

An investigation of sex differences in acoustic features in black-capped chickadee (*Poecile atricapillus*) chick-a-dee calls

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(Received 2 March 2016; revised 10 August 2016; accepted 12 August 2016; published online 9 September 2016)

Sex differences have been identified in a number of black-capped chickadee vocalizations and in the *chick-a-dee* calls of other chickadee species [i.e., Carolina chickadees (*Poecile carolinensis*)]. In the current study, 12 acoustic features in black-capped chickadee *chick-a-dee* calls were investigated, including both frequency and duration measurements. Using permuted discriminant function analyses, these features were examined to determine if any features could be used to identify the sex of the caller. Only one note type (A notes) classified male and female calls at levels approaching significance. In particular, a permuted discriminant function analysis revealed that the start frequency of A notes best allowed for categorization between the sexes compared to any other acoustic parameter. This finding is consistent with previous research on Carolina chickadee *chick-a-dee* calls that found that the starting frequency differed between male- and female-produced A notes [Freeberg, Lucas, and Clucas (2003). *J. Acoust. Soc. Am.* **113**, 2127–2136]. Taken together, these results and the results of studies with other chickadee species suggest that sex differences likely exist in the *chick-a-dee* call, specifically acoustic features in A notes, but that more complex features than those addressed here may be associated with the sex of the caller.

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Pages: 1598–1608

I. INTRODUCTION

Within a species, males and females can differ markedly in both appearance and behaviour. Observable sex differences serve as an indicator of the sex of the individual to others of the species. These indicators can be selected for as a signal or occur independent of selection as a cue. Having a consistent sign of the sex of an individual can assist organisms in quickly and effectively reacting to the presence of an unknown conspecific. Animals can thus determine if the new individual is a competitor that they should be aggressive toward or a potential mate that they should attempt to attract or pursue.

Black-capped chickadees (*Poecile atricapillus*) are a relatively common, non-migratory songbird species whose range extends across much of North America (Smith, 1991). They form mating pairs in the breeding season in a defended territory and form flocks with a structured social hierarchy over the winter months (Smith, 1991). Black-capped chickadees are sexually monomorphic to human observers and produce multiple vocalizations. Their *chick-a-dee* call is usually described as consisting of four main note types delivered in a relatively fixed order of A, B, C, and D (Ficken *et al.*, 1978); however, black-capped chickadees also produce D-hybrid (Dh) notes (Kroodsmma and Miller, 1996; Proppe and

Sturdy, 2009) that resemble an A or B note that merges with a D note (see Fig. 1). Within a given call, each note type can be produced once, multiple times, or not at all (Ficken *et al.*, 1978). This variable and highly combinatorial arrangement allows for a huge variety of call compositions and, by extension, a variety in the types of information calls can contain. For example, *chick-a-dee* calls contain information about species identity (black-capped and mountain chickadees: Bloomfield and Sturdy, 2008; black-capped and Carolina chickadees: Bloomfield *et al.*, 2003), flock membership (Mammen and Nowicki, 1981; Nowicki, 1989), individual identity (Charrier *et al.*, 2004), and predator threat level (Templeton *et al.*, 2005).

Sex differences have been described in some black-capped chickadee vocalizations such as *fee-bee* songs (Hahn *et al.*, 2013) and *tseet* calls (Guillette *et al.*, 2010a). Sex differences have also been found in the *chick-a-dee* calls of Carolina chickadees where females have been found to produce A notes with higher starting frequencies than males (Freeberg *et al.*, 2003). As birds that live in forested areas where visual contact is limited, the ability to recognize the sex of the individual from call alone is an ecologically relevant ability for chickadees. In the wild, males likely benefit from knowing if a calling bird is a male or female, compared to the biological consequences of losing resources to a competitor that should have been chased off or wasting energy defending against a bird that might have been a mate. The idea that male and female calls have acoustic differences is supported by an experiment conducted by Avey and

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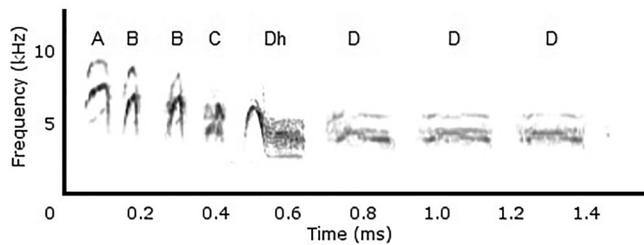


FIG. 1. Sound spectrogram of a black-capped chickadee *chick-a-dee* call (window size = 256 points, time resolution = 5.8 ms). Call was constructed from two individuals to provide exemplars of each note type.

colleagues (2008) who found that male and female black-capped chickadees showed different levels of immediate early gene expression in auditory brain regions after exposure to either male or female black-capped chickadee *chick-a-dee* calls. This difference in expression implies that there is a difference in perception such that male- and female-produced calls sound distinctive, and the sex of the perceiver affects how they react to those differences. Though the study by Avey *et al.* (2008) provides evidence for acoustic differences between the sexes, there is no indication of what those differences may be.

Bioacoustic analyses are widely employed to analyze the specific acoustic differences between the *chick-a-dee* calls of various chickadee categories (e.g., sex, flock, geographical location). Acoustic features within the individual note types are often measured and statistically analyzed to identify acoustic features that chickadees could be using to categorize calls into the different groups. In addition to individual identity, Charrier and colleagues (2004) also tested for sex differences, though the experiment was designed to identify individual and note-type differences. They determined that the maximal frequency of D notes differed between the sexes with females producing higher frequency notes, but they concluded that their sample was insufficient to state that these differences existed outside of the limited populations being investigated.

Based on the previous findings of both Avey *et al.* (2008) and Freeberg *et al.* (2003), we sought to re-examine the potential for sex differences coded in the acoustic features of the *chick-a-dee* calls of black-capped chickadees using a more comprehensive approach. Using recordings of wild-caught black-capped chickadees, we completed a bioacoustic analysis of 12 acoustic features within *chick-a-dee* calls and used permuted discriminant function analyses (pDFA) to determine if any of those features differed significantly between the sexes. We analyzed acoustic features that have been examined previously to identify individual differences in the *chick-a-dee* calls of black-capped chickadees (Nowicki and Nelson, 1990; Charrier *et al.*, 2004). We expected that one or more of the features measured in call notes would differ between the sexes, accounting for the differences in neural expression when chickadees hear male or female calls (Avey *et al.*, 2008). Based on the findings of Freeberg *et al.* (2003), which found that Carolina chickadee females produce A notes with higher starting frequencies than males, we predicted that a difference between black-

capped chickadee sexes would most likely be found in the A notes of their *chick-a-dee* calls.

II. METHODS

A. Subjects

We used *chick-a-dee* calls from 11 males and 11 females. Birds determined to be at least one year of age by examining their outer tail retrices (Pyle, 1997) were captured in Edmonton, Alberta (53.53°N, 113.53°W; 53.52°N, 113.47°W) and Stony Plain, Alberta (53.45°N, 114.01°W) between 29 February 2008 and 19 March 2012. Sex was determined by deoxyribonucleic acid (DNA) analysis (Griffiths *et al.*, 1998).

B. Housing

Chickadees were housed in individual Jupiter Parakeet cages (30 cm wide × 40 cm high × 40 cm deep; Rolf C. Hagen, Inc., Montreal, QC) that prevented individuals from being in physical contact with one another, but allowed visual and auditory communication. Chickadees were housed at the University of Alberta (Edmonton, AB) and maintained on a light:dark cycle matched to the natural cycle for Edmonton, Alberta with the temperature held constant at approximately 20 °C. While housed in the colony room, birds were provided with *ad libitum* food (Mazuri Small Bird Maintenance Diet; Mazuri, St. Louis, MO), water (vitamin supplemented on alternate days; Hagen, Rolf C. Hagen, Inc, Montreal, QC), cuttlebone, and grit. Twice per week, birds were provided with a mixture of boiled eggs and spinach or parsley; three times per week, they received one superworm; and each day they received 3–5 sunflower seeds.

C. Recordings

Birds were recorded from 20 March to 14 June 2012. Thirty minute recordings were completed between 09:00 and 13:20 for each individual bird. Birds were transported and recorded in the cage that they were housed in. Individual birds were recorded using a Marantz PMD670 (Marantz America, Mahwah, NJ) digital recorder set to a 16 bit, 44 100 Hz sampling rate, and an AKG C 1000S (AKG Acoustics, Vienna, Austria) microphone set up in 1.7 m × 0.84 m × 0.58 m sound-attenuating chambers (Industrial Acoustics Company, Bronx, NY). The microphone was positioned 30 cm above the rear center of the cage top. Digital audio files were saved to the personal computer (PC) following each recording session (see Hahn *et al.*, 2013, for further recording details). Calls were obtained from recordings over 1–4 days (average: 1.73 days) for each individual bird. There was no significant difference in the rate of call production between males and females. Males were recorded for an average of 43 min across 1.55 days and produced an average of 2.14 calls per minute while females were recorded for an average of 40 min across 1.91 days and produced an average of 2.21 calls per minute ($t = -0.085$, $p = 0.933$).

D. Acoustic measures

Each bird produced at least 17 *chick-a-dee* calls (range: 17–248 calls). Call composition was visually determined from spectrograms in SIGNAL (version 5.05.02, Engineering Design, Belmont, MA) by a single individual using [Ficken et al. \(1978\)](#) as a reference.

A random selection of 20 of each A, B, C, D-hybrid (Dh), and D notes were chosen for each bird, where possible (range A notes: 17–20; B notes: 15–20; C notes: 7–20; Dh notes: 7–20; D notes: 15–20). These served as a pool for the selection of ten notes for A, B, and D notes or selection of five notes for C and Dh notes per permutation of the discriminant function analyses (DFA). C and Dh notes were rarer than the other note types and as such the analyses were run on smaller samples. The notes were selected from one to the total number of notes available using a random number generator. Individuals that produced fewer than 11 A, B, or D notes or fewer than 6 C or Dh notes were excluded from the analysis for the respective note type. Out of 11 females and 11 males, this left 11 females and 10 males for A note analysis, 10 females and 10 males for B note analysis, 6 females and 6 males for C note analysis, 7 females and 5 males for Dh note analysis, and 11 females and 11 males for D note analysis. Individual notes were saved as separate files and standardized to a duration of 300 ms by adding equal length of silence to the beginning and end of each note using SIGNAL.

For A, B, and C notes, nine features were measured: start frequency, peak frequency, and end frequency (in Hz; measurements made from spectrograms with window size = 1024 points, frequency precision = 43.1 Hz); ascending duration, descending duration, and total duration (in ms; measurements made from spectrograms with window size = 256 points, time resolution = 5.8 ms); slope of ascending frequency modulation, which is calculated by subtracting the start frequency from the peak frequency and dividing by the ascending duration (in Hz/ms); slope of descending frequency modulation, which is calculated by subtracting the peak frequency from the end frequency and dividing by the descending duration (in Hz/ms); and maximal frequency (i.e., the loudest frequency; in Hz; measurements made from power spectra with frequency precision = 2.7 Hz; window size varied with note length). For Dh notes, 12 acoustic features were measured including the nine features used for A, B, and C notes above, as well as the D-portion duration (i.e., the length of the note segment that resembles a D note; in ms), frequency of first visible harmonic (in Hz; measurements made from power spectrums with frequency precision = 2.7 Hz; window size varied with note length), and note peak frequency (i.e., the highest frequency; in Hz; measurements made from power spectrums with frequency precision = 2.7 Hz; window size varied with note length). Four features were measured for D notes: total duration, frequency of first visible harmonic, maximal frequency, and note peak frequency, as described above. Figure 2 illustrates each of these acoustic measures and Table I summarizes the acoustic features measured for each note type.

E. Statistical analysis

To determine if the distribution of any acoustic features varied between males and females, we first calculated the coefficients of variation both within the sexes (CV_{within}) and between the sexes (CV_{between}) for each measured acoustic feature for each note type by dividing the standard deviation (SD) for the group of interest (male, female, or total sample) by the mean of that group and multiplying by 100. Then, the potential for sex coding (PSC) was calculated for each feature for each note type using the formula

$$\text{PSC} = \frac{CV_b}{\text{mean } CV_w},$$

where CV_b is the coefficient of variation between the sexes and $\text{mean } CV_w$ is the average of the coefficients of variation for males and females [i.e., $CV_{w(\text{female})}$ and $CV_{w(\text{male})}$]. PSC is an adaptation of the potential for individual coding (PIC), which can be calculated to determine if features within calls can encode individual identity (see [Sokal and Rohlf, 1995](#); [Charrier et al., 2004](#); [Hahn et al., 2013](#)). If the PSC value is greater than 1, then that feature can potentially be used for sex identification.

DFA are commonly used by bioacoustic researchers to determine if vocalizations differ between groups or individuals ([Mundry and Sommer, 2007](#)). The process investigates whether one or more features within the vocalizations can be used to accurately classify to which group the vocalizations belong ([Tabachnick and Fidell, 2007](#)). In the current study, a stepwise DFA using the leave-one-out method for cross-validation was conducted for each note type to analyze the notes in regard to the sex of the producer. The leave-one-out method involves using all but one case to determine the discriminant function, then classifying the withheld case and comparing the result to the true group status (i.e., male or female) to determine the accuracy of the discriminant function. The process is repeated until all cases are classified using derived discriminant functions ([Betz, 1987](#)). This method was chosen because it utilizes new cases (i.e., cases not used to determine the discriminant function) to test the effectiveness of the discriminant function. The analysis was conducted in R (version 3.0.3, [R Core Team, 2013](#)) with both “MASS” ([Venables and Ripley, 2002](#)) and “klaR” ([Weihs et al., 2005](#)) software packages installed.

For the DFA on A, B, and D note types, 10 notes were randomly selected and for C and Dh notes, 5 notes were randomly selected from a total sample of 20 notes produced by each individual. This randomization was repeated 100 times to account for the variation that occurs within an individual’s notes and the effect that variation has on the derived discriminant function. The average percentage of correct classifications was calculated for each note type. The binomial distribution was used to determine which features were included in the stepwise discriminant functions more than would be predicted by chance ($p = 0.01$ significance level).

[Mundry and Sommer \(2007\)](#) recommend a process called pDFA to account for the pseudoreplication that can arise when multiple vocalizations are used from each individual. In

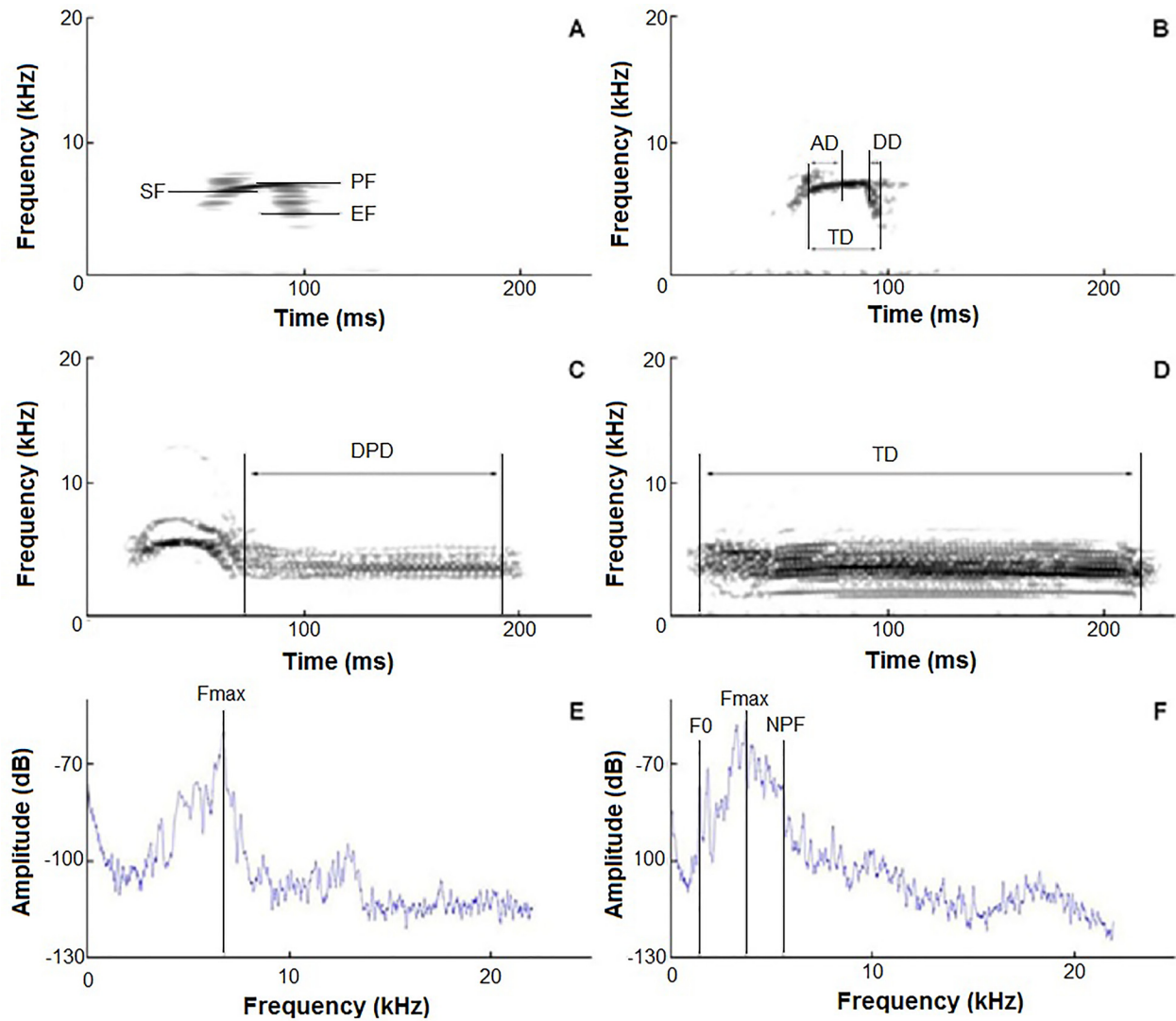


FIG. 2. (Color online) Acoustic measures made on individual note types of the chick-a-dee calls of black-capped chickadees. (A) Spectrogram of non-D notes (i.e., A, B, or C) optimized for frequency precision (window size = 1024 points, frequency precision = 43.1 Hz) for measuring start frequency (SF), peak frequency (PF), and end frequency (EF). (B) Spectrogram of non-D notes optimized for duration precision (window size = 256 points, time resolution = 5.8 ms) for measuring ascending duration (AD), descending duration (DD), and total duration (TD). (C) Spectrogram of Dh notes optimized for duration precision (window size = 256 points, time resolution = 5.8 ms) for measuring D-portion duration (DPD). (D) Spectrogram of D notes optimized for duration precision for measuring total duration (TD). (E) Power spectrum of non-D notes used to measure maximal frequency (F_{\max}). (F) Power spectrum of D notes used to measure first visible harmonic (f_0), maximal frequency (F_{\max}), and note peak frequency (NPF). Window size for power spectra varied with note length.

TABLE I. Summary of acoustic features measured from each note type (A, B, C, Dh, and D) of black-capped chickadee *chick-a-dee* calls.

Measure	Unit	Definition	Note type				
			A	B	C	Dh	D
Start frequency	Hz	Lowest frequency at the beginning of the note	X	X	X	X	
Peak frequency	Hz	Frequency when the note is no longer ascending	X	X	X	X	
End frequency	Hz	Lowest frequency at the termination of the note	X	X	X	X	
Ascending duration	ms	Length of time that the note's frequency increases	X	X	X	X	
Descending duration	ms	Length of time that the note's frequency decreases	X	X	X	X	
D-Portion duration	ms	Length of note portion that resembles a D note				X	
Total duration	ms	Length of the note	X	X	X	X	X
Slope of ascending frequency modulation	Hz/ms	Speed at which the note increases in frequency	X	X	X	X	
Slope of descending frequency modulation	Hz/ms	Speed at which the note decreases in frequency	X	X	X	X	
Frequency of first visible harmonic	Hz	Frequency of the lowest harmonic within 35 dB of the maximal frequency				X	X
Maximal frequency	Hz	Loudest or highest amplitude frequency	X	X	X	X	X
Note peak frequency	Hz	Highest frequency				X	X

this procedure, the percent correct classifications by the DFAs are compared to the null hypothesis of the distribution of a randomized data set (i.e., random notes assigned randomly as male- or female-produced) rather than assuming a normal distribution.

For the pDFA, only those features identified as being used above chance levels from the binomial test were analyzed. Again, ten notes were randomly selected for each individual for A, B, and D notes and five notes were randomly selected for each individual for C and Dh notes, and a DFA was conducted for each note type to classify based on sex. This was repeated 100 times and the average percentage of correct classifications was calculated for each note type. One thousand pDFAs were then performed with ten notes randomly selected for A, B, and D notes and five notes randomly selected for C notes. The analyses randomly assigned those notes as either female- or male-produced, with the sex of the individual held constant for that randomization (i.e., the individual was randomly assigned as male or female and all of that individual's notes were treated as being produced by that selected sex). The portion of pDFAs that correctly classified cases at a percentage equal to or greater than the classifications in the original DFAs was expressed as a *p*-value, as outlined by [Mundry and Sommer \(2007\)](#).

Five multivariate analyses of variances (MANOVA) were also conducted on the call notes using IBM SPSS for Windows (version 20.0.0; [IBM Corporation, 2011](#)). The analyses used average notes constructed for each individual from the average of each feature measured for each note type for that individual, rather than using measurements from multiple notes produced by the same individual. The MANOVA then compared the average notes produced by males and females to determine if any differences between the sexes could be detected, though not which features cause those differences.

III. RESULTS

A total of 1645 notes was analyzed (847 female, 798 male). There were 414 A notes (220 female, 194 male), 395 B notes (195 female, 200 male), 208 C notes (95 female, 113 male), 193 Dh notes (117 female, 76 male), and 435 D notes (220 female, 215 male). Calls from which the notes were randomly selected had on average 1.67 ± 0.61 A notes (range: 0–20), 0.90 ± 0.51 B notes (range: 0–14), 0.39 ± 0.30 C notes (range: 0–5), 0.22 ± 0.24 Dh notes (range: 0–1), and 3.79 ± 1.31 D notes (range: 0–14). [Table II](#) shows the means, SDs, coefficients of variation, and PSC for all acoustic features measured across all note types.

The PSC measure provides a comparison of the variation between and within the sexes. If the PSC value is above 1, this indicates that there is more variation between the sexes than there is within the sexes taken together. For A notes, the measured acoustic features that had a PSC greater than 1.00 were start frequency, peak frequency, end frequency, ascending duration, descending duration, total duration, and slope of ascending frequency modulation. The slope of ascending frequency modulation had the highest PSC (PSC = 1.09) and is therefore most likely to contribute to perceiving a difference between the sexes in A notes, though any feature with a PSC over 1.00 cannot be ruled out as contributing to the difference. For B notes, the end

frequency, slope of ascending frequency modulation, and slope of descending frequency modulation had PSC greater than 1.00, with slope of ascending frequency modulation having the greatest PSC (PSC = 1.08). The peak frequency, ascending duration, descending duration, total duration, slope of ascending frequency modulation, and slope of descending frequency modulation had PSC greater than 1.00 for C notes, with both descending duration and slope of descending frequency modulation having the largest PSC (PSC = 1.13). For Dh notes, the features with PSC greater than 1.00 were frequency of first visible harmonic, maximal frequency, start frequency, peak frequency, descending duration, D-portion duration, total duration, slope of ascending frequency modulation, and slope of descending frequency modulation. Descending duration had a PSC of 1.15, which is the highest PSC of all features across all note types. Total duration, slope of ascending frequency modulation, and slope of descending frequency modulation were the features with PSC greater than 1.00 for D notes. See [Table II](#) for a complete list of PSC values.

A. Stepwise discriminant function analysis

The stepwise DFA used to classify call notes based on the sex of the producer using all measured acoustic features yields a mean percentage of correct classification, the results of which will be presented as mean \pm SD with the range of correct classifications included. The stepwise DFA had mean percentage of correct classifications as follows: A notes: $63.46 \pm 2.27\%$ (range: 58.10%–68.10%); B notes: $64.49 \pm 2.52\%$ (range: 59.00%–71.50%); C notes: $72.73 \pm 4.49\%$ (range: 63.33%–85.00%); Dh notes: $68.52 \pm 4.68\%$ (range: 56.72%–80.60%); D notes: $60.25 \pm 2.32\%$ (range: 53.95%–65.12%). [Table III](#) lists the number of times each acoustic feature was used by the DFA to construct a discriminant function out of the 100 permutations.

B. Permuted discriminant function analysis

Using a significance level of $p < 0.01$, we conducted a binomial test to determine which acoustic features were used more often than would be expected by chance. The cutoff value was 63/100 permutations. A pDFA was conducted with only those features that remained for each note type ([Table III](#), indicated by “I”). In addition, pDFAs were conducted with only the most often used feature from each note type ([Table III](#), indicated by bold values).

For both groups of features, the pDFA process involved running a second analysis on the same data with the sex of the individuals producing the calls randomized. This created a null hypothesis distribution to compare the original data to that which took into account the multiple measures from each individual (i.e., a stand-in for a normal distribution). To interpret the results of the pDFA, the *p*-value is calculated by taking the difference in the “mean percentage of correct classification” between the normal classification and the randomized classification (i.e., how much better the normal classification performs compared to the true null hypothesis distribution, not the absolute value for the mean percentage of correct classification).

The pDFA run on the “features used greater than chance” had mean percentage of correct classifications as follows: A

TABLE II. Mean, standard deviation (SD), coefficient of variation (CV), and PSC for each acoustic feature measured in A, B, C, Dh, and D notes in black-capped chickadee *chick-a-dee* calls.

Note type	Value	Start frequency (Hz)	Peak frequency (Hz)	End frequency (Hz)	Ascending duration (ms)	Descending duration (ms)	D-portion duration (ms)	Total duration (ms)	Slope of ascending frequency modulation (Hz/ms)	Slope of descending frequency modulation (Hz/ms)	Frequency of first visible harmonic (Hz)	Maximal frequency (Hz)	Note peak frequency (Hz)
A notes	Both sexes												
	Mean	6359.27	7253.62	5463.58	25.00	11.12	—	54.34	42.35	-312.02	—	7054.83	—
	SD	626.70	410.76	949.41	10.83	8.95	—	18.29	55.20	590.00	—	503.09	—
	CV _{between}	9.85	5.66	17.36	43.10	81.16	—	33.64	130.33	-189.09	—	7.13	—
	PSC	1.03	1.02	1.01	1.02	1.03	—	1.01	1.09	1.00	—	1.00	—
	Females												
	Mean	6509.49	7306.97	5499.69	26.19	12.48	—	57.69	38.60	-288.91	—	7099.82	—
	SD	584.68	439.68	1009.46	11.78	10.05	—	17.87	72.13	623.44	—	506.86	—
	CV _{within}	8.98	6.02	18.35	45.00	80.52	—	30.97	186.86	-215.79	—	7.14	—
	Males												
	Mean	6194.02	7194.94	5423.86	23.69	9.61	—	50.66	46.61	-338.22	—	7005.33	—
	SD	632.06	358.31	877.53	9.51	7.20	—	18.08	24.14	550.05	—	495.85	—
CV _{within}	10.20	5.11	16.16	39.80	76.65	—	35.73	51.80	-162.63	—	7.07	—	
B notes	Both sexes												
	Mean	4690.60	6577.21	4618.61	18.14	10.16	—	38.47	125.71	-258.94	—	6277.57	—
	SD	984.88	782.04	925.53	6.88	5.83	—	11.24	97.32	231.83	—	787.15	—
	CV _{between}	21.03	11.89	20.08	37.98	57.47	—	29.26	77.42	-89.53	—	12.54	—
	PSC	1.00	1.00	1.01	1.00	1.00	—	1.00	1.08	1.04	—	1.00	—
	Females												
	Mean	4690.51	6549.19	4570.96	18.46	10.87	—	39.40	129.44	-257.33	—	6205.39	—
	SD	1000.04	674.16	959.96	6.93	6.00	—	10.67	118.85	269.04	—	704.70	—
	CV _{within}	21.38	10.30	21.09	37.65	55.31	—	27.13	91.82	-104.55	—	11.37	—
	Males												
	Mean	4690.71	6611.47	4676.85	17.76	9.29	—	37.34	121.35	-260.86	—	6365.78	—
	SD	969.19	894.66	880.47	6.82	5.51	—	11.83	62.64	178.20	—	868.81	—
CV _{within}	20.66	13.53	18.83	38.40	59.32	—	31.67	51.66	-68.31	—	13.65	—	
C notes	Both sexes												
	Mean	3480.43	5844.60	3686.86	20.90	11.17	—	39.07	135.43	-245.79	—	5203.30	—
	SD	763.38	804.68	586.58	8.66	4.68	—	7.95	85.08	240.71	—	729.27	—
	CV _{between}	21.85	13.66	15.80	41.36	41.90	—	20.37	62.82	-97.94	—	13.87	—
	PSC	0.99	1.09	1.00	1.01	1.13	—	1.05	1.01	1.13	—	1.11	—
	Females												
	Mean	3406.68	6100.19	3610.35	24.79	10.11	—	41.29	126.77	-310.18	—	5456.67	—
	SD	836.72	641.64	661.83	7.09	3.78	—	7.17	75.03	310.02	—	692.41	—
	CV _{within}	24.33	10.34	18.15	28.30	37.32	—	17.24	59.18	-99.95	—	12.35	—
	Males												
	Mean _{male}	3554.17	5589.01	3763.37	17.00	12.24	—	36.85	142.71	-191.66	—	4949.94	—
	SD _{male}	696.33	834.64	511.78	8.35	5.17	—	7.97	92.39	141.25	—	625.58	—
CV _{within}	19.68	14.83	13.59	47.81	42.97	—	21.59	64.74	-73.70	—	12.60	—	

TABLE II. (Continued)

Note type	Value	Start frequency (Hz)	Peak frequency (Hz)	End frequency (Hz)	Ascending duration (ms)	Descending duration (ms)	D-portion duration (ms)	Total duration (ms)	Slope of ascending frequency modulation (Hz/ms)	Slope of descending frequency modulation (Hz/ms)	Frequency of first visible harmonic (Hz)	Maximal frequency (Hz)	Note peak frequency (Hz)	
Dh notes	Both sexes													
	Mean _{sample}	4536.60	5785.95	3773.01	13.19	13.97	131.00	172.26	101.00	-183.17	2123.91	4253.84	5301.65	
	SD _{sample}	942.83	576.50	667.70	4.74	8.13	22.80	23.88	61.78	120.14	641.95	1005.93	436.05	
	CV _{between}	20.65	9.97	17.55	37.06	58.39	17.44	13.92	61.17	-65.59	29.92	23.30	8.28	
	PSC	1.02	1.06	0.99	1.00	1.15	1.04	1.07	1.05	1.06	1.03	1.03	1.00	
	Females													
	Mean	4693.44	5810.43	3919.97	13.45	14.52	132.50	175.02	86.89	-181.17	2219.92	4474.51	5243.08	
	SD	964.84	646.58	567.39	4.76	9.76	25.13	27.19	53.32	136.81	654.13	1019.06	418.77	
	CV _{within}	20.51	11.10	14.39	35.91	66.53	19.25	15.70	61.37	-75.51	28.84	22.63	8.03	
	Males													
	Mean	4317.03	5751.69	3567.28	12.82	13.19	128.91	168.38	122.74	-186.25	1989.51	3944.89	5383.65	
	SD	871.37	444.39	754.11	4.67	4.42	18.80	17.49	67.70	89.38	577.24	920.15	452.83	
	CV _{within}	20.02	7.77	20.98	38.56	34.66	14.35	10.35	55.16	-47.99	29.51	22.84	8.47	
D notes	Both sexes													
	Mean	—	—	—	—	—	—	175.62	—	—	1670.65	3587.60	5623.60	
	SD	—	—	—	—	—	—	19.55	—	—	174.28	287.42	327.56	
	CV _{between}	—	—	—	—	—	—	11.12	—	—	10.42	8.01	5.83	
	PSC	—	—	—	—	—	—	1.05	—	—	1.00	1.02	1.01	
	Females													
	Mean	—	—	—	—	—	—	177.05	—	—	1658.34	3595.32	5670.09	
	SD	—	—	—	—	—	—	22.68	—	—	168.67	319.43	327.77	
	CV _{within}	—	—	—	—	—	—	12.81	—	—	10.17	8.88	5.78	
	Males													
	Mean	—	—	—	—	—	—	173.91	—	—	1685.42	3578.35	5567.82	
	SD	—	—	—	—	—	—	15.47	—	—	181.92	246.05	332.30	
	CV _{within}	—	—	—	—	—	—	8.46	—	—	10.65	6.78	5.72	

TABLE III. Number of times measured acoustic features were used to construct a discriminant function out of 100 permutations. Values marked with a “I” were used for the pDFA analyses. The most used feature for each note type is in bold.

Note type	Start frequency	Peak frequency	End frequency	Ascending duration	Descending duration	D-portion duration	Total duration	Slope of ascending frequency modulation	Slope of descending frequency modulation	Frequency of first visible harmonic	Maximal frequency	Note peak frequency
A notes	94^I	54	82 ^I	72 ^I	53	—	46	70 ^I	49	—	74 ^I	—
B notes	98 ^I	70 ^I	56	95 ^I	66 ^I	—	69 ^I	100^I	79 ^I	—	81 ^I	—
C notes	77 ^I	76 ^I	78 ^I	76 ^I	79 ^I	—	81 ^I	84 ^I	76 ^I	—	89^I	—
Dh notes	69 ^I	82 ^I	90 ^I	81 ^I	81 ^I	91 ^I	87 ^I	84 ^I	92^I	67 ^I	72 ^I	63
D notes	—	—	—	—	—	—	60	—	—	71 ^I	56	98^I

notes: $61.66 \pm 2.84\%$ (range: 53.81%–69.05%); B notes: $62.95 \pm 2.75\%$ (range: 54.00%–70.50%); C notes: $68.18 \pm 4.91\%$ (range: 53.33%–78.33%); Dh notes: $61.25 \pm 6.20\%$ (range: 43.33%–75.00%); D notes: $58.20 \pm 2.87\%$ (range: 51.82%–66.36%). When the sex of the caller was randomized and the mean percentage of correct classifications was compared to the original percentage of correct classifications, the classifications and corresponding p -values were as follows: A notes: 56.81% ($p = 0.170$); B notes: 57.68% ($p = 0.169$); C notes: 60.12% ($p = 0.211$); Dh notes: 61.74% ($p = 0.560$); D notes: 53.70% ($p = 0.174$). For all note types except Dh notes, the original data allowed for better classification on average than the sex-randomized data; however, none of the differences were significant with a p -value ≤ 0.05 (see Table IV).

The pDFAs conducted using the “top single feature for each note type” had mean percentage of correct classifications as follows: A notes: $61.22 \pm 1.74\%$ (range: 56.19%–66.19%); B notes: $54.57 \pm 0.88\%$ (range: 51.00%–55.00%); C notes: $65.93 \pm 4.60\%$ (range: 53.33%–78.33%); Dh notes: $55.98 \pm 2.38\%$ (range: 50.00%–63.33%); D notes: $57.89 \pm 2.33\%$ (range: 60.91%–61.36%). When the sex of the caller was randomized and the mean percentage of correct classifications was compared to the original percentage of correct

classifications, the classifications and corresponding p -values were as follows: A notes: 54.07% ($p = 0.066$); B notes: 52.93% ($p = 0.396$); C notes: 55.32% ($p = 0.115$); Dh notes: 54.09% ($p = 0.500$); D notes: 52.63% ($p = 0.129$). For all note types, the original data allowed for better classification than the sex-randomized data; however, none of the differences were significant at a p -value ≤ 0.05 (see Table IV).

C. MANOVA

Results from the MANOVAs closely reflected those of the pDFA with no measured acoustic features showing significant differences between the sexes for any note type; though, as with the pDFA results, A notes were the closest to significance (A notes: $p = 0.063$; B notes: $p = 0.113$; C notes: $p = 0.434$; Dh notes: $p = 0.704$; and D notes: $p = 0.137$).

IV. DISCUSSION

Using a measure of PSC, we found that there were acoustic features in all note types that varied more between the sexes than within, indicating that these features could contribute to sex differences in the *chick-a-dee* call of black-

TABLE IV. Results of pDFA on “features used above chance” and “most used features” to discriminate between male and female produced A, B, C, Dh, and D notes from *chick-a-dee* calls showing the difference between the original data and permuted (i.e., randomized) data.

Note type	Method for choosing feature(s)	Acoustic feature(s) used	Mean percentage of correctly classified elements (original data set)	SD	Range	Mean percentage of correctly classified elements (permuted data set)	p -value
A notes	Above chance ($p = 0.01$)	F_{\max} , SF, EF, AD, SAFM	61.66%	2.84%	53.81–69.05%	56.81%	0.170
	Top used feature	SF	61.22%	1.74%	56.19–66.19%	54.07%	0.066
B notes	Above chance ($p = 0.01$)	F_{\max} , SF, PF, AD, DD, TD, SAFM, SDFM	62.95%	2.75%	54.00–70.50%	57.68%	0.169
	Top used feature	SAFM	54.57%	0.88%	51.00–55.00%	52.93%	0.396
C Notes	Above chance ($p = 0.01$)	F_{\max} , SF, PF, EF, AD, DD, TD, SAFM, SDFM	68.18%	4.91%	53.33–78.33%	60.12%	0.211
	Top used feature	F_{\max}	65.93%	4.60%	53.33–78.33%	55.32%	0.115
Dh notes	Above chance ($p = 0.01$)	f_0 , F_{\max} , NPF, SF, PF, EF, AD, DD, DND, TD, SAFM, SDFM	61.25%	6.20%	43.33–75.00%	61.74%	0.560
	Top used feature	SDFM	55.98%	2.38%	50.00–63.33%	54.09%	0.500
D notes	Above chance ($p = 0.01$)	f_0 , NPF	58.20%	2.87%	51.82–66.36%	53.70%	0.174
	Top used feature	NPF	57.89%	2.33%	60.91–61.36%	52.63%	0.129

capped chickadees. The PSC values of the descending duration of C and Dh notes were the highest (C notes: PSC = 1.13; Dh notes: PSC = 1.15), indicating that they could potentially contribute the most to differentiating the sexes. For C notes, males had longer descending durations. The opposite was true for Dh notes. However, because of differential rates of note type occurrence the analyses on these note types were conducted on half as many subjects as the other note types due to their rarity. This reduction in sample may explain why C and Dh notes were identified as likely candidates involved in sex discrimination through PSC analysis, but not in the other statistical analyses. From a biological standpoint, the fact that both C and Dh notes were rarely produced may make them poor indicators of sex (if considered alone) because the majority of calls produced would not include that information.

The results of the pDFA showed that of the five note types investigated (A, B, C, Dh, and D notes), acoustic features in A notes were the most accurate at classifying the sex of call producers. Specifically, the start frequency of A notes could be used to distinguish between male and female calls at levels that approached significance. The MANOVA results matched those of the pDFA for A note acoustic features being best able to accurately classify the caller's sex. These results suggest that there are features within A notes that signal the sex of the caller.

The difference in the results from PSC and pDFA derive from the fact that they investigate two separate concepts. PSC is a measure of variability and examines the distribution of the measures for each group and how they differ. This can be useful in discriminating between groups if the distributions do not overlap. However, this analysis is restricted to examining individual features in isolation. The pDFA, on the other hand, is concerned with determining which combination of features allow for the correct classification of items into groups. As such the two analyses could detect different ways of distinguishing between the sexes based on acoustic features in calls. It should also be noted that the PSC values observed here are lower than what have been found in similar bioacoustic analyses that examined variability in call notes. While the highest PSC in this study was 1.15, Charrier and colleagues (2004) calculated PIC up to 2.7 in the *chick-a-dee* call of black-capped chickadees. Similarly, Hahn and colleagues (2013) calculated PIC up to 1.69 in the *fee-bee* song of male and female black-capped chickadees. As there is no way to determine the "significance" of PSC and related calculations, the values merely serve as an indicator for potential use in conjunction with other statistical analyses and behavioural testing.

A notes were the most common non-D notes (i.e., A, B, C) in the sampled *chick-a-dee* calls, with the 414 A notes making up 25% of the total 1645 notes. The prevalence of A notes in *chick-a-dee* calls may suggest that sex identifying acoustic cues are likely to be found in A notes because these notes are present in the majority of calls and thus the caller's sex could be signaled often.

For A and Dh notes, but not other note types, frequency measures of females tended to be higher than the corresponding male measures. While start frequency was most

useful for A notes in discriminating male and female *chick-a-dee* calls, the end frequency of black-capped chickadee *tseet* calls were found to contribute strongly to the classification of male and female black-capped and mountain chickadees (Guillette *et al.*, 2010b). *Tseet* calls are acoustically similar to A notes of the *chick-a-dee* call, but are usually produced singly rather than in strings of notes as is often the case for A notes. Similarly, the start frequency of A notes varies between male and female calls produced by Carolina chickadees (Freeberg *et al.*, 2003), so it is possible that frequency is a sex-related acoustic feature that is conserved across Parid species. This difference in frequency across vocalization types could be a constraint of body size, as female chickadees tend to be smaller than males (Smith, 1991), and in other bird species, vocalization frequency has been linked to body size (Fitch, 1999). Hughes *et al.* (1998) found that black-capped chickadees reared in isolation were capable of producing species-typical A notes, meaning that those notes are innate (i.e., not learned). This supports the idea that sex differences within frequency measures of A notes could be at least partially due to sex differences in morphology and physiology.

Other, non-A notes of black-capped chickadees' *chick-a-dee* calls are learned, such that young birds must be exposed to the vocalizations of a conspecific tutor in order to produce species-typical vocalizations as an adult (B and C notes: Hughes *et al.*, 1998; D notes: Mammen and Nowicki, 1981; Nowicki 1983, 1989). Here, the pDFA classifying B notes using maximal frequency, start frequency, peak frequency, ascending duration, descending duration, total duration, slope of ascending frequency modulation, and slope of descending frequency modulation yielded a relatively low *p*-value, hinting at their possible involvement in sex discrimination. Our results indicate a more complex interaction in B note acoustic features because, unlike A notes where a single feature (start frequency) could be used to classify the sex of the note producer more accurately than the original analysis, when the pDFA was conducted with only slope of ascending frequency modulation for B notes, sex of the caller was classified more poorly. While we have proposed that A notes differ between the sexes due to differences in physiology and morphology, the learned nature of B notes suggests that physical factors may not completely explain any sex differences. As a learned component, sex differences could be due to differential learning, such as young males using adult males as tutors and young females using adult females as tutors.

Studies of call convergence in black-capped chickadee winter flocks has shown that the D notes of *chick-a-dee* calls change each winter such that the D notes of the individuals within a flock become more similar to each other (Nowicki, 1989). This plasticity makes it unlikely that D notes contain sex-specific information, which was supported by the non-significant pDFA and MANOVA results.

Dh notes in black-capped chickadee *chick-a-dee* calls have not been previously described as part of the species' vocal repertoire (Ficken *et al.*, 1978; Smith, 1991). Dh notes are, however, a well-documented component of Carolina chickadee calls (e.g., Bloomfield *et al.*, 2005) and mountain

chickadee calls (Bloomfield *et al.*, 2004). Of the 22 birds in our sample, 12 produced Dh notes. Our analyses revealed that none of the features measured in Dh notes could be used to discriminate male- and female-produced calls. However, Dh notes could hold information about individual identity or flock identity (similar to D notes); with its structure resembling a combination of an introductory note (A or B) and a D note, Dh notes could encode information that has been found in both categories of notes. Future bioacoustic analyses and behavioural studies can investigate the form and function of these notes, which were previously believed to be rare in black-capped chickadees (Ficken *et al.*, 1978).

With parts of this analysis yielding results just below significance, it raises the question of whether the sample is sufficiently large to detect the sought effects. While a larger sample size is rarely detrimental, the number of subjects and the number of vocalizations used per subject were comparable to those in similar experiments looking at sex differences in the *fee-bee* song of black-capped chickadees (Hahn *et al.*, 2013), antiphonal calling between white-winged vampire bats (*Diaemus youngi*; Carter *et al.*, 2008), and the examples outlined in the original pDFA paper (Mundry and Sommer, 2007), which used pDFAs and yielded significant results. Accounting for pseudoreplication with a pDFA allows for better resolution of effects that may be obscured in a traditional DFA (Mundry and Sommer, 2007). However, with the large number of acoustic measures analyzed across five note types, more subjects may assist in resolving the effects we observed.

In the same way that human speech means much more to humans than just a fluctuation of frequencies and harmonics, *chick-a-dee* calls are far more complicated than the 12 acoustic features that were measured in the current analysis. It is possible that we did not measure the acoustic feature or features that contain sex differences within the call. This analysis investigated acoustic features in single notes in isolation from the remainder of the call. To discriminate between the sexes, black-capped chickadees could use acoustic features within individual notes, within note types, or within the whole call that were not examined here. Future research will investigate if and how the composition of *chick-a-dee* calls affects the information being signaled. For example, do females tend to produce pairs of A notes before C notes, while males are more likely to produce triplets of A notes?

Carolina chickadee *chick-a-dee* calls have been shown to contain redundancy (Freeberg *et al.*, 2003) where a part of the call can be used to predict the composition of the remainder of the call. Because black-capped chickadees share many aspects of their calls with Carolina chickadees, it is reasonable to predict that black-capped chickadee *chick-a-dee* calls contain similar redundancy. This means of transmitting information is particularly useful in environments, such as forests, where part of the call may not be heard by others. In this way, a bird can hear a portion of the call and extrapolate what the rest of the call would sound like. High frequency notes like A, B, and C notes tend to weaken more quickly in dense forests than lower frequency D notes (Proppe *et al.*, 2010), meaning that non-D notes would be most useful for

transmitting information, like sex of the caller over short distances, while D notes would transmit better over longer distances. The redundancy in information contained within calls could mean that sex differences are expressed in multiple ways within a call, they could be found across multiple notes and note types, and the patterns of expression could vary by context. An even more thorough analysis of the composition of both male and female calls could identify any such trends.

ACKNOWLEDGMENTS

Research was conducted in accordance with the Canadian Council on Animal Care Guidelines and Policies with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta and the University of Calgary Life and Environmental Sciences Animal Care Committee. Research was carried out under an Environment Canada Canadian Wildlife Service Scientific permit, Alberta Fish and Wildlife Capture and Research permits, and City of Edmonton Parks Permit. This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant and Discovery Accelerator Supplement (DAS), an Alberta Ingenuity Fund (AIF) New Faculty Grant, a Canada Foundation for Innovation Infrastructure Operating Fund (IOF), and Canada Foundation for Innovation (CFI) New Opportunities Grant, along with start-up funding and CFI partner funding from the University of Alberta to C.B.S. We would like to thank our research assistant, Brittany Hope, and our volunteer, Justine Kreuger, for their help standardizing *chick-a-dee* calls.

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