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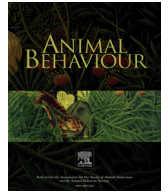
Open-ended vocal learning in Costa's hummingbird

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Here we examine whether vocal learning in Costa's hummingbird, *Calypte costae*, is open-ended. Open-ended learning is the ability of a vocal learning animal to memorize and learn to incorporate new song material into its vocal repertoire after reaching sexual maturity. Open-ended vocal learners are able to learn as adults because they have either a sensitive phase that never closes or a seasonal reopening of the sensitive phase. In prior experiments, we raised 18 individually housed male Costa's hummingbirds in isolation chambers from fledging (day 21 posthatch) until they were approximately 1 year old. During that time, they were tutored and learned song that was individually specific and stable. Here we report what happened when we moved two cohorts of eight (in 2017) and six (in 2018) of these ~1-year-old birds to communal housing in two outdoor aviaries, placing them in physical, visual and acoustic contact with other adult Costa's hummingbirds that sang songs to which each individual had never previously been exposed. The remaining four 1-year-old birds (in 2018) were instead kept in isolation for their second year as a control, then first exposed to each other at 2 years of age. Within 2 months, all of the 1-year-old birds rapidly changed their songs to produce novel songs that were unique to each aviary. The control birds that remained in isolation for a second year did not change their songs. These second-year birds then changed their songs when they were moved to the aviaries and exposed to novel song for the first time at 2 years of age. Although additional experiments are important (e.g. tutoring adults raised in the wild), our results show that Costa's hummingbirds have open-ended vocal learning through at least their third year.

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The ability to learn vocalizations based on auditory experience has been reported in a number of lineages of birds: Psittacopasserae (passerines, parrots), hummingbirds, cuckoos, shorebirds (black-headed gull, *Chroicocephalus ridibundus*), waterfowl (musk duck, *Biziura lobata*) and loons (common loon, *Gavia immer*), as well as a few groups of mammals, including humans (ten Cate, 2021). When learning takes place, it occurs on a spectrum. At one end of the spectrum, the ability to learn vocalizations is restricted to early, preadult life (Beecher & Brenowitz, 2005; Searcy et al., 2021), such as the period between fledging until arrival and settlement on the breeding grounds (Beecher & Brenowitz, 2005). Vocal learning birds that are restricted to learning vocalizations early in life are closed-ended learners (Brenowitz & Beecher, 2005). 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 et al., 1994; Marler & Slabbekoorn, 2004; Nottebohm, 1972; ten

Cate, 2021). Thus, the other end of the spectrum is 'open-ended' song learning.

In the canonical pattern of song development, vocal learning birds go through two main phases of learning (Searcy et al., 2021). The first is the sensory phase, during which species-specific vocalizations are heard and memorized (Marler & Peters, 1987). The next stage is the sensorimotor phase, during which the bird makes vocalizations and attempts to match them to the song that it memorized during the sensory phase (Marler, 1970). During the sensorimotor phase, these vocalizations go through three stages: subsong, plastic song and adult song (Beecher & Brenowitz, 2005). In closed-ended vocal learners, once a song reaches the adult song stage, the song is said to crystallize and it undergoes minimal changes later in life (Beecher & Brenowitz, 2005). In open-ended learners, two possible mechanisms could allow new song material to be added: (1) the sensitive phase never closes, allowing the bird to continuously learn new song material (Beecher & Brenowitz, 2005); (2) the sensitive phase closes each year, but it reopens, increasing neural plasticity in song nuclei and permitting learning of new material during a specific window (Beecher & Brenowitz, 2005). Evidence for open-ended vocal learning arises

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from experiments that track songs over multiple years to see whether new song material is added after the first breeding season.

From whom do learners learn? As juveniles, some passerines learn song from their father or another older tutor; thus, in close-ended learners, song is transmitted vertically or nearly so. In open-ended learning, by contrast, vocalizations are transmitted 'horizontally' between adults. In parrots, for instance, calls are learned from flockmates and reflect group identity (Smith-Vidaurre et al., 2020). In some passerines, songs are transmitted between birds on neighbouring territories, leading to the formation of 'song neighbourhoods' (Ranjard et al., 2017). Thus, open-ended learning interacts with the ecology of how song is used in song-learning species.

Hummingbirds remain a largely unexplored group of vocal learners (Baptista & Schuchmann, 1990) and seem to have evolved this ability independently of the evolution of vocal learning in Psittacopasserae, the lineage uniting parrots and passerines (Jarvis et al., 2014). To date, only one study has documented open-ended vocal learning in a hummingbird. Araya-Salas and Wright (2013) recorded songs from wild long-billed hermit, *Phaethornis longirostris*, leks over four breeding seasons. Nine out of 49 males showed song replacement, in which the replacement songs were not recorded until after the first year of measurement (Araya-Salas & Wright, 2013). But field studies do not permit ascertaining whether new acoustic components that appear late in life really are newly learned, as opposed to being learned early in development but not expressed (sung) or rarely expressed until later in life (Chaiken et al., 1994; Marler, 1997). A method that firmly addresses this criticism is to control acoustic exposure by rearing birds in an isolated acoustic setting from the nestling stage, where the acoustic stimuli to which the bird has been exposed are known.

The ability to learn past the first year has not been examined in any other hummingbird. Here we build on our two previous experiments on Costa's hummingbirds, *Calypte costae*, which revealed that their vocal ontogeny is similar to songbird vocal learning (Johnson & Clark, 2020), with a sensory phase, a sensorimotor phase, then subsong, plastic song and adult song. Costa's hummingbirds do not begin to learn song until after fledging (Johnson & Clark, 2020), and the father is not known to help in care. Thus, it seems unlikely that young males learn song from their father, and instead learn from other adult males that they encounter after leaving the nest. We also showed that their sensitive phase is wide (Johnson & Clark, 2022). Unlike passerines, males do not have a complex repertoire comprising several song types. Instead, male Costa's hummingbirds sing a single stereotyped song, which is sung repetitively in bouts (Williams & Houtman, 2008).

In this study, we performed an experiment examining open-ended learning in three cohorts of Costa's hummingbirds that were subjects of these previous two studies. The subjects ($N = 18$ males) had previously undergone two different vocal learning experiments over their first year of life. At the end of these experiments, each individual sang some sort of song. A couple of individuals sang nearly wild-type song, while a few others sang 'isolate song' (song that is the result of inadequate tutoring), while most sang songs with distinctive, individually specific idiosyncratic features. These idiosyncratic songs included features we intentionally taught them (from birds in Johnson & Clark, 2020), as well as features that we interpreted to be the result of inadequate tutoring leading to incomplete learning (birds in Johnson & Clark, 2022). In total, we had a complete record of all the songs and other Costa's hummingbirds that they had been exposed to since they were 21 days old and a complete record of all of the vocalizations each individual produced (Fig. 1). After cessation of these prior experiments, we moved these year-old birds into large communal aviaries where they were exposed to songs from other

members of their cohort, as well as other hummingbirds housed in the aviaries. This included two male Anna's × Costa's hummingbird (*Calypte anna* × *C. costae*) hybrids. (These two hybrids had been taken from a wild Costa's hummingbird nest and initially housed in isolation chambers, but once they started to sing, it became obvious they were not Costa's hummingbirds, so they were removed from that experiment and housed in the aviaries. As a result, they learned and sang isolate song from previous occupants of the aviaries). The birds in the aviaries were also exposed to free-living Anna's and Costa's hummingbirds that sang the typical song of each of these species. If Costa's hummingbirds are open-ended vocal learners, we expected that individuals previously kept in acoustic isolation would, after exposure to other individuals with different songs, then incorporate novel material (to which they had not been previously exposed) into their song.

METHODS

Animal Husbandry

Indoor husbandry procedures are summarized here; further details (including all of the equipment used) were the same as described in Johnson and Clark (2020, 2022). In brief, adults were 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.

Ethical Note

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 the University of California, Riverside (UCR) Institutional Animal Care and Use Committee protocols 20130018 and 20160039. All birds were captured at or around UCR or in the city of Riverside, minimizing transport time to the laboratory. Birds were taken by hand from nests just prior to fledging, ~21 days posthatch, when they begin to feed themselves and their husbandry becomes much easier. For enrichment, birds in isolation chambers were provided with multiple perches and given a small water dish for bathing biweekly. All cages were cleaned weekly. Birds were monitored daily for health.

The aviaries were outdoors on the UCR campus and were constructed by the same company that built the nonpublic hummingbird aviaries at the San Diego Zoo. On the UCR campus, it does not ever quite reach freezing in winter and can reach 43 °C in summer; dishes of fresh water were provided for thermoregulation especially when temperatures exceeded 37 °C. The Costa's hummingbird is a desert-adapted hummingbird that can handle these high ambient temperatures. Each aviary included locations sheltered from direct sun, high heat or rain. The aviaries also included *Ficus* for cover and to provide barriers and also had flowering plants that sometimes had flowers, for enrichment. There were seven aviaries (Fig. 1), two of which were large enough to house four birds each and four smaller aviaries that normally housed one bird each. All seven aviaries were in acoustic contact; the birds in adjacent aviaries were in visual and acoustic contact and the two large aviaries were separated by a 1.5 m walkway.

The physical environment around the aviaries is loud and not suitable for sound recording; in addition to the usual campus noises (traffic, bell-tower, etc.), it also featured nearly continuous industrial sounds from air-handling and electrical equipment. Moreover, with up to 20 hummingbirds on hand, determining which individual was vocalizing was not always possible.
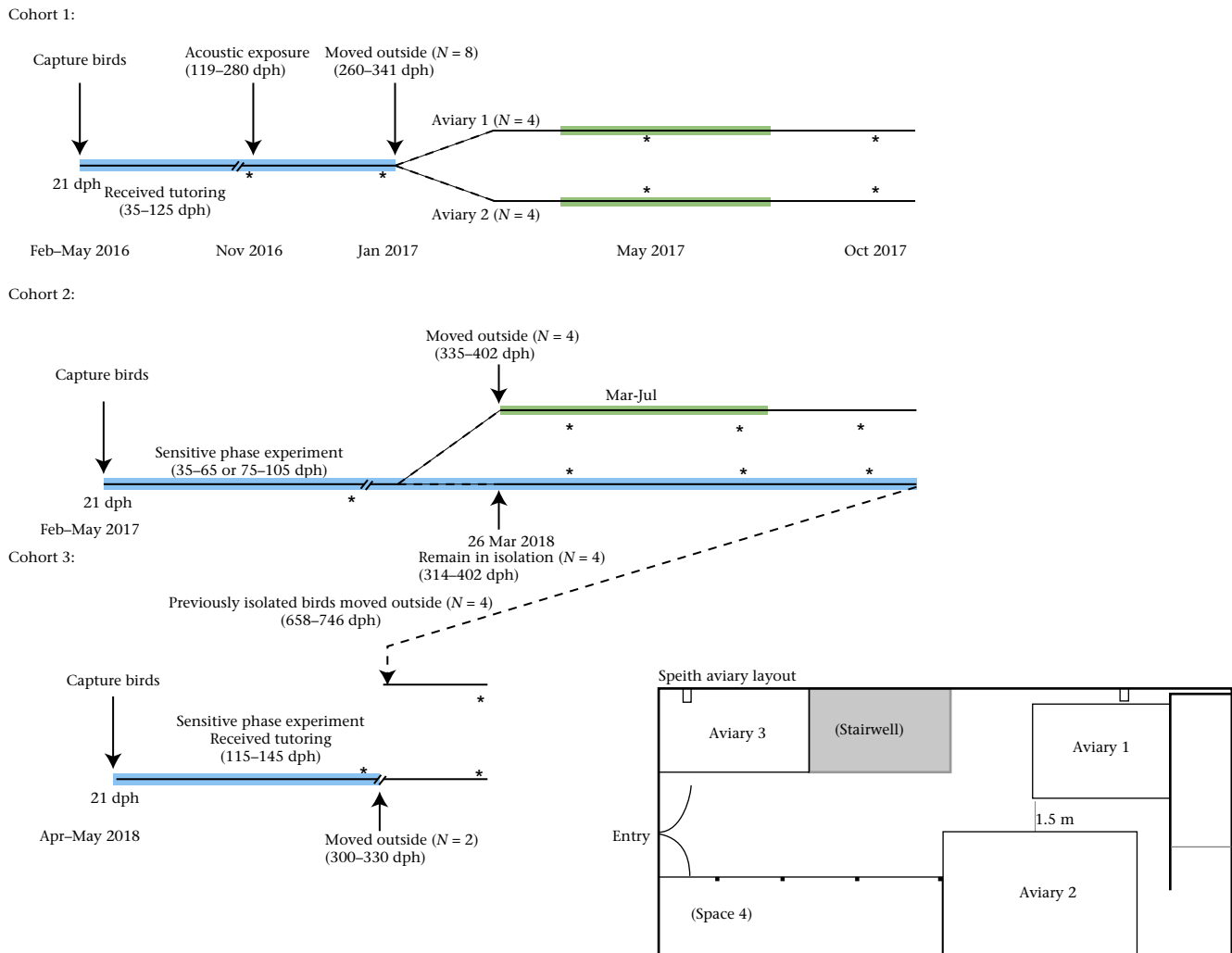
Figure 1. Timeline of events for birds used in open-ended vocal learning experiments. Three cohorts of birds were collected over three breeding seasons, 2016–2018. The timeline shows the experiments in which each cohort was used previously, whether the birds acted as adult models for another experiment (green), their treatment group for the open-ended experiment and their age (days posthatch, dph); blue denotes when birds were in acoustic isolation, asterisks denote when recordings were made. The layout of the aviaries is shown at the bottom right. Birds from these experiments were predominantly housed in Aviaries 1 and 2, while the remaining aviaries housed birds not used in this experiment, such as two male Anna's × Costa's hummingbird F1 hybrids, which were each housed in one of four smaller aviaries (denoted as 'Space 4') adjacent to Aviary 2.

Due to their aggressive behaviour, hummingbirds can be a challenge to house communally. We followed standard techniques, such as providing one feeder per bird (Krebs et al., 2002; Mobbs, 1982) and using physical barriers (e.g. *Ficus*) to encourage the birds to form stable territories (e.g. 1 bird in each corner of the aviary). We also carefully, daily monitored their behaviour to ensure that the birds were evenly matched in agonistic interactions (chases), such that no individual bird was ever deprived of the ability to feed from at least one feeder. For the first (2017) cohort of birds (see below) this worked, and we were able to stably house two groups of four birds each in the two largest aviaries, for months. By contrast, in the second and third cohorts of birds (described further below) aggression from certain individuals was a greater problem, such that we could not replicate the conditions of 2017. We were forced to change the make-up of the communally housed birds mid experiment and house the subordinate individuals in the smaller aviaries. In all of our experiments (both the isolation chambers and the aviaries), we had good survivorship (>90% annually) and relatively few health problems. At the end of the experiments, no birds were euthanized: all surviving birds were donated to the San Diego Zoo or the Fairchild Tropical Botanic Garden.

Isolation Chambers

The isolation room was the location where all song recordings took place, as well as where the isolation birds for cohort 2 were housed. The isolation room contained 12 individual isolation chambers and recording equipment as described previously (Johnson & Clark, 2020). We used the program Sound Analysis Pro (Tchernichovski et al., 2000) to acquire all song recordings. We took periodic recordings of songs produced in isolation chambers. Aviary-housed birds were captured with butterfly nets and temporarily placed in an isolation chamber for several hours and sometimes overnight (as needed; maximum time in chamber: 24 h), until they sang. After recording at least five song bouts that contained at least 10 individual songs, we returned birds to the aviary.

Open-ended experiment

Subjects (N = 18) were male Costa's hummingbirds that had previously undergone two different vocal learning experiments over their first year of life (Fig. 1). As the individual birds experienced different conditions, Fig. 1 provides an overview of the three cohorts and what they each experienced. There were three cohorts

of birds (see details below), split into five experimental groups for statistical analysis (see below), where the control group (treatment 4) included the same four birds as treatment 5.

Cohort 1

Eight Costa's hummingbird males were removed from their nests between February and June 2016 at ~21 days posthatch and housed individually, then tutored from day 35 to day 125 with either a Costa's-like song (N = 6, different birds received different songs) or Anna's hummingbird (N = 2) tutor song in the presence of an adult male Costa's hummingbird. The six birds tutored with Costa's-like songs sang individually recognizable songs, as did the two birds tutored with Anna's hummingbird song; these two produced versions of isolate song, the song that develops when a male *C. costae* is raised in acoustic isolation.

On 25 November 2016, when cohort 1 birds were on average 242 ± 31 days posthatch, we took the doors off of the isolation chambers, thus permitting acoustic contact inside the bird room. Then on 25 January 2017 (average 303 ± 31 days posthatch), we placed four birds into Aviary 1 and four birds into Aviary 2. The birds remained in these groups for 11 months, until November 2017. The groups were in acoustic and visual contact with the other group, but the two aviaries were separated by 1.5 m. All of these individuals served as adult models for the experiment described in Johnson and Clark (2022), which examined the sensitive phase of learning. This is relevant because, as they served as adult models, they were simultaneously exposed to their pupils (hatch-year Costa's hummingbird males) and to playback of typical Costa's hummingbird song. We found no evidence that acting as an adult model had any effect on the bird's songs (see Results).

Cohort 2

Eight Costa's hummingbird males captured (~21 days posthatch) in February through May 2017. These birds were given speaker playback of regular Costa's hummingbird song in the presence of an adult male (i.e. males from cohort 1) over one of two tutoring time periods: 35–65 days posthatch or 75–105 days posthatch. Birds received one hour of tutoring on 20 days over their 30-day treatment period. Even though all birds received the same tutor song (wild-type Costa's hummingbird song), each individual acquired a unique, abnormal Costa's hummingbird song, as the result of incomplete learning (Johnson & Clark, 2022).

These eight birds of cohort 2 were split into two treatment groups: an exposure group (N = 4) and an isolation group (N = 4) (average bird age at beginning of experiment: 373 ± 35 posthatch). As these individuals differed in how similar their songs were to normal Costa's hummingbird song, we assigned birds to the two groups such that each group contained a mixture of birds with songs ranging from high to low similarity to Costa's hummingbird songs. The isolation group 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 in March 2018 and were exposed to each other (plus the other stimuli in the aviaries), over their second year of life. Just like in cohort 1, the four exposure birds acted as adult models for another experiment (the birds in cohort 3), and thus were exposed to Costa's hummingbird song playback while they served as models.

Unlike cohort 1, the four exposure males could not be housed together throughout the experiment. Hummingbirds are aggressive and form dominance hierarchies in which dominant birds exclude 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, cohort 2 males were housed with different aviary-mates over the course of

the experiment (see Results). We took recordings of all individuals once a month for cohort 2.

Cohort 3

Two birds were captured between April and May in 2018 at ~21 days posthatch. Similar to the birds in 2017, these birds were given speaker playback of a Costa's hummingbird song in the presence of an adult male from day 115 to day 145 days posthatch. Both birds received 1 h of tutoring on 20 days over the 30-day treatment and developed their own unique abnormal Costa's hummingbird song, as we reported previously (Johnson & Clark, 2022). We simultaneously moved these two birds (average age = 315 ± 21 days posthatch), along with the four birds that had been in isolation for 2 years from cohort 2 (average age = 706 ± 37 days posthatch), to the outdoor aviaries in March 2019. We initially housed birds in two groups of three, but after aggression developed (as in cohort 2), the birds were split into one group of three, one pair and one bird housed singly. None of these birds was used as an adult model. Due to time constraints, cohort 3 had only one postexposure recording, which took place 1 month after the birds were moved to the outdoor aviary.

Analysis of Pre- and Postexposure Song

We compared each individual's postexposure songs with its preexposure songs with a cross-correlation test on the spectrograms (44.1 kHz, 16-bit recordings; FFT window of 256, band-pass filtered from 13 kHz to 4 kHz to remove background sound such as air pumps) in the program Raven version 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, where 0 indicates that the spectrograms have no frequency overlap and 1 indicates that the spectrograms are identical (Raven 1.5 settings: normalized, linear power). For each bird-day sampled, we selected 10 individual songs, selected randomly from multiple recordings from the same day. These 10 songs were then compared to other sets of 10 songs, for a total of 100 cross-correlations. We then took an average of these 100 cross-correlations and used this single average as the input for statistics.

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

Statistics

We predicted that, if the birds exhibit open-ended vocal learning, then their songs should change after exposure to other birds. Therefore, their self-similarity cross-correlation scores should decrease after being exposed to other birds. However, birds held in isolation might also show a decrease in self-similarity through time via drift. Therefore, we also 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. To examine whether birds' self-similarity cross-correlation scores changed from time point 1 (pre-exposure) to time point 2 (postexposure), we used ANOVA, with bird as a random factor and treatment (isolated or not) as a fixed factor and a treatment∗time point interaction effect.

We also predicted that the birds would learn to match the songs of other birds to which they were exposed. Specifically, we predicted that between-bird cross-correlation scores would increase following exposure to songs of other birds. We did not compare the songs of each bird to all other birds. Rather, we only compared the between-bird cross-correlation scores of future aviary-mates to

each other. We defined five treatments: we compared four exposed birds from cohort 1/aviary 1 to each other (treatment 1, 6 bird—bird comparisons per time point); four exposed birds from cohort 1/aviary 2 to each other (treatment 2); four exposed birds from cohort 2/all aviaries to each other (treatment 3); four nonexposed birds from cohort 2 to each other as a control (treatment 4); six exposed birds from cohort 3 to each other (treatment 5). To examine whether the birds' between-bird cross-correlation scores changed from time point 1 (pre-exposure) to time point 2 (postexposure),

we used an ANOVA, with treatment as a fixed factor and a treatment*time point interaction.

RESULTS

Cohort 1

The eight male Costa's hummingbirds, which had previously developed individually specific songs when housed in isolation for
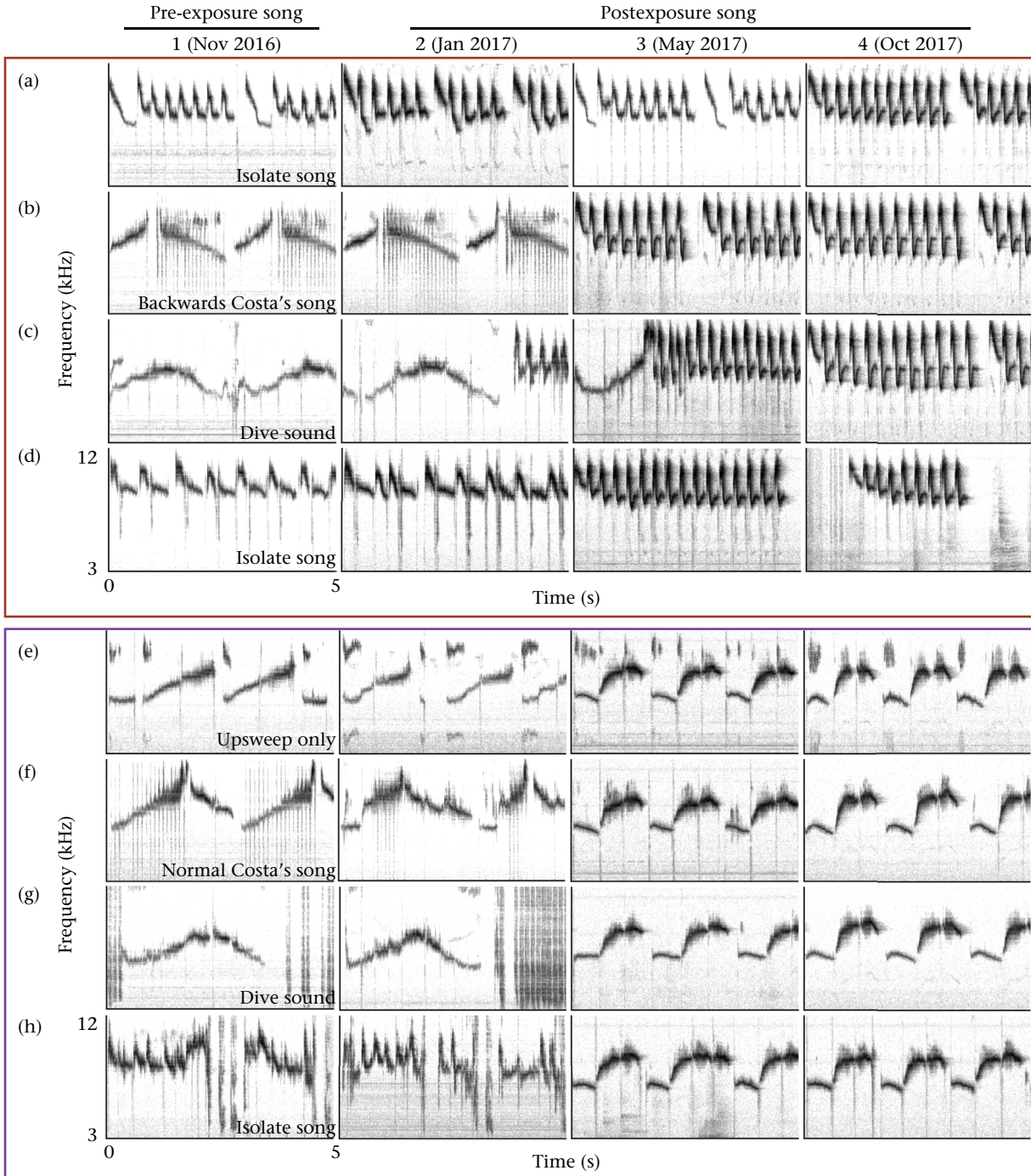

Figure 2. Song spectrograms of eight 1-year-old Costa's hummingbirds in Cohort 1, which were reared in isolation for 1 year, then housed in two groups of four for the remainder of the experiment. (a—d) Aviary 1 (red panel). (e—h) Aviary 2 (purple panel). Individuals received different experimental treatments in a prior experiment: birds in Aviary 1 were exposed to Anna's hummingbird song ('isolate song'). The birds shown here are the same ones described in Johnson and Clark (2020). Spectrogram parameters: 44.1 kHz, FFT window size of 1024 samples.

1 year, rapidly changed their songs after being exposed to each other. Specifically, in each of the two aviaries, the four males each rapidly converged to closely match the songs of their aviary-mates (Fig. 2) but not those of the other group in the adjacent aviary.

Aviary 1: all of the birds in aviary 1 started with different songs (Fig. 2a–d). Two initially sang versions of Costa's isolate song (Fig. 2a–d), a series of frequency downsweeps (Johnson & Clark, 2020), although with different organization of the downsweeps. The other two birds started with Costa's-like songs (backwards Costa's song, Fig. 2b; Costa's dive sound, Fig. 2c). At the end of the experiment (October 2017), all birds converged on a song that resembled Costa's isolate song, with a series of downsweeps, but sang with a faster cadence than 'typical' isolate song (Fig. 2). Across the four time periods, the self-similarity scores decreased for all individuals. Of note: the two birds with Costa's-like song had prior exposure to isolate song. Specifically, the previous song-learning experiment that these birds had participated in had entailed exposing the young birds to two things simultaneously: an adult model and playback of sound, and the models we used in 2016 were themselves birds that had failed to learn song (in 2015) and hence that sang isolate song (i.e. the results presented in Johnson & Clark, 2020). In total, the two birds that started with Costa's-like song (Fig. 2b and c) had been exposed to 172 bouts and 42 bouts of isolate song, produced by their adult model, while they were being tutored with playback of Costa's-like song during their first year of life.

Aviary 2: all birds started with different songs (Fig. 2e–h). After the birds were moved into the aviary, the songs of all four birds changed and collectively converged on a novel song (Fig. 2) that had little resemblance to any one individual's pre-existing song, and therefore was a new song. This song was within the normal Costa's hummingbird frequency range (6–12 kHz) but was shorter, lasting about 1.5 s compared to the duration of 2.4 s of the typical song. This song did not resemble any vocalization that any of the birds heard at any point after the age of 21 days posthatch in their life. Self-similarity score declined over time (Fig. 3). Of note: the birds in Aviary 1 and Aviary 2 were in visual and acoustic contact with birds in the other aviary, but the collective songs sung by birds in Aviary 1 and Aviary 2 were quite different.

Cohort 2

All individuals in cohort 2 started with a unique song (Fig. 4). These songs contained considerable variation and tended to be longer than typical Costa's hummingbird song (5 s or longer) and

less stereotyped than adult songs. This difference in the initial songs of cohort 2 and cohort 1 is the product of the difference in the experimental treatments they experienced in their first year (Johnson & Clark, 2022).

Isolation birds: the four birds that remained in isolation for a second year produced songs that were acoustically and spectrographically similar (Fig. 4a–d) throughout their second year. Additionally, these birds retained a high degree of within-bird song variation. It is possible these birds never reached the stereotyped 'crystallized' adult song stage; thus, their songs remained variable.

Exposure birds: the four exposure birds' songs changed after the birds were exposed to other Costa's hummingbirds (Fig. 4e–h). Unlike cohort 1, the birds were not housed together in a consistent social group for as long (see above) and the exposure birds did not all converge as tightly on a single song. By the end of the experiment, all individuals produced songs that contained mostly downsweeps (Fig. 4e–h) and that were entirely different from the songs sung by the birds in either aviary of cohort 1.

Cohort 3

Cohort 3 comprised four (isolation) birds that had been kept in isolation for their second year of life (i.e. control treatment from cohort 2), then exposed to other hummingbirds at the start of their third year (Fig. 5a–d), and two additional birds that lived in isolation only for their first year of life (Fig. 5e and f). The songs of the five birds that were housed together also converged on a unique song made up of short tonal notes that increased and decreased in an undulating pattern (Fig. 5b–d, e–f). The sixth bird, which had to be held alone, showed small changes (Fig. 5a), while the other five birds showed more signs of converging on a song. Only one recording was taken approximately 1-month postexposure due to time constraints.

Analysis

Songs of 18 Costa's hummingbirds that were reared in isolation changed after they were exposed to other Costa's hummingbirds, which caused a decline in self-similarity scores in all birds (Fig. 3). The postexposure time point self-correlation scores significantly decreased relative to the pre-exposure time point ($t_{43} = 3.16$, $P = 0.0043$), but there was no effect of treatment ($P = 0.575$) or bird (most $P > 0.1$; two birds had P values of 0.07 and 0.059) and no treatment*time point interaction effect ($P = 0.23$). Therefore, self-similarity scores of exposed and isolated birds decreased over time, regardless of whether they were exposed to other birds in their second year of life.

When we compared exposed birds' songs to each other, several variables differed significantly (Fig. 6, Table 1), including time point ($t_{78} = -8.73$, $P = 0.0001$), treatment 1 ($P = 0.0036$) and treatment 2 ($P < 0.001$), while treatment 3 ($P = 0.68$) and treatment 5 ($P = 0.25$) did not differ significantly from treatment 4 (the control) (Table 1).

There was a significant interaction effect between treatment and time point. Cross-correlation scores of the between-bird similarity analysis increased after exposure in treatments 2, 3 and 5 (treatment*time point interaction effects: $P < 0.0001$, 0.012 and 0.031, respectively) relative to the control (treatment 4) (Fig. 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 posthatch until approximately 1 year old (after moulting into their adult plumage
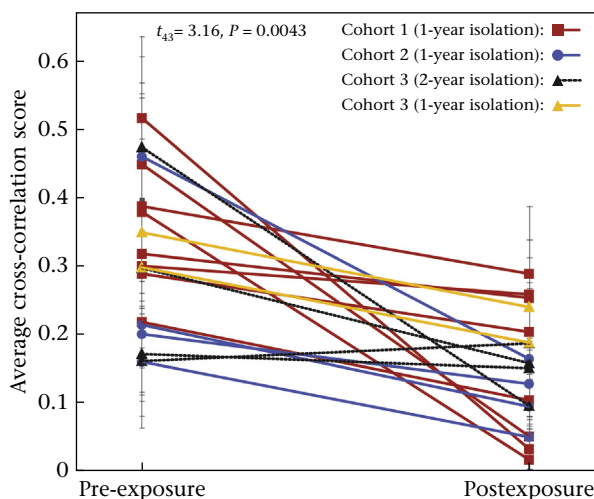

Figure 3. Self-similarity cross-correlation scores of Costa's hummingbirds that were exposed to other birds after being isolated for 1–3 years.
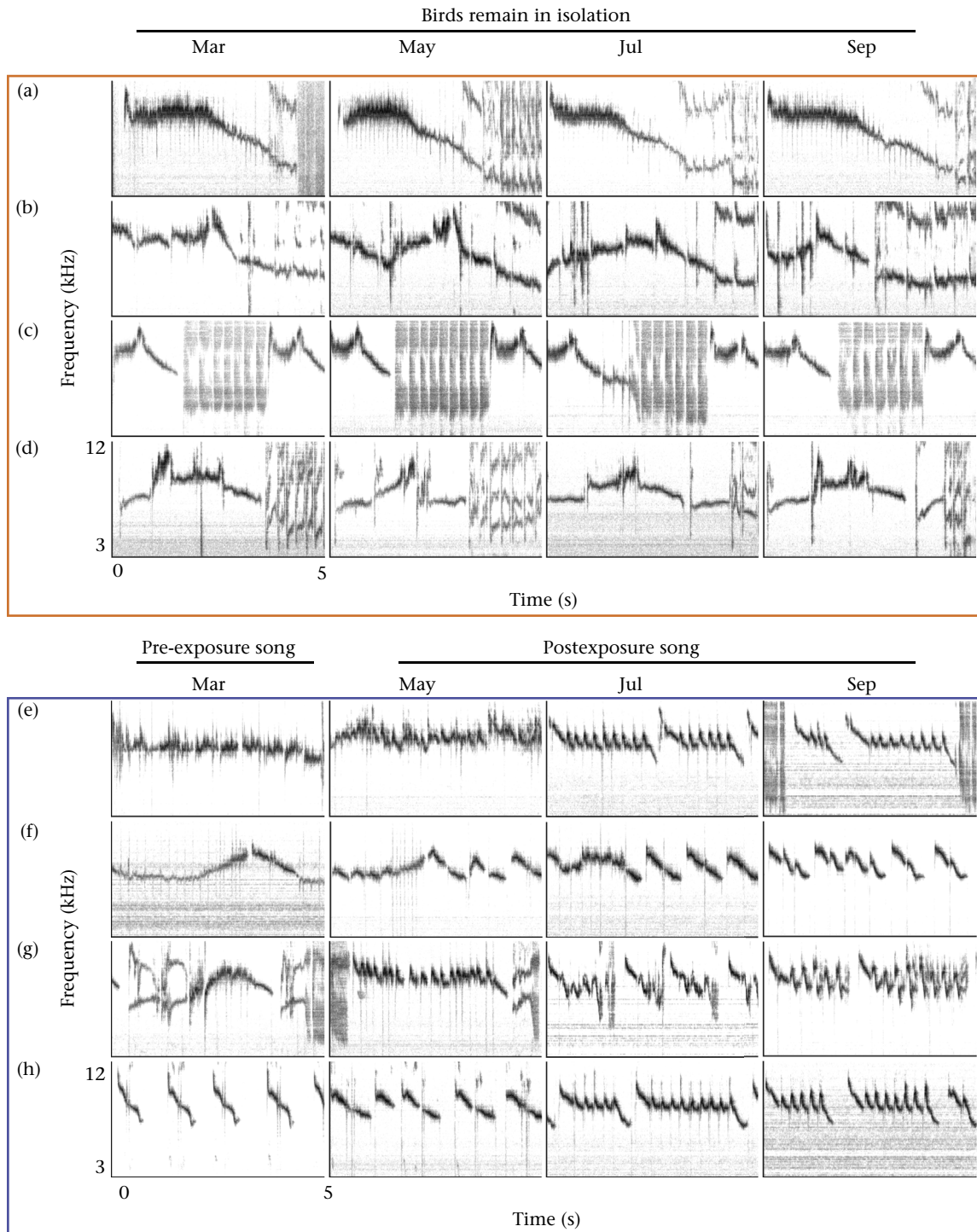
Figure 4. Song spectrograms of Costa's hummingbirds in Cohort 2, which either (a–d) remained in isolation for both their first and second year of life (treatment 4: $N = 4$, orange panel), or (e–h) were moved to an outdoor aviary and exposed to other hummingbirds in their second year of life (treatment 3: $N = 4$, blue panel). Thus, birds in treatment 3 were in physical, visual and acoustic contact at the start of their second year of life. However, unlike Cohort 1 (Fig. 2), husbandry problems in the exposure group prevented us from housing birds in treatment 3 together continuously; therefore, these four birds did not live in the same aviary or with the same cagemates throughout the experiment. Individuals received different experimental treatments in a prior experiment. The birds shown here are the same ones described in Johnson and Clark (2022). Spectrogram parameters: 44.1 kHz, FFT window size of 1024 samples.

and appearing to become sexually mature) rapidly changed their songs after being visually, acoustically and physically exposed to conspecifics (Figs 2–6). By contrast, a control group of four birds, which were housed in isolation for a second year, did not change their songs by as much (Fig. 3). In turn, these birds, after 2 years of isolation, also rapidly changed their song when housed with conspecifics for the first time. Although treatment 1 birds converged on a song that resembled isolate song, i.e. resembling songs to which they had received a small amount of exposure when young, the other exposure groups converged on a novel song to which none of
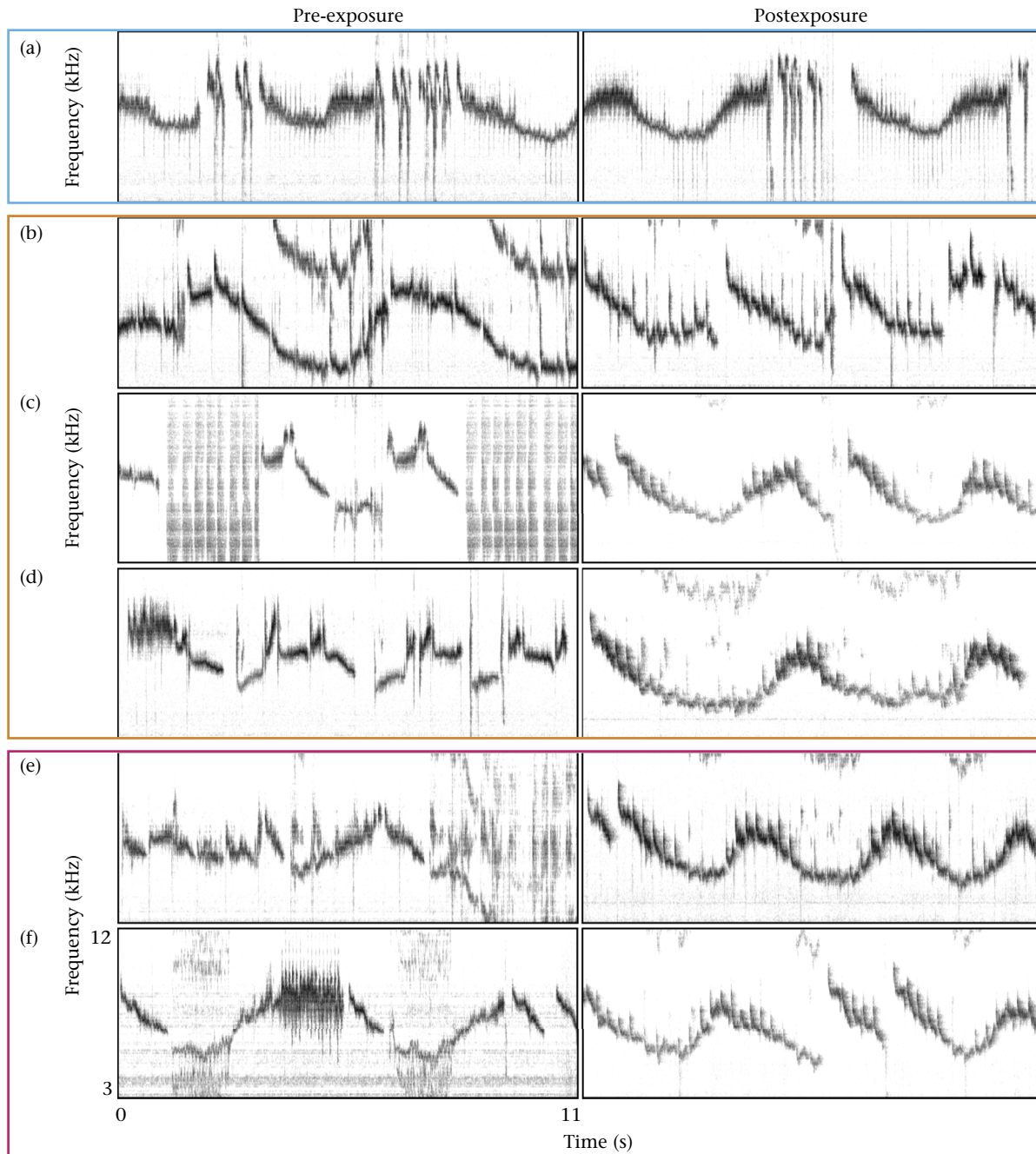
Figure 5. Song spectrograms of Costa's hummingbirds in Cohort 3 (treatment 5), which either (a–d) remained in isolation until the start of their third year (*N* = 4), or (e–f) were placed in isolation only for their first year of life (*N* = 2). In (a; blue panel), the bird was housed alone in an outdoor aviary but was acoustically exposed to other Costa's hummingbirds: 2-year isolation pre- and postexposure songs. In (b–d; orange panel), the birds were housed communally: 2-year isolation pre- and postexposure songs. In (e–f; pink panel), the birds were housed communally: 1-year isolation pre- and postexposure songs. Spectrogram parameters: 44.1 kHz, FFT window size of 1024 samples. Birds in (a–d) are the same birds as in Fig. 3a–d.

them had had prior exposure (Figs 2–4). We hypothesize that the group's song converged onto a song that resembled isolate song because one of the birds that in this group sang isolate song and was an especially vigorous singer, as might be expected if hummingbirds are statistical learners (learning the song to which they are most often exposed). Moreover, each group converged on different forms of song from the other groups, where the exact song seemed to be an amalgamation of the songs sung by the individual birds. These results cannot be explained by maturation, degradation or drift of the song. The only explanation consistent with all of

these data is open-ended song learning. Collectively these data indicate that Costa's hummingbirds are open-ended vocal learners, able to substantially change their song after reaching sexual maturity.

Birds group-housed within an aviary converged on the same song (Figs 2, Fig. 3m–p, Fig. 4j–l, q–r, Fig. 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 (Fig. 2). In previous experiments, we found that Costa's hummingbirds require acoustic, visual and physical contact for vocal
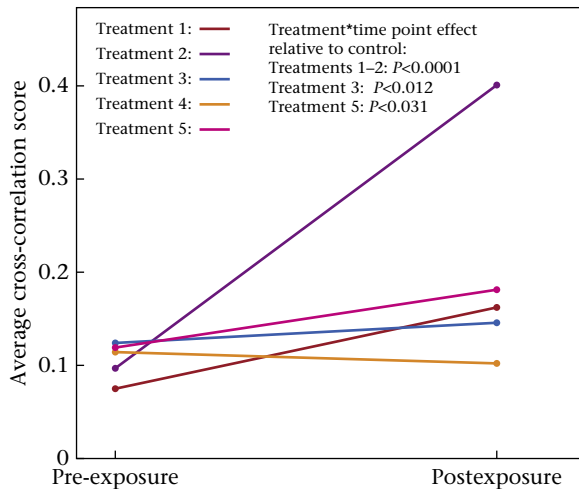
Figure 6. Cross-correlation scores of the between-bird similarity analysis. Birds were either group-housed with consistent group composition (treatments 1—2), group-housed with inconsistent group composition (treatments 3, 5), or kept in isolation (treatment 4 control birds).

Table 1
Between-bird song similarity analysis

Term	Estimate	*t* ratio	Prob>|*t*|
Intercept	0.1529861	28.02	**<0.0001**
Time point	-0.047686	-8.73	**<0.0001**
Treatment 1	-0.034098	-3.02	**0.0036**
Treatment 2	0.0966956	8.56	**<0.0001**
Treatment 3	-0.00337	-0.41	0.6806
Treatment 5	-0.014048	-1.16	0.2509
Treatment 1∗time point	0.0041209	0.36	0.7164
Treatment 2∗time point	-0.105512	-9.34	**<0.0001**
Treatment 3∗time point	0.0209466	2.57	**0.0124**
Treatment 5∗time point	0.0267339	2.20	**0.0309**

Treatment 4 was the control. Significant outcomes are shown in bold.

learning to occur. In a previous experiment, two juvenile Costa's hummingbirds raised in the same isolation chamber with only speaker playback of a tutor song matched each other's songs and failed to learn the playback song (Johnson & Clark, 2020). Instead, a live adult model was necessary for a young Costa's hummingbird to learn its tutor song (Johnson & Clark, 2020).

Our result seems to suggest that male Costa's hummingbirds match the song of neighbours with which they physically interact. During the breeding season, male Costa's hummingbirds set up individual territories within suitable habitat, then interact (fight, chase) with neighbouring males. In this respect, the social behaviour of male hummingbirds more closely resembles songbirds, rather than parrots. Many songbirds match songs of their neighbours, such as at the beginning of the breeding season (Nottebohm, 1972; Payne & Payne, 1997). In contrast, parrots live in flocks and learn new contact calls (shared with groupmates) when group composition changes (Sewall et al., 2016). Song matching is thought to aid in recognition and communication with neighbours and be important in competition via countersinging (Brown & Farabaugh, 1997). Neighbours can use song degradation to determine the location and distance of individuals, but only if they are familiar with the songs of their neighbours and can recognize whether degradation has taken place (McGregor & Krebs, 1984). These results suggest that Costa's hummingbirds might do the same thing. Moreover, as an anonymous referee for the present study pointed out, the type of open-ended learning demonstrated here, 'modification of an existing song by convergence', is essentially identical to

vocal learning of flock call types demonstrated in parrots (Sewall et al., 2016) and is not the same as open-ended learning as shown by passerines, which exhibit open-ended learning by adding or replacing individual vocal elements within their repertoires (ten Cate, 2021). Costa's hummingbirds essentially have a repertoire of one song (a simple, short song), which, as we have shown here, can be highly modified at adulthood.

This study shows that Costa's hummingbirds can change their song after spending their first year or their first two years of life in isolation. In each iteration of the experiment, we moved birds into social groups at the same time of year (~January), near the beginning of the breeding season. As a result, learning in our adult birds occurred at the same time of year, shortly after the onset of the breeding season. From their behaviour (e.g. courtship displays), we infer that our birds came into breeding condition when introduced into the aviaries. Thus, our results do not directly inform whether the sensitive phase may open or shut at different times of year. There are two proposed mechanisms for open-ended learning in songbirds (Doupe & Kuhl, 1999; Zeigler & Marler, 2008). The first is a seasonal reopening of the sensitive phase, generally prior to the breeding season (Doupe & Kuhl, 1999; Nottebohm, 1972; 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 our previous experiments (Johnson & Clark, 2022) do not indicate which mode of open-ended vocal learning is most applicable to Costa's hummingbirds. Experimental exposure of isolated birds to conspecifics outside of the normal breeding season could reveal whether the ability to learn new songs is seasonal or continuous.

Cohorts 2 and 3 (but not cohort 1) comprised birds that had not completed song learning at the outset of the experiment; at the onset of this experiment, their songs were longer than the wild-type song to which they had been exposed (Figs 3 and 4; Johnson & Clark, 2022). This atypical learning history in their first year may have affected their plasticity in subsequent years. In songbirds, birds that receive inadequate exposure to song during the critical period can be 'rescued' later in life (Gobes et al., 2019). Similar experiments have not yet been performed on hummingbirds to determine whether they show a similar rescue effect, but given how many apparent similarities there are between hummingbird song learning and oscine passerine song learning, it would not be surprising if hummingbirds show a similar rescue effect. Under this 'rescue' hypothesis, we would have predicted that cohorts 2 and 3 would show greater adult song learning than cohort 1. Our results show that the two groups of birds in cohort 1 had the greatest convergence in songs (cf. Figs 2, 3 and 4), however our data do not refute this 'rescue' hypothesis, as there was a confounding effect: we were unable to house cohorts 2 and 3 together for as long as the two groups of birds from cohort 1 that were communally housed, due to husbandry problems. An experiment testing whether wild-caught adult Costa's hummingbirds, which would have undergone natural learning, change their songs as dramatically as the birds in the present study would directly address whether the atypical learning environment in our experiments affected our results. For that matter, wild Costa's hummingbird are a song-learning enigma: despite having a nearly 160 km breeding range (from southern Nevada to the southern tip of Baja California), there are no known dialects; all wild Costa's hummingbirds sound essentially the same! The dramatic changes in song we document here (e.g. Fig. 2b—g, Fig. 4f) are not possible in wild birds, due to the uniformity of their song. It is also curious that, despite having the ability to sing complicated songs (e.g. Fig. 5a—d), the wild-type song is short and simple. There is still much to reveal about hummingbird

vocal learning. These experiments are the first laboratory-based studies of open-ended vocal learning in any hummingbird and show a second hummingbird species (in addition to wild long-billed hermits; 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 laboratory studies are needed to reveal the nuances of open-ended vocal learning in this lineage.

Author Contributions

Christopher J. Clark: Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Katherine E. Johnson:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data Availability

Data for this study are available as Supplementary material.

Declaration of Interest

The authors declare no conflicts of interest.

Acknowledgments

Supplementary Material

Supplementary material associated with this article is available, in the online version, at https://doi.org/10.1016/j.anbehav.2024.10.012.

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