Bioacoustics
iFirst article, 2012, 1–15

Songs of the Eastern Phoebe, a suboscine songbird, are individually distinctive but do not vary geographically

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(*Received 6 June 2012; revised 25 September 2012; final version received 1 October 2012*)

Animal displays may vary both within and among individuals and also within and among populations. This variation may contain important information used by animals for individual recognition. Suboscine birds are thought to develop song by fully innate mechanisms and are poorly studied relative to oscine birds, where song learning results in significant variation in song structure among individuals and the development of dialects. Recent research, however, demonstrates that suboscine song is often individually distinctive and in some cases shows signs of regional variation. We used spectrogram cross-correlation and canonical discriminant function analysis to examine individual and geographic variations in songs of Eastern Phoebes (*Sayornis phoebe*), suboscine birds with two song types. Both song types were individually distinctive and showed significantly higher cross-correlations within than among individuals. Discriminant function analysis correctly assigned 85.3% of “phee-bee” and 90.0% of “phee-b-be-bee” songs to the correct male, levels that are significantly higher than expected by chance. The individually distinctive characters of songs were also significantly repeatable among recording sessions. Eastern Phoebe song did not vary geographically between two populations separated by 640 km; permuted discriminant function analysis assigned 65% of “phee-bee” and 70% of “phee-b-be-bee” songs to the correct population, which did not differ significantly from chance expectations. Variation among males in song characteristics could be used by both males and females to discriminate among individuals. These detailed bioacoustic analyses support the idea that individual distinctive acoustic signals are widespread across suboscine birds.

Keywords: Eastern Phoebe; discriminant function analysis; geographic variation in song; individual distinctiveness; spectrogram cross-correlation; suboscine

Introduction

Variation in vocalizations and the importance of this variation for animals with vocal learning have been the focus of a large body of research (reviewed by Catchpole and Slater 2008; Bradbury and Vehrencamp 2011). Yet the function and importance of variation in the vocalizations of animals with stereotyped (and mostly innate) acoustic displays have received considerably less attention. There has been a recent surge of interest in variability of stereotyped or innate vocal displays at both individual and geographic levels (e.g. Wilczynski and Ryan 1999; Lovell and Lein 2004a; Saranathan et al. 2007; Fitzsimmons et al. 2008). These studies have led to the discovery that in many vocal

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ISSN 0952-4622 print/ISSN 2165-0586 online

http://dx.doi.org/10.1080/09524622.2012.740174
http://www.tandfonline.com

animals in diverse taxa, individuals show distinctive vocal features despite superficially similar sounding vocalizations and superficially similar appearance of spectrograms (e.g. May 1998; Bee et al. 2001; Burnett et al. 2001; Wiley 2005). Playback experiments have revealed that individually distinctive vocalizations are used by animals to discriminate among conspecifics (e.g. Bee and Gerhardt 2002; Lovell and Lein 2004b; Yovel et al. 2009). In addition, geographic variation in these vocal signals has also been demonstrated in some cases (e.g. Bretagnolle 1989; Wilczynski and Ryan 1999; Sedgwick 2001; Gillam and McCracken 2007).

The songbirds – order Passeriformes – are divided into two major suborders: Passeri, commonly referred to as the oscine songbirds and Tyranni, commonly referred to as the suboscine birds (Sibley and Alquist 1990; Barker et al. 2002). The oscine songbirds learn their songs from conspecifics (reviewed in Beecher and Brenowitz 2005), whereas the suboscines have innate songs and are generally understood to show no evidence of vocal learning (Kroodsma 1984, 1985, 1989). The oscine songbirds have been particularly well studied with respect to variation in song structure (reviewed in Falls 1982; Baker and Cunningham 1985) and communication strategies (reviewed in Todt and Naguib 2000). Until recently, suboscine song and communication behaviour has received little attention. The dawn chorus singing displays of suboscine Eastern Kingbirds (*Tyrannus tyrannus*) have recently been shown to contain information that may be used for mate choice (Dolan et al. 2007; Murphy et al. 2008). Studies of variability in the song structure of multiple suboscine birds reveal that songs are individually distinctive (e.g. Wiley 2005) and are used by both males (e.g. Lovell and Lein 2004b) and females (Seddon and Tobias 2010) to recognize conspecifics. In addition, geographic variation in song structure has been identified in several suboscine groups (Lindell 1998; Sedgwick 2001; Leger and Mountjoy 2003; Kroodsma 2005; Seddon and Tobias 2007; Fitzsimmons et al. 2008, 2011). Seasonal variation in song structure (e.g. Ríos-Chelén and Garcia 2007; Robertson et al. 2009) and variation in note composition of songs (e.g. Leger 2005) have also been observed in suboscines. Individual identification by song is important for conspecific communication in the complex communication networks in which most animals live (McGregor 2005). Describing variation in suboscine songs and determining its functional significance are an important first step to studying communication patterns in this group, and may lead to important discoveries about the evolution of vocal learning and communication behaviour.

We studied the songs of Eastern Phoebes (*Sayornis phoebe*) in two populations in Ontario, Canada, to investigate variation at both individual and geographic levels. Eastern Phoebes are small tyrant flycatchers that show no evidence of vocal learning when deprived of song tutors and raised in a laboratory from a young age (Kroodsma 1985) or when deafened and deprived of auditory feedback (Kroodsma and Konishi 1991). In addition, there is no evidence of imitation of song characters in tape-tutored juveniles (Kroodsma 1989). Eastern Phoebes sing two song forms referred to as the regularly repeated vocalization by Smith (1969). The first type sounds like “phee-bee” (sometimes identified as “RR1” or Type 1), whereas the second type sounds like “phee-b-be-bee” (sometimes identified as “RR2” or Type 2; Smith 1969). We use both spectrogram cross-correlation and discriminant function analysis from fine-scale measurements of songs to test for individual distinctiveness of songs both within and among recordings and to test for geographic variation among two populations separated by 640 km. Based on the mounting evidence that suboscine song shows more individual and geographic distinctiveness than previously recognized, we predicted that the songs of both forms would show variation at both population and individual levels.

Methods

Field recordings

We recorded the dawn chorus of male Eastern Phoebes using a Sennheiser MKH-70 microphone and a Marantz PMD-661 solid-state recorder in WAV format, at a 44.1 kHz, 16-bit sampling rate. Recordings were made at two sites. (1) From 30 April to 1 June 2010, 19 male Eastern Phoebes were recorded along Opinicon Lake Road, near Chaffey's Locks, Ontario (44°34′N, 76°19′W). This site is located approximately 50 km outside Kingston, Ontario, near Queen's University Biology Station (hereafter called population 1). Each male was recorded on a single morning. Eight of the males were recorded on an additional morning for comparison among recordings of the same male. The average delay between the first and second recordings for these eight males was 10.25 ± 0.79 days (range: 2–18). (2) From 31 May to 15 July 2011, 10 male Eastern Phoebes were recorded in Echo Bay, Ontario (46°31′N, 84°01′W). This site is located approximately 25 km outside Sault Ste Marie, ON (hereafter called population 2). Population 1 and population 2 were separated by a distance of 640 km. Recordings were collected using the same microphone and recorder and were operated by the same recordist JRF. Males were not marked for individual identification. Males were identified by location of song perch and knowledge of territory boundaries. All recordings were made during the dawn chorus when all territorial males were singing such that we could hear the males who were not being recorded and be certain that we recorded each male separately. We were confident that second recordings represented the same territorial male because second recordings were made from males singing from the same song perch and our field observations confirm that male Eastern Phoebes typically sing from the same song posts during dawn chorus bouts throughout the breeding season (based on observations of individually marked birds at dawn; J. Foote, unpublished isdata).

Song analysis

For each bird we selected the clearest 10 songs of each song type, hereafter called Type 1 songs ("phee-bee") and Type 2 songs ("phee-b-be-bee"). Songs were visualized in Syrinx PC (J. Burt, Seattle, WA, USA) and we selected songs with minimal overlapping by background noise (assessed visually from the spectrograms). The 20 songs of each male were extracted from the original field recording and pasted into individual WAV files which were identified by a random three digit number so that male identification or site was not apparent during measurement. Measurements were then conducted blind to individual and population identity.

We used two methods to classify songs at the individual and population levels. The first method of classification used was spectrogram cross-correlation, using Raven Pro v. 4.0 (Cornell Bioacoustics Lab, Ithaca, NY, USA). Spectrogram cross-correlation involves taking each song of each male and calculating the per cent of overlap with each song of all other males; songs that are very similar result in a high spectrogram cross-correlation score (values approaching 1). Before analysis, the songs were band-pass filtered to remove noise outside the frequency range of Eastern Phoebe song (Type 1: noise removed below 2000 Hz and above 6000 Hz; Type 2: noise removed below 2000 Hz and above 7000 Hz). For each song type for all of the 19 males recorded from population 1, we calculated the average cross-correlation score between each of his own songs (within male) and those of all the other 18 males (among males). We also calculated Type 1 × Type 2 cross-correlation scores both within males (between Type 1 and Type 2 songs of the same male) and among males (between Type 1 of one male and Type 2 of other males). For the eight males from population 1 that were recorded twice, we also calculated average cross-correlation values between songs from the first and second recordings. To compare between the two populations, the songs of 10 randomly selected recordings from population 1 were compared to the 10 recordings from population 2. Average cross-correlation values were calculated for each of these 20 males both with other males from the same population (within-population comparison) and males at the other population (among-population comparison). All spectrogram cross-correlations were calculated by comparing normalized spectrograms of band-pass filtered songs created with Hamming window, 512 fast fourier transform (FFT), and 87.5% overlap. All statistical comparisons of cross-correlation scores were non-parametric and unpaired because the same song entered into multiple comparisons when computing averages.

The second method of classification we used was canonical discriminant function analysis. First, fine-scale measurements of songs were taken from spectrograms viewed in Raven Pro v 4.0 using a Blackman window, FFT size 512, and a time grid overlap of 87.5%. For Type 1 and Type 2 songs, fine structural details measured are summarized in Figures 1 and 2, respectively. Frequency of maximum amplitude (FMA) values were measured using the spectrogram-slice function in Raven which gives a plot of relative amplitude versus frequency at a point in time. For both Type 1 and Type 2 songs, discriminant analyses were used to classify songs to test for (1) individual distinctiveness of songs (19 males from population 1); (2) repeatability of measurements from different recordings (8 males from population 1 recorded twice) and (3) geographic variation (10 males from population 1 and 10 males from population 2). To reduce the number of variables and to avoid problems with multi-collinearity of song features, the fine-scale song measurements (see Figures 1 and 2; Tables 1 and 2) of each song type were first subjected to a principal component analysis. All principal components with eigenvalues greater than 1.0 were used to construct the discriminant functions. (1) For the analysis of individual variation in song, jackknifed classifications were used to calculate classification

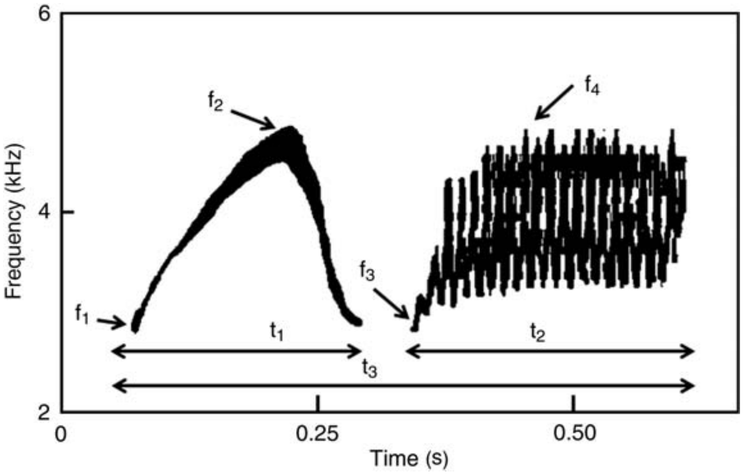

Figure 1. Sound spectrogram of an Eastern Phoebe Type 1 song showing fine structural details measured for discriminant function analysis. Measured details included length of *phee* (t_1), length of *bee* (t_2), length of song (t_3), FMA at the start of the *phee* (f_1), FMA at the peak frequency of the *phee* (f_2), bandwidth of *phee* (f_2–f_1), FMA at the start (f_3) and peak frequency of the *bee* (f_4), bandwidth of *bee* (f_4–f_3), number of modulations in the *bee* and modulation rate (number of modulations/t_2).

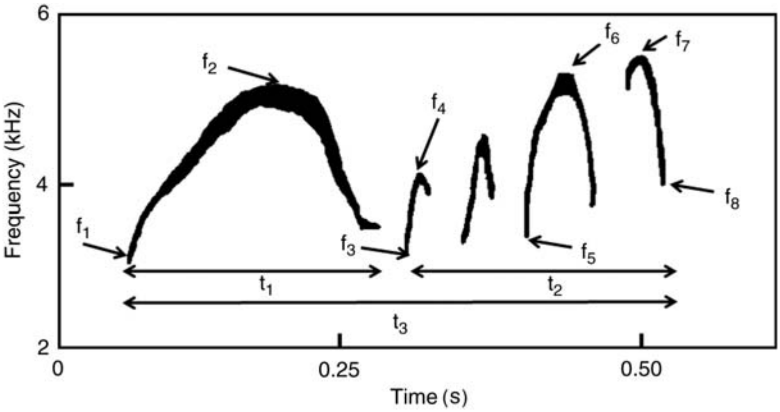

Figure 2. Sound spectrogram of an Eastern Phoebe Type 2 song showing fine structural details measured for discriminant function analysis. Measured details included length of *phee* (t_1), length of *b-be-bee* (t_2), length of song (t_3), FMA at the start of the *phee* (f_1), FMA at the peak frequency of the *phee* (f_2), bandwidth of *phee* (f_2–f_1), FMA at the start (f_3) and peak (f_4) of first introductory note of *b-be-bee* (note 1), FMA at the start (f_5) and peak frequency (f_6) of second-last note of *b-be-bee* (note 2), FMA at the peak frequency (f_7) and end (f_8) of last note of *b-be-bee* (note 3), bandwidth of the *b-be-bee* (maximum f_{3-8} − minimum f_{3-8}), number of notes in *b-be-bee*. Note: minimum number of notes in *b-be-bee* was three; all males shared one introductory note and the final two notes in this syllable so additional introductory notes were not included in the frequency measurements.

accuracy where each song was assigned to an individual or site using all the other songs in the data-set, except the song being classified. This analysis was conducted using SPSS v19 (IBM, Armonk, NY, USA). (2) For the discriminant function using two recordings of each individual, either the first or the second recording was randomly selected for each male and all 10 of the songs from the randomly selected recording were used to create the training function in JMP v10 (SAS Institute, Cary, NC, USA). The function was then applied to the songs of the second recording to estimate classification accuracy (as in Kirschel et al. 2011). (3) For the analysis of geographic variation, we used a permuted discriminant function analysis (pDFA; Mundry and Sommer 2007) to control for the pseudoreplication that can result when more than one song per individual is included in the analysis. To construct the pDFA, for both Type 1 and Type 2 songs, five principle components derived from the fine structural measurements were used (all components with eigenvalues > 1.0). These principal components explained 89.5% of the variation in Type 1 song and 77.9% of the variation in Type 2 song. The permutation-based discriminant function analyses were carried out using a script written by R. Mundry (MPI for Evolutionary Anthropology, Leipzig, Germany) in R (v2.15.1; R Development Core Team 2012) with the MASS library loaded (Venables and Ripley 2002). The pDFA used 20 subjects and five calls per subject to derive the discriminant function. Because we had 10 calls for every individual, in order to run the script with five calls per subject, we had to randomly select one individual and randomly select five or its songs to exclude from the analysis. Five (randomly selected) calls per subject were used for cross validation (total of 95 calls from 19 males). The random selection of calls to be cross-validated was repeated 100 times and the results were averaged. We ran a total of 1000 permutations for the analysis.

For our analysis of individual variation, we also calculated the potential for individual encoding (PIC) value (potential for individual coding; Vignal et al. 2004) for each fine-scale measurement following the methods of Robisson et al. (1993). We also assessed

Table 1. Mean ± SE of fine-scale song measurements for Type 1 songs from population 1 and population 2; coefficient of variation between males (CV_b) and for individual males ($CV_{i,\mathrm{mean}}$), PIC and results of ANOVA for all fine-scale song measurements of Type 1 songs from 19 males from population 1[a].

	Mean ± SE					
Variable	Population 1	Population 2	CV_b	$CV_{i,\mathrm{mean}}$	PIC	$F_{18,171}$[b]
Phee-length (s)	0.23 ± 0.007	0.24 ± 0.013	14.17	4.39	3.23	91.58
Bee-length (s)	0.26 ± 0.098	0.25 ± 0.011	16.95	4.94	3.43	109.32
Song length[c] (s)	0.53 ± 0.010	0.54 ± 0.018	8.54	3.02	2.82	73.17
FMA start *phee* (Hz)	2801 ± 22.1	2654 ± 51.4	3.52	2.31	1.52	18.10
FMA max *phee* (Hz)	4802 ± 80.4	4867 ± 91.5	7.48	2.26	3.31	76.76
FMA min *bee* (Hz)	3220 ± 50.2	2922 ± 62.8	6.97	3.53	1.97	27.03
FMA max *bee* (Hz)	4952 ± 50.5	5172 ± 67.5	4.55	1.63	2.79	67.27
Bandwidth *phee* (Hz)	2001 ± 73.1	2213 ± 71.1	16.33	6.59	2.48	54.42
Bandwidth *bee* (Hz)	1733 ± 66.8	2250 ± 42.3	17.23	8.26	2.09	35.43
Number of modulations	19.5 ± 0.71	18.9 ± 0.65	16.19	5.12	3.16	93.87
Modulation rate (mod/s)	0.013 ± 0.0001	0.013 ± 0.0002	5.85	3.14	1.86	26.12

Note: [a] Mean ± SE values for population 2 are for comparison, they were not used in the calculations of CV and PIC.
[b] All $p < 0.0001$.
[c] Does not equal *Phee*-length + *Bee*-length because of variation in presence and length of silent interval between “Phee” and “Bee” notes.

Table 2. Mean ± SE of fine-scale song measurements for Type 2 songs from population 1 and population 2; coefficient of variation between males (CV_{b}) and for individual males ($\mathrm{CV}_{i,\mathrm{mean}}$), PIC and results of ANOVA for all fine-scale song measurements of Type 1 songs from 19 males from population 1[a].

	Mean ± SE					
Variable	Population 1	Population 2	CV_{b}	$\mathrm{CV}_{i,\mathrm{mean}}$	PIC	$F_{18,171}$[b]
Phee-length (s)	0.24 ± 0.008	0.24 ± 0.013	14.61	6.01	2.43	48.50
B-be-bee length (s)	0.24 ± 0.006	0.25 ± 0.01	11.85	6.75	1.76	26.42
Song length[c] (s)	0.49 ± 0.011	0.50 ± 0.022	9.91	5.33	1.86	32.29
FMA start *phee* (Hz)	2840 ± 28.4	2818 ± 35.6	4.46	2.63	1.70	25.17
FMA max *phee* (Hz)	4774 ± 72.7	4657 ± 77.7	6.80	2.29	2.97	70.43
FMA start note 1 (Hz)	3036 ± 39.1	2978 ± 63.4	5.76	4.53	1.27	12.61
FMA max note 1 (Hz)	3734 ± 63.5	3688 ± 143.1	7.59	4.41	1.72	23.56
FMA start note 2 (Hz)	3074 ± 46.0	3064 ± 59.4	6.68	4.9	1.36	12.72
FMA max note 2 (Hz)	5390 ± 65.8	5255 ± 65.6	5.46	1.34	4.07	153.03
FMA max note 3 (Hz)	5207 ± 79.6	5149 ± 33.6	6.83	1.46	4.66	190.0
FMA end note 3 (Hz)	3462 ± 71.1	3321 ± 74.5	9.18	2.84	3.23	86.44
Bandwidth *phee* (Hz)	1933 ± 68.4	1839 ± 59.3	15.81	7.47	2.12	43.31
Bandwidth *b-be-bee* (Hz)	2566 ± 64.1	2409 ± 36.5	11.16	4.45	2.45	56.49
Number of notes in *b-be-bee*	3.88 ± 0.13	3.96 ± 0.22	15.14	7.92	1.91	25.36

Note: [a] Mean ± SE values for population 2 are for comparison, they were not used in the calculations of CV and PIC.
[b] All $p < 0.0001$.
[c] Does not equal *Phee*-length + *B-be-bee*-length because of variation in presence and length of silent interval between "Phee" and "B-be-bee" notes.

whether there was significantly more variation among than within individuals using ANOVA.

There are multiple methods for comparing sounds, each with strengths and limitations. To ensure our analysis was as rigorous as possible, we used multiple methods of comparing sounds between individuals and populations, as outlined above; multiple methods have also been used in previous investigations (e.g. Fitzsimmons et al. 2008; Lein 2008; Kirschel et al. 2009). All statistics were performed using JMP v10, SPSS v19 and R v2.15.1. Results were considered significant at $p < 0.05$. All data are reported as mean ± SE unless otherwise noted.

Results

Individual variation in song

We analysed a single recording session from each of 19 male Eastern Phoebes from population 1 using spectrogram-cross-correlation, discriminant function analysis, PIC and ANOVA. First, spectrogram cross-correlation revealed that Type 1 and Type 2 Eastern Phoebe songs were individually distinctive. For Type 1 songs, cross-correlation values of within males were significantly higher than among males (Wilcoxon test: $Z_{18} = 5.23$, $p < 0.0001$; within male: 49.9 ± 2.0% overlap; among male: 25.4 ± 1.0% overlap). For Type 2 songs, cross-correlation values of within male songs were also significantly greater than among male songs (Wilcoxon test: $Z_{18} = 5.02$, $p < 0.0001$; within male: 44.8 ± 1.7% overlap; among male: 25.9 ± 0.9% overlap). We also found that Type 1 × Type 2 cross-correlations reveal that the individually distinctive characteristics of songs hold true among song types. Type 1 × Type 2 correlations where both types were from the same male were significantly higher than were Type 1 × Type 2 cross-correlations where the two types were from different males (Wilcoxon test: $Z_{18} = 4.89$, $p < 0.0001$; within male: 43.3 ± 0.02% overlap; among male: 25.0 ± 0.01% overlap). Figure 3 shows the individual variation in Type 1 and Type 2 songs of six male Eastern Phoebes.

Discriminant function analysis corroborated these results, confirming that both Type 1 and Type 2 songs were individually distinctive. A discriminant analysis based on four principal components summarizing 79.8% of the variation in song structure was created for Type 1 song. We found that Type 1 songs were assigned to the correct male with 85.3% accuracy (162 of 190 songs assigned to the correct male), significantly greater than the

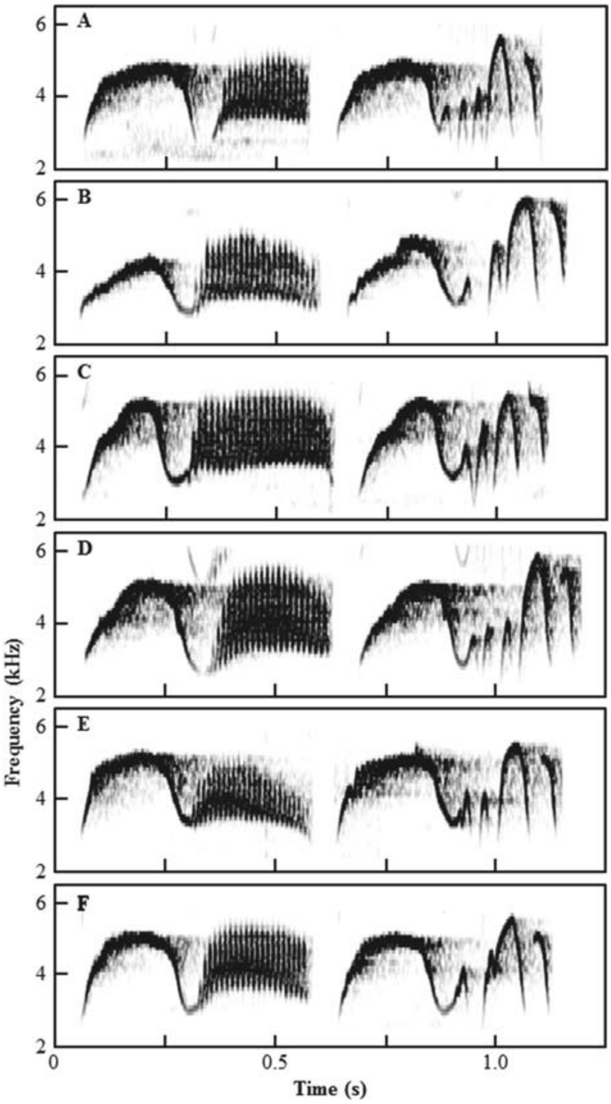

Figure 3. Song of six male Eastern Phoebes (A–F) from population 1 illustrating individual variation in Type 1 (first song in each line) and Type 2 (second song in each line) songs.

chance expectation of 5.3% (the chance probability of assigning the correct individual with $n = 19$ males; binomial test: $p < 0.0001$). A discriminant analysis based on five principal components summarizing 74.8% of the variation in song structure was created for Type 2 song. We found that 90.0% of Type 2 songs were assigned to the correct male (171 of 190 songs were assigned to the correct male), significantly greater than the chance expectation of 5.3% (binomial test: $p < 0.0001$).

All characters for both Type 1 and Type 2 songs showed PIC values (potential for individual coding) above 1.0 (Tables 1 and 2, respectively) indicating that they are more variables between than within individuals. PIC values of > 2.0 were found for 8/11 Type 1 song measurements and 7/14 Type 2 song measurements. In addition, ANOVA revealed that all measured song characteristics of both Type 1 and Type 2 songs were significantly more variable among than within males, a pattern which held true even after correction for multiple comparisons (all $p < 0.0045$ for Type 1 songs, α level for 11 comparisons, and $p < 0.0036$, for Type 2 songs, α value for 14 comparisons; Tables 1 and 2, respectively).

Individual consistency in song production

For eight males that were recorded on two separate occasions, Type 1 song spectrogram cross-correlation scores from the same male from the same recording (51.6 ± 2.7% overlap) and from different recordings (41.8 ± 2.5% overlap) did not differ significantly, while both same recording and different recording spectrogram cross-correlation scores differed significantly from spectrogram cross-correlation scores of those males with other males in the population (23.9 ± 1.8%; Kruskal–Wallis test: $F_{2,7} = 17.78$, $p < 0.001$; Dunn's multiple comparison post-test). Similarly, for Type 2 songs, cross-correlation scores of songs from the same male from the same recording (44.4 ± 1.8% overlap) and from different recordings (37.4 ± 1.9% overlap) did not differ significantly, while both same recording and different recording spectrogram cross-correlation scores differed significantly from spectrogram cross-correlation scores of those males with males in the population (25.9 ± 1.6% overlap; Kruskal–Wallis test: $F_{2,7} = 15.92$, $p < 0.0001$; Dunn's multiple comparison post-test).

For Type 1 songs, we carried out a discriminant analysis based on four principal components summarizing 85.2% of the variation in song structure of eight males that were recorded on two occasions. Songs were assigned to the correct male with 68.8% accuracy (55 of 80 songs classified correctly) based on the measurements of Type 1 songs, significantly higher than the 12.5% expected by chance (the chance probability of assigning the correct individual with $n = 8$ males; binomial test: $p < 0.0001$). For Type 2 song, we performed a discriminant analysis based on five principal components summarizing 82.3% of the variation in song structure. Type 2 songs were assigned to the correct male with 96.2% accuracy (77 of 80 songs classified correctly) significantly more than the 12.5% accuracy expected by chance (binomial test: $p < 0.0001$).

Geographic variation in song

Spectrogram cross-correlation revealed no evidence of geographic variation in song between the two sites. For Type 1 songs, within and among site cross-correlations did not differ significantly (Wilcoxon test: $Z_{19} = 1.18$, $p = 0.24$; within site: 35.1 ± 1.3% overlap; between site: 33.6 ± 1.2% overlap). For Type 2 songs, within and between site cross-correlations did not differ significantly (Wilcoxon test: $Z_{19} = 0.91$, $p = 0.36$; within site: 34.8 ± 1.0% overlap; among site: 33.0 ± 1.1% overlap).

There was no obvious differentiation between populations for Type 1 songs (pDFA: per cent correctly cross-classified calls $= 65.5\%$, $N = 95$ cross-classified calls, $p = 0.53$). The result for the calls selected to derive the discriminant function was slightly better but also did not reach significance (per cent correctly classified $= 67.3\%$, $N = 100$, $p = 0.52$). Similarly, there was no obvious differentiation between populations for Type 2 songs (pDFA: per cent correctly cross-classified calls $= 70.0\%$, $N = 95$ cross-classified calls, $p = 0.42$). The result for the calls selected to derive the discriminant function was similar and also did not reach significance (per cent correctly classified $= 70.2\%$, $N = 100$, $p = 0.463$).

Discussion

Rigorous bioacoustic analyses involving spectrogram cross-correlation, PIC analysis, analysis of variance and discriminant function analysis revealed that songs of Eastern Phoebes show significant evidence of individual distinctiveness. In addition, both spectrogram cross-correlation and discriminant function analysis reveal that songs of the same male from different recording sessions were more similar than were songs of different males, indicating that song features can be used for individual identification of Eastern Phoebes. pDFA and spectrogram cross-correlation revealed no evidence of significant geographic variation in song from two populations of Eastern Phoebes. Consequently, our results suggest that the songs of this suboscine species do not vary geographically in Ontario.

Our results demonstrate that both Type 1 and Type 2 Eastern Phoebe songs are individually distinctive. All four methods revealed significantly more variability among than within males for both song types. Kroodsma (1985, 1989) found that laboratory-raised Eastern Phoebes show individual variation in modulation rate, temporal characteristics of songs and the number of notes used in Type 2 songs. Our results expand on Kroodsma's (1985, 1989) findings by showing that wild birds also show individual differences in both frequency and temporal parameters of songs. Individually distinctive songs have also been demonstrated for other suboscine birds including Acadian Flycatchers (*Empidonax virescens*; Payne and Buddle 1979; Wiley 2005), Long-tailed Manakins (*Chiroxiphia linearis*; Trainer and McDonald 1995), Spotted Antbirds (*Hylophylax naevioides*, Bard et al. 2002), Alder Flycatchers (*E. alnorum*, Lovell and Lein 2004a), Buff-breasted Flycatchers (*E. fulvifrons*, Lein 2008), Screaming Pihas (*Lipaugus vociferans*, Fitzsimmons et al. 2008, 2011), Willow Flycatchers (*E. traillii*, Fernández-Juricic et al. 2009), Mexican Ant-thrushes (*Formicarius moniliger*, Kirschel et al. 2009, 2011) and Eastern Wood-Pewees (*Contopus virens*, Clark and Leung 2011). Taken together, these recent studies that encompass a broad range of suboscine birds suggest that individual distinctiveness is widespread in this group of non-learning birds.

Our analyses reliably discriminated between 19 individual males, a number which dramatically exceeds the number of neighbours a territory-holding Eastern Phoebe would encounter during a breeding season (maximum of four neighbours per male in our study populations). Therefore, it is likely that Eastern Phoebes could use this variation in song structure to recognize individual neighbours and mates and thus may be capable of individual recognition. Male Ochre-bellied Flycatchers (*Mionectes oleagineus*) and Alder Flycatchers discriminate between neighbours and strangers (neighbour–stranger discrimination experiments; Westcott 1997; Lovell and Lein 2004b, respectively) and Alder Flycatchers also demonstrate individual recognition of neighbours (Lovell and Lein 2005). Females of two Neotropical antbird species of the genus *Hypocnemis* (*H. peruviana* and *H. subflava*) can discriminate between songs of mates and strangers (Seddon and Tobias 2010). Mean PIC values from our study are intermediate between those of Alder Flycatchers and *Hypocnemis* antbirds (Lovell and Lein 2004a; Seddon and Tobias 2010, respectively), and thus female and male Eastern Phoebes may be capable of individual discrimination using song. Acadian Flycatchers, however, show marginal evidence of neighbour–stranger discrimination (Wiley 2005), whereas Mexican Ant-thrushes fail to discriminate among neighbours and strangers (Bard et al. 2002). More studies testing neighbour–stranger discrimination and individual recognition of neighbours in both male and female suboscines, including playback studies with Eastern Phoebes, are needed.

Average PIC values were higher for Type 1 songs, indicating that they have higher potential for individual recognition. There are only two other studies of individually distinctive songs in suboscines with multiple song types. First, Lein (2008) found similar PIC values for two types of songs of Buff-breasted Flycatchers. Second, Clark and Leung (2011) found higher PIC values for the ‘pee-ah-wee’ than the ‘wee-ooo’ songs of Eastern Wood-Pewees. Our analyses of Eastern Phoebe songs show that Type 2 songs were more reliable than Type 1 songs for individual identification and had higher classification accuracy with DFA for both the within and among recording analyses which may reflect the larger number of fine structural characteristics measured from Type 2 songs. For Eastern Phoebes, the highest PIC values for Type 2 songs were for two of the frequency variables while for Type 1 songs were for one temporal and one frequency variable. The highest PIC values of both Lein (2008) and Clark and Leung (2011) were also for frequency variables, suggesting that frequency components may be particularly important in individual discrimination throughout this group of birds.

Based on recordings of individuals sampled twice, we show that songs recorded in different sessions can be reliably assigned to the same male Eastern Phoebe. Songs from different recording sessions had cross-correlation scores that were lower but did not differ significantly from those from the same recording session, yet both same-session and different-session cross-correlation scores were significantly higher than cross-correlation scores from different males. This result indicates that although song structure may show minor variation from day to day, the individually distinctive characters persist in Eastern Phoebes at least within a breeding season. Type 2 songs were particularly consistent between recording sessions with classification accuracy of 96.2%, whereas Type 1 songs were somewhat less repeatable with a classification accuracy of 68.8%. In a similar analysis of oscine Black-capped Chickadees (*Poecile atricapillus*), songs from different recordings were also found to be more variable than those from the same recording session, and complementary playback experiments revealed that males demonstrate individual recognition based on these recordings (Wilson and Mennill 2010). Although they are oscine songbirds, Black-capped Chickadees sing a two-note song that shows little variation in structure throughout the species range (Kroodsma et al. 1999). In a comprehensive examination of among-recording classification, Kirschel et al. (2011) show that suboscine Mexican Ant-thrush songs have high classification accuracy from repeat recordings within 1 year, and also among years.

Although Eastern Phoebes show no evidence of vocal learning (Kroodsma 1985, 1989), the structural features of songs may vary between recording sessions due to variation in the singer’s vocal amplitude, the singer’s motivation or differences in vocal ontogeny; and songs may also vary due to external factors, such as changes in noise level, weather or features of the recording such as the distance to the bird (e.g. Morton 1977; Larom et al. 1997; Lengagne and Slater 2002; Araya-Ajoy et al. 2009; Francis et al. 2011). For example, male Ash-throated Flycatchers (*Myiarchus cinerascens*) singing in noisy environments have higher frequency songs than males singing in less noisy environments (Francis et al. 2011). Ocellated Antbirds (*Phaenostictus mcleannani*) increase the frequency of their songs during aggressive response to playback, demonstrating that suboscines can vary acoustic features with their motivational state (Araya-Ajoy et al. 2009). Flammulated Attilas (*Attila flammulatus*) change their songs during dawn singing bouts by varying the number of elements sung and the way they were combined in a way that suggests combinatorial song syntax (Leger 2005). Single recording sessions may underestimate the amount of variation that exists in songs and calls which may reduce between-session classification accuracy (reviewed in Ellis 2008). Nevertheless, our analysis of classification accuracy, together with similar results from Wilson and Mennill (2010) and Kirschel et al. (2011), show that between-session classification accuracy is closer to within-session classification accuracy than between-individual classification accuracy.

Our comparison of two populations separated by 640 km reveal no significant geographic variation in Eastern Phoebe songs in Ontario, Canada. Both pDFA and spectrogram cross-correlation revealed no significant evidence of geographic variation in either Type 1 or Type 2 song. Cross-validated classification accuracy of the discriminant function analysis of Type 1 song was 65.5% and Type 2 song was 70.0%, which was greater than 50% expected by chance, but not significantly different from chance expectation; future research with a larger sample sizes from more populations with different degrees of separation could help to clarify whether these classification accuracies do exceed statistical chance. Eastern Phoebes have a relatively large breeding range extending from Alberta in the west to Newfoundland in the east, and as far north as the Northwest Territories and as far south as Northern Texas (Weeks 2011). Kroodsma (1985) noted that songs from a small sample of Eastern Phoebes recorded in Ithaca, NY, did not appear to differ from those of hand-reared birds or wild recorded birds from Massachusetts, matching the pattern we report here.

Evidence of geographic variation in suboscine song is mixed. Similar to our findings, Buff-breasted Flycatcher songs do not vary between two mountain ranges separated by 100 km (Lein 2008), Long-tailed Manakin songs do not vary among three populations in Costa Rica separated by either 5 or 105 km (Trainer and Parsons 2001), and Acadian Flycatcher songs do not vary with distance over a 30 km area (Payne and Buddle 1979). In addition, in Acadian Flycatchers, songs of neighbours are not more similar to each other than to songs of more distant birds (Wiley 2005). Though we had greater than 50% assignment of songs to the correct population, our results did not represent significant geographic variation. Significant geographic variation has been documented, however, in suboscine Willow Flycatchers, where two subspecies *E. t. extimus* and *E. t. adastus* can be distinguished by song (Sedgwick 2001). At a smaller spatial scale, suboscine Screaming Piha songs show distinctive lek signatures (Fitzsimmons et al. 2008, 2011). In both Willow Flycatchers and Eastern Phoebes, songs appear structurally similar (Stein 1963; Kroodsma 1985, respectively) though fine-structural analysis reveals patterns of geographic variation in Willow Flycatchers (Sedgwick 2001) but not Eastern Phoebes (this study). Pale-breasted Spinetails (*Synallaxis albescens*) show weak geographic variation among three populations in Venezuela (Lindell 1998), with assignment values similar to those from our study. Bright-rumped Attilas (*Attila spadiceus*) demonstrate geographic variation in dawn song, but not daytime song, between Central and South American populations (although this variation may represent cryptic species; Leger and Mountjoy 2003). Chestnut-tailed Antbirds (*Myrmeciza hemimelaena*) also show variability among populations, and birds respond more intensely to playback of local versus distant songs from an isolated population (Seddon and Tobias 2007).

In conclusion, our study provides evidence of variation in the songs of Eastern Phoebes at the individual level but not at the geographic level. There is a growing body of evidence that suboscine songs are considerably more variable than previously understood. More work is needed in this group to determine the functional significance of this variation, the ontogeny of these vocal differences and the importance of this variation in the contexts of mate choice and territory defence.

Acknowledgements

We thank all the landowners in Chaffey's Locks and Echo Bay that allowed us to record birds on their property. We thank Lauren Fitzsimmons for statistical advice and Megan Lyon for song analysis assistance. We thank Roger Mundry for sharing his pDFA R script and instructions and for providing advice on data interpretation. Funding for this study was provided by the National Science and Engineering Research Council, the Algoma University Research Fund and the Canada Foundation for Innovation.

References

Araya-Ajoy Y-M, Chaves-Campos J, Kalko EKV, DeWoody JA. 2009. High-pitched notes during vocal contests signal genetic diversity in ocellated antbirds. PLoS ONE 4:e8137.

Baker MC, Cunningham MA. 1985. The biology of bird-song dialects. Behavioral and Brain Sciences 8:85–100.

Bard SC, Hau M, Wikelski M, Wingfield JC. 2002. Vocal distinctiveness and response to conspecific playback in the Spotted Antbird, a neotropical suboscine. Condor 104:387–394.

Barker FK, Barrowclough GF, Groth JG. 2002. A phylogenetic hypothesis for passerine birds: taxonomic and biogeographic implications of an analysis of nuclear DNA sequence data. Proceedings of the Royal Society of London, Series B 269:295–308.

Bee MA, Gerhardt HC. 2002. Individual voice recognition in a territorial frog (*Rana catesbeiana*). Proceedings of the Royal Society of London, Series B 269:1443–1448.

Bee MA, Kozich CE, Blackwell KJ, Gerhardt HC. 2001. Individual variation in advertisement calls of territorial male green frogs, *Rana clamitans*: implications for individual discrimination. Ethology 107:65–84.

Beecher M, Brenowitz E. 2005. Functional aspects of song learning in songbirds. Trends in Ecology and Evolution 20:143–149.

Bradbury JW, Vehrencamp SL. 2011. Principles of animal communication. 2nd ed. Sunderland (MA): Sinauer Associates.

Bretagnolle V. 1989. Calls of Wilson's storm petrel: functions, individual and sexual recognitions, and geographic variation. Behaviour 111:98–112.

Burnett SC, Kazial KA, Masters WM. 2001. Discriminating individual big brown bat (*Eptesicus fuscus*) sonar vocalizations in different recording situations. Bioacoustics 11:189–210.

Catchpole CK, Slater PJB. 2008. Bird song: biological themes and variations. 2nd ed. Cambridge: Cambridge University Press.

Clark JA, Leung J. 2011. Vocal distinctiveness and information coding in a suboscine with multiple song types: Eastern Wood-Pewee. Wilson Journal of Ornithology 123:835–840.

Dolan AC, Murphy MT, Redmond LJ, Sexton K, Duffield D. 2007. Extra-pair paternity and the opportunity for sexual selection in a socially monogamous passerine. Behavioral Ecology 18:985–993.

Ellis JMS. 2008. Decay of apparent individual distinctiveness in the begging calls of adult female White-throated Magpie Jays. Condor 110:648657.

Falls JB. 1982. Individual recognition by sounds in birds. In: Kroodsma D, Miller E, editors. Acoustic communication in birds. 2. New York: Academic Press.

Fernández-Juricic E, Del Nevo AJ, Poston R. 2009. Identification of individual and population-level variation in vocalizations of the endangered southwestern Willow Flycatcher (*Empidonax traillii extimus*). Auk 126:189–199.

Fitzsimmons LP, Barker NK, Mennill DJ. 2008. Individual variation and lek-based vocal distinctiveness in songs of the Screaming Piha (*Lipaugus vociferans*), a suboscine songbird. Auk 125:908–914.

Fitzsimmons LP, Barker NK, Mennill DJ. 2011. Further analysis supports the conclusion that the songs of Screaming Pihas are individually distinctive and bear a lek signature. Auk 128:790–792.

Francis CD, Ortega CP, Cruz A. 2011. Vocal frequency change reflects different responses to anthropogenic noise in two suboscine tyrant flycatchers. Proceedings of the Royal Society of London, Series B 278:2025–2031.

Gillam EH, McCracken GF. 2007. Variability in the echolocation of *Tadarida brasiliensis*: effects of geography and local acoustic environment. Animal Behaviour 74:277–286.

Kirschel ANG, Earl DA, Yao Y, Escobar I, Vilches E, Vallejo EE, Taylor CE. 2009. Using songs to identify individual Mexican Antthrush (*Formicarius moniliger*): a comparison of four classification methods. Bioacoustics 19:1–20.

Kirschel ANG, Cody ML, Harlow ZT, Promponas VJ, Vallejo EE, Taylor CE. 2011. Territorial dynamics of Mexican Ant-thrushes *Formicarius moniliger* revealed by individual recognition of their songs. Ibis 153:255–268.

Kroodsma DE. 1984. Songs of the Alder Flycatcher (*Empidonax alnorum*) and Willow Flycatcher (*Empidonax traillii*) are innate. Auk 101:13–24.

Kroodsma DE. 1985. Development and use of two song forms by the Eastern Phoebe. Wilson Bulletin 97:21–29.

Kroodsma DE. 1989. Male Eastern phoebes (*Sayornis phoebe*; Tyrannidae, Passeriformes) fail to imitate songs. Journal of Comparative Psychology 103:227–232.

Kroodsma DE. 2005. The singing life of birds: the art and science of listening to birdsong. New York: Houghton Mifflin.

Kroodsma DE, Konishi M. 1991. A suboscine bird (Eastern phoebe, *Sayornis phoebe*) develops normal song without auditory feedback. Animal Behaviour 42:477–487.

Kroodsma DE, Byers BE, Halkin SL, Hill C, Minis D, Bolsinger JR, Dawson J-A, Donelan E, Farrington J, Gill FB. et al., 1999. Geographic variation in Black-capped Chickadee songs and singing behavior. Auk 116:387–402.

Larom D, Garstang M, Payne K, Raspet R, Lindeque M. 1997. The influence of surface atmospheric conditions on the range and area reached by animal vocalizations. Journal of Experimental Biology 200:421–431.

Leger DW. 2005. First documentation of combinatorial song syntax in a suboscine passerine species. Condor 107:765–774.

Leger DW, Mountjoy DJ. 2003. Geographic variation in song of the Bright-rumped Attila (Tyrannidae: *Attila spacdiceus*): implications for species status. Auk 120:69–74.

Lein MR. 2008. Song variation in Buff-breasted Flycatchers (*Empidonax fulvifrons*). Wilson Journal of Ornithology 120:256–267.

Lengagne T, Slater PJB. 2002. The effects of rain on acoustic communication: tawny owls have good reason for calling less in wet weather. Proceedings of the Royal Society of London, Series B 269:2121–2125.

Lindell C. 1998. Limited geographic variation in the vocalizations of a neotropical Furnariid, *Synallaxis albescens*. Wilson Bulletin 110:368–374.

Lovell SF, Lein MR. 2004a. Song variation in a population of Alder Flycatchers. Journal of Field Ornithology 75:146–151.

Lovell SF, Lein M. 2004b. Neighbor-stranger discrimination by song in a suboscine bird, the alder flycatcher, *Empidonax alnorum*. Behavioral Ecology 15:799–804.

Lovell SF, Lein MR. 2005. Individual recognition of neighbors by song in a suboscine bird, the alder flycatcher *Empidonax alnorum*. Behavioral Ecology and Sociobiology 57:623–630.

May L. 1998. Individually distinctive Corncrake *Crex crex* calls: a further study. Bioacoustics 9:135–148.

McGregor PK. 2005. Animal communication networks. Cambridge: Cambridge University Press.

Morton ES. 1977. On the occurrence and significance of motivation-structural rules in some bird and mammal sounds. American Naturalist 111:855–869.

Mundry R, Sommer C. 2007. Discriminant function analysis with nonindependent data: consequences and alternatives. Animal Behaviour 74:965–976.

Murphy MT, Sexton K, Dolan AC, Redmond LJ. 2008. Dawn song of the Eastern kingbird: an honest signal of male quality? Animal Behaviour 75:1075–1084.

Payne RB, Buddle P. 1979. Song differences and map distances in a population of Acadian flycatchers. Wilson Bulletin 91:29–41.

R Development Core Team. 2012. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Ríos-Chelén AA, Garcia CM. 2007. Responses of a sub-oscine bird during playback: effects of different song variants and breeding period. Behavioural Processes 74:319–325.

Robertson BA, Fontaine JJ, Loomis E. 2009. Seasonal patterns of song structure variation in a suboscine passerine. Wilson Journal of Ornithology 121:815–818.

Robisson P, Aubin T, Bremond J-C. 1993. Individuality in the voice of the emperor penguin *Aptenodytes forsteri*: adaptation to a noisy environment. Ethology 94:279–290.

Saranathan V, Hamilton D, Powell GVN, Kroodsma DE, Prum RO. 2007. Genetic evidence supports song learning in the three-wattled bellbird *Procnias tricarunculata* (Cotingidae). Molecular Ecology 16:3689–3702.

Seddon N, Tobias JA. 2007. Song divergence at the edge of Amazonia: an empirical test of the peripatric speciation model. Biological Journal of the Linnean Society 90:173–188.

Seddon N, Tobias JA. 2010. Character displacement from the receiver's perspective: species and mate recognition despite convergent signals in suboscine birds. Proceedings of the Royal Society of London, Series B 277:2475–2483.

Sedgwick JA. 2001. Geographic variation in the song of Willow Flycatchers: differentiation between *Empidonax traillii adastus* and *E.t. extimus*. Auk 118:366–379.

Sibley CG, Alquist JE. 1990. Phylogeny and classification of birds. New Haven (CT): Yale University Press.

Smith WJ. 1969. Displays of *Sayornis phoebe* (Aves, Tyrannidae). Behaviour 33:283–322.

Stein RC. 1963. Isolating mechanisms between populations of Traill's Flycatcher. Proceedings of the American Philosophical Society 107:21–50.

Todt D, Naguib M. 2000. Vocal interactions in birds: the use of song as a model in communication. Advances in the Study of Behavior 29:247–296.

Trainer JM, McDonald DB. 1995. Singing performance, frequency matching and courtship success of long-tailed manakins (*Chiroxiphia linearis*). Behavioral Ecology and Sociobiology 37:249–254.

Trainer JM, Parsons RJ. 2001. Uniformity of Long-tailed Manakin songs from three localities in Costa Rica. Wilson Bulletin 113:431–434.

Venables WN, Ripley BD. 2002. Modern applied statistics with S. 4th ed. New York: Springer.

Vignal C, Mathevon N, Mottin S. 2004. Audience drives male songbird response to partner's voice. Nature 430:448–451.

Weeks Jr. HP. 2011. Eastern phoebe (*Sayornis phoebe*). In: Poole A, editor. The birds of North America Online. Ithaca (NY): Cornell Lab of Ornithology.

Westcott DA. 1997. Neighbours, strangers and male-male aggression as a determinant of lek size. Behavioral Ecology and Sociobiology 40:235–242.

Wilczynski W, Ryan MJ. 1999. Geographic variation in animal communication systems. In: Foster SA, Endler JA, editors. Geographic variation in behavior. Oxford: Oxford University Press.

Wiley RH. 2005. Individuality in songs of Acadian flycatchers and recognition of neighbours. Animal Behaviour 70:237–247.

Wilson DR, Mennill DJ. 2010. Black-capped chickadees, *Poecile atricapillus*, use individually distinctive songs to discriminate among conspecifics. Animal Behaviour 79:1267–1275.

Yovel Y, Melcon ML, Franz MO, Denzinger A, Shnitzler H-U. 2009. The voice of bats: how greater mouse-eared bats recognize individuals based on their echolocation calls. PLoS Computational Biology 5:e1000400.