An investigation of sex differences in acoustic features of the *chick-a-dee* call of black-capped chickadees (*Poecile atricapillus*)

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

Department of Psychology

University of Alberta

Abstract

The *chick-a-dee* call of the black-capped chickadee (*Poecile atricapillus*) is composed of four main note types (A, B, C, and D) that occur in a fixed order. Sex differences have been identified in a number of black-capped chickadee vocalizations (including *tseet* calls and *fee-bee* songs) and in the *chick-a-dee* calls of other chickadee species (specifically, Carolina chickadees [*P. carolinensis*]). In the current study, I investigated twelve acoustic features in black-capped chickadee *chick-a-dee* calls including frequency, duration, and amplitude measurements. Using permuted discriminant function analyses, these features were examined to determine which feature, or combination of features, could be used to identify the sex of the caller. Only one note type (A notes) allowed for the discrimination of male and female calls at levels approaching significance. In particular, the start frequency of A notes provided the best discrimination. 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 et al. 2003). Future research will investigate the behavioural response of black-capped chickadees as they discriminate male and female *chick-a-dee* calls as well as acoustically manipulated calls. The results of this and future projects will add to our knowledge of the proximate mechanisms underlying vocal communication of black-capped chickadees in particular and, more generally, will add to our knowledge of vocal communication in animals that use learned vocalizations, including humans.

Keywords: BLACK-CAPPED CHICKADEE, SONGBIRDS, VOCALIZATIONS, CALLS, BIOACOUSTICS

Preface

This thesis is an original work by Kimberley Ann Campbell. No part of this thesis has been previously published. All procedures followed the Canadian Council on Animal Care (CCAC) Guidelines and Policies and were approved by the Animal Care and Use Committee for Biosciences at the University of Alberta (AUP 108). I was responsible for the designing the concept, collection of data, data analysis, and manuscript composition. A.H. Hahn assisted with data analysis and contributed to manuscript edits. N. McMillan provided manuscript edits. C.B. Sturdy was the supervisory author and was involved with formation of concepts and revision of the manuscript.

Acknowledgements

I would like to thank Dr. Christopher Sturdy, my supervisor, for his time, encouragement, and guidance. He gave me a chance at starting in a new field of research and I couldn't be more grateful. Without his support, this project would not have been possible.

I would also like to thank my family for the support and motivation to pursue this path of education.

Thank you to my supervisory committee members: Dr. Marcia Spetch and Dr. Pete Hurd. I greatly appreciate your guidance and commitment.

Thank you to my amazing lab mates for the constant support and encouragement. I would especially like to acknowledge the contribution of both Dr. Neil McMillan and Allison Hahn teaching me all the things I needed to know to get my project off the ground.

I would also like to thank the University of Alberta Faculty of Graduate Studies and Research for awarding me the Queen Elizabeth II Graduate Scholarship – Master's level. The award provided financial support that allowed me to focus on my research and studies.

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List of Abbreviations

AD – Ascending duration

CV - coefficient of variation SF – Start frequency

DD – Descending duration

DFA – Discriminant function analysis

Dh – D-hybrid note

DPD – D-portion duration

EF – End frequency

f0 – Frequency of first visible harmonic

F_{max} – Maximal frequency

NPF – Note peak frequency

pDFA – Permuted discriminant function analysis

PF – Peak frequency

PSC – Potential for sex coding

SAFM – Slope of ascending frequency modulation

SDFM – Slope of descending frequency modulation

TD – Total duration

Introduction

Within a species, males and females can differ markedly in appearance and behaviour. Humans experience these differences on a daily basis: males tend to be larger than females with deeper voices and often more aggressive demeanor (Wood & Eagly, 2002). Similar trends can be seen in groups as diverse as mammals (McPherson & Chenoweth, 2012) and fish (Parker, 1992). Observable sex differences serve as a signal (i.e., an evident message) to others of the species indicating the individual's sex. Having a consistent signal 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.

The evolution of sexual dimorphism between the sexes of a species makes sense in the context of a species' natural environment. Males and females often occupy different roles (e.g., defending their territory or raising their young, respectively), which can lead to selection for divergent traits (McPherson & Chenoweth, 2012). The types of traits that differ can depend on the specific species and the environment in which they exist. For example, some species are sexually dimorphic in that the sexes differ in visual appearance. In some bird species, males tend to be more brightly coloured and have elaborate feather ornamentation compared to their drab female counterparts (Owens & Hartley, 1997). In some mammals such as cows (*Bos taurus*), sheep (*Ovis aries*), brown rats (*Rattus norvegicus*), and weasels (*Mustela nivalis*) males tend to be larger than females (McPherson & Chenoweth, 2012). Sexual selection leads males to evolve traits like bright colours or larger size that could otherwise be detrimental to survival because the traits make them more visible to predators, easier for predators to catch, or make tasks like foraging for food more difficult. Natural selection would not usually favour such traits, but those qualities become more common in a population because females choose the brightly coloured or larger males as mates more often (Shine, 1989).

In some environments, like dense forest or underground, it can be impossible for individuals of a species to consistently observe each other. In these cases, visual signals are far less effective for communication than non-visual signals, such as auditory signals. One mode of auditory signaling is accomplished by vocalizations, which are used to convey information (e.g., the presence of food, or mood) but can also passively contain additional information about an individual (age, health, sex, location). In addition to acting on visual traits like colour and size, sexual selection can also occur in relation to vocalizations where female preference for a vocal trait can lead to increased mating opportunities for males with that trait and a subsequent increase in that trait within that population (Andersson, 1994; Catchpole & Slater, 2008).

Songbirds

Songbirds are a taxa of birds that were grouped primarily based on their complex syringeal muscles associated with the production of their typically complex songs (Catchpole & Slater, 2008). A monophyletic suborder within the Passeriformes (the perching birds) known as *Passeri* (or Oscines), songbirds comprise approximately 4,000 of the nearly 10,000 known species of birds (Mayr, 1946). This diverse group includes species ranging from the minute ruby-crowned kinglet (*Regulus calendula*) up to the comparatively large common raven (*Corvus corax*). Found in both urban and rural areas worldwide, some of the most populous bird species on earth (including house sparrows [*Passer domesticus*] and European starlings [*Sturnus vulgaris*]) are songbirds.

Best known for their vocalizations, songbirds produce two main types: songs, used for mate attraction and territory defense; and calls, used for establishing contact, conveying messages, and warning others (for review, see Catchpole & Slater, 2008). Songbird species share many of the mechanisms of vocal production and their shared method of learning those vocalizations has been the subject of many studies.

Songbird vocalizations can either be described as either innate or learned. Innate vocalizations are those that can be produced in the absence of adult tutors. The female zebra finch (*Taeniopygia guttata*) contact call, for example, is produced even though females do not learn their vocalizations (Simpson and Vicario, 1990). Leaned vocalizations are those that require exposure to and imitation of an adult conspecific in a process termed vocal learning. Vocal learning refers to the process by which organisms modify their own vocalizations to more closely match the vocalizations of others and is required for the acquisition of species-typical vocalizations in a select number of species (Janik & Slater, 2000). Though the process of vocal learning is relatively rare in the animal kingdom, it has been documented in a number of vertebrate lineages, including cetaceans (Janik, 2014), elephants (Stoeger & Manger, 2014), pinnipeds (Reichmuth & Casey, 2014), and songbirds (Bottjer & Arnold, 1986). These groups display differing degrees of vocal learning capability, with humans demonstrating a well-developed process of vocal learning in their acquisition of language (Egnor & Hauser, 2004).

Songbirds as model systems

Language is a trait unique to humans, which makes it difficult to study its mechanisms and evolution. Rather than attempting to unravel the complex process of human language learning directly, many researchers choose to use a comparative approach. By working with a non-human model system that possesses many similar processes to those of interest in humans, a general understanding of the mechanisms underlying those processes can be attained. Songbirds have been used increasingly in recent years as a model for understanding mechanisms of communication (Doupe & Kuhl, 1999; Slater, 2003). Songbirds as a group provide a unique model taxa (especially in terms of evolutionary study) in that they are a large collection of related species that display similar but varied vocal learning abilities. This allows for extensive comparative analyses on species that are well-suited for psychological study. Songbirds tend to be small enough to be housed in captivity, they have high metabolisms, and can run multiple food-reinforced trials in a day (see Kroodsma & Miller, 1996).

A common and widely-used model species is the zebra finch. This species of finch is native to the forests and grasslands of Australia, shows clear sexual dimorphism in their feather patterning, and the males sing complicated songs learned from tutors (Zann, 1996). Simpson and Vicario (1990) showed that zebra finch contact calls are also learned, but only in males. Interestingly, despite not having the same learned component as male contact calls, female contact calls are still individually distinctive (Forstmeier, Burger, Temnow, & Derégnaucourt, 2009). The fact that only the males learn many of their vocalizations can limit the usefulness of zebra finches as a model because only one sex can be studied for mechanisms of vocal learning and production. Though they are one of the most well studied species for their song production and perception (for review, see Riebel, 2009), zebra finches are not the only viable models of vocal learning.

Black-capped chickadees

Black-capped chickadees (*Poecile atricapillus*) are another useful songbird model of vocal learning. They are one of the most common, widely recognized, and broadly-distributed songbird species in North America. Their range includes most of Canada and portions of the northern United States of America stretching from the east coast to the west coast (Smith, 1991). They are non-migratory, seed- and insect-eaters that are sexually monomorphic to human observers and have a well-studied collection of vocalizations which are acquired through imitation of adult conspecifics.

Black-capped chickadees form relatively monogamous pairs from the fall into the breeding season and the males aggressively defend their territory. Females choose their mates based on the males' perceived quality and a few pairs form transient flocks with a structured social hierarchy over the winter months (Smith, 1991).

Black-capped chickadees are a useful model species because they are small enough to be maintained in a laboratory environment, their natural history is thoroughly documented, and, unlike zebra finches, both males and females produce songs (Hahn, Krysler, & Sturdy, 2013b).

Vocalizations. Black-capped chickadees produce a number of vocalizations including a *chick-a-dee* call, *tseet* call, *gargle* call, and *fee-bee* song (see Ficken, Ficken, & Witkin, 1978 for complete vocal repertoire survey). As with many songbirds, their young often need to hear the vocalizations of adult conspecifics in order to develop species-typical vocalizations (*chick-a-dee* call: Hughes , Nowicki, & Lohr, 1998; *fee-bee* song: Shackleton & Ratcliffe, 1993; *gargle* call: Ficken et al., 1985; *tseet* call: Guillette, Bloomfield, Batty, Dawson, & Sturdy, 2010b). *Tseet* and *gargle calls* are both produced year-round; however, their uses and structures are quite varied: *tseet* calls are composed of a single note and are used to make and retain contact with conspecifics, while *gargle* calls are complex, multi-note vocalizations produced during antagonistic encounters (Ficken et al., 1978). These vocalizations are less well-studied than both *fee-bee songs* and *chick-a-dee* calls. Unlike most songbirds, who produce structurally complex songs and comparatively simple calls, the *fee-bee* song of the black-capped chickadee is a relatively simple vocalization composed of only two whistled notes (Ficken et al., 1978). This song is sung by both sexes, is produced primarily in the spring and is used for both mate attraction and territory defense.

In contrast to the *fee-bee* song, black-capped chickadees' *chick-a-dee* call is more acoustically complex. Used for a variety of purposes including expressing alarm, alerting others of food, and corresponding flock movements, this multi-note call consists of four main note types delivered in a relatively fixed order of A, B, C, and D. Within a given call, each note type can be produced once, multiple times, or not at all (Ficken et al., 1978). In some cases, black-capped chickadees are observed to produce a note that resembles an A or B note attached to a D note. This note is called a D-hybrid note (Dh) note and is produced by Carolina chickadees (*P. carolinensis*; Bloomfield, Phillmore, Weisman, & Sturdy, 2005), mountain chickadees (*P. gambeli*; Bloomfield et al., 2005), and black-capped chickadees (pers. obs). This variable and highly combinatorial arrangement allows for a huge variety of call compositions and, by extension, the information they contain. Hughes et al. found that the multi-purpose *chick-a-dee* call of the black-capped chickadee contains components that are both innate (A notes) and learned (B and C notes) which implies that though some note types can only be correctly produced when vocal learning occurs, A notes may be biologically controlled (1998). *Chick-a-dee* calls contain information on species identity (black-capped and mountain chickadees: Bloomfield & Sturdy, 2008; black-capped and Carolina chickadees: Bloomfield et al., 2005), flock membership (Mammen & Nowicki, 1981; Nowicki, 1989), and individual identity (Charrier, Bloomfield, & Sturdy, 2004).

Sex differences. With the wide variety of essential information contained within *chick-a-dee* calls, it is reasonable to expect that calls may contain information indicating the sex of the caller. As birds that live in forested areas where visual contact is limited, the ability to recognize the sex of an individual from call alone is an ecologically relevant skill. 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 scaring off a bird that could have been a mate.

In an experiment investigating immediate early gene expression in the brains of black-capped chickadees who heard male- or female-produced *chick-a-dee* calls or *fee-bee* songs, Avey and colleagues (2008) found that there was a difference in gene expression in the auditory pathway of birds who heard male and female calls. There was also a difference in expression dependent upon the sex of the bird that perceived the calls (e.g., males had different neurological reactions than female birds to male calls, and male birds had different neurological reactions to male calls than female 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. Because the birds only ever heard calls and did not have access to any additional information about the caller (e.g., visual cues), this difference between the sexes must be encoded in the acoustics of calls produced.

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 (as established in Nowicki & Nelson, 1990). This process, called a bioacoustic analysis, allows for identification of features that chickadees could be using to categorize calls into the different groups.

Using a bioacoustic analysis, Freeberg and colleagues (2003) investigated the *chick-a-dee* calls of Carolina chickadees for sex differences and found that the starting frequency of A notes within their two study populations differed between males and females. Charrier and colleagues

(2004) conducted a bioacoustic analysis on the *chick-a-dee* calls of black-capped chickadees in which they examined what acoustic features allowed for the separation of notes into their note type categories (i.e., A, B, C, Dh, and D notes) and which features had the potential for identifying individual differences within those note types. In the process, they also investigated whether any of the features differed between the sexes. They identified features with high specificity to both *chick-a-dee* call note types and also individual identity (Charrier et al., 2004). This previous bioacoustic analysis on *chick-a-dee* calls did not find acoustic differences between male and female calls; however, both *tseet* calls and *fee-bee* songs of black-capped chickadees have been shown to vary based on sex of the producer (*tseet*: Guillette, Bloomfield, Batty, Dawson, & Sturdy, 2010a; *fee-bee*: Hahn et al., 2013b).

Current study

Based on the previous findings of both Avey et al. (2008) and Freeberg et al. (2003), I sought to re-examine the potential for sex differences coded in the acoustic features of the *chick-a-dee* calls of black-capped chickadees in a more comprehensive manner than the incidental investigation by Charrier and colleagues (2004). The main focus of the experiment conducted by Charrier et al. was to identify note type and individual identifying features in *chick-a-dee* calls, rather than to identify sex differences. As such, their analysis of features for sex differences was to conduct a *t*-test on each feature to see if the measures differed between the sexes. The limitations of such an examination stem from the use of multiple tests for the same null hypothesis, which can be lead to over-correction and a reduction in statistical power when correction procedures are undertaken; multiple measures from each individual can increase both type I and II error due to individual differences confounding the group differences; not being able to determine the extent to which the groups differ, only indicating if the groups do or do not differ; and missing the interactions between features by running each feature independently (Mundry & Sommer, 2007).

A more commonly used statistical procedure in behavioural research is the discriminant function analysis (DFA). This multivariate analysis employs only a single test for each null hypothesis, which uses multiple measured features to determine not only if those features differ between two groups, but how reliably they differ (Mundry & Sommer, 2007). It addresses all but one of the limitations raised above: the effects of using multiple measures per individual, which is a common practice in behavioural study. The bias created by using multiple, non-independent measures from individuals without correction is referred to as pseudoreplication. A variant of DFA called permuted discriminant function analysis (pDFA) as described by Mundry and Sommer (2007) accounts for this pseudoreplication when multiple vocalizations from an individual are analyzed (see Hahn et al. 2013a, 2013b; Foote, Palazzi, and Mennill 2012).

Using recordings of eleven male and eleven female wild-caught black-capped chickadees, I completed a bioacoustic analysis of twelve acoustic features within *chick-a-dee* calls using a pDFA to determine if any of those features differed significantly between the sexes. These features have been used previously to identify individual differences in the *chick-a-dee* calls of black-capped chickadees (Nowicki & Nelson, 1990; Charrier, Bloomfield, & Sturdy, 2004) and include measures of duration, frequency, and amplitude. These measures serve as a rough approximation of the shape and intensity of *chick-a-dee* call notes as they are represented visually in spectrograms (graphs of frequency by time) and spectrums (graphs of amplitude by frequency). Similar featural simplifications in *fee-bee* songs of black-capped chickadees were used to identify a characteristic within the song that differed between the sexes (the *fee glissando*; Hahn et al., 2013b) and Hahn and colleagues were able to show that manipulating that feature could change chickadees' perception of the sex of a song's producer (2015). I expected that one or more of the features measured on call notes could narrow down the source of 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), I predicted that a difference between the sexes would most likely be found in the A notes of their *chick-a-dee* calls.

Methods

Subjects

I used the vocalizations from eleven males and eleven females. Birds 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. Birds were determined to be at least one year of age in the field by examining their outer tail retrices (Pyle, 1997). Sex was later determined by DNA analysis (Griffiths et al., 1998).

Housing

Chickadees were individually housed in Jupiter Parakeet cages (30 cm wide x 40 cm high x 4 cm deep; Rolf C. Hagen, Inc., Montreal, QC) that prevented individuals from being in physical contact, 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 20ºC. 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.

Recordings

Birds were recorded from 20 March to 14 June 2012. Half-hour 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 PC following each recording session. Calls were obtained from recordings over one to four days (average: 1.73 days) for each individual bird. (See Hahn et al. 2013b for further recording details).

Acoustic Measures

Each bird produced at least 17 *chick-a-dee* calls (range: 17-248 calls). Call composition was determined in SIGNAL (version 5.05.02, Engineering Design, 2013) by visual analysis of a spectrogram by a single researcher using Ficken, Ficken, & Witkin (1978) and Otter (2007) as references.

A random selection of 20 of each A, B, C, Dh, and D notes were chosen for each bird using a random number generator to select 20 numbers from one to the total number of notes available. Individuals that produced fewer than eleven A, B, or D notes or fewer than six C or Dh notes were excluded from the analysis for the respective note type. Out of 11 females and 11 males, this left 10 males and 11 females for A note analysis, 10 males and 10 females for B note analysis, 6 males and 6 females for C note analysis, 5 males and 7 females for Dh note analysis, and 11 males and 11 females for D note analysis. 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, peak, and end frequency (SF, PF, and EF in Hz; measurements made from spectrograms with window size = 1024 points, frequency precision = 43.1 Hz); ascending, descending, and total duration (AD, DD, and TD in ms; measurements made from spectrograms with window size = 256 points, time resolution = 5.8 ms); slope of ascending frequency modulation (SAFM) 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 (SDFM) 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; F_{max} in Hz; measurements made from power spectrums 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 (SF, PF, EF, AD, DD, TD, SAFM, SDFM, F_{max}) as well as the D-portion duration (i.e., the length of the note segment that resembles a D note; DPD in ms), frequency of first visible harmonic (f0 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; NPF in Hz; measurements made from power spectrums with frequency precision = 2.7 Hz; window size varied with note length). Only four features were measured for D notes: TD, f0, F_{max}, and NPF, as described above. Figure 1 illustrates each of these acoustic measures and Table 1 summarizes the acoustic features measured for each note type.

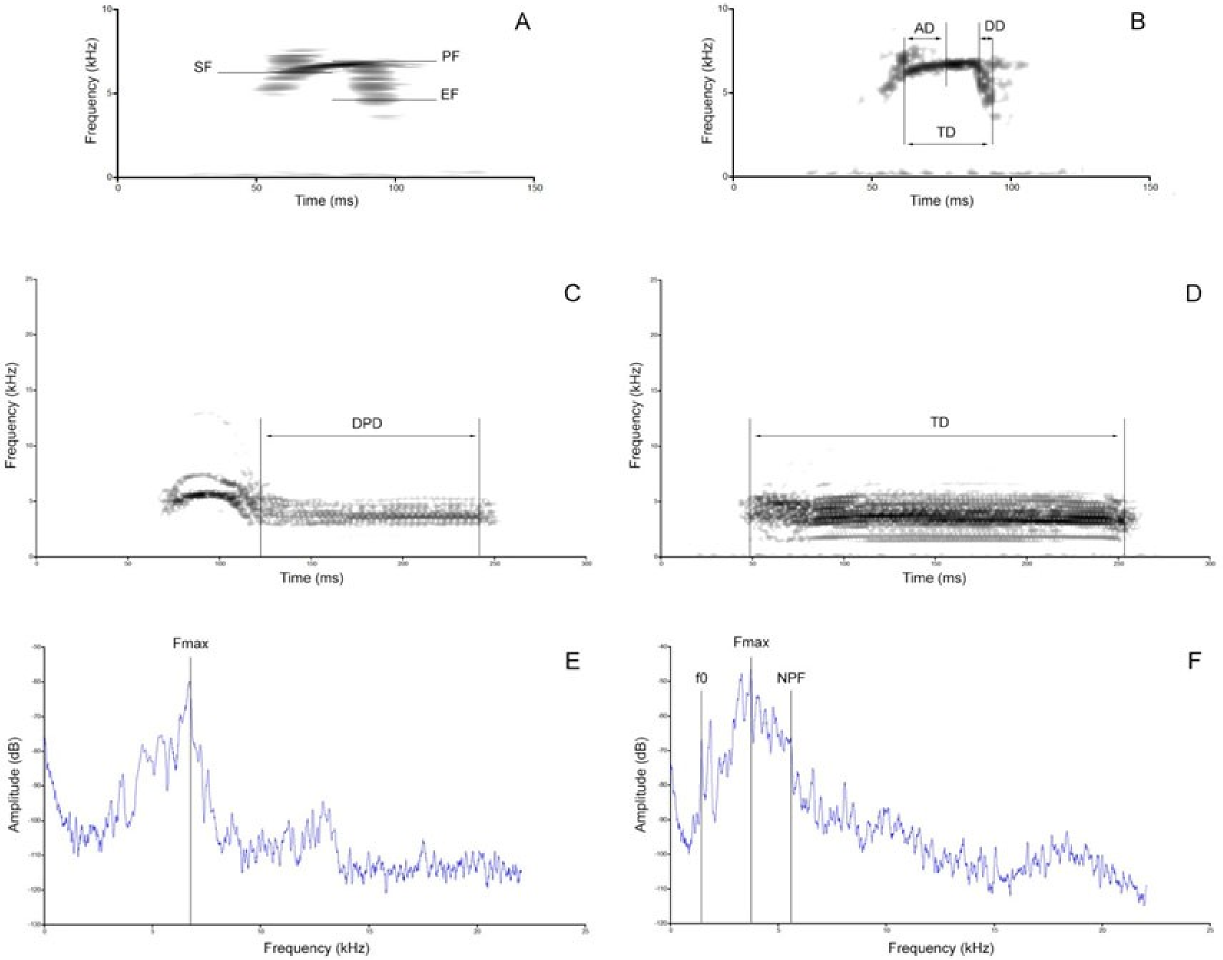

Figure 1. Acoustic measures made on individual note types of the *chick-a-dee* calls of black-capped chickadees. (A) Spectrogram of non-D notes (i.e., A, B, or C) resolved for frequency precision (window size = 1024 points, frequency precision = 43.1 Hz) for measuring start frequency (SF), peak frequency (PF), and end frequency (EF). (B) Spectrogram of non-D notes resolved 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 resolved for duration precision (window size = 256 points, time resolution = 5.8 ms) for measuring D-portion duration (DPD). (D) Spectrogram of D notes resolved 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 (f0), maximal frequency (F_{max}), and note peak frequency (NPF). Window size for power spectra varied with note length.

Table 1. Summary of acoustic features measured from each note type (A, B, C, Dh, and D) of black-capped chickadee *chick-a-dee* calls.

Measure	Abbr.	Measure	Definition	Note Type				
				A	B	C	Dh	D
Start Frequency	SF	Hz	Lowest frequency at the beginning of the note	X	X	X	X	
Peak Frequency	PF	Hz	Frequency when the note is no longer ascending	X	X	X	X	
End Frequency	EF	Hz	Lowest frequency at the termination of the note	X	X	X	X	
Ascending Duration	AD	ms	Length of time that the note's frequency increases	X	X	X	X	
Descending Duration	DD	ms	Length of time that the note's frequency decreases	X	X	X	X	
D-Portion Duration	DPD	ms	Length of note portion that resembles a D note				X	
Total Duration	TD	ms	Length of the note	X	X	X	X	X
Slope of Ascending Frequency Modulation	SAFM	Hz/ms	Speed at which the note increases in frequency	X	X	X	X	
Slope of Descending Frequency Modulation	SDFM	Hz/ms	Speed at which the note decreases in frequency	X	X	X	X	
Frequency of First Visible Harmonic	f0	Hz	Frequency of the lowest harmonic within 35 dB of the F_{max}				X	X
Maximal Frequency	F_{max}	Hz	Loudest or highest amplitude frequency	X	X	X	X	X
Note Peak Frequency	NPF	Hz	Highest frequency				X	X

Statistical Analyses

To determine if any measured acoustic features varied between males and females, I first calculated the coefficients of variation both within the sexes ($\mathrm{CV}_{\mathrm{within}}$) and between the sexes ($\mathrm{CV}_{\mathrm{between}}$) for each of the measured acoustic features for each note type. CVs are a useful means of determining how variation in different samples (here, males and females) compares. The coefficient of variation within the sexes was calculated with the following formula:

$$\mathrm{CV_w} = \frac{\mathrm{SD_w}}{\mathrm{mean}} \times 100$$

where $\mathrm{SD_w}$ is the standard deviation and mean is the average, both of which are calculated using the measures from all notes from the sex of interest. The coefficient of variation between the sexes was calculated with the following formula:

$$\mathrm{CV_b} = \frac{\mathrm{SD_b}}{\mathrm{mean}} \times 100$$

where $\mathrm{SD_b}$ is the standard deviation and mean is the average calculated using the measures from all notes from the entire sample.

Then, the potential for sex coding (PSC) was calculated for each feature for each note type using the formula:

$$\mathrm{PSC} = \frac{\mathrm{CV_b}}{\mathrm{mean\ CV_w}}$$

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

Discriminant function analyses (DFA) are commonly used by bioacoustic researchers to determine if vocalizations differ between groups or individuals (Mundry & 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 & Fidell, 2007). In this case, a DFA was run for each note type using all features measured for that note type to determine if any feature or features could be used to accurately classify the notes by the sex of their producer.

A stepwise DFA was run on each note type to analyze the notes in regards to the sex of the producer. The analysis was conducted in R (version 3.0.3, R Foundation for Statistical Computing, 2013) with both “MASS” (Venables & Ripley, 2002) and “klaR” (Weihs et al., 2005) software packages installed using the leave-one-out method for cross-validation. This 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 to determine the accuracy of the discriminant function. The process is repeated until all cases are classified using derived discriminant functions (Bertz, 1987). This is a useful method because it uses new cases (i.e., cases not used to determine the discriminant function) to test the effectiveness of the discriminant function.

For the DFA on A, B, and D note types, ten notes were randomly selected for each individual. Due to their rarity, only five C notes and five Dh notes were randomly chosen for 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 permuted DFA to account for pseudoreplication and the associated issues that arise when multiple vocalizations are used from each individual. In this procedure, the percent correct classifications for 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 this stage, only those features identified as being used above chance levels were analyzed. Again, ten notes were randomly selected for each individual for each note type (with five being selected for C and Dh notes) and the randomization was repeated 100 times with the average percentage of correct classifications 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 and Dh notes. The analyses randomly assigned the notes of an individual as either male- or female-produced, independent of the individual's actual 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). A significant *p*-value indicated that the non-randomized data performed better than the randomized data at classifying the sex of a call-producer.

Five multivariate analyses of variances (MANOVA) were also conducted using the data in IBM SPSS for Windows (version 20.0.0; IBM Corp., 2011), one for each note type. The analyses was conducted using the average of all features measured for each individual for each note type, rather than using measurements from multiple notes produced by the same individual to avoid issues associated with pseudoreplication. The MANOVA then compared the average notes produced by males and females. A significant result would indicate that there were sex differences within a note type and serve as a confirmation of results obtained from the pDFA.

Results

A total of 1645 notes were 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). Recorded calls from which the notes were randomly selected had on average 1.67 ± 0.61 A notes (range: 0 to 20), 0.90 ± 0.51 B notes (range: 0 to 14), 0.39 ± 0.30 C notes (range: 0 to 5), 0.22 ± 0.24 Dh notes (range: 0 to 1), and 3.79 ± 1.31 D notes (range: 0 to 14).

Table 2 shows the means, standard deviations, coefficients of variance, and potential for sex coding for all acoustic features measured across all note types. It shows that between notes, A notes tended to have the highest start, peak, and end frequencies, followed closely by B notes, with C notes having the lowest average values for those measures. Dh notes had measures equivalent to the start frequencies of B notes and to the peak and end frequencies of C notes. This corresponds to previous findings that A notes are perceived by chickadees as similar to B notes and B notes as similar to C notes, showing the relatedness between the three note types (Sturdy, Phillmore, & Weisman, 2000). A notes had the longest ascending duration, Dh notes had the shortest, and B and C notes were intermediate. Dh notes had a longer descending duration than any of the non-D notes. Dh and D notes were longer than A notes, which were longer than both B and C notes.

Both A and Dh notes tended to have higher start frequencies than end frequencies, meaning they formed asymmetrical inverted U's; B and C notes had approximately equal start and end frequencies, forming symmetrical inverted U's. A, B, and C notes all displayed longer ascending than descending durations, meaning the notes tended to take more time to rise to their peak frequency than to drop from it. Dh notes had approximately equal ascending and descending durations. A, B, C, and Dh notes all showed a larger magnitude SAFM than SDFM; the difference between the two measures got smaller as note order progressed (i.e., A notes had the largest difference and Dh notes had the smallest) (Table 2).

For A, B, C, and Dh notes, the largest magnitude CV was SDFM for both sexes combined and for each sex individually with the exception of males' Dh notes in which SAFM was marginally larger (SAFM: CV = 55.16; SDFM: CV = 47.99). For D note, TD was the largest magnitude CV for both sexes combined and for females (CV = 11.12 and 12.81, respectively), however f0 was largest for males (CV= 10.65). A and B notes both had SAFM as the largest PSC value with 1.09 and 1.08, respectively; C notes had a PSC of 1.13 for both SS and SDFM; Dh notes had a DD PSC of 1.15; and D notes had a PSC of 1.05 for TD (Table 2).

Stepwise Discriminant Function Analysis

The stepwise DFAs used to classify calls based on the sex of the producer using the measured acoustic features from each of the note types yielded a mean percentage of correct classification of withheld notes which is presented as mean ± SD with the range of correct classifications included. The stepwise DFAs for all note types had mean percentage of correct classifications as follows: A notes:63.46 ± 2.27% (range: 58.10 – 68.10%); B notes: 64.49 ± 2.52% (range: 59.00 – 71.50%); C notes: 72.73 ± 4.49% (range: 63.33 – 85.00%); Dh notes: 68.52 ± 4.68% (range: 56.72 – 80.60%); D notes: 60.25 ± 2.32% (range: 53.95 – 65.12%). Table 3 lists the number of times each acoustic feature was used by the DFA to construct a discriminant function out of the 100 conducted permutations.

Table 2. Acoustic feature measures from all note types of black-capped chickadee *chick-a-dee* calls.

Note Type	Value	Start Frequency (SF in Hz)	Peak Frequency (PF in Hz)	End Frequency (EF in Hz)	Ascending Duration (AD in ms)	Descending Duration (DD in ms)	D-Portion Duration (DPD in ms)	Total Duration (TD in ms)	Slope of Ascending Frequency Modulation (SAFM Hz/ms)	Slope of Descending Frequency Modulation (SDFM Hz/ms)	Frequency of First Visible Harmonic (f0 in Hz)	Maximal Frequency (F_{max} in Hz)	Note Peak Frequency (NPF in Hz)
A Notes	**Both sexes**												
	Mean$_{sample}$	6359.27	7253.62	5463.58	25.00	11.12	-	54.34	42.35	-312.02	-	7054.83	-
	SD$_{sample}$	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$_{female}$	6509.49	7306.97	5499.69	26.19	12.48	-	57.69	38.60	-288.91	-	7099.82	-
	SD$_{female}$	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$_{male}$	6194.02	7194.94	5423.86	23.69	9.61	-	50.66	46.61	-338.22	-	7005.33	-
	SD$_{male}$	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$_{sample}$	4690.60	6577.21	4618.61	18.14	10.16	-	38.47	125.71	-258.94	-	6277.57	-
	SD$_{sample}$	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$_{female}$	4690.51	6549.19	4570.96	18.46	10.87	-	39.40	129.44	-257.33	-	6205.39	-
	SD$_{female}$	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$_{male}$	4690.71	6611.47	4676.85	17.76	9.29	-	37.34	121.35	-260.86	-	6365.78	-
	SD$_{male}$	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$_{sample}$	3480.43	5844.60	3686.86	20.90	11.17	-	39.07	135.43	-245.79	-	5203.30	-
	SD$_{sample}$	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$_{female}$	3406.68	6100.19	3610.35	24.79	10.11	-	41.29	126.77	-310.18	-	5456.67	-
	SD$_{female}$	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	-
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$_{female}$	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$_{female}$	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$_{male}$	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$_{male}$	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$_{sample}$	-	-	-	-	-	-	175.62	-	-	1670.65	3587.60	5623.60
	SD$_{sample}$	-	-	-	-	-	-	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$_{female}$	-	-	-	-	-	-	177.05	-	-	1658.34	3595.32	5670.09
	SD$_{female}$	-	-	-	-	-	-	22.68	-	-	168.67	319.43	327.77
	CV$_{within}$	-	-	-	-	-	-	12.81	-	-	10.17	8.88	5.78
	Males												
	Mean$_{male}$	-	-	-	-	-	-	173.91	-	-	1685.42	3578.35	5567.82
	SD$_{male}$	-	-	-	-	-	-	15.47	-	-	181.92	246.05	332.30
	CV$_{within}$	-	-	-	-	-	-	8.46	-	-	10.65	6.78	5.72

Table 3. 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 bolded.

Note Type	**Start Frequency** (SF)	**Peak Frequency** (PF)	**End Frequency** (EF)	**Ascending Duration** (AD)	**Descending Duration** (DD)	**Total Duration** (TD)	**Slope of Ascending Frequency Modulation** (SAFM)	**Slope of Descending Frequency Modulation** (SDFM)	**Frequency of First Visible Harmonic** (f0)	**Maximal Frequency** (F_{max})	**Note Peak Frequency** (NPF)
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}	87^{I} (91^{I}DPD)	84^{I}	**92**^{I}	67^{I}	72^{I}	63
D Notes	-	-	-	-	-	60	-	-	71^{I}	56	**98**^{I}

Permuted Discriminant Function Analyses

Using a $p < 0.01$, I 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 permuted DFA (pDFA) was conducted with only those features that remained for each note type. In addition, pDFAs were conducted with only the most used feature from each note type (A notes: SF; B notes: SAFM; C notes: F_{max}; Dh notes: SDFM; and D notes: NPF). The results of these analyses are summarized in Table 4.

The mean percentage of correctly classified elements from the original data (i.e., the data where sex was not randomized) for the above chance features ranged from 58.20% (D notes) to 68.18% (C notes). The mean percent of correctly classified elements for the randomized data had a range of 53.70% (D notes) to 61.74% (Dh notes). A and B notes had the lowest p-values ($p = 0.170$ and $p = 0.169$, respectively) and Dh notes had the highest ($p = 0.560$). These results show no significant difference between the measured acoustic features of female and male calls for any note type.

A pDFA was conducted using both those features that were used more than 63% of the time and on the most used feature for each note type. The mean percentage of correctly classified elements from the original data for the top used features ranged from 54.57% (B notes) to 65.93% (C notes). The randomized data produced a mean percent of correctly classified elements range of 52.63% (D notes) to 54.09% (Dh notes). A notes had the lowest p-value ($p = 0.066$) and Dh notes had the highest ($p = 0.500$). In the pDFA for A note start frequency, a p-value of 0.066 means that for 66 times out of the 1000 permutations, the percent correct classification for the randomized data was equal to or better than the percent correct classification of the original data (i.e., only 6.6% percent of the cases with randomized sexes were classified at a rate equivalent to the original data). In this case, females produced A notes with higher start frequency than males did.

MANOVA

Results from the MANOVAs closely reflected those of the pDFAs 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: $p = 0.137$).

Table 4. Results of permuted discriminant function analyses on features used above chance levels 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)**	f0, 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)**	f0, 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

Discussion

In this study, I measured acoustic features within each note type of the *chick-a-dee* call of the black-capped chickadee as part of a bioacoustic analysis to determine which, if any, features could be used to discriminate male- and female-produced calls. The potential for sex coding (PSC) calculated for each acoustic feature measured for each note type showed almost equal variance between groups (here, between the sexes) as within the groups (within males and within females), meaning that none of the features stood out as a clear signal of the producer's sex. Of all the note types, C notes had the most acoustic feature PSC values over 1 (the point of equal variance between and within groups), but the values were not significantly different and the results were not corroborated by the permuted discriminant function analysis (pDFA).

The results of the pDFA showed that of the five note types that were investigated (A, B, C, Dh, and D notes), acoustic measures within 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 could imply that there are features related to start frequency in A notes that could signal the sex of the caller.

A notes were the most common non-D notes (i.e., A, B, C) that were produced in the sampled *chick-a-dee* calls. The prevalence of A notes in *chick-a-dee* calls may suggest that A notes would be a good place for sex differences to be encoded because they are present in the majority of calls and thus the caller's sex could be signaled often. Freeberg et al. (2003) found a similar result to those of this study in Carolina chickadees in which the beginning frequency of A notes varied between the sexes in their two study populations. Charrier and colleagues (2004) conducted a bioacoustic analysis on the same acoustic features as in the current study and found that start frequency was one of the acoustic features useful in the discrimination of non-D notes (i.e., being able to tell A notes from B notes and C notes). These results suggest that the start frequency of a note (specifically A notes) could be used to determine not only the type of note being produced, but also the sex of the caller producing that note.

B notes also had a relatively low *p*-value for the pDFA computed based on acoustic features that were used in discriminant functions above chance levels, but unlike the case with A notes in which the discrimination performed closer to standard significance levels when only the top feature was used, the discriminant functions for B notes performed more poorly when the pDFA was re-run with only the top used feature. Thus, neither B notes nor any of the other notes could be used to reliably discriminate male and female calls. With no statistically significant difference in features between males and females, it appears that *chick-a-dee* calls may be more sexually monomorphic than other vocalizations of black-capped chickadees (*tseet*: Guillette et al., 2010a; *fee-bee*: Hahn et al., 2013b) and other songbird species (contact calls in Bengalese finches (*Lonchura striata domestica*): Okanoya, 1993; zebra finches: Simpson & Vicario, 1990) that have been analyzed previously.

Many of the other vocalizations that have been shown to be sexually dimorphic in songbirds are either contact calls (e.g., *tseet* calls) which serve to maintain contact between birds in flocks and pairs (Ficken et al., 1978), or songs (e.g., *fee-bee* songs) which are used for mate attraction and territory defense (Ficken et al., 1978): two cases where knowing the sex of a conspecific is essential to an individual's fitness. *Chick-a-dee* calls are not often used in situations where knowing the sex of an unknown individual appears to be critical; instead, they are involved in flock coordination, predator mobbing, and alerting conspecifics (Ficken et al., 1978). Therefore, features signaling the sex of the caller may be less prominent in *chick-a-dee* calls than other vocalizations where the sex of the caller is linked to the function of that vocalization.

Given that *chick-a-dee* calls serve multiple signaling functions, it is unsurprising that the notes of the call have been found to contain information on individual identity (A, B, and C notes: Charrier et al., 2004; D notes: Mammen, & Nowicki, 1981), geographic location (A, C, and D notes in Carolina chickadees: Freeberg et al., 2003), and flock identity (D notes: Nowicki, 1989). The method of acquisition for this variety of information has been debated. Are *chick-a-dee* calls learned (i.e., only properly produced when young birds can hear and imitate adults) or innate (i.e., genetically encoded and properly produced even in the absence of tutors)? Freeberg et al. (2003) suggest that the differences due to geography in their samples are likely learned because the populations being investigated are not geographically distant enough for genetic differences to account for the vocal divergence. Flock identity is also a learned feature which has been found in the vocal plasticity of D notes of both wild (Mammen & Nowicki, 1981) and captive (Nowicki, 1989) black-capped chickadees (called flock convergence).

Hughes et al. (1998) reared black-capped chickadee chicks in isolation from adult vocalizations, but not other similarly isolated conspecifics. In this situation, B and C notes of isolate-reared birds developed abnormally, whereas A and D notes did not. This implies that a conspecific tutor was necessary for the development of species-typical B and C note production. However, the A notes of the isolate birds were featurally indistinguishable from those of wild-caught birds. This means that the A notes of *chick-a-dee* calls have a strong innate component and are not learned from adult conspecifics. With B, C, and D notes of *chick-a-dee* calls being either learned or flexible, they do not provide an ideal platform for conveying a consistent signal such as the sex of the caller. A notes, which are not learned, are thus more likely to be stable across time and would be able to reliably convey the sex of an individual.

Within *chick-a-dee* calls of black-capped chickadees, Dh notes have not yet been investigated for any encoded information. In fact, the literature does not make reference to them as part of the vocal repertoire of black-capped chickadees at all (Ficken et al., 1978; Smith, 1991). They are, however, a well-documented component of Carolina chickadee calls (e.g., see Bloomfield et al., 2005). Of the 22 birds in my sample, 12 produced Dh notes. My analyses revealed that none of the features measured in Dh notes could be used to discriminate male- and female-produced calls ($p = 0.560$). However, Dh notes could hold information about individual identity or could be involved in flock convergence and therefore flock identity (similar to D notes); with its structure resembling a combination of an introductory note (A, B, or C) and a D note, Dh notes could encode information that had been found in both categories of note, or neither. 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.

Limitations

It must be considered that all note types are known to contain features that vary with different naturally-occurring categories (e.g., identity, flock, geography). This additional information can complicate the search for sex differences. The pDFA attempted to account for these differences using the permutation procedure, but any additional, uncontrolled variables can complicate an analysis and make it more difficult to find statistical significance in subtle differences.

Chickadees perceive their vocalizations aurally; however, my analysis was based on visual representations of the acoustic structures. This discrepancy could mean that my analysis was unable to detect or failed to quantify one or more features within the calls that the chickadees use to discriminate the sex of callers. In the same way that human speech is 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. The birds could be attending to a combination of features within a note, between notes, and/or across note types. This restriction of a bioacoustic analysis limits the detection of differences because it cannot provide evidence that the birds perceive the difference that is analytically identified. For this reason, bioacoustic analyses tend to be followed up with behavioural, perceptual experiments, such as operant conditioning experiments, to determine which aspects of the calls are actually attended to using the findings of the bioacoustic analyses as a starting point.

Future Research

The questions raised by this study can drive investigation of whether sex differences between the notes of *chick-a-dee* calls of black-capped chickadees are innate or learned. Research can also address if and how the composition of the *chick-a-dee* calls themselves can affect the information being signaled. For example, do females tend to produce pairs of A notes before C notes, while males prefer triplets of A notes?

Since it has been shown that chickadees have different neural reactions differently to male and female calls (Avey et al., 2008), but the present analyses were unable to definitively predict what features they are attending to, future studies employing operant conditioning could assist in localizing the information. An experiment in which birds are rewarded for responding to the *chick-a-dee* call notes of one sex (for example, males) while being punished for responding to the notes of the other sex (here, females) could illustrate the chickadees' ability to discriminate the sexes. If they are able to learn the discrimination for all note types, it would suggest that all note types can signal the sex of the caller. If only one note type can be consistently discriminated, an additional discrimination could be performed where calls are manipulated such that one note type within a call is replaced by notes from the other sex. For example, a male call could be modified by replacing only the A notes with A notes from a female call.

Beyond the limits of individual notes and note features, future studies could focus on the composition of entire *chick-a-dee* calls. Carolina chickadee calls 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 their close relatives, the 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 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. This redundancy could mean that sex differences are encoded multiple places within a call and could even be stored across multiple notes and note types. A thorough analysis of the composition of both male and female calls could identify any such trends.

Conclusions

The *chick-a-dee* call of black-capped chickadees is an acoustically complex vocalization that contains variable features that can be used to discriminate among individuals, flocks, and geographic locations. The current study did not identify any features within the note types of the call that could be used to differentiate male- and female-produced calls, but the results suggest that at least one acoustic feature in A notes may differ between the sexes. Future research should focus on the potential for sex differences within the compositions of calls rather than just on features within the individual note types. If differences are observed, the behavioural aspects of discriminating male and female calls can be investigated to gain a better understanding of what the chickadees are attending to when they listen to male- and female-produced calls.

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