Parallel evolutionary forces influence the evolution of male and female songs in a tropical songbird

BRENDAN A. GRAHAM* iD, DANIEL D. HEATH*†, RYAN P. WALTER†‡,
MELISSA M. MARK§ & DANIEL J. MENNILL* iD

*Department of Biological Sciences, University of Windsor, Windsor, ON, Canada
†Great Lakes Institute of Environmental Research, University of Windsor, Windsor, ON, Canada
‡Department of Biological Science, California State University Fullerton, Fullerton, CA, USA
§Doris Duke Conservation Scholars Program, Northern Arizona University, Flagstaff, AZ, USA

Abstract

Given the important role that animal vocalizations play in mate attraction and resource defence, acoustic signals are expected to play a significant role in speciation. Most studies, however, have focused on the acoustic traits of male animals living in the temperate zone. In contrast to temperate environments, in the tropics, it is commonplace for both sexes to produce complex acoustic signals. Therefore, tropical birds offer the opportunity to compare the sexes and provide a more comprehensive understanding of the evolution of animal signals. In this study, we quantified patterns of acoustic variation in Rufous-and-white Wrens (*Thryophilus rufalbus*) from five populations in Central America. We quantified similarities and differences between male and female songs by comparing the role that acoustic adaptation, cultural isolation and neutral genetic divergence have played in shaping acoustic divergence. We found that males and females showed considerable acoustic variation across populations, although females exhibited greater population divergence than males. Redundancy analysis and partial-redundancy analysis revealed significant relationships between acoustic variation and ecological variables, genetic distance, and geographic distance. Both ambient background noise and geographic distance explained a high proportion of variance for both males and females, suggesting that both acoustic adaptation and cultural isolation influence song. Overall, our results indicate that parallel evolutionary forces act on male and female acoustic signals and highlight the important role that cultural drift and selection play in the evolution of both male and female songs.

Introduction

Variation in the acoustic signals of animals can have profound evolutionary implications (Boughman, 2002). Acoustic signals play an important role in attracting mates and defending resources (Bradbury & Vehrencamp, 2011), and therefore, changes in acoustic structure may promote reproductive isolation between populations (Jones, 1997; Irwin *et al.*, 2001; Lemmon, 2009). Given that acoustic signals may play a role in speciation, questions remain about the forces that influence the evolution of acoustic signals (Wilkins *et al.*, 2013). Do acoustic signals reflect phylogenetic history and evolve in unison with neutral genetic variation? Or do acoustic signals evolve independently of genetic evolution as a result of cultural evolution or plasticity induced through some form of environmental change (MacDougall-Shackleton & MacDougall-Shackleton, 2001; Wright *et al.*, 2005; Prohle *et al.*, 2006; Campbell *et al.*, 2010; Clegg & Phillimore, 2010; González *et al.*, 2011)?

The songs of birds are acoustic traits that vary geographically (Catchpole & Slater, 2008). Most research on the evolution of bird song focuses on temperate-

Correspondence: Brendan A. Graham, University of Windsor, 401 Sunset Avenue, Biology Building, Windsor, ON N9B3P4, Canada.
Tel.: 519-253-3000x4725; e-mail: b.graham001@gmail.com

breeding birds where songs are primarily sung by males (reviewed in Podos & Warren, 2007; but see Garamszegi *et al.* 2007). Neotropical birds differ from temperate birds in key life history traits (Stutchbury & Morton, 2008; Martin, 2015), which impact the evolution of acoustic traits. Whereas many temperate species undergo annual long-distance migrations between their breeding and wintering grounds, the majority of tropical birds defend territories year-round (Stutchbury & Morton, 2008). Female song and male–female duets are widespread in the tropics, whereas these behaviours are rare or absent in the North Temperate Zone (Slater & Mann, 2004). Relatively little is known about the behavioural ecology and evolutionary biology of female song (Langmore, 1998; Riebel *et al.*, 2005), in spite of the fact that female song is a widespread and ancestral trait in songbirds (Odom *et al.*, 2014).

Reduced dispersal is considered a key driver of speciation at tropical latitudes (Claramunt *et al.*, 2012). Unlike temperate species, the majority of tropical birds show strong philopatry (Stutchbury & Morton, 2008) and often disperse short distances from natal territories (Yáber & Rabenold, 2002; Woltmann *et al.*, 2012). A low propensity for dispersal across ecological barriers promotes reproductive isolation in understory birds of the Neotropics (Burney & Brumfield, 2009) and can reduce gene flow and play a role in cultural isolation (i.e. divergence in learned traits that arises from a lack of social interaction between populations; Lynch & Baker, 1994). Cultural drift (i.e. changes in the acoustic structure of songs due to stochastic processes that arise during vocal learning, such as copy errors or improvisation) influences acoustic variation in animals that learn their songs (Lynch, 1996), and likely acts in concert with dispersal patterns and song-learning behaviour to promote acoustic divergence (Ellers & Slabbekoorn, 2003). Although links between population structure (examined using neutral markers) and acoustic divergence have been demonstrated in animals that do not learn their acoustic signals (e.g. Isler *et al.*, 2005; Campbell *et al.*, 2010), there is little evidence to suggest that neutral genetic divergence and acoustic variation are linked in animals that learn their acoustic signals (Soha *et al.*, 2004; Wright *et al.*, 2005; Leader *et al.*, 2008; Yoktan *et al.*, 2011; Ortiz-Ramírez *et al.*, 2016; but see Baker *et al.*, 1982; MacDougall-Shackleton & MacDougall-Shackleton, 2001).

Changes in the composition and structure of songs may also arise as a result of responses to selection differences between populations (Collins *et al.*, 2009). Natural, social and sexual selection have all been shown to influence acoustic variation (Jones, 1997; Tobias & Seddon, 2009; Cardoso & Atwell, 2011; Danner *et al.*, 2011; Wilkins *et al.*, 2013). Just as drift plays an important role in the evolution of learned traits like song, so too does selection, given that animals can adjust their songs or learn specific songs in response to different selection pressures (Ellers & Slabbekoorn, 2003). For example, song learning may be facilitated by female choice (Wilkins *et al.*, 2013). Females often respond more intensely to local dialects and mates that produce local dialects, and the inability to sing local dialects may affect male reproductive success (Macdougall-shackleton *et al.*, 2002; Danner *et al.*, 2011).

In addition to dispersal, drift and selection, ecological features are one local source of selection that influence acoustic variation (Burney & Brumfield, 2009; Wilkins *et al.*, 2013). Acoustic differences between populations may arise as a result of acoustic adaptation, where signals become optimized for transmission through different environments (Morton, 1975). Habitat structure (e.g. Hunter & Krebs, 1979; Handford & Lougheed, 1991; Slabbekoorn & Smith, 2002), ambient noise (e.g. Hanna *et al.*, 2011; Mockford *et al.*, 2011) and climate (e.g. Forrest, 1994; Brumm & Naguib, 2009) all influence the transmission and structure of acoustic signals. For example, habitat and noise can influence the evolution of song frequency: low-frequency sounds transmit more efficiently than high-frequency sounds through densely vegetated habitats (Morton, 1975; Forrest, 1994), and many animals change the frequency of their vocalizations to prevent masking by background noise (Slabbekoorn & Peet, 2003; Hanna *et al.*, 2011). Given the role that ecological features play in the evolutionary process, combining ecological data with genetic and acoustic data will provide greater insight into evolutionary patterns and acoustic differences between populations (Manel *et al.*, 2003; Kozak *et al.*, 2008).

In this study, we examine acoustic variation in Rufous-and-white Wrens (*Thryophilus rufalbus*). This species is a year-round resident of the tropics with a distribution that extends from southern Mexico through Central America and into Colombia and Venezuela. Both males and females sing in this species, songs show structural differences between sexes, and both males and females possess repertoires of up to 15 song types (Mennill & Vehrencamp, 2005; Harris *et al.*, 2016). The pattern of song learning has not been studied in Rufous-and-white Wrens, but our observations of song structure suggest that males learn songs primarily from males, and females learn songs primarily from females. The pattern of song transmission in birds where both sexes sing has been studied in very few species; in Superb-fairy Wrens (*Malurus cyaneus*), young males and females appear to acquire song elements from both male and female tutors (Evans & Kleindorfer, 2016), whereas in Plain Wrens (*Cantorchilus modestus*) and Eastern whipbirds (*Psophodes olivaceous*), young males appear to acquire songs from other males, and young females appear to acquire songs from other females (Mennill & Rogers, 2006; Marshall-ball & Slater).

To investigate the factors that contribute to acoustic variation in male and female Rufous-and-white Wrens,

we studied five populations along a 500 km transect in Central America. Our study sites vary in habitat structure and climate, allowing us to examine the role that habitat and environment play in shaping acoustic variation. We compare acoustic variation with ecological variables, geographic distance and genetic distance (using both biparentally inherited microsatellite markers and maternally inherited mitochondrial DNA sequences as measures of gene flow among populations) to examine whether acoustic differences between populations are influenced by acoustic adaptation, cultural isolation or genetic isolation (Table 1). For example, a significant positive relationship between acoustic distance and genetic distance would suggest that acoustic variation is influenced by gene flow, whereas a negative or nonsignificant relationship would indicate that acoustic variation and gene flow are not linked. To date, examples of how female songs vary geographically are rare (but see Mennill & Rogers, 2006) and given that both male and female Rufous-and-white Wrens sing, this study system allows us to examine whether males and females show similar patterns of acoustic divergence and whether parallel evolutionary forces act on the design of male and female acoustic signals.

Materials and methods

We studied Rufous-and-white Wrens in five populations in Central America (Fig. 1). Four populations were located in Costa Rica (Sector Santa Rosa of the Guanacaste Conservation Area: 10.85°N, 85.60°W; Sector Rincón de la Vieja of the Guanacaste Conservation Area: 10.78°N, 85.35°W; University of Georgia Campus in the San Luis Valley near Monteverde: 10.28°N, 84.79°W; Central Valley: 9.90°N, 84.25°W) and one population was located in Nicaragua (Reserva Miraflora: 13.27°N, 86.31°W). We monitored birds in Costa Rica from 2012 to 2014, and birds in Nicaragua from 2004 to 2008. In each population, we captured birds using

mist-nets and banded each individual with a unique band combination that included three colour bands and one numbered aluminium band. From each bird, we collected a small blood sample (~100 μL) from the brachial vein and stored blood samples in 95% ethanol or Queen's Lysis Buffer (Seutin *et al.*, 1991). We determined the sex of individuals based on the presence of a brood patch (only females incubate in this species) and by singing behaviour (sexes can be distinguished based on fine-structural differences in songs; Mennill & Vehrencamp, 2005).

Acoustic measurements

We recorded birds between April and July of each year, a time of year when vocal output is high for this species (Topp & Mennill, 2008). We collected most of our recordings (60%) using focal recording methods, where we followed each bird around its territory for several hours during the morning (between 0445 h and 1100 h) and confirmed the bird's identity by observing its leg bands. We collected focal recordings using a solid-state digital recorder (Marantz PMD-660; 44.1 KHz sampling rate; 16-bit accuracy; WAVE format) and a shotgun microphone (Sennheiser MKH70). We supplemented these recordings with recordings from automated recorders (Song Meter SM2, Wildlife Acoustics Inc., Concord, Massachusetts, USA; sampling frequency: 44.1 KHz; full equipment details in Mennill *et al.*, 2012). We placed these recorders near the centre of the territories of each focal pair, usually within 10 m of the pair's nest. We confirmed that the songs collected with automated recorders came from the intended birds by re-sighting the focal individuals in their territory after automated recording sessions, and by matching the songs collected by the automated recorders to the songs collected during focal recordings (see Harris *et al.*, 2016, for further details). Although the majority of birds used in this study were banded,

Table 1 Description of the hypotheses tested in this investigation, to determine which factors play an important role in the evolution of male and female Rufous-and-white wren songs. We present all of these hypotheses for completeness, but recognize that some of these hypotheses are not mutually exclusive.

Hypothesis	Predictions
Acoustic Adaptation	If acoustic divergence between populations is influenced by acoustic adaptation, we predicted that we would find a significant positive relationship between acoustic distance (a measure of acoustic differences between populations) and differences in ecological differences (i.e. habitat or environmental noise). Under this scenario, acoustic divergence arises because songs are selected for optimal transmission through different types of habitats.
Cultural Isolation	If acoustic divergence between populations is influenced by cultural isolation (e.g. a lack of dispersal between populations), we predicted that we would find a significant relationship between acoustic distance and geographic distance. Under this scenario, acoustic divergence may arise as a result of neutral processes (for example, changes in the structure of songs via inaccurate song learning) or due to differences in sexual or social selection between populations.
Neutral Genetic Divergence	If acoustic divergence between populations is influenced by neutral genetic divergence, we predicted that we would find a significant positive relationship between acoustic distance and genetic distance. Under this scenario, acoustic divergence arises because cultural evolution and biological evolution are linked.
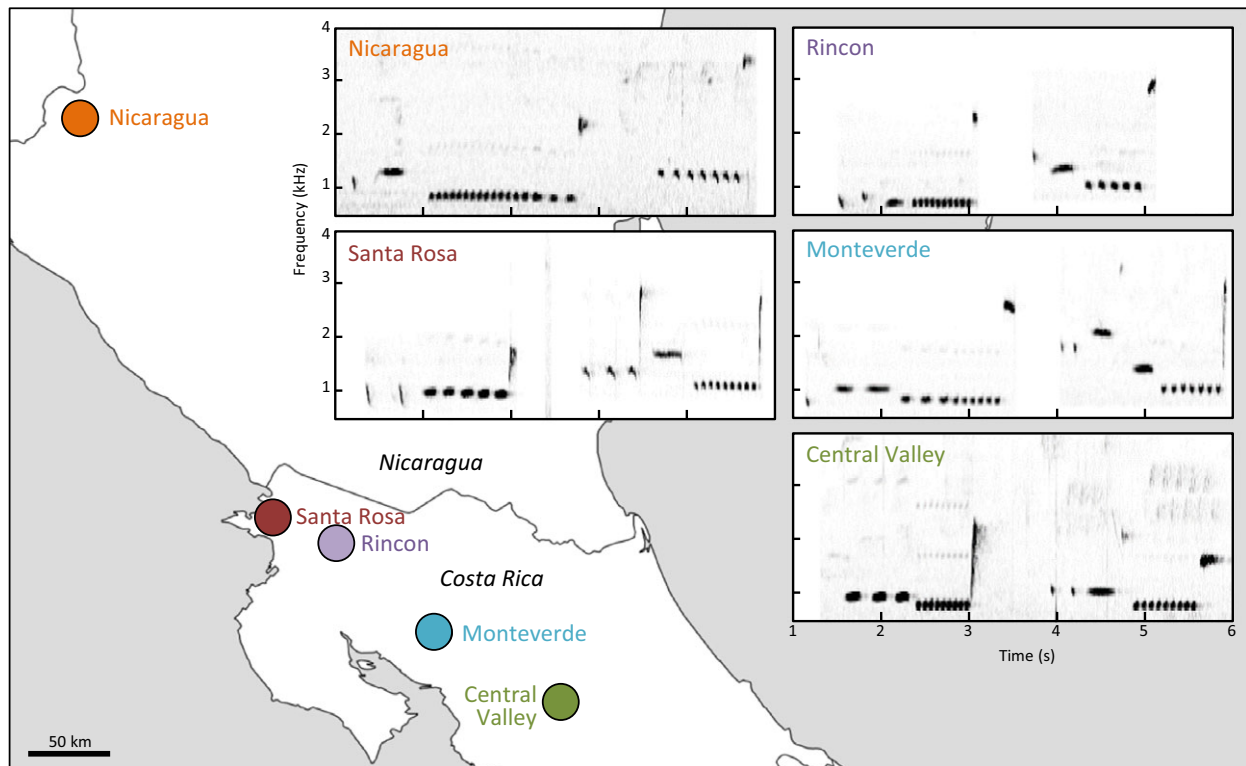
Fig. 1 Map of the location of five populations of Rufous-and-white Wrens where we studied acoustic, ecological and genetic variation. Sound spectrograms show examples of male songs (on the left side of each spectrogram) and female songs (on the right side of each spectrogram) recorded from each of the five populations.

we also used songs from six unbanded males and 17 unbanded females. In all instances, the unbanded animal's breeding partner was banded, which facilitated identification. We distinguished unbanded birds from each other based on their location and their affiliation with a banded individual. As with our approach with banded birds, we compared songs across all recordings of unbanded birds to validate that we were recording the same individual throughout the breeding season.

We annotated all audio files using SYRINX-PC sound analysis software (J. Burt, Seattle, Washington, USA). For each male and female, we built a song library of all the songs that each bird sang. Once we had created a song library for each bird, we selected songs that had a high signal-to-noise ratio and we collected fine-structural measurements from these songs. In this species, males and females both produce song types (i.e. songs that are composed of a stereotyped sequence of elements that are often shared between birds; Mennill & Vehrencamp, 2005; Harris *et al.*, 2016). For each bird, we measured up to five exemplars of each song type (males: average of 2.63 exemplars, range 1–5; females: average of 2.07 exemplars, range 1–5), and calculated a mean measurement of eight different spectro-temporal features (see below) for each song type for each

individual. Whenever possible, we included songs from multiple recording sessions, measuring no more than three exemplars of each song type per recording.

To quantify geographic variation in the songs of male and female Rufous-and-white Wrens, we measured eight different temporal and spectral features of their songs (Fig. 2): (A) duration of the song (s), (B) number of syllables in each song, (C) duration of all the pauses in the song (s; the silent interval between one syllable and the next syllable), (D) dominant frequency of the trill (Hz), (E) minimum frequency of the song (Hz), (F) maximum frequency of the song (Hz), (G) duration of the terminal syllable (s) and (H) the bandwidth of the terminal syllable (Hz; calculated by subtracting the minimum frequency of the terminal syllable from the maximum frequency of the terminal syllable). We used the automated parameter measurement tool in AviSoft-SASLab Pro (version: 5.2.04; R. Sprecht; Berlin, Germany) to measure the fine-structural measurements of all songs, thereby minimizing human subjectivity in collecting these measurements. Songs were resampled to 8000 Hz, which allowed maximal spectral resolution in AviSoft (the maximum frequency of Rufous-and-white Wren songs in this data set was < 4000 Hz). For each song, we created a sound spectrogram with an

Fig. 2 Sound spectrogram of the song of a male Rufous-and-white Wren, showing the eight fine-structural measurements made for each male and female song: (A) song length (s); (B) number of syllables; (C) intersyllable interval (s; the average duration of silence between each syllable); (D) dominant frequency of the trill (Hz); (E) minimum frequency of the song (Hz); (F) maximum frequency of the song (Hz); (G) length of terminal syllable (s); and (H) bandwidth of the terminal syllable (Hz).
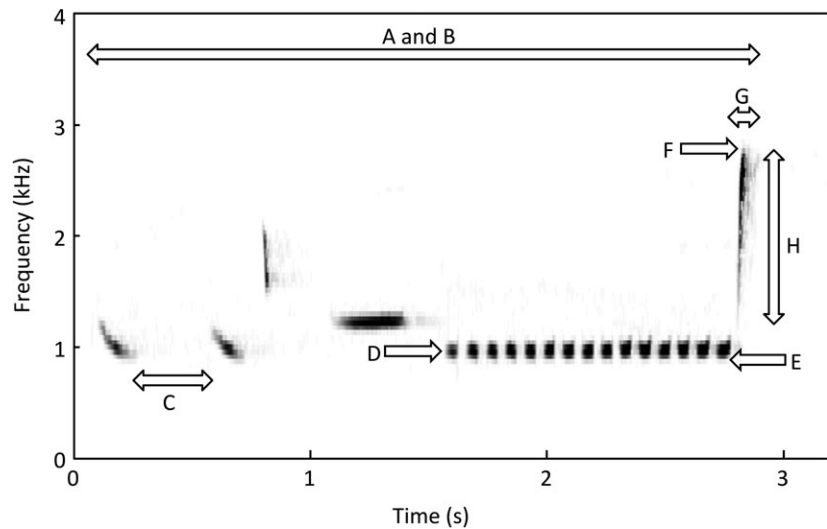
effective resolution of 8 Hz and 4 ms (settings: transform size: 1024 Hz; overlap: 96.86%; window: Hamming). We used a high-pass filter of 500 Hz to remove any low-frequency background noise. The measurements used for all statistical analysis represent population means for each song type (males: an average of 4.88 individuals per song type, range = 1–28; females: 3.81 individuals per song type; range = 1–17). We obtained population means for each song type by analysing the individual means for each song type in an individual's repertoire (see above). Overall, we measured 1669 male songs representing 134 song types from 91 individuals from five populations in Nicaragua and Costa Rica, and 670 female songs representing 79 song types from 71 individuals from four populations in Costa Rica. We collected too few recordings of female songs in Nicaragua to quantify variation in female songs in this population, and therefore, our analysis of female songs is restricted to the four Costa Rican populations.

Genetic analysis

We used microsatellite and mtDNA markers to assess genetic population structure at different temporal scales. Microsatellite loci evolve relatively quickly (mutation rates = 10^{-3} to 10^{-4} per generation; Weber & Wong, 1993), which allowed us to examine the role of contemporary gene flow (Primmer *et al.*, 1996), whereas mtDNA evolves much slower (approximately 2.3% per million years; Smith & Klicka, 2010) and therefore allowed us to examine the influence of shared ancestry/historical gene flow.

We extracted DNA from blood samples using a Wizard Extraction Kit (Promega) and genotyped 211 individuals (129 males and 81 females plus 1 individual from Nicaragua whose sex was as unknown) at 10

microsatellite loci. We used four existing microsatellite primer sets *ThPl 14, ThPl 20, ThPl 30* (Brar *et al.*, 2007) and *RWWR 2c* (Herman Mays personal communication), and we developed six new microsatellite primer sets (*Tru 08, Tru 11, Tru 18, Tru 20, Tru 24* and *Tru 25;* Table S1) following a modified method of the Fischer & Bachman (1998) microsatellite enrichment procedure detailed in Walter *et al.* (2007). All PCR reactions were conducted in 12.5 μL reactions with 1 μL of genomic DNA. PCR cocktails contained 1.25 μL of 10× PCR buffer (Applied Biosystems), 0.5 μL of MgCl$_2$ (2.5 mM), 0.45 μL of dNTPs (0.2 mM), 0.05 μL of bovine serum albumin and 0.5 U of Taq (Genscript, Applied Biosystems). For the primer sets *Tru 08, Tru 11, Tru 18, Tru 20, Tru 24, Tru 25* and *RWWR 2c*, we included 1 μM each of an M13 tailed-forward primer (see Table S1), reverse primer, and a 5' IR-dye-labelled M13 primer (GTAAAACGACGGCCAGT). For the remaining three primer sets (*ThPl 14, ThPl 20,* and *ThPl 30*), we used 1 μM each of the forward primer and the IR-dye-labelled reverse primer. PCR conditions for *ThPl 14, ThPl 20* and *ThPl 30* followed those described in Douglas *et al.* (2012), whereas for the remaining primer sets, we used the following PCR conditions: one cycle of 94.0 °C for 2 min, followed by 34 cycles of 94.0 °C for 10 s, 50.0 °C for 10 s, 72.0 °C for 30 s, followed by a final extension cycle of 72.0 °C for 90 s, although for the primer set *Tru 24*, we increased the annealing temperature (T_2) to 54.0 °C to eliminate stutter. PCR products were visualized using a LiCor 4300 DNA analyser (LiCor Biosciences, Inc.), and allele sizes were scored using GeneImagIR 4.05 (Scanalytics, Inc., Rockville, MD). To ensure consistent sizing and scoring across gels, we ran controls with known size standards on each run.

We tested for deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium for each

population × locus combination using GenePop version 4.0.10 (Raymond & Rousset, 1995) and corrected for multiple tests using sequential Bonferroni corrections (Rice, 1989). We calculated allelic richness (A_R), observed heterozygosity (H_O) and expected heterozygosity (H_E) using FSTAT version 2.9.2.3 (Goudet, 1995; Table S2). Two of the three population × locus combinations that were not in HWE were found at Santa Rosa; to ensure that departures from HWE were not driving the observed patterns, we performed our analysis with all 10 loci and then repeated the analyses without the two loci (*ThPl 14* and *ThPl 30*) that showed significant departures from HWE at Santa Rosa. We used the full microsatellite data set for all analyses, because removing these loci did not change our results.

We sequenced the full NADH dehydrogenase 2 mitochondrial gene (ND2; 1041 bp) for 57 Rufous-and-white Wrens from five sites in Central America. We amplified ND2 sequences using previously designed primers (forward primer L5215; TATCGGGCCCATACCCC GAAAAT; Hackett, 1996; reverse primer H1064 CTTTGAAGGCCTTCGGTTTA; Drovetski *et al.*, 2004). All PCR reactions were conducted in 25 μL reactions with 1 μL of genomic DNA. PCR cocktails contained 2.5 μL of 10× PCR Buffer (Applied Biosystems), 1.0 μL of MgCl$_2$ (2.5 mM), 0.9 μL of dNTP (0.2 mM), 1 μM each of the forward and reverse primer and 1.0 U of Taq (Genscript, Applied Biosystems). PCR thermocycler conditions used the following conditions: one cycle of 94.0 °C for three minutes, followed by 35 cycles of 94.0 °C for 40 s, 50.0 °C for 40 s, 72 °C for one minute, followed by a final extension cycle of 72 °C for 3 min. PCR amplicons were sequenced using the forward primers at the McGill University and Génome Quebec Innovation Center. Sequences were aligned and trimmed to their respective lengths (1041 bp) using Mega 5.0 (Tamura *et al.*, 2007). In addition, we calculated the number of haplotypes, haplotype diversity and nucleotide diversity using DNAsp 5.0 (Librado & Rozas, 2009; Table S3)

Ecological measurements

We turned to existing databases for measurements of ecological variables at each of our five study sites. We obtained climate data from the WorldClim database (http://worldclim.org) and extracted data using QGIS. The extracted climate data include mean values from over 50 years (1950–2004; Hijmans *et al.*, 2005) and with a spatial resolution of ~1 km (Table S4). Rather than creating a single ecological distance variable (as has been used in other studies; e.g. Ruegg *et al.*, 2006), we used the raw data for four ecological variables to explore the role of acoustic adaptation. Habitat is known to influence the transmission and design of acoustic signals (Morton, 1975); accordingly, in this analysis, we included two biotic factors, mean annual

precipitation (mm) and mean annual temperature (°C), and one abiotic factor, altitude (m), because these factors influence habitat composition. In the absence of vegetation density measurements for each site, we treated these variables as a proxy for habitat density, based on the assumption that cooler, wetter habitats were likely to have greater vegetation density than drier, warmer habitats (Table S4). Altitude and mean annual temperature were strongly correlated with each other ($r = -1.0$), so we tested the influence of these variables separately in our model.

As a separate ecological variable, we measured background noise. Our justification for including background noise as an ecological variable is that background noise arises from biological, abiotic and urban noise sources, and can influence the evolution of acoustic signals in many bird species (Slabbekoorn & Peet, 2003; Hanna *et al.*, 2011; Luther & Derryberry, 2012) including Rufous-and-white Wrens (Graham *et al.*, 2017a). Many animals shift the frequency of their vocalizations to avoid background masking, and acoustic differences could reflect such noise differences between sites (Slabbekoorn & Peet, 2003). We focused on background noise in the range between 0 and 1 kHz, because many of the introductory and trill syllables of male and female Rufous-and-white Wrens are produced between 500 Hz and 1 kHz, and this frequency spectrum is expected to experience heavy overlapping by background noise (Slabbekoorn, 2004). To quantify background noise, we classified sites as having low, moderate or high background noise, by visually inspecting spectrograms of focal recordings (Table S4). We previously quantified background noise at three of the five studies examined in this study (see Graham *et al.*, 2017a,b), and together with this previous knowledge, we considered the source of background noise following our initial visual inspection of spectrograms. Abiotic noise sources such as wind and flowing water and anthropogenic noise sources are known to mask acoustic signals (e.g. Slabbekoorn & Peet, 2003; Hanna *et al.*, 2011; Bradbury & Vehrencamp, 2011; Luther & Derryberry, 2012). We assessed noise to be high in the Central Valley because both flowing water and anthropogenic noise were consistently present in all sound recordings. Wind was present at all sites, but was considerably higher at Monteverde and therefore we distinguished background noise to be greater at this location than both Santa Rosa and Nicaragua where high winds were far less frequent during recordings across the breeding season.

Statistical analysis

Acoustic variation
We analysed acoustic data using two methods. First, we used a discriminant function analysis (hereafter 'discriminant analysis') on male and female songs

separately to evaluate whether source populations (the site where the bird was recorded) were distinguishable based on the eight fine-structural measurements of songs (see supplemental Table S3 for canonical axis loadings). We ran separate discriminant analyses for each sex. For this analysis, we used the leave-one-out classification approach, and we report the percentage of songs assigned to the correct group using the cross-validation approach in SPSS (version 23.0, SPSS Inc., Chicago, IL, USA). Given that we were unable to measure songs from females in the Nicaragua population, we performed a second, parallel analysis for males wherein we excluded the males of the Nicaragua population, so that we could directly compare patterns between males and females. We used χ^2 tests to evaluate whether our discriminant analysis successfully assigned songs to the correct population at a level that exceeded chance. Second, we used a multivariate analysis of variance (MANOVA) to examine if acoustic variables were significantly different among populations. Prior to analysis, acoustic variables were tested for intercorrelations using a Pearson correlation analysis; no correlations (r) exceeded 0.7; and therefore, all variables were included in both analyses (Ruegg *et al.*, 2006). We also checked for normality using Shapiro–Wilks tests and by visually inspecting the Q-plots of the residuals for each acoustic variable. Four acoustic variables for males (bandwidth of terminal syllable, length of terminal syllable, duration of all pauses in the song and number of syllables) and three acoustic variables for females (bandwidth of terminal syllable, length of terminal syllable and number of syllables) were log-transformed prior to testing for normality. Finally, we conducted *post hoc* comparisons using Tukey's honest significant differences tests to assess which populations differed in their acoustic variables. All values throughout the paper represent the mean and standard error.

Acoustic divergence

We used redundancy analysis (Legendre & Legendre, 1998) to test the role of acoustic adaptation (ecological variables), dispersal (geographic distance), genetic drift (microsatellite or mtDNA genetic distance) or a combination of these factors (Wang & Summers, 2010) on acoustic divergence in male and female Rufous-and-white Wrens. Although other studies have used Mantel and partial Mantel tests to examine linear relationships, this approach has received criticism and may lack the power to detect overall patterns compared to other approaches such as redundancy analysis (Legendre & Fortin, 2010). Redundancy analysis is a form of multivariate multiple regression that uses constrained ordination techniques to test the effects of explanatory variables on a set of response variables. We analysed acoustic divergence at the song-type level, similar to the approach used by Potvin & Clegg (2015). For this analysis, we used the first two canonical axes of our

discriminant analysis for each song type as our response variable. We analysed songs at the song-type level rather than the population level to improve our power to measure the factors that influence acoustic divergence. Further, male and female Rufous-and-white Wrens possess song repertoires and display considerable variation among song types. Therefore, we analysed patterns at the song-type level to capture the full variation of songs at each population, whereas this variation would be reduced and thereby lost if we had only compared population means, as used in many population-level analyses.

We included seven variables in our redundancy analysis: (i) geographic distance (a proxy for the degree of cultural isolation between populations), (ii) microsatellite genetic distance, (iii) mtDNA genetic distance, and the four previously mentioned ecological variables, (iv) mean annual precipitation, (v) mean annual temperature, (vi) altitude and (vii) ambient background noise. We used latitude and longitude as our vector coordinates to measure geographic distance between paired sites (as in Pilot *et al.*, 2006), whereas we calculated Nei's genetic distance between paired sites for mtDNA markers in GenAlex 6.5 (Peakall & Smouse, 2012) and Cavalli-Sforza & Edwards (1967) chord distance between paired sites for microsatellites in GenoDive 2.0b23 (Meirmans & Van Tienderen, 2004). To test the effects that genetic distance have on acoustic patterns, we transformed our distance matrices into vector coordinates using a Principal Coordinate Analysis in GenAlex, with an intent to transform the measured genetic distances into continuous variables that we could include in our analyses (as in He *et al.*, 2013; Potvin & Clegg, 2015). For microsatellite and mtDNA genetic distance, we used only the first principal coordinates as an explanatory variable because the first principal coordinate explained the majority of variance for both distances. In addition to redundancy analysis, we also performed partial-redundancy analysis, where we tested the effects of one explanatory variable while controlling for at least one other variable. All redundancy analysis and partial-redundancy analysis models were performed in R using the Vegan package (R Development Core team, 2014).

Results

Acoustic variation: Males

We observed substantial acoustic variation in male songs across five populations of Rufous-and-white Wrens in Central America. Discriminant analysis assigned songs to the correct population at a level that exceeded chance ($\chi^2 = 38.0$, d.f. = 16, $P = 0.002$; Table 2; Fig. 3), although only 36.6% of songs were assigned to the correct population. The Central Valley (48.5%) and Santa Rosa (43.8%) songs had the highest

percentage of correct song assignment, whereas Nicaragua (15.4%) and Monteverde (23.1%) had the lowest percentage of correct song assignment. When we

Table 2 Percentage of male and female songs assigned to the correct population using discriminant analysis. N equals the number of songs used for the analysis. For males, we performed two separate analyses. The first analyses included all five populations, whereas the second analyses excluded all Nicaragua songs. The purpose of this second analysis was to allow direct comparison between the assignment of male and female songs, because we did not measure any female songs from Nicaragua.

	All males		Males excluding Nicaragua		All females	
	N	Population, %	*N*	Population, %	*N*	Population, %
Nicaragua	13	15.4	–	–	–	–
Santa Rosa	32	43.8	32	68.8	32	84.4
Rincon	30	36.7	30	50.0	19	47.4
Monteverde	26	23.1	26	23.1	18	11.1
Central Valley	33	48.5	33	36.4	10	40.0
Overall	134	36.6	121	45.5	79	53.2

excluded Nicaragua songs from our discriminant analysis, 45.5% of male songs were assigned to the correct population, again at a level that exceeded chance ($\chi^2 = 42.6$, d.f. = 9, $P < 0.001$). Removing Nicaragua improved our ability to assign songs correctly to both Santa Rosa (68.8%) and Rincon (50.0%), whereas the number of songs correctly assigned to the Central Valley decreased slightly (36.4%).

We found significant differences in the fine-structural features of male songs among populations using multivariate analysis of variance (Wilks' lambda = 0.44; $F_{451,32} = 3.56$, $P < 0.001$, partial $\eta^2 = 0.19$). Three fine-structural measurements showed significant differences between populations: duration of terminal syllables ($F_{129,4} = 13.05$, $P < 0.001$, partial $\eta^2 = 0.29$), bandwidth of terminal syllables ($F_{129,4} = 3.07$, $P = 0.02$, partial $\eta^2 = 0.09$) and minimum frequency of songs ($F_{129,4} = 3.45$, $P = 0.01$, partial $\eta^2 = 0.10$; Table 3). Five of ten *post hoc* pairwise comparisons were significant for the duration of terminal syllables; terminal syllables from Nicaragua (0.06 ± 0.03 s) were significantly shorter than terminal syllables from the Central Valley (0.20 ± 0.02 s), Monteverde (0.14 ± 0.07 s) and
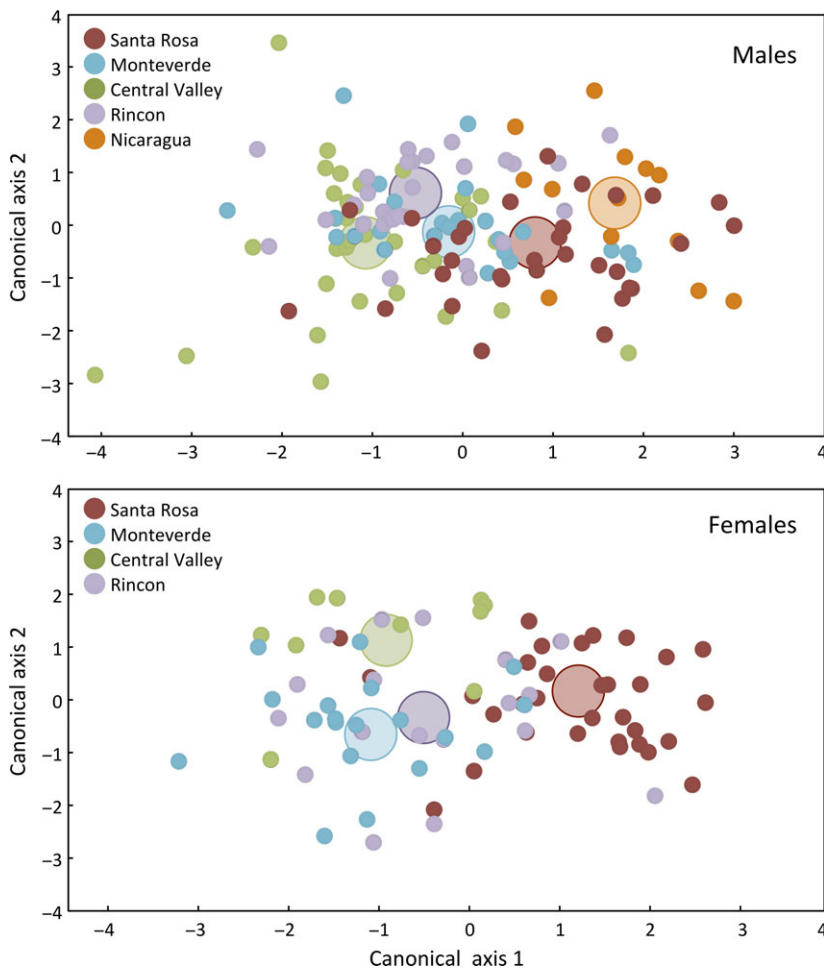
Fig. 3 Plots of the first two canonical axes from discriminant function analyses based on measurements of Rufous-and-white Wren songs from Central America. Male songs (top) were recorded from five populations in Nicaragua and Costa Rica; female songs (bottom) were recorded from four populations in Costa Rica. Large circles represent the mean centroid for each population.

Rincon (0.20 ± 0.02 s); and Santa Rosa terminal syllables were significantly shorter (0.11 ± 0.01 s) than terminal syllables from Rincon and the Central Valley. For the bandwidth of the terminal syllable, only one of ten pairwise comparisons was significantly different: terminal syllables from Santa Rosa spanned a larger bandwidth (470 ± 91 Hz) than at Rincon (175 ± 58 Hz). Similarly, only one of ten pairwise comparisons was significant for minimum frequency: Central Valley songs had a higher minimum frequency (803 ± 13 Hz) than Rincon (735 ± 14 Hz).

Acoustic variation: Females

For female Rufous-and-white Wrens, we also observed substantial acoustic variation in the structure of songs among four populations. Discriminant analysis assigned female songs to the correct population at levels that exceeded chance ($\chi^2 = 36.1$, d.f. = 9, $P < 0.001$; Table 2; Fig. 3). Overall, 53.2% of female songs were assigned to the correct population. By comparison, discriminant analysis correctly assigned more female songs than male songs to the correct population (36.6% using all five populations; 45.5% when we excluded Nicaragua male songs and analysed all sites where we recorded both sexes). For females, Monteverde had the lowest percentage of songs correctly assigned (11.1%; two of 18 songs), whereas Santa Rosa had the highest percentage of songs correctly assigned (84.4%; 27 of 32 at Santa Rosa).

As was the case for males, multivariate analysis of variance demonstrated that female songs vary significantly between populations (Wilks lambda = 0.36, $F = 3.46$, $P < 0.001$, partial $\eta^2 = 0.29$; Table 3). Three variables were significantly different between populations: terminal syllable bandwidth ($F = 13.62$, $P < 0.001$, partial $\eta^2 = 0.35$), dominant frequency of the trill ($F = 2.98$, $P = 0.04$, partial $\eta^2 = 0.11$) and minimum frequency of the song ($F = 5.68$, $P = 0.001$, partial $\eta^2 = 0.19$). Post hoc analyses revealed that terminal syllables from Santa Rosa covered a larger bandwidth (746 ± 95 Hz) than terminal syllables from any of the other populations. Furthermore, dominant frequency of the song was significantly different ($P = 0.048$) between Santa Rosa (1029 ± 25 Hz) and the Central valley (1163 ± 44 Hz). Similarly, Santa Rosa (872 ± 26) and Central Valley (1087 ± 46) also showed significant differences for minimum frequency of the song ($P = 0.001$).

Acoustic divergence

Both redundancy and partial-redundancy analysis models of individual song types indicated that geographic distance, genetic distance and environmental variables significantly predicted acoustic variation (Table 4), although geographic distance and background noise accounted for more variation than genetic distance or the remaining three environmental variables (annual precipitation, temperature and altitude) for both males and females. Males and females showed similar patterns for both redundancy and partial-redundancy models, although there were subtle differences (see Table 4).

Discussion

We explored the relationship between acoustic variation, genetic variation and ecological variation in five populations of Rufous-and-white Wrens in Central America, focusing on both males and females. Our results support greater roles for both acoustic adaptation and cultural isolation on acoustic divergence than neutral genetic divergence in both sexes. Acoustic patterns were correlated with genetic distance, but acoustic changes appeared to occur independently of genetic changes, suggesting that cultural drift and cultural selection have a stronger influence on the evolution of acoustic signals than neutral genetic divergence (Wright et al., 2008; Byers et al., 2010; Lin et al., 2015; Potvin & Clegg, 2015). With respect to the acoustic adaptation hypothesis, ambient noise differences rather than habitat differences between populations had a greater effect on acoustic divergence. Our comparison of males and females shows similar patterns of acoustic divergence for the sexes, suggesting that similar evolutionary processes act on the evolution of male and female songs (Price, 2015). This is one of the first such analyses to reveal parallel processes of evolution of vocal signals in males and females.

Our results indicate a link between acoustic variation and geographic distance, which is not surprising given that geographic distance is considered a key component of acoustic variation in many animals, especially for animals that exhibit strong philopatry and limited dispersal (Isler et al., 2005; Prohle et al., 2006; Ortiz-Ramírez et al., 2016). Insectivorous understory birds, like wrens, are generally thought to have limited dispersal (Stouffer & Bierregaard, 1995; Sekercioğlu et al., 2002; Moore et al., 2005), and genetic patterns as well as banding data indicate that this is the case for Rufous-and-white Wrens (Graham et al., 2017b). Given that dispersal and gene flow are limited in this species, these factors are likely to reinforce within-population cultural differences and further drive acoustic divergence (Nelson et al., 2001; Podos & Warren, 2007).

Acoustic differences may arise between populations as a result of neutral song variation or they may reflect different selection pressures or mating preferences at each site (Podos & Warren, 2007; Collins et al., 2009). Neutral song variation occurs as a result of improvisation or inaccurate song learning by young birds, where copying errors introduced during the song-learning process may change song structure and drive cultural differences between populations (Lynch, 1996; Ellers &

Table 3 Mean (±SE) values of male and female solo songs fine-structural measurements from each of the five populations. R^2 represents the total percentage of variance attributed to each variable (values presented are the adjusted R^2 values). F represents the F-statistic for each variable and p represents the P value. Bold values indicate variables that were significant at $P < 0.05$, and letters indicate those values that were significantly different following *post hoc* comparisons. Four variables for males (number of syllables, intersyllable interval, terminal syllable length and bandwidth of the terminal syllable) and three variables for females (number of syllables, intersyllable interval and bandwidth of the terminal syllable) were log-transformed for the analysis but are presented here untransformed for comparison.

Variable	Population					R^2, %	d.f.	F	p
	Central Valley	Monteverde	Rincón	Santa Rosa	Nicaragua				
Males									
Song Length (s)	2.13 ± 0.09 (a)	2.19 ± 0.10 (a)	2.14 ± 0.09 (a)	2.35 ± 0.09 (a)	2.31 ± 0.04 (a)	2.90	4, 129	0.97	0.43
Number of Syllables	14.60 ± 1.15 (a)	13.60 ± 0.82 (a)	15.52 ± 1.30 (a)	14.63 ± 0.56 (a)	13.69 ± 1.46 (a)	1.60	4, 129	0.52	0.72
Intersyllable interval (s)	0.10 ± 0.01 (a)	0.10 ± 0.01 (a)	0.09 ± 0.01 (a)	0.12 ± 0.01 (a)	0.10 ± 0.01 (a)	5.60	4, 129	1.9	0.11
Dominant Frequency of the Trill (Hz)	894 ± 12 (a)	885 ± 13 (a)	870 ± 12 (a)	917 ± 12 (a)	903 ± 19 (a)	5.80	4, 129	1.99	0.10
Minimum Frequency of Song (Hz)	**803 ± 13 (a)**	**773 ± 15 (ab)**	**735 ± 14 (b)**	**756 ± 13 (ab)**	**768 ± 21 (ab)**	**9.70**	4, 129	**3.45**	**0.01**
Maximum Frequency of Song (Hz)	1859 ± 82 (a)	2010 ± 92 (a)	1834 ± 86 (a)	1890 ± 83 (a)	1869 ± 130 (a)	1.70	4, 129	0.57	0.68
Length of Terminal Syllable (s)	**0.20 ± 0.02 (a)**	**0.14 ± 0.07 (ab)**	**0.20 ± 0.02 (a)**	**0.11 ± 0.01 (bc)**	**0.06 ± 0.01 (c)**	**28.80**	4, 129	**13.05**	**< 0.001**
Bandwidth of Terminal Syllable (Hz)	**348 ± 87 (ab)**	**374 ± 99 (ab)**	**175 ± 58 (a)**	**470 ± 91 (b)**	**336 ± 116 (ab)**	**8.70**	4, 129	**3.07**	**0.02**
Females									
Song Length (s)	2.08 ± 0.11 (a)	1.84 ± 0.09 (a)	1.84 ± 0.08 (a)	1.90 ± 0.06 (a)	—	4.30	3, 75	1.12	0.35
Number of Syllables	14.32 ± 1.79 (a)	10.50 ± 0.82 (a)	11.96 ± 1.11 (a)	11.52 ± 0.83 (a)	—	5.30	3, 75	1.41	0.25
Intersyllable interval (s)	0.10 ± 0.01 (a)	0.11 ± 0.01 (a)	0.10 ± 0.01 (a)	0.12 ± 0.01 (a)	—	3.60	3, 75	0.92	0.44
Dominant Frequency of the Trill (Hz)	**1163 ± 44 (a)**	**1037 ± 33 (ab)**	**1100 ± 32 (ab)**	**1029 ± 25 (b)**	—	**10.60**	3, 75	**2.98**	**0.04**
Minimum Frequency of Song (Hz)	**1087 ± 46 (a)**	**945 ± 34 (ab)**	**947 ± 34 (ab)**	**872 ± 26 (b)**	—	**18.50**	3, 75	**5.68**	**0.001**
Maximum Frequency of Song (Hz)	2511 ± 174 (a)	2256 ± 129 (a)	2334 ± 126 (a)	2340 ± 97 (a)	—	1.80	3, 75	0.46	0.71
Length of Terminal Syllable (s)	0.17 ± 0.02 (a)	0.13 ± 0.02 (a)	0.14 ± 0.01 (a)	0.15 ± 0.02 (a)	—	6.10	3, 75	1.61	0.19
Bandwidth of Terminal Syllable (Hz)	**297 ± 123 (a)**	**326 ± 120 (a)**	**91 ± 20 (a)**	**746 ± 95 (b)**	—	**35.30**	3, 75	**13.63**	**< 0.001**

Table 4 Results of redundancy analyses and partial-redundancy analyses testing the relationship between acoustic variation and genetic distance, geographic distance and ecological variables for male and female Rufous-and-white Wrens (Inertia measures of the amount of variation in the data set; R^2 = the proportion of variation explained by each variable; F = calculated F-scores; d.f. = degrees freedom in the model). For males, we performed an analysis with all five populations and a second analysis without Nicaragua, for the purpose of comparing patterns with females because we were unable to measure songs for females from this site. Italicized letters indicate the hypothesis tested from Table 1 to determine the role of that variable on acoustic divergence. All tests were considered significant at $P < 0.05$.

Variable	Males: five population analyses						Males: four population analyses						Females					
	Inertia	R^2, %	F	d.f.	P		Inertia	R^2, %	F	d.f.	P		Inertia	R^2, %	F	d.f.	P	
Cultural Isolation																		
Geographic distance	0.62	21.88	18.35	1,132	0.001		0.67	24.99	19.66	2,118	0.001		1.1	34.67	20.17	2,76	0.001	
Geographic distance | background noise	0.14	5.05	4.7	2,129	0.002		0.06	1.92	3.41	1,117	0.032		0.06	1.94	2.4	1,75	0.090	
Geographic distance | microsatellite	0.14	4.92	4.14	2,130	0.004		0.43	15.89	12.75	2,117	0.001		0.74	23.54	14.51	2,75	0.001	
Geographic distance | mtDNA	0.2	6.93	5.96	2,130	0.001		0.54	20.08	16.12	2,117	0.001		0.99	31.28	19.28	2,75	0.001	
Geographic distance | habitat*	0.58	20.63	19.43	2,129	0.001		0.17	6.51	10.54	1,117	0.001		0.27	8.64	10.65	1,75	0.001	
Acoustic Adaptation																		
Noise	0.73	25.66	22.6	2,132	0.001		0.67	25.20	19.87	2,118	0.001		1.18	37.23	22.54	2,76	0.001	
Altitude	0.11	3.71	5.09	1,132	0.012		0.37	13.97	19.33	1,119	0.001		0.74	2.34	23.52	1,77	0.001	
Annual precipitation	0.28	9.74	14.25	1,132	0.001		0.31	11.55	15.53	1,119	0.001		0.73	23.14	23.18	1,77	0.001	
Mean.temperature	0.11	3.87	5.31	1,132	0.007		0.33	12.45	16.92	1,119	0.001		0.73	22.95	22.94	1,77	0.001	
Habitat*	0.31	10.89	8.007	2,131	0.001		0.55	20.61	15.31	2,118	0.001		0.97	30.53	16.7	2,76	0.001	
Background noise | Geographic distance	0.25	8.82	8.21	2,129	0.001		0.06	2.12%	3.41	1,117	0.03		0.14	4.49	5.54	1,75	0.006	
Background noise | microsatellite	0.25	0.0882	7.83	2,130	0.001		0.43	15.89	12.75	2,117	0.001		0.74	23.54	14.51	2,75	0.001	
Background noise | mtDNA	0.32	11.36	10.37	2,130	0.001		0.54	20.08	16.12	2,117	0.001		0.99	31.28	19.28	2,75	0.001	
Background noise | habitat*	0.55	19.40	17.95	2,129	0.001		0.17	6.51	10.54	1,117	0.001		0.27	8.64	10.65	1,75	0.001	
Habitat* | Geographic distance	0.27	9.64	9.076	2,129	0.001		0.06	2.12	3.41	1,117	0.001		0.14	4.50	5.54	1,75	0.005	
Habitat* | background noise	0.13	4.64	4.29	2,129	0.007		0.05	1.92	3.08	1,117	0.04		0.06	1.94	2.4	1,75	0.10	
Habitat* | mtDNA	0.18	6.31	5.39	2,130	0.001		0.54	20.08	16.12	2,117	0.04		0.99	31.28	19.28	2,75	0.001	
Habitat | microsatellite	0.25	8.96	7.965	2,130	0.001		0.43	15.89	12.75	2,117	0.001		0.74	23.54	14.51	2,75	0.001	
Neutral Genetic Divergence																		
mtDNA	0.49	17.46	27.93	1,132	0.001		0.19	7.04	9.01	1,119	0.002		0.49	15.63	14.27	1,77	0.001	
mtDNA | Geographic distance	0.07	2.51	4.32	1,130	0.03		0.06	2.14	3.41	1,117	0.040		0.14	4.49	5.54	1,75	0.004	
mtDNA | background noise	0.09	3.16	5.77	1,130	0.004		0.05	1.92	3.08	1,117	0.060		0.06	1.94	2.4	1,75	0.100	
mtDNA | microsatellite	0.05	1.79	2.93	1,131	0.05		0.24	8.97	13.27	1,118	0.001		0.44	13.91	15.01	1,76	0.001	
mtDNA | habitat*	0.37	12.89	21.99	1,130	0.001		0.17	6.51	10.45	1,118	0.001		0.27	8.64	10.65	1,75	0.001	
Microsatellite	0.51	17.96	28.91	1,132	0.001		0.3	11.23	15.06	1,119	0.001		0.25	7.89	6.6	1,77	0.003	
Microsatellite | Geographic distance	0.03	0.99	1.68	1,130	0.190		0.06	2.12	3.41	1,117	0.030		0.14	4.50	5.54	1,75	0.007	
Microsatellite | background noise	0.03	1.13	2	1,130	0.120		0.05	1.92	3.08	1,117	0.050		0.06	1.94	2.4	1,75	0.010	
Microsatellite | mtDNA	0.07	2.29	3.75	1,131	0.020		0.35	13.17	19.47	1,118	0.001		0.68	21.65	23.36	1,76	0.001	
Microsatellite | habitat	0.45	16.03	28.51	1,130	0.001		0.17	6.51	10.45	1,118	0.001		0.27	8.64	10.65	1,75	0.001	

*For the term habitat, we included two variables annual precipitation, and altitude in this model. Mean annual temperature was not included because it was significantly correlated with altitude. Bold values indicate values that are significantly different at $P < 0.05$.

Slabbekoorn, 2003). Although we found a significant relationship between acoustic variation and neutral genetic variation, our results suggest that cultural isolation, and not genetic divergence, drives patterns of acoustic variation. Both acoustic variation and genetic variation exhibit an isolation-by-distance pattern (Graham, 2016), however, when we controlled for geographic distance in our analyses, genetic distance explained relatively little variation in acoustic patterns. This result is not surprising given that there is little evidence to suggest that genetic drift and acoustic variation are linked in animals that exhibit vocal learning (Soha *et al.*, 2004; Wright *et al.*, 2005; Leader *et al.*, 2008; Yoktan *et al.*, 2011; Ortiz-Ramírez *et al.*, 2016; but see Baker *et al.*, 1982; MacDougall-Shackleton & MacDougall-Shackleton, 2001). Previous studies have also suggested that acoustic patterns reflect vicariance or historical genetic patterns (González *et al.*, 2011; Sosa-López & Mennill, 2014). Similar to other studies, our results indicate that mtDNA genetic patterns were not linked with acoustic variation (Wright & Wilkinson, 2001; Leader *et al.*, 2008; Ribot *et al.*, 2012). This result is not surprising for males, given that mtDNA is maternally inherited and therefore reflects the movement of matrilineal lines historically. The mismatch between acoustic and mtDNA patterns for females further suggests the role that cultural drift has on acoustic patterns in Rufous-and-white Wrens.

The decoupling of cultural variation and genetic variation likely arises as a result of both cultural drift and cultural selection. Although it is difficult to determine which factor exerts greater influence in this study, both cultural drift and selection influence learned traits like song, given that songbirds are able to adjust their songs via learning (Ellers & Slabbekoorn, 2003). With respect to selection, acoustic variation may arise as a result of intrasexual and intersexual selection. For example, intrasexual selection may influence male birds to learn the songs of their neighbours (Kenyon *et al.*, 2017), whereas intersexual selection may cause males to learn local songs because it increases their ability to attract mates and reproduce (Danner *et al.*, 2011). Under both scenarios, songs can diverge quickly and independently of genetic changes (Ellers & Slabbekoorn, 2003). Furthermore, individual mating preferences may not only influence changes in song structure, but also the song types that are used in populations (Cardoso & Atwell, 2011). For example, some tropical species follow duet codes when coordinating their songs to produce duets with their mates (Logue, 2006; Templeton *et al.*, 2013a). In these species, males and females respond to each other's songs consistently with the same song types (Logue, 2007; Templeton *et al.*, 2013b). The phenomenon of duetting may apply its own cultural pressures if animals follow these duet codes. Individuals may learn specific song types because they are culturally selected or sexually selected, and in both cases, the resulting population-level patterns can be independent of genetic patterns.

Ecological selection is considered an important driver of acoustic divergence. While acoustic structure is often correlated with habitat structure (Boncoraglio & Saino, 2007), other ecological factors such as ambient noise, and acoustic competition with other species, may influence acoustic structure (Handford & Lougheed, 1991; Dingle *et al.*, 2008; Luther, 2009; Azar *et al.*, 2014; Hart *et al.*, 2015). In this study, we found background noise to be a significant predictor of acoustic variation. This result matches previous findings at three of the five populations used in this study (Santa Rosa, Rincon, and Monteverde), where transmission properties of both male and female songs varied among sites due to significant differences in the level of ambient noise (Graham *et al.*, 2017a). Our analysis of the fine-scale structure of songs further indicates a role for acoustic adaptation due to ambient noise differences. We observed differences between sites in both the minimum frequency of a song and dominant frequency of the trill of male and female songs. Minimum frequency and dominant frequency were higher at our Central Valley population than other populations. The Central Valley site is located in the middle of a heavily populated urban area; animals are known to produce signals at a higher frequency in urban areas, so that their signals can be heard above traffic or other sources of anthropogenic noise (e.g. Slabbekoorn & Peet, 2003; Hanna *et al.*, 2011; Luther & Derryberry, 2012). These differences likely reflect ambient noise differences among sites rather than habitat structure as suggested in other studies (Mockford *et al.*, 2011).

Previous studies have demonstrated that habitat influences acoustic divergence (Slabbekoorn & Smith, 2002; Slabbekoorn & Smith, 2002; Ruegg *et al.*, 2006; Caro *et al.*, 2013). Although we observed a significant effect of environment on acoustic variation in our study, background noise accounted for a greater proportion of the observed variance among populations and song types across our different ecological variables for both our redundancy and partial-redundancy models. Overall, the effects of habitat were less apparent in our study than in previous studies. One possible explanation is that our ecological variables (altitude, mean annual temperature and mean annual precipitation) may not accurately reflect habitat differences among our study sites. Alternatively, habitat differences may be more difficult to detect in our study, because Rufous-and-white Wrens strictly inhabit forests (Stotz *et al.*, 1996), and their songs are adapted for optimal transmission through forests (Barker *et al.*, 2009). By comparison, previous studies have compared acoustic variation among broadly distributed species that live in drastically different habitats (e.g. open grasslands vs. densely vegetated forested habitats; Handford & Lougheed, 1991).

In our study, a greater proportion of female songs were correctly assigned to their original population than the proportion of male songs that were correctly assigned. Previous work on Eastern Whipbirds (*Psophodes olivaceus*) found that females exhibited greater geographic variation than males (Mennill & Rogers, 2006). Overall, males and females showed similar patterns, given that both background noise and geographic distance influenced acoustic variation for both sexes. These results indicate that similar evolutionary forces act on acoustic variation in Rufous-and-white Wrens. Given that female song is the ancestral trait in oscine birds (Odom *et al.*, 2014), further studies are necessary to examine female song at both local and geographic scales (Riebel *et al.*, 2005). Furthermore, including female song in future studies will help to provide greater insight into the evolution of acoustic signals (Price, 2015).

Conclusion

We examined the relationship between acoustic divergence and geographic distance, genetic distance, and ecological differences in male and female Rufous-and-white Wrens in Central America. Acoustic patterns were generally concordant between sexes, and our results indicate that cultural isolation and acoustic adaptation influence acoustic variation of male and female song. Although acoustic structure and population genetic structure exhibit some similar patterns, acoustic variation is not linked with genetic variation, further emphasizing the role that both cultural drift and selection play in the evolution of acoustic signals. Patterns were generally congruent for both sexes, although we did observe some differences. Between-sex differences may be indicative of different evolutionary forces acting on songs of males and females, but the increased variation more likely reflects that fewer female songs were analysed overall, as well as the absence of measured songs from Nicaragua. For example, the percentage of variation explained by habitat doubled for males when we analysed patterns without Nicaragua. Despite there being small differences in our analyses of four male populations vs. all five populations, patterns were generally congruent across both analyses. Few studies to date have examined female song, and our study highlights the challenges of studying female song; female Rufous-and-white Wrens sing fewer songs overall, less frequently, and more quietly than males, resulting in fewer recordings of high-quality songs from females than males (500 vs 1600 male songs in our data set, in spite of the same recording effort). Our study emphasizes the importance of studying female song, because studying male and female song patterns together may help to provide greater insight into the ecology and evolution of acoustic variation in tropical animals.

Acknowledgments

References

Azar, J.F., Bell, B.D. & Borowiec, M. 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.

Baker, M.C., Thompson, D.B., Sherma, G.L., Michael, A. & Tomback, D.F. 1982. Allozyme frequencies in a linear series of song dialect populations. *Evolution* **36**: 1020–1029.

Barker, N.K.S., Dabelsteen, T. & Mennill, D.J. 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.

Boncoraglio, G. & Saino, N. 2007. Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. *Funct. Ecol.* **21**: 134–142.

Boughman, J.W. 2002. How sensory drive can promote speciation. *Trends Ecol. Evol.* **17**: 571–577.

Bradbury, J.W. & Vehrencamp, S.L. 2011. *Principles of Animal Communication*. Sinauer Associates, Sunderland, MA.

Brar, R. K., Schoenle, L. A., Stenzler, L. M., Hall, M. L., Vehrencamp, S. L. & Lovette, I. J. 2007. Eleven microsatellite loci isolated from the banded wren (*Thryothorus pleurostictus*). *Mol. Ecol. Notes* **7**: 69–71.

Brumm, H. & Naguib, M. 2009. Environmental acoustics and the evolution of bird song. *Adv. Study Behav.* **40**: 1–33.

Burney, C.W. & Brumfield, R.T. 2009. Ecology predicts levels of genetic differentiation in neotropical birds. *Am. Nat.* **174**: 358–368.

Byers, B. E., Belinsky, K. L. & Bentley, R. A. 2010. Independent cultural evolution of two song traditions in the chestnut-sided warbler. *Am. Nat.* **176**: 476–489.

Campbell, P., Pasch, B., Pino, J.L., Crino, O.L., Phillips, M. & Phelps, S.M. 2010. Geographic variation in the songs of neotropical singing mice: testing the relative importance of drift and local adaptation. *Evolution* **64**: 1955–1972.

Cardoso, G.C. & Atwell, J.W. 2011. Directional cultural change by modification and replacement of memes. *Evolution* **65**: 295–300.

Caro, L.M., Caycedo-Rosales, P.C., Bowie, R.C.K., Slabbekoorn, H. & Cadena, C.D. 2013. Ecological speciation along an elevational gradient. *J. Evol. Biol.* **26**: 357–374.

Catchpole, C.K. & Slater, P.J. 2008. *Bird Song: Biological Themes and Variations*. University Press, Cambridge, UK.

Cavalli-Sforza, L. L. & Edwards, A. W. 1967. Phylogenetic analysis: models and estimation procedures. *Evolution* **21**: 550–570.

Claramunt, S., Derryberry, E.P., Remsen, J.V. & Brumfield, R.T. 2012. High dispersal ability inhibits speciation in a continental radiation of passerine birds. *Proc. Biol. Sci.* **279**: 1567–1574.

Clegg, S.M. & Phillimore, A.B. 2010. The influence of gene flow and drift on genetic and phenotypic divergence in two species of Zosterops in Vanuatu. *Philos. Tran. R. Soc. Lond. B. Biol. Sci.* **365**: 1077–1092.

Collins, S.A., de Kort, S.R., Pérez-Tris, J. & Tellería, J.L. 2009. Migration strategy and divergent sexual selection on bird song. *Proc. Biol. Sci.* **276**: 585–590.

Danner, J.E., Danner, R.M., Bonier, F., Martin, P.R., Small, T.W. & Moore, I.T. 2011. Female, but not male, tropical sparrows respond more strongly to the local song dialect: implications for population divergence. *Am. Nat.* **178**: 53–63.

Dingle, C., Halfwerk, W. & Slabbekoorn, H. 2008. Habitat-dependent song divergence at subspecies level in the grey-breasted wood-wren. *J. Evol. Biol.* **21**: 1079–1089.

Douglas, S. B., Heath, D. D. & Mennill, D. J. 2012. Low levels of extra-pair paternity in a neotropical duetting songbird, the Rufous-and-white Wren (*Thryothorus rufalbus*). *Condor* **114**: 393–400.

Drovetski, S.V., Zink, R.M., Rohwer, S., Fadeev, I.V., Nesterov, E.V., Karagodin, I. *et al.* 2004. Complex biogeographic history of a Holarctic passerine. *Proc. Biol. Sci.* **27**: 545–551.

Ellers, J. & Slabbekoorn, H. 2003. Song divergence and male dispersal among bird populations: a spatially explicit model testing the role of vocal learning. *Anim. Behav.* **65**: 671–681.

Evans, C. & Kleindorfer, S. 2016. Superb fairy-wren (*Malurus cyaneus*) sons and daughters acquire song elements of mothers and social fathers. *Front. Ecol. Evol.* **4**: 1–10.

Fischer, D. & Bachman, K. 1998. Microsatellite enrichment in organisms with large genomes (*Allium cepa* L.). *Biotechniques* **24**: 196–800.

Forrest, T.G. 1994. From sender to receiver: propagation and environmental effects on acoustic signals. *Am. Zool.* **34**: 644–654.

Garamszegi, L. Z., Pavlova, D. Z., Eens, M. & Moller, A. P. 2007. The evolution of song in female birds in Europe. *Behav. Ecol.* **18**: 86–96.

González, C., Ornelas, J. & Gutiérrez-Rodriguez, C. 2011. Selection and geographic isolation influence hummingbird speciation: genetic, acoustic and morphological divergence in the wedge-tailed sabrewing (*Campylopterus curvipennis*). *BMC Evol. Biol.* **11**: 38.

Goudet, J. 1995. F STAT version 1.2.: a computer program to calculate F-statistics. *J. Hered.* **86**: 485–486.

Graham, B.A. 2016. Cultural evolution in rufous-and-white wrens (Thryophilus rufalbus): insights from acoustic and genetic analyses. University of Windsor [PhD Thesis].

Graham, B.A., Sandoval, L., Dabelsteen, T. & Mennill, D.J. 2017a. A test of the acoustic adaptation hypothesis in three types of tropical forest: degradation of male and female Rufous-and-white Wren songs. *Bioaccoustics* **26**: 37–61.

Graham, B.A., Heath, D.D. & Mennill, D.J. 2017b. Dispersal influences genetic and acoustic spatial structure for both males and females in a tropical songbird. *Ecol. Evol.* **7**: 10089–10102.

Hackett, S.J. 1996. Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). *Mol. Phylogenet. Evol.* **5**: 368–382.

Handford, P. & Lougheed, S.C. 1991. Variation in duration and frequency characters in the song of the rufous-collared sparrow, *Zonotrichia capensis*, with respect to habitat, trill dialects and body size. *Evolution* **93**: 644–658.

Hanna, D., Blouin-Demers, G., Wilson, D.R. & Mennill, D.J. 2011. Anthropogenic noise affects song structure in red-winged blackbirds *Agelaius phoniceus*. *J. Exper. Biol.* **214**: 3549–3556.

Harris, A.J., Wilson, D.R., Graham, B.A. & Mennill, D.J. 2016. Estimating repertoire size in songbirds: a comparison of three techniques. *Bioacoustics* **25**: 211–224.

Hart, P.J., Hall, R., Ray, W., Beck, A. & Zook, J. 2015. Cicadas impact bird communication in a noisy tropical rainforest. *Behav. Ecol.* **26**: 839–842.

He, Q., Edwards, D.L. & Knowles, L.L. 2013. Integrative testing of how environments from the past to the present shape genetic structure across landscapes. *Evolution* **67**: 3386–3402.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* **25**: 1965–1978.

Hunter, M.L. & Krebs, J.R. 1979. Geographic variation in the song of the great tit Parus major in relation to ecological factors. *J. Anim. Ecol.* **48**: 759–785.

Irwin, D.E., Bensch, S. & Price, T.D. 2001. Speciation in a ring. *Nature* **409**: 333–337.

Isler, M.L., Isler, P.R. & Brumfield, R.T. 2005. Clinal variation in vocalizations of an antbird (Thamnophilidae) and implications for defining species limits. *Auk* **122**: 433–444.

Jones, G. 1997. Acoustic signals and speciation: the roles of natural and sexual signals in the evolution of cryptic species. *Adv. Study Behav.* **26**: 217–354.

Kenyon, H.L., Alcaide, M., Toews, D.P.L. & Irwin, D.E. 2017. Cultural isolation is greater than genetic isolation across an avian hybrid zone. *J. Evol. Biol.* **30**: 81–95.

Kozak, K.H., Graham, C.H. & Wiens, J.J. 2008. Integrating GIS-based environmental data into evolutionary biology. *Trends Ecol. Evol.* **23**: 141–148.

Langmore, N.E. 1998. Functions of duet and solo songs of female birds. *Trends Ecol. Evol.* **13**: 136–140.

Leader, N., Geffen, E., Mokady, O. & Yom-Tov, Y. 2008. Song dialects do not restrict gene flow in an urban population of the orange-tufted sunbird, *Nectarinia osea*. *Behav. Ecol. Sociobiol.* **62**: 1299–1305.

Legendre, P. & Fortin, M. 2010. Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. *Mol. Ecol. Res.* **10**: 831–844.

Legendre, P. & Legendre, L. 1998. *Numerical Ecology*. Elsevier, Amsterdam, the Netharlands.

Lemmon, E.M. 2009. Diversification of conspecific signals in sympatry: geographic overlap drives multidimensional reproductive character displacement in frogs. *Evolution* **63**: 1155–1170.

Librado, P. & Rozas, J. 2009. DNASP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451–1452.

Lin, A., Jiang, T., Kanwal, J. S., Lu, G., Luo, J., Wei, X. *et al.* 2015. Geographical variation in echolocation vocalizations of the Himalayan leaf-nosed bat: contribution of morphological variation and cultural drift. *Oikos* **124**: 364–371.

Logue, D.M. 2006. The duet code of female black-bellied Wren. *Condor* **108**: 326–335.

Logue, D.M. 2007. How do they duet? Sexually dimorphic behavioural mechanisms structure duet songs in the black-bellied wren. *Anim. Behav.* **73**: 105–113.

Luther, D.A. 2009. The influence of the acoustic community on songs of birds in a neotropical rain forest. *Behav. Ecol.* **20**: 864–871.

Luther, D.A. & Derryberry, E.P. 2012. Birdsong keeps pace with city life: changes in song over time in an urban songbird affects communication. *Anim. Behav.* **83**: 1059–1066.

Lynch, A. 1996. The population memetics of birdsong. In: *Ecology and Evolution of Acoustic Communication in Birds* (D. E. Kroodsma, E. H. Miller, eds), pp. 181–197. Cornell University Press, Ithaca, NY.

Lynch, A. & Baker, A.J. 1994. A population memetics approach to cultural evolution in chaffinch song : differentiation among populations. *Evolution* **48**: 351–359.

Macdougall-shackleton, E. A., Derryberry, E. P. & Hahn, T. P. 2002. Nonlocal male white-crowned sparrows have lower paternity and higher parasite loads than males singing local dialect. *Behav. Ecol.* **13**: 682–689.

MacDougall-Shackleton, E.A. & MacDougall-Shackleton, S.A. 2001. Cultural and genetic evolution in mountain white-crowned sparrows: song dialects are associated with population structure. *Evolution* **55**: 2568–2575.

Manel, S., Schwartz, M.K., Luikart, G. & Taberlet, P. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends Ecol. Evol.* **18**: 189–197.

Marshall-ball, L. & Slater, P. J. B. 2008. Repertoire sharing by the individual and the pair : insights into duet function and development in the plain wren *Thryothorus modestus*. *J. Avian Biol.* **39**: 293–299.

Martin, T.E. 2015. Age-related mortality explains life history strategies of tropical and temperate songbirds. *Science* **349**: 966–970.

Meirmans, P.G. & Van Tienderen, P.H. 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Mol. Ecol. Notes* **4**: 792–794.

Mennill, D. J., Battiston, M., Wilson, D. R., Foote, J. R. & Doucet, S. M. 2012. Field test of an affordable, portable, wireless microphone array for spatial monitoring of animal ecology and behaviour. *Methods Ecol. Evol.* **3**: 704–712.

Mennill, D.J. & Rogers, A.C. 2006. Whip it good! Geographic consistency in male songs and variability in female songs of the duetting eastern whipbird *Psophodes olivaceus*. *J. Avian Biol.* **37**: 93–100.

Mennill, D.J. & Vehrencamp, S.L. 2005. Sex differences in singing and duetting behavior of Neotropical rufous-and-white wrens *Thryothorus rufalbus*. *Auk* **122**: 175–186.

Mockford, E.J., Marshall, R.C. & Dabelsteen, T. 2011. Degradation of rural and urban great tit song: testing transmission efficiency. *PLoS ONE* **6**: e28242.

Moore, I.T., Bonier, F. & Wingfield, J.C. 2005. Reproductive asynchrony and population divergence between two tropical bird populations. *Behav. Ecol.* **16**: 755–762.

Morton, E.S. 1975. Ecological sources on section on song. *Am. Nat.* **109**: 17–34.

Nelson, D.A., Khanna, H. & Marler, P. 2001. Learning by instruction or selection : implications for patterns of geographic variation in bird song. *Behaviour* **138**: 1137–1160.

Odom, K.J., Hall, M.L., Riebel, K., Omland, K.E. & Langmore, N.E. 2014. Female song is widespread and ancestral in songbirds. *Nat. Commun.* **5**: 1–6.

Ortiz-Ramírez, M.F., Andersen, M.J., Zaldívar-Riverón, A., Ornelas, J.F. & Navarro-Sigüenza, A.G. 2016. Geographic isolation drives divergence of uncorrelated genetic and song variation in the Ruddy-capped Nightingale-Thrush (*Catharus frantzii*; Aves: Turdidae). *Mol. Phylogenet. Evol.* **94**: 74–86.

Peakall, R. & Smouse, P.E. 2012. GenAlEx 6.5: genetic analysis in excel. Population genetic software for teaching and research-an update. *Bioinformatics* **28**: 2537–2539.

Pilot, M., Jedrzejewski, W., Branicki, W., Sidorovich, V.E., Jedrzejewska, B., Stachura, K. *et al.* 2006. Ecological factors influence population genetic structure of European grey wolves. *Mol. Ecol.* **15**: 4533–4553.

Podos, J. & Warren, P.S. 2007. The evolution of geographic variation in birdsong. *Adv. Study Behav.* **37**: 403–458.

Potvin, D.A. & Clegg, S.M. 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 about the evolution of bird song and other sexually dimorphic signals. *Front. Ecol. Evol.* **3**: 1–6.

Primmer, C.R., Møller, A.P. & Ellegren, H. 1996. A wide-range survey of cross-species microsatellite amplification in birds. *Mol. Ecol.* **5**: 365–378.

Prohle, H., Koshy, R.A., Mueller, U., Rand, A.S. & Ryan, M.J. 2006. Geographic variation of genetic and behavioral traits in northern and southern túngara frogs. *Evolution* **60**: 1669–1679.

R Development Core team. 2014. *R: A Language and Environment for Statistical Computing*. R foundation for statistical computing, Vienna, Austria. URL http://www.R-project.org/

Raymond, M. & Rousset, F. 1995. GENEPOP version 1.2.: population genetics software for exact tests and ecumenicism. *J. Hered.* **86**: 248–249.

Ribot, R.F.H., Buchanan, K.L., Endler, J.A., Joseph, L., Bennet, A.T.D. & Berg, M.L. 2012. Learned vocal variation is associated with abrupt cryptic genetic change in a parrot species complex. *PlosOne* **7**: 1–9.

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223–225.

Riebel, K., Hall, M.L. & Langmore, N.E. 2005. Female songbirds still struggling to be heard. *Trends Ecol. Evol.* **20**: 419–420.

Ruegg, K., Slabbekoorn, H., Clegg, S. & Smith, T.B. 2006. Divergence in mating signals correlates with ecological variation in the migratory songbird, Swainson's thrush (*Catharus ustulatus*). *Mol. Ecol.* **15**: 3147–3156.

Sekercioğlu, C. H., Ehrlich, P. R., Daily, G. C., Aygen, D., Goehring, D. M. & Sandi, R. F. 2002. Disappearance of insectivorous birds from tropical forest fragments. *Proc. Natl. Acad. Sci. U. S. A.* **99**: 263–267.

Seutin, G., White, B.N. & Boag, P.T. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Can. J. Zool.* **69**: 82–90.

Slabbekoorn, H. 2004. Singing in the wild: the ecology of birdsong. In: *Nature's Music: The Science of Birdsong* (P. Marler & H. Slabbekoorn, eds), pp. 178–205. Elsevier Academic Press, New York, NY.

Slabbekoorn, H. & Peet, M. 2003. Ecology: birds sing at a higher pitch in urban noise. *Nature* **424**: 267.

Slabbekoorn, H. & Smith, T.B. 2002. Habitat dependent song divergence in the little greenbul: an analysis of environmental selection pressures on acoustic signals. *Evolution* **56**: 1849–1858.

Slater, P.J.B. & Mann, N.I. 2004. Why do the females of many bird species sing in the tropics? *J. Avian Biol.* **35**: 289–294.

Smith, B.T. & Klicka, J. 2010. The profound influence of the late Pliocene Panamanian uplift on the exchange, diversification, and distribution of new world birds. *Ecography* **33**: 333–342.

Soha, J.A., Nelson, D.A. & Parker, P.G. 2004. Genetic analysis of song dialect populations in Puget Sound white-crowned sparrows. *Behav. Ecol.* **15**: 636–646.

Sosa-López, J.R. & Mennill, D.J. 2014. Continent-wide patterns of divergence in acoustic and morphological traits in the house wren species complex. *Auk* **131**: 41–54.

Stotz, D.F., Fitzpatrick, J.A., Parker, T.A. III & Moskovits, D.K. 1996. *Neotropical Birds: Ecology and Conservation*. University of Chicago Press, Chicago, IL.

Stouffer, P.C. & Bierregaard, R.O., Jr. 1995. Use of Amazonian forest fragments by understory insectivorous birds. *Ecology* **76**: 2429–2445.

Stutchbury, B.J.M. & Morton, E.S. 2008. Recent advances in the behavioural ecology of tropical birds. *Wilson J. Ornithol.* **120**: 26–37.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24**: 1596–1599.

Templeton, C.N., Ríos-Chélan, A.A., Quirós-Guerrero, E., Mann, N.I. & Slater, P.J.B. 2013a. Female happy wrens select songs to cooperate with their mates rather than confront intruders. *Biol. Lett.* **9**: 20120863.

Templeton, C.N., Mann, N.I., Ríos-Chélan, A.A., Quirós-Guerrero, E., Macías-Garcia, C. & Slater, P.J.B. 2013b. An experimental study of duet integration in the happy wren, *Pheugopedius felix*. *Anim. Behav.* **86**: 821–827.

Tobias, J.A. & Seddon, N. 2009. Signal design and perception in hypocnemis antbirds: evidence for convergent evolution via social selection. *Evolution* **63**: 3168–3189.

Topp, S.M. & Mennill, D.J. 2008. Seasonal variation in the duetting behaviour of rufous-and-white wrens (*Thryothorus rufalbus*). *Behav. Ecol. Sociobiol.* **62**: 1107–1117.

Walter, R.P., Ovenden, J.R. & Heath, D.D. 2007. Characterization of four tetranucleotide and six dinucleotide microsatellite markers for use in the tropical freshwater fish *Telmatherina antoniae* and related species. *Mol. Ecol. Res.* **7**: 651–653.

Wang, I.J. & Summers, K. 2010. Genetic structure is correlated with phenotypic divergence rather than geographic isolation in the highly polymorphic strawberry poison-dart frog. *Mol. Ecol.* **19**: 447–458.

Weber, J.L. & Wong, C. 1993. Mutation of human short tandem repeats. *Hum. Mol. Gen.* **2**: 1123–1128.

Wilkins, M.R., Seddon, N. & Safran, R.J. 2013. Evolutionary divergence in acoustic signals: causes and consequences. *Trends Ecol. Evol.* **28**: 156–166.

Woltmann, S., Sherry, T.W. & Kreiser, B.R. 2012. A genetic approach to estimating natal dispersal distances and self-recruitment in resident rainforest birds. *J. Avian Biol.* **43**: 33–42.

Wright, T.F. & Wilkinson, G.S. 2001. Population genetic structure and vocal dialects in an amazon parrot. *Proc. Biol. Sci.* **268**: 609–616.

Wright, T.F., Rodriguez, A.M. & Fleischer, R.C. 2005. Vocal dialects, sex-biased dispersal, and microsatellite population structure in the parrot *Amazona auropalliata*. *Mol. Ecol.* **14**: 1197–1205.

Wright, T.F., Dahlin, C.R. & Salinas-Melgoza, A. 2008. Stability and change in vocal dialects of the yellow-naped amazon. *Anim. Behav.* **76**: 1017–1102.

Yáber, M.C. & Rabenold, K.N. 2002. Effects of sociality on short-distance, female-biased dispersal in tropical wrens. *J. Anim. Ecol.* **71**: 1042–1055.

Yoktan, K., Geffen, E., Ilany, A., Yom-Tov, Y., Naor, A. & Leader, N. 2011. Vocal dialect and genetic subdivisions along a geographic gradient in the orange-tufted sunbird. *Behav. Ecol. Sociobiol.* **65**: 1389–1402.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Table S1 Primer list and characteristics for the 10 microsatellite loci and 1 mtDNA gene used to genotype Rufous-and-white Wrens in this study; #alleles = total number of alleles for each locus, and T_A = Annealing temperature.

Table S2 Number of individuals (N) genotyped at mtDNA (ND2 gene) and 10 microsatellite loci.

Table S3 Correlation coefficients between the canonical axes (CA) arising from Discriminant Function Analyses and the spectro-temporal measurements of male and female Rufous-and-white Wren songs.

Table S4 Environmental variables and latitude and longitude by population.