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Cultural and morphological divergence of Darwin's cactus finches (Geospiza scandens) across Galápagos Islands

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Contents

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2. Abstract

Understanding the divergence of cultural traits, such as bird song, provides critical insights into evolutionary processes, particularly in isolated populations. Darwin's cactus finches (*Geospiza scandens*) on the Galápagos Islands serve as a model for examining the interplay between genetic, morphological, and cultural factors in speciation. This study investigates divergence in song syllable types, acoustic characteristics, singing behavior, and morphology between allopatric cactus finch populations on Floreana and Santa Cruz Islands. We compiled a syllable type library and analyzed acoustic characteristics and singing behavior in 50 males, alongside morphological measurements from 36 males collected over several years. Acoustic analyses focused on frequency- and time-related parameters, while syllable diversity and singing behavior were evaluated through syllable type repertoires and song structure metrics. Our findings revealed significant divergence in syllable repertoires, with no shared syllable types between islands, and distinct acoustic differences, including broader frequency bandwidths and higher peak frequencies in Floreana males. Morphological analysis showed smaller beak sizes in Floreana males, consistent with acoustic differences, but no significant differences in body size. Despite morphological constraints, singing behavior - such as syllable repetition rates and the number of syllables per song - was comparable across populations. Cultural processes, including social learning and drift, likely contribute to the observed acoustic divergence. This study adds to a growing body of information on the role of geographic separation in the accumulation of both cultural and morphological differences between populations, leading to divergence between populations that could affect speciation scenarios in cactus finches if populations cease to interbreed after secondary contact in the future.

3. Zusammenfassung

Das Verständnis der Divergenz kultureller Merkmale, wie des Vogelgesangs, bietet entscheidende Einblicke in evolutionäre Prozesse, insbesondere in isolierten Populationen. Charles Darwin's Kaktusfinken (*Geospiza scandens*) auf den Galápagos-Inseln stellen ein ideales Modell dar, um das Zusammenspiel genetischer, morphologischer und kultureller Faktoren bei der Entstehung neuer Arten zu erforschen. Diese Studie untersucht die Divergenz in Bezug auf Liedsilbentypen, akustische Charakteristika, Gesangsverhalten und Morphologie zwischen allopatrischen Kaktusfinken-Populationen auf den Inseln Floreana und Santa Cruz. Wir erstellten einen Katalog der vorherrschenden Silbentypen, analysierten akustische Parameter und das Gesangsverhalten von 50 Männchen sowie morphologische Merkmale von 36 Männchen, die über mehrere Jahre hinweg gesammelt wurden. Die akustischen Analysen konzentrierten sich auf frequenz- und zeitbezogene Parameter, während die Silbendiversität und das Gesangsverhalten anhand von Silbentyp-Repertoires und Metriken zur Gesangsstruktur bewertet wurden. Unsere Ergebnisse zeigten eine deutliche Divergenz in den Silbenrepertoires, ohne gemeinsame Silbentypen zwischen den Inseln, sowie signifikante Unterschiede in den akustischen Parametern, wie etwa breitere Frequenzbandbreiten und höhere Spitzenfrequenzen bei den Männchen von Floreana. Die morphologische Analyse ergab kleinere Schnabelgrößen bei den Männchen von Floreana, was mit den akustischen Unterschieden übereinstimmt, jedoch keine signifikanten Unterschiede in der Körpergröße. Trotz dieser morphologischen Unterschiede im Schnabel war das Gesangsverhalten – etwa die Silbenwiederholungsraten und die Anzahl der Silben pro Lied – zwischen den Populationen vergleichbar. Kulturelle Prozesse wie soziales Lernen und Drift tragen wahrscheinlich zur beobachteten akustischen Divergenz bei. Diese Studie erweitert

das Wissen über die Bedeutung geografischer Isolation bei der Entstehung kultureller und morphologischer Unterschiede zwischen Populationen. Solche Unterschiede könnten die Artbildung bei Kaktusfinken beeinflussen, insbesondere wenn sich die Populationen nach einem erneuten Kontakt nicht mehr miteinander fortpflanzen.

4. Introduction

Allopatric speciation, the process by which geographically isolated populations diverge into distinct species, is a cornerstone of evolutionary biology (Mayr, 1942). Central to this process is the interplay between genetic drift, morphological divergence, and changes in culturally transmitted traits such as vocalizations (Colombelli-Négrel & Kleindorfer, 2021). In birds, song divergence presents a particularly valuable opportunity for study as it reflects both genetic and cultural evolution, influenced by morphological constraints and social learning (Nowicki & Searcy, 2005; Searcy et al., 2021; Vernes et al., 2021).

In vocal production learning birds, morphological traits, such as those associated with beak shape, impose biomechanical constraints on sound production, creating a link between morphology and song structure (Podos & Nowicki, 2004; Huber & Podos, 2006; Christensen et al., 2006; Podos et al., 2009). Birds with larger beaks often produce slower or simpler songs due to the physical challenges of generating rapid or complex notes (Podos, 2001). During singing, birds modify the length of their vocal tract - and consequently its resonant frequency - by opening and closing their beaks to emphasize different frequencies (Podos & Nowicki, 2004). Larger beaks, however, may be slower to open and close, limiting the speed and precision with which frequency modulation can occur. As a result, birds with larger beaks are often constrained to produce songs with narrower frequency bandwidths and slower repetition rates (Podos, 2001; Podos et al., 2004; Huber & Podos, 2006). Additionally, body size can also influence song characteristics (Ryan & Brenowitz, 1985; P. R. Grant, 1986; Brumm, 2009; Martin et al., 2011). Body size affects syrinx volume, which in turn influences the fundamental frequency of the song, as demonstrated in Darwin's finches (Bowman, 1983)

and other bird species (Badyaev & Leaf, 1997; Kirschel et al., 2009).

Above and beyond morphological constraints, vocal production learning involves the longitudinal transmission of syllable types through a process of exposure, attention, imitation and reproduction. Thus, there are many opportunities for error or variance as different syllable types are attended to, memorized, produced and transmitted across generations, making bird song an ideal subject for the study of cultural evolution (Lipkind & Tchernichovski, 2011; Aplin, 2019; Williams & Lachlan, 2022). Cultural evolution (Whiten, 2019) in vocalizations is increasingly documented across vocal production learning systems, including whales (Garland & McGregor, 2020), bats (Lin et al., 2015), and seals (Reichmuth & Casey, 2014). Bird song represents one of the prime models for investigating such intergenerational behavioral change, where cultural evolutionary change is defined as a behavioral trait that is shared across generations and passed on through social learning (Klump et al., 2021). Since bird songs are learned and passed across generations, divergence in songs can occur independently of genetic or morphological factors.

Geographic variation in song has been reported in a variety of songbird populations over different spatial distances (Galeotti et al., 1996; Kroodsma, 1985; Leader et al., 2000; Slabbekoorn et al., 2003; Reyes et al., 2024). This variation typically results from a combination of natural isolation by geographical barriers and song-learning dynamics (Podos & Warren, 2007). Over time, cultural evolution can drive the emergence of new song types and the formation of local dialects, resulting in distinct vocal patterns (Slater, 2003; Parker et al., 2010; Aplin, 2016). For instance, isolated populations of swamp sparrows (*Melospiza georgiana*) exhibit distinct song patterns that persist through cultural transmission, even in

the absence of environmental or ecological differences (Lachlan et al., 2018). Such distinct vocalizations can function as prezygotic barriers, reducing interbreeding between populations and highlighting the potential for cultural evolution to reinforce reproductive isolation, which can ultimately drive speciation (Grant & Grant, 1996; Colombelli-Négrel & Kleindorfer, 2021).

Darwin's finches, with their geographical isolation and diverse beak morphologies adapted to different ecological niches, represent a particularly compelling model for studying the dynamics of speciation. In addition to their morphological variation, Darwin's finches have also been shown to exhibit song divergence across geographically separated populations (Grant & Grant, 1997a, 1997b; Colombelli-Négrel & Kleindorfer, 2021; Colombelli-Négrel et al., 2023), and this divergence has been documented to be linked to speciation (P. R. Grant & Grant, 2009; Podos & Schroeder, 2024). Finch song typically consists of repeated renditions of a single syllable type, and these syllables are culturally transmitted. Juvenile males learn their syllable type from a male tutor, usually their father or another nearby male (Bowman, 1979, 1983; Millington & Price, 1985; Grant & Grant, 1996, 2018; Christensen & Kleindorfer, 2007; Peters & Kleindorfer, 2018). Once learned, a finch's syllable generally remains unchanged throughout its adult life, as observed in ground finches (Gibbs, 1990; Grant & Grant, 1996) and in small tree finch males (*Camarhynchus parvulus*) over a two-year period (Christensen et al., 2006). The combination of morphological diversity and vocal variation makes Darwin's finches an excellent system for exploring the interplay between morphology, cultural drift, and acoustic divergence. In this study, we investigate the potential divergence in song, singing behavior, and morphology between two geographically isolated populations of Darwin's cactus finches (*Geospiza scandens*).

The aim of this project is to identify the range of song syllable types, differences in acoustic characteristics of syllables, male singing behavior and male morphology across two allopatric populations of Darwin's cactus finches on Floreana Island and Santa Cruz Island, Galápagos Archipelago. First, we compile a library of syllable types within each population. Second, we compare acoustic characteristics of syllables between island populations. Third, we compare the singing behavior and, fourth, the morphological measurements between the two islands. If there is cultural divergence in song between the two islands, we predict 1) shared and unshared syllable types on each island, and 2) differences in acoustic characteristics of syllable types between islands. If there are island differences in song production or morphological constraints to song production, then we predict 3) interisland differences in singing behavior (number of syllable types per song, number of syllables per song, trill rate per song), and 4) interisland differences in morphological measurements.

5. Methods

Study species

The common cactus finch (*Geospiza scandens*) belongs to the tanager family, Thraupidae (Passeriformes), and is one of the 17 species known as Darwin's finches, which are only found on the Galápagos Islands, Ecuador and with one species on Cocos Island, Costa Rica (P. R. Grant & Grant, 2007). *Geospiza scandens* inhabits most of the major Galápagos Islands, including Pinta, Santa Fé, Floreana, Santa Cruz, Isabela, Pinzón, Marchena, Santiago, and Rábida. However, on islands where it is absent (such as Fernandina, Genovesa, Española, Darwin, and Wolf), it is replaced by its close relative, the Española cactus finch (Kleindorfer, Fessl, et al., 2019; Jaramillo & D. A. Christie, 2020). The natural habitats of cactus finches are dry scrublands and arid woodlands in lowland areas of these islands, with a particularly strong association with the prickly pear cactus (*Opuntia*), which provides both food and nesting sites (Boag & Grant, 1984).

Cactus finches belong to the medium-sized ground finches, measuring 12–14 cm in length and weighing 20–24 grams (Abbott et al., 1977). They have longer bills that are deep at the base and taper to a point, adapted for feeding on *Opuntia* pulp, seeds, and flowers (Abbott et al., 1977; B. R. Grant & Grant, 1981; Millington & Grant, 1983). The plumage of cactus finches can indicate the age of males: yearling males, like females, display brown-gray feathers, but with each annual molt, they develop progressively more black feathers, becoming fully black-bodied at around the age of five years (B. R. Grant & Grant, 1987).

The cactus finch uses *Opuntia* not only as a food source but also as a preferred nesting site,

building dome-shaped nests in the dense parts of the cactus. Breeding typically begins in January or February, following the onset of the heavier rains (Boag & Grant, 1984). During this period, males build display nests and sing actively to attract mates and defend their territories (B. R. Grant & Grant, 1996).

Study sites

Data collection was conducted during the Darwin's finch breeding season in March 2024, with recording sites located in the lowlands of Santa Cruz (Puerto Ayora: 0°44´S, 90°18´W, Garrapatero: 0°40´S, 90°13´W) and Floreana Island (1°,16´S, 90°29´W) in the Galápagos Archipelago (Figure 1). The arid lowland sites on both islands are primarily characterized by vegetation such as shrubs, dwarf trees, and cacti from the genera *Opuntia* and *Candelabra* (Jackson, 1993). Santa Cruz, centrally located within the archipelago, is the second-largest island, covering 986 km² and supporting a population of over 15,000 people (INEC 2015). In comparison, Floreana Island is smaller, with an area of 173 km² and approximately 140 permanent inhabitants.

Recordings

To examine differences in song characteristics and syllable type occurrence between the two cactus finch populations, we recorded 25 males on each island, totaling 50 individuals. In 14 of the 25 males on Floreana and all 25 males on Santa Cruz, we broadcast sympatric conspecific song to elicit a male song using a maximum of six songs per broadcast. The playback was used if the male was observed in the territory, but not singing. The one-minute playback stimuli were broadcast with a JBL Clip 4 Bluetooth speaker (JBL Inc., USA) and

consisted of six evenly spaced songs from the same individual, high-pass filtered at 1500 Hz, with normalized peak amplitude, and saved as 16-bit WAV files. In our analyses, we accounted for playback use to control for any potential influence on the measured song parameters. Recordings were made using a Zoom F3 Field Recorder (Zoom Corporation, Japan) and a Sennheiser MKE 600 directional microphone (Sennheiser electronic GmbH & Co. KG, Germany) at a sampling rate of 48 kHz and 32-bit float resolution. We aimed to record at least 10 high-quality songs per male (Floreana: mean = 11.6, SD = 2.77; Santa Cruz: mean = 13.8, SD = 2.98).

Acoustic analysis

All recordings were first visualized using Audacity 3.4.2 (Audacity Team, 2024) and exported at a 48 kHz sampling rate with 16-bit depth. We then used Raven Pro 1.6.5 (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology, 2024) to create spectrograms of the recordings (Hann window, window length = 512, contrast = 50, brightness = 50). For analysis, we selected songs with a high signal-to-noise ratio, yielding a total of 635 songs (Floreana: 289 songs, Santa Cruz: 346 songs). To define the start and end of each song, we classified a vocalization as a song if the time interval between the last syllable of the previous song and the first syllable of the next song was minimum two seconds apart.

After saving the songs as separate files, all further analyses were conducted in RStudio (Posit team, 2024). Using the *warbleR*/*ohun* packages (Araya-Salas & Smith-Vidaurre, 2017) / (Araya-Salas et al., 2023) we employed energy-based detection to mark syllable start and end points within each song, based on energy and time characteristics. To prevent low-frequency noise from affecting relative amplitude, within the *energy_detector* function, we applied a

bandpass filter (1.5–8 kHz). Each song's detections were visually verified on labeled spectrograms using the *label_spectro* function, with arguments *threshold*, *smooth*, and *hold.time* adjusted as needed for accuracy. This process identified a total of 2001 syllables: 888 syllables across 289 songs from Floreana and 1113 syllables across 346 songs from Santa Cruz.

To obtain frequency and time-related features of each syllable, we used functions from the *warbleR* package. The *freq_ts* function was applied to extract dominant frequency contours as time series, allowing us to calculate parameters such as minimum and maximum dominant frequency, bandwidth, and slope of the dominant frequency. Further, we used the function *spectro_analysis* to derive additional features, including duration, mean frequency, standard deviation, median frequency, first and third quartile frequencies, interquartile frequency range, median time, first and third quartile times, interquartile time range, skewness, kurtosis, spectral entropy, time entropy, and spectrographic entropy (see package manual for detailed descriptions). After filtering syllables above 1500 Hz, we created mean frequency spectra using the *meanspec* function from the *seewave* package (Sueur et al., 2008) to measure peak frequency, representing the frequency with the peak amplitude for each sound file. Song duration was determined by measuring the time interval between the start of the first detected syllable and the end of the last detected syllable within each song during energy-based syllable detection. To quantify trill rates, we counted the number of syllables in each song and divided this by the song duration.

Categorization of syllable types

Using the spectrograms generated in Raven Pro 1.6.5, two researchers first categorized the

syllables into syllable types by consensus on the basis of structural similarities. To assess the generalizability of this categorization across researchers, five people were asked to classify the syllable types into categories. For this task, we used R's *spectro* function from the *seewave* package to create spectrograms at the same scale, enhancing background-to-signal contrast. Participants received two printed spectrograms per syllable type, accompanied by instructions to create unique syllable type categories and/or match syllable type pairs for each island based on perceived image similarity.

Morphological measurements

To examine morphological differences between the islands, we analyzed measurements from 36 cactus finch males (17 from Floreana and 19 from Santa Cruz) at the time of capture and banding as part of our long-term mist-netting between 2000 and 2024 (Table 1). The measured parameters included beak length culmen (tip of beak to base of feathers, mm), beak width measured at the culmen (mm), beak depth measured at the culmen (mm), tarsus length (mm), and flattened wing length (mm).

Data analysis

Acoustic structure

To investigate differences in the acoustic structure of syllable types, we conducted a principal component analysis (PCA) using 21 frequency- and time-related measurements with the *prcomp* function from R's base *stats* package (R Core Team, 2024).The first four principal components (PC1–PC4), each with an Eigenvalue greater than 1, collectively explained 86% of the variance and were selected for further analysis (Table 2). PC factor loadings for all 21 acoustic variables were calculated (Table 3).

PC1–PC4 were then used to conduct linear mixed models (LMMs), with each principal component as the response variable using *lmer* function from the package *lme4* (Bates et al., 2015). Island and playback use (yes/no) were included as fixed effects, while bird ID and syllable type were included as random effects.

Singing behavior

To evaluate potential differences in male singing behavior between the two islands, we analyzed the number of syllables per song, the number of syllable types per song, and the trill rate per song.

Generalized linear mixed models (GLMMs) from the *lme4* package (Bates et al., 2015) were used for the number of syllables per song and the number of syllable types per song as response variables, with island and playback use included as fixed effects. Random effects differed by model: for the number of syllables per song, both bird ID and syllable type were included, while for the number of syllable types per song, only syllable type was used due to

negligible variance attributable to bird ID. A Poisson error distribution with log link function was applied to both GLMMs.

To assess variations in trill rate per song between island populations, a LMM (package *lme4*) was applied, including island and playback as fixed effects and bird ID and syllable type as random effects.

Morphology

For examination of morphological differences between the islands, a principal component analysis was performed on the three beak measurements (beak length, width, depth), with the first principal component having an Eigenvalue greater than 1 and explaining approximately 61% of the variance; it was therefore taken as our primary measure for beak size dimension (Table 4). A separate PCA for body measurements (tarsus and wing length in mm) identified PC1 as the primary body size measure, explaining 60% of the variance (Eigenvalue = 1.19, Table 4). Both $PC1_{beak}$ and $PC1_{body}$ were normally distributed with homogenous variance, as confirmed by the Shapiro-Wilk test and Levene's test, respectively. Independent two-tailed t-tests were then performed on $PC1_{beak}$ and $PC1_{body}$ to assess differences in males between the islands.

6. Results

Syllable types

From visual inspection of the spectrograms, we identified 12 syllable types per island, resulting in a total of 24 syllable types, with syllable type 14 divided into subtypes 14a and 14b. Validation by five independent reviewers yielded an overall agreement of 97% with this classification. Syllable types 1–12 were exclusive to Floreana Island, while types 13–24 occurred only on Santa Cruz. No syllable types were shared between the two islands (Figure 2).

Of the 50 males, the songs of 7 individuals had two different syllable types in 12-100% of their songs, while the other 43 males (86%) had song with one syllable type. Figure 3 shows examples of spectrograms for the males with two syllable types. On Floreana, 5 of 25 males had songs with two syllable types, and on Santa Cruz, 2 of 25 males had songs with two syllable types (Table 5).

On Floreana, syllable type 2 was sung in combination with syllable type 11 by 2 males but was also used by 3 males with only one syllable type. On Santa Cruz, syllable type 13 occurred in combination with syllable type 23 in 1 male but was also sung by another male who used only one syllable type in his songs. The subtypes of syllable type 14 (14a and 14b) were sung separately by two males each and together by one male.

Half the syllable types on Floreana (6/12) were each sung by 7-17% of males, and six syllable types were each sung by fewer than 5% of males (Table 6). Over half the syllable types on

Santa Cruz (7/12) were each sung by 7-22% of males, and each of the other five syllable types by fewer than 5% of males (Table 6).

Differences in the acoustic structure of the syllable types

The linear mixed models showed significant differences between islands for PC1 (higher factor loadings for frequency bandwidth) and PC4 (dominated by peak frequency). Males from Floreana produced syllable types with broader frequency bandwidth (estimate = -3.56, SE = 0.81, t = -4.42, $p < 0.001$) and higher peak frequencies (estimate = -0.716, SE = 0.34, t = -2.12, p = 0.0339) compared to males from Santa Cruz. However, no significant differences were found between the islands in terms of time-related variables (PC2) or spectral shape features (PC3).

Playback had a positive effect on spectral shape (estimate = -0.205, SE = 0.10, t = -2.03, p = 0.0425), but did not significantly influence the other acoustic features measured.

Visualization of PC1 and PC2 revealed not only differences between islands (Figure 4a) but also variation among syllable types overall. Each syllable type clustered recognizably according to frequency bandwidth (PC1) and time (PC2) (Figure 4b).

Differences in singing behavior

The models used to assess potential variations in male singing behavior between the islands revealed no significant differences. Males did not differ in the number of syllables per song (estimate = 0.041, SE = 0.19, p = 0.825), the number of syllable types per song (estimate = -0.108, SE = 0.12, p = 0.345), or the trill rate per song (estimate = 0.082, SE = 0.50, t = 0.165, p

= 0.869) between the two islands.

Differences in morphological measurements

Analysis of beak measurements (PC1_beak) showed significant differences between the islands. Cactus finch males from Santa Cruz had significantly larger beaks than males from Floreana (two-tailed t-test: t = -2.24, n = 17 of Floreana, n = 19 of Santa Cruz, p = 0.033; Table 7). There were no significant differences in body measurements (PC1_body) between the islands (two-tailed t-test: t = -1.27, n = 17 of Floreana, n = 19 of Santa Cruz, p = 0.215; Table 7).

7. Discussion

We investigated the range of song syllable types, differences in acoustic characteristics of syllables, male singing behaviors, and male morphology between two geographically separated populations of Darwin's cactus finches on Floreana and Santa Cruz Islands in the Galápagos Archipelago. Our analysis revealed that the two populations exhibited clear differences in their syllable repertoires, as well as some evidence of divergence in acoustic parameters and morphology. In total, we identified 24 syllable types across 50 males (25 males from each island), with 12 unique types on each island. Importantly, there were no shared syllable types between the two islands, highlighting a significant divergence in syllable repertoire. In our sample of 50 males, 7 individuals (14%) were observed producing songs that included two syllable types.

Our analysis of acoustic parameters revealed significant differences between the two populations. Males from Floreana produced syllables with broader frequency bandwidths and higher peak frequencies compared to males from Santa Cruz. These differences may be attributed to the influence of morphology on song production. Beak size, for instance, has been shown to constrain the frequency range and syllable repetition rate, as birds with larger beaks are less capable of opening and closing their beaks rapidly (Podos, 2001; Podos et al., 2004; Huber & Podos, 2006). Consistent with these findings, we found that males from Santa Cruz had bigger beaks than males from Floreana. Moreover, while no difference in trill rate was observed, syllable frequency bandwidth was narrower in the population with larger beaks. The narrower frequency bandwidths produced by Santa Cruz males may reflect a compensatory adaptation to biomechanical limitations associated with their larger beaks. As

a result, song divergence may emerge as a byproduct of morphological differences of the beak (Podos, 2001; Badyaev et al., 2008). Besides the beak size differences, no significant differences in body size between males from Floreana and Santa Cruz were found. Furthermore, no differences were detected in syllable repetition rates, the number of syllables per song, or the number of syllable types per song between the two populations, indicating comparable singing behavior in males across populations.

In addition to the differences in beak morphology, cultural processes might also be driving the observed acoustic divergence across populations. Genetic and cultural divergence can occur when populations become isolated or experience bottlenecks. Divergence in acoustic characteristics within a species can arise through various mechanisms, including cultural innovation (the introduction of new song or syllable types through mutations; Marler, 1970; Mundinger, 1980), cultural drift (the gradual and random alteration of song or syllable types; (Potvin & Clegg, 2015; Williams, 2021; Williams & Lachlan, 2022), and biases in social learning (Aplin, 2016). For birds, these cultural processes can lead to rapid changes in acoustic characteristics (Aplin, 2019).

Young Darwin's finches acquire their songs through a culturally mediated learning process from nearby males, including their fathers or close neighbors (Bowman, 1979, 1983; Millington & Price, 1985; B. R. Grant & Grant, 1996; P. R. Grant & Grant, 2018; Christensen & Kleindorfer, 2007; Peters & Kleindorfer, 2018). The transmission of songs in Darwin's finches is not error-free, similar to the inheritance of song patterns in zebra finches (Böhner, 1983, 1990; Zann, 1990; Mann & Slater, 1994). The process of vocal production learning involves several distinct stages: initial exposure to adult songs, memorization of these vocal patterns,

and eventual practice leading to the crystallization of a mature song (Immelmann, 1969; Marler, 1970; Marler & Peters, 1982a, 1982b; Podos et al., 2009). Each of these stages is prone to errors, which can arise due to inaccuracies in auditory perception, imperfect memory retention, or variability during vocal practice (Marler & Tamura, 1964; B. R. Grant & Grant, 1996; Podos & Warren, 2007). These errors may accumulate over time and introduce subtle, yet potentially significant, deviations between the learned and original songs. In Darwin's finches, these culturally transmitted variations can propagate within populations, contributing to the emergence of novel syllable types or unique song patterns (Slater, 2003; Parker et al., 2010; Aplin, 2016; Williams, 2021).

The cultural nature of song transmission enables rapid adaptation to environmental and social contexts. Darwin's finch females often prefer males with songs that differ from their fathers' song types, a preference that could function to reduce inbreeding (Gibbs & Grant, 1989; B. R. Grant, 1984; B. R. Grant & Grant, 1989, 1996; P. R. Grant & Grant, 1995). This mechanism can also promote syllable diversity within populations, as males with uncommon or distinct song characteristics may gain a reproductive advantage (Colombelli-Négrel et al., 2023). As a result, song can be a flexible trait that evolves independently of genetic changes, highlighting the significant role of cultural evolution in the speciation and diversification of Darwin's finches.

Another mechanism potentially driving the divergence of these populations is hybridization with the closely related medium ground finch (*Geospiza fortis*). Previous studies have documented that interbreeding between cactus finches and medium ground finches is not uncommon (P. R. Grant & Grant, 1997a; P. R. Grant et al., 2003, 2004; Thackeray, 2024). Medium ground finches are characterized by shorter and broader beaks compared to cactus

finches. Hybrid offspring may exhibit altered beak morphology and modified song characteristics, introducing variability into populations (Peters & Kleindorfer, 2018; Kleindorfer et al., 2019)

During data collection on Floreana, nest monitoring was conducted alongside song recording. Data from 25 nests belonging to 20 cactus finch males were collected. While cactus finches are strongly associated with *Opuntia* cacti, which serve as both food sources and nesting sites, our observations revealed that only 3 of the 25 cactus finch nests were built in *Opuntia*. The majority of nests (15) were found in *Candelabra* cacti, while 7 were located in trees (e.g., *Palo Santo*, *Acacia*) or hanging from vines. Interestingly, medium ground finches, typically described as nesting in shrubs or low trees, were often observed actively defending nests in *Opuntia*. These observations in cactus finches and medium ground finches on Floreana Island may already indicate a shift in nesting behavior.

Such behavioral changes were not observed on Santa Cruz during data collection; however, comprehensive nest monitoring was not conducted on Santa Cruz Island. While hybridization between cactus finches and medium ground finches has likely occurred on both islands, its effects may be more pronounced in smaller populations like the one on Floreana (P. R. Grant et al., 2003). Small populations are particularly susceptible to hybridization with closely related species due to limited mate choice (P. R. Grant & Grant, 1997a).

On Floreana, the close coexistence of cactus finches and medium ground finches might increase the likelihood of interbreeding. If such interbreeding is occurring, this may influence song learning, as young males often imitate the songs of nearby adults, sometimes also of

other species (B. R. Grant & Grant, 1996).

Introgressive hybridization, as documented in Darwin's finches, can have profound effects on morphology and behavior, including changes in beak shape and song characteristics (P. R. Grant et al., 2004; Kleindorfer et al., 2014; Peters & Kleindorfer, 2018; Dudaniec et al., 2025). In small populations, such as the cactus finches on Floreana, the influx of medium ground finch genes could accelerate divergence, creating distinct differences in both morphological traits and acoustic parameters compared to larger or more stable populations, such as those on Santa Cruz. Further research is needed to investigate the genetic basis of these population-level differences.

The observed differences in syllable repertoires and beak morphology between the two cactus finch populations revealed divergence between the island populations. We suggest that these differences are likely shaped by a combination of genetic factors, cultural transmission, and presumed hybridization, underscoring the need for further studies to confirm these mechanisms and better understand their interplay in driving population divergence. Such research could provide valuable insights into evolutionary and speciation processes while also informing conservation strategies aimed at protecting the unique ecosystems of the Galápagos Islands. Planned follow-up studies, including playback experiments, will investigate whether male cactus finches respond to allopatric song variants, shedding light on the extent of song-based behavioral divergence between the populations.

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9. Appendix

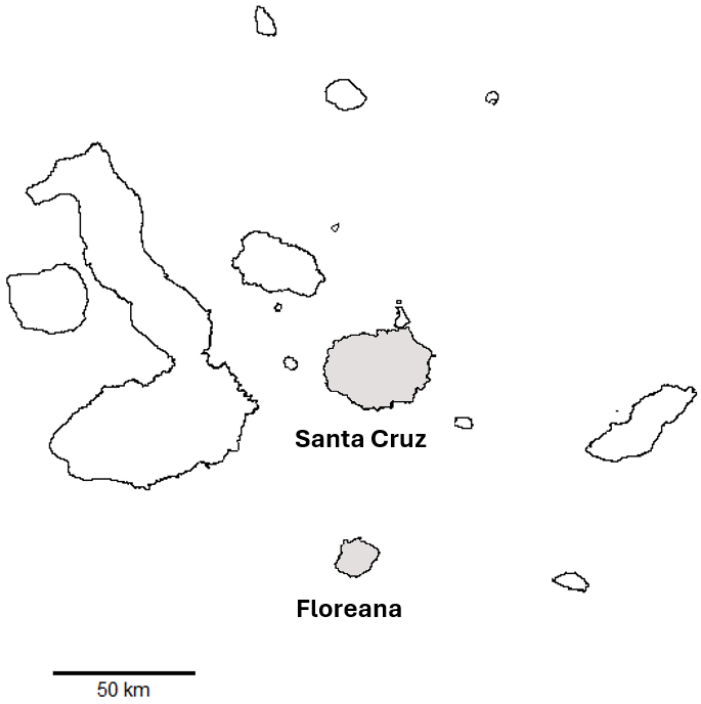


FIGURE 1 Map of the Galápagos Archipelago, with Santa Cruz and Floreana highlighted. The two islands are located approximately 50 km apart.

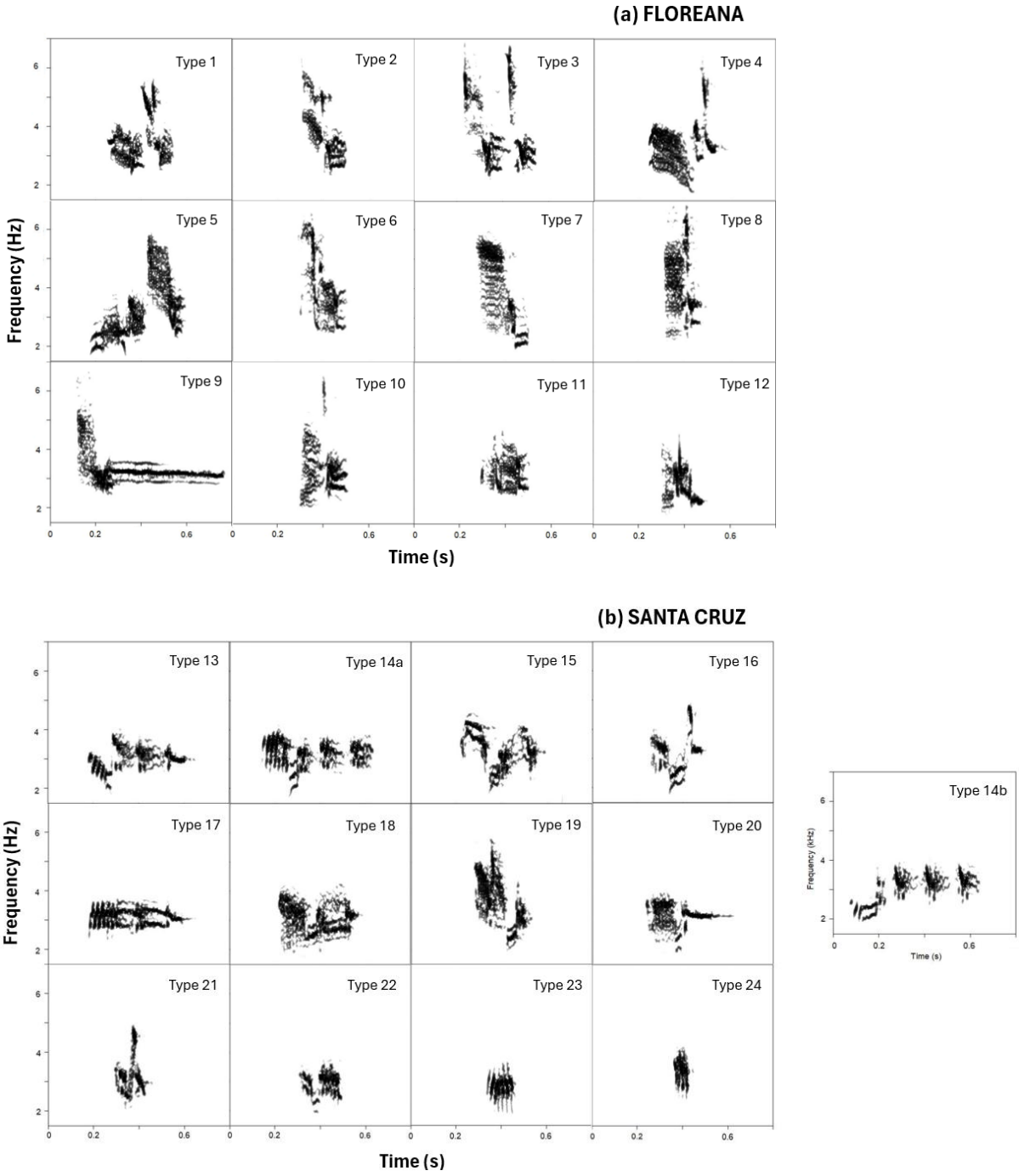
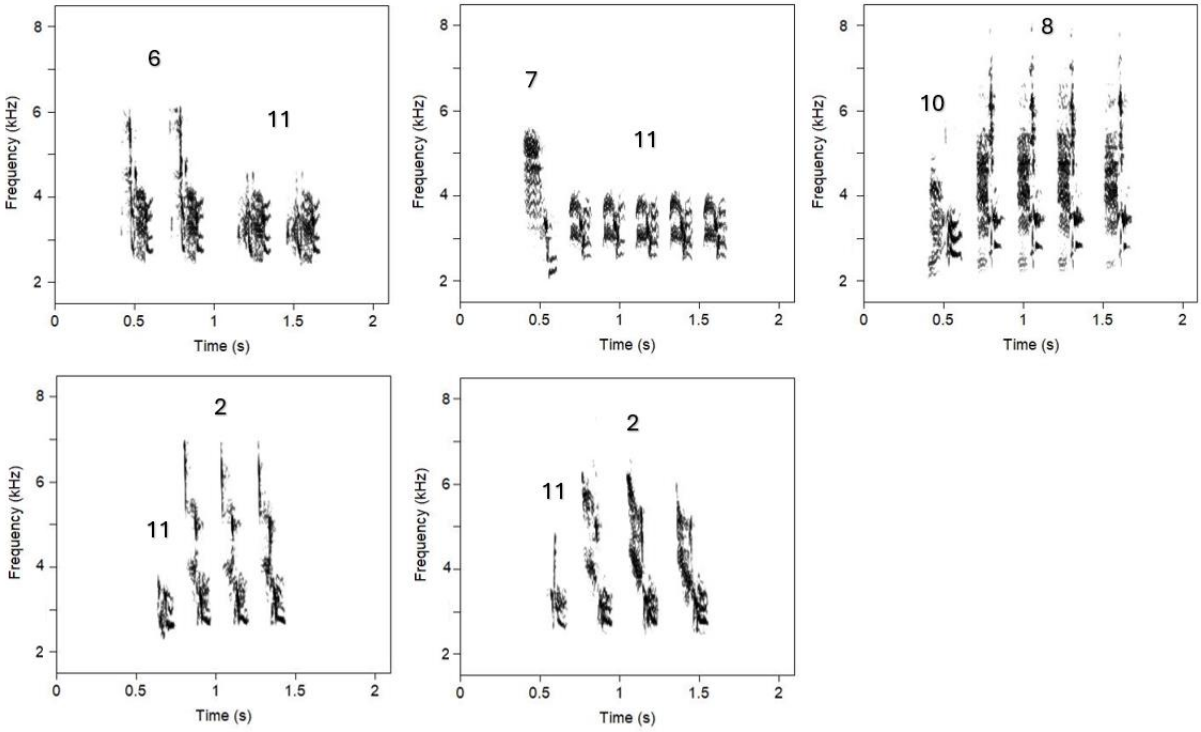


FIGURE 2 Examples of syllable types on (a) Floreana Island and (b) Santa Cruz: Syllable types 1-12 were found exclusively on Floreana Island, while syllable types 13-24 were unique to Santa Cruz. No syllable types were shared between the islands. Syllable type 14b lacks the introductory element present in type 14a.

(a) FLOREANA



(b) SANTA CRUZ

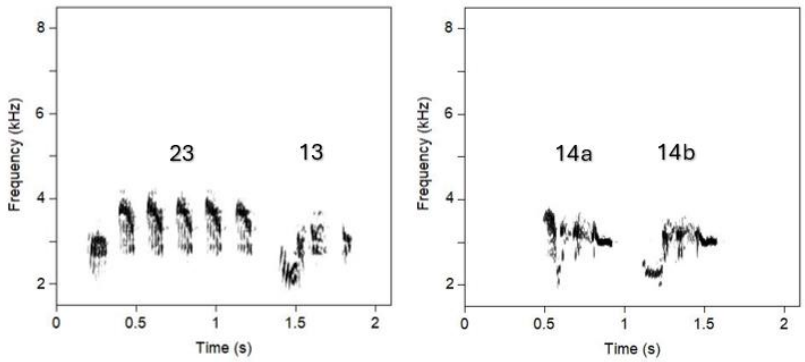
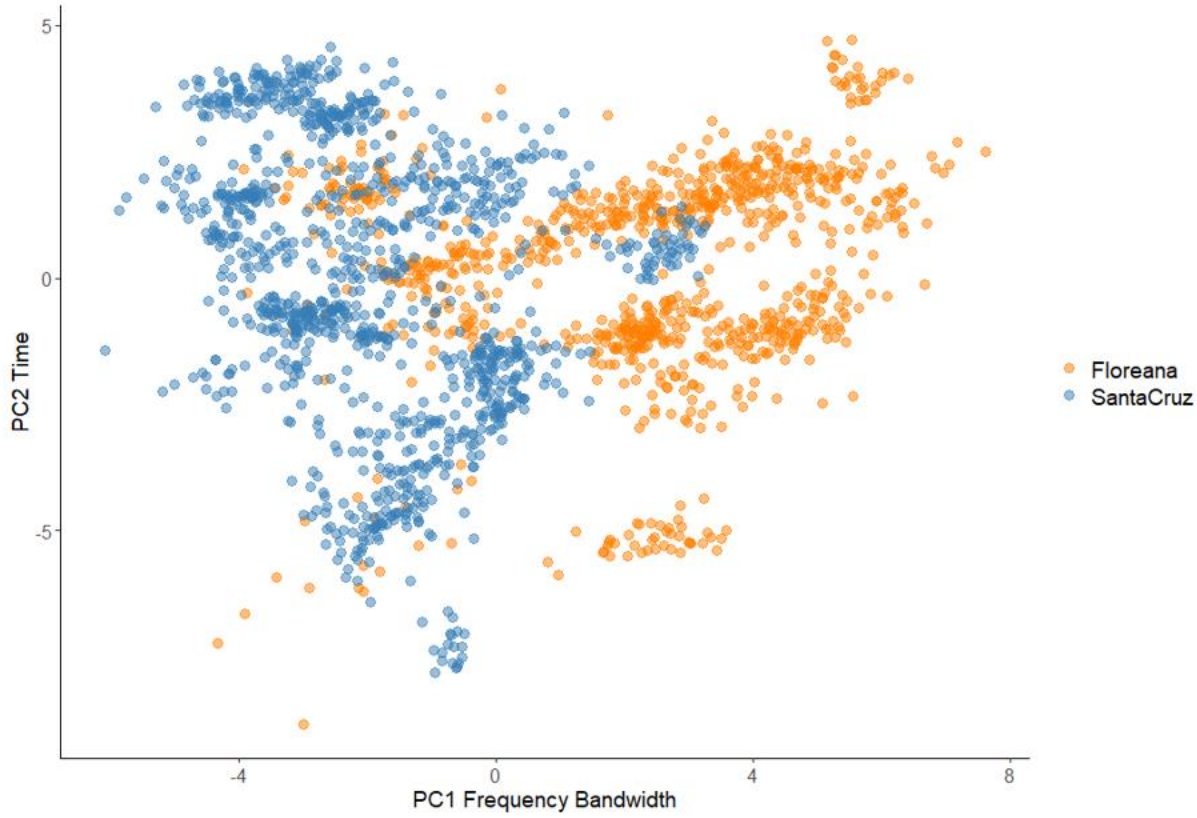


FIGURE 3 Spectrograms showcasing songs with two syllable types, including examples from the five males on Floreana Island (a) and the two males on Santa Cruz Island (b) that produced song with two syllable types in this study.

(a)


5
0
-5
PC2 Time
-4
0
4
8
PC1 Frequency Bandwidth
Floreana
SantaCruz

(b)

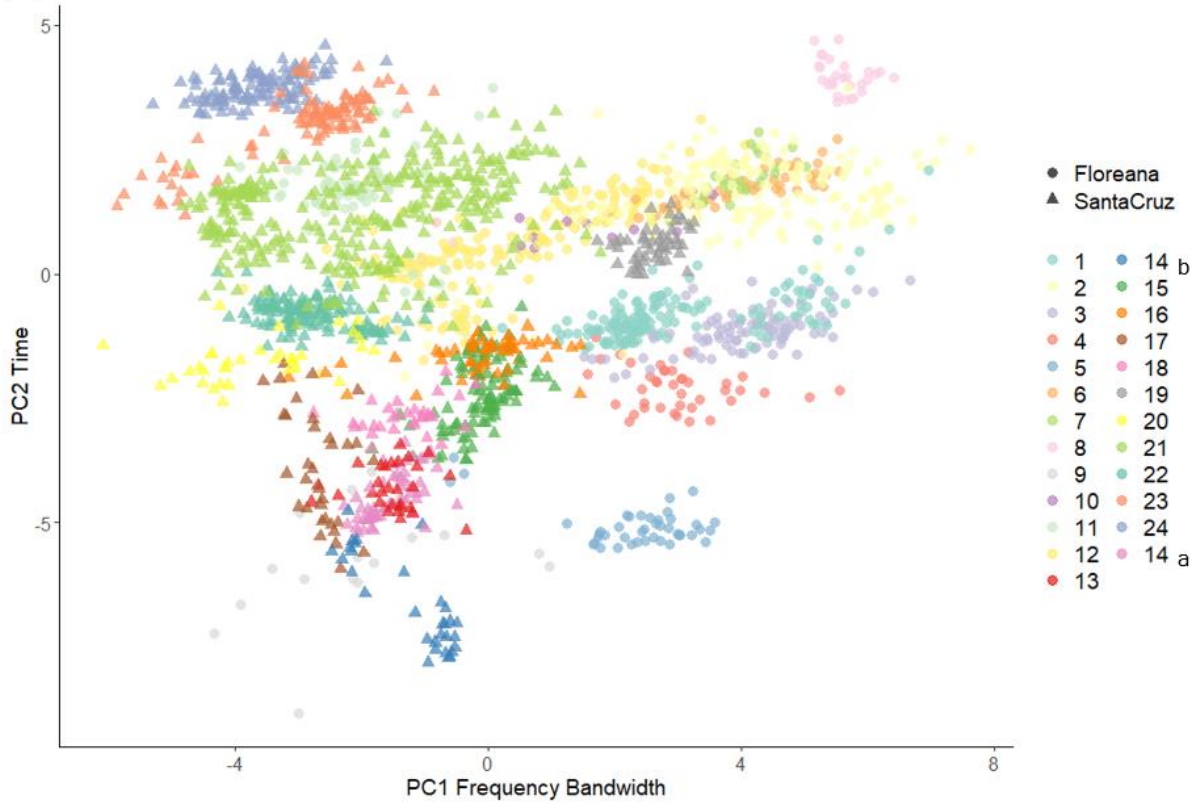

5
0
-5
PC2 Time
-4
0
4
8
PC1 Frequency Bandwidth
Floreana
SantaCruz
1
2
3
4
5
6
7
8
9
10
11
12
13
14 b
15
16
17
18
19
20
21
22
23
24
14 a

FIGURE 4 Variation in the acoustic structure of syllable types based on the first two components PC1 (dominated by frequency bandwidth) and PC2 (dominated by time-related variables) of the PCA analyzing acoustic characteristics. **(a)** Santa Cruz males ● produced songs with broader frequency bandwidth than Floreana males ●. **(b)** Each syllable type (1-12 for Floreana ● and 13-24 for Santa Cruz ▲) clustered in an identifiable way according to frequency bandwidth (PC1) and time (PC2).

TABLE 1 shows morphological measurements of 36 male cactus finches (17 from Floreana, 19 from Santa Cruz) collected between 2000 and 2024. PC1_beak represents the first principal component from beak measurements (length, depth, width in mm), while PC1_body reflects the first principal component from body measurements (tarsus and flattened wing length in mm).

Year	Island	Male ID	Beak length culmen	Beak depth	Beak width	Tarsus length	Flattened wing length	PC1_beak	PC1_body
2005	Floreana	F1	20.2	9.2	8.2	21.4	69	-0.24	-0.87
2005	Floreana	F2	21.2	10.1	9.3	21.7	68.5	1.93	-0.82
2005	Floreana	F3	19.6	10	8.7	20.7	68	0.90	-1.62
2005	Floreana	F4	21	9.6	8.7	23	72.5	0.82	1.20
2016	Floreana	F5	20	9.3	7.8	24	66	-0.53	-0.08
2016	Floreana	F6	19	8.7	7.6	23.7	74	-1.59	2.09
2016	Floreana	F7	21.5	9.5	8.7	24	70	0.84	1.11
2016	Floreana	F8	18.5	8.8	7.5	23.4	69	-1.69	0.42
2020	Floreana	F9	27.3	9.3	8.3	22.1	68	1.70	-0.71
2020	Floreana	F10	19.1	9.2	8.2	22.9	72	-0.51	0.99
2020	Floreana	F11	16.5	8.5	8.2	23	68	-1.88	-0.13
2020	Floreana	F12	21	9.7	8.5	23.4	72	0.75	1.31
2024	Floreana	F13	17.7	10.7	8.85	20.2	67	1.31	-2.24
2024	Floreana	F14	17.7	9.5	7.4	20.4	70.1	-1.24	-1.19
2024	Floreana	F15	16.5	8.6	6	22.1	69	-3.71	-0.42
2024	Floreana	F16	18.5	9.1	6.7	21.7	72	-2.08	0.21
2024	Floreana	F17	16.4	8.9	7.1	21.7	69	-2.45	-0.68
2000	Santa Cruz	SC1	21.5	10.2	9.5	21.9	71	2.28	0.05
2000	Santa Cruz	SC2	20.3	9.8	9.3	21.3	73.5	1.39	0.40
2001	Santa Cruz	SC3	20.8	9.4	9	22.5	70	0.82	0.14
2001	Santa Cruz	SC4	19.1	8.4	7.6	20.5	68.5	-1.88	-1.60
2002	Santa Cruz	SC5	22	10.1	9.4	22	71	2.21	0.11
2002	Santa Cruz	SC6	20.5	9.6	9.2	20.9	71	1.14	-0.60
2004	Santa Cruz	SC7	19.1	10.5	8.8	21.3	70	1.39	-0.64
2004	Santa Cruz	SC8	21.8	9.4	9.2	23.8	73.5	1.24	2.01
2016	Santa Cruz	SC9	25.6	9.1	8.4	23.5	70	1.16	0.78
2016	Santa Cruz	SC10	21.7	9.6	7.9	23	74	0.29	1.64
2016	Santa Cruz	SC11	22.2	10	9	23.3	72	1.80	1.24
2016	Santa Cruz	SC12	22.3	9.9	8.5	23.5	76	1.28	2.56
2016	Santa Cruz	SC13	19.9	9	8	24	67	-0.70	0.22
2016	Santa Cruz	SC14	23	9.9	8.8	23	72	1.72	1.05
2016	Santa Cruz	SC15	22.4	8.3	7.1	22.6	69	-1.61	-0.09
2016	Santa Cruz	SC16	19.2	9.8	8.2	20.3	72	0.15	-0.69
2016	Santa Cruz	SC17	19.8	9.7	7.2	21.4	69	-0.69	-0.87
2016	Santa Cruz	SC18	19.9	9.8	89.2	23.1	74	0.32	1.71
2016	Santa Cruz	SC19	20	9.5	7.8	22.8	69	-0.32	0.03

TABLE 2 Eigenvalues, proportion and cumulative variances for the first 4 components of the principal component analysis of the frequency- and time-related measurements

PC	Eigenvalue	Proportion Variance	Cumulative Variance
PC1	8.61	0.41	0.41
PC2	6.08	0.29	0.70
PC3	2.20	0.10	0.80
PC4	1.18	0.06	0.86

TABLE 3 Factor loadings for the first 4 principal components from the PCA of 21 acoustic variables. Shading highlights the highest loadings for each PC (values > 0.3).

Factor	PC1_freq_bandwidth	PC2_time	PC3_spectral_shape	PC4_peak_freq
Duration (sec)	-0.08	0.38	0.13	0.12
Minimum dominant frequency (kHz)	0.04	-0.25	0.27	0.04
Maximum dominant frequency (kHz)	-0.31	-0.04	0.11	-0.16
Bandwidth (kHz)	-0.31	0.04	0.02	-0.17
Peak frequency (kHz)	-0.08	-0.08	0.29	0.53
Slope	0.16	0.15	0.02	-0.36
Mean frequency (kHz)	-0.29	-0.15	0.21	-0.04
Standard deviation (kHz)	-0.3	-0.02	-0.08	-0.31
Frequency median (kHz)	-0.25	-0.17	0.23	0.18
Frequency Q25 (kHz)	-0.13	-0.21	0.43	0.17
Frequency Q75 (kHz)	-0.31	-0.1	0.11	-0.16
Frequency IQR (kHz)	-0.31	-0.03	-0.05	-0.25
Time median (sec)	-0.08	0.37	0.13	0.04
Time Q25 (sec)	-0.1	0.35	0.09	0.05
Time Q75 (sec)	-0.09	0.38	0.13	0.08
Time IQR (sec)	-0.08	0.37	0.14	0.1
Skew	0.22	0.02	0.41	-0.33
Kurtosis	0.16	0.02	0.45	-0.39
Spectral entropy	-0.31	0.05	-0.17	0
Time entropy	0.16	-0.32	0	-0.02
Spectrographic entropy	-0.29	-0.12	-0.2	-0.02

TABLE 4 Loading coefficients of the principal component analysis of the beak and body measurements

Factor	Loading coefficient to PC1_beak
Beak length culmen	0.48
Beak depth	0.57
Beak width	0.67
Eigenvalue	1.83
%Variance	61%

Factor	Loading coefficient to PC1_body
Tarsus length	0.71
Flattened wing length	0.71
Eigenvalue	1.19
%Variance	60%

TABLE 5 Details of seven Darwin's cactus finch males (of 50 males recorded during 2024) that produced a song with two syllable types; all other males (86 %) had song with one syllable type. The table includes male ID and Island, the number of song recordings (n) per male, the percentage of songs with one syllable type versus two syllable types, the syllable type combinations, and three syntax examples for each male.

Male ID	Island	#Recordings (n)	% Song with 1 syllable type	% Song with 2 syllable types	Syllable types present	Syntax examples
Male 1	Floreana	17	88	12	6 & 11	6-6-6-6; 11-11-11; 6-6-11-11-6-6
Male 2	Floreana	14	29	71	7 & 11	7-7; 7-11-11-11-11; 7-7-11-11-11-11-7-7
Male 3	Floreana	10	0	100	10 & 8	10-8-8-8; 10-8-8-8-8; 10-8
Male 4	Floreana	8	0	100	11 & 2	11-2-2-2-2-2-2; 11-2-2-2-2-11-2; 11-2-2-2-2
Male 5	Floreana	12	0	100	11 & 2	11-2-2-2; 11-2-2; 11-2-2-2-2-2
Male 6	Santa Cruz	21	71	29	23 & 13	23-23; 23-23-23-23-13; 23-23-23-23-23-13-13
Male 7	Santa Cruz	20	50	50	14a & 14b	14a; 14a-14a-14b; 14a-14b

TABLE 6 Characteristics of syllable types on **(a) Floreana** and **(b) Santa Cruz** Islands. Mean values and standard deviation of the measured parameters including duration, minimum dominant frequency, maximum dominant frequency, bandwidth and peak frequency. Data include 25 males per island whereby 5 males had songs with two syllable types on Floreana Island and two males had songs with two syllable types on Santa Cruz. Syllable types are listed in order of their percentage occurrence, with the number of males producing each syllable type indicated in parentheses.

(a) FLOREANA

Syllable type	% occurrence	Duration (sec)[a]	Minimum dominant frequency (kHz)[a]	Maximum dominant frequency (kHz)[a]	Bandwidth (kHz)[a]	Peak frequency (kHz)[a]
1	16.7 (5)	0.28 (0.02)	2.61 (0.12)	5.49 (0.61)	2.88 (0.69)	3.13 (0.19)
2	16.7 (5)	0.22 (0.04)	2.66 (0.08)	6.02 (0.53)	3.36 (0.53)	3.52 (0.89)
12	16.7 (5)	0.21 (0.03)	2.25 (0.08)	4.3 (0.53)	2.05 (0.53)	2.68 (0.56)
11	13.3 (4)	0.14 (0.03)	2.72 (0.14)	3.79 (0.3)	1.07 (0.28)	3.1 (0.29)
3	10.0 (3)	0.31 (0.02)	2.7 (0.12)	6.18 (0.28)	3.48 (0.32)	3.3 (0.09)
5	6.7 (2)	0.42 (0.01)	1.99 (0.18)	5.32 (0.15)	3.33 (0.25)	2.54 (0.15)
4	3.3 (1)	0.33 (0.02)	2.08 (0.13)	5.69 (0.25)	3.61 (0.31)	3.38 (0.22)
6	3.3 (1)	0.21 (0.01)	2.73 (0.03)	6.09 (0.18)	3.36 (0.19)	3.51 (0.14)
7	3.3 (1)	0.21 (0.01)	2.15 (0.04)	5.33 (0.1)	3.18 (0.12)	4.69 (0.75)
8	3.3 (1)	0.17 (0.01)	2.98 (0.3)	6.04 (0.68)	3.06 (0.55)	3.53 (0.36)
9	3.3 (1)	0.6 (0.06)	2.78 (0.13)	5.05 (0.27)	2.27 (0.31)	3.25 (0.06)
10	3.3 (1)	0.2 (0.01)	2.57 (0.12)	5.3 (0.72)	2.73 (0.74)	2.84 (0.23)
	100.0 (30)					[a]Mean (SD)

(b) SANTA CRUZ

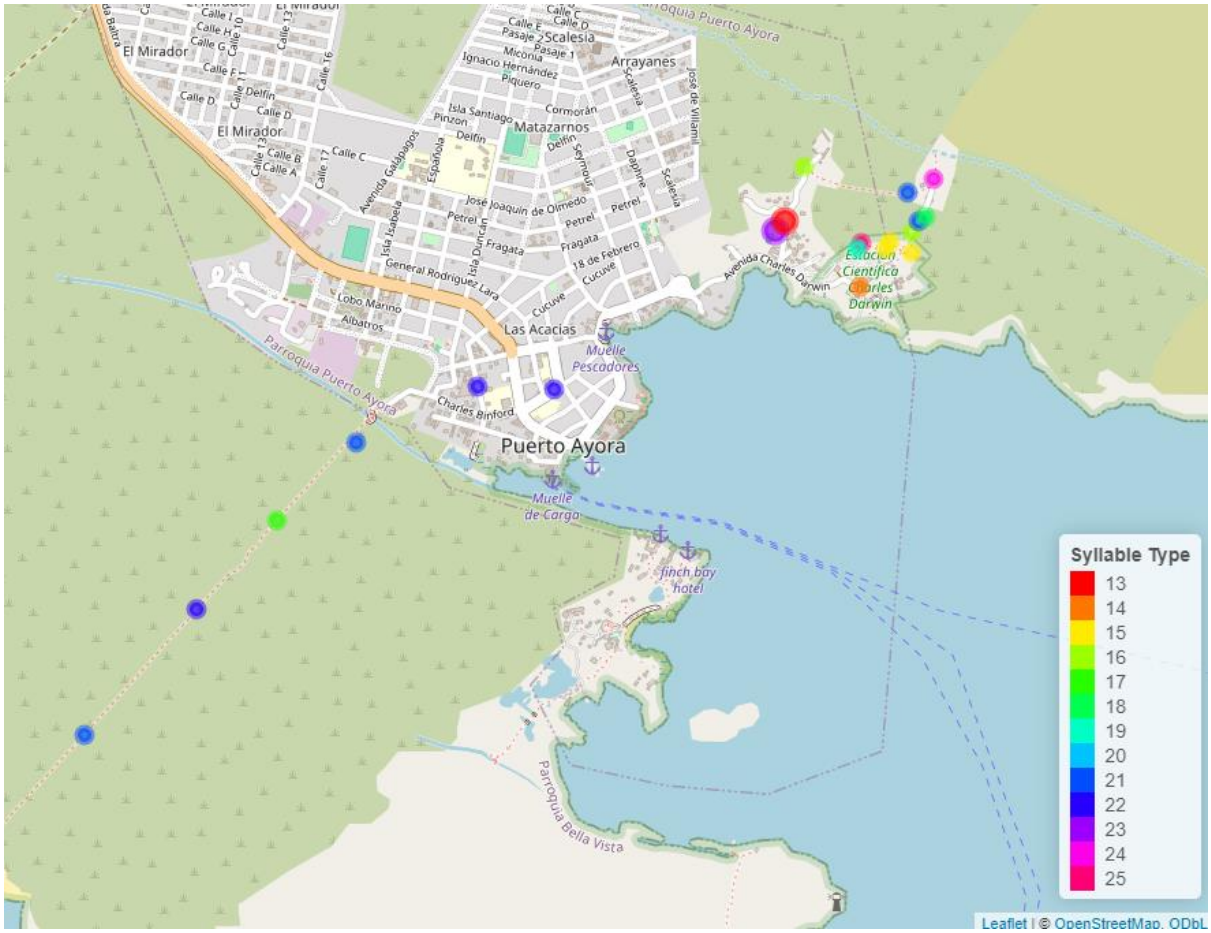
Syllable type	% occurrence	Duration (sec)	Minimum dominant frequency (kHz)[a]	Maximum dominant frequency (kHz)[a]	Bandwidth (kHz)[a]	Peak frequency (kHz)[a]
21	22.2 (6)	0.16 (0.03)	2.59 (0.26)	4.09 (0.65)	1.5 (0.75)	3.2 (0.18)
15	11.1 (3)	0.33 (0.03)	2.26 (0.18)	4.28 (0.15)	2.02 (0.18)	3.67 (0.5)
22	11.1 (3)	0.21 (0.02)	2.31 (0.11)	3.33 (0.12)	1.02 (0.15)	3.09 (0.09)
13	7.4 (2)	0.45 (0.03)	2.38 (0.12)	3.61 (0.3)	1.23 (0.25)	3.04 (0.08)
14a	7.4 (2)	0.48 (0.02)	2.36 (0.09)	3.68 (0.06)	1.33 (0.11)	3.18 (0.16)
14b	7.4 (2)	0.51 (0.05)	2.1 (0.16)	3.57 (0.16)	1.47 (0.25)	3.18 (0.12)
16	7.4 (2)	0.27 (0.02)	2.28 (0.16)	4.72 (0.3)	2.44 (0.32)	3.33 (0.17)
17	7.4 (2)	0.45 (0.04)	2.47 (0.29)	3.34 (0.07)	0.87 (0.24)	3.04 (0.14)
18	3.7 (1)	0.35 (0.01)	2.47 (0.05)	3.76 (0.12)	1.29 (0.13)	3.21 (0.2)
19	3.7 (1)	0.24 (0.01)	2.29 (0.09)	4.93 (0.1)	2.64 (0.1)	3.56 (0.52)
20	3.7 (1)	0.28 (0.02)	2.4 (0.11)	3.59 (0.12)	1.19 (0.17)	3.13 (0.03)
23	3.7 (1)	0.11 (0.01)	2.94 (0.23)	3.68 (0.33)	0.74 (0.25)	3.51 (0.32)
24	3.7 (1)	0.08 (0.01)	3.09 (0.17)	3.65 (0.13)	0.56 (0.19)	3.3 (0.08)
	100.0 (27)					[a]Mean (SD)

Table 7 Morphological data for 17 Floreana and 19 Santa Cruz males collected between 2000 and 2024. Table shows mean values and standard error of beak size (length, depth, width in mm) and body size (tarsus, wing length in mm) per island. Beak measurements' PCA (PC1_beak) revealed significantly larger beaks in Santa Cruz males (p = 0.033), while body size shows no significant differences between islands.

	Floreana	Santa Cruz	
Morphological Variables	*Mean ± SE*	*Mean ± SE*	*p*
Beak length culmen	19.5 ± 0.6	21.1 ± 0.4	
Beak depth	9.3 ± 0.1	9.6 ± 0.1	
Beak width	8.0 ± 0.2	8.5 ± 0.2	
PC1_beak	-0.5 ± 0.4	0.6 ± 0.3	**0.033**
Tarsus length	22.3 ± 0.3	22.4 ± 0.3	
Flattened wing length	69.7 ± 0.5	71.2 ± 0.6	
PC1_body	-0.1 ± 0.3	0.4 ± 0.3	0.215

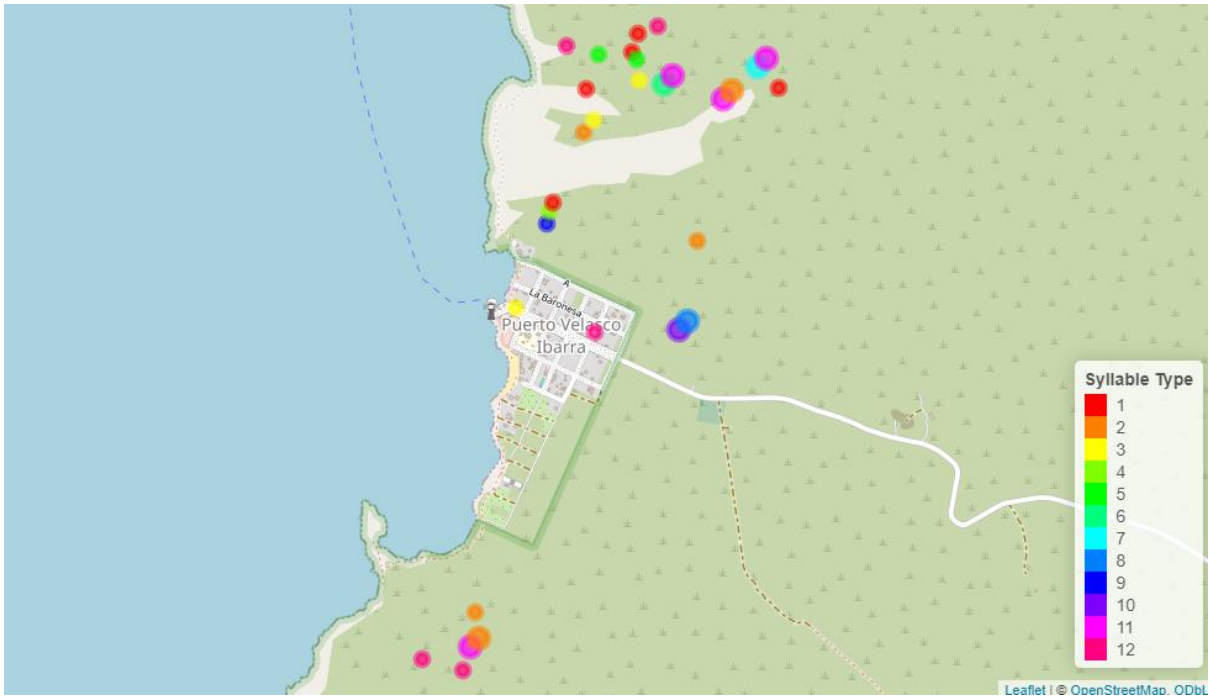
10. Supplementary

S 1 Map of Santa Cruz showing song recording sites in Puerto Ayora and Garrapatero for a total of 25 males. Each dot represents a recorded syllable, with each color corresponding to a different syllable type. In total, 12 syllable types (numbered 13 to 25) were recorded on this island.



S 2 Magnified map of Puerto Ayora, Santa Cruz, showing the recording sites. Each dot represents a recorded syllable, with each color corresponding to a different syllable type. A total of 25 males were recorded on Santa Cruz, 19 of which were from Puerto Ayora. One of these 19 males had songs containing two syllable types, indicated by a larger-diameter dot.

S 3 Magnified map of Garrapatero, Santa Cruz, showing the song recording sites. Each dot represents a recorded syllable, with each color corresponding to a different syllable type. A total of 25 males were recorded on Santa Cruz, with 6 of them from Garrapatero. One of these 6 males had songs containing two syllable types, indicated by a larger-diameter dot.



S 4 Map of Floreana showing song recording sites inside and outside of Puerto Velasco Ibarra for a total of 25 males. Each dot represents a recorded syllable, with each color corresponding to a different syllable type. Five of the 25 males had songs containing two syllable types, indicated by larger-diameter dots. In total, 12 syllable types (numbered 1 to 12) were recorded on this island.