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Black-capped chickadees categorize songs based on features that vary geographically

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The songs of many songbird species vary geographically, yet, the songs of black-capped chickadees (*Poecile atricapillus*) show remarkable consistency across most of the species' North American range. Previous research has described subtle variations in the song of this species by comparing songs produced by males at distant parts of the species' range (British Columbia and Ontario). In the current study, we used an operant discrimination task to examine if birds classify the songs produced by males in these two previously-studied locations as belonging to distinct open-ended categories. In both experiments, when birds were presented with new songs, they continued to respond to songs from the same geographic location as the songs that were reinforced during initial discrimination training, suggesting that birds were using open-ended categorization. Additionally, we presented birds with songs in which we manipulated acoustic features in order to examine the acoustic mechanisms used during discrimination; results provide support that birds use the duration of the song when discriminating, but the results also suggest that birds used additional acoustic features. Taken together, these experiments show that black-capped chickadees classify songs into open-ended, geography-based categories, and provide compelling evidence that perceptible acoustic differences exist in a vocalization that is seemingly consistent across the species' range.

Keywords: acoustic discrimination, black-capped chickadee, categorization, geographic variation, song, operant conditioning

Many animals' vocal signals vary geographically, including anurans (Pröhl et al. 2007; Ryan and Wilczynski 1991), birds (Baker and Cunningham, 1985; Wright, 1996), and mammals (Mitani et al. 1999; Campbell et al. 2010; Kershenbaum et al. 2012). For songbirds, the pattern of geographic variation differs across species (for review see Catchpole and Slater 2008; Podos and Warren 2007). For example, in some species, neighboring males share song types, but song types vary across different populations of males (e.g., white-crowned sparrow, *Zonotrichia leuophrys*, Marler and Tamura 1962). Because male song in passerines is a sexual signal, used for territory defense and mate attraction, the ability to discriminate among vocalizations on the basis of geographic variation would be advantageous if, for example, local adaptations exist (Kawecki and Ebert 2004) that make it beneficial for females to preferentially mate with local males.

When variations in vocal signals exist, animals may benefit by the ability to detect the acoustic differences in vocalizations. In fact, the results of playback studies suggest that birds can perceive geographic variation in acoustic signals. Males have stronger behavioural responses (e.g., approach and singing) to local songs compared to songs produced by males from more distant populations (e.g., white-crowned sparrow, Milligan and Verner 1971; corn bunting, *Emeriza calandra*, McGregor 1983; Darwin's ground finches, *Geospiza spp.*, Ratcliffe and Grant 1985; song sparrow, Searcy et al. 1997). Females, too, exhibit preferences for male songs based on geographic information (e.g., white-crowed sparrow, Baker et al. 1981; rufous-collared sparrow, *Zonotrichia capensis*, Danner et al. 2011). In order for songbirds to respond differently to different vocal signals, there must be perceptible acoustic variation within the vocalizations being compared. If there are perceptual differences, there are several mechanisms the birds could employ to discriminate the vocalizations.

One mechanism that birds could use when discriminating among vocalizations is category perception. Using this mechanism, birds would respond similarly to vocalizations that belong to the same perceptual category. Acoustic discrimination via a category perceptual mechanism has been demonstrated in songbirds (e.g., European starlings, *Sturnus vulgaris*, Braaten 2000; Gentner and Hulse 1998; zebra finches, *Taeniopygia guttata*, Sturdy et al. 1999; black-capped chickadees, *Poecile atricapillus*, Bloomfield and Sturdy 2008; Hahn et al., 2015). Another mechanism that animals could use when discriminating among vocalizations is rote memorization. In order to discriminate sounds using rote memorization, an animal would need to have experience with, and memorize the specific acoustic features of the sound that is to-be-remembered. However, by using perceptual categories, an individual could distinguish between a local or non-local animal without memorizing all song types produced by local animals. Field-based playback studies provide evidence that birds recognize individual neighbors using cues from song and location (e.g., Falls and Brooks 1975) and perceive different song types as produced by the same individual (e.g., Searcy et al. 1995).

Operant conditioning techniques are useful for studying category perception, because animals in the laboratory can be trained to discriminate among sounds, and subsequently presented with novel sounds to test for generalization, where the pattern of response to novel sounds can be used as evidence for category perception. Through a process of open-ended classification (see Herrnstein, 1990), animals can learn a categorization “rule” (based on the common features of signals that belong to the category) which would also allow animals to quickly classify new signals. In contrast, animals relying on rote memorization are not able to learn a general categorization rule when discriminating among signals.

In contrast to the geographic variation that is found in the songs produced by other songbird species (e.g., swamp sparrow, *Melospiza georgiana*, Marler and Pickert 1984 song sparrows, *M. melodia*, Searcy et al. 2003), black-capped chickadee *fee-bee* songs are considered to show remarkably little variation across localities. *Fee-bee* songs are a two-note vocalization, with the first note (i.e., *fee*) sung at a higher frequency than the second note (i.e., *bee*), and this frequency relationship between the two notes remains relatively consistent across song bouts (Horn et al. 1992; Weisman et al. 1990). *Fee-bee* songs contain acoustic features indicating individual identity (Christie et al. 2004a; Hahn et al. 2013b) and field (Wilson and Mennill 2010) and laboratory (Phillimore et al. 2002) studies have demonstrated that chickadees can discriminate among individuals based on their songs. Although the black-capped chickadees' range extends across most of North America (Smith 1991), little geographic variation has been described in this song (although, variation has been found in geographically-isolated populations of chickadees, where some animals produce unusual songs; see Gammon and Baker 2004; Kroodsma et al. 1999). Recently, Hahn et al. (2013a) conducted a bioacoustic analysis on songs produced by birds from different geographic locations (northern British Columbia and eastern Ontario). For this bioacoustic analysis, six acoustic features were analyzed, similar to previous bioacoustic analyses of *fee-bee* songs (e.g., Christie et al., 2004a,b; Hoeschele et al., 2010): (1) the total duration of the song, (2) the proportional duration of the *fee* note (i.e., duration of the *fee* note divided by the total song duration), (3) the frequency decrease in the *fee* note (called the *fee* glissando), (4) the frequency ratio between the two notes (i.e., end frequency of the *fee* note divided by the start frequency of the *bee* note), (5) the relative amplitude of the two notes, and (6) the relative loudness of the *fee* note to the entire song. Using a permuted discriminant function analysis (Mundry and Sommer, 2007) to determine which acoustic features vary with

geographic location, Hahn et al. (2013a) found that birds from northern British Columbia produced songs that were longer in total duration compared to birds from eastern Ontario. In addition, the acoustic cues that are associated with a male's dominance rank vary between these two locations (i.e., variation in the consistency of the interval ratio in songs from Ontario, Christie et al. 2004b; variation in the consistency of the relative amplitude in songs from British Columbia, Hahn et al. 2013a; Hoeschele et al. 2010) indicating additional geographic variation that occurs in *fee-bee* songs.

In the current study, we used an operant go/no-go task to address two questions: (1) Do black-capped chickadees perceive acoustic differences and categorize songs based on geographic location? (2) If so, what are the acoustic mechanism(s) that chickadees use to perform this discrimination? To compare chickadees' abilities using open-ended categorization versus rote memorization, we employed a true category/pseudo category paradigm (similar to Bloomfield et al. 2008; Hahn et al. 2015). Within this task, birds were divided into "true category" or "pseudo category" discrimination groups. Birds in the true category group discriminated between songs recorded in two separate geographic locations (i.e., British Columbia or Ontario). If songs produced by birds within each geographic region are perceptually similar to one another, birds could learn a general category rule (e.g., respond to any song from British Columbia) and use this category rule when making a response. Birds in the pseudo category group discriminated between the same songs as the true category group, but the songs were assigned to random "categories." In other words, responses made to half of the British Columbia and half of the Ontario songs were reinforced, while the other half were non-reinforced. Therefore birds in the pseudo category group could not use a category rule when responding, and had to rely on rote memorization to remember each reinforced and non-reinforced song. Following discrimination

training, we presented all birds with novel songs from each location. If birds in the true category group continued to respond to novel songs based on the initial training contingencies (i.e., based on geographic location), this would be further evidence that birds were using open-ended categorization. In contrast, we expected birds in the pseudo category discrimination group to respond non-differentially to the novel songs because birds in this group will not have learned to respond based on a category rule during discrimination.

In Experiment 2, we presented birds with songs that we experimentally manipulated to understand the specific acoustic mechanisms that birds used when performing these discriminations. Specifically, we wanted to examine whether birds used the overall song duration (i.e., the acoustic feature that shows the most variation between these populations; Hahn et al. 2013a) as a cue when discriminating. We also examined if birds used acoustic features within either the first (i.e., *fee*) or second (i.e., *bee*) note to discriminate between British Columbia and Ontario songs, by presenting birds in the true category groups with songs that we edited to include one note from each of the two locations. This manipulation allowed us to examine if birds used features within one of the song notes to discriminate between the geographic locations. In this study, our subject chickadees originated from a geographic region (i.e., central Alberta) distant from the two regions where the stimulus songs were recorded (i.e., British Columbia or Ontario). Geographic variation in *fee-bee* songs produced by birds in these three locations (i.e., Alberta, British Columbia, and Ontario) have not been examined, so we had no predication of how birds may respond to the songs from each location based on the acoustic structure of local songs (and how the local song structure compares to songs from these more distant locations).

General methods

Subjects

Black-capped chickadees were captured in Edmonton (North Saskatchewan River Valley, 53.53°N, 113.53°W; Mill Creek Ravine, 53.52°N, 113.47°W), Stony Plain (53.46°N, 114.01°W), or Kananaskis Country (51.02°N, 115.03°W), Alberta, Canada. At time of capture, birds were identified as at least one year of age by plumage (Pyle 1997). Sex was determined by DNA analysis (Griffiths et al., 1998).

Before the experiment, birds were housed in individual cages (30 × 40 × 40 cm, Rolf C. Hagen, Inc., Montreal, QB, Canada) with visual and auditory contact with conspecifics. Birds were kept under the natural light cycle for Edmonton, Alberta. Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St Louis, MO, USA), water (vitamin supplemented three days a week; Prime vitamin supplement; Hagen, Inc.), grit, and cuttlebone. Birds were provided the following nutritional supplements: three to five sunflower seeds daily, one superworm (*Zophobas morio*) three times a week, and a mixture of eggs and greens (spinach or parsley) twice a week.

Apparatus

For a detailed description of the apparatus see Sturdy and Weisman (2006). Each bird was tested in a modified cage (30 × 40 × 40 cm) that was housed individually in a ventilated, sound-attenuating chamber illuminated by a 9 W, full spectrum fluorescent bulb. Birds had access to a motor-driven feeder (see Njegovan et al. 1994) through an opening (11 cm × 16 cm) on one side of the cage. The position of the bird was monitored via infrared beams in the feeder

and the perch closest to the feeder (i.e., request perch; see Sturdy and Weisman [2006] for diagram of the apparatus). A personal computer connected to a single-board computer (Palya and Walter 2001) set up trials and recorded a bird's responses. Stimuli were played from a CD through either a Cambridge A300 or 640A Integrated Amplifier (Cambridge Audio, London, England) or an NAD310 Integrated Amplifier (NAD Electronics, London, England) and a speaker to the side of the feeder (Fostex FE108 Σ or Fostex FE108E Σ full-range speaker; Fostex Corp., Japan; frequency response range 80-18 000 Hz). In each cage there were three perches, a water bottle, cuttlebone, and grit cup. During testing, birds were kept on the natural light cycle and completed trials throughout the daylight period. Typically, birds completed 700-1900 trials a day, depending on the number of daylight hours (which ranged from approximately 7.5 h to 16 h). Birds were provided one superworm twice a day; however, during the operant discrimination task, Mazuri was only available as a reward for correct responding.

Acoustic stimuli

Songs used as stimuli in Experiments 1 and 2 were recorded from banded populations of chickadees during the dawn chorus period at the University of Northern British Columbia (Prince George, British Columbia) between 27 April and 14 May 2000-2004 and Queen's University Biological Station (near Kingston, Ontario) between 25 April and 10 May 1999-2001. Songs from the University of Northern British Columbia were recorded with a Sennheiser MKH70 or ME67 microphone (Sennheiser Electronic, Old Lyme, CT) or an Audio-Technica ATB815a microphone (Audio-Technica U.S., Stow, OH) and a Marantz PMD430 (Marantz America, Mahwah, NJ) tape recorder. Songs recorded at the Queen's University Biological Station were recorded with a Sennheiser MKH70 or Audio-Technica AT815a microphone and

Sony Walkman Professional WM-D6C (Sony, Tokyo, Japan) or Marantz PMD222 tape recorder. Field recordings were digitized at 22 050 Hz but were resampled from 22 050 to 44 100 Hz using SIGNAL 5.10.29 software (Engineering Design, Berkeley, CA, USA) in order to be used as experimental stimuli. In Experiment 2, we also used songs recorded during the dawn chorus period at a field station in the John Prince Research Forest (Fort St. James, British Columbia) between 28 April and 16 May 2006, 2008 and 2009. Songs from the John Prince Research Forest were recorded with a Sennheiser MKH70, Sennheiser ME67, or Audio-Technica ATB815a microphone and a Marantz PMD430 tape recorder or Marantz PMD671 digital recorder. Songs from all populations were of high quality (i.e., no audible interference such as other conspecific or heterospecific vocalizations) and low background noise when viewed on a spectrogram with amplitude cutoffs of -35 to 0 dB relative to song peak amplitude. Stimuli were bandpass filtered outside the range of the songs to remove background noise using GoldWave version 5.58 (GoldWave, Inc., St. John's, NL, Canada). Using SIGNAL, songs were edited from longer audio files to contain 5 ms of silence before and after each song, the stimuli were tapered to remove transients, and amplitude was equalized. During the experiment, stimuli were presented at ~75 dB as measured by a Radio Shack Sound Level Meter (Radio Shack, Fort Worth, TX, USA) or Brüel & Kjær Type 2239 (Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark; A weighting, slow response) at the approximate height and position of a bird's head when on the request perch.

Experiment 1

Methods

Subjects

Eleven black-capped chickadees (six males and five females) were tested between October 2011 and February 2012. Four birds (two males, two females) had previous operant experience discriminating *chick-a-dee* calls or synthetic tones (Guillette et al. 2011; Hoeschele et al. 2013); eight birds (three females: one in each of three discrimination groups; five males: one in each of two true category discrimination groups and three males in the pseudo category discrimination group; see “Discrimination training” below for group descriptions) had previous experience discriminating *fee-bee* songs; however, none of the birds had experience with the particular songs used as stimuli for the current experiment.

Acoustic stimuli

A total of 40 *fee-bee* songs (20 recorded at the University of Northern British Columbia, British Columbia; 20 recorded at Queen’s University Biological Station, Ontario) were used as stimuli in Experiment 1. From our sample of song recordings, we randomly selected one song produced by a given individual to be used as a stimulus during Experiment 1. In addition, because some subjects had prior experience with songs recorded at these two locations, we ensured that birds did not have prior experience with the songs used as exemplars in the current experiment.

Procedure

Pretraining. The aim of *Pretraining* was to ensure birds remained on the request perch during the entire duration of the song, responded at a high level to all songs, and responded non-

differentially to the S+ stimuli (i.e., rewarded songs) and S- stimuli (i.e., unrewarded songs) that would be presented later during *Discrimination training*. Once the bird learned to use the request perch and feeder, *Pretraining* began. To initiate a trial, the bird had to land and remain on the request perch for between 900-1100 ms, after which a randomly-selected song played without replacement. A trial was considered interrupted if the bird left the request perch before the song finished playing. This resulted in a 30-s timeout with the houselight turned off. Once a song finished playing, if the bird entered the feeder within 1 s, it received access to food for 1 s, followed by a 30-s intertrial interval, during which the houselight remained on. Remaining on the request perch during the song presentation and 1 s after the song finished playing resulted in a 60-s intertrial interval with the houselight on, but this intertrial interval ended once the bird left the request perch. This increased the probability that a bird would make a response (i.e., leave the request perch following the presentation of a song) on a given trial. Birds continued on *Pretraining* until they completed six 200-trial blocks of $\geq 60\%$ responding and at least four 200-trial blocks $\leq 3\%$ difference in responding to future rewarded (S+) and unrewarded (S-) stimuli.

Discrimination training. During this phase the procedure from *Pretraining* was maintained; however, only ten British Columbia songs and ten Ontario songs were presented. Half of the songs were now assigned to be S+ (i.e., rewarded) and half as S- (i.e., unrewarded). Which songs were S+ and S- depended on the group that each chickadee was assigned to. Responses to S- songs now resulted in a 30-s intertrial interval with the houselight off. Responses to S+ songs resulted in 1 s access to food.

Black-capped chickadees were randomly assigned to a true category discrimination group ($N = 6$) or pseudo category discrimination group ($N = 5$). Birds in the true category discrimination group were divided into two subgroups: one group discriminated rewarded British Columbia songs from unrewarded Ontario songs (referred to hereafter as British Columbia S+ group; one male and two females), while the other group discriminated rewarded Ontario songs from unrewarded British Columbia songs (referred to hereafter as Ontario S+ group; two males and one female). Birds in the pseudo category discrimination group (three males and two females) discriminated five randomly-selected S+ British Columbia songs and five randomly-selected S+ Ontario songs from five S- British Columbia songs and five S- Ontario songs.

Transfer training. This phase was identical to *Discrimination training*, except that an additional ten British Columbia songs and ten Ontario songs were presented. Responses to these transfer songs continued to be reinforced based on the same contingencies as in *Discrimination training* (i.e., based on geographic location or pseudorandomized).

Response measures

A discrimination ratio (DR) is a measure of how well birds discriminate rewarded (S+) songs from unrewarded (S- songs). In order to calculate a DR, first, we calculated the percent response for each stimulus exemplar using the following formula: $(R+/(N-I)) \times 100$, where $R+$ is the number of trials that the bird visited the feeder, N is the total number of trials, and I is the number of trials that the bird left the perch before the song finished playing (i.e., interrupted trials). The DR was calculated by dividing the average percent response to the S+ stimuli by the

average percent response to the S+ stimuli plus the average percent response to the S- stimuli. If a bird responded at chance level (i.e., equally to S+ and S- stimuli), the DR = 0.5. If a bird only responded to S+ stimuli (i.e., perfect discrimination) the DR = 1.0. *Discrimination training* continued until birds completed three 200-trial blocks with a DR ≥ 0.75 with the last two blocks being consecutive. Following *Discrimination training*, birds completed *Transfer training* which ended once birds completed three 200-trial blocks with a DR ≥ 0.75 with the last two blocks being consecutive. One bird (a male in pseudo category group) died during this phase; however, in our analysis we examined responding during the first block of *Transfer training*, so we included this bird in the analysis.

Statistical analyses

To determine whether birds in the two true category discrimination groups differed in their speed of acquisition, we conducted an independent samples *t*-test on the number of 200-trial blocks to reach criterion. We conducted a similar independent samples *t*-test on the number of trials to reach criterion to compare the true and pseudo category groups. We conducted analyses of variance (ANOVA) on the proportion of response to the different stimulus types (i.e., Discrimination S+ songs, Discrimination S- songs, Transfer S+ songs, Transfer S- songs) during the first 200-trial block of *Transfer training*. This allowed us to determine if birds in the true category group continued to respond to the *Transfer training* songs based on the contingencies from *Discrimination training*. We conducted this analysis using the arcsine square-root transformed proportion of response (to correct for non-normality) using the following formula:

$\arcsin[\sqrt{x}]$, where x equals the untransformed proportion of responding. Figures depict the untransformed data. All statistics were conducted in Statistica v12 (StatSoft, Inc).

Ethical note

Throughout the experiments, birds were monitored daily, provided with free access to water, grit and cuttlebone and each bird was given two superworms. During the experiments, birds were housed in the testing apparatus, which minimized the transport and handling of the birds. When testing was complete, birds were returned to the colony room to be used in future studies. All studies were conducted with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta (AUP 108). All procedures were in accordance with the Canadian Council on Animal Care (CCAC) Guidelines and Policies and the ABS Guidelines for the Use of Animals in Research. Chickadees were captured and research was conducted under an Environment Canada Canadian Wildlife Service Scientific permit, Alberta Fish and Wildlife Capture and Research permits, and City of Edmonton Parks Permit.

Results

Trials to criterion

Data from three birds (one female in the Ontario S+ group and two males in the pseudo category group) had to be removed from the analysis due to equipment failure during *Discrimination training*, resulting in data from seven subjects ($N = 3$ for British Columbia S+ group; $N = 2$ for Ontario S+ group; $N = 3$ for pseudo group). Independent samples t -tests on the

number of 200-trial blocks to complete *Discrimination training* revealed no significant difference between the two true category (i.e., British Columbia S+; Ontario S+) groups ($t = 3.00$, $P = 0.058$), but found a significant difference between the true and pseudo category groups ($t = 7.36$, $P = 0.0003$), with the pseudo category group requiring more than twice as many trial blocks to complete the discrimination (average number of trial blocks = 8.0 and 19.33, for true and pseudo category groups, respectively). See Figure 1.

Transfer training

During *Transfer training*, all birds ($N = 6$) in the true category discrimination groups met criterion within the first three 200-trial blocks. Birds ($N = 4$) in the pseudo category discrimination group took an average of 26.5 blocks to reach criterion (range = 12-57 blocks). We conducted a Discrimination group (British Columbia S+; Ontario S+, Pseudo) \times Stimulus set (Discrimination S+ songs, Discrimination S- songs, Transfer S+ songs, Transfer S- songs) ANOVA on the proportion of response to training and test songs during the first 200-trial block of *Transfer training* (first five presentations of each Transfer song). For this analysis, the Discrimination S+ and Discrimination S- songs refer to the rewarded and unrewarded (respectively) songs presented during *Discrimination training* and the Transfer S+ and Transfer S- songs refer to the new songs (i.e., not heard during *Discrimination training*) that were rewarded and unrewarded (respectively) during *Transfer training*. There was a significant main effect of Stimulus type ($F_{3,21} = 114.96$, $P < 0.001$) and a significant main effect of Discrimination group ($F_{2,7} = 5.49$, $P = 0.037$). There was also a significant Stimulus Type \times Discrimination Group interaction ($F_{6,21} = 10.61$, $P < 0.001$). We conducted planned comparisons

to assess the significant interaction. For each group, we compared the Discrimination S+ songs to the Discrimination S- songs and the Transfer S+ songs to the Transfer S- songs. Birds in all groups responded significantly more to the Discrimination S+ songs compared to the Discrimination S- songs (British Columbia S+ group, $t = 6.49$, $P < 0.001$; Ontario S+ group, $t = 6.49$, $P < 0.001$; Pseudo category group, $t = 5.80$, $P < 0.001$). Birds in the true category groups responded significantly more to the Transfer S+ songs compared to the Transfer S- songs (British Columbia S+ group, $t = 13.51$, $P < 0.001$; Ontario S+ group, $t = 12.16$, $P < 0.001$), while there was no significant difference in the response by birds in the pseudo category group ($t = -0.79$, $P = 0.458$). See Figure 2.

Experiment 2

Results from Experiment 1 suggested that birds could discriminate between songs produced by birds from different geographic locations. Because birds in the true category group continued to respond to the novel songs presented during *Transfer training* based on the contingencies from *Discrimination training*, it suggests that birds in the true category group were not simply memorizing individual songs in order to complete the discrimination. In Experiment 2, we tested a new group of black-capped chickadees on geographically-based song discrimination using stimuli from the same two locations as Experiment 1. However, in Experiment 2 we made three important changes from Experiment 1: (1) we included two pseudo category groups (S+ songs for one group were the S- songs for the second group, and vice versa), (2) we included a *Generalization* phase in which we presented novel songs without reinforcement to the true category and pseudo category groups (in Experiment 1, novel songs

presented during *Transfer training* were reinforced), and (3) we included songs that we experimentally manipulated in order to examine the perceptual mechanisms for the discrimination (true category group only).

Methods

Subjects

Sixteen black-capped chickadees (eight male, eight female) were tested between July 2012 and January 2013. Three birds had previous experience using the request perch and experimental feeder; one bird had previous experience discriminating synthetic tones (Hoeschele et al. 2013); the remaining birds ($N = 12$) were naïve to the experimental apparatus.

Acoustic stimuli

Natural stimuli. Ten British Columbia songs and ten Ontario songs were used as stimuli during *Discrimination training*. An additional seven songs from each location were used as natural stimuli during *Generalization* and *Transfer training*. From our sample of song recordings, we randomly selected song stimuli, so that only one song recorded from a given individual was used during *Discrimination training* and *Generalization*. Following *Transfer training*, birds completed test sessions in which they were presented with natural, unmanipulated songs ($N = 10$) recorded at John Prince Research Forest, British Columbia (one randomly-selected song from a given individual) and manipulated songs. Songs were prepared and presented in the same way as in Experiment 1. Songs manipulated and used during additional test sessions were further prepared as described below.

Spliced songs. We created spliced song stimuli to test if birds were preferentially using information in one of the two notes to discriminate songs produced by birds from the two geographic locations. Songs ($N = 16$) were bandpass filtered in Goldwave and the *fee* and *bee* notes from eight British Columbia and eight Ontario songs were edited into individual WAV files using SIGNAL. Four types of spliced songs were created: British Columbia-British Columbia (*fee* and *bee* notes from two songs from British Columbia), Ontario-Ontario (*fee* and *bee* notes from two songs from Ontario), British Columbia-Ontario (*fee* note from a British Columbia song and *bee* note from an Ontario song), and Ontario-British Columbia (*fee* note from an Ontario song and *bee* note from a British Columbia song). When creating the spliced songs, we made the internote interval constant for all stimuli at 100 ms, which is similar to the internote interval in natural songs (e.g., $X_{\text{internote}} = 135$ ms, Ficken et al. 1978) and the internote interval used by other studies manipulating song features (e.g., $X_{\text{internote}} = 100$ ms, Hahn et al. 2015; Hoeschele et al. 2012). We changed the start frequency of the *fee* note to match the start frequency of the *fee* note that was being replaced in the song using the “sliding time scale/pitch shift” effect in Audacity 1.3.12. This manipulation resulted in songs with an average \pm SD internote interval frequency ratio of 1.13 ± 0.036 (range = 1.07-1.186) which corresponds to the internote interval ratios found in a larger sample of songs ($N = 360$) from these two geographic locations (average \pm SD: 1.13 ± 0.022 ; range: 1.047-1.206; Hahn et al. 2013a) and corresponds to the average internote interval ratio reported previously (i.e., 1.13; Weisman et al. 1990)..

Total duration manipulated songs. We created stimuli in which we manipulated the total duration of the songs to determine if birds were using the total duration to discriminate between songs produced by birds from the two geographic locations. To create the total duration manipulated stimuli, we used four British Columbia songs and four Ontario songs. We manipulated each song to increase and decrease (by lengthening or shortening the note and interval lengths) its total duration by approximately 3 SD (i.e., ± 38.2 ms) away from the mean of all songs presented during *Discrimination training*; each song was also presented unmanipulated. These manipulations were completed using the “change tempo” effect in Audacity 1.3.12; frequencies of the songs were not altered. This resulted in six different types of total duration manipulated songs: British Columbia and Ontario songs with a total duration decreased, British Columbia and Ontario songs unmanipulated, and British Columbia and Ontario songs with a total duration increased.

Procedure

Pretraining. The procedure for *Pretraining* remained the same as in Experiment 1. Birds remained on *Pretraining* until they had consistently high response rates (six blocks of $\geq 60\%$ responding to all stimuli and four blocks $\geq 60\%$ responding to test stimuli) and four blocks with $\leq 3\%$ difference in responding to future S+ and S- stimuli.

Discrimination training. The procedure and criterion for *Discrimination training* remained the same as Experiment 1. As in Experiment 1, birds were randomly assigned to either a true category discrimination group ($N = 12$; British Columbia S+ Group: three males, three females;

Ontario S+ Group: three males, three females) or pseudo category discrimination group ($N = 4$; two males, two females). Birds in the pseudo category discrimination group were divided into two subgroups (one male and one female in each subgroup). Each subgroup discriminated five randomly-selected S+ British Columbia songs and five randomly-selected S+ Ontario songs from five different S- British Columbia songs and five different S- Ontario songs. The S+ songs for one subgroup were the S- songs for the other subgroup, and vice-versa.

Pretesting. This phase was identical to *Discrimination training*, except S+ songs were reinforced with a reduced probability (i.e., $P = 0.85$). On 15% of trials, when an S+ stimulus played, entering the feeder resulted in a 30-s intertrial interval with the houselight on, but no access to food. *Pretesting* was used to prepare birds for *Generalization* and manipulated test trials during which responses to test stimuli were neither reinforced nor punished. This phase continued until birds completed two consecutive 200-trial blocks with a $DR \geq 0.75$.

Generalization. During *Generalization*, the stimuli and reinforcement contingencies from *Pretesting* were maintained. In addition, 14 songs not heard during *Discrimination training* (seven British Columbia and seven Ontario songs) were introduced. *Generalization* stimuli were each presented once during a 214-trial block (songs from *Pretesting* were each presented 10 times, randomly-selected without replacement). Responses to generalization stimuli resulted in a 30-s intertrial interval with the houselight on, but no access to food. All birds completed a minimum of three blocks of *Generalization* and these were included for analysis.

Transfer training. Following *Generalization*, all birds in the true category groups (British Columbia S+ and Ontario S+) continued onto *Transfer training*. During this phase, the 20 songs from *Discrimination training* were presented and the 14 songs used during *Generalization* were also included as S+ or S- stimuli (contingencies based on their location of origin). These 34 stimuli were each presented six times, randomly-selected without replacement, during a 204-trial block. This was to increase the pool of stimuli used during the subsequent test sessions. As in *Pretesting*, all S+ stimuli were reinforced with reduced probability ($P = 0.85$). *Transfer training* continued until birds completed three 204-trial blocks with a DR ≥ 0.75 with the last two blocks being consecutive.

Additional test sessions. The remaining test stimuli (i.e., 10 John Prince Research Forest songs; 16 spliced songs; 24 songs used during the manipulated total duration tests) were divided into four test sessions. During each test session, the stimuli and contingencies from *Transfer training* were maintained (i.e., the 34 songs from *Transfer training* were each presented six times for a total of 204 song presentations); in addition, two or three John Prince Research Forest songs, four spliced songs, and six total duration manipulated songs were each presented once in a block, resulting in a 216- or 217-trial block. For each test session, a minimum of three trial blocks were completed and these were included in the analysis. After each test session, birds completed one block of *Transfer training* with a DR ≥ 0.75 before moving onto the next test session. The order of the test sessions was pseudorandomized between discrimination group and sex. An individual song was manipulated in multiple ways (i.e., duration increased, duration decreased, and unmanipulated); however, only one manipulation of an individual song was included in a single test session (i.e., the same song manipulated to be increased in duration and decreased in

duration was not included in the same block of trials), so numerous stimuli were presented between birds hearing different manipulations of the same song.

Response measures

We calculated DRs using the same method as in Experiment 1. To compare the responses to generalization and manipulated stimuli, we scaled the proportion of response for each subject by rescaling the highest proportion of response to a test stimulus to 1.0 and rescaling the proportion of response to all other stimuli as a ratio of the highest proportion of response. With this rescaling we accounted for individual differences in response levels among subjects. Rescaling was conducted separately for generalization stimuli, John Prince Research Forest songs, spliced songs, and total duration manipulated songs.

Statistical analyses

We conducted an ANOVA on the number of trials to criterion to determine if birds in the two true category groups differed in their speed of acquisition during *Discrimination training*. We also conducted similar analyses to determine if birds in the true and pseudo category groups differed in their speed of acquisition during *Discrimination training*. We conducted additional ANOVA and Tukey's planned comparisons on the proportion of response to the test stimuli using the arcsine square-root transformed data. All statistics were conducted in Statistica v12 (StatSoft, Inc).

Results

Trials to criterion

Two females in the British Columbia S+ group were not included in the analysis of *Discrimination training* due to equipment failure during this phase, this resulted in data from 14 subjects ($N = 4$ for British Columbia S+ group; $N = 6$ for Ontario S+ group; $N = 4$ for pseudo category group).

A Sex \times Discrimination Group (British Columbia S+, Ontario S+) ANOVA on the number of 200-trial blocks to complete *Discrimination training* for birds in the two true category groups found no significant main effects or interaction (all $F \leq 1.22$, all $P \geq 0.31$), indicating that there was no significant difference in the number of trials to reach criterion for birds rewarded for responding to British Columbia versus Ontario songs.

An independent samples t -test on the number of 200-trial blocks to reach criterion for the two pseudo category groups revealed no significant difference in the speed of acquisition between the two pseudo category groups ($t = 0.11$, $P = 0.92$), so we combined the two groups in the remaining analyses.

We conducted a Sex \times Discrimination Group (True, Pseudo) ANOVA on the number of 200-trial blocks to complete *Discrimination training*. There were no significant main effects or interactions (all $F \leq 0.685$, all $P \geq 0.43$), indicating no significant difference in discrimination performance between the true and pseudo category groups. See Figure 1.

Generalization

We conducted a Sex \times Discrimination Group (British Columbia S+, Ontario S+, Pseudo) \times Stimulus Type (British Columbia song, Ontario song) ANOVA on the scaled proportion of responses to examine the response to untrained British Columbia and Ontario songs. There was a significant main effect of Stimulus Type ($F_{1,10} = 6.14, P = 0.033$), a significant Discrimination Group \times Stimulus Type interaction ($F_{2,10} = 30.38, P < 0.001$), and a significant Sex \times Discrimination Group \times Stimulus Type interaction ($F_{2,10} = 6.22, P = 0.018$). We conducted a planned comparison to examine the Discrimination Group \times Stimulus Type interaction. Birds in the British Columbia S+ group responded significantly more to British Columbia songs ($t = 2.75, P = 0.020$), birds in the Ontario S+ group responded significantly more to Ontario songs ($t = 7.85, P < 0.001$), while birds in the pseudo category group did not respond significantly differently to the two song types ($t = 0.38, P = 0.71$; see Figure 3).

Additional test sessions

John Prince Research Forest songs. We conducted a t -test to examine the percentage of response by the two true category groups (British Columbia S+, Ontario S+) to songs from John Prince Research Forest, British Columbia. There was no significant difference between the two groups ($t = 0.56, P = 0.59$), indicating that when tested with songs from a third location, birds responded similarly to the new songs regardless of which geographic location was the S+ category during *Discrimination training* (i.e., British Columbia or Ontario).

Spliced songs. We conducted a Sex \times Discrimination Group (British Columbia S+, Ontario S+) \times Stimulus Type (British Columbia-British Columbia, Ontario-Ontario, British Columbia-Ontario,

Ontario-British Columbia) ANOVA on the scaled proportion of response to the spliced songs. This analysis revealed a significant Discrimination Group \times Stimulus Type interaction ($F_{3,24} = 7.29, P = 0.001$). There were no other significant main effects or interactions (all $F \leq 0.41$, all $P \geq 0.55$). We conducted planned comparisons on the scaled proportion of response to the different types of spliced songs by birds in the British Columbia S+ group. For this analysis, we compared the spliced control (British Columbia-British Columbia) stimuli to the other three types. Birds responded significantly more to the British Columbia-British Columbia songs compared to the Ontario-British Columbia songs ($t = 2.70, P = 0.027$). We also conducted planned comparisons on the scaled proportion of response to the different types of spliced songs by birds in the Ontario S+ group. For this analysis, we compared the spliced control (Ontario-Ontario) stimuli to the other three types. Birds responded significantly more to the Ontario-Ontario songs compared to the British Columbia-British Columbia songs ($t = 2.79, P = 0.023$). See Figure 4.

Total duration manipulated songs. We conducted a Sex \times Discrimination Group (British Columbia S+, Ontario S+) \times Stimulus Type (British Columbia and Ontario songs with a total duration decreased, British Columbia and Ontario songs unmanipulated, and British Columbia and Ontario songs with a total duration increased) ANOVA on the scaled proportion of response to the songs in which the song duration was manipulated. This analysis revealed a significant Stimulus Type \times Discrimination Group interaction ($F_{5,40} = 14.59, P < 0.001$). There were no other significant main effects or interactions (all $F \leq 1.70$, all $P \geq 0.16$). We conducted planned comparisons on the scaled proportion of response to the different total duration-manipulated songs by birds in the British Columbia S+ group. For this analysis, we compared the control stimuli (British Columbia unmanipulated songs) to the other manipulated song types. Birds

responded significantly more to British Columbia unmanipulated songs compared to all Ontario song stimuli (decreased total duration, $t = 4.34$, $P = 0.002$; unmanipulated, $t = 3.78$, $P = 0.005$; increased total duration, $t = 2.86$, $P = 0.021$). We conducted similar planned comparisons for the responding by birds in the Ontario S+ group, by comparing the control stimuli (Ontario unmanipulated songs) to the other song types. Birds responded significantly more to Ontario unmanipulated songs than to British Columbia unmanipulated songs ($t = 2.74$, $P = 0.025$) and British Columbia songs increased in total duration ($t = 2.59$, $P = 0.032$). See Figure 5.

Acoustic analysis

The results from Experiment 1 revealed that birds in the true category group learned the discrimination in fewer trials compared to birds in the pseudo category group, while the results from Experiment 2 revealed no significant difference in the number of trials to reach criterion between the true category and pseudo category groups. In order to examine the acoustic variation between the rewarded and unrewarded songs for each discrimination group (i.e., true category and pseudo category) in each experiment, we conducted an acoustic analysis on the stimuli and multivariate analyses of variance (MANOVA) to compare acoustic features in the rewarded and unrewarded songs. For these analyses, we used nine acoustic measures as our dependent variables. We used the six acoustic measures that were analyzed in Hahn et al. (2013a): total duration, *fee* proportional duration, *fee* glissando, interval ratio, relative amplitude, and relative loudness of the *fee* note. In addition, we included three frequency measurements: start frequency of the *fee* note, start frequency of the *bee* note, and end frequency of the *fee* note. We conducted a separate MANOVA for the true category and pseudo category groups for each experiment. For

the true category group in Experiment 1, there was a significant difference in the total duration between the rewarded and the unrewarded songs ($F_{1,18} = 10.21, P = 0.005$). For the pseudo category group in Experiment 1, there was a significant difference in the *fee* glissando between the rewarded and unrewarded songs ($F_{1,18} = 4.96, P = 0.039$). In Experiment 2, there were no significant differences between the rewarded and unrewarded songs for either the true category or pseudo category groups; however, the smallest P -value was associated with the difference between the total duration of the rewarded and unrewarded songs for the true category group ($F_{1,18} = 3.12, P = 0.094$).

Discussion

In the current study, we report on the ability of black-capped chickadees to discriminate among songs produced by conspecifics from different geographic locations. Overall, the results reveal that songs belong to perceptual categories based on the singer's geographic origin. Results from the manipulated song tests reveal that while birds may use the total duration of the songs when discriminating, they also use other acoustic features to discriminate among songs based on geographic origin.

Perceptual categorization

In Experiment 1, we found that birds in the true category group learned the discrimination in fewer trials compared to birds in the pseudo category group, suggesting that birds in the true category group perceived songs as belonging to separate perceptual categories

while birds in the pseudo category did not. However, in Experiment 2, there was no significant difference in the number of trials to complete *Discrimination training* for the true and pseudo category groups. It is possible that, in Experiment 2, the pseudo category S+ songs happened to be more acoustically similar to one another simply by chance than to the S- songs and vice versa, thus creating “categories” of songs that the birds could use when discriminating (i.e., birds in pseudo category group used open-ended categorization when discriminating). It is also possible that the song stimuli used in Experiment 2 were not ideal exemplars of geographic origin, making it difficult for birds in the true category group to detect a categorical difference; therefore, birds in both the true and pseudo category groups were using rote memorization, resulting in no difference in how long it took the groups to learn the task. In order to examine these possibilities, we conducted an acoustic analysis of the song stimuli that were used in each experiment. The results from these analyses provide support that birds may have used the total duration of the songs to perform the discrimination in Experiment 1, as the total duration was found to be significantly different between the rewarded and unrewarded songs for the true category group. In addition, there was an acoustic feature (*fee* glissando) that was significantly different between the rewarded and unrewarded songs for the pseudo category group; however, in spite of this acoustic difference between rewarded and unrewarded songs, birds learning the pseudo category discrimination did not do so as fast as birds that learned the true category discrimination. This suggests that the total duration of the songs was an especially salient cue during the discrimination in Experiment 1. In Experiment 2, there were no significant differences in any of the measured acoustic parameters between the rewarded and unrewarded songs. This coincides with no significant difference in learning rate between true and pseudo category groups. Taken together, these results provide evidence that a true category group can learn the

category discrimination in significantly fewer trials compared to a pseudo category group when there are significant differences in the acoustic parameters between rewarded and unrewarded songs. However, in Experiment 2, even though the discrimination stimuli did not contain significant acoustic differences between the rewarded and unrewarded songs (likely leading to no observed difference between the true and pseudo category groups), the responding of birds in the true category group still generalized to novel stimuli (Figure 3).

In addition to examining differences in responding between true category and pseudo category groups, categorization abilities can also be tested by presenting the individuals with novel song exemplars. If the individuals continue to respond to the novel song exemplars based on the perceptual categories learned during *Discrimination training*, it suggests that birds are using categorization. The results from *Transfer training* (Experiment 1) and *Generalization* (Experiment 2) suggest that birds in the true category group were using open-ended categorization, as birds in the true category groups continued to respond to novel songs based on the contingencies from *Discrimination training*, while birds in the pseudo category group responded to the novel songs similarly regardless of geographic location.

In Experiment 2, we presented birds with novel songs from a third geographic location (i.e., John Prince Research Forest) to examine whether songs from this location would be perceived as similar to British Columbia or Ontario songs. Previous bioacoustic analyses indicate that songs from the John Prince Research Forest are more similar to songs recorded at the University of Northern British Columbia compared to songs recorded at the Queen's University Biological Station in Ontario (Hahn et al. 2013a). However, in Experiment 2, when we tested birds with novel songs from John Prince Research Forest, both true category S+ groups responded similarly to the songs, suggesting that songs from this location (although also

recorded in British Columbia) were perceived as distinct from the songs produced by birds in the other two locations. Although the bioacoustic analyses suggest acoustic similarities among the songs from these two British Columbia populations (Hahn et al. 2013a), the current results suggest that birds are perceiving acoustic differences in songs recorded in geographic regions that are relatively close (133 km separated the two British Columbia sites). In addition, birds perceive acoustic differences in songs recorded in geographic regions that are further apart (i.e., over 3,460 km separated the Ontario site from the British Columbia sites). Similarly, white-crowned sparrows produce one song type that varies geographically and geographic variation is evident for locations that are relatively close (3.2 km) and locations that are further apart (160 km; Marler and Tamura 1962).

While it is less likely that non-migratory birds would encounter individuals from the extremes of their geographic range (as we tested in the current study), black-capped chickadees disperse (e.g., up to 11 km for juvenile dispersal reported in Weise and Meyer 1979) and movements of longer distances have also been reported (e.g., 50-2,000 km; Brewer et al. 2006) so birds may encounter individuals originating from different geographic regions. Chickadees from different habitat types (high- and low-quality habitat, Grava et al. 2012) or habitats with different levels of anthropogenic noise (Proppe et al. 2012) produce songs with acoustic differences. In addition, the habitat-of-origin of both the singer and the song receiver influence how the singer is perceived (Grava et al. 2013). In mountain chickadees (*Poecile gambeli*), acoustic features in song vary between high and low habitat elevations (Branch and Pravosudov 2015) and high-elevation females prefer high-elevation males, suggesting that females prefer locally adapted males (Branch et al. 2015).

In humans, speech can be divided into categories using acoustic cues in accents to indicate different social groups for people speaking the same language. Adults will rate a speaker with an accent similar to their own (i.e., a native accent) as having more positive attributes compared to someone speaking the same language with a different accent (Anisfeld et al. 1962). Even prelinguistic children exhibit preferences for speakers with a native accent (Kinzler et al. 2007), demonstrating that accents are a mechanism by which people can perceive others as belonging to the same social group. Acoustic cues that enable group cohesion also exist in the vocalizations of other species such as primates (Byrne 1981), cetaceans (Ford 1989), bats (Boughman and Wilkinson 1998), psittaciformes (Wright 1996), and songbirds (Brown 1985; Feekes 1982), including black-capped chickadees. For example, the *chick-a-dee* call of the black-capped chickadee is a vocalization used to maintain flock cohesion (Ficken et al. 1978). If flock membership changes, there is convergence in acoustic parameters in the calls among flock members (Mammen and Nowicki 1981) which chickadees may use as an acoustic mechanism to discriminate flock members from non-members (Nowicki 1983). Geographic differences in *fee-bee* songs would allow chickadees to determine the geographic origin of conspecifics and distinguish a local bird from a bird that originated from a more distant geographic region.

Acoustic mechanisms

In Experiment 2, following *Discrimination training* and *Generalization*, we presented chickadees in the true category group with songs that we manipulated in order to examine the acoustic mechanism for the geography-based discrimination of songs. Specifically, we presented spliced songs and songs in which we altered the total duration.

While bioacoustic analyses may reveal certain acoustic features that vary among vocalizations, these analyses are limited to the actual features that are measured, and natural vocalizations contain a rich variety of possible acoustic cues that could be used by the animals themselves. The response of songbirds, including black-capped chickadees (Hahn et al. 2015; Hoeschele et al. 2012), is influenced by acoustic cues other than the features measured by bioacousticians. For example, song sparrows discriminate between the songs of males from local and distant populations (Searcy et al. 2002). Although song sparrow songs contain acoustically distinct note types, by manipulating song element composition, Searcy et al. (2003) found that song sparrows do not use specific element composition to discriminate local from more distant songs, suggesting that perceptual categories formed by researchers may not be perceptually distinct categories to birds (Searcy et al. 2003).

The current results provide support that birds were using the total duration of the songs when discriminating. When the duration of Ontario songs was increased, birds in the British Columbia S+ group responded more to these increased songs; however, birds still responded significantly less to these songs compared to unmanipulated British Columbia songs (Figure 5). Similarly, when British Columbia songs were decreased in duration, the responding to these songs by birds in the Ontario S+ group was not statistically different from the response to unmanipulated Ontario songs. These results are in line with the previous bioacoustic analyses, indicating that British Columbia songs are longer in duration compared to Ontario songs (Hahn et al. 2013a). In addition, the results suggest that birds were using other acoustic cues within the songs. Chickadees in both S+ groups responded more to S+ associated songs regardless of the manipulation (e.g., birds in the British Columbia S+ group responded similarly to unmanipulated songs, British Columbia songs increased in length, and British Columbia songs decreased in

length). One possibility is that birds were using acoustic information within one of the two song notes. To examine this possibility, we presented birds with spliced songs that contained one song note from each population. When presented with these spliced songs, birds in the British Columbia S+ group responded significantly less when an Ontario note was presented first in the song, providing evidence that birds rewarded for responding to British Columbia songs were relying on acoustic information in the *fee* note. However, birds in the Ontario S+ group responded similarly to songs containing one British Columbia note and one Ontario note and songs containing two Ontario notes, suggesting that birds were responding based on acoustic information within either song note. Responding similarly to the different spliced songs may also indicate that there are acoustic similarities among the notes from different locations. If the individual note types from each location are acoustically similar, birds likely used a combination of acoustic features when multiple features were available (i.e., when discriminating among natural songs).

In addition to acoustic cues within each song note, there could also be relevant information in the internote interval of natural songs. Corn buntings (*Emberiza calandra*) recognize dialects using components of both the song elements and silence portion between notes (Pellerin 1982), demonstrating the importance of the song composition as a whole and not a single feature within the acoustic song elements. In *fee-bee* songs, important acoustic information exists in the relationship between notes. For example, acoustic cues for male dominance exists in the frequency ratio (Christie et al. 2004b) and the amplitude ratio (Hoeschele et al. 2010) between the two song notes. The biological relevance of the two notes in combination with one another may be a reason that birds do not only rely on information in one of the notes.

In other songbird species, birds may rely on acoustic features in only one portion of the song in order to perceive geographic differences. For example, in one subspecies of white-crowned sparrow (*Zonotrichia leuophrys pugetensis*) males use the terminal trill portion of the song as an acoustic cue for geographic variation more than the introductory components of the song (Nelson and Soha 2004); however in another subspecies of white-crowned sparrow (*Z. l. nuttalli*) males rely on the introductory components of the song when distinguishing between local and foreign dialects (Thompson and Baker 1993). In contrast, female response is not mediated by a single component of the song alone, but females rely on a combination of acoustic cues (Baker et al. 1987), demonstrating that the mechanisms used to perceive geographic differences in songs can also vary within a single species. These differences in perception may be related to biological relevance. In the current study, we presented chickadees with songs from two distant geographic regions, but chickadees were not tested with local songs. Acoustic similarities or differences between the songs produced in the subjects' location of origin (i.e., central Alberta) and the songs used as stimuli (i.e., songs from northern British Columbia and eastern Ontario) may influence the biological salience of the songs. Further work is required to examine how songs produced by birds in the local (i.e., Alberta) population compare to songs produced by birds in British Columbia and Ontario; however, birds from Alberta are from the same subspecies as birds from British Columbia (*Poecile atricapillus septentrionalis*), while birds from Ontario are from a different subspecies (*P.a. atricapillus*; Pyle, 1997) so it is likely that local songs may be acoustically similar to songs produced by birds of the same subspecies. European starlings discriminate between variation in geographic dialects and show stronger responses (i.e., vocalize more often and with a shorter latency) to a familiar song dialect (Adret-Hausberger 1982), suggesting that local songs are a more salient acoustic signal. Further studies

should examine how chickadees would respond if tested with local songs and if it would be easier for chickadees to distinguish local songs from songs originating from more distant locations.

Conclusions

Our results provide evidence that black-capped chickadees can discriminate among songs produced by males at distant parts of the species' range using perceptual categorization. For black-capped chickadee *fee-bee* songs, although there is little overall structural variation across geographic regions for non-isolated groups (Kroodsma et al. 1999), the results from the current experiments suggest that songs contain acoustic variation that chickadees can perceive. In both experiments, when chickadees were presented with novel song stimuli from the two locations, only birds in the true category groups continued to respond to songs based on the contingencies learned during *Discrimination training*, which suggests that these birds were using open-ended categorization. In contrast, chickadees in the pseudo category groups responded to the novel songs non-differentially, which suggests that birds in these groups were relying on rote memorization to learn the task during the initial *Discrimination training*. Tests with manipulated song stimuli (spliced songs and total duration manipulated songs) revealed that birds were likely using multiple acoustic features when discriminating. In addition, the results suggest that the particular songs initially discriminated during acquisition (including which songs were reinforced) can influence the initial discrimination performance, as well as, the specific acoustic features that birds use when discriminating.

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Figure legends

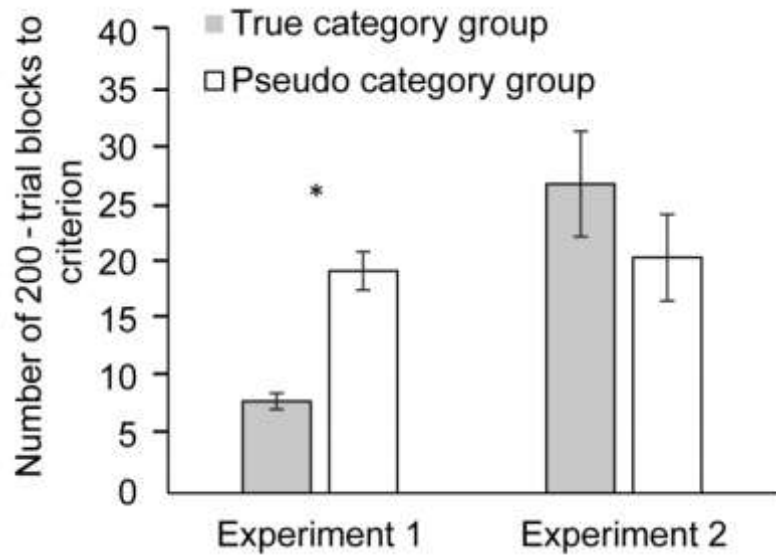


Figure 1. The average \pm SE number of 200-trial blocks to reach the *Discrimination training* criterion for birds in the true category group (gray bars) and pseudo category group (white bars) in each experiment. (*) indicates a significant difference in the number of trial blocks to reach criterion between the true and pseudo category groups ($P \leq 0.05$).

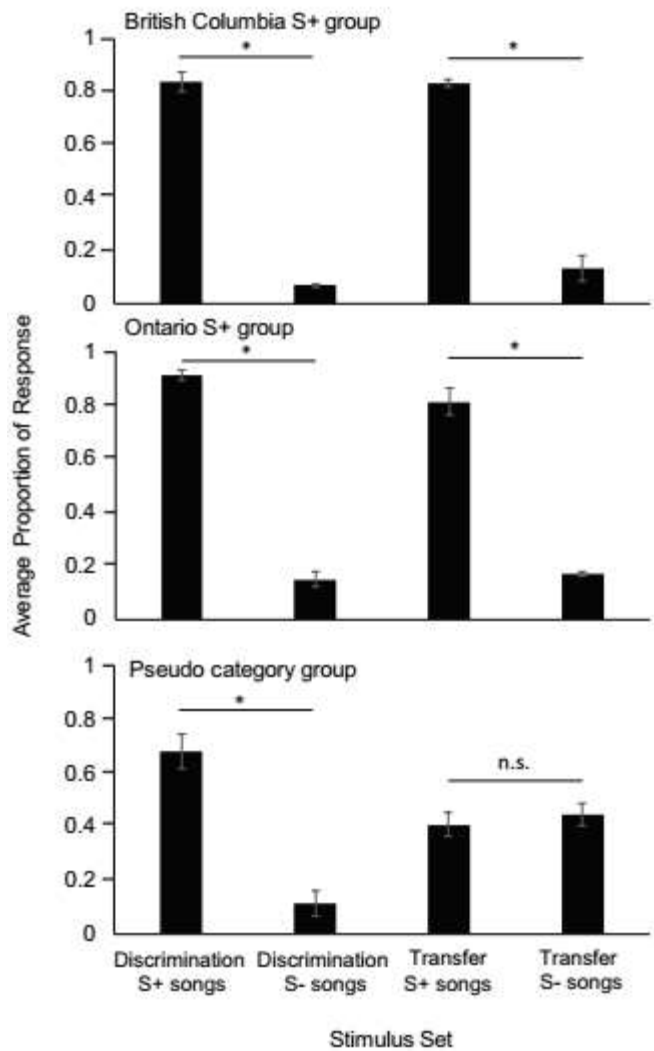


Figure 2. Average \pm SE proportion of response to Discrimination S+ songs, Discrimination S- songs, Transfer S+ songs, and Transfer S- songs during the first 200-trial block of *Transfer training* for each group in Experiment 1. Lines indicate the comparisons that were made (*) indicates a significant difference in responding by that discrimination group; n.s. indicates no significant difference; $P \leq 0.05$).

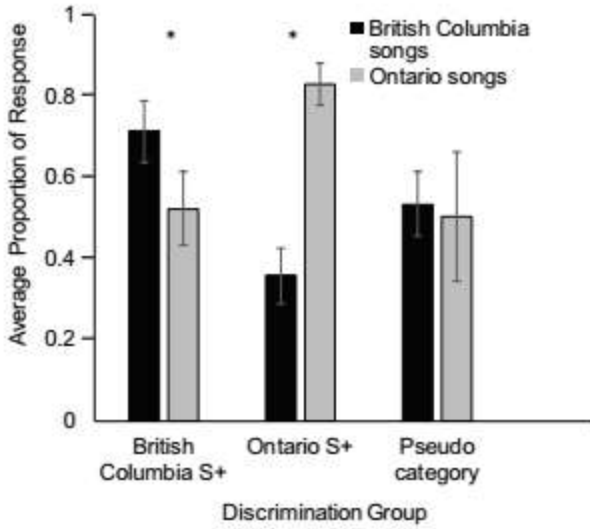


Figure 3. Average \pm SE proportion of response for each discrimination group during *Generalization* in Experiment 2. (*) indicates a significant difference in response to British Columbia and Ontario songs by that discrimination group ($P \leq 0.05$).

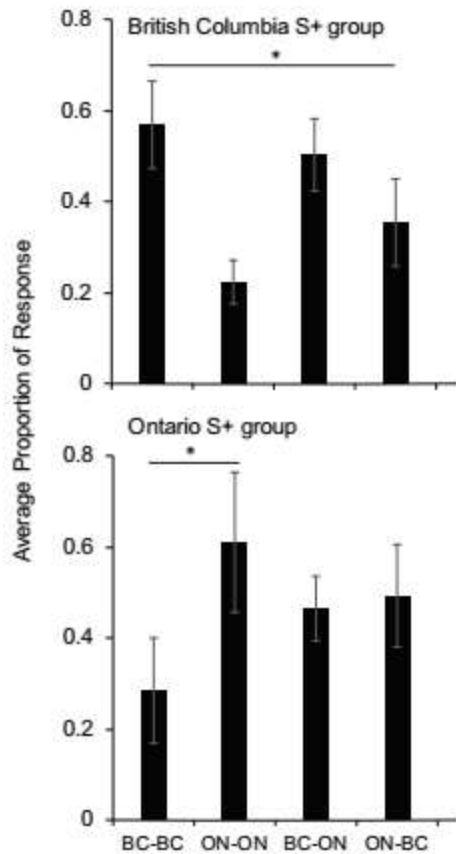


Figure 4. Average \pm SE proportion of response to spliced songs in Experiment 2. The different stimulus types were: British Columbia *fee* and *bee* notes (BC-BC), Ontario *fee* and *bee* notes (ON-ON), British Columbia *fee* note and Ontario *bee* note (BC-ON), and Ontario *fee* note and British Columbia *bee* note (ON-BC). The top panel displays the response by birds in the British Columbia S+ discrimination group; the bottom panel displays the response by birds in the Ontario S+ discrimination group. (*) indicates a significant difference in response ($P \leq 0.05$).

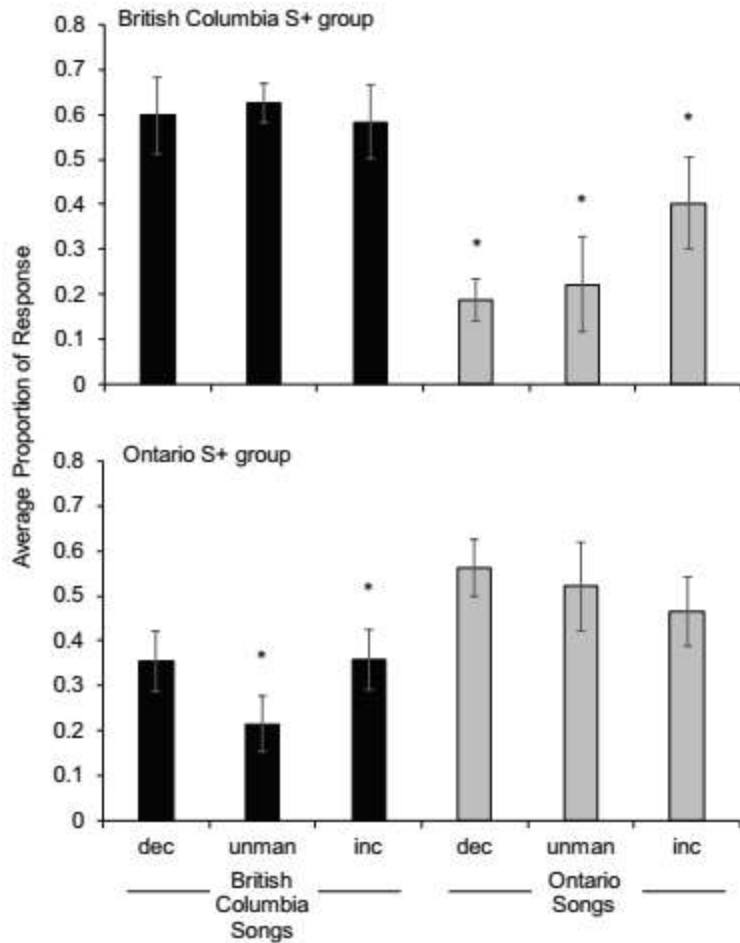


Figure 5. Average \pm SE proportion of response during Experiment 2 to total duration manipulated songs. Black bars are British Columbia songs and gray bars are Ontario songs. Stimuli were presented in three ways: decreased in duration (dec), unmanipulated (unman) and increased in duration (inc). (*) indicates a significant difference in response compared to the unmanipulated control songs (unman British Columbia songs for birds in British Columbia S+ discrimination group; unman Ontario songs for birds in Ontario S+ discrimination group; $P \leq 0.05$).