Individual acoustic differences in female black-capped chickadee (*Poecile atricapillus*) *fee-bee* songs

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Running title: Individual differences in female songbird song

ABSTRACT

In songbirds, song has traditionally been considered a vocalization mainly produced by males. However, recent research suggests that both sexes produce song. While the function and structure of male black-capped chickadee (*Poecile atricapillus*) *fee-bee* song have been well-studied, research on female song is comparatively limited. Past discrimination and playback studies have shown that male black-capped chickadees can discriminate between individual males via their *fee-bee* songs. Recently, we have shown that male and female black-capped chickadees can identify individual females via their *fee-bee* song even when presented with only the *bee* position of the song. Our results using discriminant function analyses (DFA) support that female songs are individually distinctive. We found that songs could be correctly classified to the individual (81%) and season (97%) based on several acoustic features including but not limited to *bee*-note duration and *fee*-note peak frequency. In addition, an artificial neural network (ANN) was trained to identify individuals based on the selected DFA acoustic features and was able to achieve 90% accuracy by individual and 93% by season. While this study provides a quantitative description of the acoustic structure of female song, the perception and function of female song in this species requires further investigation.

Keywords: Black-capped chickadee; communication; female song; song; songbirds

I. INTRODUCTION

Within oscines (the true songbirds), songs are traditionally considered a sexually selected signal, produced primarily by males, and serving two main functions, territorial defense and mate attraction (Catchpole and Slater, 2008). Prior studies examining songbird vocalizations suggest that females lack song (Langmore, 1998; Riebel, 2003). Nonetheless, there is an increasing number of studies of female song in songbirds, further supporting the argument that females do produce song and that their song serves a function (Langmore, 1998, Riebel, 2003). A review of songbird species (Odom, Hall, Riebel, Omland, & Langmore, 2014), reported that female song is present in 71% of the reviewed 323 species and unknown in the remaining 29% of songbird species. Recent studies have shown that female black-capped chickadees (*Poecile atricapillus*) also sing, however the function of female song in this species is currently unknown (Hahn, Krysler, & Sturdy, 2013; Montenegro et al., 2020).

The black-capped chickadee *fee-bee* song is a two-note vocalization that is primarily used for territorial defense and mate attraction and traditionally thought to only be used by males (Ficken et al., 1978; Smith 1991). However, there are several reports of females singing songs that are acoustically similar to male *fee-bee* songs (i.e., songs are tonal and contain two notes) in the laboratory (Hahn et al., 2013b) and field (Dwight 1987; Hill & Lein 1987). As in male black-capped chickadees, the first note in the female song (*fee*-note) is produced at a higher frequency than the second note (*bee*-note) and the frequency of the *fee*-note decreases over the duration of the note (referred to as the *fee* glissando; Weisman, Ratcliffe, Johnsrude, & Hurly, 1990; Hahn et al., 2013b). A bioacoustic analysis of several acoustic features showed that the *fee* glissando is less pronounced in males than it is in females (Hahn et al., 2013b). A follow up operant go/no-go discrimination task suggested that black-capped chickadees are able to identify the sex of an individual using the *fee* glissando within their *fee-bee* song (Hahn et al., 2015). In addition, female song production is more variable acoustically, with inter-note intervals ranging from 1.5-8.0s, while male song is produced more regularly, with inter-note intervals running from 2.5-5.0s (Kobrina, Hahn, Mercado, Sturdy, 2019).

Being able to determine the sex of an individual via song, and the ability to identify individuals via song, is advantageous in distinguishing among conspecifics to discriminate mate from non-mate, and among flockmates. In several species, discriminating between individuals via acoustic signals has been shown to facilitate identification of a familiar conspecific (e.g., Song Sparrow (*Melospiza melodia*); Stoddard et al., 1990) or a mate (e.g., great tits (*Parus major*); Lind, Dabelsteen, & McGregor, 1996). A recent study has suggested that the *fee-bee* song in the black-capped chickadee may be used for mate recognition (Hahn et al., 2013b), and in order to be used for mate recognition, the *fee-bee* song would need to contain information concerning individual identity. Previous studies have indicated that male black-capped chickadee song contains information regarding individual identity (Phillmore et al. 2002; Christie et al., 2004a; Hoeschele et al. 2010, Wilson & Mennill 2010; Hahn et al., 2015). A previous study examining *fee-bee* songs suggests that the total duration and the interval ratio is used to identify individual males (Christie et al. 2004a). In addition, males and female chickadees eavesdrop on male singing contests and use song to identify successful and unsuccessful conspecifics and their quality (Mennill, Ratcliffe, & Boag, 2002; Christie et al. 2004b; Mennill & Ratcliffe 2004).

Prior operant go/no-go discrimination tasks (Phillmore et al. 2002) and playback studies (Wilson & Mennill 2010) have also indicated that male black-capped chickadees can discriminate between individual males via their *fee-bee* songs. And a recent operant task showed that male and female chickadees can discriminate between females via their *fee-bee* songs

(Montenegro et al., 2020). A bioacoustic analysis of male *fee-bee* songs indicates that songs are more distinct and variable between individuals rather than within individuals, with song length, *fee-note* duration, and the *fee* glissando being the most variable features (Wilson & Mennill, 2010). Furthermore, during playback of the above analyzed song, wild chickadees remained within their testing area and sang significantly longer in response to *fee-bee* songs from different recorded males compared songs from the same recorded individual male, further suggesting the ability to discriminate between individuals based on song (Wilson & Mennill, 2010). To date, the particular acoustic differences between individual female *fee-bee* songs is unknown.

Here we measured 13 acoustic features in female black-capped chickadee *fee-bee* songs, including frequency and duration measurements, to investigate which acoustic features in song might be used to identify individual females. We completed a bioacoustic analysis analyzing these 13 acoustic features using both discriminant function analyses and artificial neural networks to determine if the acoustic features measured could be used to identify the individual producing a specific song. Previous research has shown male and female black-capped chickadees can identify individual females via their *fee-bee* song even when presented with only the *bee* position of the song (Montenegro et al., 2020). Therefore, we predicted that the source of acoustic differences between female black-capped chickadee song would most likely be found in the *bee* note portion of their *fee-bee* songs.

II. METHODS

A. Subjects

We used *fee-bee* songs from six females (Female A-Female F) used in a previous study focused on individual identification of female chickadees (Montenegro et al., 2020). Sex was determined by DNA analysis of blood samples (Griffiths et al. 1998). Birds were captured in

Edmonton (North Saskatchewan River Valley, 53.53°N, 113.53°W; Mill Creek Ravine, 53.52°N, 113.47°W), Alberta, Canada, in January 2010-2014. All birds were at least one year of age at capture, verified by examining outer tail rectrices (Pyle 1997). All birds were individually housed in parakeet cages (30 × 40 × 40 cm; Rolf C. Hagen, Montreal, Quebec, Canada) in colony rooms. Birds had visual and auditory, but not physical, contact with each other. Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St. Louis, Missouri, USA), water with vitamins supplemented on alternating days (Prime Vitamin Supplement; Rolf C. Hagen), grit, and a cuttlebone. Additional nutritional supplements included 3–5 sunflower seeds daily, one superworm (*Zophabas morio*) 3 times a week, and a mixture of hard-boiled eggs and greens (spinach or parsley) twice a week. The colony rooms were maintained at ~20°C and on a light:dark cycle that followed the natural light cycle for Edmonton, Alberta, Canada.

B. Recordings of acoustic stimuli

Of the six birds, four were recorded in Spring 2012 (Female A, B, E, F) and two birds were recorded in Fall 2014 (Female C, D). A recording session for an individual bird lasted ~1 hr and all recordings took place at 0815 hours after colony lights turned on at 0800 hours. Birds were recorded individually in their colony room cages, which were placed in sound-attenuating chambers (1.7m × 0.84 m × 0.58 m; Industrial Acoustics, Bronx, New York, USA). Recordings were made using an AKG C 1000S (AKG Acoustics, Vienna, Austria) microphone connected to a Marantz PMD670 (Marantz America, Mahwah, New Jersey, USA) digital recorder (16-bit, 44,100 Hz sampling rate). The microphone was positioned 0.1 m above and slightly behind the cage. Following a recording session, audio files were analyzed and cut into individual files using SIGNAL 5.03.11 software (Engineering Design, Berkley, California, USA).

C. Acoustic measures

Each female provided 24 *fee-bee* songs, amounting to 144 *fee-bee* songs in total. Song composition was visually determined from spectrograms in SIGNAL (version 5.05.02, Engineering Design, Belmont, MA) by a single individual (CM) using Ficken et al. (1978) as a reference. All vocalizations were of high quality (i.e., no audible interference) and were bandpass filtered (lower bandpass: 500 Hz, upper bandpass: 14,000 Hz) using GoldWave 6.31(GoldWave, St. John's, Newfoundland, Canada) to reduce any background noise. For each stimulus, 5 ms of silence was added to the leading and trailing portion of the vocalization to standardize duration. Individual songs were then saved as separate (.WAV) files.

For each song we measured 13 acoustic features examined previously in studies of identification in chickadee song (Christie et al., 2004a; Hahn et al., 2013a; Hahn et al., 2013b Hoeschele et al., 2010; Otter & Ratcliffe, 1993) and calls (Campbell et al., 2016; Guillette et al., 2010). Measurements included: (1) total duration of song, (2) *fee*-note duration, (3) the proportion of song duration occupied by the *fee*-note (*fee*-note duration divided by the total duration of the song), (4) *bee*-note duration, (5) the proportion of song duration occupied by the bee-note (bee-note duration divided by the total duration of the song), (6) *fee*-note start frequency, (7) *fee*-note peak frequency, (8) *fee*-note end frequency, (9) *fee* glissando (decrease in frequency across the duration of the fee-note, calculated by dividing the start frequency of the *fee*-note by the end frequency of the *fee*-note), (10) *bee*-note start frequency, (11) *bee*-note peak frequency, (12) *bee*-note end frequency, (13) the internote interval between the notes (calculated by subtracting the *fee*- and *bee*-note duration from total song duration). The above acoustic features were measured from sound spectrograms using SIGNAL. Sound spectrograms of a *fee-bee* song were used for all duration (time resolution 5.8 ms) measurements and frequency

(frequency resolution 172.3Hz) measurements. See Figure 1 for how the acoustic features were measured.

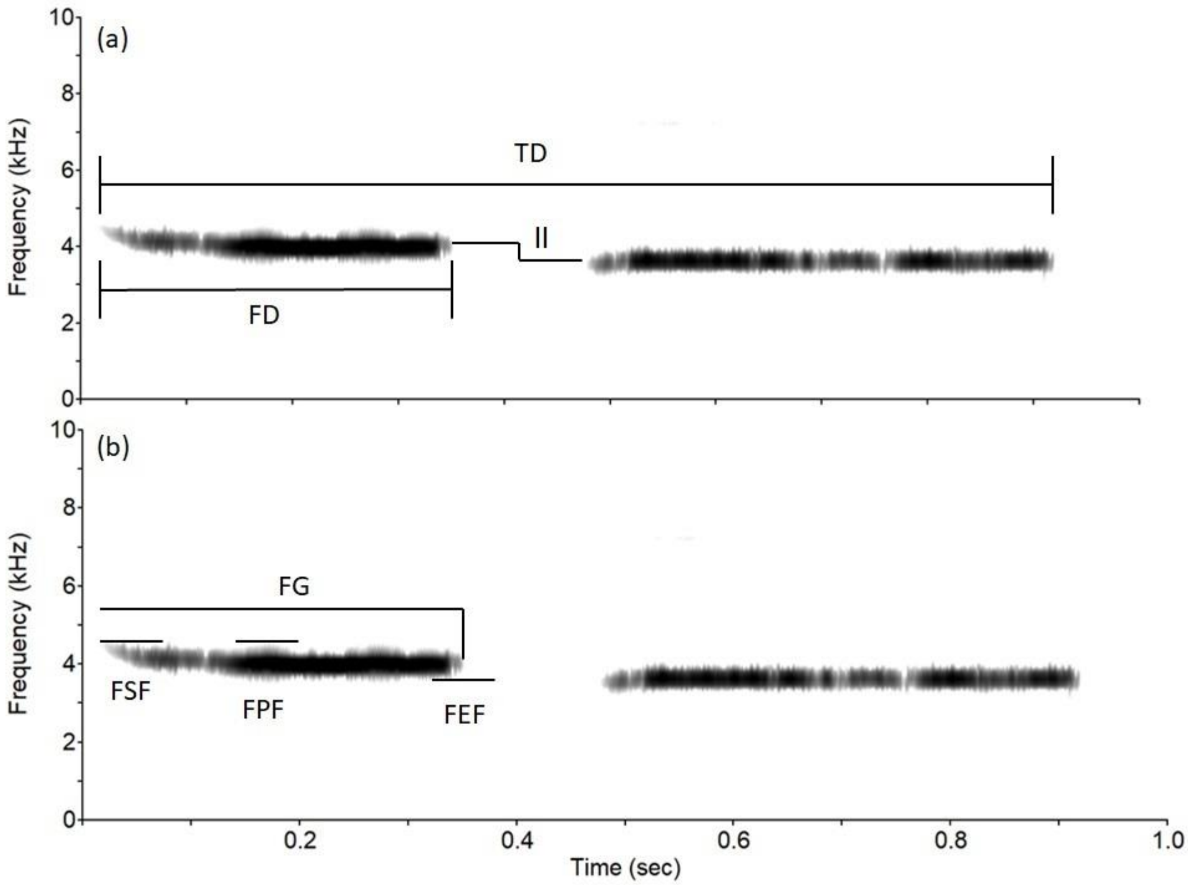

Figure 1. Sound spectrogram depicting acoustic measurements performed in *fee-bee* songs. All measurements depicted for *fee*-notes were measured similarly for *bee*-note measurements. (a) Sound spectrogram (time resolution 5.8 ms) of a *fee-bee* song. Measurements shown: total duration of song (TD) and fee-note duration (FD). (b) Sound spectrogram (frequency resolution 172.3Hz) of the same *fee-bee* song. Measurements shown: *fee* glissando (ratio of frequency decrease within fee-note) (FG), internote interval (II) (frequency ratio between the notes), *fee* start frequency (FSF), *fee* peak frequency (FPF), *fee* end frequency (FEF).

A total of 144 songs was analyzed (24 songs from six female black-capped chickadees). Table I shows the mean, standard deviation, coefficients of variation between individuals (CV_b), coefficients of variation within an individual (CV_w), and potential for individual coding value (PIC) for all acoustic features measured across each female. We calculated the coefficients of variation between individuals (CV_b) using the following formula:

$$CV_b = \left(\frac{SD}{MEAN}\right) \times 100$$

here the SD is the standard deviation and mean is the average for the total sample, and we calculated the coefficient of variation within an individual (CV_w) using the formula:

$$CV_w = \left(\frac{SD}{MEAN}\right) \times 100$$

here the SD and mean are calculated from each individual's songs (Sokal and Rohlf, 1995; Charrier et al., 2004; Hahn et al., 2013b; Campbell et al., 2016). For each acoustic feature, the PIC value is the ratio CV_b/mean CV_w, where mean CV_w is the average CV_w calculated for all individuals (Charrier et al., 2004; Hahn et al., 2013b; Campbell et al., 2016). If we observe a PIC value greater than 1, then that particular acoustic feature may be used for individual identification.

D. Statistical analysis

Discriminant function analysis (DFA) is commonly used in bioacoustic research to discriminate the vocalizations of groups or individuals based on specific acoustic features and can also suggest which features are used for identification via classification (Mundry & Sommer, 2007). If the acoustic features previously measured in the *fee-bee* songs vary among individuals, then a DFA can use the features to accurately classify the songs to each individual (Tabachnick & Fidell, 2007). Thus, we used a stepwise DFA and the leave-one-out method of cross-validation where one case is withheld at a time and the discriminant function is derived from the remaining cases. Then using the discriminant function that was derived, the withheld case is then classified. These steps are repeated until all cases have been classified in this manner (Betz, 1987). We report the cross-validated percentage of correct classifications, the standardized coefficients, and eigenvalues for the discriminant functions derived from our analyses. Cross-validation can provide an estimate for how well the derived discriminant function can predict group membership with a new sample. The standardized coefficients express the relative importance of each variable to the discriminant score. A greater contribution is associated with a standardized coefficient with a larger magnitude. In addition, as the standardized coefficient's magnitude increases it represents a closer relationship between the variable and the discriminant function (Klecka, 1980). We also report Cohen's *Kappa*; this index was calculated in order to assess if the model's performance differed from expectations based on chance (Titus, Mosher, & Williams, 1984). Following the DFA, we conducted a corresponding repeated measures multivariate analysis of variance (MANOVA) using the acoustic features to compare songs produced by each individual for significant differences. All statistical analyses were conducted using SPSS (Version 20, Chicago, SPSS Inc.).

Artificial neural networks are widely used in bioacoustic research to identify species-specific signals and to identify specific individuals within a species by determining the distinct features within a vocalization (Parsons & Jones, 2000; Mcgregor, 2002; Pozzi, Gamba, & Giacoma, 2008; Hahn et al., 2013a). The networks used in the current study used similar settings as those described in Nickerson et al. (2006), Guillette et al. (2010), and Hahn et al. (2015). We trained the network using the Rosenblatt program (Dawson, 2004), and each network had an input unit for each acoustic feature which was connected to one of six output units. Each of the input units corresponded to one acoustic feature within the *fee-bee* song. The output units used a sigmoid-shaped logistic equation to transform the sum of the weighted signals from each input into an activity value that ranged between 0 and 1. The learning rate was set at 0.5, and we continued training until the output unit produced a 'hit' (defined as an activity level of 0.9 or higher when the correct response was to turn 'on' (i.e., correct bird), or an activity level of 0.1 or lower when the correct response was to turn 'off' (i.e., incorrect bird)). Prior to training, the connection weights for each network were set to a random weight between −0.1 and 0.1, so each network served as one 'subject'.

III. RESULTS

A. Acoustic analysis

A correlation matrix showed that *fee* start frequency and *fee* peak frequency ($r(144) = 0.934$, $p < 0.001$), and *bee* start frequency and *bee* peak frequency ($r(144) = 0.897$, $p < 0.001$) are highly correlated. In addition, the *fee* proportion of the total song length was highly correlated to the *bee* proportion of the total song length ($r(144) = -0.875$, $p = < 0.001$). Thus, the acoustic features of *fee* start frequency, *bee* start frequency, *fee* proportion were removed from further DFA and MANOVA analyses, leaving 10 acoustic features.

Results for the coefficients of variation between individuals (CV_b) suggests that the duration measurements (total and individual note duration, CV_bs > 38.32) of female song were more variable compared to the frequency measurements (peak and end frequencies for both notes & *fee* glissando, CV_bs > 5.46). Also more variable than frequency measurements was the *bee*-note proportion measurement ($CV_b = 23.15$) and internote interval measurement ($CV_b = 28.08$).

The potential for individual coding (PIC) value provides a comparison of the variation between and within the individual female birds by each acoustic feature measured. All 10 acoustic features had PIC greater than 1.0, indicating that they may contain cues of individual identification and aid in classification of songs to individual females. Duration measurements for individual *fee-bee* song had the greatest PIC (*bee*-note duration, PIC = 2.54; *fee*-note duration, PIC = 2.05; total duration, PIC = 1.91) and are most likely to contribute to differences in individual female song. The proportion of song duration occupied by the *bee*-note had a high PIC value (PIC = 1.61). In addition, all frequency measurements (with the exception of the *fee* glissando) followed the above duration measurements in terms of PIC (PICs > 1.35), and also alternated notes (in order of PIC; *fee*-note & *bee*-note peak frequency; *fee*-note & *bee*-note end frequency). The two features which had the lower PIC values included the internote interval (PIC = 1.28) and the *fee* glissando (PIC = 1.26). However, we should note that any feature with a PIC over 1.00 cannot be ruled out as contributing to the differences between individuals. See Table I for all PIC values by acoustic feature.

B. DFA, MANOVA, & ANN (by individual)

The stepwise DFA used to classify songs based on the individual female producing the song used 10 measured acoustic features. In total, one stepwise analysis with six steps was performed. Stepwise analysis showed that *bee*-note duration, *fee*-note peak frequency, *bee*-note proportion, *fee*-note end frequency, internote interval, and *bee*-note peak frequency can be used to classify 80.55% of songs by the individual female based on cross-validated classifications. The overall Cohen's *Kappa* coefficient was high (0.81), which indicates good model performance. See Table II for predicted group membership distributions by DFA and ANN. See Table III for Wilks' lambdas, F statistics, and p values for all acoustic features. See Table IV for standardized coefficients, eigenvalues, percentage of variance, and canonical correlations for the discriminant functions.

Results from the repeated measures MANOVA revealed significant differences between all six female chickadees based on the measured acoustic features, ($F_{(45, 584)} = 23.797$, $p < 0.001$, partial $\eta^2 = 0.606$). While the vocalizations of these females were significantly different, the repeated measures MANOVA cannot determine which acoustic features cause these differences. See Table V for significant differences between individual females. See Figure 2 for centroid plots for all females.

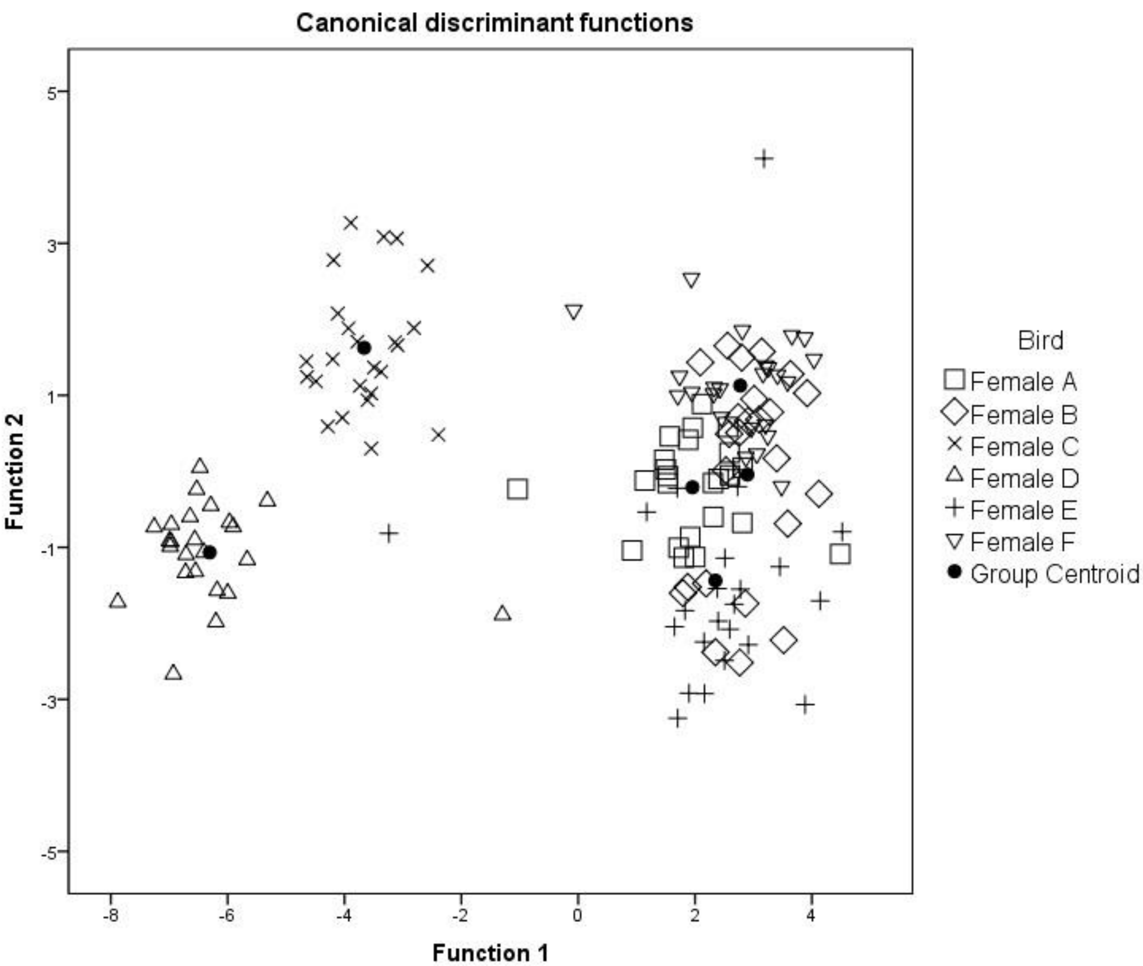

Figure 2. Centroid plot for all females showing the distribution of each song in relation to all songs. Each female, A-F has each of their classified songs plotted, remaining. Circles denote the group centroid for each bird.

For the ANN, pilot testing with female song stimuli indicated that the network never learned to classify the 144 songs to the six individual females based on the 10 measured acoustic stimuli with 100% accuracy, therefore we could not use perfect performance as the criterion to stop training. As a result, we stopped training the network after 30,000 training sweeps, which was approximately the number of sweeps that the artificial neural network reached its maximum number of hits ($\overline{x}$=783). Since each of the six female chickadees contributed 24 songs, there were 864 total measurements that could be used to identify one female. The 30,000 sweeps showed that 783 individual measurements were correctly classified (90% accuracy). See Table II for predicted group membership distributions.

C. DFA, MANOVA, & ANN (by season)

While our analysis of acoustic stimuli by the individual was highly accurate, results also showed a strong difference between the songs of the four individual females recorded in the Spring and the two individual females recorded in the Fall. Thus, we performed a separate DFA, MANOVA, and complimentary ANN, for the vocalizations sorted by season (i.e., Fall vs. Spring based on the measured acoustic features).

The stepwise DFA used to classify songs based on season of female-produced song (Fall vs. Spring) used the identical 10 measured acoustic features as the above analysis by individual. In total, one stepwise analysis with three steps was performed. Stepwise analysis showed that *bee*-note duration, *bee*-note peak frequency, and *fee* glissando can be used to classify 97.15% of songs by the season they were produced based on cross-validated classifications. Our overall Cohen's *Kappa* showed high accuracy (0.96), indicating good model performance. See Table II for predicted group membership distributions by DFA and ANN. See Table III for Wilks' lambdas, *F* statistics, and *p* values for all acoustic features. See Table IV for standardized coefficients, eigenvalues, percentage of variance, and canonical correlations for the discriminant functions.

Results from the MANOVA revealed significant differences between Fall and Spring songs based on the measured acoustic features ($F_{(9, 134)} = 133.595$, $p < 0.001$, partial $\eta^2 = 0.900$). While the songs of these females by season were significantly different, the repeated measures MANOVA cannot determine which acoustic features cause these differences. See Table V for significant differences between seasons.

For the ANN, we stopped training the network after 40,000 training sweeps, which was approximately the number of sweeps that the network reached its maximum number of hits ($\overline{x}$=268). As each of the six female chickadees contributed 24 songs by season, there were 288 total measurements that could be attributed to one season. The 40,000 sweeps showed that 268 individual measurements were correctly classified (93%). See Table II for predicted group membership distributions.

IV. DISCUSSION

Overall, using discriminant function analyses and artificial neural networks we were able to classify individual female-produced *fee-bee* songs to a high degree of accuracy; although some female birds showed overlap and we also observed an impact of season. The analyses identified many acoustic features which differed significantly between individuals. Several acoustic features including *bee*-note measurements and the *fee* glissando (for season only), were found to be in-line with previous research on individual identification in male and female black-capped chickadees.

While a previous study (Montenegro et al., 2020) found that the *bee*-note half of the female *fee-bee* song is more important for individual identification, the results of the DFA showed that = acoustic features of the *fee*-note and the *bee*- were most accurate at classifying the individual female singer. Specifically, *bee*-note duration, *fee*-note peak frequency, *bee*-note proportion, *fee*-note end frequency, internote interval, and *bee*-note peak frequency could be used to classify individual females. Results indicated that while *bee*-note measurements (*bee*-note duration) were most important in classifying song, *fee*-note frequency measurements were also important. The artificial neural networks (ANN) were used to confirm correct and incorrect classification of songs identified by the DFA. Both methods of classification, DFAs and ANNs, did find a degree of overlap between the songs of Female B and Female F and showed the highest number of errors when classifying Female B and Female F.

The MANOVA results showed significant differences between individual females based on acoustic features identified by the DFA, again including acoustic features of both *fee*- and *bee*-notes. Tukey's *post-hoc* analysis and centroid plots revealed that not all the six identified acoustic features were significantly different between the females. Some females overlapped more with other females and some overlapped less with other females, and not all acoustic features were significantly different between individuals, suggesting individual differences in acoustic features between the female birds. For example, *bee*-note duration was significantly different between Female C and all other birds, (p = 0.001), but *bee*-note duration for Female A was only significantly different from Female C and D (p = 0.001). The centroid plot (Figure 2) shows the overlap between each song from each bird. Female C and Female D are shown as clusters separate from each other and from all other birds; comparatively, Female, A, B, E, F are closely clustered together. These two birds are distinct from the rest of the four birds, thus, the DFA and ANN were able to classify songs produced by Female C (DFA, 95.8%; ANN, 96%) and Female D (DFA, 100%; ANN, 97%) to highest degree of accuracy.

Female C and D were recorded in a different year and season (Fall 2014) than the rest of the females (Spring 2012). While the previous operant study using these vocalizations showed no difference in response or ability to discriminate based on year of recording or season (Montenegro et al., 2020), we ran a separate DFA, MANOVA, and ANN in order to investigate identification via season of female-produced song. The DFA showed that *bee*-note duration, *bee*-note peak frequency, and the *fee* glissando were the most important features in classifying individuals by Fall vs. Spring and could be used to classify female song to a high degree (97.15%). However, the MANOVA showed significant differences between *bee*-note duration and *fee* glissando but not the *bee*-note peak frequency. The ANN was able to confirm that our female-produced *fee-bee* songs could be classified to a high degree (93%). Interestingly, the *fee* glissando in chickadee song has previously been associated with sex discrimination (Hahn et al., 2015) and all songs in this prior study were previously recorded in the Spring. A prior acoustic analysis has also suggested that male- and female-produced songs do differ by season (Campbell, Thunburg, & Sturdy, 2019). Perhaps the difference in the *fee* glissando in female song that we observe in the current study mirrors the biological functions of male song (i.e., mate attraction, territory defense, solicitation of extrapair copulations), which are more profound in the spring, the black-capped chickadee breeding season (Avey, Quince, & Sturdy, 2007). Notably, a previous study on seasonal plasticity in chickadees and other songbirds used auditory evoked potentials to find that there are seasonal changes in the auditory processing systems of chickadees, and that these changes match the acoustic properties of songs during and outside of the breeding season (Vélez, Gall, & Lucas, 2015). That said, there appears to be no difference in the song system based on season and the *fee-bee* song (Smulders et al., 2006). Overall, while these possible functions compliment the current proposed function of female song (Langmore,

1998) and past literature on song and season, we must still consider that male and female songs do differ in form and function in this species.

The DFA results suggest that there are features within the latter half of the *fee-bee* song that signal the identity of the singer as well as features that match prior studies on male identification. A previous study found that female and male chickadees were able to identify individual females when listening to only the *bee*-note portion of their respective *fee-bee* songs. When discriminating between *fee*-note portions, the chickadees were no longer able to perform the discrimination (Montenegro et al., 2020), thus supporting the acoustic features identified by the DFA. In addition, it has been previously shown that the internote interval is used by chickadees when discriminating between individual males via their song (Christie et al., 2004a). Internote interval was also identified by the current DFA and ANN as being an important feature in classifying female songs by individual. While the *fee* glissando was only significant when classifying female song by season, some *fee*-note acoustic features such as peak frequency, and end frequency were identified as significantly different among females. In addition, a past study has shown that female and male black-capped chickadees show no difference in frequency sensitivity, specifically that female and male chickadees exhibit the greatest sensitivity to frequencies between 2 and 4 kHz, as evidenced by auditory evoked potentials (Wong & Gall, 2015). These evoked potential results show that not only is the auditory system of both sexes sensitive in the frequency region of fee-bee song, but also suggest that song is important to both sexes. Considering we found parallels between female and male individual identification via song, perhaps the functions of song are similar in both sexes. Or perhaps the features that the current DFA selected for classification of individuals is evidence of overall voice recognition simply because many black-capped chickadee vocalizations lend themselves to individual identification. Prior research has shown that black-capped chickadees can identify individual chickadees by their *chick-a-dee* calls (Mammen & Nowicki, 1981, Charrier & Sturdy, 2005) and possibly by *tseet* calls (Guillette, Bloomfield, Batty, Dawson, & Sturdy 2010).

Collectively, our findings suggest the classification of female black-capped chickadees via female-produced *fee-bee* song is not note dependent or season dependent. While *bee*-note features were identified as significantly different between females and previously shown to be important to chickadees when discrimination between females, our analyses suggests that some *fee*-note features were also involved in recognition, and these differences in acoustic features differ between seasons. Further studies manipulating acoustic features of female *fee-bee* songs can aid in further determining which features are most important for individual identification and how they may work together. In addition, further exploring female song use can also benefit identifying which acoustic features are used in discriminating individuals. Depending on how females use song, whether for territorial defense, mate attraction, or another function entirely, the way in which black-capped chickadees interpret the song may differ and thus the important acoustic features may differ.

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TABLES

Table I. Summary of acoustic features measured including means, SDs, coefficient of variation between individuals, coefficient of variation within individuals, and potential for individual coding for all acoustic features measured across each female black-capped chickadee.

Bird	Value	Total duration (ms)	*Fee*-note duration (ms)	*Bee*-note duration (ms)	*Bee*-note proportion (%)	*Fee*-note peak (Hz)	*Fee*-note end (Hz)	*Fee* glissando	*Bee*-note peak (Hz)	*Bee*-note end (Hz)	Internote interval (ms)
All birds	**Mean**	895	399	392	43	4352	3432	1.24	3813	3117	104
	SD	343	272	157	10	331	187	0.09	365	405	29
	$CV_{between}$	38.32	68.09	40.16	23.15	7.60	5.46	7.53	9.58	12.98	28.08
	PIC	1.91	2.05	2.54	1.61	1.57	1.38	1.26	1.55	1.35	1.28
Female A Spring	**Mean**	1004	432	469	47	4312	3571	1.18	3946	3306	99
	SD	73	56	43	4	116	90	0.06	55	89	19
	CV_{within}	7.23	12.93	9.28	8.54	2.69	2.52	4.85	1.39	2.98	18.93
Female B Spring	**Mean**	1032	389	511	50	4347	3446	1.23	3741	3089	132
	SD	103	84	24	4	272	201	0.07	123	114	18
	CV_{within}	9.96	21.60	4.69	8.31	6.27	5.84	5.79	3.29	3.69	13.61
Female C Fall	**Mean**	857	546	224	29	4594	3511	1.27	3767	3050	90
	SD	613	604	54	9	229	98	0.08	113	679	27
	CV_{within}	71.47	111.05	24.13	31.28	4.98	2.79	6.48	21.57	22.25	29.72
Female D Fall	**Mean**	411	183	146	35	3985	3253	1.64	3970	3262	82
	SD	68	56	47	7	99	113	0.07	76	110	34
	CV_{within}	16.49	30.62	32.42	19.91	2.49	3.46	5.95	1.91	3.37	41.02
Female E Spring	**Mean**	1082	462	495	46	4175	3292	1.25	3646	2972	125
	SD	95	47	74	5	374	229	0.10	213	638	25
	CV_{within}	8.80	10.08	14.98	10.29	8.95	6.95	7.81	5.84	21.44	19.93
Female F Spring	**Mean**	986	382	507	51	4701	3516	1.34	3808	3021	97
	SD	60	49	48	4	169	79	0.07	117	123	9

	CV_{within}	6.08	12.87	9.43	7.86	3.59	2.25	4.96	3.08	4.07	8.82

Table II. Predicted group membership percentages by individual (A) and by season (B). First line includes cross-validated percentages by DFA. Second line includes predicted group membership percentages by ANN results for comparison.

A

	Predicted group membership by individual					
Bird & Season	**Female A**	**Female B**	**Female C**	**Female D**	**Female E**	**Female F**
Female A **Spring**	83.3 92.0	4.2 3.0	4.2 1.0	0.0 0.0	0.0 0.0	8.3 3.0
Female B **Spring**	4.2 5.0	54.2 79.0	0.0 0.0	0.0 1.0	4.2. 14.0	37.5 3.0
Female C **Fall**	0.0 2.0	0.0 0.0	95.8 97.0	0.0 1.0	4.2 0.0	37.5 0.0
Female D **Fall**	0.0 1.0	0.0 1.0	0.0 5.0	100.0 94.0	0.0 0.0	0.0 0.0
Female E **Spring**	8.3 3.0	0.0 1.0	4.2 5.0	0.0 0.0	87.5 90.0	0.0 1.0
Female F **Spring**	8.3 5.0	20.8 19.0	4.2 4.0	0.0 0.0	4.2 2.0	62.5 70.0

B

	Predicted group membership by season	
Bird	Fall	Spring
Fall	96.6 93.0	3.1 7.0
Spring	2.1 1.0	97.9 99.0

Table III. Acoustic features that are used in the analysis at each step by DFA results showing relative importance of each feature in discriminating between individual female chickadees via their *fee-bee* song (A) and in discrimination between season via female *fee-bee* song (B).

A

Step	Variable	Wilk's lambda	*F* statistic	Significance
1	*Bee*-note duration	0.100	248.854	< 0.001
2	*Fee-note* peak frequency	0.049	96.506	< 0.001
3	*Bee*-note (proportion)	0.27	67.642	< 0.001
4	*Fee*-note end frequency	0.018	52.465	< 0.001
5	Internote interval	0.015	42.117	< 0.001
6	*Bee*-note peak frequency	0.012	36.319	< 0.020

B

Step	Variable	Wilk's lambda	*F* statistic	Significance
1	*Bee*-note duration	0.128	969.814	< 0.001
2	*Bee*-note peak frequency	0.121	512.093	< 0.001
3	*Fee* glissando	0.114	362.079	< 0.001

Table IV. Reported values for the five discriminant functions via individual female bird (A), including standardized coefficients, eigenvalues, percentage of variance, and canonical correlations, and for the one discriminant function via season (B).

A

	Function				
Standardized coefficients	**1**	**2**	**3**	**4**	**5**
Bee-note duration	1.31	0.24	-0.25	-0.37	-0.47
Bee-note (proportion)	-0.40	-0.72	0.84	0.68	0.57
Fee-note peak	0.04	0.75	-0.02	0.80	-0.02
Fee-note end	0.44	0.22	0.26	-0.78	0.60
Bee-note peak	-0.47	0.17	0.20	-0.44	-0.30
Internote interval	0.15	-0.35	-0.55	0.24	0.77
Eigenvalue	14.25	1.39	0.53	0.39	0.11
% of variance	85.5	8.3	3.1	2.4	0.6
Canonical correlation	0.969	0.762	0.587	0.531	0.309

B

	Function
Standardized coefficients	**1**
Bee-note duration	-0.27
Bee-note peak frequency	1.07
Fee glissando	-0.264
Eigenvalue	7.759
% of variance	100.0
Canonical correlation	0.941

Table V. Repeated measures MANOVAs reported mean differences and significance by individual female chickadee (A) and by season (B) based on acoustic features.

A

Comparison		Mean difference	Significance
Female A	Female B	45.446	0.106
	Female C	50.828	0.071
	Female D	185.134	< 0.001*
	Female E	12.465	0.656
	Female F	89.324	0.002*
Female B	Female C	5.382	0.848
	Female D	139.688	< 0.001*
	Female E	-32.981	0.240
	Female F	43.878	0.119
Female C	Female D	134.306	< 0.001*
	Female E	-38.363	0.172
	Female F	38.496	0.171
Female D	Female E	-172.669	< 0.001*
	Female F	-95.810	0.001*
Female E	Female F	76.859	0.007*

B

Comparison		Mean difference	Significance
Fall	Spring	81.172	< 0.001