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Cultural divergence and morphological variation of isolated remnant populations of the endangered Floreana mockingbird

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ABSTRACT

Context. Cultural divergence refers to changes over time in behavioural traits. These cultural changes could have important implications for conservation planning, and impact the success of conservation efforts such as translocations. **Aims.** Here, we investigate the extent, and potential impacts, of cultural divergence in two isolated populations of the Floreana mockingbird (*Mimus trifasciatus*) on the Galápagos Islands. **Methods.** Using contemporary recordings of vocalisations we test for spatial vocal differentiation between mockingbird populations. Furthermore, we explore the potential drivers of change using morphological measurements and historical recordings of the species. **Key results.** We found evidence of spatial and temporal differentiation in vocalisations between the two populations of the Floreana mockingbird. We accurately classified over 75% of the birds to the correct populations based on multivariate measures of audio recordings using canonical analysis of principal coordinates (CAP). We also found significant differences in morphometrics between populations; specifically, beak depth was associated with frequency modulation, an acoustic measure that is significantly different between populations. Furthermore, we found evidence of change in the complexity of the vocalisations over a period of 57 years. **Conclusions.** Cultural divergence was found in the two remaining populations of this endangered species. Factors such as changes in morphology and cultural drift might have been influenced the change in vocalisation across time and populations. **Implications.** We highlight the importance of considering behavioural factors when planning reintroductions of endangered species where there is a need to minimise the risk of assortative mating so as to maximise genetic diversity.

Keywords: conservation, cultural divergence, cultural drift, Floreana mockingbird, Galapagos, morphology, reintroduction, vocalisations.

Introduction

Avian vocalisations are a well-studied example of complex cultural traits in non-human animals. Three main groups of birds are known to culturally transmit calls and songs: (1) hummingbirds; (2) parrots; and (3) oscine songbirds (Jarvis *et al.* 2000). Nevertheless, recent evidence of vocal learning has also been found in a hand-reared Australian musk duck (*Bizioura lobata*) showing clear similarities with the vocal learning capability of songbirds and parrots (ten Cate and Fullagar 2021). Although defining the differences between learned calls and song is still debated, bird song has been defined as vocalisations with a high degree of complexity (Rose *et al.* 2022). Many functions has been attributed to complex learned vocalisations including the 'dual-purpose' hypothesis of mate attraction and territory defence (Catchpole and Slater 2003) and signal of reproductive quality (for example, Slabbekoorn and Smith 2002; de Kort *et al.* 2009). However, complex vocalisations have also been associated with functions beyond courtship and territory defence, such as social cohesion (Loning *et al.* 2023) and communication (Peckre *et al.* 2019).

Commonly used measures of song and call complexity include repertoire size (diversity), delivery (songs per minute), switching (proportion), variation (short term diversity), duration, bandwidth (frequency bandwidth), and inflections per second (Benedict and Najar 2019).

Here, we consider complexity as a change in the syllable repertoire, song structure and delivery of song, based on the measurements mentioned above. Overall complexity of bird vocalisations may be influenced by multiple ecological factors. Song complexity may vary with the age of the bird, where vocalisations of the juveniles are simpler than those of the adults due to the learning process (O'Loghlen and Rothstein 1995; Kipper and Kiefer 2010). Sociality can also influence song complexity, where highly social and cooperative-breeding birds show variation in vocalisations related to social status (Voigt *et al.* 2007). Additionally, complexity of vocalisations has been shown to relate to the complexity of the habitat (Hill *et al.* 2017), breeding stage (Demko and Mennill 2019), and differences in brain size between sexes (Garamszegi *et al.* 2005).

Geographical variation in songs has been observed in many songbird populations at various spatial scales (Kroodsma 1985; Galeotti *et al.* 1996; Leader *et al.* 2000; Bell *et al.* 2003). This variation usually arises from a combination of dynamic song-learning processes and isolation by natural barriers (Podos and Warren 2007). Over time, cultural evolution (Mesoudi 2017) can lead to the development of new song types and the emergence of local dialects, such as new types of song (Slater 2003; Laland and Janik 2006; Parker *et al.* 2010, 2012; Garland *et al.* 2011; Aplin 2016). Bird vocalisations may change over time due to founder effects and cultural drift in a similar way to genetic drift, though potentially more rapidly because new songs are transmitted and learned within and among extant generations (Whitehead *et al.* 2004; Laiolo and Jovani 2007). Demographic factors can also play an important role in the evolution of song, as they do for non-cultural evolution. For instance, larger, or denser populations often have greater song repertoires; whereas, decreasing populations, or those prone to extinction tend to have smaller repertoires (Baker 1975; Laiolo and Tella 2007; Laiolo *et al.* 2008; Crates *et al.* 2021). Thus, the correlation between population size and song diversity mirrors the effects of small populations and genetic inbreeding (Spielman *et al.* 2004; O'Grady *et al.* 2006).

Cultural evolution may not be the only process driving vocalisations divergence over time. Morphological variation among populations may also drive the development of new dialects. Traits such as the morphology of beaks, vocal tracts (Podos and Nowicki 2004; Huber and Podos 2006; Snowberg and Benkman 2007; Derryberry *et al.* 2012, 2018; Kazemi *et al.* 2023) and body size may affect vocalisations via mechanical constraints on song production (Podos 1997; García and Tubaro 2018). Nevertheless, in some endangered species, new local culture can develop as a consequence of conservation practices. For instance, the use of different species as fostering parents and the reduced number of individuals in breeding facilities has been shown to influence the development of culture (Martínez and Logue 2020; Crates *et al.* 2021). In some cases, divergence of vocalisations can lead to cultural barriers to reproduction between populations (Irwin *et al.* 2001; Slabbekoorn and Smith 2002).

Small populations are often exposed to the negative effects of inbreeding. Translocation of individuals among populations can mitigate this problem and improve genetic diversity (Tracy *et al.* 2011). However, cultural and behavioural factors may hamper attempts to introduce genetic diversity to populations via translocations. For example, assortative mating based on local dialect preferences could compromise the reproductive success of mixed founder populations and the success of the translocation itself (Parker *et al.* 2012; Bradley *et al.* 2014). Recently, the potential effects of cultural evolution in small or endangered populations have received attention. For instance, Crates *et al.* (2021) showed that a loss of cultural diversity is associated with a reduction in individual fitness during breeding. Identifying scenarios that support the retention of cultural diversity may become an integral component of a conservation plan (Ryan 2006). For human-assisted conservation efforts, such as translocations, regional dialects can lead to assortative mating, where females prefer to mate with 'familiar' males due to a strong preference for familiar song types (Rowe and Bell 2007; Martins *et al.* 2018). Thus, different responses to signals from different conspecific populations represent evidence of a degree of reproductive isolation (Irwin *et al.* 2001), decreasing the chances of a successful translocation by inhibiting genetic and cultural mixing when multiple source populations are transferred.

In this study, we investigated the vocalisations and morphology of the Floreana mockingbird (*Mimus trifasciatus*), an endangered cooperative breeding (one dominant pair and several subordinate birds including offspring's of previous breeding season and unrelated females) songbird living in two remnant populations that have been isolated since the extinction of the main population on Floreana Island (Grant *et al.* 2000). Although the genetic consequences of isolation and divergence are well known for the species (Hoeck *et al.* 2010*a*, 2010*b*), little is known about the degree of cultural evolution and divergence that may affect any attempts to reintegrate these populations.

The two aims of our study were to: (1) compare vocalisations from the two mockingbird populations to quantify cultural divergence, while controlling for factors such as sex and age of the individuals; and (2) identify potential drivers of any cultural divergence between the populations. We hypothesised that: (a) complexity of vocalisations is partially explained by morphological differences between populations that underpin cultural divergence between populations; and (b) the complexity of mockingbird vocalisations within the smaller of the two population has declined over a 57-year period due to cultural drift. We tested hypothesis (b) on one population because of the availability of historical recordings and because this population has experienced a quantifiable degree of genetic drift since the extinction of the main population on Floreana (Hoeck *et al.* 2010*a*).

Materials and methods

Study site

Our study was conducted on Champion (9.4 ha) and Gardner-by-Floreana (76.5 ha) islets located at 0.8 and 8 km off the Coast of Floreana Island, respectively. Detailed descriptions of the study sites can be found in Reyes *et al.* (2022).

Recordings of wild Floreana mockingbirds

During the non-breeding season in August 2019, we opportunistically recorded vocalisations of seven individual birds on Champion (180 h) and 14 individuals on Gardner (60 h). The birds on Champion, and most of the birds within the Gardner study area (12 ha), were colour banded for individual recognition. All recordings were made using a directional shotgun microphone (Sennheiser ME66, Sennheiser, Germany) and a portable solid-state recorder (Marantz Professional PMD 661, Marantz Professional, Cumberland, USA) with 24-bit sampling precision and 48 kHz sampling rate. *Mimus trifasciatus* live in territorial groups of six or more individuals (EMR Reyes, pers. obs.). Within these groups, dominant males and females typically sing regularly from specific high perches along with submissive individuals on lower perches (EMR Reyes, pers. obs.). Recordings were initiated when a bird was located and ended when the bird stopped singing or vacated the perch. Recordings were made on calm days (0 and 1 in Beaufort scale), and the unidirectional microphone was positioned to minimise wind noise. The lack of anti-predator behaviour of the Floreana mockingbird meant that all recordings could be made within 5 m of the focal bird. For each recording, we documented the GPS location, date, time of the day, unique colour band combination (when possible) and behaviour. Individuals were generally recorded more than once during each day and through different days to account for temporal variation. Individuals were identified using a Nikon D90 digital camera with 70–300 mm lens and Eagle Optics Ranger 10 × 42 binoculars. For the analysis, we only used recordings of birds that could be identified by their bands.

Vocalisation categories

In general, mockingbirds (family Mimidae) are considered open-ended learners (Gammon 2020) and their vocalisations are complex and consist of sequences of different types of syllables that can be combined in multiple ways to produce a variety of vocalisation types (Botero *et al.* 2009; Gammon 2014). The only known descriptions of the behavioural context of vocalisations for mockingbirds in Galápagos are from Vitousek *et al.* (2007) and Fusani *et al.* (1994). In this study, we classified the Floreana mockingbird vocalisations into three main categories matching the terminology used for the vocal array of the northern mockingbird (*Mimus polyglottos*) (Derrickson and Breitwisch 1992): (1) submissive call or 'begging', which is a short repetitive syllable given by juveniles and submissive birds in the proximity of an adult or a bird higher in the family hierarchy; (2) alarm or 'hew calls', which comprise two types of calls, one in response to Galapagos terrestrial snakes (*Pseudalsophis biserialis*) consisting of a short rasp call, sometimes repetitive, and the other high amplitude short call for threats including owls and observers; and (3) 'chats', which consist of single vocalisation or 'single chats', and continuous 'chats' that we define as 'chatbursts'. The behavioural contexts of the different types of 'chats' we observed were not studied in detail because of time and resource constraints. Nevertheless, the behavioural context of these vocalisations was the same for both islands, and breeding stage was controlled for in the analysis. For the purpose of our research, we excluded from our dataset the 'begging' and 'hew calls' due to insufficient numbers of recordings of these types for population comparisons as many of the recordings were from unidentified birds. Hence, we focus on single 'chats' and 'chatburst' because of the potential of these vocalisations to be a conspecific recognition signals directed to individuals inside and outside the social structure of this cooperative breeding birds (family groups) (Logan *et al.* 1983; Logan 1985; Botero and Vehrencamp 2007).

Acoustic analysis

We visualised the recordings as spectrograms (Fig. 1) using the open software KOE (Fukuzawa *et al.* 2020), with an FFT window of 512. We retained for analysis only high-quality recordings (i.e. high signal-to-noise ratio songs with minimal background noise and that did not overlap with other birds' songs). Recordings were segmented into units or syllables, defined as one or more element between less than 15 ms interval between elements (i.e. a single note or sound trace on spectrogram) repeated in a specific temporal unit and frequency (Wildenthal 1965; Kershenbaum *et al.* 2016). In KOE, for each recording, we extracted the following seven acoustic variables using the 'extract features' option: (1) duration; (2) spectral bandwidth mean; (3) frequency modulation mean; (4) amplitude modulation mean; (5) goodness of pitch mean; (6) entropy mean; and (7) harmonic ratio mean. We chose these variables because they are the most commonly used in the bioacoustics literature and because temporal, frequency and amplitude characteristics of vocalisations are used as a measure of complexity (Kershenbaum 2014; Benedict and Najar 2019; Rose *et al.* 2022). Using ordination by *t*-distributed stochastic neighbour embedding (*t*-SNE; using a perplexity of 30 and 5000 iterations) and similarity indices, we categorised syllables

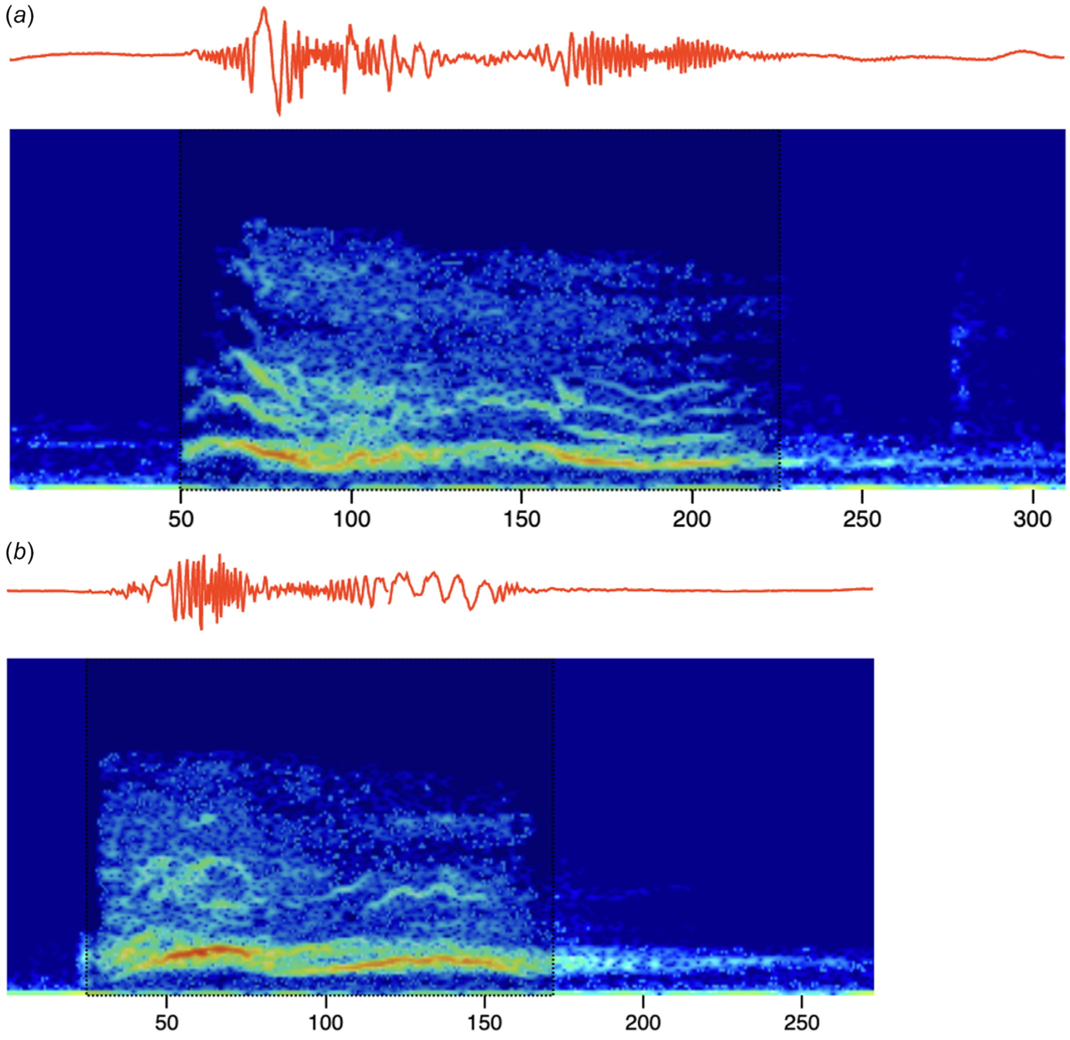

Fig. 1. Spectrograms of the *Mimus trifasciatus* 'chats' vocalisation for the two different populations: (*a*) Champion population; and (*b*) Gardner population.

based on identifiable clusters of syllable types (see Fukuzawa *et al.* (2020) for details).

Syllables were plotted by individual (see Supplementary material A) to check for individual variation, and outliers were not removed due to the small sample size. For each of the seven acoustic variables extracted from *KOE*, we averaged the values across all the vocalisations for an individual, giving a single mean value for each acoustic variable for each individual. Each variable was then normalised to have a mean of zero and a standard deviation of one (using the 'normalisation' routine in PRIMER *v7*; Clarke and Gorley 2015). We then calculated a matrix of between-individual Euclidean distances using all seven normalised variables. We checked for homogeneity of multivariate dispersions (distance of observations to their centroids) using PERMDISP with 9999 permutations for each variable (Anderson 2006). Then we used Canonical Analysis of Principal coordinates (CAP, 9999 permutations; Anderson and Willis 2003) with leave-one-out cross validation to determine whether individuals could be correctly assigned to the two populations based on the acoustic variables. We used a one-factor non-parametric multivariate analysis of variance (PERMANOVA) with 9999 permutations (Anderson 2001) using the Euclidean distance matrix to test for differences in centroids between the two populations. This analysis was repeated to examine the effects of origin, age, and sex. The transitions in social status from subordinates/helpers to dominants/breeders may induce the development of new song types (Voigt *et al.* 2007). There is evidence that song variation is related to the social status in cooperative breeding mockingbirds (Derrickson 1987; Botero *et al.* 2009). Here, we did not control for social status, but we controlled for age and sex, factors that play an important role in the social status in the Galápagos mockingbird (*M. parvulus*), a related species of

Floreana mockingbird (Curry 1988*a*, 1988*b*). PERMDISP, CAP and PERMANOVA analyses were conducted in PRIMER-e *v7* and PERMANOVA+ software.

Morphology and vocalisation analysis

We used morphological measurements of individuals captured since 2010 (92 individuals from Champion, and 315 from Gardner) as a part of a long-term project monitoring the population dynamics of the species. Individuals were captured, banded and morphological body measurements were taken during the banding process: (1) mass to the nearest 0.5 g with a Pesola spring balance; (2) beak depth in vertical plane in the middle of the nares; (3) beak width in the upper mandible in a horizontal plane in the anterior edge of the nares; (4) tarsus from the intertarsal joint to the foot joint; (5) wing chord with the wing in a natural arc and at 90° angle with the radius/ulna; (6) head-beak from the upper beak tip to the nape; and (7) tail length. All measurements were taken to the nearest 0.1 mm. We averaged the measurements of individual birds recaptured through time. Age was estimated based on the date of banding until 2019 and sex was assigned using the criteria described in Reyes *et al.* (2022). Morphological data were assessed for normality and plotted outliers were removed from the dataset.

To test for the possible influence of morphology on the vocal variables, we used the morphological and acoustic data of the 21 individuals by performing a series of linear regressions using the methods described in Huber and Podos (2006). To remove the influence of body size metrics on beak metrics we regressed each of the three beak measurements against the first axis (70.2% of variation) of the Principal Component Analysis (PCA) of tarsus, wing and weight. PCA was run using the package *Factoextra* and *FactoMineR* (Lê *et al.* 2008; Kassambara and Mundt 2019) in R (R Core Team 2013). Furthermore, we regressed each acoustic variable against the residuals of the each beak measures-PC1 body size regression using a linear regression to quantify the relationship between beak traits and acoustic variables. Then we examined the beak related morphological traits using non-metric multidimensional (nMDS) in R (R Core Team 2013).

Analysis of historical recordings

We obtained four digitised recordings of vocalisations of the Floreana mockingbird from January 1962 from the Macaulay Library (Cornell Lab of Ornithology). Recordings were made by a single person (R.L. Bowman) on Champion Islet using a NAGRA III tape recorder connected to an American D-33 microphone in a 76.2 cm aluminium parabola set on the mono channel. We assume that each recording was made of a different individual bird. We discarded one recording due to it being *hew calls*. From the remaining three 'chatburst' recordings, we extracted 1224 syllables using *KOE* (Supplementary material B). For the comparison with the contemporary vocalisations, we only used a subset of three *chatburst* recordings from Champion that matched the historical recordings. We were aware of potential differences added by possible tape degradation of historical recordings over time. Nevertheless, recordings made on the Galapagos by the same person at the same time showed high sound quality (Goodale and Podos 2010). Hence, we did not conduct any test to assess tape degradation described in Derryberry (2007) because of our small sample size. We used *KOE* to extract the same set of acoustic variables described above. We created a data matrix combining the values of the acoustic variables from the historical recordings and the acoustic values of Champion 'chatburts'. We ran the same statistical analysis described for interpopulation vocalisations for this new data set (PERMDISP, CAP and PERMANOVA).

Results

We analysed 2559 syllables from seven individuals on Champion and 3856 syllables from 14 individuals on Gardner, representing the 7% of the current estimated population. The average number of syllables by individuals was 305.47 (±143.77). The minimum number of syllables analysed were three and the maximum were 2014 for individuals.

Interpopulation vocalisations

We found no significant difference in multivariate dispersions of the acoustic variables between populations (PERMDISP, $F = 0.42$, $P = 0.60$; Champion mean dispersion = 1.95 ± 0.51, $n = 7$; Gardner mean dispersion = 2.29 ± 0.28, $n = 14$) or by sex (PERMDISP, $F = 0.01$, $P = 0.92$; female mean dispersion = 2.29 ± 0.39, $n = 8$; male mean dispersion = 2.24 ± 0.31, $n = 13$), or between ages (PERMDISP, $F = 1.60$, $P = 0.87$).

The CAP analysis produced a single axis to discriminate between the two populations ($\delta_1{}^2 = 0.81$). The CAP allocated 76% of individuals to the correct population based on the acoustic data using a leave-one-out cross-validation procedure (Fig. 2). PERMANOVA analysis found a significant difference in centroids between populations (pseudo-$F_{1,19} = 2.20$, $P = 0.04$) (Table 1). No significant differences in the vocalisations were found for age (pseudo-$F_{5,15} = 1.66$, $P = 0.12$) or sex (pseudo-$F_{1,19} = 1.30$, $P = 0.25$).

Morphology and vocalisations

Differences between populations were found in four of the six morphological traits (Table 2, Fig. 3). In the regression analysis between acoustic variables and beak morphology, we found that the following acoustic variables were positively correlated with the width of the beak: duration ($P = 0.048$), goodness of pitch ($P = 0.012$) and harmonic ratio ($P = 0.0056$)

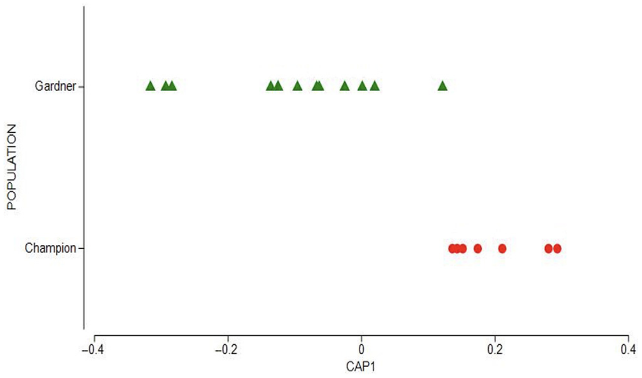

Fig. 2. CAP analysis plot showing the Gardner population and the Champion population vs the first canonical axis. The CAP plot choses an axis that best discriminate the song complexity data. Triangles

Table 1. Descriptive statistics of the acoustic parameters for both populations (11 males and 10 females).

Acoustic variables	Champion			Gardner			*t*-value
	N	Mean	s.d.	*N*	Mean	s.d.	
Duration	7	117.95	18.72	14	116.71	18.66	0.88
Spectral bandwidth	7	2550.71	143.43	14	2542.23	179.37	0.9
Frequency mod.	7	0.31	0.13	14	0.46	0.11	**0.02**
Amplitude mod.	7	−0.004	0.009	14	−0.03	0.12	0.97^{A}
Goodness of pitch	7	0.14	0.02	14	0.14	0.006	1^{A}
Entropy	7	−5.87	0.36	14	−5.64	0.78	0.79^{A}
Harmonic ratio	7	0.21	0.11	14	0.18	0.07	0.51

Bold number represent statistically significant differences between population using a *t*-test.

^{A}Results computed using a Wilcoxon Test.

(Fig. 4*a*, *d*, *f*). Meanwhile, frequency modulation ($P = 0.049$) was negatively correlated with depth and entropy ($P = 0.088$) (Fig. 4*c*, *e* respectively) were negatively correlated width of the beak.

Historical vs contemporary vocalisations

We analysed 1224 historical syllables from three individuals and 2498 syllables from four contemporary individual's recordings. The average number of syllables by individuals was 531.71 (±331.09). The minimum number of syllables by induvial analysed were 27 and the maximum were 2387. Vocalisations from 1962 were not more variable than recent recordings vocalisations (PERMDISP, $F = 2.93$, $P = 0.30$; historical mean dispersion $= 1.74 \pm 0.40$, $n = 3$; contemporary mean dispersion $= 1.05 \pm 0.19$, $n = 4$). A single canonical axis was calculated for the CAP analysis ($\delta_1^2 = 0.89$). The leave-one-out cross-validation allocation of observations assigned an individual to a time period with a 100% (7/7) accuracy. PERMANOVA results showed a significant difference between the centroids from historic and contemporary recordings (pseudo-$F_{1,5} = 9.06$, $P = 0.03$; Table 3).

Discussion

We investigated differences in song characteristics and morphological traits between two isolated populations of Floreana mockingbird from the Galápagos. We found significant differences in the acoustic characteristics of songs of these two extant populations, and that individuals could be accurately assigned to a specific population based on these features. Using historic sound recordings, we found some evidence of divergence in acoustic variables in the mockingbird's vocalisations over a period of 57 years on the Champion Islet population. We also found correlations between beak morphology and song parameters, however only one of these traits (beak depth, not width) differed between populations. Divergence in both cultural and genetic structures can arise when populations become isolated and/or undergo bottlenecks. Such divergence is likely to be due to cultural processes including cultural drift (random deletion of song types), cultural innovation (mutations that result in new song types; Marler 1970; Mundinger 1980), and genetic changes that drive morphological variations (Wyles *et al.* 1983). For birds, acoustic characteristics can change quickly due to cultural process such as drift, innovation, and the influence of social interactions (Aplin 2019). Nonetheless, genetic factors can also influence song characteristics through mechanisms such as genetic predispositions for certain vocalisations or morphological traits that impact sound production through biomechanical effects (Podos and Nowicki 2004).

The frequency modulation parameter in our mockingbird populations was specifically linked to beak depth, which could be the only morphological trait responsible for the acoustic divergence in these populations. The avian beak provides the final modifications to vocal signals and is used in coordination with the vocal tract and the tongue to influence frequency and tempo (Huber and Podos 2006; Friis *et al.* 2022; Krishnan 2023). However, precisely how beak depth alters frequency modulation requires further study. Even though beak width also varied between populations, it did not correlate with any of the seven acoustic variables studied, providing evidence that other acoustic differences are more likely to be driven by cultural processes. Furthermore, frequency modulation did not differ between extant and historical recordings leading us to speculate that this divergence in acoustic characteristics has been driven by cultural processes rather than morphological changes.

Divergence in acoustic characteristics within a species can arise through a number of pathways including cultural processes such as social learning and song innovation, cultural drift due to random loss of song variants (Williams and Lachlan 2022), and through acoustic adaptation to

Table 2. Descriptive statistic of the Floreana mockingbird morphometry.

Parameter	**Champion**			**Gardner**			***t*-value**	***u***
	N	**Mean (s.d.)**	**Range**	***N***	**Mean (s.d.)**	**Range**		
Weight (g, M + F combined)	92	58.62 (4.80)	47–70	315	62.10 (5.78)	46–77	**<0.001**	**<0.001**
Male	57	60.27 (4.08)	51–70	136	64.65 (5.46)	48–77	**<0.001**	**<0.001**
Female	35	57.61 (4.96)	47–70	179	60.17 (5.26)	46–74	**0.001**	**0.001**
Head (mm, M + F combined)	92	57.64 (1.27)	54.5–60.3	315	58.09 (1.60)	53.7–62.4	**0.006**	**0.01**
Male	57	58.16 (1.11)	55.5–60.3	136	58.82 (1.62)	53.7–62.4	**0.006**	**0.007**
Female	35	57.31 (1.27)	54.5–60.1	179	57.52 (1.35)	53.7–60.7	0.3	0.21
Width (mm, M + F combined)	92	6.70 (0.62)	5.3–8.1	315	6.75 (0.69)	5.1–8.5	0.53	0.37
Male	57	6.9 (0.56)	5.4–8.1	136	6.88 (0.64)	5.1–8.4	0.89	0.98
Female	35	6.57 (0.63)	5.4–8.1	179	6.65 (0.71)	5.1–8.5	0.47	0.34
Depth (mm, M + F combined)	92	6.53 (0.35)	5.9–7.5	315	6.20 (0.33)	5.4–7	**<0.001**	**<0.001**
Male	57	6.66 (0.30)	6–7.3	136	6.31 (0.30)	5.5–7	**<0.001**	**<0.001**
Female	35	6.45 (0.36)	5.9–7.5	179	6.12 (0.33)	5.4–7	**<0.001**	**<0.001**
Tarsus (mm, M + F combined)	92	39.64 (1.53)	36–42.7	315	39.64 (1.49)	35.9–43.2	0.97	0.9
Male	57	40.72 (1.32)	36.4–42.6	136	40.39 (1.36)	36.2–43.2	0.19	0.14
Female	35	38.98 (1.25)	36–42.7	179	39.07 (1.31)	35.9–42.3	0.68	0.59
Wing (mm, M + F combined)	92	117.52 (4.98)	104–128.7	315	118.90 (1.96)	105–131.5	**0.02**	0.06
Male	57	122 (2.02)	120–128.7	136	123.60 (2.90)	119.5–131.5	**0.01**	0.07
Female	35	114 (3.58)	104–119	179	115.33 (2.69)	105–119	0.1	0.16

Bold numbers represent statistically significant differences between populations. *T*-test and Wilcoxon test are pooled together for comparison.

local habitats (AAH) (Morton 1975; Ey and Fischer 2009). These changes in culture can happen in short time frames and across variable spatial scales (Otter *et al.* 2020). In our study populations, several of these factors may have contributed to the cultural divergence we quantified. Floreana mockingbirds are highly social and have a cooperative breeding system, although they tend to be in smaller groups during the breeding season (Per. obs.). Larger group sizes were measured on Gardner, probably due to the habitat availability on this island and high density of individuals (Reyes 2021). In cooperative breeding birds, there is a correlation between song complexity and social group size (Freeberg 2006; Freeberg *et al.* 2012). All four species of Galápagos mockingbirds show different degrees of group structure and social organisation, even between populations of the same species (Curry 1989). However, the differences in the group structure among populations of the Floreana mockingbirds have not been studied.

Divergence in vocalisations between populations can also result from adaption to local habitat structure; the acoustic adaptation hypothesis (AAH) (Morton 1975; Ey and Fischer 2009). One of the main predictions of the AAH is that vocalisations will be characterised by lower frequencies in areas of denser vegetation as higher frequencies degrade over shorter distances than lower frequencies (Morton 1975). While the dominant vegetation communities on Champion and Gardner are both dominated by *Opuntia* cactus (*Opuntia megasperma*), there are some differences in density and phenotypic features (i.e. *Opuntia* cactus are arboreal on Champion, while on Gardner *Opuntia* are shrubby), which vary between the islets (Curry 1986; S. Ascencio, unpubl. data). This might influence the habitat structure and thus the song transmission in the respective acoustic environment (Derryberry *et al.* 2018). Habitat structure has been shown to influence song complexity and song sharing in vocally complex songbirds. For example, in the tūī (*Prosthemadera novaeseelandieae*), an endemic honeyeater from New Zealand) and the skylark (*Alauda arvensis*), habitat structure influences song sharing and song complexity (Briefer *et al.* 2010; Hill *et al.* 2017). To date, empirical evidence for the AAH across a wide range of taxa is inconsistent and the aspects of vocal behaviour adapted to environmental conditions may vary with species

Fig. 3. Unconstrained nMDS ordination of beak morphological traits of Floreana mockingbirds. Red circles, Champion birds (*N* = 92: 35 males, 57 females); teal circles, Gardner birds (*N* = 315: 136 males, 179 females). Scale in the nMDS are arbitrary. nMDS stress value of 0.03 indicate a fair representation of the reduced dimensions. The variation in sizes between sexes might account for the overlap of points.

and local habitats (Hardt and Benedict 2021). To test whether the AAH has driven differences in vocalisation characteristics between our two populations, future studies could experimentally test the efficiency of signal transmission (natural and synthesised calls) in the different habitats where the two populations have persisted over generations.

Geographic isolation and differences in habitat structure can lead to genetic differentiation in morphological traits, especially in multipurpose structures such as the avian beak, as we found here. Such differentiation can be more pronounced between populations when they vary in specialised trophic niches and climates (Slarkin 1985; Grant and Grant 1989, 1993; Friedman *et al.* 2019). Morphological differentiation among populations has been reported previously for this species (Deem *et al.* 2011). In our study we found a significant difference in four of six morphological traits between populations. Two of these four traits were related to beak, specifically, the depth and head-beak measurements.

Morphological variation in beak traits has been demonstrated in other Galápagos birds (Bollmer *et al.* 2003; Santiago-Alarcon *et al.* 2006). More generally, studies have shown correlations between beak morphology and song variation. For example, Demery *et al.* (2021) found in a phylogenetical level analysis of the tanagers (Thraupidae) that beak size and shape influence distinct elements of song, independently of the covariation of body size, with beak size influencing temporal variables, and beak shape influencing trill rate. Similarly, Derryberry *et al.* (2018) in their study with ovenbirds (Furnaridae) showed that both body mass and beak size influence frequency and temporal aspects of the song. In contrast, Slabbekoorn and Smith (2000) did not find a correlation between beak size and song in the African finch (*Pyrenestes ostrinus*), a species with highly polymorphic beaks. To confirm the role of different beak morphological characteristics on song traits in our two Floreana Mockingbirds population, further research using larger sample sizes and genetic analysis are required.

Overall, the findings from our study support the conclusion of a primarily cultural influence on the acoustic characteristics of Floreana mockingbirds, with a secondary influence of genetic changes via beak morphology. The key processes that have likely contributed to the cultural divergence in the Floreana mockingbird are founder effect, and ongoing isolation and small population size. When Gardner islet separated from Floreana Island due to the sea-level rise of the inter-glacial cycle (Geist 1996), only a portion of the acoustic diversity from Floreana was likely retained on Gardner. In contrast, the population on Champion remained connected to Floreana Island because of its closer proximity (800 m), facilitating the exchange of cultural traits through migration of individuals between the islands until the extinction of the Floreana population around 1880 (Curry 1986). Syllables change at a slower rate in isolated populations due to a lack of immigration, thereby strengthening founder effects (Baker *et al.* 2001). Finally, unlike Gardner, Champion is a small islet (9.4 ha) with a small population. This could induce a strong effect of cultural drift, accelerating the cultural divergence by loss of complexity, in parallel with the genetic drift reported for these populations (Hoeck *et al.* 2010*a*). This idea is further supported by a reduction of song complexity in the Champion Island population between 1962 and 2019. We acknowledge that the strength of our conclusions is limited by our small sample size taken from only two time points; unfortunately, historical recordings are scarce. Nevertheless, this result is consistent with losses of cultural complexity over time in other small, insular populations of birds (Tanimoto *et al.* 2017; Paxton *et al.* 2019). Further studies are needed to clarify the relation between genetic differences, morphology, local adaptation, and song in this species.

Implications for conservation reintroductions

Floreana mockingbirds are proposed for reintroduction to Floreana Island (Ortiz-Catedral 2018) as a part of the Floreana Island Restoration Project. To date, only genetic factors and basic ecological requirements have been considered when planning the reintroduction of this species. Accordingly, the proposed Floreana Island population would source birds from both existing populations to maximise genetic diversity (Hoeck *et al.* 2010*a*; Bozzuto *et al.* 2017). Behaviour (cultural) traits of the species have not been considered until now, nor have any potentially negative consequences of moving birds with different vocalisations. To the best of our knowledge, the only modern case of observed interactions between

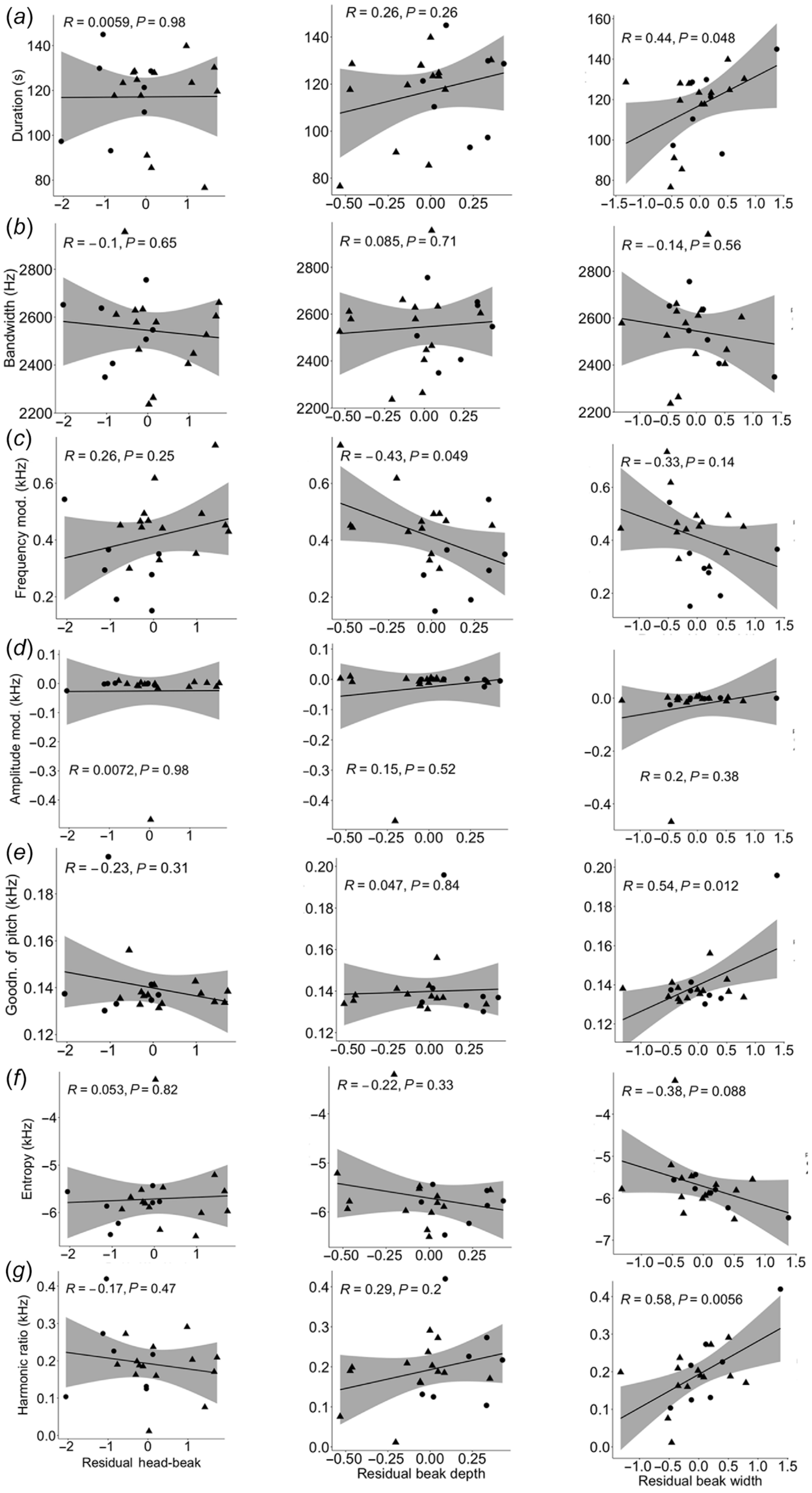

Fig. 4. Relationship between the three residual beak measures and vocal parameters for (*a*) duration, (*b*) bandwidth, (*c*) frequency, (*d*) amplitude modulation, (*e*) goodness of pitch, (*f*) entropy, and (*g*) harmonic ratio. Circles, Champion; triangles, Gardner populations.

mockingbirds with different vocalisations has been between the Floreana mockingbird and San Cristobal mockingbird (*Mimus melanotis*), where interspecific aggression was observed (Ortiz-Catedral *et al.* 2021). Nevertheless, there is evidence of temporary co-occurrence of two species of mockingbirds (*M, parvulus* and *M. melanotis*) on a single island, showing that Galápagos mockingbirds could potentially hybridise (Nietlisbach *et al.* 2013).

Table 3. Descriptive statistics of the acoustic parameters for both historical and recent recordings of the Champion population.

Acoustic variables	Historical			Recent			*t*-value
	N	Mean	s.d.	*N*	Mean	s.d.	
Duration	3	94.52	49.64	4	129.23	68.88	0.08
Spectral bandwidth	3	4615.83	772.60	4	2538.39	342.20	**0.01**
Frequency mod.	3	0.26	0.15	4	0.35	0.19	0.23
Amplitude mod.	3	0.004	0.24	4	−0.01	0.17	0.63[A]
Goodness of pitch	3	0.12	0.02	4	0.14	0.04	**0.02**
Entropy	3	−4.36	0.90	4	−5.78	0.68	**0.005**
Harmonic ratio	3	0.66	0.18	4	0.22	0.15	0.06[A]

Bold number represent statistically significant differences between population using a *t*-test.

[A]Results computed using a Wilcoxon Test.

It is well known that assortative mating can arise as a problem in translocated populations. For example, the North Island Kokako (*Callaeas wilsoni*), an endemic duetting songbird of New Zealand, has been translocated from multiple source populations, with a high rate of assortative mating between birds from the same source populations having been observed (Bradley *et al.* 2014). Nevertheless, cultural divergence is not always an impediment to gene flow between populations. Research on both closed- (only juveniles learn new songs) and open-ended song learners (birds learn new songs throughout their life) have been found that specific dialects are not correlated with a specific genetic structure (Leader *et al.* 2008; Ortiz-Ramírez *et al.* 2016). Mockingbirds are considered open-ended song learners (Gammon 2020) and both sexes are known for their ability to mimic vocalisations, even those of other species (i.e. 'heterospecific' songs; Howard 1974; Botero and Vehrencamp 2007; Gammon 2014; Gammon and Stracey 2022). For Floreana mockingbird, mimicry and open-ended learning have not been reported or studied to date. Nevertheless, mimicry seems an unlikely cause of vocal divergence, because of both islets share the same diversity of bird's species. These two factors of vocal plasticity could play an important role in minimising the effects of assortative mating if a reintroduction is attempted.

Our study highlights cultural differences that may reduce gene flow and undermine the success of the planned reintroductions. The success of the species reintroduction will depend on the methodology employed. If the single individual approach is preferred (Bozzuto *et al.* 2017) over the group harvesting suggested in Reyes (2021), it is possible that the effects of assortative mating may be stronger. Specifically, in a low-quality habitat as is currently the case on Floreana Island, single birds may form 'groups' with individuals of similar vocalisation to increase the probability of survival (Reyes 2021). We recommend that simple playback experiments could be done before reintroducing Floreana mockingbirds, to measure the response to the songs of each population. Conservation efforts, such as multi-source translocations of Floreana mockingbirds to Floreana Island, rely on identifying potential barriers to maximising the genetic diversity of the founding population. Our study highlights the importance of considering behavioural aspects when planning species reintroduction and will set a precedent for the multi-species reintroduction of 13 extirpated species planned to Floreana Island in the Galapagos Archipelago.

Supplementary material

Supplementary material is available online.

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Data availability. The datasets used in preparing this paper are available from the corresponding author on reasonable request. Recordings used in this study have been archived at The Macaulay Library at the Cornell Lab of Ornithology. Lab of Ornithology and Xeno-Canto webpage.

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