

Black-capped Chickadees (*Poecile atricapillus*) can identify individual females by their fee-bee songs

Carolina Montenegro,¹ William D. Service,¹ Erin N. Scully,¹ Shannon K. Mischler,¹ Kimberley A. Campbell,¹ and Christopher B. Sturdy^{1,2,a,*}

¹Department of Psychology, University of Alberta, Edmonton, Alberta, Canada

²Neuroscience and Mental Health Institute, University of Alberta, Edmonton, Alberta, Canada

^aCurrent address: Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

*Corresponding author: csturdy@ualberta.ca

ABSTRACT

Individual recognition is a social behavior that occurs in many bird species. A bird's ability to discriminate among familiar and unfamiliar conspecifics is critical to avoid wasting resources such as time and energy during social interactions. Black-capped Chickadees (*Poecile atricapillus*) are able to discriminate individual female and male chick-a-dee calls, potentially male and female tseet calls, and male fee-bee songs. In the current study, we used an operant discrimination go/no-go paradigm to determine whether female and male chickadees could discriminate between fee-bee songs produced by individual female chickadees as well as test which song component(s) enable this discrimination. Birds trained on natural categories—the songs of different females—learned to respond to rewarded stimuli more quickly than birds trained on random groupings of female songs and were able to transfer this learning to new songs from the same categories. Chickadees were also able to generalize their responding when exposed to the bee note of the fee-bee song of rewarded individuals; they did not generalize to fee notes. Our results provide evidence that Black-capped Chickadees can use female-produced fee-bee songs for individual recognition. However, the acoustic features underlying individual recognition require further investigation.

Keywords: Black-capped Chickadee, communication, female song, individual recognition, *Poecile atricapillus*, operant conditioning, song

LAY SUMMARY

- The current study used an operant conditioning paradigm to test whether Black-capped Chickadees can distinguish between the songs of individual female Black-capped Chickadees.
- Research on female song in Black-capped Chickadees has shown that female song differs from male song in sound and perception, and chickadees can distinguish between male and female song.

- We found that male and female chickadees can distinguish between females by listening to their song, and can do so using the whole song and only part of the song.
- A chickadee's ability to distinguish song by sex as well as by the individual female suggests that female song does serve a function in Black-capped Chickadees.

INTRODUCTION

In temperate bird species, song is considered a sexually selected signal generally used predominantly by males, serving 2 main functions: (1) territorial defense and (2) mate attraction (Catchpole and Slater 2008). Most studies examining temperate songbird vocalizations have concentrated on males, leading to the misconception that females lack song (Langmore 1998, Riebel 2003). However, reports and studies of female song in temperate species are ever increasing, and in many species, it is now recognized that both males and females can and do produce song (Langmore 1998, Riebel 2003, Odom and Benedict 2018, Riebel et al. 2019). In a review of 323 songbird species, it was reported that female song is present in 71% of species, including Yellow Warblers (*Dendroica petechia*; Hobson and Sealy 1989), European Starlings (*Sturnus vulgaris*; Sandell and Smith 1997), Red-winged Blackbirds (*Agelaius phoeniceus*; Yasukawa 1989), and the focus of the current study, Black-capped Chickadees (*Poecile atricapillus*; Hahn et al. 2013). This is not to say that females of the remaining 29% of species do not sing, only that females have not been documented singing (Odom et al. 2014).

The Black-capped Chickadee fee-bee song is a simple 2-note vocalization that is used primarily by males for territorial defense and mate attraction (Ficken et al. 1978, Smith 1991). There are several accounts of females singing songs that are acoustically similar to male fee-bee songs (i.e. songs are tonal and contain 2 notes) both in field (Dwight 1897, Hill and Lein 1987) and laboratory settings (Hahn et al. 2013). While observations and studies on female song in Black-capped Chickadees are very sparse, the song seems to serve a similar purpose. Early observations of breeding pairs indicate that while males produce the fee-bee song more often than females, both sexes produce the song to protect territories during the spring (Odum 1942). The first note in the song (fee) is produced at a higher frequency compared to the second note (bee) and the frequency of the fee note decreases over the duration of the note (referred to as the fee glissando; Hahn et al. 2013). The fee glissando is less pronounced in males than in females. In male songs, the fee glissando is highly stereotyped (Christie et al. 2004b); however, the stereotypy of the female fee glissando has yet to be examined. Black-capped Chickadees are able to identify the sex of an individual using the fee glissando within their fee-bee song (Hahn et al. 2015). In addition to being able to determine the sex of an individual via song, the ability to identify individuals via song is also beneficial in distinguishing between friend or foe, whether conspecific, heterospecific, or predator. In several species, discriminating between individuals via acoustic signals has been shown to facilitate identification of a familiar conspecific (e.g., Song Sparrow [*Melospiza melodia*]; Stoddard et al. 1990) or a mate (e.g., Zebra Finch [*Taeniopygia guttata*]; Miller 1979). A recent study has suggested that the fee-bee song in the Black-capped Chickadee is used for mate recognition (Hahn et al. 2013) and in order to be used

for mate recognition the fee-bee song would need to contain information concerning individual identity.

For Black-capped Chickadees specifically, previous studies have indicated that male Black-capped Chickadee song contains information regarding individual identity (Phillmore et al. 2002, Christie et al. 2004b, Hoeschele et al. 2010, Wilson and Mennill 2010, Hahn et al. 2015). A study examining fee-bee songs produced by Black-capped Chickadees in eastern Ontario suggested that the total duration of song is used by conspecifics to identify individual males and may encode male quality (Christie et al. 2004b). In addition, Black-capped Chickadee males and females eavesdrop on male singing contests suggesting that both males and females attend to song bouts and use song to identify successful and unsuccessful conspecifics and their quality (Mennill et al. 2002, Christie et al. 2004b, Mennill and Ratcliffe 2004). Past operant go/no-go discrimination tasks (Phillmore et al. 2002) and playback studies (Wilson and Mennill 2010) have indicated that male Black-capped Chickadees can discriminate between individual males via fee-bee songs. Recently researchers have proposed that one of the functions of female song includes advertising individual quality (Langmore 1998, Odom and Benedict 2018, Riebel et al. 2019). The ability to differentiate between individual females could aid in assessing quality and identifying rank.

In the current study, we use an operant go/no-go paradigm to determine (1) if male and female Black-capped Chickadees can discriminate between individual female Black-capped Chickadee fee-bee songs, and (2) the song component(s) that enable this discrimination. We trained Black-capped Chickadees in an operant discrimination task and tested the birds using both unmanipulated songs and acoustically manipulated songs. We were interested in examining whether chickadees could identify individual female chickadees based on song and whether they use acoustic features in one or both notes within the song when discriminating between individuals. The results add to a growing body of literature on female song in temperate songbird species and provide valuable insights into sex-based discrimination of songs in this species and the function of the fee-bee song in females.

METHODS

Subjects

Eighteen Black-capped Chickadees (9 males and 9 females) were tested between February and June, 2019. In total, 16 Black-capped Chickadees (8 males and 8 females) completed the experiment. One female failed to learn non-discrimination training (see description below) and was removed from the experiment, and one male died of natural causes (see Ethical Note). Sex was determined by deoxyribonucleic acid analysis of blood samples (Griffiths et al. 1998). Birds were captured in Edmonton (North Saskatchewan River Valley, 53.53°N, 113.53°W; Mill Creek Ravine, 53.52°N, 113.47°W), Alberta, Canada, in January 2018. All birds were at least one year of age at capture, verified by examining outer tail rectrices (Pyle 1997).

Prior to the experiment, birds were individually housed in parakeet cages ($30 \times 40 \times 40$ cm; Rolf C. Hagen, Montreal, Quebec, Canada) in colony rooms. Birds had visual and auditory, but not physical, contact with each other. Birds had ad libitum access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St. Louis, Missouri, USA), water with vitamins supplemented on alternating days (Prime Vitamin Supplement; Rolf C. Hagen), grit, and a cuttlebone. Additional nutritional supplements included 3–5 sunflower seeds daily, one superworm (*Zophabas morio*) 3 times a week, and a mixture of hard-boiled eggs and greens (spinach or parsley) twice a week. The colony rooms were maintained at $\sim 20^{\circ}\text{C}$ and on a light:dark cycle that followed the natural light cycle for Edmonton, Alberta, Canada.

All birds had previous experience with at least one operant experiment involving chick-a-dee calls (7 birds with 1 experiment and 9 birds with 2 experiments) but none of the birds had previous experimental experience with Black-capped Chickadee-produced fee-bee songs in any experimental paradigm.

Apparatus

During the experiment birds were housed individually in modified colony room cages ($30 \times 40 \times 40$ cm) placed inside a ventilated, sound-attenuating operant chamber. The chambers were lit with a full spectrum LED bulb (3W, 250 lm E26, Not-Dim, 5000 K; Lohas LED, Chicago, Illinois, USA) and the natural light/dark cycle for Edmonton, Alberta, was maintained throughout the experiment. Each cage contained 2 perches in addition to a perch fitted with an infrared sensor (i.e. request perch), a water bottle, grit cup, and cuttlebone. Birds had ad libitum access to water (with vitamins supplemented on alternating days), grit, and cuttlebone and were provided 2 superworms daily (one in the morning and one in the afternoon). An opening (11×16 cm) on the left side of the cage allowed the birds to access a motorized feeder, which was also equipped with an infrared sensor (Njegovan et al. 1994). Food was only available as a reward for correct responses to auditory stimuli during the operant discrimination task. A personal computer connected to a single-board computer (Palya and Walter 1993) scheduled trials and recorded responses to stimuli. Stimuli were played from a personal computer hard drive through a Cambridge Integrated Amplifier (model A300 or Azur 640A; Cambridge Audio, London, England). Stimuli played in the chamber through a Fostex full-range speaker (model FE108 Σ or FE108E Σ ; Fostex, Japan; frequency response range: 80–18,000 Hz) located beside the feeder. See Sturdy and Weisman (2006) for a detailed description of the apparatus.

Recordings of Acoustic Stimuli

Six female Black-capped Chickadees' fee-bee song recordings were used for the current study; 4 birds were recorded in spring 2012 and 2 birds were recorded in fall 2014. Birds were captured in Edmonton (North Saskatchewan River Valley, 53.53°N , 113.53°W ; Mill Creek Ravine, 53.52°N , 113.47°W), Alberta, Canada, in January 2010, 2011, 2012, and 2014. All birds were at least one year of age at capture, verified by examining outer tail rectrices (Pyle 1997). A recording session for an individual bird lasted ~ 1 hr and all recordings took place at 0815 hours after colony lights turned on at 0800 hours. Birds were recorded individually in their colony room cages, which were placed in sound-attenuating chambers ($1.7\text{m} \times 0.84\text{m} \times 0.58\text{m}$;

Industrial Acoustics, Bronx, New York, USA). Recordings were made using an AKG C 1000S (AKG Acoustics, Vienna, Austria) microphone connected to a Marantz PMD670 (Marantz America, Mahwah, New Jersey, USA) digital recorder (16-bit, 44,100 Hz sampling rate). The microphone was positioned 0.1 m above and slightly behind the cage. Following a recording session, audio files were analyzed and cut into individual files using SIGNAL 5.03.11 software (Engineering Design, Berkley, California, USA).

Acoustic Stimuli

A total of 156 vocalizations were used as stimuli in the current experiment composed of 26 fee-bee songs produced by each of 6 female Black-capped Chickadees. Of the 26 songs produced by each bird, 24 songs were used without modification and 2 songs were edited to create spliced songs using SIGNAL. Spliced songs contained either a fee note or a bee note from the original song and, respectively, either a bee note or a fee note from another bird not included in the study. When creating the spliced songs, the internote interval was held constant at 100 ms, similar to the internote interval in natural songs (e.g., Xinternote = 135 ms; Ficken et al. 1978) and the internote interval used by other studies manipulating song features (e.g., Xinternote = 100 ms; Hoeschele et al. 2010, Hahn et al. 2015). Since songs are sung over a range of absolute frequencies (Weisman et al. 1990, Horn et al. 1992, Mennill and Otter 2007), frequencies of replacement notes were manipulated using Audacity 2.2.2 software so that the start frequency of the fee note used matched the start frequency of the fee note that was being replaced in the song.

All vocalizations were of high quality (i.e. no audible interference) and were bandpass filtered (lower bandpass: 500 Hz, upper bandpass: 14,000 Hz) using GoldWave 6.31 (GoldWave, St. John's, Newfoundland, Canada) to reduce any background noise. For each stimulus, 5 ms of silence was added to the leading and trailing portion of the vocalization and tapered to remove transients (in order to reduce “popping” from transients during stimulus playback) and amplitude was equalized using SIGNAL 5.03.11 software. During the experiment, stimuli were presented at ~75 dB as measured by a Brüel and Kjær Type 2239 (Brüel and Kjær Sound and Vibration Measurement A/S, Nærum, Denmark) sound pressure meter (A-weighting, slow response).

Procedure

Non-discrimination training. Pretraining began once the bird learned to use the request perch and feeder to obtain food. During pretraining, birds were trained to respond to a tone (1,000 Hz) 1 s in length to receive access to food. Acclimatization to the chamber, feeder, and speaker occurred over an approximately 15-day period. The average time to complete non-discrimination training ranged from 10 days to 41 days (mean = 21.43, SD = 9). During non-discrimination training, birds received food for responding to all fee-bee song stimuli. Each trial began when a bird landed on the request perch and remained for 900–1,100 ms, at which point a randomly selected stimulus played. Songs were presented in random order from trial to trial until all 156 stimuli had been heard without replacement; once all 156 stimuli were used, a new random sequence was generated and initiated. If the bird left the request perch during a stimulus presentation, the trial was considered interrupted, resulting in a 30-s lights out period. If the bird entered the feeder within 1 s after the stimulus played, it was given 1 s access to food, followed by a 30-s intertrial

interval. If a bird remained on the request perch during the stimulus presentation and the 1 s following the completion of the stimulus, it received a 60-s intertrial interval with the lights on, but this interval ended if the bird left the request perch. The above procedure engenders a high level of responding on all trials. Birds continued on non-discrimination training until they completed six 468-trial blocks at $\geq 60\%$ responding on average to all stimuli, at least four 468-trial blocks at $\leq 3\%$ difference in responding to future rewarded vs. unrewarded discrimination stimuli, at least four 468-trial blocks at $\leq 3\%$ difference in responding to future rewarded vs. unrewarded transfer stimuli, and at least four 468-trial blocks at $\leq 3\%$ difference in responding to spliced stimulus types (fee replaced vs. bee replaced). Following a day of free feed, birds completed a second round of non-discrimination training in which they complete at least one 468-trial block that met each of the above requirements. A 468-trial block consisted of the bird experiencing each of the 156 stimuli 3 times. Non-discrimination training is necessary in order to expose the bird to all the stimuli that will be used in the experiment and to ensure the birds treat the stimuli equivalently. See Figure 1 for flowchart of study stages.

Discrimination training. The discrimination training procedure was similar to previous training; however, of the original 156 stimuli, only 60 training stimuli were presented, and responses to these stimuli were differentially reinforced. In particular, responses to half of the stimuli (“rewarded stimuli”, S+) were positively reinforced with 1 s access to food, as before, and responses to the other half (“unrewarded stimuli”, S-) were instead punished with a 30-s intertrial interval with lights off. Discrimination training continued until birds completed six 360-trial blocks with a discrimination ratio (DR) between S+ and S- of >0.80 with the last 2 blocks being consecutive. For DR calculations see Response Measures below.

Subjects were randomly assigned to either a True category discrimination group ($n = 10$) or Pseudo category discrimination group ($n = 6$). Black-capped Chickadees in the True category discrimination group were divided into 2 subgroups: (1) True 1 ($n = 5$) discriminated between 30 rewarded fee-bee songs produced by 3 individual chickadees (S+) and 30 unrewarded fee-bee songs produced by another 3 individual chickadees (S-); and (2) True 2 ($n = 5$) discriminated between the same songs with opposite rewards such that the 30 rewarded (S+) fee-bee songs were the S- from True 1 and the 30 unrewarded (S-) fee-bee songs were the S+ from True 1.

The Pseudo category discrimination group was also divided into 2 subgroups: (1) Pseudo 1 ($n = 3$) discriminated between 30 randomly selected rewarded (S+) fee-bee songs and 30 randomly selected unrewarded (S-) fee-bee songs; and (2) the second subgroup Pseudo 2 ($n = 3$) discriminated between the same songs with opposite reward structure such that the 30 rewarded (S+) fee-bee songs were the S- from Pseudo 1 and the 30 unrewarded (S-) fee-bee songs were the S+ from Pseudo 1 (S+) fee-bee songs and 30 randomly selected unrewarded (S-) fee-bee songs. The purpose of the Pseudo groups was to include a control in which subjects were not trained to categorize according to individual chickadee and would instead be required to memorize each vocalization independent of the producer.

Discrimination-85 training. This procedure was identical to discrimination training except that rewarded songs were reinforced with a reduced probability (i.e. $P = 0.85$). On 15% of trials when a rewarded stimulus was played and a bird entered the feeder, no access to food was granted. A

30-s intertrial interval ensued, during which the lights remained on. This was done to prepare birds for probe trials in which some stimuli were neither rewarded nor unrewarded. Discrimination-85 training continued until birds completed 2 consecutive 360-trial blocks with a DR of at least 0.80.

Probe I. During probe I the reinforcement contingencies from discrimination-85 training were maintained. In addition to the 60 stimuli from discrimination training, this stage included 12 novel fee-bee songs, 2 from each of the 6 individual females. For True groups, 6 of these novel songs were categorized as P+ and the other 6 as P−, based on whether they were produced by the same birds as the S+ or the S− training stimuli. For Pseudo groups, the novel songs were not assigned to categories. For both groups, the 12 novel stimuli were neither rewarded nor unrewarded. The birds completed six 72-trial blocks in which the 60 familiar discrimination stimuli repeated once per block and the 12 probe sequences played once per block.

Transfer trainings. The transfer training procedures were generally the same as the discrimination trainings except as noted. Stimuli used were replaced by 60 new songs (recorded from the same 6 females). Responses to half of these stimuli (S−) were unrewarded with a 30-s intertrial interval with lights off. Transfer training continued until birds completed six 360-trial blocks with a discrimination ratio (DR) ≥ 0.80 with the last 2 blocks being consecutive. Subjects in the True 1 and True 2 groups discriminated between 30 new rewarded (S+) fee-bee songs produced by the same 3 individual chickadees heard in discrimination training (S+) and 30 new unrewarded fee-bee songs produced by the same 3 individual chickadees heard in discrimination training (S−). Subjects in the Pseudo 1 and Pseudo 2 groups discriminated between 30 new randomly selected, rewarded (S+) fee-bee songs and 30 new randomly selected unrewarded (S−) fee-bee songs. The transfer-85

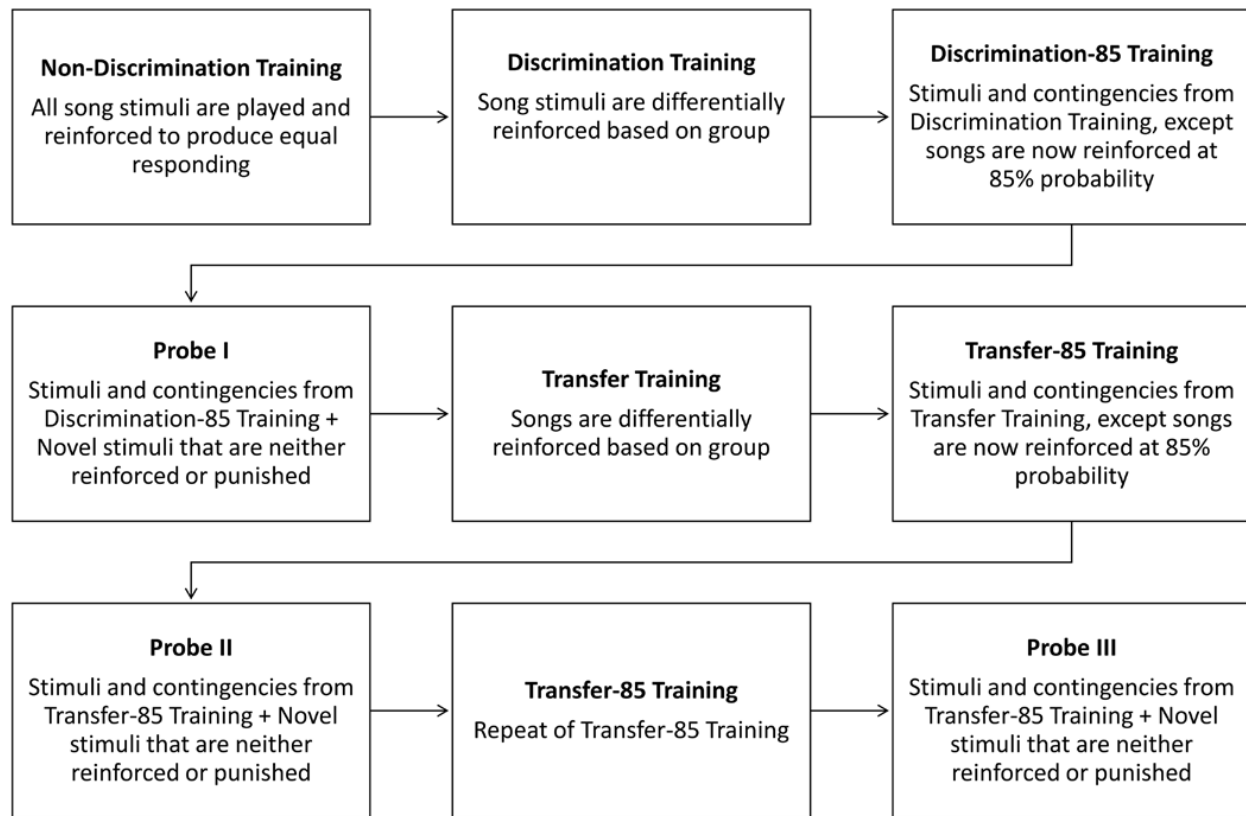


FIGURE 1. Flowchart depicting the stages of the experimental procedure including the name of each stage followed by a short description (see text for details).

training procedure was identical to discrimination-85 training but used songs from the first transfer training.

Probe II. During probe II the reinforcement contingencies from transfer-85 training were maintained. In addition to the 60 stimuli from transfer training, this stage included 12 novel fee-bee songs, 2 from each of the 6 individual females. As in probe I, the 12 novel stimuli were neither rewarded nor unrewarded, in order to assess how the birds responded to novel stimuli. P+ and P- were assigned in a similar manner to probe I. The birds completed six 72-trial blocks in which the 60 familiar transfer stimuli repeated once per block and the 12 probe sequences played once per block.

Probe III. Prior to probe III, subjects were given an additional stage of transfer-85 training in case their response rates had decreased during probe II. In probe III, reinforcement contingencies from transfer-85 training were maintained, and in addition to the 60 stimuli from transfer training, this stage included the 12 novel spliced fee-bee songs, including 2 songs derived from each individual chickadee: one using a fee note from a previously rewarded female bird and a bee note from another bird not included in the study, and one using a fee note from another bird not included in the study and a bee note from a previously rewarded female bird. For True groups, probe III stimuli were separated into 4 groups: (1) fee note from a rewarded bird; 3 songs

(Fee+); (2) bee note from a rewarded bird; 3 songs (Bee+); (3) fee note from an unrewarded bird; 3 songs (Fee-) and; (4) bee note from an unrewarded bird; 3 songs (Bee-). The 12 novel stimuli were neither rewarded nor unrewarded. The birds completed six 72-trial blocks in which the 60 familiar transfer stimuli repeated once per block and the 12 probe sequences played once per block.

Response measures. For each 360-block trial during training, a percent response was calculated ($R+/(N-I)$): $R+$ is the number of trials in which the bird went to the feeder, N is the total number of trials, and I is the number of interrupted trials in which the bird left the perch before the entire stimulus played. For discrimination training, a DR was calculated by dividing the mean percent response to all $S+$ stimuli by the mean percent response to $S+$ stimuli plus the mean percent response to $S-$ stimuli. A DR of 0.50 indicates equal response to rewarded ($S+$) and unrewarded ($S-$) stimuli; a DR of 1.00 indicates perfect discrimination between stimuli.

Statistical analyses. We conducted an analysis of variance (ANOVA) comparing the number of trials needed to reach criterion and the DRs between the True and Pseudo groups during discrimination training, an ANOVA comparing the number of trials needed to reach criterion and the DRs between True and Pseudo groups during transfer training, and an ANOVA comparing the number of trials needed to reach criterion and the DRs in True and Pseudo groups between discrimination training and transfer training. We also conducted a repeated measures ANOVA comparing responding to training stimuli and probe stimuli for probes I, II, and III. We also conducted post hoc tests to test for differences in the number of trials to reach criterion during discrimination training and responding to probe stimuli for probes I, II, and III. All statistical analyses were conducted using SPSS 20 (SPSS, Chicago, Illinois, USA).

Ethical note. Throughout the experiment, birds remained in the testing apparatus to minimize the transport and handling of each bird. One male subject died from natural causes during operant training. Following the experiment, healthy birds were returned to the colony room for use in future experiments. All procedures were conducted in accordance with the Canadian Council on Animal Care (CCAC) Guidelines and Policies with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta (AUP 1937), which is consistent with the Animal Care Committee Guidelines for the Use of Animals in Research. Birds were captured and research was conducted under an Environment Canada Canadian Wildlife Service Scientific permit (#13-AB-SC004), Alberta Fish and Wildlife Capture and Research permits (#56066 and #56065), and a City of Edmonton Parks permit.

RESULTS

Discrimination and Transfer Training

In discrimination training, True group birds reached criterion (i.e. learned to discriminate) significantly faster than did Pseudo group birds based on DRs ($F_{1,14} = 12.022$, $P = 0.004$, $\eta^2 = 0.462$). There were no significant differences in trials to criterion by sex ($F_{1,8} = 0.870$, $P = 0.870$, $\eta^2 = 0.004$). Tukey's post hoc analysis showed no significant difference between True

groups 1 and 2 ($P = 0.963$), and no significant difference between Pseudo 1 and Pseudo 2 ($P = 0.761$).

In transfer training, True group birds again learned to discriminate significantly faster than did Pseudo group birds based on DRs ($F_{1,14} = 15.981$, $P = 0.001$, $\eta^2 = 0.533$). Here too, there were no significant differences in trials to criterion by sex ($F_{1,8} = 0.621$, $P = 0.453$, $\eta^2 = 0.072$). Tukey's post hoc analysis showed no significant difference between True groups 1 and 2 ($P = 0.979$), and no significant difference between Pseudo 1 and Pseudo 2 ($P = 0.271$).

Comparing results from discrimination training and transfer training, True groups learned to discriminate transfer training stimuli to criterion in fewer blocks compared to discrimination training stimuli based on DRs ($F_{1,8} = 11.786$, $P = 0.009$, $\eta^2 = 0.596$). In contrast, Pseudo groups showed no difference in the rate of learning during discrimination training vs. transfer training ($F_{1,4} = 0.040$, $P = 0.851$, $\eta^2 = 0.010$). See Figure 2 for results of discrimination and transfer training.

Probe I

In probe I, percent response in True groups differed across the 4 stimulus types: rewarded discrimination stimuli, unrewarded discrimination stimuli, rewarded probe I stimuli, and unrewarded probe I stimuli ($F_{1,9} = 44.002$, $P < 0.001$, $\eta^2 = 0.830$). Tukey's post hoc analyses revealed a significant difference in responding between discrimination training S+ and S- ($P < 0.001$), with higher responding to S+ stimuli, and a significant difference in responding between probe I stimuli ($P < 0.001$), with higher responding to P+ stimuli, suggesting birds were able to generalize their responding (Figure 3). There were no significant differences within Pseudo groups by stimulus type ($F_{1,5} = 1.211$, $P = 0.340$, $\eta^2 = 0.195$).

Probe II

In probe II, percent response in True groups differed across the 4 stimulus types: rewarded transfer stimuli, unrewarded transfer stimuli, rewarded probe II stimuli, and unrewarded probe II stimuli ($F_{1,9} = 63.487$, $P < 0.001$, $\eta^2 = 0.876$). Tukey's post hoc analyses showed a significant difference in responding between transfer training S+ and S- ($P < 0.001$), with higher responding to S+ stimuli, and a significant difference in responding between probe II stimuli ($P < 0.001$), with higher responding to P+ stimuli, suggesting birds were able to generalize their responding (Figure 4). There were no significant differences within Pseudo groups by stimulus type ($F_{1,5} = 0.027$, $P = 0.876$, $\eta^2 = 0.005$).

Probe III: Spliced Songs

In probe III percent responding in the True groups differed across the 6 stimulus types: rewarded transfer stimuli, unrewarded transfer stimuli, and the Fee+,

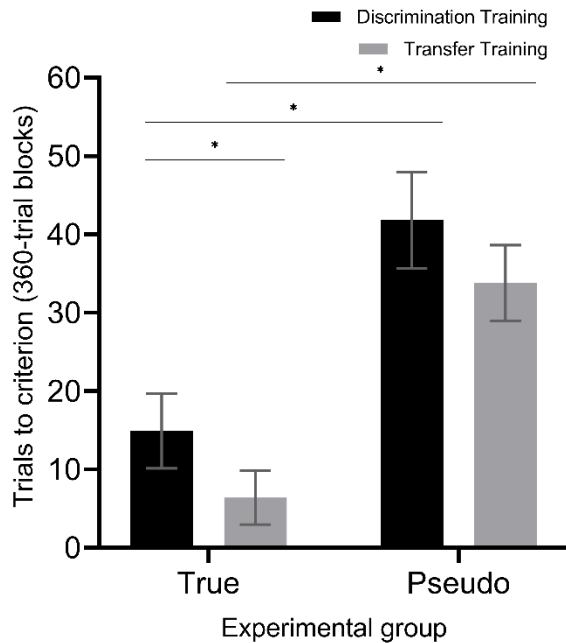


FIGURE 2. Trials to criterion by True groups and Pseudo groups in discrimination training and transfer training. The following differences were significant (indicated by asterisks): True groups vs. Pseudo groups in discrimination training (ANOVA, $P = 0.004$), True groups vs. Pseudo groups in transfer training ($P = 0.001$), and True groups in discrimination vs. transfer training ($P = 0.009$). Error bars represent standard error.

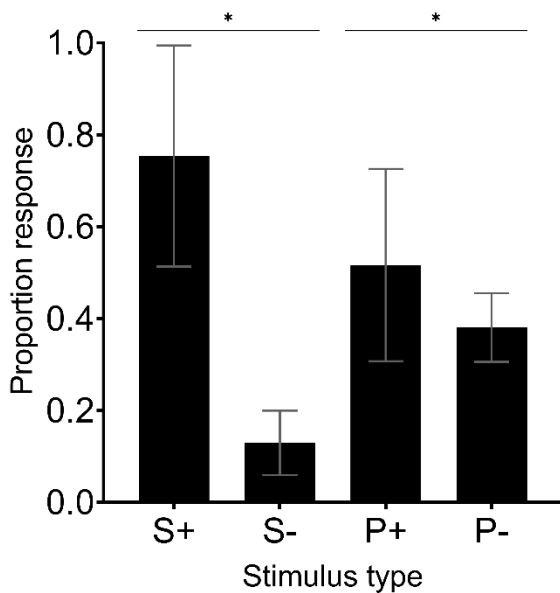


FIGURE 3. Percent response by True groups in probe I trials. The following differences were significant (indicated by asterisks): rewarded (S+) vs. unrewarded (S-) song stimuli (repeated measures ANOVA: $P < 0.001$), and rewarded (P+) vs. unrewarded (P-) probe stimuli ($P = 0.001$). Error bars represent standard error.

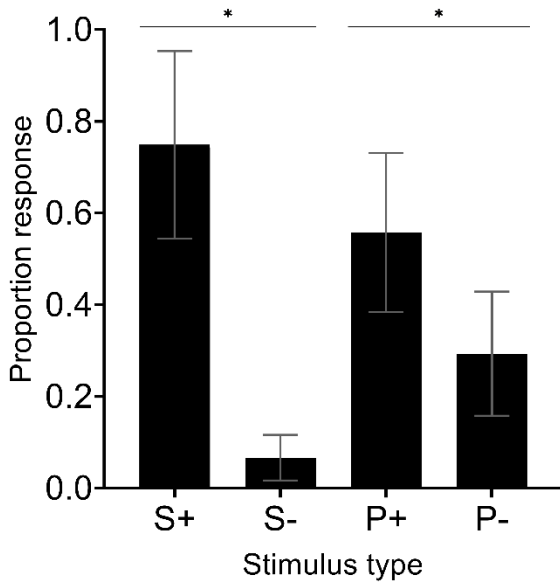


FIGURE 4. Percent response by True groups in probe II trials. The following differences were significant (indicated by asterisks): rewarded (S+) vs. unrewarded (S-) song stimuli (repeated measures ANOVA: $P < 0.001$), and rewarded (P+) vs. unrewarded (P-) probe stimuli ($P = 0.001$). Error bars represent standard error.

Bee+, Fee-, Bee- stimuli ($F_{1,9} = 47.878$, $P < 0.001$, $\eta^2 = 0.842$). Tukey's post hoc analyses showed a significant difference in responding between transfer training S+ and S- ($P < 0.001$), with higher responding to S+ stimuli. Analysis also showed a significant difference in responding between probe III stimuli ($P = 0.003$), with higher responding to Bee+ stimuli compared to Bee- stimuli, and no significant differences between Fee+ vs. Fee- stimuli ($P = 0.242$) or Fee+ vs. Bee+ ($P = 0.708$) (Figure 5). Results showed significant differences within Pseudo groups between stimulus types ($F_{1,5} = 17.531$, $P = 0.009$, $\eta^2 = 0.778$). However, Tukey's post hoc analyses showed no significant difference in responding between discrimination training S+ and S- ($P = 0.056$), and no significant differences in responding between all probe III stimuli ($P = 0.111$).

DISCUSSION

Previous research has shown Black-capped Chickadees are capable of identifying individual chickadees by their chick-a-dee call (Mammen and Nowicki 1981, Charrier and Sturdy 2005) and possibly by tseet calls (Guillette et al. 2010). In addition, Black-capped

Chickadees can identify individual males by their fee-bee songs (Phillmore et al. 2002, Christie et al. 2004a, Wilson and Mennill 2010). Our study shows that female and male Black-capped Chickadees are able to discriminate between individual females based on fee-bee song. But the acoustic cues behind identification require further investigation.

Results showed that during discrimination training birds in the True groups (i.e. birds trained to respond to songs of particular individual females) were quicker to discriminate between rewarded female song and unrewarded female song when compared to Pseudo group birds (i.e. birds trained on random sets of female songs), suggesting True category discriminations were easier to learn vs. memorizing randomly selected rewarded songs. During transfer training, birds in the True groups were quicker to learn to respond correctly to rewarded female song stimuli compared to Pseudo group birds, again suggesting True category discriminations were easier to learn. Our data indicates that birds in the True groups used open-ended categorization while birds in the Pseudo groups used rote memorization in order to respond correctly in our tests. In addition, results from probe I and II trials showed that the categories learned by the True birds could be generalized to novel stimuli. Pseudo birds continued to show no difference in responding as they were not assigned stimuli to generalize to.

Probe III data showed that there was no significant difference between Fee+ and Bee+ stimuli in True groups, meaning birds did not respond differentially to either the fee note or bee note from their previously rewarded female. Birds showed no other differences in responding except for the Bee+ vs. the Bee- stimuli, with higher responding to the Bee+ stimuli (bee notes from previously rewarded females). This suggests that the bee note is sufficient for discriminating between individual females. However, research has shown that in males the fee glissando remains constant at different absolute pitches (Christie et al. 2004b) and is necessary for individual recognition (Shackleton et al. 1992). In females the fee glissando shows a greater change in frequency compared to males (Hahn et al. 2013) and may be used by chickadees to tell the difference between female and male conspecifics (Hahn et al. 2015) and perhaps for individual recognition. Past research has also shown that these 2 note songs are rich in information about the producer including information such as individual identity (Phillmore et al. 2002, Christie et al. 2004a), sex (Hahn et al. 2013), rank (Christie et al. 2004b), and quality (Otter and Ratcliffe 1993, Christie et al. 2004b). Further research is necessary to determine how chickadees are able to use the fee-bee song for individual identification.

We also observed no differences between sexes in speed of acquisition during discrimination training or transfer training. Recognition of individual females based on the fee-bee song might be equally important to both male and female chickadees. Individual recognition based on song is advantageous in that the listener can correctly identify an individual as a neighbor or an invading individual, especially when considering the typical uses of song in the current species, including mate attraction and territorial defense (Smith 1991). While the purpose of song in

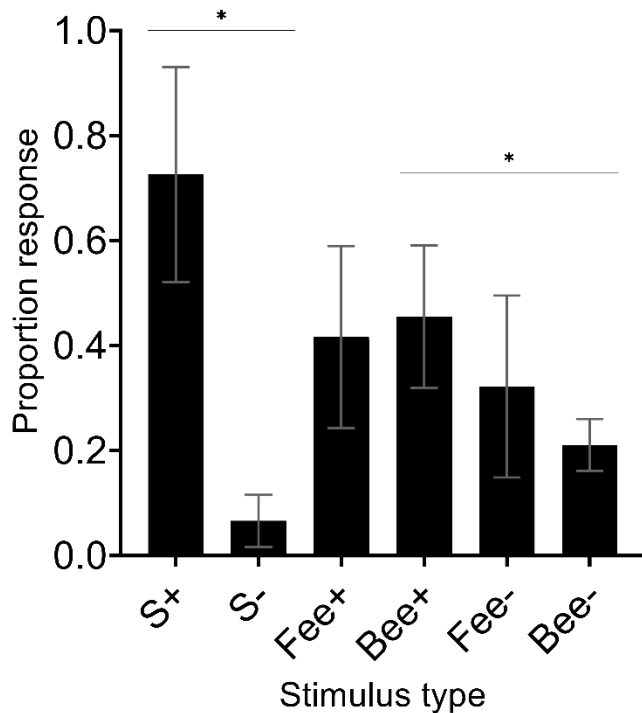


FIGURE 5. Percent response by True groups in probe III trials. The following differences were significant (indicated by asterisks): rewarded (S⁺) vs. unrewarded (S⁻) song stimuli (repeated measures ANOVA: $P < 0.001$), and rewarded (Bee⁺) vs. unrewarded (Bee⁻) probe stimuli ($P = 0.003$). Error bars represent standard error.

female Black-capped Chickadees is less understood, research has highlighted its potential use in aggressive female–female interaction, and future female–male mating interactions (Montenegro et al. personal communication). The current study showed no differences in responding between females and males, suggesting they are attending to the same features and both receive relevant information. The ability to quickly identify an individual female would be beneficial in saving time and energy if females are vocalizing to defend territories or to communicate with a mate or potential mate.

Our results suggest that discrimination of individual females based on fee-bee song is easiest when both notes can be used but that the bee note is sufficient for such discrimination (Figure 5). Because fee-bee songs are not the only vocalization used by Black-capped Chickadees to identify individuals, voice characteristics might be a mechanism that aids in identification. Perhaps all vocalizations from an individual share distinguishing features that allow for discrimination. If the categorization that we observed was due to voice recognition, such distinguishing features might be more prominent in bee notes than in fee notes. Given what we know about song in male and female Black-capped Chickadees, future research should first explore both the fee and bee note portion of the fee-bee song. While past research has focused on the fee note and shown that the fee glissando differs between males and females (Hahn et al.

2013), it appears that the bee note is also important for identification. A more fine-grained comparison of acoustic differences in female song would also be worth exploring. Overall, further research with additional song manipulations is necessary to examine the acoustic mechanisms behind individual recognition via female-produced fee-bee songs as well as the function of female song.

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Data availability: Analyses reported in this article can be reproduced using the data provided by Montenegro et al. (2020).

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