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Dominance and geographic information contained within black-capped chickadee (*Poecile atricapillus*) song

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Abstract

In songbirds, male song is an acoustic signal used to attract mates and defend territories. Typically, song is an acoustically complex signal; however, the *fee-bee* song of the black-capped chickadee is relatively simple. Despite this relative simplicity, two previous studies (Christie et al., 2004b; Hoeschele et al., 2010) found acoustic features within the *fee-bee* song that contain information regarding an individual's dominance rank; however each of these studies reported a different dominance-related acoustic cue. Specifically, the relative amplitude of the two notes differed between the songs of dominant and subordinate males from northern British Columbia, while the interval pitch ratio differed between the songs of dominant and subordinate males from eastern Ontario. In the current study, we examined six acoustic features within songs from both of the chickadee populations (northern British Columbia and eastern Ontario) examined in these previ-

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ous studies and used bioacoustic analyses and discriminant function analyses to determine whether there is a consistent dominance-related acoustic cue across both, or in each of these populations. Consistent with the previous findings, the current results indicate that relative amplitude differs based on dominance status in the songs from British Columbia; however, our results failed to reach significance with songs from Ontario. These results suggest that acoustic cues that signal a male's dominance in this species vary with geographic location. Furthermore, examining songs from these two locations and one additional location in northern British Columbia, we found that discriminant function analyses could correctly classify songs based on geographic location. Considering the broad extent of the species' range, black-capped chickadee song is considered relatively invariant; however, our results suggest that there is geographic variation in songs, although the differences are subtle compared to geographic song variation in other species.

Keywords
black-capped chickadees, *fee-bee* song, dominance, geographic variation, song structure.

1. Introduction

In songbirds, male song is primarily used for mate attraction and territory defense (Catchpole & Slater, 2008); however, in many species additional information is contained within song, including individual identity (see Catchpole & Slater, 2008 for review), geographic origin (e.g., swamp sparrows, *Melospiza georgiana*, Marler & Pickert, 1984; white-crowned sparrows, *Zonotrichia leucophrys nuttalli*, Baker & Thompson, 1985; song sparrows, *Melospiza melodia*, Searcy et al., 2003), male quality (dusky warblers, *Phylloscopus fuscatus*, Forstmeier et al., 2002), and dominance rank (black-capped chickadees, *Poecile atricapillus*, Christie et al., 2004b; Hoeschele et al., 2010).

For animals that live in social groups with dominance hierarchies, a male's condition may influence his dominance rank or fighting ability and acoustic cues can signal this dominance status. For example, dominant males may vocalise more frequently than subordinates (domestic chicken, *Gallus gallus domesticus*, Leonard & Horn, 1995; black-capped chickadee, *Poecile atricapillus*, Otter et al., 1997). In general, dominance hierarchies are established based on the outcomes of competitive dyadic interactions over access to resources (Drews, 1993; Ratcliffe et al., 2007). Because dominance rank is established through dyadic encounters, and is not an absolute trait of an individual, dominance is a relative measure (Drews, 1993); however, in many species that live in stable social groups, dominance hierarchies are also stable (Wiley et al., 1999). Established dominance hierarchies diminish the need for frequent confrontations to maintain position within the social group.

Individuals do not need prior experience with one another to determine a conspecific's dominance rank if other rank-identifying cues are present (e.g., morphological or vocal characteristics). In this way, the maintenance of stable dominance hierarchies does not require individual recognition (Drews, 1993).

Black-capped chickadees are songbirds that live in winter flocks with linear dominance hierarchies. The dominance hierarchies are stable, persisting from flock establishment in winter through flock break-up and territorial establishment in spring (Smith, 1991; Ratcliffe et al., 2007). Birds of different dominance status exhibit fitness differences; dominant males have greater over-winter survival, more readily acquire a territory during the breeding season (Desrochers et al., 1988), obtain larger territories, which contain more resources (Mennill et al., 2004), and have higher lifetime reproductive success (Schubert et al., 2007). Whereas visual cues correlate with dominance status in some avian species (e.g., badge size in house sparrows, *Passer domesticus*, Møller, 1987), including chickadees (plumage colouration and reflectance, Mennill et al., 2003), acoustic cues can indicate dominance status when visual contact is not available, as is common for many territorial animals during the breeding season.

Compared to the complex songs of many oscines (Catchpole & Slater, 2008), the *fee-bee* song of the black-capped chickadee appears to be a relatively simple long-distance acoustic signal (Dixon & Stefanski, 1970; Ficken et al., 1978; Mennill & Otter, 2007), consisting of two whistled notes, with the first note (i.e., the *fee* note) sung at a higher frequency than the second note (i.e., the *bee* note). All male chickadees produce *fee-bee* songs across a range of absolute pitches (Weisman & Ratcliffe, 1989; Weisman et al., 1990; Horn et al., 1992). During a singing bout, a male can increase or decrease the absolute frequency of his song, a behaviour known as 'pitch shifting' (Ratcliffe & Weisman, 1985; Hill & Lein, 1987) and males will often pitch shift in order to match the frequency of another male's song (Horn et al., 1992). Two previous studies (Christie et al., 2004b; Hoeschele et al., 2010) found acoustic features within *fee-bee* songs that indicate a male's dominance rank; however each of these studies reported a different dominance-related cue. Examining songs from eastern Ontario, Christie et al. (2004b) found that dominant males maintain a more consistent interval ratio (i.e., the frequency difference between the *fee* and *bee* notes) over multiple renditions of songs as they shift the absolute pitch of the song than do subordinate males. In contrast, subordinate male songs' interval ratio decreases as the absolute pitch of the song is increased (Christie et al., 2004b). Examining songs from northern British Columbia, Hoeschele et al. (2010), found that within a song, the amplitude ratio of the *fee* and the *bee* notes is produced in a more consistent manner in dominant males' songs compared to the songs of subordinate males and these results indicate that relative amplitude is an acoustic feature that could indicate a male's rank within a single song exemplar. In general, song consistency in songbirds may be related to male age, dominance status, and social context (for review see Sakata & Vehrencamp, 2012). For black-capped chickadees, although the amplitude of the *fee* note relative to the entire song has been measured in Ontario songs (i.e., Christie et al., 2004a, b), these studies did not examine the relative amplitude between the *fee* and the *bee* note, as was shown to be important in the British Columbia study. Therefore, relative amplitude, which differs between dominant and subordinate songs in northern British Columbia (Hoeschele et al., 2010), may also serve as an acoustic dominance marker in songs produced by birds from eastern Ontario. The results of these previous studies (Christie et al., 2004a, b; Hoeschele et al., 2010) have indicated that certain features within songs are possible dominance markers within a population; whether these dominance markers are consistent across populations, or whether dominance-related cues vary by the birds' geographic origin has not been examined.

In the current study, we examined acoustic features in *fee-bee* songs that may be used as cues for dominance status or geographic origin. To examine possible dominance-related cues, we measured six acoustic features in the songs from two populations of chickadees. We examined each of these two populations separately using discriminant function analyses to determine which acoustic feature(s) may be associated with a male's dominance status. Two previous studies, looking at songs from two separate populations, examined potential dominance cues. Christie et al. (2004b) found a potential dominance cue in *fee-bee* songs as males shifted the absolute frequency of their songs, while Hoeschele et al. (2010) reported a cue that could indicate dominance with information from a single song exemplar. In the current study, we try to clarify these previous results to determine if there is a consistent acoustic feature, found within an individual song, which could be used as a dominance cue in both populations of black-capped chickadees.

In contrast to the geographic variation found in the songs of many songbird species (see Podos & Warren, 2007 for review), the overall structure of the *fee-bee* song has been considered to be relatively invariant across the species' range (Hailman, 1989; Kroodsma et al., 1999), with few exceptions in geographically-isolated island populations and isolated mainland chickadee populations (Kroodsma et al., 1999; Gammon & Baker, 2004). The different dominance-related features found by previous studies led us to also examine if there is overall geographic variation within the song. Using discriminant function analyses, we examined if acoustic features vary between songs produced by birds from different geographic origins. We examined songs produced by dominant and subordinate birds together and each dominance status independently (i.e., only dominant or only subordinate) in terms of geographic origin to examine potential geographic differences in *fee-bee* songs.

2. Methods

2.1. Recordings

Fee-bee songs were recorded in the field during the dawn chorus period at the University of Northern British Columbia (Prince George, BC, Canada) (53°54′N, 122°50′W) between 27 April and 14 May 2000–2004, at a field station in the John Prince Research Forest (Fort St. James, BC, Canada) (54°40′N, 124°24′W) between 28 April and 16 May 2006, 2008–2009 and at the Queen's University Biological Station (near Kingston, ON, Canada) (44°34′N, 76°19′W) between 25 April and 10 May 1999–2001. The songs were from banded populations of chickadees, and the dominance rank of the birds was known. Dominance assessments were made based on a set of standardised observed behaviours (e.g., supplants, chases; see Smith, 1991; Ratcliffe et al., 2007 for details). For the purpose of this study, a male was considered dominant if it was the highest-ranking male within a flock. A male was considered subordinate if it was the lowest-ranking male within a flock (i.e., second-ranking male in flocks with two males; third-ranking male in flocks with three males). Middle-ranked male songs were not analysed. Dominance ranks were assessed by observing multiple interactions between male flock-mates at temporary feeding stations (see Mennill et al., 2004; van Oort et al., 2006 for more information on dominance assessment). The interactions used to establish the relative dominance relationships were collected prior to recording male choruses in the spring.

Songs obtained from birds in the University of Northern British Columbia, BC population were recorded using either a Sennheiser MKH70 or ME67 (Sennheiser Electronic, Old Lyme, CT, USA) or an Audio-Technica ATB815a microphone (Audio-Technica U.S., Stow, OH, USA) and a Marantz PMD430 (Marantz America, Mahwah, NJ, USA) tape recorder. Songs were digitised at 22 050 Hz (16-bit format) using AviSoft SASLAB Pro 4.40 software (Avisoft Bioacoustics, Berlin, Germany), highpass filtered between 2.8 kHz and 3.0 kHz (depending on song frequency) and lowpass filtered at 4.5 kHz or 4.6 kHz. Songs obtained from birds in the John Prince Research Forest, BC population were recorded using a Sennheiser MKH70 or ME67 or Audio-Technica ATB815a microphone and a Marantz PMD430 audiocassette recorder or Marantz PMD671 digital recorder. Songs were digitised at 44 100 Hz (16-bit format) using AviSoft SASLAB Pro 4.40 software. Songs obtained from birds at the Queen's University Biological Station, ON population were recorded using either a Sennheiser MKH70 or Audio-Technica AT815a microphone, Sony Walkman Professional WM-D6C (Sony, Tokyo, Japan) or Marantz PMD222 tape recorder, and digitised at 22 050 Hz (16-bit format) using Syrinx-PC sound analysis software (J. Burt, Seattle, WA, USA).

2.2. *Bioacoustic analyses*

Fee-bee songs were analyzed using Signal 5.10.29 software (Engineering Design, Berkeley, CA, USA). Songs for measurement were randomly selected from longer recordings. Spectrograms were generated with an intensity range of −35 to 0 dB relative to song peak amplitude, so low amplitude noise would not be visualised, and songs were only included if both the *fee* and *bee* notes were distinguishable for measuring via sound spectrogram (i.e., not masked by background noise). We measured six acoustic features examined previously in studies of dominance cues in chickadee song (e.g., Christie et al., 2004b; Hoeschele et al., 2010). We examined two temporal measurements: (1) total duration of song and (2) the proportion of song duration occupied by the *fee* note (*fee* note duration divided by the total duration of the song), two spectral measurements: (3) *fee* glissando (decrease in frequency across the duration of the *fee* note, calculated by dividing the start frequency of the *fee* note by the end frequency of the *fee* note) and (4) the interval ratio between the notes (calculated by dividing the end frequency of the *fee* note by the start frequency of the *bee* note), and two amplitude

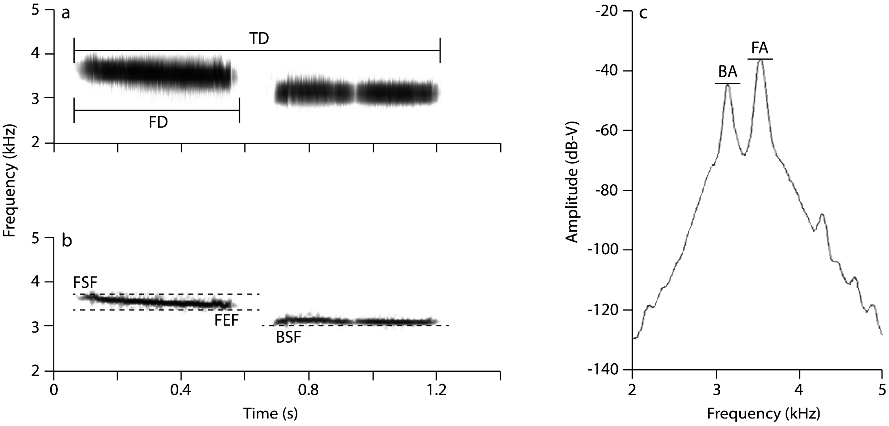

Figure 1. Sound spectrogram and power spectrum depicting acoustic measurements performed in *fee-bee* songs. (a) Sound spectrogram (time resolution 5.8 ms) of a *fee-bee* song. Measurements shown: total duration of song (TD) and *fee* note duration (FD). (b) Sound spectrogram (frequency resolution 43.1 Hz) of a *fee-bee* song. Measurements shown: *fee* start frequency (FSF), *fee* end frequency (FEF), and *bee* start frequency (BSF). (c) Power spectrum (FFT window 32768; 88 Hz smoothing). Measurements shown: *bee* note amplitude (BA) and *fee* note amplitude (FA).

measurements: (5) relative amplitude of the *fee* and *bee* note (calculated by dividing the maximum amplitude of the *bee* note by the maximum amplitude of the *fee* note) and (6) the root mean squared (RMS) amplitude ratio (calculated as the RMS amplitude of the *fee* note divided by the RMS ratio of the entire song; see Figure 1 for spectrograms showing measurements). We accounted for the different sampling rate of songs (i.e., 22 050 Hz or 44 100 Hz) by obtaining the duration measurements with a spectrogram window size of either 128 points or 256 points (for 22 050 and 44 100 Hz, respectively), both producing a time resolution of 5.8 ms, and the frequency measurements with a spectrogram window size of either 512 points or 1024 points (for 22 050 and 44 100 Hz, respectively), both producing a frequency resolution of 43.1 Hz.

We collected the above measurements from a random sample of 180 *fee-bee* songs from the University of Northern British Columbia, BC and 180 *fee-bee* songs from Queen's University Biological Station, ON (in each population, nine songs produced by ten dominant individuals and nine songs produced by ten subordinate individuals). Fewer songs were available from the John Prince Research Forest, BC population, where we analysed 72 *fee-bee* songs (nine songs produced by four dominant individuals and nine songs produced by four subordinate individuals). Songs that occurred immediately before or after a pitch shift were not included in the analysis. Some songs used in the current analysis from the University of Northern British Columbia, BC (Hoeschele et al., 2010) and Queen's University Biological Station, ON (Christie et al., 2004b) have been previously examined in terms of dominance status (95.6 and 40%, respectively), but the current analysis examines the same six acoustic features within songs from both of these populations using a different statistical technique compared to the previous analyses (i.e., discriminant function analysis with a permutation procedure; details below). We also examined song features in terms of geographic origin. In spite of comparison of gross differences in *fee-bee* songs across the continent (Kroodsma et al., 1999; Gammon & Baker, 2004), there has not been a direct comparative geographic analysis conducted on the simple *fee-bee* song using detailed spectrotemporal measurements.

2.3. Statistical analyses

We conducted discriminant function analyses in R (version 2.14.1, R Foundation for Statistical Computing, Vienna, Austria) using the software packages "MASS" (Venables & Ripley, 2002) and "klaR" (Weihs et al., 2005). We conducted discriminant function analyses to examine if the six acoustic features we measured could be used to classify songs based on (1) the dominance status of birds from British Columbia, (2) the dominance status of birds from Ontario, or (3) the geographic location of origin of the singer. For the first analyses, we included songs from 20 individuals from the University of Northern British Columbia, BC and classified the songs based on dominance. We measured the acoustic features of nine songs per individual, but conducted a stepwise discriminant function analysis, using the leave-one-out method, that included six randomly selected songs per individual ($N = 120$) to classify songs based on the dominance status or location of origin of the producer. With the leave-one-out method of cross-validation, one case is withheld at a time and the discriminant function is derived based on the remaining cases; the withheld case is then classified using the discriminant function that was derived, and the process is repeated until all cases have been classified in this manner (Betz, 1987). Results from classification using the leave-one-out method are useful as an estimate for how well the discriminant function derived from all cases can predict group membership with a new sample (i.e., a different set of cases than those used to derive the discriminant function). We did not separate our sample into two groups (one to create the discriminant function and one to classify), because we had a small sample of individuals and we wanted to evaluate the accuracy of the discriminant function analyses with as many songs as possible. We repeated this randomisation process 100 times, because even within an individual, song features can vary each time the song is produced, so the specific songs included in the analysis can affect the results. We then calculated the mean percentage of correct classifications for these 100 iterations. By performing multiple discriminant function analyses on randomly selected songs, we can determine how well the discriminant function can classify the songs on average and which feature(s) are used by the functions more than would be expected by chance. To determine which acoustic features were used by significantly more of these stepwise discriminant functions than would be expected by chance, we conducted binomial tests and using only these acoustic features, we conducted permuted discriminant function analyses, as suggested by Mundry & Sommer (2007). We conducted the permuted discriminant function analyses because our data set contained more than one song per individual, which can result in pseudoreplication. As before, we randomly selected six songs per individual, repeated this 100 times, and calculated an average percentage of correct classifications. We then conducted 1000 permuted discriminant function analyses, in which we randomly selected six songs per individual and randomly assigned the songs produced by an individual to one of the classification groups (i.e., one of two dominance status or one of three locations of origin). We then calculated a p-value by finding the proportion of permuted discriminant function analyses that resulted in a percent correctly-classified equal to or greater than the average percentage of correct classifications of the original data set. With permuted discriminant function procedures, the null hypothesis is that the discriminant functions analysing the original data set does not classify better than the discriminant functions classifying the permuted data (Mundry & Sommer, 2007).

We repeated these procedures and conducted discriminant function analyses with the permutation procedure, classifying based on dominance six randomly-selected songs from each of 20 individuals from Queen's University Biological Station, ON ($N = 120$ songs). Because we had a small sample of individuals from John Prince Research Forest, BC, we did not classify these songs in terms of dominance status. We conducted additional discriminant function analyses to determine how well songs could be classified based on location of origin using six randomly-selected songs from 20 individuals from the University of Northern British Columbia, BC, 20 individuals from Queen's University Biological Station, ON, and eight individuals from John Prince Research Forest, BC ($N = 288$ songs). To further analyse whether any geographic differences are being driven by songs produced by only dominant or subordinate birds, we conducted discriminant function analyses classifying only dominant songs by location of origin ($N = 144$) and we conducted discriminant function analyses classifying only subordinate songs by location of origin ($N = 144$).

Using the six features we measured, we calculated the average song features for each of the 48 individual birds (20 individuals from both the University of Northern British Columbia, BC and Queen's University Biological Station, ON, and 8 individuals from John Prince Research Forest, BC). These average songs were examined in SPSS (version 19.0.0, SPSS, Chicago, IL, USA) using a multivariate analysis of variance (MANOVA) to compare songs from these three locations.

3. Results

3.1. Acoustic difference by dominance rank in British Columbia

For songs recorded at the University of Northern British Columbia, BC, the average percentage of correct classifications based on dominance status by the stepwise discriminant function analyses was 64.8 ± 2.9%; range: 56.7–71.7%; for all analyses, the percentage of correct classifications is given as mean ± SD and the range of percent correct classifications for the 100 iterations is reported. Binomial tests revealed that *fee* glissando ($z = 6.6$, $p < 0.001$), interval ratio ($z = 4.4$, $p < 0.001$), and relative amplitude ($z = 9.6$, $p < 0.001$) were used in significantly more discriminant function analyses than would be expected by chance (chance = 0.50); using only these features, we performed a permuted discriminant analysis. Results revealed that there was no difference in these acoustic features between dominant and subordinate songs (average percentage of correct classifications for the original data set = 65.3 ± 2.6%; range 58.3–72.5%; 138/1000 of the permuted data sets had a percent correctly-classified equal to or greater than the average percent correctly-classified of the nonrandomised data; $p = 0.14$).

We also conducted permuted discriminant function analyses using only relative amplitude, because this feature was used by almost all discriminant function analyses (98/100) that we conducted on the original data set (compared to 83/100 and 72/100, for *fee* glissando and interval ratio, respectively). Results revealed a significant difference in the relative amplitude of dominant and subordinate songs (average percentage of correct classifications for the original data set = 63.2 ± 2.7%; range 57.5–71.7%; 40/1000 of the permuted data sets had a percent correctly-classified equal to or greater than the average percent correctly-classified of the original data set; $p = 0.04$).

3.2. Acoustic differences by dominance rank in Ontario

For songs recorded at Queen's University Biological Station, ON, the average percentage of correct classifications based on dominance status by the stepwise discriminant function analyses was 62.8 ± 2.4%; range: 57.5–67.5%. Binomial tests revealed that total duration ($z = 9.4$, $p < 0.001$), *fee* proportional duration ($z = 7.8$, $p < 0.001$), interval ratio ($z = 9.0$, $p < 0.001$), relative amplitude ($z = 3.8$, $p < 0.001$), and RMS ratio ($z = 6.0$, $p < 0.001$) were used in significantly more discriminant function analyses than would be expected by chance; using only these features, results from the permuted discriminant function analysis revealed that there was no difference in these acoustic features between dominant and subordinate songs (average percentage of correct classifications for the original data set = 62.6 ± 3.1%; range: 55.0–72.5%; 325/1000 of the permuted data sets had a percentage of correct classifications equal to or greater than the average percentage of correct classifications of the original data set; $p = 0.33$).

We also conducted permuted discriminant function analyses using only total duration and interval ratio, because these features were used by almost all discrimination function analyses (97/100 and 95/100, respectively) that we conducted on the original data set (compared to 89/100, 69/100, and 80/100, for *fee* proportional duration, relative amplitude, and RMS ratio, respectively). Results from this permuted discriminant function analysis revealed there was no difference in these acoustic features between dominant and subordinate songs (average percentage of correct classifications for the original data set = 61.2 ± 1.9%; range 56.7–65.8%; 281/1000 of the randomised data sets had a percent correctly-classified equal to or greater than the average percent correctly-classified of the original data set; $p = 0.28$).

3.3. Acoustic differences by geographic origin

To examine differences in songs based on the geographic origin of the singer, we conducted discriminant function analyses and a MANOVA on songs recorded at the University of Northern British Columbia, BC, Queen's University Biological Station, ON, and John Prince Research Forest, BC. The average percentage of correct classifications based on geographic origin by stepwise discriminant function analyses was 65.5 ± 1.3%; range 62.2–69.1%. Binomial tests revealed that total duration ($z = 10.0$, $p < 0.001$), interval ratio ($z = 9.0$, $p < 0.001$), and RMS ratio ($z = 9.4$, $p < 0.001$) were used in significantly more discriminant function analyses than would be expected by chance; using only these features we performed a permuted discriminant function analysis. Results revealed a significant difference in the acoustic features between the songs from different geographic locations (average percentage of correct classifications for the original data set = 65.5 ± 1.6%; range 61.8–69.1%; no permuted data sets had a percentage of correctly-classified songs equal to or greater than the average percent correctly-classified of the original data set; $p < 0.001$).

Examining the average misclassifications from the 100 discriminant function analyses on the original data set reveals that the majority of songs from the University of Northern British Columbia, BC (75.9%) and Queen's University Biological Station, ON (75.4%) were correctly-classified, while the majority of songs from the John Prince Research Forest, BC (62.9%) were misclassified as University of Northern British Columbia, BC songs (Table 1). Results from the MANOVA revealed that total duration ($F_{2,45} = 13.80$, $p < 0.001$, partial $\eta^2 = 0.38$, observed power = 0.997) and relative amplitude ($F_{2,45} = 3.36$, $p = 0.04$, partial $\eta^2 = 0.13$, observed power = 0.605) were significantly different between the three locations. A Tukey post-hoc comparison revealed that the total duration of songs from Queen's University Biological Station, ON were significantly different from the other two locations ($p \leqslant 0.009$), with Ontario songs being shorter compared to the British Columbia songs, while songs from the University of Northern British Columbia, BC and John Prince Research Forest, BC were not significantly different from one another ($p = 0.73$). For relative amplitude, a Tukey post-hoc comparison revealed that songs from Queen's University Biological Station, ON were significantly different from songs from John Prince Research Forest, BC ($p = 0.03$), with the relative amplitude ratio of the two notes being closer to 1 in the Ontario songs. The other four features did

Table 1.
Average predicted group membership of 100 discriminant function analyses classifying songs from the original data set by geographic origin.

Actual group	Predicted group membership		
	UNBC	QUBS	JPRF
UNBC	75.85*	22.98	1.17
QUBS	22.27	75.38*	2.35
JPRF	62.94	22.40	14.67*

An asterisk (*) indicates a correct classification (in percentages). Misclassifications (in percentages) are in corresponding rows and columns. Overall, 65.5% of cases are correctly classified. Songs from both dominant and subordinate birds are included. UNBC, University of British Columbia, BC; QUBS, Queen's University Biological Station, ON; JPRF, John Prince Research Forest, BC.

not differ significantly between the three locations ($F_{2,45} \leqslant 2.91$, $p \geqslant 0.07$, partial $\eta^2 \leqslant 0.12$, observed power $\leqslant 0.541$). Table 2 gives the means and standard deviations for the six acoustic features for each of the three locations.

Additional discriminant function analyses were conducted to classify *fee-bee* songs by geographic origin, separately for each dominance status. For dominant individuals, the average percent of correct classification based on geographic origin by stepwise discriminant function analyses was 61.5 ± 1.8%; range 56.3–66.7%. Binomial tests revealed that total duration ($z =$

Table 2.
Mean and standard deviation (SD) of the six acoustic features measured in the bioacoustic analysis for *fee-bee* songs originating from each of the three locations.

	UNBC (mean ± SD)	QUBS (mean ± SD)	JPRF (mean ± SD)
Total duration (ms)*	1049.22 ± 47.03	958.51 ± 56.69	1031.27 ± 74.85
Fee proportional duration	0.42 ± 0.01	0.41 ± 0.02	0.42 ± 0.01
Fee glissando	1.09 ± 0.01	1.08 ± 0.01	1.09 ± 0.02
Interval ratio	1.13 ± 0.01	1.14 ± 0.02	1.15 ± 0.02
Relative amplitude*	1.04 ± 0.07	1.02 ± 0.05	1.08 ± 0.05
RMS ratio	1.59 ± 0.36	1.42 ± 0.28	1.69 ± 0.35

Songs from both dominant and subordinate birds are included. Significant differences (indicated by MANOVA) are indicated by an asterisk (*). UNBC, University of British Columbia, BC; QUBS, Queen's University Biological Station, ON; JPRF, John Prince Research Forest, BC.

10.0, $p < 0.001$), *fee* proportional duration ($z = 1.8$, $p = 0.0359$), interval ratio ($z = 2.8$, $p = 0.0026$), relative amplitude ($z = 9.0$, $p < 0.001$), and RMS ratio ($z = 5.8$, $p < 0.001$) were used in significantly more discriminant function analyses than would be expected by chance; using only these features we performed a permuted discriminant function analysis. Results revealed a significant difference in the acoustic features between the songs from different geographic locations (average percentage of correct classifications for the original data set = 60.6 ± 1.5%; range 57.6–64.6%; 40/1000 of the permuted data sets had a percent correctly-classified equal to or greater than the average percent correctly-classified of the permuted data sets; $p = 0.04$).

For subordinate individuals, the average percentage of correct classifications based on geographic origin by the stepwise discriminant function analyses was 75.1 ± 2.1%; range: 69.4–81.3%. Binomial tests revealed that total duration ($z = 10.0$, $p < 0.001$), *fee* proportional duration ($z = 4.4$, $p < 0.001$), interval ratio ($z = 10.0$, $p < 0.001$), relative amplitude ($z = 6.2$, $p < 0.001$), and RMS ratio ($z = 8.8$, $p < 0.001$) were used in significantly more discriminant function analyses than would be expected by chance, using only these features we conducted permuted discriminant function analyses. Results revealed a significant difference in the acoustic features between the songs from different geographic locations (average percentage of correct classifications for the nonrandomised data = 74.0 ± 1.9%; range: 68.1–78.5%; no randomised data sets had correct-classification percentages equal to or larger than the average nonrandomised data; $p < 0.001$).

4. Discussion

We used bioacoustic analyses to examine six acoustic features of the *fee-bee* songs of male black-capped chickadees, and conducted discriminant function analyses to examine how these features vary between songs produced by birds of different dominant status or with different locations of origin. Results from discriminant function analyses suggest that (1) there is variation in the songs between dominant and subordinate birds, (2) the acoustic features that convey information about dominance rank differ between geographic origins, and (3) there is geographic variation in the *fee-bee* song, regardless of dominance status. We used permuted discriminant function analyses to examine the songs from two populations (i.e., eastern Ontario and northern

British Columbia) and determine if features within the songs vary with dominance status. Permuted discriminant function analyses allowed us to analyse which song features varied between dominant and subordinate birds, and by testing against the true null hypothesis distribution (i.e., the permuted data set), we could determine how accurately the two groups could be distinguished and the true probability of the results.

Two previous studies, that each examined one of two geographically separated populations of black-capped chickadees, found different acoustic features within *fee-bee* songs that varied based on dominance status (Christie et al., 2004b; Hoeschele et al., 2010). Our results are in agreement with previous findings (Hoeschele et al., 2010), demonstrating that the dominance status of birds from northern British Columbia can be predicted based on the relative amplitude between the two notes. In a population of birds in eastern Ontario, Christie et al. (2004b) found that dominant males maintain a consistent interval ratio across song pitches, while subordinate birds do not; however, the relative amplitude between the two notes was not examined. In our analysis of eastern Ontario songs, results from the discriminant function analysis failed to reach significance with all acoustic features, suggesting that consistency in the amplitude between the two notes within a single song does not contain dominance-related information in songs from this population. In the current analysis, we examined acoustic features within a song, but we did not examine consistency of song features among songs of varying pitch produced by individual males. Dominance information is contained within the songs produced by birds from this Ontario population when pitch-shifting behaviour is taken into account (Christie et al., 2004b). Our results do not reveal a consistent dominance-related acoustic feature that is found across populations. However, since the current study did not examine the consistency of the interval ratio across a male's songs in the northern British Columbia population, as Christie et al. (2004b) measured in songs produced by birds in eastern Ontario, we cannot rule out the possibility that populations share this acoustic feature used to identify rank.

The current findings suggest that, in addition to subtle but consistent differences in the acoustic features of songs produced by dominant and subordinate birds, there is also subtle acoustic variation depending on geographic location, with the total duration of songs varying among the three geographic locations (Table 2). Songs recorded from the University of Northern British Columbia, BC are, on average, 9% longer than the songs recorded at Queen's

University Biological Station, ON. It is noteworthy that the average percentage difference in duration between our main study populations is below the threshold for duration detection reported for other avian species, which ranges between 10 and 20% for synthetic tonal stimuli (Dooling & Haskell, 1978; Maier & Klump, 1990; Weisman et al., 1999). However, using natural zebra finch calls, Lohr et al. (2006) demonstrated that zebra finches (*Taeniopygia guttata*) and budgerigars (*Melopsittacus undulatus*) could detect temporal changes as brief as 1–2 ms. Determining duration detection thresholds for both tonal stimuli and natural stimuli in chickadees and determining whether chickadees can perceive these differences in duration requires further direct examination. In isolated populations of black-capped chickadees, geographic variation in *fee-bee* songs, including novel introductory notes and multiple song types, has been found (Kroodsma et al., 1999; Gammon & Baker, 2004). However, this type of variation is strikingly different from the highly-stereotypic song found in the majority of black-capped chickadee populations, and is more similar to abnormal song produced by black-capped chickadees that are tape-tutored and reared in the absence of adult conspecifics (Shackleton & Ratcliffe, 1993; Kroodsma et al., 1995).

Environmental factors, such as habitat, can affect the evolution of visual and acoustic signals (Wilczynski & Ryan, 1999), including sexual signals (e.g., those that correlate with male quality or dominance). For black-capped chickadees, whose range extends across North America with great variety in habitat type and climate (Smith, 1991), subtle acoustic differences within the songs of birds from different populations may arise due to differences in the local habitat characteristics. Depending on characteristics within the habitat, sounds will propagate differently through the environment and the acoustic adaptation hypothesis holds that songs will evolve to maximise transmission properties in their native habitat (Morton, 1975; Hansen, 1979; Rothstein & Fleischer, 1987). The two British Columbia sites occur within the same ecosystem zone (Montane Cordillera; Marshall, 1999), and have greater similarities in dominant tree species to each other than either location does to the Ontario population in this study (Boreal Shield ecosystem). These slight differences in dominant tree species may contribute to slight differences in sound transmission properties, and could account for subtle differences in songs between broad regions.

Grava et al. (2012) found that when comparing the songs produced by black-capped chickadee males of similar dominance rank, but from varying habitat quality, males in young forests (lower-quality habitat) produced songs with less consistent interval ratios compared to birds from mature forests (higher-quality habitat). Previous studies have found no difference in song transmission through these adjacent habitat patches (Hansen et al., 2005), suggesting that condition-related features of songs influence song structure within and between regions.

In the current study, geographic differences were evident when discriminant function analyses were used to classify songs produced by individuals of one dominance status, with the discriminant function analysis classifying songs produced by dominant birds having a lower percentage of correct classifications compared to the discriminant function classifying only subordinate songs. Dominant birds' songs may have species-typical acoustic features which vary less between geographic locations. In this way, differences in song output could act as an honest indicator of male quality (Zahavi, 1975). Dominant males may produce songs that contain features that are costly to produce, while subordinate birds are unable to consistently produce vocally-challenging songs and consistency in vocal performance may be an honest signal of male quality (for review see Sakata & Vehrencamp, 2012). In dusky warblers, males that produce physiologically-challenging (i.e., maintaining high amplitude) songs were more likely to have extra-pair offspring (Forstmeier et al., 2002) and in other songbird species, females give more copulation solicitation displays in response to male songs that are vocally difficult (i.e., high trill rate and broad frequency bandwidth) to produce (canary, *Serinus canaria*, Drăgănoiu et al., 2002; swamp sparrow, Ballentine et al., 2004). For songbirds with a repertoire of songs, Lambrechts & Dhondt (1988) propose an anti-exhaustion hypothesis to explain why birds switch between song types. The notion is that repeating the same song type requires the syringeal and respiratory muscles to be moved in a repetitive way possibly leading to fatigue. However, more work is needed to determine if producing consistent songs across renditions may be more physiologically-demanding for individuals with one song type, such as the black-capped chickadee.

In a behavioural assay, captive female black-capped chickadees from Alberta produced more vocalisations and were more active when presented with songs produced by dominant males from British Columbia (Hoeschele et al., 2010). This further supports the idea that the feature(s) within *fee-bee* songs that identify dominant birds are stable and can be discriminated by birds from a different geographic location. To test this hypothesis, black-capped chickadees from Alberta should be tested in the same paradigm as in Hoeschele et al. (2010) but with songs from the Ontario population. Using an operant discrimination paradigm, we can examine if black-capped chickadees can discriminate songs produced by males of different dominance status or geographic location; similar experiments have been conducted to examine chickadees' perception of species-based differences in their calls (e.g., Bloomfield et al., 2008; Bloomfield & Sturdy, 2008; Guillette et al., 2010). Additionally, with an operant discrimination task, we can manipulate the acoustic features to make the songs more-or-less dominant based on acoustic features identified in this study, and by examining how birds respond, we can gain valuable insight into what acoustic features birds use to differentiate between songs produced by males of different dominance status or geographic origin.

The current results, along with previous behavioural tests (Hoeschele et al., 2010) and bioacoustic comparisons (Christie et al., 2004b; Hoeschele et al., 2010), suggest that *fee-bee* songs contain acoustic features that differ between songs produced by dominant and subordinate males. In addition, our results indicate that there are acoustic features within *fee-bee* songs that vary with geographic location. These results demonstrate that within a relatively simple signal, significant acoustic variation exists, and future research should focus on how chickadees perceive these acoustic differences.

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