended). Each recording was band pass filtered from 4 to 13kHz to remove background sounds (e.g., air pumps). These 10 songs were the base unit that were then compared to other sets of 10 songs using the cross-correlation function in Raven. Therefore, each comparison of two sample units to each other compared ten songs from the first sample unit with ten songs from the second sample unit for a total of 100 cross-correlations. I then took an average of these 100 cross correlations and used this single average cross-correlation value as the input for statistics.

The comparisons were of two types: either I compared the same bird to itself at a different timepoint (hereafter, self-similarity), or I compared that bird to a different birds at the same timepoint (hereafter, between-bird similarity).

Statistics:

I hypothesized that, if the birds exhibited open-ended vocal learning, that their songs would change after exposure to other birds. Therefore, their self-similarity cross-correlation scores would decrease after being exposed to other birds. However, birds held in isolation might also decrease in self-similarity score. Therefore, I predicted that there would be an interaction effect between treatment and isolation-- birds held in isolation would change their songs less than birds exposed to other birds. I tested whether the bird's cross correlation scores decreased from timepoint 1 (pre-exposure) to timepoint 2 (post-exposure) with an ANOVA, with bird as a random factor, treatment (isolated or not) as a fixed factor, and a treatment x timepoint interaction effect to test my hypothesis.

I also hypothesized that, if the birds exhibited open ended vocal learning, that they would learn to match the songs of the other birds to which they were exposed. Therefore, I predicted that, when compared to the songs of other aviary-mates, that the between-bird cross-correlation scores of birds would go up after exposure, relative to the between-bird cross correlation scores prior to exposure. Out of 18 birds, I did not compare the songs of each of the 17 other birds. Rather, I only compared the scores of future aviary-mates to each other. I compared 4 exposed birds from Experiment 1, aviary 1 to each other (treatment 1) (6 comparisons per timepoint); 4 exposed birds from experiment 1 aviary 2 to each other (treatment 2); 4 exposed birds from experiment 2 all aviaries to each other (treatment 3), 4 non-exposed birds to each other as a control (treatment 4), and 6 exposed birds from experiment 3 to each other (treatment 5). I tested whether the bird's cross correlation scores increased from timepoint 1 (pre-exposure) to timepoint 2 (post-exposure) with an ANOVA, treatment as a fixed factor, and a treatment x timepoint interaction effect to test my hypothesis that the control treatment (#4) would not show the same effect as the other groups.

Results:

Experiment 1

The eight male Costa's, previously housed in isolation for a year (in which they developed different song types), and then moved to an outdoor aviary and separated into two stable groups, changed their song after being exposed to each other and tended to match the songs of aviary mates (Figure 2).

Aviary #1: All of the birds in aviary 1 started with different songs (Figure 2, A-B). Two of the birds began the experiment singing Costa's isolate song, though with different organization of the down-sweeps (Figure 2, A and D) and the other two birds started with Costa's like songs, one singing backwards Costa's song (Figure 2, B) and the other singing Costa's dive sound (Figure 2, C). At the end of the experiment, all birds converged on a song very similar to Costa's isolate song, a series of down-sweeps (Figure 2, column 4).

This first experiment proceeded in a different way than the other experiments. There was a brief period when the birds were able to hear, but not see or physically interact with each other. The second recording (Figure 2, 2) show song produced after birds were in acoustic contact with each other. The self-similarity scores decreased for all individuals (Figure 2, self-similarity scores lower right corner of spectrogram). The following two recordings (Figure 2, columns 3 and 4) show further changes in song after the birds were in visual, auditory, and physical contact. The two birds that started with Costa's like song were exposed to isolate song (Figure 2, B (heard 172 bouts) and C (heard 42 bouts), produced by the adult model, while they were being tutored during their first year of life.

Aviary #2: All birds started with different songs (Figure 2, E-H). At the second recording the songs began to change with all birds having a lower self-similarity score then before exposure (Figure 2, column 2). After birds were moved into the aviary, songs changed and converged on a novel song (Figure 2, column 3 and 4) that had little resemblance to any one individual's song, and therefore is a new song. This song is within the normal Costa's frequency range, from 6-12 kHz, but is shorter, lasting about 1.5 seconds compared to the typical duration of 2.4 seconds. It begins with a short down-sweep, followed by a short upsweep, and finishes with a very short down-sweep. This song does not resemble any vocalization that any of the birds heard at any point after the age of 21 dph in their life.

Experiment 2

All individuals in experiment 2 started with a unique song (Figure 3). These songs contained considerable variation and were not stereotyped adult songs with little variation song to song, as the songs of birds in experiment 1 were, presumably due to not receiving tutoring for a sufficient amount of time (see Chapter 2).

Isolation birds: the birds that remained in isolation produced songs that were acoustically and spectrographically similar (Figure 3) throughout the entire experiment. Additionally, these birds retained a high degree of within bird song variation. These birds never reached the stereotyped 'crystallized' adult song stage thus their songs have remained variable.

Exposure birds: The four exposure birds' songs changed after being exposed to other Costa's hummingbirds (Figure 3). Unlike experiment 1, the exposure birds did not all converge on a single song, possibly because they could not be housed in consistent groups. All individuals produced songs that contained mostly down-sweeps, similar to Costa's isolate song.

Experiment 3:

Experiment 3 was comprised of four (isolation) birds from experiment 2, which were kept in isolation for their second year of life (Figure 4, A-D), and two additional birds that lived in isolation for just their first year of life (Figure 4, E-F). The songs of the five birds housed together were made up of short tonal notes that increase and decrease in an undulating pattern (Figure 4, B-F). Despite the decrease in self-similarity correlation score, the changes are small for the bird that was housed alone (Figure 4, A).

Analysis:

Songs of Costa's hummingbirds that were reared in isolation during their first or first and second year of life changed after aviary exposure to other Costa's hummingbirds (Figure 5). To measure changes in song for all of the birds in the experiments ($n = 18$), I first examined each bird's self-similarity scores-- did their post-exposure songs resemble their pre-exposure songs. The timepoint 2 self-cross-correlation scores significantly decreased relative to timepoint 1 ($p = 0.0043$, $t= 3.16$, $df = 43$), but there was no effect of treatment ($p = 0.575$), bird (most $p > 0.1$; 2 birds had p values of 0.07 and 0.059), or a treatment x timepoint interaction effect ($p = 0.23$). Therefore, both exposed and isolated birds had their self-similarity scores decrease over time, irrespective of whether or not they were exposed to other birds in their second year of life.

In the second analysis, where we compared different exposed bird's songs to each other, several variables were significant (Figure 6, Table 1). Timepoint was significant ($p = 0.0001$, $t = -8.73$, $df = 78$), as were treatments 1 ($p = 0.0036$) treatment 2 ($p < 0.001$), while treatments 3 ($p = 0.68$) and treatment 5 ($p = 0.25$) were not statistically significant (Table 1).

There was a significant interaction effect between experimental treatment and timepoint. Cross-correlation scores of the between-bird similarity analysis increased after exposure in experimental treatments 2, 3, and 5 (experimental treatment x timepoint interaction effects, $p < 0.0001$, 0.012 and 0.031 respectively) relative to the control (experimental treatment #4) (Figure 6, Table 1). Therefore, exposing multiple hummingbirds that originally sang different songs to each other resulted in their songs converging on each other.

Discussion:

Costa's hummingbirds isolated from day 21 post-hatch until approximately one year of age changed their songs after being visually, acoustically, and physically exposed to conspecifics (Figure 2, 3, 4, 5, and 6). Additionally, Costa's hummingbirds isolated from day 21 post-hatch until approximately two years of age also changed their song after being exposed to conspecifics for the first time (Figure 4). Therefore, Costa's hummingbirds are open-ended vocal learners.

In order to demonstrate that the change in song was the result of open-ended learning and not due to maturation, degradation, or drift of the song, I performed an open-ended learning experiment with a control group that remained in isolation during its second year of life (Figure 3, orange panel). The results of this experiment are difficult to interpret. While subjectively the songs of isolation birds do not appear to have changed (Figure 3), self-similarity cross-correlation scores indicate that they did (Figure 5).

Further, there was no difference between the scores of isolation versus exposure birds (Figure 5). In other words, the results do not support the hypothesis that songs of exposure birds would change and those of isolation birds would not (Figure 3, Figure 5). Possibly the intra-bird song variation may be obscuring differences between isolation exposure self-similarity scores. A future experiment could use birds that received sufficient tutoring and developed a stereotyped adult song, unlike the birds used in this experiment.

While the results of experiment 2 were inconclusive, an additional result suggests that the changes in song post-exposure are not simply maturation, degradation, or drift of the song, but open-ended song learning. Birds group-housed within an aviary for extended periods converged on the same song (Figure 2, Figure 3 (blue panel), Figure 4 (B-F), Figure 6), while birds housed in adjacent aviaries (in acoustic and visual but not physical contact) did not converge on the song types produced in the adjacent aviary (Figure 2). Based on previous experiments, Costa's require acoustic, visual, and physical contact for vocal learning to occur. In previous experiment, two juvenile Costa's raised in the same isolation chamber with only speaker playback of a tutor song will match each other's song and not the playback song (Chapter 1). Moreover, an adult model is necessary for a young Costa's to learn its tutor song (Chapter 1). Many songbirds also require a live tutor to learn (Marler & Slabbekoorn, 2004).

The tendency of male Costa's hummingbirds to match the song of neighbors may reveal aspects of the species' ecology. Some parrots and songbirds are known for their abilities to match calls or songs of their neighbors. Parrots learn new contact calls when group composition changes (Sewall, Young, & Wright, 2016) and many songbirds match neighbors' songs at the beginning of the breeding season (Nottebohm et al., 1986; Payne & Payne, 1997). One possible explanation is to allow birds to learn to recognize and sing the song of new territorial neighbors (Brenowitz, 1997; Nordby, Campbell, & Beecher, 2002). Song matching is thought to aid in recognition and communication with neighbors, as well as being important in competition via counter-singing (Brown & Farabaugh, 1997). It is thought that neighbors can use song degradation as a way of determining location and distance of individuals, however, this only works if birds share the song, and can recognize if degradation has taken place (McGregor & Krebs, 1984). These results suggest that Costa's Hummingbirds might do the same thing.

This study shows that Costa's hummingbirds can change their song after spending their first, or first and second, year of life in isolation. However, the results do not inform about the sensitive phase of learning. There are thought to be two mechanisms for open-ended learning in songbirds (Doupe & Kuhl, 1999; Zeigler & Marler, 2008). The first is a seasonal re-opening of the sensitive phase, generally prior to the breeding season (Doupe & Kuhl, 1999; Nottebohm et al., 1986; Zeigler & Marler, 2008). At other times the adult bird is incapable of learning new song material (Doupe & Kuhl, 1999). The second mode is a persistent sensitive phase allowing learning of new song material at any time (Beecher & Brenowitz, 2005; Doupe & Kuhl, 1999). The results from these and previous experiments (Chapter 2) do not indicate which mode of open-ended vocal learning is most applicable to Costa's hummingbirds. In all experiments, isolation birds were exposed to other *C. costae* during the breeding season. Experimental exposure of isolated birds to conspecifics outside of the normal breeding season could reveal if the ability to learn new songs is seasonal or continuous.

There is still much to reveal about hummingbird vocal learning. These experiments are the first lab-based studies of open-ended vocal learning in any hummingbird and show a second hummingbird species (in addition to *Phaethornis longirostris*, studied in the field (Araya-Salas & Wright, 2013)) to be an open-ended vocal learner. It is unknown whether open-ended learning is ubiquitous in the hummingbird clade, or as in songbirds, only found in some species. More field and lab studies are needed to reveal the nuances of open-ended vocal learning in this lineage.

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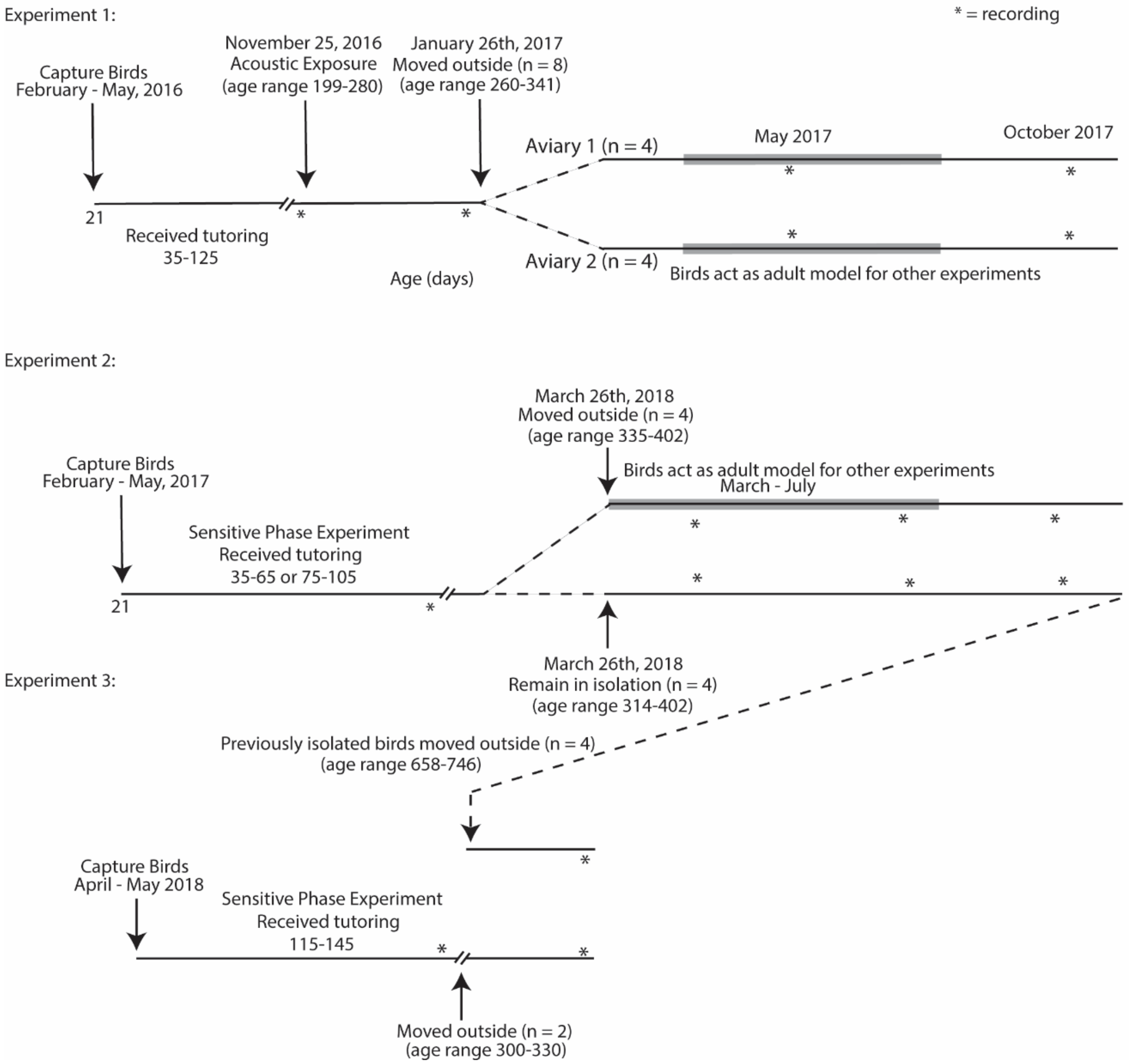

Figure 3.1: Timeline of events for birds used in open-ended vocal learning experiments. Birds used for these open-ended vocal learning experiments were collected over three breeding seasons, 2016-2018. The timeline includes which experiments the birds were involved in previously, if they acted as an adult model for another experiment, and what treatment group they were in for the open-ended experiment. Unlabeled numbers are days post hatching

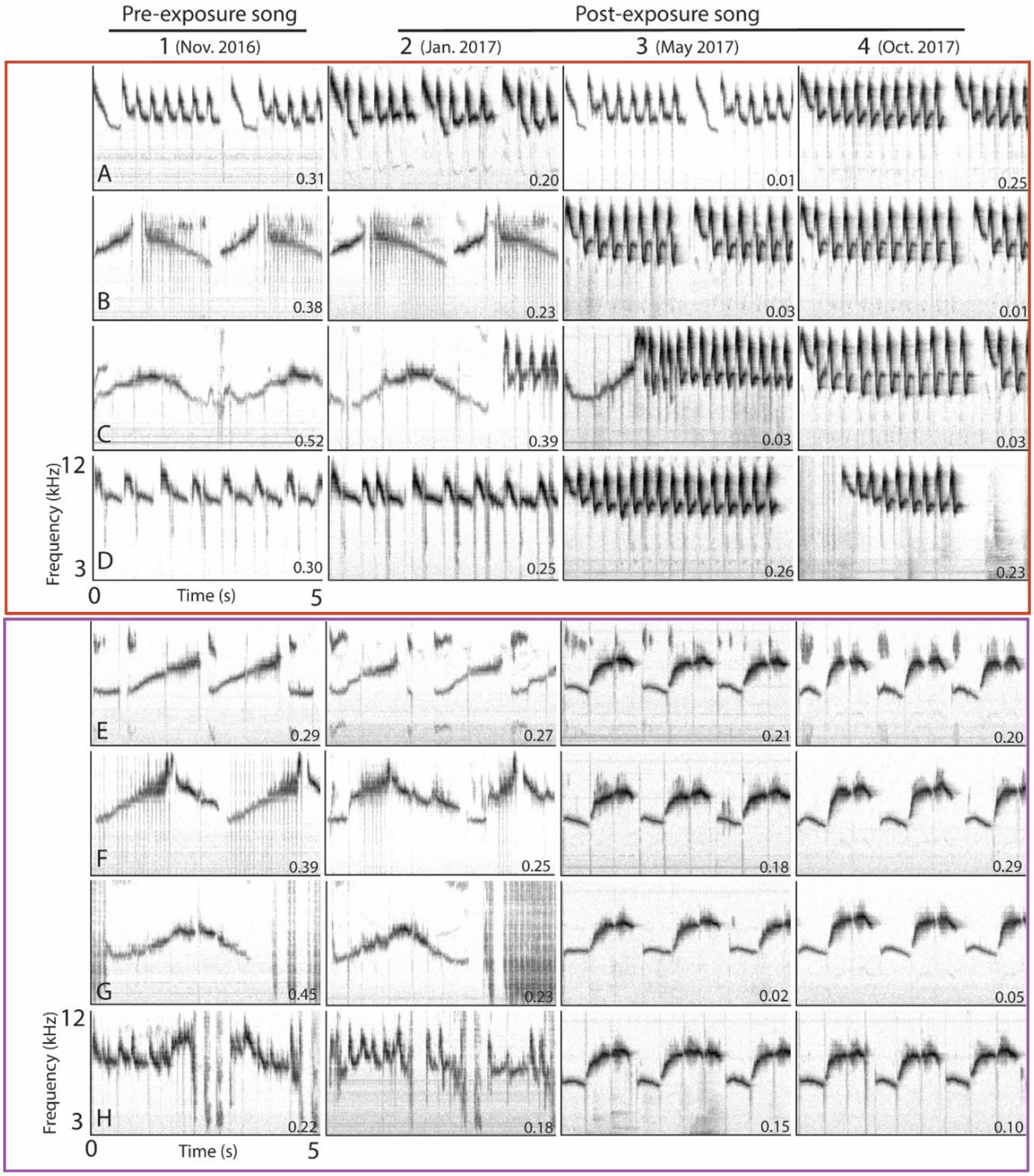

Figure 3.2: Experiment 1 results: Spectrograms of eight Costa's hummingbirds reared in isolation for 1 year then housed in two groups of 4, converge their songs with their cage-mates. Top (red): Aviary #1: A-D; Bottom (purple): Aviary #2: E-H. The two aviaries were in acoustic and visual but not physical contact. Birds within an

aviary remained within the same group throughout the entire experiment (unlike Experiment 2, shown in Fig. 3, and Experiment 3, shown in Fig 5). Note: Different individual birds received different experimental treatments in a prior experiment. Goose and Rocky, unlike the other birds, were both tutored with wild-type Costa's song; Goose's song in particular (F1) was initially a very good rendition of a 'typical' wild-type song of this species. Self-similarity cross-correlation scores are located in the lower right corner of each spectrogram. Spectrograms parameters (all figures): 44.1 kHz, FFT window size of 1024 samples. All spectrograms are 5 seconds, 3 – 12 kHz

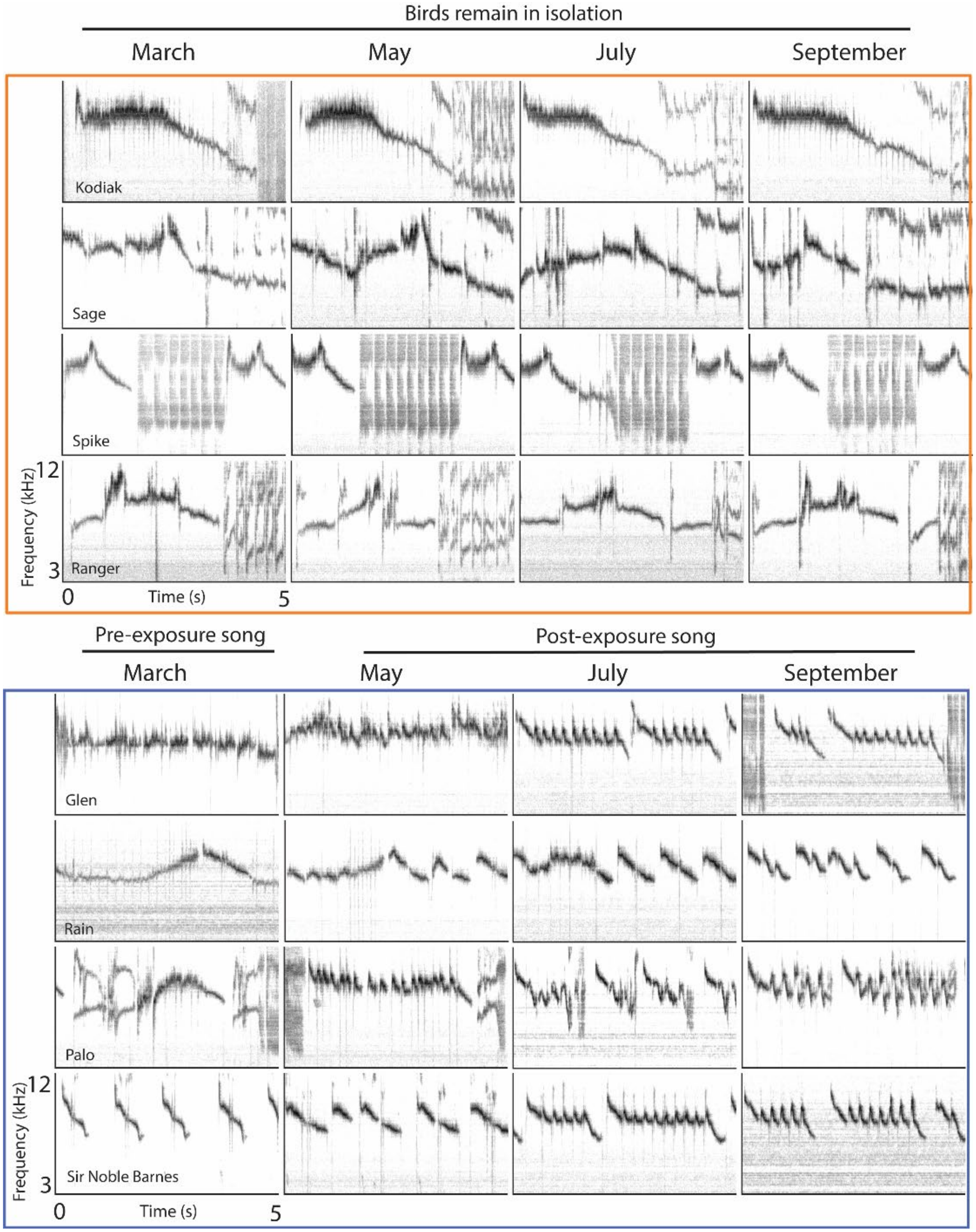

Figure 3.3: Experiment 2 results: Spectrograms of Costa's Hummingbirds' songs that remained in isolation for both their first and second year of life (N = 4, orange

panel) or that were exposed to other hummingbirds in their second year of life (N = 4, blue panel). Orange panel, songs of isolation birds appear to remain unchanged in any significant way. Blue panel are birds that were moved to an outdoor aviary and were thus in physical, visual, and acoustic contact. Unlike Experiment 1 (Figure 2), husbandry problems in the exposure group prevented us from housing the four birds together continuously, therefore these four birds did not live in the same aviary or with the same cage-mates through the experiment. First column of songs is the pre-exposure song, and columns 2-4 are post-exposure songs. Note: Different individual birds received different experimental treatments in a prior experiment. For wild-type song, see Figure 2 (Goose). Spectrograms parameters (all figures): 44.1 kHz, FFT window size of 1024 samples. All spectrograms are 5 seconds, 3 – 12 kHz. Note: recordings in Figure 2 span 11 months, whereas these recordings span 7 months.

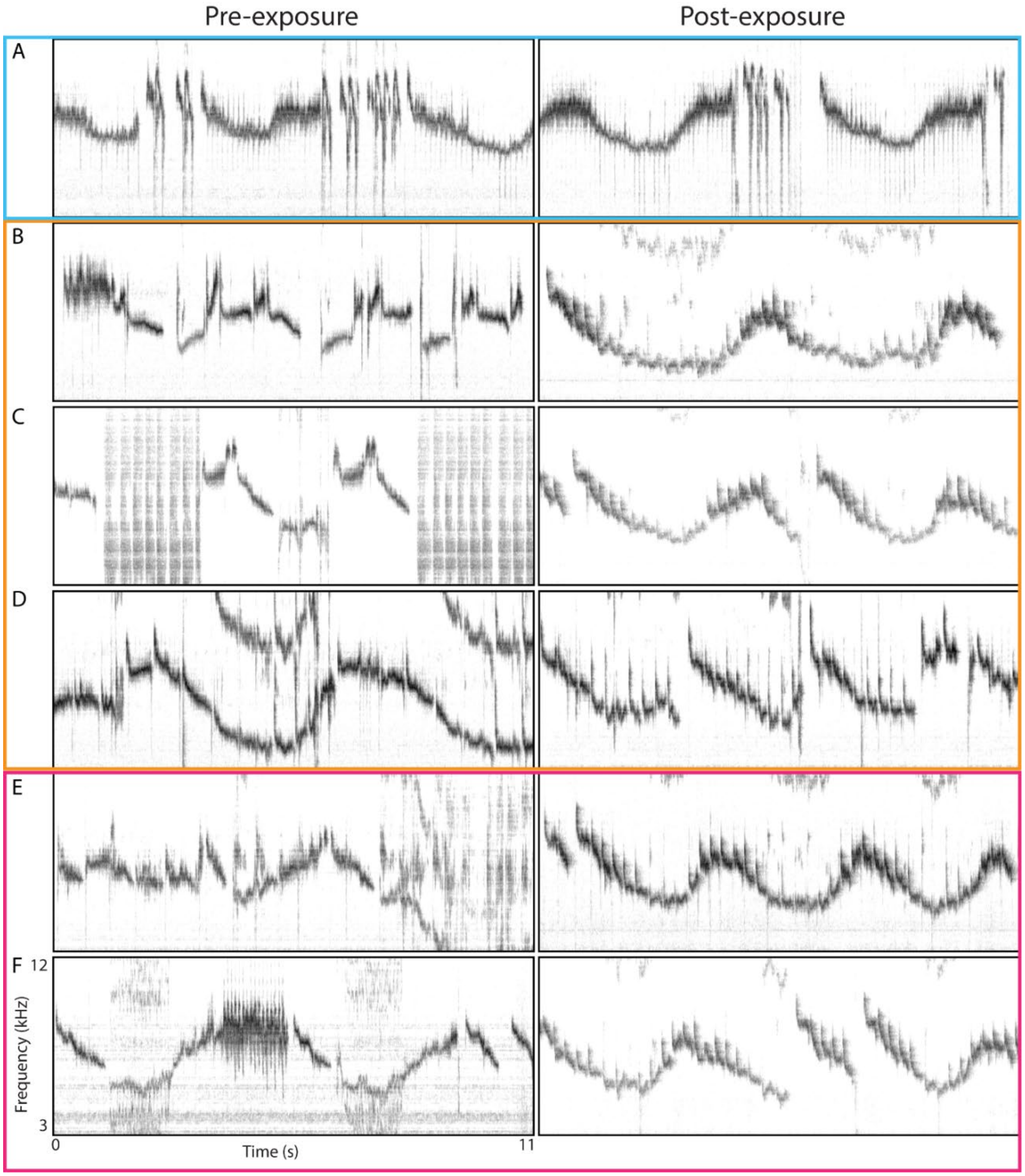

Figure 3.4. Experiment 3 results: Pre- and post-exposure Spectrograms of Costa's Hummingbirds' songs that remained in isolation for both their first and second year of life (n = 4, blue and orange panel) or placed in isolation for just their first year of life (n = 2, pink panel). Blue panel: 2-year isolation male pre- and post-exposure songs,

housed by himself in the outdoor aviary, thus being acoustically exposed to other Costa’s hummingbirds, but not in physical or visual contact. Orange panel: 2-year isolation males pre- and post-exposure songs, housed with other Costa’s. Pink panel: 1-year isolation males pre- and post-exposure songs. Spectrograms parameters (all figures): 44.1 kHz, FFT window size of 1024 samples. All spectrograms are 11 seconds, 3 – 12 kHz.

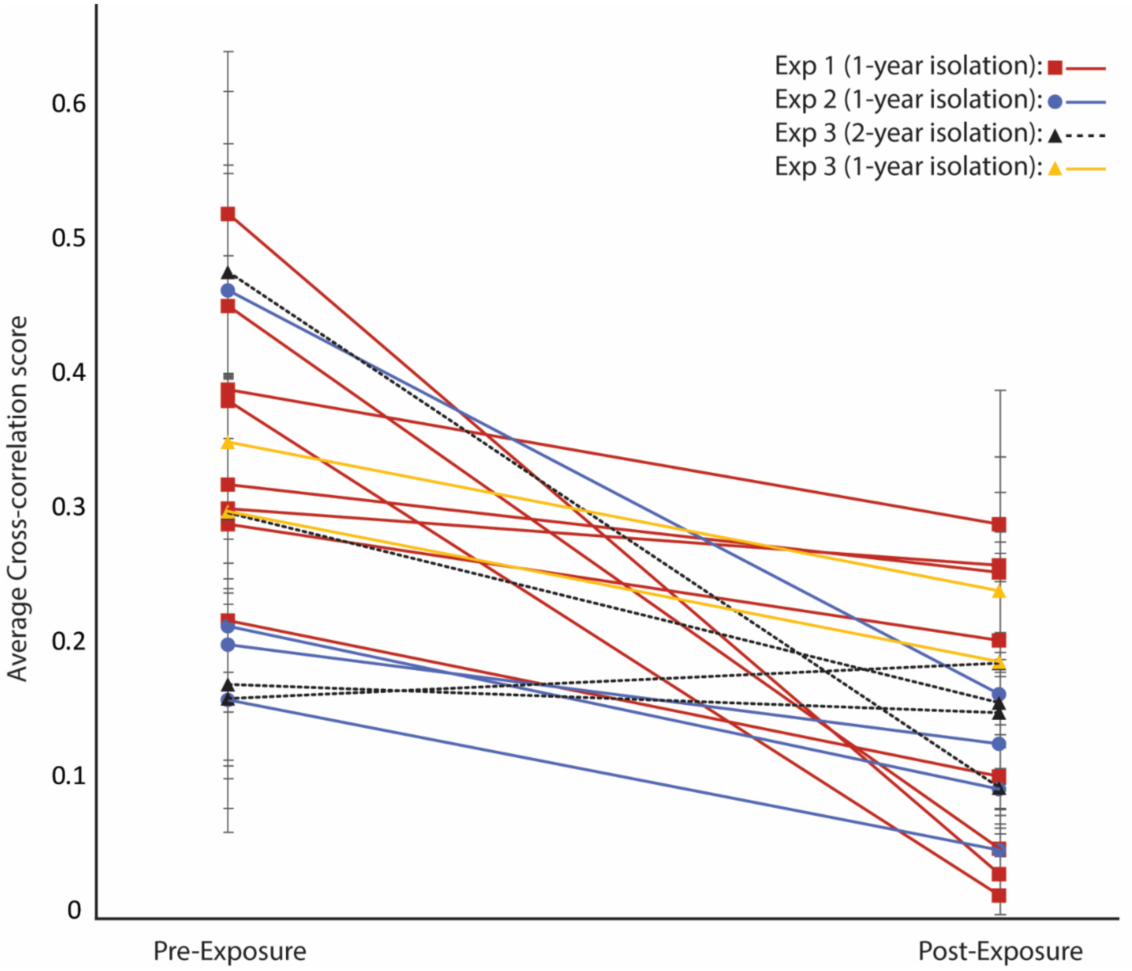

Figure 3.5: Self-similarity cross-correlation scores decrease after previously isolated Costa's are exposed to other birds. Birds from experiment 1, 2, and 3 all have self-similarity scores that significantly decreased after being exposed to other birds ($p = 0.0043$, $t= 3.16$, $df = 43$).

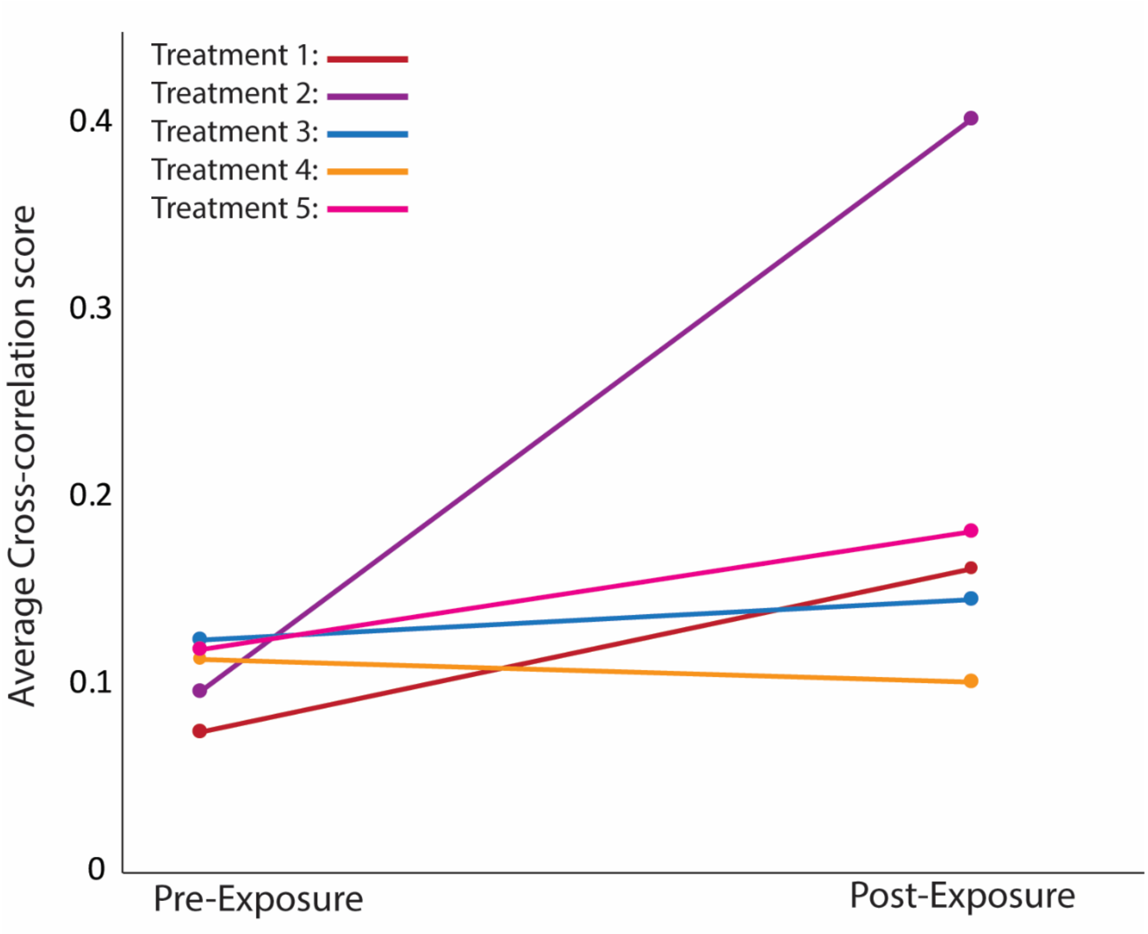

Figure 3.6: Birds that are housed together, learn to match each other's songs. Treatments 1-3, and 5: Birds were housed in either constant groups (Treatment 1 and 2) or housed in inconsistent group composition (Treatment 3 and 5)   contained birds that were housed with other birds. Treatment 4: control birds were kept in isolation. Cross-correlation scores of the between-bird similarity analysis increased after exposure in experimental treatments 2, 3, and 5 (experimental treatment x timepoint interaction effects, $p < 0.0001$, 0.012 and 0.031 respectively) relative to the control (experimental treatment #4)

Table 3.1: Between bird song similarity analysis

Term	Estimate	T Ratio	Prob>|t|
Intercept	0.1529861	28.02	<.0001*
Timepoint	-0.047686	-8.73	<.0001*
Treatment 1	-0.034098	-3.02	0.0036*
Treatment 2	0.0966956	8.56	<.0001*
Treatment 3	-0.00337	-0.41	0.6806
Treatment 5	-0.014048	-1.16	0.2509
Treatment 1*timepoint	0.0041209	0.36	0.7164
Treatment 2*timepoint	-0.105512	-9.34	<.0001*
Treatment 3*timepoint	0.0209466	2.57	0.0124*
Treatment 5*timepoint	0.0267339	2.20	0.0309*

Note: Treatment 4 is the control

Conclusion:

Hummingbirds represent a new opportunity to explore the mechanisms and evolutionary origins of vocal learning. Prior to this dissertation, there were very few lab-based experiments exploring hummingbird vocal learning. Here I show that the techniques used for decades to examine vocal learning in songbirds can be adapted to hummingbirds. By using isolation experiments, I confirmed that Costa’s hummingbirds are vocal learners. Male Costa’s hummingbirds removed from the nest at 21 days post-hatch and raised in isolation produce an abnormal ‘isolate’ song largely consisting of ‘down-sweep’ notes. Isolate songs from all individuals were very similar, suggesting that down-sweeps may be a 'species universal' portion of the Costa’s song, similar to what is observed in passerines (Marler, 1997). The species universal portion of the song is postulated to be innate (Marler & Slabbekoorn, 2004). Possibly, this helps a vocal-learning species to direct attention to their own species’ song and not that of another species (Marler & Slabbekoorn, 2004).

Additionally, I found that Costa’s need to have a social tutor in isolation experiments for learning to take place. Playback of adult song through speakers alone is insufficient. Costa’s hummingbirds need auditory, visual, and/or tactile interaction with an adult male in order for song learning to occur. Young males that interact with a live adult model during playback of an appropriate song learn to sing the playback song. However, unlike some songbirds, they will not learn a heterospecific song, even that of a close relative, regardless of whether a live model is present. Pupils did faithfully copy subtle features of slightly atypical conspecific tutor songs (e.g., a *C. costae* song played backwards). This is most consistent with memorization of tutor song and rejects the hypothesis that hearing a Costa’s-like song is a ‘releaser’ sufficient to elicit production of an innate species-specific song.

My experiments generated the first preliminary timeline of song ontogeny for any hummingbird. Costa’s have a sensitive phase that starts sometime after fledging but prior to the commencement of singing (around day 50) and are capable of learning if kept in acoustic isolation until day 115. Accordingly, the sensitive phase extends at least to day 115 and probably later. This confirms overlap of the sensory and sensory motor periods. The ontogeny of song in Costa's hummingbirds closely resembles songbird song ontogeny with a subsong, plastic song, and crystalized song stages.

Costa’s hummingbirds isolated from day 21 post-hatch until approximately one year of age change their songs after being visually, acoustically, physically exposed, and housed with other male Costa’s hummingbirds. Results from control groups indicate that these changes are not due to maturation, degradation, or drift, but specifically to exposure to another Costa’s hummingbirds. Birds group-housed within an aviary for extended periods converge on the same song, while birds housed in adjacent aviaries (in acoustic and visual but not tactile contact) do not.

The number of apparent similarities between hummingbird vocal learning and songbird vocal learning is surprising, as these lineages diverged over 60 million years ago (Prum et al., 2015). Convergent evolution often follows different 'paths' to the same end point, and details of mechanism often differ between convergent clades. While additional studies on hummingbird vocal learning will likely uncover differences relative to passerines, the number of common features between hummingbirds and passerines suggest that vocal learning has deep origins of trait in all birds.

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