

RESEARCH ARTICLE

Female and male song exhibit both parallel and divergent patterns of cultural evolution: A long-term study of song structure and diversity in tropical wrensBrendan A. Graham,^{1,a,*} Daniel D. Heath,^{1,2} and Daniel J. Mennill^{1,©}¹ Department of Integrative Biology, University of Windsor, Windsor, Ontario, Canada² Great Lakes Institute of Environmental Research, University of Windsor, Windsor, Ontario, Canada* Corresponding author: b.graham001@gmail.com[©] Current address: Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada

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ABSTRACT

Animal culture changes over time through processes that include drift, immigration, selection, and innovation. Cultural change has been particularly well-studied for animal vocalizations, especially for the vocalizations of male animals in the temperate zone. Here we examine the cultural change in the vocalizations of tropical Rufous-and-white Wrens (*Thryophilus rufalbus*), quantifying temporal variation in song structure, song type diversity, and population-level distribution of song types in both males and females. We use data from 10 microsatellite loci to quantify patterns of immigration and neutral genetic differentiation over time, to investigate whether cultural diversity changes with rates of immigration. Based on 11 yr of data, we show that the spectro-temporal features of several widely-used persistent song types maintain a relatively high level of consistency for both males and females, whereas the distribution and frequency of particular song types change over time for both sexes. Males and females exhibit comparable levels of cultural diversity (i.e. the diversity of song types across the population), although females exhibit greater rates of cultural change over time. We found that female changes in cultural diversity increased when immigration is high, whereas male cultural diversity did not change with immigration. Our study is the first long-term study to explore cultural evolution for both male and female birds and suggests that cultural patterns exhibit notable differences between the sexes.

Keywords: birdsong, cultural diversity, cultural evolution, drift, female song, song learning

LAY SUMMARY

- Animal culture changes over time, and here we present the first long-term study to explore cultural evolution for both male and female birds.
- To study animal culture, we recorded the songs of male and female Rufous-and-white Wrens living in Guanacaste, Costa Rica over an 11 yr period.
- We show that the structure of several widely-used persistent song types maintain a high level of consistency for both males and females, whereas the distribution and frequency of particular song types change over time for both sexes.
- Males and females exhibit comparable levels of cultural diversity, although females exhibit greater rates of cultural change over time.
- We collected genetic samples to measure immigration, and we found female cultural diversity increases when immigration is high, whereas male cultural diversity does not change with immigration.
- Our results suggest that cultural patterns exhibit some similarities, and some notable differences, between the sexes.

Los cantos de la hembra y el macho muestran tanto patrones paralelos como divergentes de evolución cultural: Un estudio de largo plazo de la estructura y la diversidad del canto en *Thryophilus rufalbus***RÉSUMÉ**

La cultura de un animal cambia a lo largo del tiempo a través de procesos que incluyen deriva, inmigración, selección e innovación. El cambio cultural ha sido particularmente bien estudiado para las vocalizaciones animales, especialmente para las vocalizaciones de animales macho de zonas templadas. Aquí examinamos el cambio cultural en las vocalizaciones de la especie tropical *Thryophilus rufalbus*, cuantificando la variación temporal en la estructura del canto, la diversidad de tipos de cantos y la distribución a nivel de población de tipos de cantos en machos y hembras. Usamos datos de 10 loci de microsatélites para cuantificar los patrones de inmigración y diferenciación genética neutral a lo largo del tiempo,

para investigar si la diversidad cultural cambia con las tasas de inmigración. Basados en 11 años de datos, mostramos que los rasgos espectro-temporales de varios tipos de cantos persistentes ampliamente usados mantienen un nivel relativamente alto de consistencia tanto para machos como para hembras, mientras que la distribución y la frecuencia de tipos de cantos particulares cambian a lo largo del tiempo para ambos sexos. Los machos y las hembras muestran niveles comparables de diversidad cultural (i.e. la diversidad de tipos de cantos a lo largo de la población), aunque las hembras muestran tasas mayores de cambio cultural a lo largo del tiempo. Encontramos que los cambios de las hembras en diversidad cultural aumentan cuando la inmigración es alta, mientras que la diversidad cultural de los machos no cambió con la inmigración. Nuestro trabajo es el primer estudio de largo plazo que explora la evolución cultural tanto en aves macho como hembra y sugiere que los patrones culturales exhiben diferencias notables entre los sexos.

Palabras clave: aprendizaje del canto, canto de aves, canto de la hembra, deriva, diversidad cultural, evolución cultural

INTRODUCTION

Birds produce diverse and complex acoustic signals, including both innate and learned vocalizations (Jarvis 2004). The presence of learned vocal traits has made birds a model system for studying the evolution of animal culture (Laland and Janik 2006, Catchpole and Slater 2008, Irwin 2012, Whiten 2019). By documenting temporal changes in the features of bird songs, we gain insight into the evolutionary forces that influence cultural evolution, including drift, immigration, selection, and innovation (e.g., copying errors or improvisations; Lynch 1996). Some studies show that acoustic signals remain stable across time (Byers et al. 2010, Lachlan et al. 2018), while other studies show that acoustic signals may evolve quickly, over both short timespans (<10 yr) and longer timespans (30 yr and beyond; e.g., Ince et al. 1980, Payne et al. 1981, Gammon and Baker 2006, Wright et al. 2008, Byers et al. 2010, Goodale and Podos 2010, Williams et al. 2013, Azar et al. 2014, García et al. 2015).

Several aspects of avian vocalizations have been examined in the context of cultural evolution. For example, several studies have shown that the temporal and spectral structure of songs changes over time (Byers et al. 2010, Williams et al. 2013, Azar et al. 2014), while other studies have shown that some structural aspects of song persist over time and that the geographic boundaries of avian dialects also remain consistent across time (Harbison et al. 1999, Wright et al. 2008, Byers et al. 2010, García et al. 2015). Other studies have focused on the frequency, distribution, and persistence of syllables or song types for those species that possess song type or syllable repertoires (Ince et al. 1980, Payne et al. 1981, Lachlan et al. 2018). Much of this work has shown that song types and syllables persist across time with great consistency; although recent work has shown how quickly new song variants can arise within populations and spread at the continental scale (Otter et al. 2020).

The study of cultural evolution in birds has focused primarily on male acoustic signals, with a heavy emphasis on species living in north-temperate latitudes (Riebel et al. 2005, Podos and Warren 2007). Fewer studies have been conducted in the tropics (although see Wright et al. 2008,

García et al. 2015), where female song is prominent (Slater and Mann 2004, Riebel et al. 2005). Although female song is uncommon in north-temperate species and has been poorly studied overall, recent research reveals that it is the ancestral state among songbirds (Odom et al. 2014). Given that female song is more widespread than previously thought, and that female song shows patterns of complexity that match that of male song, we now recognize that it is an important priority to conduct more rigorous studies of species in which females sing (Odom and Benedict 2018). Furthermore, by studying the vocalizations of females in tandem with males, we can develop more holistic insights into animal culture, and the evolution and function of animal vocalizations (Price 2015).

Here we examine cultural evolution in a tropical songbird, the Rufous-and-white Wren (*Thryophilus rufalbus*), a year-round resident of Central and South America. Both male and female Rufous-and-white Wrens sing complex and musical songs, and possess song repertoires (individuals sing up to 15 distinct song types; Mennill and Vehrencamp, 2005, Harris et al. 2016). In comparison to males, females sing less often, possess smaller repertoires, and share fewer songs with neighbors (Mennill and Vehrencamp 2005). Female Rufous-and-white Wrens may exhibit lower cultural diversity (i.e. the frequency and distribution of song types in the population) in comparison to males, because vocal output and repertoire size are lower for females, but this hypothesis has not been tested rigorously. Since both males and females sing, this species allows us to study song types across time and to quantify patterns of cultural diversity and evolution within and between sexes.

We based our analysis of cultural evolution in male and female Rufous-and-white Wren songs on 11 yr of field data. We focused on 2 aspects of cultural evolution, including changes in the acoustic structure of songs (i.e. the spectral and temporal features of songs) and as well as changes in the cultural diversity of songs (i.e. the frequency-of-occurrence and distribution of song types across the population). In conjunction with our acoustic analyses, we analyzed genetic differentiation and variation across the same period using microsatellite data, which allowed us to explore patterns of immigration and to measure the level of

neutral genetic diversity and neutral genetic differentiation over time. Overall, we had 3 goals. First, we sought to examine the spectral and temporal characteristics of a subset of song types and quantify changes in these features over time. Second, we sought to examine cultural diversity for both males and females. Specifically, we wanted to quantify song-type richness (i.e. the number of song types present in the population), and song-type frequency (i.e. the frequency with which song types are sung) to determine whether males and females exhibit similar levels of cultural diversity over time, and whether males and females exhibit parallel or divergent cultural patterns. Third, we sought to examine the relationship between cultural variation and immigration; with 11 yr of genetic data, we combined acoustic and genetic datasets to characterize changes in songs while quantifying rates of immigration.

METHODS

From 2003 to 2013 we studied a population of Rufous-and-white Wrens in the mature dry forest habitat of Sector Santa Rosa of the Guanacaste Conservation Area (10.85°N, 85.60°W; 300 m.a.s.l.) in northwestern Costa Rica. From 2003 to 2007 we studied individuals across an area of 125 ha, and in 2008 we expanded our study site to 240 ha (details in [Woodworth et al. 2018](#)). Each year we captured birds using mist-nets and banded individuals with a unique combination of 3 color bands and a numbered aluminum band. From each bird, we collected a small blood sample (~100 µL) from the brachial vein and stored blood samples in Queen's Lysis buffer ([Seutin et al. 1991](#)) or 95% ethanol. We determined the sex of each bird based on the presence of a brood patch (females) and singing behavior (sexes can be distinguished based on spectro-temporal features of their songs; [Mennill and Vehrencamp 2005](#)). Over the 11 yr of this study our estimate of average longevity was 2.2 ± 0.1 yr (mean \pm standard error [SE]) for males, and 1.9 ± 0.2 yr for females (the longest-lived birds were 2 males that lived for 7 yr and a female that lived for 8 yr). Therefore, we studied multiple generations of birds throughout this investigation.

In each year we recorded birds from April to July, a period of high vocal output for this species ([Topp and Mennill 2008](#)). We collected the majority of our recordings (~60%) during focal recording sessions, when we followed individually marked birds around their territories for 60–90 min each morning (between 0445 and 1100 hr) and confirmed the birds' identities based on their color bands. We collected at least 2 focal recordings from each individual each year using a digital recorder (Marantz PMD-660 or PMD-661; 44.1 kHz sampling rate; 16-bit accuracy; WAVE format) and a shotgun microphone (Sennheiser MKH70 or ME67/K6). We collected the remaining recordings

passively by placing automated digital recorders ([Mennill et al. 2012](#)) within the center of each territory and recording the intended pair throughout the morning for several days (see [Harris et al. 2016](#) for details). We confirmed that the intended birds were sampled by the automated recorders by re-sighting the focal pair within the respective territory after each recording period, and by matching the songs collected by automated recorders with songs recorded during focal recordings ([Harris et al. 2016](#)). From each male, we recorded on average 1928 ± 128 songs (range: 260–11,691) and from each female, we recorded on average 390 ± 65 songs (range: 65–1839). Based on saturation curves generated for individuals from this population during 3 previous analyses ([Mennill and Vehrencamp 2005](#), [Harris et al. 2016](#), [Moser-Purdy et al. 2019](#)), and the extensive number of recordings of each bird in our dataset, we are confident that we sampled the complete repertoire, or the vast majority of the repertoire, for all of the birds in this analysis.

Song Type Assignment

For our analyses of cultural patterns, we analyzed song type variation across years for both males and females. We visualized and organized field recordings using Syrinx-PC sound analysis software (J. Burt, Seattle, Washington, USA) and constructed a song library for each bird (138 males and 102 females) as well as a population-level song library of all male and female songs that were recorded across the 11 yr study period ([Online Supplementary Material Figures S1 and S2](#)). We used visual classification to categorize songs, differentiating between song types based on their spectro-temporal characteristics ([Harris et al. 2016](#)); previous research in this study population by [Barker \(2008\)](#) revealed that visual classification based on fine-structural details (duration of song, maximum frequency, minimum frequency, and inter-syllable interval) match the result achieved by discriminant analysis. Following the approach outlined in [Graham et al. \(2017a\)](#), we considered 2 songs to be the same song type when 1) they featured the same sequences and frequencies (i.e. pitch) of introductory syllables; 2) they featured trills composed of the same type of elements (i.e. the same shape on the sound spectrogram), produced at similar frequencies (≤ 100 Hz difference) and delivered at a similar rate (≤ 2 syllable s^{-1} difference); and 3) they featured similar terminal syllables (i.e. similar shape on the sound spectrogram). We placed extra emphasis on the trill and introductory portions of the songs because these are the most variable portions of Rufous-and-white Wren songs. Songs were classified and song libraries were organized by multiple observers between 2003 and 2013 and double-checked by BAG and DJM. We tested the repeatability of the song type classification by randomly selecting 10 song types for

males and females and asking 4 different observers to classify the songs. Song types were blindly classified (the year and individual identity were withheld from the observer) and there was 100% agreement on song type classification across the reviewers (details in Moser-Purdy et al. 2019).

Acoustic Structure

For males, we measured 1,327 songs representing 8 different song types from 61 different males (average number of songs measured per male: 21.8 songs; range: 7–46 songs). For females, we measured 333 songs representing 13 different song types from 55 females (average number of songs measured per female: 6.1 songs; range: 1–25 songs). We analyzed 4 extra song types for females compared to males because females sing less often than males; by including the extra song types we could increase the number of female songs in our analysis. We chose the particular 10 male song types and 14 female song types because they were common in the population, they were present across all the years that we analyzed, and we had sufficient recordings of the song types from multiple individuals to analyze. We measured the spectro-temporal features of songs from 3 different years: 2003, 2007, and 2013 (the 3 yr were chosen based on our genetic analyses; we chose these points because 2003 and 2013 were markedly different from each other based on our genetic measurements, whereas 2007 was intermediate between 2003 and 2013; see Results). Although the vast majority of individuals were present in only one of these 3 time periods, 5 individuals were recorded during 2 of these time periods (3 males were recorded in both 2003 and 2007, one male and one female were recorded in both 2007 and 2013). Whenever possible we included songs from multiple recordings (i.e. from a different day) and measured up to 3 exemplars for each song type per recording. All measurements used for all statistical analyses represent means for each song type. This gave rise to measurements for 310 male (2003: $n = 74$; 2007: $n = 91$; 2013: $n = 145$) and 165 female (2003: $n = 66$; 2007: $n = 59$; 2013: $n = 81$) songs in our analyses.

To quantify variation for several widely-used song types of male and female Rufous-and-white Wrens we measured 10 temporal and spectral measurements: 1) the duration of the song (s); 2) the number of syllables; 3) element rate of the trill (the number of elements per second in the trill portion of the song); 4) dominant frequency of the trill (Hz); 5) length of the terminal syllable (s); 6) bandwidth of the terminal syllable (Hz); 7) dominant frequency of the terminal syllable (Hz); 8) duration of all the pauses in the song (s; we considered a pause as the space between the end of one syllable and the beginning of the next syllable); 9) minimum frequency of the song (Hz); and 10) maximum frequency of the song (Hz). We used the automated parameter measurements tool in AviSoft SASLab Pro (PAUP* 5.2.04; R. Sprecht;

Berlin, Germany) to measure these features, minimizing human subjectivity. Sound files containing the songs were resampled to 8,000 Hz, which allowed maximum frequency resolution in AviSoft (the maximum frequency of Rufous-and-white Wren songs in this dataset was <4,000 Hz). For each song, we created a spectrogram with an effective resolution of 8 Hz and 4 ms (settings: transform size: 1,024 Hz; overlap: 96.86%; window: Hamming). We used a high-pass filter of 500 Hz to remove any low-frequency background noise from the sound files. We tested for intercorrelations between all spectro-temporal features using a Pearson correlation analysis; none of the intercorrelations (r) exceeded 0.7 and, therefore, we included all 10 variables in our analyses (Ruegg et al. 2006). We log-transformed the length of the terminal syllable before testing variables for normality using a Shapiro-Wilks test and by inspecting Q-plots of the residuals; all 10 variables passed the normality test. We corrected for multiple comparisons in our models using a Tukey test. We conducted a principal component analysis (PCA) to reduce the measured acoustic variables into fewer composite variables for our analysis. We analyzed males and females together and performed our PCA on all measured male songs and female songs. For the PCA we performed a direct oblimin rotation because this method allowed for correlations between components, and retained all principal components with Eigenvalues above 1.0. The first 4 principal components explained 83.2% of the variance for male and female song types (Supplementary Material Table S1). To determine if songs varied between years, we performed 4 separate linear mixed models on the 4 retained principal components, using principal component scores as our dependent variable and year as our independent variable. We also included sex as a fixed factor, and because we had multiple songs from the same individual and measured different song types, we included individual and song type as random effects in each of our 4 models. We ran these analyses using the *lme4* package in R (R Core Development Team 2020).

Measurements of Cultural Diversity

To quantify cultural diversity, we measured song-type richness and song-type abundance both within years and across years. To compare patterns of song-type richness across years for each sex, we calculated Chao-2 indices for males and females for each year, using the software package *estimateS* 9.1.0 (Colwell 2013). We used extrapolation and rarefaction techniques to generate estimates of song-type richness (as in Potvin and Clegg 2015), treating each song type as a distinct unit. This approach is typically used to estimate the number of species within a community but has been used effectively to estimate syllable diversity among songbird populations (Potvin and Clegg 2015). For this analysis we used “year” as our sample and the number

of individuals singing each song-type as our incidence-based frequency count; for example, if 10 birds sang the same song-type in year one, we recorded the frequency value for this song-type as 10. We used this approach because it accounts for sample size differences, and is especially reliable for estimating diversity when some classes are under-represented (Chao 1984), thereby allowing us to make comparisons of diversity among years. The ability of this method to account for sample size when estimating diversity was especially important, given that sampling was unequal across years due to variation in recording effort (number of recordists varied across years) as well as fluctuations in population size (details in Woodworth et al. 2018). Therefore, this approach allowed us to account for the likelihood that song-type diversity was underestimated, especially in years with smaller sample sizes. We estimated the number of song types using 2,000 randomizations without replacement. We chose this number because it was approximately triple the highest number of songs (i.e. the sum of song types recorded from every individual sampled in a given year) that we analyzed in a year ($n = 701$ overall for males and females in 2012). We used extrapolation-rarefaction techniques to calculate 95% confidence intervals (CI) for each year for each sex and we compared song-type richness within and between sexes to determine if song-type richness was equal across years and between sexes. In addition, we used an ANOVA to compare annual estimates of song-type richness between males and females.

In addition to estimating overall song-type richness, we also attempted to quantify cultural transmission patterns (i.e. the pattern of song learning from conspecific animals) from the perspective of how many song-types are shared across our population each year. To examine cultural transmission patterns, we calculated the estimated number of song types shared between years within each sex by calculating the number of shared observations (in this case song-types) between each year using the *EstimateS* software. In addition to comparing patterns for males and females separately, we also calculated the estimated number of song types shared between males and females across years. We then plotted the number of songs shared with time (i.e. the number of years between points-of-comparison) and ran a spearman's R correlation to determine the relationship of sharing across time. We ran these tests in *Past* 3.0 (Hammer et al. 2001).

To complement the aforementioned analysis of cultural transmission, we also calculated the observed values of song sharing within and across years. We calculated repertoire sharing among individuals using an adjusted Jaccard's coefficient (S_j) of sharing with the following formula (Tracey and Baker 1999):

$$S_j = c / ((a + b + c) / d)$$

where a is the number of songs in individual A's repertoire but not individual B's, b is the number of songs in individual B's repertoire but not individual A's, c is the number of songs shared between 2 individuals, and d is the difference in repertoire size between individual A and B. Song-sharing values reflect the average percentage of songs that an individual shares with all members of the same sex within the study population. We performed a linear mixed model in R using the *lme* package to compare patterns of within-year song sharing within and between sexes across years. We included within-year song sharing as our dependent variable, and sex and year as our independent variables. We also included individual as a random effect, given that the majority of individuals were sampled repeatedly, in 2 or more years of our study.

Next, we calculated cultural distance between years, to quantify how song-type abundance changed across the 11 yr study period. This allowed us to determine whether the same songs are learned with similar frequencies over time. We calculated Morisita indices of sharing between years to quantify cultural distance. This index measures the dispersion (i.e. frequency and distribution) of observations and, therefore, we chose to use this index in order account for how common a specific song type was within each year, as opposed to whether or not it was present (like that of the modified Jaccard's index explained above). Given that the Morisita index quantifies sharing, we subtracted the calculated Morisita index value from one to measure cultural distance between 2 time periods. We did this for each sex, and compared cultural distance across years between sexes using a Mann-Whitney U test.

Microsatellite Genotyping and Analyses

We extracted DNA from blood samples using a Wizard Extraction Kit (Promega), and genotyped 213 individuals (123 males and 90 females; Online Supplementary Material Table S2) at 10 microsatellite loci. We used the 10 microsatellite primers outlined in Graham et al. (2017a), and all PCR protocols followed the methods outlined in that study. Our goal in conducting microsatellite analysis was to establish genetic measures of immigration, genetic diversity, and genetic differentiation for comparing to our measurements of cultural evolution. Microsatellites are neutral genetic markers and not subject to selection, which allowed us to quantify immigration, genetic diversity, and changes in allele frequencies (Selkoe and Toonan 2006) across the same time period that we examined changes in the song.

We tested for deviations from Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium in *GenePop* PAUP* 4.0.10 (Raymond and Rousset 1995), and corrected for multiple tests using sequential Bonferroni corrections (Rice 1989). We calculated allelic richness

(A_R) and inbreeding coefficient (F_{IS}) using FSTAT PAUP* 2.9.2.3 (Goudet 1995), and calculated observed (H_O) and expected heterozygosity (H_E) in GenALeX 6.501 (Peake and Smousall 2006, 2012). For these analyses, we treated each year separately, so that we could analyze patterns across years. Although the per-year sample sizes may seem small, it has been shown that 25–30 samples are adequate for estimating allele frequencies within a population (Hale et al. 2012). As the pooled sample size for each year (males and each female combined) are above or close to these totals, we feel that our genetic diversity estimates are accurate. Although female samples are lower than this threshold in most years, we feel that they are accurate given that estimates of heterozygosity, and allelic richness are comparable with values estimated for males. We compared genetic diversity patterns within years and among years, using Kruskal-Wallis tests, and between sexes using Mann-Whitney U tests. To estimate and compare patterns of dispersal between sexes we calculated annual assignment indices for each sex. This index uses allele frequencies to calculate the probability of a genotype originating in the population from which it was sampled. Individuals with low and negative values are less likely to have been born in the population where they were sampled, whereas individuals with a high positive value are more likely to have been locally recruited (Paetkau et al. 1995). Assignment indices were generated for each individual each year in GENECLASS 2.0 (Piry et al. 2004) using Bayesian genotype assignment (Rannalla and Mountain 1997) and Monte Carlo resampling (10,000 replicates; Paetkau et al. 2004). We then calculated the mean assignment index for each sex in each year of the study. Although assignment indices are often used in multi-population studies, they can also be used in a single population to measure and quantify the probability of an individual possessing a local or non-local genotype (as in Mossman and Waser 1999, Liebgold et al. 2013, Vangestel et al. 2013). We used assignment indices as a proxy for immigration based on the principle that years with negative values would indicate there is a larger presence of immigrants and years with positive values would indicate high local recruitment. We compared mean annual values for each year and overall between sexes using a two-tailed t -test ($P < 0.10$).

To examine genetic differences across time, we tested for differences in allele frequency distributions among years using pairwise exact tests in *GenePop* (1000 dememorization steps for 10,000 iterations). Multiple tests were corrected for using sequential Bonferroni corrections ($P < 0.001$). To visualize and further assess genetic differentiation across years we used the discriminant analysis of principal components (DAPC; Jombart et al. 2010) method using the *ADEGENET* 2.01 package (Jombart and Ahmed 2011) in R 3.2.4 software (R Core team 2020).

DAPC transforms the data using principal components and then performs discriminant analysis on the retained principal components. For our analysis, we used the *optim.a.score* function in *ADEGENET* to choose the optimal number of principal components, and thereby avoid over-fitting the data; in the end, we retained the first 27 principal components (which accounted for over 70% of the observed variation), and plotted the first 2 discriminant functions.

Correlation Between Acoustic and Genetic Patterns

To examine whether changes in cultural diversity are linked with changes in genetic diversity or immigration, we used Spearman's rank correlation tests. We used the annual change in song richness as our cultural diversity index. To calculate this index, we used our Chao-2 song richness estimates and subtracted the previous year's estimates from the current year's estimate. We generated 10 data points for each sex, and began by calculating the difference between 2004 and 2003 and continued up to 2013, with the final data point representing the difference in song richness between 2012 and 2013. To quantify immigration, we used our genetic assignment index values. Again, we calculated the annual change in values across time for each sex by subtracting the previous year's score from the current year. To quantify neutral genetic diversity, we created a new variable for each sex in every year of our study. We created our genetic diversity variable by performing a principal component analysis on observed heterozygosity and allelic richness. This approach, outlined by Garner et al. (2004) and later employed by Ficetola et al. (2007), creates a single measurement of genetic diversity that is a composite variable based on multiple measurements, as opposed to the arbitrary use of a single measurement to estimate genetic diversity. The first principal component explained 64.0% of the variance. We also explored the 2-way interactions between sex and genetic diversity and between sex and assignment index. For this second analysis, we again calculated the annual change in genetic diversity by subtracting the previous year's score from the current year. For our Spearman's rank correlation analyses, we first examined males and females together to explore an overall link between measures and then examined the relationship between estimated song diversity and genetic diversity and assignment index within each sex. The purpose of these secondary analyses was to examine the potential for differences between sexes.

Finally, we tested if changes in song frequencies (i.e. whether the same song types continue to be produced over time) were linked with genetic changes and time. We measured the relationship between cultural distance, genetic distance, and time for each sex (across all 11 yr), using

Mantel and partial Mantel tests. The cultural distance was calculated as listed above, and we calculated time as the number of years between 2 time periods. We measured the genetic distance between time periods by calculating Cavalli-Sforza and Edwards (1967) chord distance for each sex, which we calculated in *Genodive* 3.03 (Meirmans and Van Tienderen 2004). For these analyses, we also accounted for the level of individuals present each year, given that many individuals were present across multiple years. We calculated how similar the population (which we refer to hereafter as “population turnover”) of males and females each year was based on the number of individuals shared between years, using a Jaccard index (J). We then created a dissimilarity index by subtracting using the formula $1-J$. All Mantel, and partial Mantel tests were run in *Genodive*.

RESULTS

Acoustic Variation

We observed differences between male ($n = 310$) and female ($n = 165$) Rufous-and-white Wrens in the spectro-temporal features of certain widespread persistent song types: sex differences were evident in 4 of the linear models (for principal component 1, “terminal syllable length and pitch”, males showed longer and lower-pitched terminal syllables: $F = 22.4$, $P < 0.001$; for principal component 2, “syllable number and rate”, males uttered syllables at a faster rate: $F = 5.7$, $P = 0.02$; for principal component 3, “song frequency”, male songs were lower in pitch: $F = 69.3$, $P < 0.001$; and for principal component 4, “song length”, male songs were longer: $F = 56.1$, $P < 0.001$). Our finding that males and females show sex differences in songs of several widely-used song types—where males sing longer, and lower-pitched songs with faster syllable repetition rates—matches the finding of a previous investigation that had a much smaller sample size (Mennill and Vehrencamp 2005).

Across years, the spectro-temporal features of a select subset of widely-used persistent song types showed high consistency for both male and female songs, although female songs from 2013 were uttered at a faster rate than songs in 2007 (PC2: $F = 4.8$, $P < 0.001$). Additionally, there was a year \times sex interaction ($F = 4.4$, $P < 0.001$); females in 2013 uttered syllables at a faster rate than females in 2003 and 2007. We found no year \times sex interaction for our analyses of PC1 ($F = 1.2$, $P = 0.32$), PC3 ($F = 0.3$, $P = 0.72$), or PC4 ($F = 2.7$, $P = 0.07$). In our comparison of several widespread song types between males and females, female songs were shorter than male songs for 8 of the 9 comparisons ($P < 0.01$), although there were no differences across years within each sex. Furthermore, our plot of the first 2 principal components revealed considerable overlap

in songs across years for both males and females (Figure 1). Although we observed no strong overall pattern of acoustic changes through time, we did observe some changes in syllable composition across time (Supplementary Material Figures S1 and S2), associated with birds singing new song types.

We analyzed song data for 138 males and 102 females and identified 110 song types throughout the study (69 male song types, and 59 female song types). Of the 69 male and 59 female song types, 18 song types were shared between males and females. The 2 most common male song types in the population were also the 2 most common female song types. Twelve of the 15 most common male song types, and 10 of the 15 most common female song types were shared by both sexes, whereas the remainder of the most-common song types were sex-specific. Overall, 32% of male song types and 36% of female song types were unique to a single individual, and a typical male song type was sung by 14% of the individuals analyzed (range: 1–87%), while a typical female song was sung by 12% of the individuals analyzed (range: 1–70%; Supplementary Material Figure S3).

The male song type with the longest detection period was detected across all 11 yr of the study, and similarly, the longest female song type was detected across all 11 yr; overall 22% of male song types and 28% of female song types were detected in all 11 yr. The shortest time period a song type was detected was a single year; 10% of male song types and 15% of female song types were detected in a single year. The majority of song types that were detected in a single year were unshared song types, although 28% of single-year male song types were shared between 2 individuals. On average, a male song type was detected for 6.1 ± 0.4 yr, while a female song type was detected for 6.5 ± 0.5 yr.

Song-type richness, calculated using rarefaction and extrapolation technique, was similar between the sexes and across years, although annual estimates of song-type richness were higher for males than females ($F = 12.8$, $P < 0.001$; Table 1, Figure 2). All of our annual estimates using rarefaction and extrapolation techniques reached an asymptote, suggesting that song-type richness was accurately estimated, with the exception of the estimates of male song-type richness in 2008 (Figure 2); song-type richness may have been underestimated during that year. Overall, estimated song-type richness ranged from 37.6 ± 3.8 to 60.3 ± 17.6 for females, and 48.0 ± 6.8 to 95.8 ± 31.9 for males.

The number of estimated songs shared within each sex varied with time (Figure 3), with fewer songs shared between years as time interval increased (males and females combined: $r_s = -0.42$, $P < 0.001$; males: $r_s = -0.26$, $P = 0.05$; females: $r_s = -0.62$, $P < 0.001$; Figure 3). By contrast, we observed no relationship between the number of estimated songs shared across years between sexes ($r_s = -0.15$,

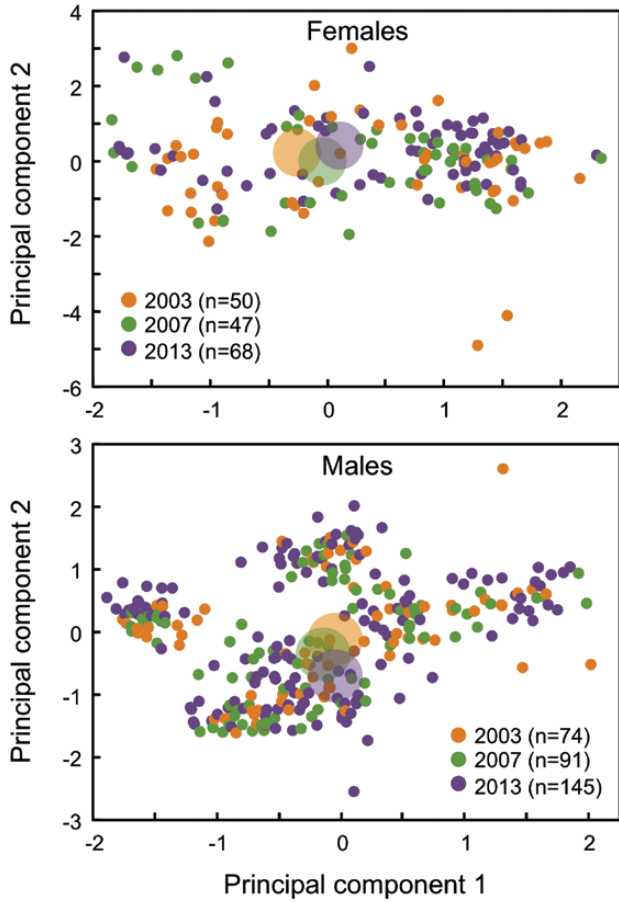


FIGURE 1. Principal component analysis of female (top; $n = 165$) and male (bottom; $n = 310$) Rufous-and-white Wren song types reveal no temporal clustering based on 10 spectro-temporal measurements. Large circles represent the mean centroids for each year. Plotted are the first principal component (representing pitch and terminal syllable length) and second principal component (representing syllable rate and syllable numbers).

$P = 0.11$; Figure 3). Our analysis of observed song sharing revealed that males exhibit greater within-year song sharing than females during the 11 yr study period: males shared $47 \pm 1\%$ of their songs, whereas females shared only $27 \pm 1\%$ of their songs ($F_{1,243} = 389.90, P < 0.001$; Figure 4). Within-year song sharing varied across time ($F_{10,243} = 6.28, P < 0.001$) for both sexes ($F_{10,243} = 12.79, P < 0.001$), ranging from 39 to 50% for males and from 24 to 36% for females (parameter estimate: $-0.19 \pm 0.01, t = -18.64, P < 0.001$). The sex-differences in song-sharing observed in this study matches those results reported in previous studies of this population (Graham et al 2017a, 2018a).

Overall, cultural distance (our measurement of how dissimilar the frequency-of-use and distribution of song types are between years) was greater for females than males; male song-type frequency-of-use changed $\sim 4\%$ per yr (range: 1–9%), while female song-type frequency-of-use changed $\sim 12\%$ per yr (range: 3–22%). These results indicate that

TABLE 1. Annual summary statistics of male and female song-type richness and song-type sharing. Number of birds recorded (N); song richness (S) represents the total number of song types detected each year; Chao-2 is the estimated song-type richness ($\pm SD$) accounting for unsampled song types using extrapolation and refraction techniques; song sharing represents the average within year song sharing percentage for each sex.

Year	Males				Females			
	N	S	Chao-2	Song sharing	N	S	Chao-2	Song sharing
2003	16	33	63.0 ± 19.0	0.49	11	35	64.5 ± 19.2	0.26
2004	20	35	56.6 ± 11.0	0.50	18	40	74.9 ± 20.8	0.24
2005	24	31	48.4 ± 9.3	0.50	15	34	40.0 ± 4.4	0.27
2006	22	38	52.9 ± 8.0	0.48	18	37	45.9 ± 6.7	0.34
2007	19	39	74.6 ± 17.0	0.46	15	28	43.2 ± 11.0	0.35
2008	25	39	79.6 ± 15.3	0.46	19	35	50.9 ± 10.2	0.27
2009	33	48	79.0 ± 11.01	0.39	20	34	49.9 ± 10.2	0.25
2010	29	46	79.5 ± 12.5	0.41	14	32	57.1 ± 17.8	0.36
2011	30	42	72.4 ± 12.3	0.45	22	34	37.6 ± 2.8	0.24
2012	47	46	56.0 ± 4.4	0.49	24	39	47.1 ± 5.8	0.26
2013	37	38	49.3 ± 6.4	0.50	23	37	46.3 ± 6.8	0.27
							Chao-2 95% CI	
							26.8–102.2	
							34.2–115.6	
							31.4–48.6	
							32.7–59.1	
							21.7–64.8	
							31.0–70.9	
							30.0–69.8	
							22.3–91.9	
							32.1–43.0	
							35.7–58.5	
							33.0–59.6	

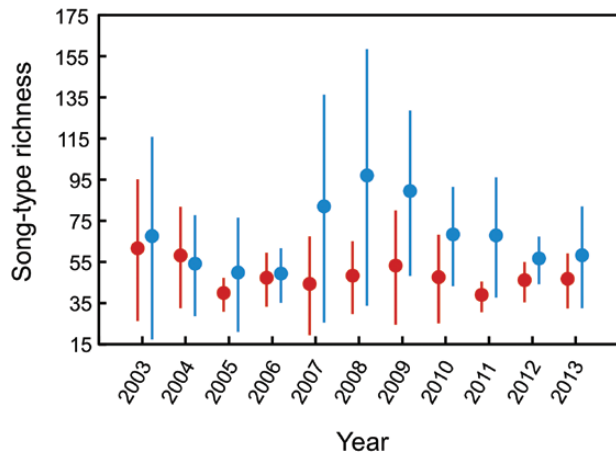


FIGURE 2. Annual estimates ($\pm 95\%$ CI) of song-type richness for female (red circles) and male (blue circles) Rufous-and-white Wrens based on extrapolation and rarefaction techniques. Richness is considered similar between the sexes when 95% CIs overlap.

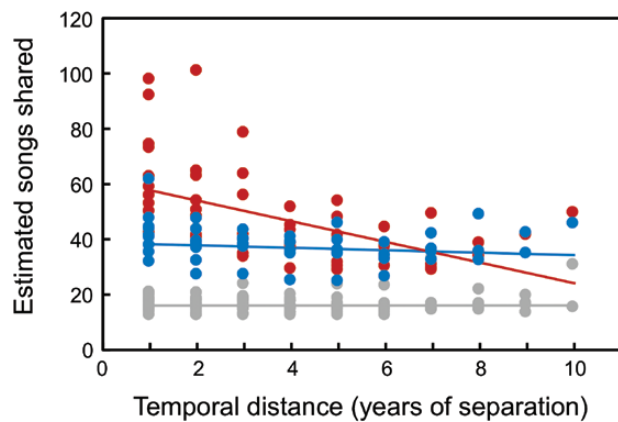


FIGURE 3. Number of songs shared between female (red circles) and male (blue circles) Rufous-and-white Wrens decreased with temporal distance (i.e. years of separation between comparison groups). The number of songs shared between both males and females (grey circles), however, showed no relationship with time. Lines show the relationship for song types shared between females (red line: $r = -0.62$, $P < 0.001$), between males (blue line: $r = -0.26$, $P = 0.05$), and between both sexes (grey line: $r = -0.15$, $P = 0.11$).

females exhibited greater levels of cultural change across time than males.

Genetic Estimates of Immigration and Diversity

We genotyped 213 individual Rufous-and-white Wrens (123 males and 90 females). The 10 microsatellites used in our study showed high variation across all years. 3 of 110 tests showed deviations from Hardy-Weinberg Equilibrium, while 2 of 45 loci combinations showed evidence of linkage disequilibrium following corrections for multiple comparisons. We included these loci in all analyses,

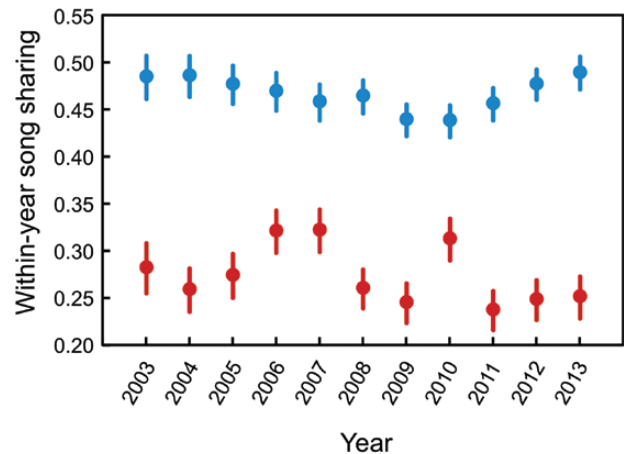


FIGURE 4. Annual rates of within-year song sharing (mean \pm SE) for male (blue circles) and female (red circles) Rufous-and-white Wrens.

because running the analyses without these loci produced similar results. We observed similar levels of genetic diversity across years (A_R , F_{IS} , H_o , or H_E ; [Supplementary Material Table S2](#)), and within each sex ($P > 0.17$, Kruskal–Wallis). Between sexes, only A_R was different, with males showing greater allelic richness than females ($U = 5106.5$, $z = -2.0$, $P < 0.05$).

We found differences in allele frequencies for 13 of the 55 pairwise comparisons (Fisher's exact tests: $P < 0.001$). The first 3 yr were different from later years; 2003 was different from all years from 2008 to 2013, 2004 was different from both 2010 and 2011, whereas 2005 was different from all years from 2009 to 2013. Similar to our results detected using Fisher's exact tests, DAPC suggested similar patterns of temporal genetic differentiation ([Supplementary Material Figure S4](#)). Again, the first 3 yr (2003–2005) were more similar to each other than later years, while later years (2008–2013) clustered more closely together.

Comparisons of dispersal between the sexes revealed that females are the more dispersive sex, matching previous analyses from this population ([Graham et al. 2017a](#); [Supplementary Material Table S2](#)). Assignment indices varied between sexes, with negative assignment values for females and positive assignment values for males (two-tailed t -test: $t_{1,212} = 1.72$, $P = 0.09$; females-assignment index = -0.33 ; males-assignment index = 0.24). Females had a negative assignment index for 7 of the 11 yr, although assignment indices were different between sexes for only a single year (2009; $t_{1,48} = -2.21$, $P = 0.03$), when females were the more dispersive sex (-0.97 versus 0.56 for males).

Cultural Evolution, Immigration, and Genetic Diversity

Changes in estimated song-type richness were not correlated with neutral genetic diversity when data from

TABLE 2. Summary of Mantel and partial Mantel tests examining the relationship (coefficient = Mantel's r) between cultural distance, genetic distance, and time for both male and female Rufous-and-white Wrens. Given that many individuals were present across multiple years, we used the variable "population turnover" to account for how different the composition of individuals in the population was between 2 points in time. Lines are used to distinguish the variable that was controlled for in the partial Mantel tests.

Hypothesis tested	Male		Females	
	r	P	r	P
Cultural distance Genetic distance	0.84	<0.001	0.64	<0.001
Cultural distance Time	0.89	<0.001	0.91	<0.001
Cultural distance Population turnover	0.78	<0.001	0.83	<0.001
Genetic distance Time	0.67	<0.001	0.65	<0.001
Genetic distance Population turnover	0.68	0.004	0.59	<0.001
Population turnover Time	0.80	0.001	0.82	<0.001
Cultural distance Genetic distance Time	0.64	0.004	0.13	0.25
Cultural distance Time Genetic distance	0.81	0.001	0.85	<0.001
Cultural distance Genetic distance Population turnover	0.61	<0.001	0.32	0.05
Cultural distance Time Population turnover	0.72	<0.001	0.74	<0.001

the sexes were pooled ($r_s = 0.17$, $P = 0.47$), but when the sexes were treated separately we observed differences between males and females. Song-type richness increased with increases in genetic diversity for females ($r_s = -0.81$, $P = 0.04$), while for males song-type richness did not vary with changes in genetic diversity ($r_s = -0.50$, $P = 0.14$). For our comparison of estimated song-type richness with immigration (i.e. the genetic assignment index), changes in song-type richness were not correlated with immigration when the sexes were pooled ($r_s = -0.35$, $P = 0.13$). We did observe sex-specific differences though when the sexes were examined separately; changes for estimated song-type richness were highest for females when local recruitment was low (i.e. song-type diversity was greater when immigration rates were high), but not for males ($r_s = 0.27$, $P = 0.45$). This pattern suggests that immigration may have a lower impact on male song-type richness than females.

For both males and females cultural distance was correlated with both neutral genetic distance and time, and neutral genetic distance was correlated with time (Table 2). Our partial Mantel tests, however, suggest that cultural changes are more closely related to time than genetic distance; cultural distance and neutral genetic distance were correlated when we controlled for time for males (partial Mantel test: $r = 0.64$, $P = 0.004$) but not for females ($r = 0.13$, $P = 0.25$). When we controlled for neutral genetic distance, however, cultural distance and time remained correlated for both males and females (partial Mantel test: males: $r = 0.80$, $P = 0.001$; females: $r = 0.82$, $P < 0.001$). As one would expect, population turnover increased across time and cultural and genetic distance increased in response to population turnover for both males and females over time (cultural distance: males: $r = 0.78$, $P < 0.001$; females: $r = 0.83$, $P < 0.001$; genetic distance: males: $r = 0.68$, $P < 0.004$; females: $r = 0.59$, $P < 0.001$). When we controlled for population turnover, cultural distance

remained correlated with both time and genetic distance for both sexes.

DISCUSSION

Our investigation of cultural variation in Rufous-and-white Wrens is the first long-term study to examine and quantify cultural change for both male and female birds. Previous studies of this species reported sex differences in vocal output, repertoire size, and song sharing (Mennill and Vehrencamp 2005, Topp and Mennill 2008), and here we show that males and females exhibit similar levels of song-type richness. Previous studies of cultural variation have focused on males (Wheelwright et al. 2008, García et al. 2015). Given the widespread occurrence of female song (Odom et al. 2014), our analyses provide a more complete representation of the evolution of animal acoustic traits (Price 2015). Although we observed only small changes in the spectro-temporal features of the subset of song types that we measured across our study period, we did observe changes in the frequency and distribution of song types across our 11 yr study. Despite the observed changes in allele frequencies across time, the cultural changes we reported occur independently of neutral genetic changes. Our results do suggest that increases in cultural diversity are associated with increases in immigration, as has been suggested in previous studies (Stewart and MacDougall-Shackleton 2008, Fayet et al. 2014). This pattern appears to be sex-specific, however, because females exhibited higher song-type richness when immigration was high, whereas male song-type richness did not vary with immigration.

Within the subset of widely-used persistent male and female song types we measured, we found relatively few changes in the spectral and temporal characteristics of songs over an 11 yr period. Similar to our results, many

other studies have observed temporal consistency in the overall acoustic structure of learned vocal signals across time (Gammon and Baker, 2006, Wright et al. 2008, Byers et al. 2010, Goodale and Podos, 2010, Azar et al. 2014, Williams et al. 2013, García et al. 2015), although Azar et al. (2014) demonstrated changes in the frequency characteristics of the syllables of songs. It is important to note that the habitat at our study site has remained fairly consistent across the duration of our study, although there are temperature and precipitation differences across years (Woodworth et al. 2018). Given that the songs of both males and females are optimized for transmission through forest habitats (Barker et al. 2009, Graham et al. 2017b), it is not surprising to observe little change in the spectral and temporal features of song, unlike other systems where acoustic changes over time are associated with changes to the acoustic environment (Luther and Baptista 2009). Although the spectral and temporal characteristics of songs remained mostly unchanged across sampling periods in many studies of bird vocalizations, some studies observed small changes in the syllable composition of learned vocal signals (Harbison et al. 1999, Wright et al. 2008, Byers et al. 2010, García et al. 2015). These changes in syllable composition or the reorganization of songs via neutral song evolution (i.e. copying errors leading to cultural drift) have been shown to give rise to new songs (Ince et al. 1980, Payne et al. 1981). Similar to these previous studies, we observed subtle changes in syllable composition of song types, although in the majority of cases these changes were minor. While these small differences may not change the overall structure of songs in a population, they are further evidence for how songs may change over time. In addition to neutral song evolution, these changes may result from cultural selection where the use of specific syllables may improve an individual's ability to attract and acquire mates (Rehsteiner et al. 1998, Vallet et al. 1998) or enhance the transmission properties of the song (Potvin and Clegg 2015). Given that Rufous-and-white wrens have fairly large song repertoires, our analysis of song-types in the population focused on the most common songs: analysis of all song-types in the population would be tremendously time-consuming, but might yield further insights into how the song changes over time.

Across the 11 yr of our study, we found that some song types became more common, while others disappeared from the population via cultural extinction, coinciding with predictions and observations from other studies (Ince et al. 1980, Payne et al. 1981, Payne 1996, Nelson et al. 2004, Byers et al. 2010, O'Laghlan et al. 2011). This raises the question: why do some song types survive longer than others? Payne et al. (1981) suggested that some songs are more easily copied or altered, and therefore survive longer and are less likely to change in structure. Peters et al.

(2012) found that acoustic structure may influence song type survival because young birds learn the least degraded songs; young birds may not be able to hear and reproduce those songs that transmit poorly through the environment. Other songs may survive longer because they are used more often, resulting in a higher likelihood of young birds learning these songs from song-tutors (Wheelwright et al. 2008). This last hypothesis is especially interesting for our study system, given that both males and females sing. It is possible that some song types occur with greater consistency across time because both males and females share and produce these song types, thereby creating more opportunities for young birds to learn them. Other evolutionary pressures, including duetting (where males and females combine solo songs to produce a coordinated acoustic signal), may influence temporal acoustic variation. For instance, Kōkako (*Callaeus wilsoni*) choose partners from the same dialect, possibly because it is easier to produce duets with such partners (Bradley et al. 2014). Additionally, several duetting species adhere to syntactic rules and duet codes, where males and females combine their songs non-randomly to produce duets (Logue 2006, Wright and Dahlin 2007, Templeton et al. 2013) and the propensity to respond to an individual's mate in this manner may, in turn, restrict temporal acoustic variation. In this manner, selection may favor song consistency, rather than promoting variation, as has been suggested in previous studies for non-duetting species (Byers et al. 2010, Wilkins et al. 2013). Further studies testing this idea would expand our knowledge of the diverse forces shaping male and female songs.

Our finding that song-type richness is similar between the sexes (based on Chao-2 estimates) underscores the value of studying animals where both sexes sing to expand our ideas about sexually dimorphic social signals (Price 2015, Odom and Benedict 2018). Although male and female Rufous-and-white Wrens exhibited similar levels of song-type richness, only 16% of the identified song types were shared between sexes. The limited song-type sharing between sexes observed in this study is a pattern that has been observed in other duetting species (Hall 2004). These results imply independent cultural patterns for each sex (Riebel 2003). The presence of independent song behaviors and culture for each sex has been observed in other species (Illes 2014), therefore we should not be surprised by the observed pattern in our study. It does, however, reinforce the idea that female birds, and not just male birds, produce complex and diverse songs, which can follow independent trajectories of cultural evolution. Therefore, current models of cultural evolution should incorporate both sexes and account for the potential for different patterns of cultural evolution in males and females (Price 2015, Graham et al. 2017a).

Although there are many similarities in culture between male and female Rufous-and-white Wrens, we did observe some sex-specific cultural patterns. Therefore we must ask: how is it that different cultural patterns evolve for males and females of the same species? Sex differences in life history may provide the answer. In our study population, males share more songs with neighbors than non-neighbors, resulting in greater spatial structure within the breeding population with respect to song sharing (Graham et al. 2017a). Although females also share more songs with neighbors than non-neighbors, their overall level of song sharing is much lower. This pattern reflects dispersal differences between sexes: females disperse farther from natal territories as juveniles and disperse more often between breeding territories as adults. Although more research is needed to comprehend the song ontogeny of female Rufous-and-white wrens, previous studies suggest a close relationship between culture and dispersal (Graham et al. 2017a). The close relationship between culture and dispersal has led us to hypothesize that females may be exposed to more song tutors than males while they search for breeding partners following dispersal from their natal territories (Graham et al. 2017a), which may explain differences in the level of cultural change exhibited between sexes.

Sex-specific differences in cultural evolution may reflect differences in patterns of song use by males and females. The Social Adaptation Hypothesis predicts that birds learn songs to match those of their neighbors following settlement in a territory (Payne, 1981). To date this hypothesis has been explored almost exclusively in male songbirds (e.g., Yoktan et al. 2011). Hall et al. (2015) experimentally tested the function of female song in Banded Wrens (*Thryophilus pleurostictus*), a closely related congener to our study species, and found that female birds did not use their songs to counter-sing with rival females or attract mates. While songs may be used to defend territories, females responded to rival pairs in combination with their male partner. Hall et al. (2015) hypothesize that the main function for a female song in Banded Wrens is in-pair communication. If this is the case in Rufous-and-white Wrens, the frequency and distribution of female song types may change faster, because females are not attempting to match their songs with rivals, and therefore selection pressures may be relaxed for females, because there is less incentive for them to copy their neighbor's songs as accurately as males.

Across the 11 yr period, we observed genetic changes, however, cultural differentiation did not appear to be correlated with neutral genetic differentiation. These results are not surprising given that other studies, over short time periods, have demonstrated no relationship between cultural and genetic evolution in bird species that learn

their songs (Wright et al. 2005, Leader et al. 2008, Yoktan et al. 2011). Across our long-term study of Rufous-and-white Wrens, we have observed inter-annual fluctuations in population size (Woodworth et al. 2018), yet we see no changes in cultural diversity. Previous research has shown that changes in population size (in particular reductions in population size via bottlenecks and founder effects) can result in reductions of cultural diversity (Laiolo and Tella, 2007, Laiolo et al. 2008, Parker et al. 2012).

In this study, we observed a relationship between cultural diversity and immigration, similar to other studies that have examined the relationship between immigration and cultural diversity (Stewart and MacDougall-Shackleton, 2008, Fayet et al. 2014). In Rufous-and-white Wrens cultural diversity decreased with declines in immigration; males and females, however, exhibited contrasting patterns. Female cultural diversity increased with immigration, whereas male cultural diversity did not vary with immigration. The relationship for males is not surprising, given that other studies have suggested that cultural diversity is highest when local recruitment is high (Beecher 2017). For females, we have previously shown that dispersal is sex-biased in this population; females settle in breeding territories further from natal territories and change breeding territories between years more often than males (Graham et al. 2017a). Female Rufous-and-white wrens learn songs post-dispersal from song tutors in their breeding territories (Graham et al. 2018a), yet the low song-sharing and weak spatial acoustic structure observed for females in this population likely arises as a byproduct of dispersal (Graham et al. 2017a).

We observed genetic differentiation over time at our long-term study site using neutral genetic markers. Changes in allele frequencies could result from genetic drift, migration, and mutations. Given the strong relationship between time and genetic distance, our results emphasize the role that drift plays in influencing genetic differentiation, even over relatively short time periods. Tropical birds are known to exhibit strong patterns of philopatry (Stutchbury and Morton, 2001), and the reduced levels of dispersal associated with philopatry and year-round territoriality reduce gene flow and thereby enhance the effects of drift on genetic variation and differentiation (Francisco et al. 2007, Smith et al. 2014). Previous research on our study population has demonstrated that although rates of immigration are relatively low, there is gene flow between our study population and other nearby populations (Graham et al. 2018a).

Our study demonstrates cultural evolution for both male and female song. Males and females exhibit comparable levels of cultural diversity, but cultural patterns are sex-specific. In addition to cultural changes, we observed

neutral genetic changes. Although these processes are occurring simultaneously, these patterns are occurring independently of each other. Additionally, we found that cultural diversity increased with immigration for females but not males. Changes in cultural patterns are occurring despite song-type richness and spectral and temporal characteristics of songs remaining relatively consistent across an 11 yr study period, thereby suggesting a role for drift in the temporal variation of song, a culturally-inherited trait. Similarly, drift is an important driving factor of temporal genetic differentiation as well. Our analyses do not allow us to differentiate whether sex-differences in song function, or drift, are the dominant force; we speculate that both forces are at play. Our research provides greater insight into the evolution of male and female acoustic signals, suggesting that similar processes influence the evolution of male and female songs (Graham et al. 2018b) even though males and females exhibit different cultural patterns. This study represents the first long-term study to quantify cultural evolution of female song; however, more studies are necessary to improve our comprehension of female song in birds and provide deeper insight into the evolution of acoustic signals in wild animal populations.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Ornithology* online.

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Ethics statement: This research was reviewed and approved by the Animal Care Committee at the University of Windsor.

Author contributions: DJM initiated the long-term study and with the assistance of his students collected all the acoustic recordings and blood samples. BAG conducted all acoustic and genetic analyses. All authors shared in the design, writing, and editing of the paper.

Conflict of interest statement: The authors state no conflict of interest for this submission

Data availability: All data used for the acoustic and genetic analyses are available from Graham et al. (2021).

LITERATURE CITED

- Azar, J. F., B. D. Bell, and M. Borowiec (2014). Temporal change of the song of a local population of the Grey Warbler (*Gerygone igata*): Has its song changed over time? *Emu* 114:80–85.
- Barker, N. K. (2008). Effective Communication in Tropical Forests: Song Transmission and the Singing Behaviour of Rufous-and-White Wrens (*Thryophilus rufalbus*). Master’s thesis, University of Windsor, Windsor, Canada.
- Baker, M. C., D. B. Thompson, G. L. Sherman, M. A. Cunningham, and D. F. Tomback (1982). Allozyme frequencies in a linear series of song dialect populations. *Evolution; International Journal of Organic Evolution* 36:1020–1029.
- Barker, N. K., T. Dabelsteen, and D. J. Mennill (2009). Degradation of male and female Rufous-and-white Wren songs in a tropical forest: Effects of sex, perch height, and habitat. *Behaviour* 146:1093–1122.
- Beecher, M. D. (2017). Birdsong learning as a social process. *Animal Behaviour* 124:233–246.
- Bradley, D. W., L. E. Molles, and J. R. Waas (2014). Post-translocation assortative pairing and social implications for the conservation of an endangered songbird. *Animal Conservation* 17:197–203.
- Byers, B. E., K. L. Belinsky, and R. A. Bentley. (2010). Independent cultural evolution of two song traditions in the chestnut-sided warbler. *The American Naturalist* 176:476–489.
- Catchpole, C. K., and P. J. B. Slater (2008). *Bird Song: Biological Themes and Variation*. Cambridge University Press, Cambridge, UK.
- Cavalli-Sforza, L. L., and A. W. F. Edwards (1967). Phylogenetic analysis: Models and estimation procedures. *American Journal of Human Genetics* 19:233–257.
- Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics* 11:265–270.
- Colwell, R. K. (2013). EstimateS: Statistical Estimation of Species Richness and Shared Species from Samples, version 9. <http://purl.oclc.org/estimates>
- Fayet, A. L., J. A. Tobias, R. E. Hintzen, and N. Seddon (2014). Immigration and dispersal are key determinants of cultural diversity in a songbird population. *Behavioural Ecology* 25:744–753.
- Ficetola, G. F., T. W. Garner, and F. De Bernardi. (2007). Genetic diversity, but not hatching success, is jointly affected by postglacial colonization and isolation in the threatened frog, *Rana latastei*. *Molecular Ecology* 16:1787–1797.
- Francisco, M. R., H. L. Gibbs, M. Galetti, V. O. Lunardi, and P. M. Galetti, Jr. (2007). Genetic structure in a tropical lek-breeding bird, the blue manakin (*Chiroxiphia caudata*) in the Brazilian Atlantic Forest. *Molecular Ecology* 16:4908–4918.
- Gammon, D., and M. Baker (2006). Persistence and change of vocal signals in natural populations of chickadees: Annual sampling of the gargle call over eight seasons. *Behaviour* 143:1473–1509.
- García, N. C., R. S. Arrieta, C. Kopuchian, and P. L. Tubaro (2015). Stability and change through time in the dialects of a Neotropical songbird, the Rufous-collared Sparrow. *Emu* 115:309–316.
- Garner, T. W., P. B. Pearman, and S. Angelone. (2004). Genetic diversity across a vertebrate species’ range: a test of

- the central-peripheral hypothesis. *Molecular Ecology* 13:1047–1053.
- Goodale, E., and J. Podos. (2010). Persistence of song types in Darwin's finches, *Geospiza fortis*, over four decades. *Biology Letters* 6:589–592.
- Goudet, J. (1995). Fstat version 1.2.: A computer program to calculate F-statistics. *Journal of Heredity* 86:485–486.
- Graham, B. A., D. D. Heath, and D. J. Mennill. (2017a). Dispersal influences genetic and acoustic spatial structure for both males and females in a tropical songbird. *Ecology and Evolution* 7:10089–10102.
- Graham, B. A., D. D. Heath, and D. J. Mennill (2021) Female and male song exhibit both parallel and divergent patterns of cultural evolution: A long-term study of song structure and diversity in tropical wrens. *Ornithology* 138:1–16. <https://doi.org/10.5061/dryad.3tx95x6dt>
- Graham, B. A., D. D. Heath, R. P. Walter, and D. J. Mennill (2018a). Immigrant song: Males and females learn songs after dispersal in a tropical songbird. *Behavioural Ecology* 29:711–723.
- Graham, B. A., D. D. Heath, R. P. Walter, M. M. Mark, and D. J. Mennill. (2018b). Parallel evolutionary forces influence the evolution of male and female songs in a tropical songbird. *Journal of Evolutionary Biology* 31:979–994.
- Graham, B. A., L. Sandoval, T. Dabelsteen, and D. J. Mennill (2017b). A test of the acoustic adaptation hypothesis in three types of tropical forest: Degradation of male and female Rufous-and-white Wren songs. *Bioacoustics* 26:37–61.
- Hale, M. L., T. M. Burg, and T. E. Steeves. (2012). Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. *PLoS One* 7:e45170.
- Hall, M. L., M. R. D. Rittenbach, and S. L. Vehrencamp (2015). Female song and vocal interactions with males in a Neotropical wren. *Frontiers in Ecology and Evolution* 3:1–13.
- Hammer, Ø., D. A. T. Harper, and P. D. Ryan (2001). Paleontological statistics software package for education and data analysis. *Paleontology*. *Electronica* 4:9. https://palaeelectronica.org/2001_1/past/past.pdf
- Harbison, H., D. A. Nelson, and T. P. Hahn (1999). Long-term persistence of song dialects in the mountain White-crowned Sparrow. *The Condor* 101:133–148.
- Harris, A. J., D. R. Wilson, B. A. Graham, and D. J. Mennill (2016). Estimating repertoire size in songbirds: A comparison of three techniques. *Bioacoustics* 25:211–224.
- Illes, A. (2014). Context of female bias in repertoire size, singing effort, and singing independence in a cooperatively breeding songbird. *Behavioural Ecology and Sociobiology* 69:139–150.
- Ince, S. A., P. J. B. Slater, and C. Weismann (1980). Changes with time in the songs of a population of chaffinches. *The Condor* 82:285–290.
- Irwin, D. E. (2012). Culture in songbirds and its contribution toward the evolution of new species. In *Creating Consilience: Integrating the Sciences and the Humanities* (E. Slingerland and M. Collard, Editors). Oxford University Press, Oxford, UK. pp. 163–178.
- Jarvis, E. D. (2004). Learned birdsong and the neurobiology of human language. *Annals of the New York Academy of Sciences* 1016:749–777.
- Jombart, T., and I. Ahmed. (2011). adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* (Oxford, England) 27:3070–3071.
- Jombart, T., S. Devillard, and F. Balloux (2010). Discriminant analysis of principle components: A new method for the analysis of genetically structured populations. *BMC Genetics* 11:1.
- Lachlan, R. F., O. Ratmann, and S. Nowicki (2018). Cultural conformity generates extremely stable traditions in bird song. *Nature Communications* 9:2417.
- Laiolo, P., and J. L. Tella (2007). Erosion landscapes of animal cultures in fragmented. *Frontiers in Ecology and Environment* 5:68–72.
- Laiolo, P., M. Vögeli, D. Serrano, and J. L. Tella (2008). Song diversity predicts the viability of fragmented bird populations. *PLoS One* 3:e1822.
- Laland, K. N., and V. M. Janik (2006). The animal cultures debate. *Trends in Ecology & Evolution* 21:542–547.
- Leader, N., E. Geffen, O. Mokady, and Y. Yom-Tov (2008). Song dialects do not restrict gene flow in an urban population of the Orange-tufted Sunbird, *Nectarina osea*. *Behavioural Ecology and Sociobiology* 62:1299–1305.
- Liebig, E. B., N. M. Gerlach, and E. D. Ketterson. (2013). Similarity in temporal variation in sex-biased dispersal over short and long distances in the dark-eyed junco, *Junco hyemalis*. *Molecular Ecology* 22:5548–5560.
- Logue, D. M. (2006). The duet code of the female Black-bellied Wren. *The Condor* 108:326–335.
- Luther, D., and L. Baptista (2009). Urban noise and the cultural evolution of bird songs. *Proceedings of the Royal Society of London Series B: Biological Sciences* 277:469–473.
- Lynch, A. (1996). The population memetics of bird song cultural evolution of bird song. In *Ecology and Evolution of Acoustic Communication in Birds* (D. E. Kroodsma and E. H. Miller, Editors). Cornell University Press, Ithaca, NY, USA. pp. 180–197.
- Meirmans, P. G., and P. H. Van Tienderen (2004). GENOTYPE and GENODIVE: Two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* 4:792–794.
- Mennill, D. J., and S. L. Vehrencamp (2005). Sex differences in singing and duetting behavior of Neotropical Rufous-and-white Wrens *Thryothorus rufalbus*. *The Auk* 122:175–186.
- Mennill, D. J., M. Battiston, D. R. Wilson, J. R. Foote, and S. M. Doucet (2012). Field test of an affordable, portable, wireless microphone array for spatial monitoring of animal ecology and behaviour. *Methods in Ecology and Evolution* 3:704–712.
- Moser-Purdy, C., Z. A. Kahn, B. A. Graham, and D. J. Mennill (2019). Male and female Rufous-and-white Wrens do not match song types with same sex rivals during simulated territory intrusions. *Journal of Avian Biology* 50:1–10.
- Mossman, C. A., and P. M. Waser (1999). Genetic detection of sex-biased dispersal. *Molecular Ecology* 8:1063–1067.
- Nelson, D. A., K. I. Hallberg, and J. A. Soha (2004). Cultural evolution of Puget Sound White-crowned Sparrow song dialects. *Ethology* 110:879–908.
- O'Loughlen, A. L., V. Ellis, D. R. Zaratian, L. Merrill, and S. I. Rothstein (2011). Cultural evolution and long-term song stability in a dialect population of Brown-headed Cowbirds. *The Condor* 113:449–461.

- Odom, K. J., and L. Benedict (2018). A call to document female bird songs: Applications for diverse fields. *The Auk: Ornithological Advances* 135:314–325.
- Odom, K. J., M. L. Hall, K. Riebel, K. E. Omland, and N. E. Langmore (2014). Female song is widespread and ancestral in songbirds. *Nature Communications* 5:3379.
- Otter, K. A., A. McKenna, S. E. LaZerte, and S. M. Ramsey (2020). Continental-wide shifts in song dialects of White-throated Sparrows. *Current Biology* 30:1–5.
- Paetkau, D., R. Slade, M. Burden, and A. Estoup (2004). Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology* 13:55–65.
- Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck (1995). Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* 4:347–354.
- Parker, K. A., M. J. Anderson, P. F. Jenkins, and D. H. Brunton (2012). The effects of translocation-induced isolation and fragmentation on the cultural evolution of bird song. *Ecology Letters* 15:778–785.
- Payne, R. B. (1996). Song traditions in indigo buntings: Origin, improvisation, dispersal, and extinction in cultural evolution. In *Ecology and Evolution of Acoustic Communication in Birds* (D. E. Kroodsma and E. H. Miller, Editors). Cornell University Press, Ithaca, NY, USA. pp. 198–220.
- Payne, R. B., W. L. Thompson, K. L. Fiala, and L. L. Sweany (1981). Local song traditions in Indigo Buntings: Cultural transmission of behavior patterns across generations. *Behaviour* 77:199–221.
- Peakall, R., and P. E. Smouse (2006). GenAlEx 6: Genetic analysis in excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6:288–295.
- Peakall, R., and P. E. Smouse (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics (Oxford, England)* 28:2537–2539.
- Peters, S., E. P. Derryberry, and S. Nowicki (2012). Songbirds learn songs least degraded by environmental transmission. *Biology Letters* 8:736–739.
- Piry, S., A. Alapetite, J. M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup (2004). GENECLASS2: A software for genetic assignment and first-generation migrant detection. *The Journal of Heredity* 95:536–539.
- Podos, J., and P. S. Warren (2007). The evolution of geographic variation in bird song. *Advances in the Study of Behaviour* 37:403–458.
- Potvin, D. A., and S. M. Clegg (2015). The relative roles of cultural drift and acoustic adaptation in shaping syllable repertoires of island bird populations change with time since colonization. *Evolution* 69:368–380.
- Price, J. J. (2015). Rethinking our assumptions of bird song and other sexually dimorphic signals. *Frontiers in Ecology and Evolution* 3:1–6.
- R Core Team (2020). R: A language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.r-project.org/>
- Rannala, B., and J. L. Mountain (1997). Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the United States of America* 94:9197–9201.
- Raymond, M., and F. Rousset (1995). GENEPOP version 1.2: Population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86:248–249.
- Rehsteiner, U., H. Geisser, and H. Reyer. (1998). Singing and mating success in water pipits: one specific song element makes all the difference. *Animal Behaviour* 55:1471–1481.
- Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution; International Journal of Organic Evolution* 43:223–225.
- Riebel, K. (2003). The “mute” sex revisited; vocal production and perception learning in female songbirds. *Advances in the Study of Behavior* 33:49–86.
- Riebel, K., M. L. Hall, and N. E. Langmore (2005). Female songbirds still struggling to be heard. *Trends in Ecology & Evolution* 20:419–420.
- Ruegg, K., H. Slabbekoorn, S. Clegg, and T. B. Smith (2006). Divergence in mating signals correlates with ecological variation in the migratory songbird, Swainson’s thrush (*Catharus ustulatus*). *Molecular Ecology* 15:3147–3156.
- Selkoe, K. A., and R. J. Toonan (2006). Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecology Letters* 6:615–629.
- Seutin, G., B. N. White, and P. T. Boag (1991). Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology* 69:82–90.
- Slater, P. J. B., and N. I. Mann (2004). Why do the females of many bird species sing in the tropics? *Journal of Avian Biology* 35:289–294.
- Smith, B. T., J. E. McCormack, A. M. Cuervo, M. J. Hickerson, A. Aleixo, C. D. Cadena, J. Pérez-Emán, C. W. Burney, X. Xie, M. G. Harvey, et al. (2014). The drivers of tropical speciation. *Nature* 515:406–409.
- Stewart, K. A., and E. A. MacDougall-Shackleton (2008). Local song elements indicate local genotypes and predict physiological condition in Song Sparrows *Melospiza melodia*. *Biology Letters* 4:240–242.
- Stutchbury, B. J. M., and E. S. Morton (2001). *Behavioural Ecology of Tropical Birds*. Academic Press, London, UK.
- Templeton, C. N., A. A. Ríos-Chelén, E. Quirós-Guerrero, N. I. Mann, and P. J. Slater (2013). Female happy wrens select songs to cooperate with their mates rather than confront intruders. *Biology Letters* 9:20120863.
- Topp, S. M., and D. J. Mennill (2008). Seasonal variation in the duetting behaviour of Rufous-and-white Wrens (*Thryothorus rufalbus*). *Behavioural Ecology and Sociobiology* 62:1107–1117.
- Tracy, T. T., and M. C. Baker (1999). Geographic variation in syllables of House Finch songs. *The Auk* 116:666–676.
- Vallet, E., I. Beme, and M. Kreutzer. (1998). Two-note syllables in canary songs elicit high levels of sexual display. *Animal Behaviour* 55.2:291–297.
- Vangestel, C., T. Callens, V. Vandomme, and L. Lens (2013). Sex-biased dispersal at different geographical scales in a cooperative breeder from fragmented rainforest. *PLoS One* 8:e71624.
- Wheelwright, N., M. Swett, I. I. Levin, D. Kroodsma, C. Freeman-Gallant, and H. Williams (2008). The influence of different tutor types on song learning in a natural bird population. *Animal Behaviour* 75:1479–1493.

- Whiten, A. (2019). Cultural evolution of animals. *Annual Review of Ecology, Evolution, and Systematics* 50:27–48.
- Wilkins, M. R., N. Seddon, and R. J. Safran (2013). Evolutionary divergence in acoustic signals: Causes and consequences. *Trends in Ecology & Evolution* 28:156–166.
- Williams, H., I. I. Levin, D. R. Norris, A. E. M. Newman, and N. T. Wheelwright (2013). Three decades of cultural evolution in Savannah Sparrow songs. *Animal Behaviour* 85:213–223.
- Woodworth, B. K., D. R. Norris, B. A. Graham, Z. A. Kahn, and D. J. Mennill (2018). Hot temperatures during the dry season reduce survival of a resident tropical bird. *Proceedings of the Royal Society of London B: Biological Sciences* 285:20180176.
- Wright, T. F., and C. R. Dahlin (2007). Pair duets in the Yellow-naped Amazon (Psittaciformes: *Amazona auropalliata*): Phonology and syntax. *Behaviour* 144:207–228.
- Wright, T. F., A. M. Rodriguez, and R. C. Fleischer. (2005). Vocal dialects, sex-biased dispersal, and microsatellite population structure in the parrot *Amazona auropalliata*. *Molecular Ecology* 14:1197–1205.
- Wright, T. F., C. R. Dahlin, and A. Salinas-Melgoza (2008). Stability and change in vocal dialects of the yellow-naped amazon. *Animal Behaviour* 76:1017–1027.
- Yoktan, K., E. Geffen, A. Ilany, Y. Yom-Tov, A. Naor, and N. Leader (2011). Vocal dialect and genetic subdivisions along a geographic gradient in the Orange-tufted Sunbird. *Behavioural Ecology and Sociobiology* 65:1389–1402.