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 whether 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 geographical location as the songs that were reinforced during initial discrimination training, suggesting that birds were using open-ended categorization. We also 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.

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Many animals' vocal signals vary geographically, including anurans (Pröhl, Hagemann, Karsch, & Höbel, 2007; Ryan & Wilczynski, 1991), birds (Baker & Cunningham, 1985; Wright, 1996) and mammals (Campbell et al., 2010; Kershenbaum, Ilany, Blaustein, & Geffen, 2012; Mitani, Hunley, & Murdoch, 1999). For songbirds, the pattern of geographical variation differs across species (for review see: Catchpole & Slater, 2008; Podos & Warren, 2007). For example, in some species, neighbouring males share song types, but song types vary across different populations of males (e.g. white-crowned sparrow, *Zonotrichia leuophrys*: Marler & Tamura, 1962). Because male song in passerines is a sexual signal, used for territory defence and mate attraction, the ability to discriminate among vocalizations on the basis of geographical variation would be advantageous if, for example, local adaptations exist (Kawecki & 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 geographical 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 & Verner, 1971; corn bunting, *Emeriza calandra*: McGregor, 1983; Darwin's ground finches, *Geospiza* spp.: Ratcliffe & Grant, 1985; song sparrow: Searcy, Nowicki, & Hughes, 1997). Females, too, show preferences

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for male songs based on geographical information (e.g. white-crowed sparrow: Baker, Spitler-Nabors, & Bradley, 1981; rufous-collared sparrow, *Zonotrichia capensis*: Danner et al., 2011). 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 that birds could use 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 & Hulse, 1998; zebra finches, *Taeniopygia guttata*: Sturdy, Phillmore, Price, & Weisman, 1999; black-capped chickadees, *Poecile atricapillus*: Bloomfield & Sturdy, 2008; Hahn et al., 2015). Another mechanism that animals could use when discriminating among vocalizations is rote memorization. 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 nonlocal animal without memorizing all song types produced by local animals. Field-based playback studies provide evidence that birds recognize individual neighbours using cues from song and location (e.g. Falls & Brooks, 1975) and perceive different song types as produced by the same individual (e.g. Searcy, Podos, Peters, & Nowicki, 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 classify new signals quickly. In contrast, animals relying on rote memorization are not able to learn a general categorization rule when discriminating among signals.

In contrast to the geographical variation that is found in the songs produced by other songbird species (e.g. swamp sparrow, *Melospiza georgiana*: Marler & Pickert, 1984; song sparrows, *Melospiza melodia*: Searcy, Nowicki, & Peters, 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, Leonard, Ratcliffe, Shackleton, & Weisman, 1992; Weisman, Ratcliffe, Johnsrude, & Hurly, 1990). Fee-bee songs contain acoustic features indicating individual identity (Christie, Mennill, & Ratcliffe, 2004a; Hahn, Krysler, & Sturdy, 2013), and field (Wilson & Mennill, 2010) and laboratory (Phillmore, Sturdy, Turyk, & Weisman, 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 geographical 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 & Baker, 2004; Kroodsma et al., 1999). Recently, Hahn, Guillette, et al. (2013) conducted a bioacoustic analysis on songs produced by birds from different geographical locations (northern British Columbia and eastern Ontario). For this bioacoustic analysis, six acoustic features were analysed, similar to previous bioacoustic analyses of

fee-bee songs (e.g. Christie et al., 2004a; Christie, Mennill, & Ratcliffe, 2004b; 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 & Sommer, 2007) to determine which acoustic features vary with geographical location, Hahn, Guillette, et al. (2013) 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, Guillette, et al., 2013; Hoeschele et al., 2010), indicating that additional geographical variation 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 geographical 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, Farrell, & Sturdy, 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 geographical locations (i.e. British Columbia or Ontario). If songs produced by birds within each geographical 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 nonreinforced. 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 nonreinforced 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 geographical 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 nondifferentially to the novel songs because birds in this group would 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, Guillette, et al., 2013) as a cue when discriminating. We also examined whether 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 whether birds used features within one of the song notes to discriminate between the

geographical locations. In this study, our subject chickadees originated from a geographical region (i.e. central Alberta) distant from the two regions where the stimulus songs were recorded (i.e. British Columbia or Ontario). Geographical 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 1 year of age by plumage (Pyle, 1997). Sex was determined by DNA analysis (Griffiths, Double, Orr, & Dawson, 1998).

Before the experiment, birds were housed in individual cages (30 × 40 × 40 cm, Rolf C. Hagen, Inc., Montreal, QC, 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, U.S.A.), water (vitamin supplemented 3 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, Hilhorst, Ferguson, & Weisman, 1994) through an opening (11 × 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 & 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, U.K.) or an NAD310 Integrated Amplifier (NAD Electronics, London, U.K.) and a speaker to the side of the feeder (Fostex FE108 Σ or Fostex FE108E Σ full-range speaker; Fostex Corp., Tokyo, 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 per day, depending on the number of daylight hours (which ranged from approximately 7.5 to 16 h). Birds were provided one superworm twice per 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, BC, Canada) between 27 April and 14 May 2000–2004 and Queen's University Biological Station (near Kingston, ON, Canada) 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, U.S.A.) or an Audio-Technica ATB815a microphone (Audio-Technica U.S., Stow, OH, U.S.A.) and a Marantz PMD430 (Marantz America, Mahwah, NJ, U.S.A.) 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, U.S.A.) 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, BC) 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 band-pass 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, U.S.A.) 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, 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, Reddon, Hoeschele, & Sturdy, 2011; Hoeschele, Weisman, Guillette, Hahn, & Sturdy, 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 that 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 rewarded songs (i.e. S+ stimuli) and the un-rewarded songs (i.e. S− stimuli) 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 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 time-out with the house light 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 house light 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 house light 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 at least 60% responding and at least four 200-trial blocks no more than a 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 10 British Columbia songs and 10 Ontario songs were presented. Half of the songs were now assigned to be S+ (i.e. rewarded) and half as S− (i.e. unre-warded). 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 house light off. Re-sponses 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 discrimi-nation group were divided into two subgroups: one group (one male, two females) discriminated rewarded British Columbia songs from unrewarded Ontario songs (referred to hereafter as British Columbia S+ group), while the other group (two males, one female) discriminated rewarded Ontario songs from unrewarded British Columbia songs (referred to hereafter as Ontario S+ group). Birds in the pseudo category discrimination group (three males, two fe-males) 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 10 British Columbia songs and 10 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 geographical location or pseudorandomized).

Response measures
A discrimination ratio (DR) is a measure of how well birds discriminate rewarded (S+) songs from unrewarded (S− songs). To calculate a DR, first, we calculated the percentage 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 percentage response to the S+ stimuli by the average percentage response to the S+ stimuli plus the average percentage 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 $\geq$ 0.75 with the last two blocks being consecutive. Following discrimination training, birds completed transfer training, which ended once the birds completed three 200-trial blocks with a DR $\geq$ 0.75 with the last two blocks being consecutive. One bird (a male in the pseudo category group) died during this phase; how-ever, 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 discrimi-nation 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 ANOVAs 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 whether birds in the true category group continued to response 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 for-mula: $\arcsin[\mathrm{sqrt}(x)]$, where x equals the untransformed proportion of responding. Figures depict the untransformed data. All statistics were conducted in Statistica v.12 (StatSoft, Inc., Tulsa, OK, U.S.A.).

Ethical note
Throughout the experiments, birds were monitored daily, pro-vided 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 at the University of Alberta (AUP 108) and the University of Calgary Life and Environmental Sciences Animal Care Committee. All procedures were in accordance with the Canadian Council on Animal Care (CCAC) Guidelines and Pol-icies and the ASAB/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 a 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 groups (i.e. British Columbia S+, Ontario S+; $t_3 = 3.00$, $P = 0.058$), but found a
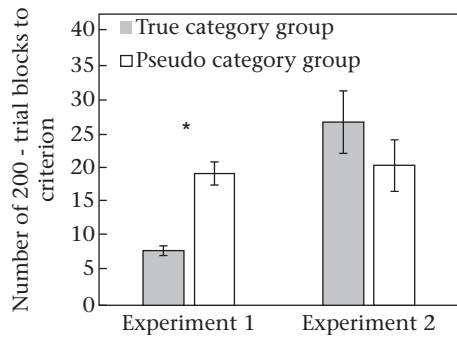
Figure 1. Mean ± SE number of 200-trial blocks to reach the discrimination training criterion for black-capped chickadees in the true category group and the pseudo category group in each experiment. *Indicates a significant difference in the number of trial blocks to reach criterion between the true and pseudo category groups ($P \leq 0.05$).

significant difference between the true and pseudo category groups ($t_6 = 7.36$, $P = 0.0003$), with the pseudo category group requiring more than twice as many trial blocks to complete the discrimination (mean trial blocks: true category group: 8.0; pseudo category group: 19.33; Fig. 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) × 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∗ 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 ($t = -0.79$, $P = 0.458$; Fig. 2).

EXPERIMENT 2

Results from experiment 1 suggested that birds could discriminate between songs produced by birds from different geographical 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
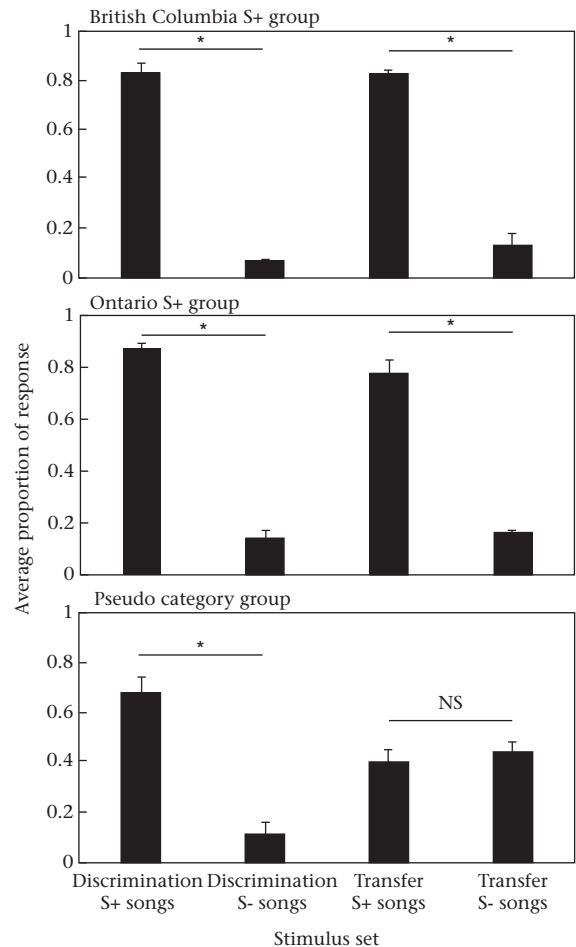

Figure 2. Mean ± SE proportion of response to Discrimination S+ songs, Discrimination S− songs, Transfer S+ songs and Transfer S− songs during the first 200-trial block of transfer training for each group of black-capped chickadees in experiment 1. *Indicates a significant difference ($P \leq 0.05$) in response.

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 males, eight females) 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. We used 10 British Columbia songs and 10 Ontario songs 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 whether birds were preferentially using information in one of the two notes to discriminate songs produced by birds from the two geographical locations. Songs ($N = 16$) were band-pass 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. mean$_{internote} = 135$ ms; Ficken, Ficken, & Witkin, 1978) and the internote interval used by other studies manipulating song features (e.g. mean$_{internote} = 100$ ms; Hahn et al., 2015; Hoeschele, Guillette, & Sturdy, 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 (http://audacity.sourceforge.net). This manipulation resulted in songs with a mean $\pm$ 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 geographical locations (mean $\pm$ SD $= 1.13 \pm 0.022$; range$1.047-1.206$; Hahn, Guillette, et al., 2013) 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 whether birds were using the total duration to discriminate between songs produced by birds from the two geographical locations. To create the total-duration manipulated stimuli, we used four British Columbia songs and four Ontario songs. We manipulated each song (by lengthening or shortening the note and interval lengths, respectively) to increase or decrease total duration by approximately 3 SD (i.e. $\pm$ 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 total duration decreased, total duration increased and total duration unmanipulated.

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 no more than a 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 a 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 house light 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 ≥ 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 songs 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 house light 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+, Ontario S+) continued on to 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 on to 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 whether birds in the two true category groups differed in their speed of acquisition during discrimination training. We also conducted similar analyses to determine whether 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 v.12.

Results

Trials to criterion

Two females in the British Columbia S+ group were not included in the analysis of discrimination training because of 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 that were 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 ($t_2 = 0.11$, $P = 0.92$), 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 (Fig. 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*stimulus type interaction ($F_{2,10} = 30.38$, $P < 0.001$) and a significant sex*discrimination group*stimulus type interaction ($F_{2,10} = 6.22$, $P = 0.018$). We conducted a planned comparison to examine the discrimination group*stimulus type interaction. Birds in the British Columbia S+ group responded significantly more to British Columbia songs ($t_{10} = 2.75$, $P = 0.020$), birds in the Ontario S+ group responded significantly more to Ontario songs ($t_{10} = 7.85$, $P < 0.001$), while birds in the pseudo category group did not respond significantly differently to the two song types ($t_{10} = 0.38$, $P = 0.71$; Fig. 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 ($t_{10} = 0.56$, $P = 0.59$), indicating that when tested with songs from a third location, birds responded similarly to the new songs regardless of which geographical 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*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
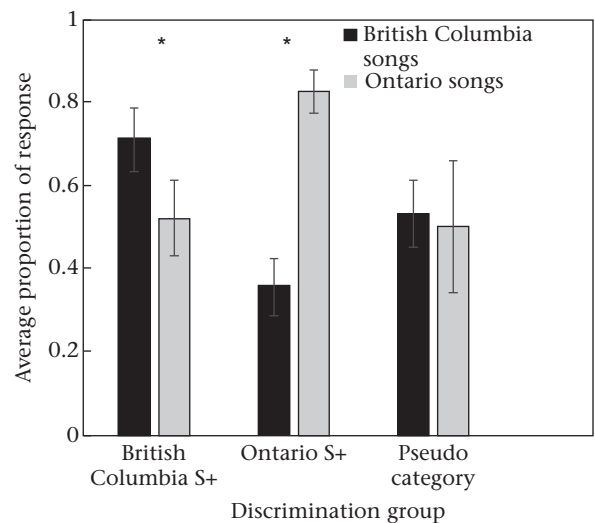

Figure 3. Mean ± SE proportion of response for each discrimination group of black-capped chickadees during generalization in experiment 2. *Indicates a significant difference ($P \leq 0.05$) in response to British Columbia and Ontario songs by that discrimination group.

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 ($t = 2.79$, $P = 0.023$; Fig. 4).

Total-duration manipulated songs. We conducted a sex × discrimination group (British Columbia S+, Ontario S+) × stimulus type (British Columbia and Ontario songs with total duration decreased, unmanipulated British Columbia and Ontario songs, and British Columbia and Ontario songs with 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∗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 (unmanipulated British Columbia songs) to the other manipulated song types. Birds responded significantly more to unmanipulated British
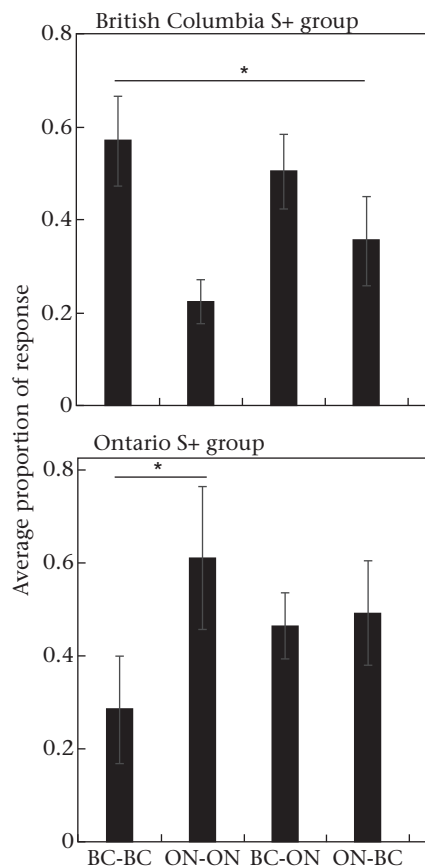

Figure 4. Mean ± SE proportion of response to spliced songs by black-capped chickadees in the British Columbia S+ discrimination group and in the Ontario S+ discrimination group in experiment 2. The different stimulus types were: British Columbia fee and bee notes (BC−BC), Ontario fee and bee notes (ON−ON), British Columbia fee note and Ontario bee note (BC−ON), and Ontario fee note and British Columbia bee note (ON−BC). *Indicates a significant difference ($P \leq 0.05$) in response.
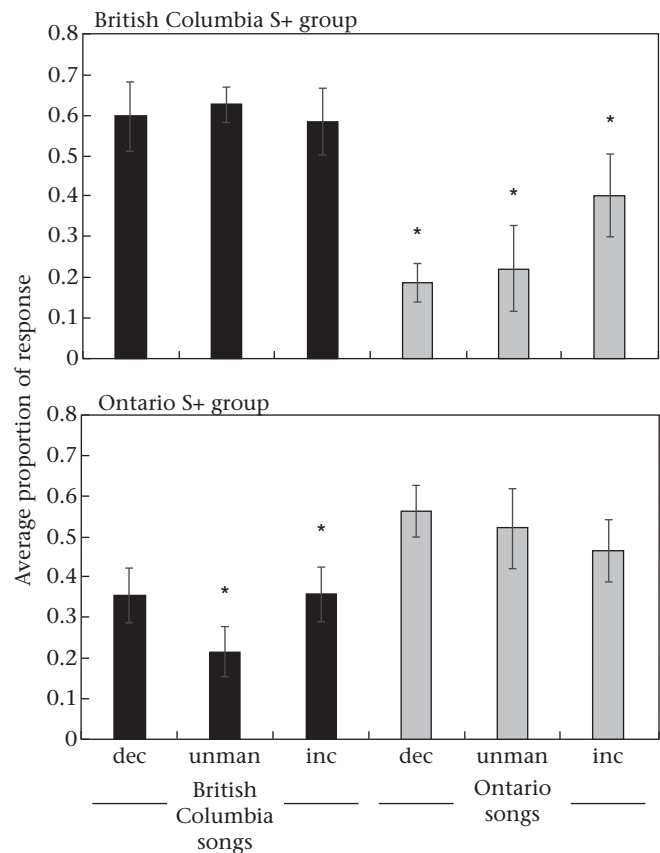

Figure 5. Mean ± SE proportion of black-capped chickadees' response to British Columbia songs (black bars) and Ontario songs (grey bars) relative to total-duration manipulated songs during experiment 2. Stimuli were presented in three ways: decreased in duration (dec), unmanipulated (unman) and increased in duration (inc). *Indicates a significant difference ($P \leq 0.05$) in response compared to the unmanipulated control songs (unmanipulated British Columbia songs for birds in British Columbia S+ discrimination group; unmanipulated Ontario songs for birds in Ontario S+ discrimination group).

Columbia songs than 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 (unmanipulated Ontario songs) to the other song types. Birds responded significantly more to unmanipulated Ontario songs than to unmanipulated British Columbia songs ($t = 2.74$, $P = 0.025$) or British Columbia songs with total duration increased ($t = 2.59$, $P = 0.032$; Fig. 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. 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 MANOVAs 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 analysed in Hahn, Guillette, et al. (2013): 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 geographical locations. Overall, the results reveal that songs belong to perceptual categories based on the singer's geographical 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 geographical origin.

Perceptual Categorization

In experiment 1, we found that birds in the true category group learned the discrimination in fewer trials than 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 geographical 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. To examine these possibilities, we conducted an acoustic analysis of the song stimuli that we 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 than 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 (Fig. 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 geographical location.

In experiment 2, we presented birds with novel songs from a third geographical 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, Guillette, et al., 2013). 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, Guillette, et al., 2013), the current results suggest that birds are perceiving acoustic differences in songs recorded in geographical regions that are relatively close (133 km separated the two British Columbia sites). In addition, birds perceive acoustic differences in songs recorded in geographical regions that are farther apart (i.e. over 3460 km separated the Ontario site from the British Columbia sites). Similarly, white-crowned sparrows produce one song type that varies geographically, and geographical variation is evident for locations that are relatively close (3.2 km) and locations that are farther apart (160 km; Marler & Tamura, 1962).

While it is less likely that nonmigratory birds would encounter individuals from the extremes of their geographical range (as we tested in the current study), black-capped chickadees disperse (e.g. up to 11 km, for juvenile dispersal reported in Weise & Meyer, 1979) and movements of longer distances have also been reported (e.g. 50–2000 km; Brewer, Diamond, Woodsworth, Collins, & Dunn, 2006), so birds may encounter individuals originating from different geographical regions. Chickadees from different habitat types (high- and low-quality habitat; Grava, Grava, & Otter, 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, Grava, & Otter, 2013). In mountain chickadees, *Poecile gambeli*, acoustic features in song vary between high- and low-elevation habitats (Branch &

Pravosudov, 2015) and high-elevation females prefer high-elevation males, suggesting that females prefer locally adapted males (Branch, Kozlovsky, & Pravosudov, 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, Bogo, & Lambert, 1962). Even prelinguistic children exhibit preferences for speakers with a native accent (Kinzler, Dupoux, & Spelke, 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 & 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 & Nowicki, 1981), which chickadees may use as an acoustic mechanism to discriminate flock members from nonmembers (Nowicki, 1983). Geographical differences in fee-bee songs would allow chickadees to determine the geographical origin of conspecifics and distinguish a local bird from a bird that originated from a more distant geographical 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, Nowicki, Hughes, & Peters, 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 than to unmanipulated British Columbia songs (Fig. 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 than Ontario songs (Hahn, Guillette, et al., 2013). 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 that were 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 probably 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 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 geographical 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 geographical variation more than the introductory components of the song (Nelson & Soha, 2004); however, in another subspecies of white-crowned sparrow (*Zonotrichia leuophrys nuttalli*) males rely on the introductory components of the song when distinguishing between local and foreign dialects (Thompson & 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, Spitler-Nabors, Thompson, & Cunningham, 1987), demonstrating that the mechanisms used to perceive geographical 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 geographical 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 (*P. a. 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 geographical 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 geographical regions for nonisolated 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 nondifferentially, 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.

Acknowledgments

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