Information contained within a simple acoustic signal: The *fee-bee* song of the black-capped chickadee (*Poecile atricapillus*)

by

Allison Helene Hahn

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Department of Psychology
University of Alberta

Abstract

Black-capped chickadees (*Poecile atricapillus*) are a common North American songbird that produce numerous types of vocalizations with various functions. The vocal repertoire of black-capped chickadees have been the focus of numerous vocal production and perception studies. Black-capped chickadees make an excellent model for studying acoustic communication because their vocal repertoire has been so well-studied. In addition to producing a learned song, parts of their *chick-a-dee* call are also learned. In many species of songbird, the species' song is a long, complex vocalization, while the species' calls are short and acoustically simple. In contrast to this, the *fee-bee* song of black-capped chickadees is a short and relatively simple two-note tonal vocalization, while their *chick-a-dee* call is a long and relatively complex vocalization. Although the acoustic structure of *fee-bee* songs is relatively simple, the function of male songs is similar to the function of other songbird songs: mate attraction and territory defense. In addition, male songs contain information regarding the individual producing the signal, including cues about the male's dominance rank. In Chapter 2, I conducted a bioacoustic analysis on male songs recorded in different geographic locations. I found that the acoustic features that indicate a male's dominance rank vary with geographic location, in addition, I found other subtle features within the song that vary with geographic location. Next, I used two operant conditioning techniques (i.e., choice preference task and a go/no-go discrimination task) to examine the perception of dominance cues in male *fee-bee* songs (Chapter 3). The results suggest that preference and discrimination performance varies depending on the location-of-origin of the singer, the sex of the signal receiver, and the category of songs that is rewarded during the task. I also examined the perception of geography-based acoustic cues in male *fee-bee* songs using a go/no-go operant discrimination task (Chapter 4). Results from this study provide evidence that male songs contain geography-related cues that are perceived by chickadees. In addition to examining acoustic cues in male songs, I also examined the production of songs by male and female chickadees. In many temperate songbirds, including black-capped chickadees, research has focused on male-produced song. However, in many temperate songbirds it is now recognized that both males and females produce song. To examine the production of songs by female black-capped chickadees, both males and females were recorded and a bioacoustic analysis was conducted on their songs (Study 4), revealing that while male and female songs have overall structural similarity (i.e., two notes), at least one acoustic feature (*fee* glissando) varies between the sexes. An operant conditioning task revealed that male and female songs belong to separate perceptual categories, but the biological salience of the songs affects the discrimination performance of the birds. In addition, this study revealed that acoustic features within the song's first note (*fee* note) likely contain information regarding the singer's sex (Study 5). Taken together, these studies reveal that a relatively simple vocalization, the *fee-bee* song, contains multiple types of information and birds can use this information when discriminating among songs; however, the biological relevance of the acoustic signal influences the discrimination performance of the birds.

Preface

All procedures followed the Canadian Council on Animal Care (CCAC) Guidelines and Policies and were approved by the Animal Care and Use Committee for Biosciences at the University of Alberta (AUP 108).

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Chapter 1 General Introduction

Animal communication

In animal communication, an individual, termed a 'signaler', produces a signal containing information, and the animal receiving the signal, termed the 'receiver', uses this information when deciding how to respond (Bradbury & Vehrencamp, 1998). The information conveyed by a signal can vary. For example, signals can include information regarding the external environment (e.g., food source, a potential predator, or nest site). When these signals are produced, they are often context-specific, for example the type of alarm call produced (e.g., vervet monkeys, *Chlorocebus pygerythrus,* Seyfarth, Cheney, & Marler, 1980) or the acoustic features within the alarm (e.g., black-capped chickadees, *Poecile atricapillus,* Templeton, Green, & Davis, 2005) vary depending on the type of predator present. When the signal is conveying information regarding an external context (e.g., predator presence), the information is relevant to a broader audience. For example, red-breasted nuthatches (*Sitta canadensis*) display stronger mobbing behaviour in response to a signal produced by a black-capped chickadee indicating the presence of a high-threat predator (Templeton & Greene, 2007), suggesting that predator information is relevant and perceived by multiple species.

In addition to containing information regarding external events, a signal can convey information regarding the signaller. This includes information regarding species, sex, quality, or individual identity. When a signal contains information regarding the individual, the information that is relevant and assessed will vary depending on the signal receiver. In some cases, information regarding the individual signaller is relevant to heterospecific species. For example, signals containing species information are used to form mixed-groups (Suzuki, 2012), or to identify heterospecifics that are not resource competitors (Li et al., 2014). Avian brood parasites may choose which nest to parasitize based on signals indicating the individual's quality (Parejo

& Avilés, 2007). These examples demonstrate that interspecific information transfer occurs with signals containing information regarding the individual signaller. However, information regarding the individual signaller is extremely important for conspecifics. For example, signals indicating sex can ensure that an individual is responding to a possible mate, while information regarding individual quality can ensure an individual is choosing a high-quality mate or avoiding a confrontation with a conspecific that is a better fighter.

Production and perception of song

For communication in which information is being transferred among individuals, not only must there be a signaller who is producing the signal, but there also must be a receiver who recognizes the signal and responds appropriately. While signals can be produced in many domains (e.g., chemical, olfactory, visual, auditory), acoustic communication is one mode of communication commonly used by songbirds. Most acoustic signals can travel long distances, can be heard in multiple directions, and can allow an animal to communicate without visual contact. Many songbird species produce numerous vocalizations (often these vocalizations are divided into two categories: 'calls' and 'songs') that can serve a multitude of functions. For most songbirds, male song is considered a sexually selected signal that follows the dual functionality described in Darwin's theory of sexual selection (1859): males are the sex that produces elaborate traits for intrasexual competition and intersexual attraction. Songs produced by male songbirds are an example of these elaborate signals, because male song indicates territory to rival males and indicates a potential mate to females (for review see Catchpole & Slater, 2008).

These dual functions have driven the evolution of song through selection pressures of intrasexual competition and intersexual mate choice. Because songs serve these dual roles, the relevant song traits will vary depending on the sex of the signal receiver. For example, females should assess a male based on his potential as a high-quality reproductive partner (i.e., a mate that will provide direct or indirect benefits), while males should assess a rival male based on the likelihood of attack or his fighting ability. As these example show, the relevant information varies depending on who is receiving the signal. Some song characteristics (e.g., repertoires or song rate, reviewed in Collins, 2004) can both attract mates and repel rivals, while in some species males may produce different songs for mate attraction and territory defense (e.g., great reed warbler, *Acrocephalus arundinaceus*, Catchpole, 1983; dusky warbler, *Phylloscopus fuscatus,* Forstmeier & Balsby, 2002). In other species, the same song is produced for both intra- and intersexual communication, but the relevant features of the song vary depending on the sex of the receiver (e.g., chaffinch, *Fringilla coelebs,* Leitáo & Riebel, 2003; Riebel & Slater, 1998). However, for both territory defense and mate attraction, in order for songs to function as communication signals, the signal receiver needs to recognize and respond appropriately to the song (e.g. recognition of territory neighbours, Brooks & Falls, 1975; recognition of mates, Lind, Dabelsteen, & McGregor, 1996). Producing species-typical songs can also affect an individual's fitness by conveying information about the individual. For example, songs can act as an honest indicator of the stress a male encountered during development, which can indicate his phenotypic or genotypic quality (Nowicki, Peters, & Podos, 1998).

Male song has been widely studied; however, not only males produce songs. In many tropical species, females produce songs or males and females sing duets (Slater & Mann, 2004); however, more recent evidence reveals that in many temperate species, females also sing (Garamszegi, Pavlova, Eens, & Møller, 2007; Odom, Hall, Riebel, Omland, & Langmore, 2014). In species where both males and females produce songs, songs should contain information regarding the sex of the signaller. For example, being able to quickly determine the sex of a singing individual is beneficial for birds in order to quickly identify a potential mate or rival. Discernible acoustic differences in songs is a mechanism that would allow birds to recognize the sex of a singing individual. In addition, the relevance of the signal will depend on the receiver (e.g., a male song, but not a female song, is relevant for a female looking for a mate). Differences in the acoustic features of the songs produced by each sex have been examined in several species to date (e.g., white-crowned sparrows, *Zonotrichia leucophrys*, Baptista, Trail, DeWolfe, & Morton, 1993; bellbirds, *Anthornis melanura*, Brunton & Li, 2006) and these acoustic differences allow individuals to differentiate the sex of a singing individual without visual cues (Hoelzel, 1986).

Study species: Black-capped chickadee (*Poecile atricapillus*)

Black-capped chickadees are a common temperate songbird found throughout most of North America (Smith 1991). Black-capped chickadees produce at least ten vocalizations with various functions (see Ficken, Ficken, & Witkin 1978) and their vocal repertoire has been the focus of many vocal production and perception studies (for review see Sturdy, Bloomfield, Charrier, & Lee, 2007). Black-capped chickadees produce a learned song (Shackleton & Ratcliffe, 1993) and parts of their calls are also learned (e.g., *gargle* call, Ficken, Ficken, & Apel, 1985; *chick-a-dee* call, Hughes, Nowicki, & Lohr, 1998). Compared to the calls of many songbird species, black-capped chickadees' *chick-a-dee* call is a long, complex vocalization (i.e., consists of multiple note types that can be repeated or omitted), while their *fee-bee* song is relatively short and acoustically simple (i.e., *fee-bee* song contains two tonal notes). While the acoustic structure of black-capped chickadee vocalizations deviate from the traditional characteristics of a 'call' that is short and simple and a 'song' that is complex, the *chick-a-dee* call is used in specific contexts, similar to the calls of other songbird species and the *fee-bee* song is important for mate attraction and territory defense, similar to the songs of other species.

In songbird species other than black-capped chickadees, males may produce multiple song types (Catchpole & Slater, 2008); male black-capped chickadees however, produce only one *fee-bee* song type, which is conserved across most of the species range (see Kroodsma et al. 1999; Gammon & Baker, 2004 for rare exceptions) and is a long-distance signal (Dixon & Stefanski, 1970; Ficken et al., 1978; Mennill & Otter, 2007). The *fee-bee* song of the black-capped chickadee contains two notes, with the first note (i.e., *fee*) produced at a higher frequency compared to the second note (i.e., *bee*). During the spring, there is an increase in song production at dawn (Avey, Quince, & Sturdy, 2008), and high-ranking males will begin singing earlier, sing for longer durations, and sing at higher rates compared to low-ranking individuals (Otter, Chruszcz, & Ratcliffe, 1997). Male chickadees produce songs across a range of absolute pitches (Horn, Leonard, Ratcliffe, Shackleton, & Weisman, 1992; Weisman & Ratcliffe, 1989; Weisman, Ratcliffe, Johnsrude, & Hurly, 1990), and during a singing bout, an individual will increase or decrease the absolute frequency of the *fee-bee* song, a behaviour called ‘pitch shifting’ (Hill & Lein, 1987; Ratcliffe & Weisman, 1985) in order to match the frequency of another male’s song, an agonistic signal (Horn et al., 1992; Mennill & Ratcliffe, 2004).

Although the overall, two-note structure of the *fee-bee* song is relatively simple, information including individual identity is conveyed within this acoustic signal (Christie, Mennill, & Ratcliffe, 2004a; Wilson & Mennill, 2010). Black-capped chickadees live in flocks with stable dominance hierarchies that persist from flock establishment in winter to flock break-up in spring (Ratcliffe, Mennill, & Schubert, 2007; Smith, 1991). For many species that live in social groups with dominance hierarchies, a male’s condition may influence his dominance rank and acoustic cues can signal this status. For example, dominant males may vocalize more frequently than subordinates (domestic chicken, *Gallus gallus domesticus*, Leonard & Horn, 1995; black-capped chickadee, Otter et al., 1997). Dominance hierarchies are often established based on the outcomes of dyadic interactions over access to resources (Drews, 1993; Ratcliffe et al., 2007). Since dominance rank is established through dyadic encounters, dominance is not an absolute trait of an individual (Drews, 1993). However, stable dominance hierarchies, like those found in black-capped chickadee flocks, reduce the need for constant confrontations to maintain position within the social group. In order for stable hierarchies to be maintained, there must be cues available to identify an individual's rank. If rank-identifying cues are present (e.g., vocal cues), individuals would not need prior experience with one another to determine dominance rank.

In black-capped chickadees, previous studies have found potential cues within the *fee-bee* songs that indicate a male's dominance status. For example, Christie, Mennill, and Ratcliffe (2004b), found that dominant males are better able to maintain a consistent interval ratio (i.e., the frequency ratio between the *fee* and *bee* notes), as they shift the absolute frequency of their song, compared to subordinate males. In addition, Hoeschele et al., (2010) found that within a song, the amplitude ratio of the *fee* and *bee* notes is produced in a more consistent manner in songs produced by dominant males compared to subordinate males. Importantly, playback studies revealed that female black-capped chickadees will respond differentially to song playback based on a male's dominance rank. However, whether one of these two particular dominance cues identified in black-capped chickadees is stable across geographic locations requires further examination.

Another aspect of black-capped chickadee vocal production and perception that has not been thoroughly examined is song production by females. While female temperate songbirds have traditionally been considered not to produce song (for reviews see Langmore, 1998; Riebel, 2003), there has been an increase in reports of female song within the literature (for review see Garamszegi et al., 2007). In black-capped chickadees, song has been considered a male-produced vocalization; however, there have been field reports of female black-capped chickadees producing song (e.g., Dwight, 1897; Hill & Lein 1987) and personal observation of our captive females indicates that females do produce a two-note *fee-bee* song with similar amplitude as male song. The production of female song requires further examination, including if there are acoustic cues that vary between male and female songs, and if birds perceive acoustic differences.

While the vocal repertoire of black-capped chickadees has been extensively studied (e.g., Ficken et al., 1978; Smith, 1991), including *fee-bee* songs (e.g., Ratcliffe & Weisman, 1985; Weisman et al., 1990) more work is needed to understand other types of variation that exists within the *fee-bee* song and what acoustic mechanisms chickadees use for perception. My dissertation research focuses on examining other acoustic cues that exist within the black-capped chickadee *fee-bee* song and examining the perceptual mechanisms that chickadees use when discriminating among songs by using behavioural tests (i.e., operant conditioning). Black-capped chickadees make an exceptional model species as they are a common vocal-learning songbird, whose behaviour and vocalizations have been well-studied. By increasing our understanding of how chickadees' perceive cues within their songs we can increase their use as a model species for examining vocal learning and acoustic communication.

Current studies

My first objective was to investigate whether *fee-bee* songs produced by male black-capped chickadees originating from different locations and different populations contain dominance markers that are consistent across populations or vary with geographic location. Bioacoustic analyses are a necessary first step in examining acoustic features in order to describe and analyze acoustic differences that vary with other traits of the song producer. In Chapter 2, I conducted extensive bioacoustic analyses on songs produced by dominant and subordinate males originating from three geographic locations. I found that the acoustic variation that was previously reported in one of the populations (Hoeschele et al. 2010) did not exist in the songs from a second population. In addition, I found that acoustic features varied based on geographic location. The results of this study suggest that dominance-related cues may not be consistent across geographic regions. In addition, the results suggest that there is distinct geographic variation in acoustic features (i.e., total duration) of *fee-bee* songs. This study is significant because it indicates previously undescribed variation in a signal that has been considered to contain remarkably little variation across most of the species' range (Kroodsma et al. 1999; Gammon & Baker, 2004).

In Chapter 3, I used two operant conditioning tasks to investigate the perception of dominance-based cues in *fee-bee* songs. First, birds completed a choice preference task, in which birds selected which songs to hear by landing on different perches (Gentner & Hulse, 2000). The second task was an instrumental learning task, in which birds were trained on a go/no-go discrimination of dominant and subordinate male songs. The goal of the first task (i.e., the choice preference task) was to determine if birds had a preference for hearing dominant or subordinate songs. The second task (i.e., the instrumental conditioning task) had two main goals: (1) determine if dominant and subordinate songs belonged to separate signal categories perceived by chickadees, and (2) determine the acoustic mechanism used for the discrimination. In addition, I examined the relationship between the responses on the two operant tasks, to determine if a bird's acoustic preference could be predicted by measures of learning speed and performance accuracy during the instrumental conditioning task.

The goal of Chapter 4 was to determine if chickadees could perceive the geographic acoustic differences that I uncovered in my initial bioacoustic analysis (Chapter 2). For this study, I used operant go/no-go discrimination tasks (similar to the task in Chapter 3) to train chickadees to discriminate among songs recorded in two geographic locations (i.e., British Columbia and Ontario). Results from Chapter 4 suggest that birds were using perceptual categorization when discriminating between the locations. In addition, chickadees' responses to test stimuli suggest they use multiple acoustic features when discriminating.

The studies in Chapters 2-4 exclusively examined songs produced by male black-capped chickadees. Overall, research on black-capped chickadee song has focused primarily on male song. Males and females will produce a *faint fee-bee* song when near the nest, but the lower amplitude of this vocalization distinguishes it from normal *fee-bee* songs (Ficken et al., 1978). While females singing normal *fee-bee* songs have been briefly mentioned in some studies (e.g., Hill & Lein, 1987) and the neural response to female song has been examined (Avey, Kanyo, Irwin, & Sturdy, 2008), no study has quantitatively examined female song production in this species. The focus on male-produced song is not exclusive to black-capped chickadees, for many other songbird species, the focus of research has been on male song and it is often overgeneralized that song is a signal produced by males during the breeding season. However, more recently, there has been an increase in research indicating that females of many songbird species (including temperate songbirds) produce song (Garamszegi et al., 2007; Odom et al., 2014). The studies in Chapters 5 and 6 examine the production of song by female black-capped chickadees (Chapter 5) and the perception of male and female songs (Chapter 6).

In Chapter 5, I conducted bioacoustic analyses (similar to Chapter 2) comparing male and female songs that I recorded in the laboratory. This study is significant because it is the first quantitative description of female song in this species. In this study, I show that females of this species *do* produce songs. In addition, the songs that they produce have overall structural similarity to male songs (i.e., contain two whistled notes), but also contain acoustic differences that could be used by listening birds when discriminating between male and female songs. The results from this bioacoustic analysis reveal possible acoustic mechanisms that chickadees may use when determining the sex of a singing individual, which can be examined in subsequent studies.

Next, I examined the perceptual abilities of black-capped chickadees when discriminating among male and female songs. In Chapter 6, I conducted an operant go/no-go discrimination task (similar to Chapters 3 and 4) to examine these perceptual abilities. In addition, I manipulated song features to gain a better understanding of the underlying mechanisms for the perception of male and female songs. I conducted two operant discrimination studies with chickadees, in which I trained chickadees on a category-based discrimination of male and female songs. I then presented birds with novel male and female songs and songs that I manipulated in order to examine the mechanism behind the discrimination. I also trained an artificial neural network on an analogous task, to examine responses in the absence of biological or experiential factors, which cannot be parsed out when training chickadees. Results from the artificial neural networks suggest that features within the song's first note (i.e., *fee* note) contain sex-specific features;

however, results from the chickadee studies suggest that there are other features within the song that also contain discriminable features between the sexes.

Finally, Chapter 7 is a general discussion that summarizes the findings from the preceding chapters. In the general discussion I describe how the studies in this thesis relate to the current knowledge of black-capped chickadee song, and more generally songbird song. I also suggest future directions based on the current results.

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Chapter 2 Dominance and geographic information contained within black-capped chickadee (*Poecile atricapillus*) song[1]

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Introduction

In songbirds, male song is primarily used for mate attraction and territory defense (Catchpole and Slater 2008); however, in many species additional information is contained within song, including individual identity (see Catchpole and Slater 2008 for review), geographic origin (e.g., swamp sparrows, *Melospiza georgiana*, Marler and Pickert 1984; white-crowned sparrows, *Zonotrichia leucophrys nuttalli,* Baker and 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 vocalize 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 coloration 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 behavior 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; 2004b), 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; 2004b; 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.

Methods

Recordings

Fee-bee songs were recorded in the field during the dawn chorus period at the University of Northern British Columbia, Prince George, British Columbia (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, Ontario (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 standardized 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 analyzed. 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 Corporation, Old Lyme, CT) or an Audio-Technica ATB815a microphone (Audio-Technica U.S., Inc., Stow, OH) and a Marantz PMD430 (Marantz America, Inc., Mahwah, NJ) tape recorder. Songs were digitized 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 digitized 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 Corporation, Tokyo, Japan) or Marantz PMD222 tape recorder, and digitized at 22,050 Hz (16-bit format) using Syrinx-PC sound analysis software (J. Burt, Seattle, WA).

Bioacoustic analyses

Fee-bee songs were analyzed using Signal 5.10.29 software (Engineering Design, Berkeley, CA). 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 visualized, 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 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 2-1 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 analyzed 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%; 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.

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 randomization 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 1,000 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 analyzing 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 analyze 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, Inc, Chicago, IL) using a multivariate analysis of variance (MANOVA) to compare songs from these three locations.

Results

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 nonrandomized 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; $p = 0.04$).

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 randomized data sets had a percent correctly-classified equal to or greater than the average percent correctly-classified of the original data set; $p = 0.28$).

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 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 2-1). 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 ($p \leq 0.009$), 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 not differ significantly between the three locations ($F(2,45) \leq 2.91$, $p \geq 0.07$, partial $\eta^2 \leq 0.12$, observed power ≤ 0.541). Table 2-2 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 = 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 nonrandomized data = 74.0±1.9%; range: 68.1-78.5%; no randomized data sets had correct-classification percentages equal to or larger than the average nonrandomized data; p < 0.001).

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 analyze 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 behavior 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 2-2). 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 maximize 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 vocalizations 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.

Table 2-1. Average predicted group membership of 100 discriminant function analyses classifying songs from the original data set by geographic origin. 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.

	Predicted Group Membership		
Actual Group	UNBC	QUBS	JPRF
UNBC	**75.85**	22.98	1.17
QUBS	22.27	**75.38**	2.35
JPRF	62.94	22.40	**14.67**

Table 2-2. 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. 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.

	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

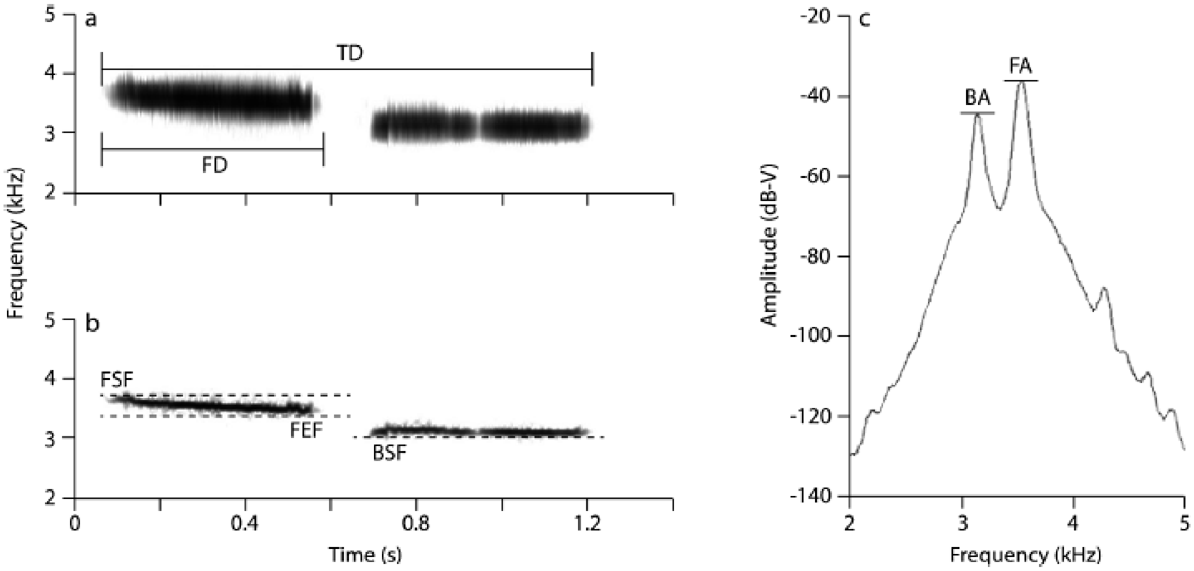

Figure 2-1. Sound spectrogram and power spectrum depicting acoustic measurements performed in *fee-bee* songs. (a) Sound spectrogram (time resolution 5.8 ms) of a *fee-bee* song. Measurements shown: total duration of song (TD) and *fee* note duration (FD). (b) Sound spectrogram (frequency resolution 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).

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Chapter 3 Dominance cues in male black-capped chickadee song: Preferences and mechanisms of discrimination

Introduction

Dominance ranks were first described by Schjelderup-Ebbe (1922/1975) studying the hierarchical social structure of group-living domestic chickens (*Gallus gallus*). However, dominance ranks and social group interactions have since been studied in numerous species (Allee, 1942/1975; Dewsbury, 1982). Dominance ranks are often established through dyadic interactions, and these interactions usually occur during disputes about access to resources. Although dominance is a relative trait (i.e., an individual may be dominant over another individual, while also being subordinate to a third individual; Drews, 1993), in stable social groups, the dominance hierarchy is often stable (Wiley, Steadman, Chadwick, & Wollerman, 1999). When the social hierarchy is stable, individuals do not need to engage in constant confrontations to assert their dominance over other individuals. In the case of chickens, for example, the dominant individual does not need to continue to act aggressively toward lower-ranking individuals once the dominance hierarchy is established (Schjelderup-Ebbe, 1922/1975). Instead, once the dominance rank is established, few confrontations need to occur, and time and energy can be spent on other activities that are necessary for survival (e.g., foraging or mating).

Although familiarity with opponents can lead to this stability (Archawaranon, Dove, & Wiley, 1991; Wiley et al., 1999), the formation and maintenance of dominance hierarchies does not imply individual recognition alone. Instead, individuals may possess a status signal that allows opponents to characterize an individual's dominance rank in the absence of individual recognition. These status signals are a possible mechanism allowing animals that live in large social groups to recognize the dominance status of another individual without needing to recognize and remember each individual and their social status, which would increase cognitive load as the number of individuals within the social group increases. Status signals are also a mechanism that would allow large social groups with dominance hierarchies to remain stable without individuals needing to engage in constant confrontations among individuals for social rank positions.

A status signal may be a visual trait that indicates dominance status (e.g., antler size in woodland caribou, *Rangifer tarandus caribou*, Barrette & Vandal, 1986; badge size in house sparrows, *Passer domesticus*, Møller, 1987), acoustic features in an auditory signal (e.g., spotted hyena, *Crocuta crocuta*, Mathevon, Koralek, Weldele, Glickman, & Theunissen, 2010; rock hyrax, *Procavia capensis*, Koren & Geffen, 2009; fallow deer, *Dama dama*, Vannoni & McElligott, 2008), or a behavioural trait, such as dominant individuals vocalizing more frequently compared to subordinates (domestic chicken, Leonard & Horn, 1995; black-capped chickadee, *Poecile atricapillus*, Otter, Chruszcz, & Ratcliffe, 1997).

If a signal indicates an individual's dominance status, what that signal means will depend on who is receiving the signal. Traits that indicate individual's fighting ability or quality is central to the theory of sexual selection (Darwin 1859). Birdsong is an example of a signal that serves this dual role. In general, male birdsong, when directed at other males, acts as a signal of territoriality, while male birdsong, when directed at females, acts as a signal to attract a mate. However, male birdsong often contains information regarding the individual that is signaling and, for species that live in social groups with dominance hierarchies, songs can contain information regarding a male's dominance status. In some species, song is essential for the establishment of dominance relationships (e.g., brown-headed cowbirds, *Molothrus ater*, Duffy, 1986). Although subordinate males are capable of singing the song preferred by females, only the dominant male sings these preferred songs (West, King, & Eastzer, 1981). If information regarding a male's dominance rank means something different for a listening female compared to a listening male, how the sexes respond to the signal should also vary. In addition, what the signaler produces may also vary depending on who is present (Dalziell, & Cockburn, 2008).

Black-capped chickadees are a common North American songbird that live in winter flocks with linear dominance hierarchies. Within a flock, males will usually dominate females, and when the flocks break-up in spring, the highest-ranking male will form a mating pair with the highest-ranking female (Smith, 1991). High-ranking birds have preferential access to resources, including food sources and local territories (Smith, 1991), and females seek extrapair copulations with high-ranking males (Mennill, Ramsay, Boag, & Ratcliffe, 2004). Males (Mennill & Ratcliffe, 2004) and females (Mennill, Ratcliffe, & Boag, 2002) eavesdrop on male song contests and male dominance cues are contained within the songs (Christie, Mennill, & Ratcliffe, 2004; Hahn et al., 2013, Hoeschele et al., 2010).

Since male chickadee songs contain dominance cues, one mechanism that birds could use to discriminate between the songs of dominant and subordinate males is through open-ended categorization (Herrnstein, 1990). If there is an acoustic cue that varies among dominant and subordinate songs, by using that acoustic cue, birds could quickly identify a singing male as dominant or subordinate and respond. If songs do not belong to perceptual categories, open-ended categorization is not possible; however, birds can still discriminate among vocalizations using rote memorization. In contrast to open-ended categorization, rote memorization requires an individual to have prior experience with and memorize each signal (e.g., a bird would need to have prior experience with the vocalizing individual, evaluate the male’s dominance rank, and recognize his song characteristics). Individual recognition may aid in rank identification; however, birds may also use rank-identifying visual (Mennill, Doucet, Montgomerie, & Ratcliffe, 2003) or acoustic (Christie et al., 2004; Hahn et al., 2013, Hoeschele et al., 2010) cues to determine a male's dominance rank. Categorization is a mechanism that birds could use to assess dominance rank without previous experience with the singing individual.

In addition to the ability to categorize songs based on acoustic dominance cues, birds may also have a preference for listening to dominant or subordinate songs. Preference tests using conspecific song as the reinforcer, have demonstrated that in other songbird species, females prefer certain characteristics in male song (e.g., song complexity, chaffinch, *Fringilla coelebs*, Leitão, ten Cate, & Riebel, 2006; song bout length, European starling, *Sturnus vulgaris*, Gentner & Hulse, 2000).

In the current study, we use a series of operant conditioning tasks to examine (1) chickadees' preference for dominant or subordinate songs, (2) the abilities of chickadees to discriminate songs based on the singer's dominance rank and (3) how acoustic preference relates to performance on the instrumental learning task. First, we use a choice preference task (similar to Gentner & Hulse, 2000) to examine chickadees' preference for dominant or subordinate songs. Next, we use a go/no-go instrumental learning task (see Sturdy & Weisman, 2006) using a true category/pseudo category paradigm. The objective for the instrumental learning task was to examine if birds could use a category perception-like mechanism to discriminate songs based on the singer's dominance rank. Following the initial discrimination, we presented birds with novel songs recorded in other geographic regions to examine how birds would categorize songs produced by dominant and subordinate males from other locations and we presented birds with songs in which we artificially manipulated an acoustic feature (i.e., relative amplitude) that varies between dominant and subordinate songs (Hahn et al., 2013; Hoeschele et al., 2010). Last, we were interested in examining how a bird's underlying preference (as measured in the choice preference task) related to its performance in the instrumental learning task, by examining learning speed (defined as the number of trials to criterion) and performance accuracy (defined as the discrimination ratio on the final day of discrimination training and the first day of pretesting).

Experiment 1: Choice preference task

Methods

Subjects

Nineteen black-capped chickadees (ten males and nine females) were tested between March and May 2012. Sex was determined by DNA analysis (Griffiths, Double, Orr & Dawson, 1998); visual examination of the gonads post mortem have revealed that this method of DNA analysis is accurate (e.g., Hahn, Guillette, Lee, McMillan, Hoang, & Sturdy, 2015). Birds were captured in Edmonton (North Saskatchewan River Valley, 53.53˚N, 113.53˚W; Mill Creek Ravine, 53.52˚N, 113.47˚W), or Stony Plain (53.46˚N, 114.01˚W), Alberta, Canada between January and March 2012 and were at least one year of age at capture (verified by examining color and shape of the outer tail retrices; Pyle, 1997).

When not in the experimental apparatus, birds were housed singly in cages (30 cm × 40 cm × 40 cm, Rolf C. Hagen, Inc., Montreal, QB) in colony rooms within the visual and auditory range of conspecifics. Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St Louis, MO), water (vitamin supplemented on alternating days; Prime vitamin supplement; Hagen, Inc.), grit, and cuttlebone. Birds received daily nutritional supplements, including: 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. The colony rooms were kept on a light:dark cycle that mimicked the natural light cycle for Edmonton, Alberta, Canada. Birds were naïve to all experimental procedures.

Apparatus

Birds were individually tested in a sound-attenuating chamber (117 cm × 120 cm × 200 cm; Industrial Acoustics Company, Bronx, NY). The testing space measured 67 cm × 116 cm × 116 cm. In the chamber, there were three perches (1.75 cm in diameter and 10 cm in length) each in front of a Fostex FE108E Σ full-range speaker (Fostex Corp., Japan; frequency response range 80-18,000 Hz). The back wall and each side wall had one speaker mounted to it. Each perch was located 100 cm from the floor of the testing area. Each perch contained an infrared beam used to monitor when a bird landed on the perch. A single-board computer (Palya & Walter, 2001) and personal computer controlled which stimuli played and recorded responses. Stimuli were played from a CD through a Cambridge Azur 640A Integrated Amplifier (Cambridge Audio, London, England) to the speakers within the chamber. During the testing session, birds had *ad libitum* access to Mazuri and water located on the floor of the testing space.

Acoustic stimuli

A total of 160 black-capped chickadee *fee-bee* songs were used as stimuli in the choice preference task. Songs were recorded from banded populations of black-capped chickadees at the University of Northern British Columbia, British Columbia and Queen's University Biological Station, Ontario (80 songs from each location; see Hahn et al., 2012 for details about the recordings). The dominance rank of male flockmates were assessed in the field (prior to audio recordings) by observing numerous interactions at winder feeding stations (see Mennill et al., 2004; van Oort, Otter, Fort, & Holschuh, 2006 for more information on dominance assessment). Within a flock, the highest-ranking male was considered a dominant male, and the lowest-ranking male was considered a subordinate male (i.e., second-ranking male in flocks with two males; third-ranking male in flocks with three males).

The field recordings were resampled from 22,050 Hz to 44,100 Hz (SIGNAL 5.10.29 software; Engineering Design, Berkeley, CA) and bandpass filtered (2,000-6,000 Hz) to remove background noise (GoldWave v5.58; GoldWave, Inc., St. John's, NL). The beginning and ending 5 ms prior to the song was tapered and amplitude was normalized using SIGNAL. Each stimulus track on the CD contained two songs produced by the same individual and 1 s of silence separated the two songs. In total, there were 40 songs (20 stimulus tracks) from each of the following groups: dominant songs from British Columbia, subordinate songs from British Columbia, dominant songs from Ontario, and subordinate songs from Ontario. Four songs produced by a given male were used (i.e., two stimulus tracks each containing two songs).

Procedure

The 160 stimuli songs were divided into two stimulus sets determined by the geographic origin of the songs (i.e., British Columbia or Ontario songs). Both stimulus sets contained songs produced by dominant and subordinate males. During the experiment, dominant songs were assigned to one perch, subordinate songs were assigned to a second perch, while the last perch had no acoustic stimuli associated with it. During a test session, birds only heard songs from one of the two geographic locations (for example, dominant and subordinate songs from British Columbia). The order that birds heard songs from each location was counterbalanced. Birds were tested until they met criteria on one stimulus set (i.e., songs from one geographic location) before being tested with the other stimulus set (i.e., songs from the second geographic location; see below for criteria). With three perches there are six possible configurations for stimuli (dominant, subordinate, or silent) to be associated with the perches. We randomly assigned the perch configuration for the first stimulus set presented. During the second test session (i.e., when a bird was tested with a new geographic location), we changed which perch was associated with dominant songs, subordinate songs and silence, so that no perch was associated with the same category of song for both geographic stimulus sets (i.e., the dominant, subordinate, and silent perches varied for the British Columbia and Ontario stimulus sets).

Each chickadee was tested in 2 h sessions (a bird never had more than one session per day) between 0900 and 1600 hours. For testing, a bird was transported from the colony room to the testing room, removed from its home cage, and released into the testing chamber. After the 2 hr testing session ended, the bird was placed back in its home cage and returned to the colony room.

During the experimental session, when a bird landed on an acoustic (i.e., non-silent) perch, breaking the infrared beam, one stimulus track (i.e., two songs) played until completion. The stimulus track that played was sampled at random without replacement from the group of stimuli that were associated with that perch. If a bird remained on the perch after the songs had finished playing, songs would not play continuously (a bird had to leave the perch and re-land on it, breaking the infrared beam, before another stimulus would play). The number of times a bird landed on each of the three perches and the duration of time spent on perch was recorded by the computer. A bird was tested in daily sessions until it had spent a total of at least 1 hr on the acoustic perches and heard all stimuli at least 5 times, or had completed a maximum of 5 experimental sessions. Once a bird had met these criteria with one stimulus set, it began sessions with the second stimulus set with a minimum of one day of rest in between testing (mean ± SD number of days between testing with two stimulus set = 3.4 ± 0.92). The order that birds heard the two stimulus sets (British Columbia and Ontario) was randomized across birds.

Response measures

We recorded the absolute amount of time spent perched and the absolute number of visits that each bird made to the three perches. Because the number of sessions required for a bird to reach criterion varied, we took the absolute amount of time that each bird spent on each of the three perches and divided it by the number of two-hour sessions that it completed in order to get a per session rate for each individual. We calculated a similar per-session rate for the number of visits made by each individual. We calculated these response measures separately for British Columbia songs and Ontario songs. One bird (a male) was found dead in the colony room (see Ethical note below) before finishing the experiment, so we could only include data from the British Columbia song set for this bird. The silent perch was not working during one of the sessions for two females on the British Columbia song sets, one female on the Ontario song set, and one male on the Ontario song set, so these birds could not be included on the analyses examining all three perches.

In order to quantify acoustic preference we used two dependent measures: (1) proportion of time on the acoustic perches, and (2) proportion of visits to the acoustic perches. We calculated a preference score by taking the amount of time spent on the dominant song perch and dividing it by the amount of time spent on the two acoustic perches. We calculated a similar preference score by dividing the number of visits to the dominant song perch and dividing it by the number of visits to both acoustic perches. A preference score close to 1 indicates a preference for the dominant song perch, a score near 0.5 indicates no preference, and a score near 0 indicates a preference for the subordinate song perch. We calculated each of these response measures separately for British Columbia songs and Ontario songs.

Statistical analyses

For each stimulus set (i.e., British Columbia songs or Ontario songs), we conducted a repeated measures ANOVA to examine the amount of time spent on each of the three perches. We conducted a similar repeated measures ANOVA to examine the number of visits to each of the three perches. To examine acoustic preference, we determined if the preference scores for each sex differed from chance level (i.e., 0.5) with a one-sample t test. To correct for non-normality, we also conducted analyses of the acoustic preferences using the arcsine square root transformed proportions with the formula arcsin(sqrt(x)), where x equals the untransformed proportion. The analysis of the transformed data results in a similar level of significance in most instances; we report any differences in the analysis of the untransformed and transformed data.

Ethical note

Studies were conducted with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta (AUP 108) and all procedures were in accordance with the Canadian Council on Animal Care (CCAC) Guidelines and Policies and the ABS Guidelines for the Use of Animals in Research. During experiment 1, birds were in the testing apparatus for two hours a day, during which they had free access to food and water. Birds were then returned to their home cage and the colony room. During experiment 2, birds were provided with free access to water, grit and cuttlebone and given two superworms daily. Birds were monitored at least two times a day. One bird was found dead in the colony room during experiment 1; a post-mortem conducted by the University of Alberta veterinarian determined that the bird had a tumor on its lung. One bird was found dead during experiment 2; a post-mortem conducted by the veterinarian suggested that the bird died from a heart defect. In both cases, these deaths were unrelated to the experimental procedures. All other birds remained healthy during the experimental procedures. Following experiment 2, birds were returned to the colony room to be used in future studies. Chickadees were captured under an Environment Canada Canadian Wildlife Service Scientific permit, Alberta Fish and Wildlife Capture and Research permits, and City of Edmonton Parks Permit.

Results

Responses to all three perches

We conducted a repeated measures ANOVA with the amount of time on each perch (dominant, subordinate, silent) as the dependent variable and sex and presentation order (British Columbia songs heard first or Ontario songs heard first) as between subject factors. For British Columbia songs, there was a significant main effect of perch ($F_{2,26} = 4.84$, $p = 0.016$. A Tukey's post-hoc test revealed that birds spent significantly more time on the silent perch compared to the dominant perch ($p = 0.013$) and subordinate perch ($p = 0.049$). For Ontario songs, there was a significant main effect of perch ($F_{2,24} = 4.29$, $p = 0.026$). A Tukey's post-hoc test revealed that birds spent significantly more time on the silent perch compared to the dominant perch ($p = 0.011$). See Figure 3-1.

We conducted a repeated measures ANOVA with number of visits to each perch (dominant, subordinate, silent) as the dependent variable and sex and presentation order (British Columbia songs heard first or Ontario songs heard first) as between subject factors. For British Columbia songs, there was no significant difference in the number of visits to the three perches or any interactions (Fs ≤ 1.41, ps ≥ 0.26). For Ontario songs, there was no significant difference in the number of visits to the three perches or any interactions (Fs ≤ 1.03, ps ≥ 0.37).

Acoustic preference

Time on acoustic perches. We conducted a one-sample t test to determine if the preference score for the proportion of time spent on the acoustic perches differed significantly from chance (i.e., 0.5) for each sex. For the British Columbia songs, the average (± SE) preference score for male and female birds (respectively) was 0.43 ± 0.08 and 0.46 ± 0.09, and neither of these preference scores differed significantly from chance, males: $t_9 = -0.85$, $p = 0.42$, females: $t_8 = -0.47$, $p = 0.65$. However, analysis of the transformed data revealed that female birds spent significantly more time on the subordinate song perch ($t_8 = 2.5$, $p = 0.04$). For the Ontario songs, the average (± SE) preference score for male and female birds (respectively) was 0.58 ± 0.08 and 0.42 ± 0.10, and neither of these preference scores differed significantly from chance, males: $t_8 = 0.91$, $p = 0.39$, females: $t_8 = -.08$, $p = 0.45$. However, the analysis of the transformed data revealed that male birds spent significantly more time on the dominant song perch ($t_8 = 3.99$, $p = 0.004$). See Figure 3-2.

Visits to acoustic perches. We conducted a one-sample t test to determine if the preference score for the proportion of visits to the acoustic perches differed significantly from chance (i.e., 0.5) for each sex. For the British Columbia songs, the average (± SE) preference score for male and female birds (respectively) was 0.40 ± 0.09 and 0.48 ± 0.10, and neither of these preference scores differed significantly from chance, males: $t_9 = -1.11$, $p = 0.29$, females: $t_8 = -0.23$, $p = 0.82$. For the Ontario songs, the average (± SE) preference score for male and female birds (respectively) was 0.54 ± 0.08 and 0.45 ± 0.09, and neither of these preference scores differed significantly from chance, males: $t_8 = 0.50$, $p = 0.63$, females: $t_8 = -0.58$, $p = 0.58$. However, the analysis of the transformed data found that male birds visited the dominant song perch significantly more than expected by chance ($t_8 = 3.56$, $p = 0.007$). See Figure 3-3.

Experiment 2: Instrumental learning task

Methods

Subjects

The same birds (*n* = 18) used in experiment 1, served as subjects in experiment 2. All housing conditions prior to experiment 2 remained the same.

Apparatus

During experiment 2, birds were individually housed in modified home cages (30 cm × 40 cm × 40 cm) placed inside a ventilated, sound-attenuating chamber. Each chamber was illuminated by a 9 W, full spectrum fluorescent bulb following the natural light cycle for Edmonton, Alberta. The cage contained three perches, water (vitamin supplemented three times a week), grit, and cuttlebone. Birds were provided two superworms daily (one in the morning and one in the afternoon), otherwise food (i.e., Mazuri; provided by a motor-driven feeder, Njegovan, Hilhorst, Ferguson, & Weisman, 1994) was only available following correct responding during the task. An opening (11 cm × 16 cm) on the side of the cage, gave the bird access to the feeder. The position of the bird was monitored by infrared cells in the feeder and request perch (perch closest to the feeder) and a personal computer connected to a single-board computer scheduled trials and recorded a bird's responses. Stimuli were played from a CD through an amplifier (Cambridge A300 or 640A Integrated Amplifier, Cambridge Audio, London, England; or NAD310 Integrated Amplifier, NAD Electronics, London, England) and a full-range speaker located in the sound-attenuating chamber (Fostex FE108 Σ or FE108E Σ, Fostex Corp., Japan; frequency response range 80-18,000 Hz). For a diagram and detailed description of the experimental apparatus see Sturdy and Weisman (2006).

Acoustic stimuli

A total of 74 (38 dominant; 36 subordinate) *fee-bee* songs were used as stimuli in the current experiment. Details regarding the song recordings used in the current experiment remained the same as in experiment 1. Songs were recorded at the same locations (i.e., University of British Columbia and Queen's University Biological Station) and songs were produced by some of the same individuals as the stimuli presented during experiment 1; however, unique song exemplars were used in both experiments (i.e., no stimuli exemplars used in experiment 1 were used in experiment 2). In addition, we presented birds with songs recorded between 28 April and 16 May 2006, 2008 and 2009 during the dawn chorus period at a field station in the John Prince Research Forest (Fort St. James, British Columbia). Songs were recorded with an Audio-Technica ATB815a, Sennheiser MKH70 or ME67 microphone and a Marantz PMD430 tape recorder or Marantz PMD671 digital recorder.

During the experiment, stimuli were presented at approximately 75 dB as measured by a Brüel & Kjær Type 2239 (Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark) decibel meter (A weighting, slow response) at the approximate position of a bird's head when on the request perch. See below for specific details regarding further stimulus preparation.

Natural Stimuli. Ten dominant and ten subordinate songs recorded at the University of Northern British Columbia (Prince George, British Columbia) were used as stimuli during discrimination training and an additional five dominant and three subordinate songs recorded from this location were used as natural (unmanipulated) stimuli during testing. There were two additional test sessions that contained natural stimuli: one test session contained ten dominant and ten subordinate songs recorded at the Queen's University Biological Station (near Kingston, Ontario) and one test session contained ten dominant and ten subordinate songs recorded at the John Prince Research Forest (Fort St. James, British Columbia). Only one vocalization produced per individual was used as a stimulus during discrimination training and the natural song test sessions. Vocalizations were of high quality (i.e., no audible interference and low background noise when viewed on a spectrogram with amplitude cutoffs of -35 to 0 dB relative to song peak amplitude). Songs were bandpass filtered (2,500-4,800 Hz; outside the frequency range of songs) using GoldWave version 5.58 (GoldWave, Inc). For each stimulus, songs were edited to include 5 ms at the leading and trailing end of the song, which was then tapered to remove transients, and amplitude was equalized using SIGNAL 5.10.29 software (Engineering Design).

Manipulated Songs. Three dominant and three subordinate songs recorded at the University of Northern British Columbia were used during manipulated song tests. For these test stimuli, we manipulated the relative amplitude between the first and second note of the songs, because previous bioacoustic analyses have shown that the relative amplitude between the two song notes varies with dominant status in birds from this population (Hahn et al. 2013; Hoeschele et al. 2010). The relative amplitude is a measurement calculated by dividing the maximum amplitude of the *bee* note by the maximum amplitude of the *fee* note. We used the standard deviations (SD) for the relative amplitude of the two notes within the song for the dominant and subordinate songs that were measured in the previous bioacoustic analysis to determine manipulations of the relative amplitude during the test session (the SD for dominant and subordinate songs was 0.083 and 0.123, respectively). To create the manipulated songs, we separated the two song notes and increased or decreased the amplitude of the *bee* note, so that the relative amplitude would decrease (i.e., become a smaller ratio) or increase (i.e., become a larger ratio), respectively. During the test session, each song was presented in five ways: (1) the relative amplitude was decreased by 1.5 SD, (2) the relative amplitude was increased by 1.5 SD, (3) the relative amplitude was manipulated to be equal, (4) the song was presented unmanipulated, and (5) sham manipulated: the two notes of the song were edited to separate the notes (similar to the manipulated songs), and then simply concatenated without altering the relative amplitude, with all manipulations the original internote conserved. Songs that were manipulated were not previously heard by the subjects, but the subjects were presented with other songs exemplars produced by these same individuals during the natural test session.

Procedure

Pretraining. Once the bird learned how to use the equipment to obtain food, pretraining began. During pretraining, birds were presented with all songs that would be presented throughout the experiment. Birds were reinforced for responding to all stimulus exemplars to ensure a high level of responding to all songs before the start of discrimination training. In order for a trial to begin, the bird had to land and remain on the request perch for 900-1100 ms. Once the stimulus finished playing, if the bird entered the feeder within 1 s, it was given 1-s access to food and then a 30-s intertrial interval (ITI), during which the houselight remained on, but a new trial could not be initiated. An interrupted trial occurred if the bird left the request perch before the stimulus finished playing, which resulted in a 30-s timeout with the houselight turned off. If a bird stayed on the request perch during the song presentation and the 1 s after the song finished, a 60-s ITI occurred with the houselight on; however, this ITI ended if the bird left the request perch. During pretraining, data was collected in blocks containing 294 trials (each stimulus played three times); the order that the stimuli played during each block was randomly-selected. Birds continued on pretraining until they completed six 294-trial blocks with ≥ 60% responding to all stimuli, at least four 294-trial blocks with ≤ 3% difference in responding to future rewarded and unrewarded stimuli and at least four 294-trial blocks with ≥ 60% feeding on future test stimuli. One bird (a female) was found dead during this phase of the experiment, which resulted in an *n* = 17 for the remaining phases of the experiment (see Ethical note above).

Discrimination training. Following pretraining, each bird moved onto discrimination training. The procedure remained the same, but only 10 dominant and 10 subordinate songs were presented and responding to half of these songs (i.e., unrewarded songs) now resulted in a 30-s ITI with the houselight off. Responding to the remaining 10 songs (i.e., rewarded songs) resulted in 1 s access to food. Discrimination training continued until birds completed three 200-trial blocks with a discrimination ratio (DR) ≥ 0.75 with the last two blocks being consecutive. See response measures, below, for information regarding the DR calculations.

Black-capped chickadees were randomly assigned to either a true category (*n* = 12) or pseudo category discrimination group (*n* = 5). The true category discrimination group contained two subgroups: six birds discriminated rewarded dominant songs from unrewarded subordinate songs (dominant song group; three males and three females), and six birds discriminated rewarded subordinate songs from unrewarded dominant songs (subordinate song group; three males and three females). The pseudo category discrimination group was also separated into two subgroups. Each subgroup discriminated five rewarded dominant and five rewarded subordinate songs from five unrewarded dominant and five unrewarded subordinate songs. The rewarded songs for one subgroup were the unrewarded songs for the other subgroup, and vice versa. There were two males and one female in one subgroup and one male and one female in the other subgroup.

Pretesting. Pretesting was the same as discrimination training, with the exception that rewarded songs were reinforced with a reduced probability (i.e., $p = 0.85$). On trials in which a song in the rewarded category were not reinforced, a bird received no access to food and a 30-s ITI with the houselight on. The purpose of pretesting was to introduce trials that were neither rewarded nor punished to prepare birds for test sessions (see below). Birds continued on pretesting until they completed two consecutive 200-trial blocks with a DR ≥ 0.75.

Natural test session: University of Northern British Columbia. Following pretesting, all birds completed this test session. The rationale for this test session was to examine if birds would continue to respond to new stimuli based on the contingencies from discrimination training (i.e., would birds in the true category group show generalization). The stimuli and reinforcement contingencies from pretesting were continued during test sessions; however, additional songs not heard during discrimination or pretesting were also presented. These new (i.e., test) stimuli included five dominant songs and three subordinate songs recorded at the same location as the songs used during discrimination (i.e., University of Northern British Columbia). Each test stimulus was presented once during a 208-trial block. Responses to these test songs s resulted in a 30-s ITI with the houselight on, but no access to food. All birds completed at least three blocks of this test session, followed by one 200-trial block of pretesting with a DR ≥ 0.75 before moving onto the next test session.

Natural test sessions: Queen's University Biological Station and John Prince Research Forest. We also presented birds with other natural (i.e., unmanipulated) song stimuli, but these songs were recorded at two different locations (i.e., Queen's University Biological Station and John Prince Research Forest). In one test session we presented ten dominant and ten subordinate songs recorded at Queen's University Biological Station, Ontario, and in the other test session we presented ten dominant and ten subordinate songs recorded at the John Prince Research Forest, British Columbia. The order that these two probe sessions were presented was randomized between subjects. During these test sessions, the stimuli and contingencies from pretesting remained and each test stimulus was presented once in a 220-trial block. Birds completed a minimum of three blocks, followed by one 200-trial block of pretesting with a DR ≥ 0.75 before moving onto the next test session.

Manipulated song test session. The remaining test stimuli ($n = 30$) were presented in the last test session. Similar to the other test sessions, each test stimulus was presented once and the stimuli from pretesting were presented ten times each. This resulted in a 230-trial block and birds completed a minimum of three blocks before completing the experiment. One bird (a male in the subordinate song group) only completed two bins of the manipulated song test and these were included in the analysis.

Response measures

We calculated a percentage response for each stimulus exemplar with the formula: $R+/(N-I) \times 100$, in which we divided the number of trials where the bird went to the feeder (R+) by the total number of trials (N) not including trials in which the bird left the perch before the entire stimulus played (I). We calculated a discrimination ratio (DR) during discrimination training and pretesting by dividing the average percent response to all rewarded stimuli by the average percent response to all stimuli (i.e., rewarded and unrewarded). Perfect discrimination (i.e., responses only to rewarded songs) is designated by a DR of 1.0, while a DR of 0.50 represents equal responding to rewarded and unrewarded songs.

We modified the two-tailed 95% confidence interval (CI) using the mean (M) and standard deviation (SD) of the percentage response to all unrewarded songs on the final day of discrimination training with the formula: CI = $M \pm 1.96 \times \text{SD}$. Using this measure, we determined how many rewarded songs were well discriminated. If the percentage response to an individual rewarded song was ≥ 95% CI, it suggests that birds were significantly discriminating that rewarded stimulus from the unrewarded stimuli.

Individual subjects varied in their response levels to the test stimuli, so we scaled the proportion of response for each individual subject by taking the highest proportion of response to a test stimulus within a test session and rescaling it to 1. The proportions of response to all other stimuli within that test session were then rescaled as a ratio of the highest proportion of responding. We rescaled each test session separately.

Statistical analyses

We conducted an ANOVA on the number of trials to criterion and DRs for the true category and pseudo category groups during discrimination training. Birds required a varying number of trials to complete discrimination training. In order to compare acquisition during discrimination, we Vincentized the acquisition curves to 14 blocks of data (the minimum number of blocks for a bird to reach criterion). To calculate the Vincentized blocks, we took the number of trial blocks it took an individual bird to reach criterion and divided it by 14. The actual number of blocks that the bird completed was then divided into the Vincentized blocks equal to the whole-number quotient, while any remaining blocks were placed into the middle two Vincentized blocks (i.e., the 7th and 8th Vincentized blocks). For example, if an individual bird took 32 blocks to reach criterion, each Vincentized block would consist of 2 actual blocks (i.e., 32/14 = 2 with a remainder of 4), and the remaining blocks (i.e., 4) would be divided between the 7th and 8th Vincentized blocks. The middle Vincentized blocks would each have 2 + 2 = 4 actual blocks grouped into it. The DRs for the trial blocks that were grouped into a Vincentized block were averaged (see Hahn et al., 2015; Hoeschele, Guillette, & Sturdy, 2012; Kling & Riggs, 1971).

In order to determine if the number of song exemplars that were responded to during discrimination training differed among groups, we examined the number of rewarded songs responded to ≥ 95% CI. We conducted additional ANOVA and Tukey's planned comparisons on the proportion of responding to the test stimuli and conducted corresponding analyses using the square root transformation (to correct for non-normality) on the proportion of responses. In most instances, analysis of transformed and untransformed data produced the same pattern of results and levels of significance, so only the untransformed data is reported. In cases where there was a difference between the analysis of transformed and untransformed data, we indicate the differences in the sections below. All statistics were conducted in Statistica v12 (StatSoft, Inc).

Results

Comparison of true and pseudo category discriminations

Trials to criterion. The average number of 200-trial blocks required for birds in each discrimination group to complete discrimination training was: 42 and 25.67 for males and females (respectively) in the dominant song group, 25.67 and 31 for males and females (respectively) in the subordinate song group, and 46 and 22.5 for males and females (respectively) in the pseudo category group. To determine whether males and females in the two true category discrimination groups (dominant song group and subordinate song group) differed in their speed of acquisition, we conducted a sex × group (dominant song, subordinate song) ANOVA on the number of 200-trial blocks required to complete discrimination training. There were no significant main effects or interactions (*F*s ≤ 2.36, *p*s ≥ 0.16), revealing that birds rewarded for responding to dominant songs learned the discrimination as quickly as birds rewarded for responding to subordinate songs.

To determine whether males and females in the two pseudo category groups differed in their speed of acquisition, we conducted a main effects ANOVA with sex and group (pseudo group 1, pseudo group 2) as categorical predictors and the number of 200-trial blocks to complete discrimination training as the dependent variable. There was a significant main effect of sex ($F_{1,2} = 70.90$, $p = 0.014$) with females requiring fewer trials to complete discrimination training. There was no main effect of group ($F_{1,2} = 0.02$, $p = 0.89$), so we combined the two pseudo groups in the remaining analyses.

In order to compare the speed of acquisition of birds in the true and pseudo category groups, we conducted a sex × group (dominant song, subordinate song, pseudo) ANOVA on the number of 200-trial blocks to complete discrimination training. There was a main effect of sex ($F_{2,11} = 4.98$, $p = 0.0474$), but no other main effects or interactions. Overall, females learned the discrimination in fewer trials compared to males (average ± SE for males = 37.89 ± 4.57; females = 26.88 ± 2.98). See Figure 3-4.

Acquisition performance. We compared task acquisition across Vincentized blocks using a repeated measures ANOVA with sex and group (dominant song, subordinate song, pseudo) as categorical predictors and the DR calculated for each Vincentized block (1-14) as dependent variables. There was a significant main effect for Vincentized block $F_{13,\,143} = 73.52$, $p < 0.001$. The DRs increased across Vincentized blocks increased. There was also a significant main effect of group $F_{2,\,11} = 4.41$, $p = 0.039$. A Tukey's post hoc test for the group main effect revealed that the DRs were significantly different between the dominant song group and the subordinate song group ($p = 0.034$), with the subordinate song group having overall higher DRs. See Figure 3-5.

Final day performance. We conducted a sex × group (dominant song, subordinate song, pseudo) ANOVA on the DR on the final day of discrimination training. There were no significant differences (Fs ≤ 1.11, all ps ≥ 0.37) suggesting that by the final day of discrimination training all birds had reached a similar level of performance.

Number of rewarded songs responded to ≥ 95% confidence interval (CI). To determine how many of the rewarded stimuli were well-discriminated, we examined how many rewarded songs were responded to ≥ 95% CI during the final block of discrimination training. We conducted a sex × group (dominant song, subordinate song, pseudo) ANOVA to determine if the true category and pseudo category groups differed in the number of exemplars responded to ≥ 95% CI. There were no significant main effects or interactions, Fs ≤ 0.57, ps ≥ 0.58. Out of 10 rewarded stimuli, the means ± SE for the number of stimuli responded to ≥ 95% CI were: dominant song group, 7.67 ± 2.33and 10 ± 0, for male and female subjects, respectively; subordinate song group, 8 ± 1.0 and 7.3 3± 2.67, for male and female subjects respectively; and pseudo category group, 9.67 ± 0.33and 9 ± 1.0, for male and female subjects, respectively.

Test sessions

Natural songs: University of Northern British Columbia, British Columbia. Following pretesting, birds in the true category and pseudo category discrimination conditions were presented with dominant and subordinate songs not heard during acquisition. We conducted a sex × group (dominant song, subordinate song, pseudo) × stimulus type (dominant song, subordinate song) ANOVA on the scaled proportion of response. There were no significant main effects or interactions Fs ≤ 3.00, ps ≥ 0.11. See Figure 3-6.

Natural songs: Queen's University Biological Station, Ontario. We conducted a sex × group (dominant song, subordinate song) × stimulus type (dominant song, subordinate song) on the scaled proportion of response to the songs recorded in Ontario. There was a significant stimulus type × group interaction $F_{1,8} = 93.73$, $p < 0.001$. To examine the interaction, we conducted a Tukey's post hoc analysis. Birds in the dominant song group responded significantly more to dominant songs ($X = 0.48$) compared to subordinate songs ($X = 0.34$), $p = 0.0006$. Birds in the subordinate song group responded significantly more to subordinate songs ($X = 0.66$) compared to dominant songs ($X = 0.53$), $p = 0.001$. See Figure 3-7.

Natural songs: John Prince Research Forest, British Columbia. We conducted a sex × group (dominant song, subordinate song) × stimulus type (dominant song, subordinate song) on the scaled proportion of response to the songs recorded at John Prince Research Forest, British Columbia. There were no significant main effects or interactions $F\text{s} \leq 4.87$, $p\text{s} \geq 0.058$.

Manipulated songs. We conducted planned comparisons (Tukey's, $p \leq 0.05$) on the average proportion of response to the rewarded discrimination songs presented during the manipulated song tests (reinforced 85% of the time) compared to average proportion of response to each of the ten manipulated song types (i.e., dominant songs and subordinate songs with: relative amplitude increased, relative amplitude decreased, equal relative amplitude, cut songs, natural songs) We also conducted planned comparisons (Tukey's, $p \leq 0.05$) on the average proportion of response to the unrewarded discrimination songs presented during the manipulated song tests compared to average proportion to each of the ten manipulated song types. Because the training and the test songs were presented a different number of times during each bins (i.e., test songs are each presented once during a bin, while training songs were each presented ten times), we scaled the training songs separately from the test stimuli. We compared the manipulated songs to the discrimination songs because the results from the test session with songs from the University of Northern British Columbia suggest that the discrimination may not generalize to novel songs, and we wanted to examine how responding to unmanipulated and manipulated song exemplars would compare to the responding to the training songs.

For birds in the dominant song group, birds responded significantly more to the rewarded discrimination songs compared to all manipulated song types (all ts ≥ 4.50; all ps ≤ 0.002). Birds in the dominant song group responded significantly less to unrewarded discrimination songs compared to dominant songs cut ($t = 2.84$, $p = 0.019$), dominant songs with the relative amplitude increased ($t = 2.30$, $p = 0.047$), subordinate songs cut ($t = 2.66$, $p = 0.026$), natural subordinate songs ($t = 3.45$, $p = 0.007$), and subordinate songs with equal relative amplitude ($t = 2.38$, $p = 0.041$) When the same analysis was conducted with the arcsine transformed data, the comparison between unrewarded discrimination songs and dominant songs with the relative amplitude increased was no longer significant ($t = 2.21$, $p = 0.054$)

For birds in the subordinate song group, bird responded significantly more to the rewarded discrimination songs compared to all manipulated song types (all ts ≥ 2.55; all ps ≤ 0.029). Analysis of the arcsine transformed data revealed that birds did not respond significantly differently between subordinate songs with the relative amplitude increased and the rewarded discrimination songs (t = 2.22, p = 0.051). Birds in the subordinate song group responded significantly less to unrewarded discrimination songs compared to subordinate songs with relative amplitude decreased ($t = 2.84$, $p = 0.018$), subordinate song with relative amplitude increased ($t = 3.04$, $p = 0.012$), subordinate songs cut ($t = 3.09$, $p = 0.012$), and subordinate songs with equal relative amplitude ($t = 3.03$, $p = 0.013$). When the same analysis was conducted with the arcsine transformed data, the comparisons between unrewarded discrimination songs and subordinate songs with relative amplitude decreased ($t = 1.81$, $p = 0.11$) was no longer significant. See Figure 3-8.

Relationship between acoustic preference and performance on the instrumental learning task

To determine if a bird’s underlying preference for dominant or subordinate songs, as measured by the choice preference task (experiment 1) could be predicted by the bird’s performance during the instrumental learning task (experiment 2), we conducted multiple regression analyses. We conducted two multiple regression analyses to predict our two measures of preference strength: (1) proportion of time on the acoustic perches, and (2) proportion of visits to the acoustic perches. For these analyses, we used the preference strength for the British Columbia stimulus set, because the songs used during discrimination training in experiment 2 were from this location. For the dependent variables, we used the proportion of time or the proportion of visits to the acoustic perch associated with the rewarded song category during the instrumental learning task; therefore, we only included birds in the true category groups in this analysis. By examining the data in the way, we could determine if the preference strength for a particular category of songs could be predicted by learning speed or performance accuracy when that category of songs was reinforced with food during the instrumental learning task. We included sex as an independent variable, along with the following learning scores from the instrumental learning task: one measure of learning speed (the number of 200-trial bins to complete discrimination training) and two measures of performance accuracy (the DR during the final bin of discrimination training and the DR during the first bin during pretesting). Previous studies have used the number of trials to criterion as a measure of learning speed and DRs as a measure of performance accuracy (e.g., Guillette et al. 2015).

Results

A multiple regression analysis was conducted to predict preference strength as measured by the proportion of time spent on each acoustic perch, using the measures of learning speed and performance accuracy as independent variables. The regression equation was significant, $R^2 = 0.80$, adjusted $R^2 = 0.69$, $F_{4,7} = 7.20$, $p = 0.013$. The DR during the first bin of pretesting was the only variable that significantly predicted the time on perch ($\beta = -0.87$, $p = 0.002$). Birds that had stronger preference ratios towards the song category that was rewarded during acquisition (i.e., closer to 1) had lower DRs during the first bin of pretesting. See Figure 3-9.

We also conducted a multiple regression analysis to predict preference strength as measured by the proportion of visits to each acoustic perch. We used the same measures of learning speed and performance accuracy as our independent variables as the analyses described above. The overall regression equation was not significant, $R^2 = 0.54$, adjusted $R^2 = 0.27$, $F_{4,7} = 2.04$, $p = 0.19$.

Discussion

In the current study, we report the results from two different operant conditioning tasks examining chickadees' responses to conspecific songs that vary with singer dominance rank. The first task was a choice preference task that allowed us to examine active responses by birds (i.e., selection of songs) in the absence of food reinforcement. The second task was an instrumental learning task, during which birds were differentially reinforced with food for responding to certain songs. We also examined the relationship between individual variation in performance during the instrumental learning task and individual variation in acoustic preference. Overall, our results suggest sex differences in acoustic preference and learning speed, with females learning to discriminate among songs faster than males. We also found that performance accuracy varied depending on which category of songs was rewarded (i.e., when subordinate songs were rewarded birds achieved higher DRs) and we found evidence that a bird's acoustic preference correlates with its performance accuracy during the instrumental learning task.

Experiment 1: Choice preference task

In the current study, we tested chickadees with songs that were recorded from other geographic locations (i.e., chickadees originating in Alberta were tested with songs recorded in British Columbia and Ontario). There are discriminable geographic differences in the songs (Hahn et al., submitted), and there are also acoustic differences indicating a male's dominance rank that vary with geographic location (Hahn et al., 2013). Using a passive playback experimental design (i.e., birds are presented with different playback stimuli but do not actively choose what they hear) Hoeschele et al. (2010) found that female chickadees (originating from the same locations as the birds tested in the current study and tested with songs from one of the two locations used in the current study, i.e, the University of Northern British Columbia) vocalized more when presented with dominant male songs compared to subordinate male songs. The rationale for using a choice preference task in the current study is that the birds actively select which songs to listen to as opposed to measuring responses to song playback. Hoeschele et al. (2010) found that females responded differentially to songs produced by dominant and subordinate males; in the current study, we found that females preferentially listened to subordinate male songs from British Columbia greater than would be expected by chance. However, when tested with songs from Ontario, the responses by female subjects did not differ from chance. For male subjects, we found differing results, with male birds demonstrating no preference for dominant or subordinate British Columbia songs, but demonstrating a preference for dominant songs produced by birds from Ontario.

These results suggest that acoustic preference for dominant or subordinate songs varies with geographic location of the singer and the sex of the receiver. Dominance hierarchies form within a population of birds and acoustic variation exists in the songs produced by males from different geographic regions (Hahn et al., 2013), different habitat types (Grava, Grava, & Otter, 2012), and different habitat elevations (Branch & Pravosudov, 2015). Acoustic differences in songs are also evident when comparing males of similar socially dominant status; for example, Grava, Grava, & Otter (2013) found that songs produced by socially dominant males are perceived differently depending on the habitat-of-origin of the singer and the song receiver.

Further investigation is required to understand how acoustic variation in songs relates to dominance rank in the population of chickadees from which our subjects came (i.e., central Alberta) and this may provide an explanation as to why we see different acoustic preferences depending on the singer's location. For example, females from Alberta may have demonstrated a preference for subordinate songs from British Columbia because those songs were acoustically similar to local Alberta songs. In mountain chickadees (*Poecile gambeli*), some females show a preference for males from their same habitat elevation, supporting the idea that females have a preference for locally adapted males (Branch, Kozlovsky, & Pravosudov, 2015).

Experiment 2: Instrumental learning task

While choice preference tasks can examine what birds actively choose to listen to and may be an indicator of female mate choice (Holveck & Riebel, 2007), categorization tasks can be used to examine acoustic mechanisms that birds may employ when discriminating between signals (e.g., Bloomfield, Farrell, & Sturdy 2008a; Hahn et al., 2015). In experiment 2, we used a true category/pseudo category discrimination task to examine if birds treated dominant and subordinate songs as open-ended categories.

Group differences. One indication that animals are using open-ended categorization is to compare the number of trials required to complete discrimination training for birds in a true category group compared to birds in a pseudo category group, with birds in the former group completing the discrimination faster if in fact they are perceiving stimuli as open-ended categories. However, this predicted difference in learning speed assumes that there is a benefit (in terms of how long it takes to learn the task) for a bird to use open-ended categorization. In the current study, we found no difference in the number of trials to complete discrimination training for birds in the true category group compared to birds in a pseudo category group. It is possible that the acoustically simple structure of the songs result in all birds (regardless of group assignment) to perform similarly. In other words, regardless of whether birds are using categorization or rote memorization, both mechanisms are equally efficient in terms of how long it takes to learn the task (e.g., Hahn et al., 2015). Similar to this, it is possible that all birds were using rote memorization which would also result in no difference in how many trials to reach criterion between the groups.

While it is possible that using more song exemplars during discrimination training would allow us to tease apart the use of categorization versus rote memorization during discrimination training, previous studies using more song exemplars (i.e., 30 rewarded and 30 unrewarded songs in Hahn et al., 2015), found no difference in trials to criterion for chickadees discriminating *fee-bee* songs. However, a similar task using fewer *chick-a-dee* call stimuli during discrimination (i.e., 20 rewarded and 20 unrewarded calls in Bloomfield, Farrell, & Sturdy, 2008b) found a significant difference in the number of trials to reach criterion between true category and pseudo category groups. Considering this previous work with chickadees, it seems likely that the salience of the stimuli (i.e., a sexually-selected signal with simple acoustic structure) is influencing the responding during the task, rather than the number of exemplars used.

In line with the notion that the biological salience of the signal was influencing responses, we found that during discrimination training, birds rewarded for subordinate songs had higher DRs compared to birds rewarded for dominant songs. This indicates that birds in the subordinate song group were preforming more accurately across trials (i.e., responding to proportionally more of the rewarded songs compared to the unrewarded songs) compared to birds in the dominant song group. However, by the final day of discrimination, there was no difference in the DRs for birds in the dominant song and subordinate song groups, indicating that by the final day of training, all birds were preforming similarly. One aspect that requires further examination is uncovering why one category of songs (i.e., subordinate songs) led to greater performance accuracy compared to the other category (i.e., dominant songs), when the only difference between the two discriminations were which songs were rewarded. Previous studies examining the categorization of calls by chickadees found a similar result, with chickadees requiring more trials to reach criterion when heterospecific calls were the rewarded stimulus class and conspecific calls were the unrewarded stimulus class (Bloomfield et al., 2008b) or chickadees continuing to respond to conspecific calls even when they belong to the unrewarded category (Bloomfield, Sturdy, Phillmore, & Weisman, 2003). However, in the current study, both categories of songs were produced by conspecific males.

One explanation for the lower performance accuracy by birds in the dominant song group is the biological relevance of the signal. In this task, we required birds to fly to the feeder following a certain category of songs to obtain food; however, in the wild, dominant males have preferential access to a food source and will chase away or supplant lower ranking males at these food sources (Smith, 1991). It is possible that birds may have had more difficulty going to a food source following a signal that typically indicates that a bird risks being chased away from food, thus leading a bird to not enter the feeder as often following dominant songs. In line with this, we also found that males in the dominant song group required more trials to reach training criterion than males in the subordinate song group. In a similar go/no-go discrimination task, Bloomfield et al. (2003) found that chickadees had more difficulty inhibiting their response (i.e., not flying to the feeder) following conspecific *chick-a-dee* calls, a signal that is used in the wild to indicate a food source (Ficken, 1981).

Sex differences. Although there was no difference in the responding between the true category and pseudo category groups, we did find that females in the pseudo category group reached criterion faster compared to males. We also found that overall females reached criterion faster compared to males; however, this difference appears to be mostly driven by males in the dominant song group and pseudo category group (see Figure 3-4). Go/no-go discrimination tasks using *chick-a-dee* calls as stimuli have reported no difference in responses when comparing male and female subjects (e.g., Bloomfield et al., 2008a; Charrier, Lee, Bloomfield, & Sturdy, 2005; Guillette, Redden, Hurd, & Sturdy, 2009). However, go/no-go discrimination tasks using *fee-bee* songs as stimuli (like in the current study) have found differences in responding by male and female subjects (e.g., female chickadees discriminate more accurately compared to males by responding to more rewarded songs, Hahn et al., 2015, and attaining higher DRs; Hoeschele et al., 2012).

Songs are signals used in intersexual and intrasexual communication; in species in which the same song types function in both types of communication, there may be differing levels of costs or benefits associated with responding to certain songs that vary with the sex of the receiver. For example, male songs are a signal for intersexual attraction and females may choose high-quality mates in order to gain direct (e.g., better nesting territory; more parental care) or indirect (e.g., better genetic fitness for young) benefits. For females, mating with a male that is less-than-ideal is a costly mistake (e.g., she may not gain direct or indirect benefits). In comparison, male songs are also used for intrasexual competition, but if a male makes a territorial response to another male who was not a threat, the costs are relatively low (e.g., wasted time or energy responding to a non-rival; Ratcliffe & Otter, 1996). If the costs associated with mistakenly responding to a song are higher for females, females should be more expert discriminators. The results from the current study support this idea; females may have discriminated faster because they are more expert at distinguishing between male signals that vary with male quality or dominance rank.

Acoustic mechanisms. Another indication that animals are using open-ended categorization is examining how birds respond to novel song exemplars; if birds continue to respond to novel song exemplars based on the contingencies from training, it is a strong indicator that birds were using open-ended categorization.

Birds did not demonstrate generalization during transfer; however, the lack of generalization may have been due to the small number of transfer stimuli (i.e., five dominant songs and three subordinate songs). In general, chickadees respond less to novel songs presented in generalization (Bloomfield et al., 2008a, Hahn et al., 2015); in the current study, even if the birds were discriminating acoustic variation in songs this responding may not generalize without a larger sample of novel song exemplars. However, when presented with songs from Ontario, birds in both true category groups continued to respond based on the contingencies during discrimination training (see Figure 3-7). This result suggests that there is an acoustic cue within the songs that birds were attending to that is similar among males of the same dominance rank across the two geographic locations. This finding supports the use of categorization, because birds continued to respond to these novel songs based on the singer's dominance status. However, more work is needed to determine the specific acoustic feature(s) that birds used during the discrimination. Dominance-based categorization was not evident when birds were tested on songs from John Prince Research Forest, suggesting that acoustic similarity within the songs produced by males of similar rank may only persist across some geographic locations.

Previous bioacoustic analyses and playback studies suggest that the relative amplitude varies between dominant and subordinate male songs, indicating that this acoustic feature may be used when distinguishing a male's dominance rank (Hahn et al., 2013; Hoeschele et al., 2010). In the current study, we tested this feature as a possible acoustic mechanism by manipulating this song feature in dominant and subordinate songs. Overall, birds responded significantly less to all manipulated test songs compared to the rewarded training songs, suggesting that the rewarded songs from discrimination training achieved a high degree of stimulus control over the birds' responding. Two results from the manipulated test session are worth noting: (1) Birds in the dominant song group responded significantly more to dominant songs with increased relative amplitude (compared to the unrewarded training songs), while there was no difference in the proportion of response by birds in this group for songs with a decreased relative amplitude. (2) Birds in the subordinate song group responded significantly more to subordinate songs with decreased relative amplitude (compared to the unrewarded training songs); however, birds in this group also responded significantly more to subordinate songs with increased relative amplitude, suggesting that birds were responding to other acoustic features, either in combination with relative amplitude or instead of relative amplitude. Birds generalized their responding to songs from Ontario, but did not respond to songs with a manipulated relative amplitude in a way that would be predicted based solely on variations in relative amplitude between dominant and subordinate songs. These results suggest that birds were using other acoustic features within the song when discriminating.

Relationship between choice preference and instrumental learning

An individual's traits can influence acoustic preference, such as a bird's sex (e.g., Gentner & Hulse, 2000) and early experience (e.g., Riebel, 2000; Farrell, Neuert, Cui, & MacDougall-Shackleton, 2015) and acoustic preference remains consistent across different tests (e.g., Holveck & Riebel, 2007; Riebel, 2000). Acoustic preference can also be related to the perceiver's quality. Holveck and Riebel (2010) found that low-quality female zebra finches preferred low-quality males, and when both members of a mated pair were of similar quality (i.e., the male and female were both low-quality or both high-quality) the latency until the first egg was laid was shorter compared to mated pairs that were of differing quality. This suggests that there are fitness benefits to finding a mate of similar quality, which would lead to individual variation in mate preference.

In black-capped chickadees, individual variation in exploration style correlates with learning speed and performance accuracy in an instrumental discrimination task (Guillette, Hahn, Hoeschele, Przyslupski, & Sturdy, 2015; Guillette, Reddon, Hoeschele, & Sturdy, 2011; Guillette, et al., 2009). In the current study, we found that a measure of performance accuracy, specifically, the DR during the first bin of pretesting, was a significant predictor of a bird's preference strength during the choice preference task. Pretesting is the first stage in which the reinforcement probability of the rewarded songs is reduced. The current study provides evidence that a bird's preference strength for a category of songs was inversely related to its performance accuracy once this change in reinforcement probability was introduced. This suggests that for birds that were being food reinforced for songs that they preferred, when the preferred songs were presented with a lower probability of reinforcement (i.e., rewarded songs are reinforced on 85% of trials), they start responding with more variability (i.e., start responding to rewarded and unrewarded songs at a proportionally similar level). Variability in responding occurs after extinction is implemented (Neuringer, 2002) and when the probability of reward decreases (Gharib, Gade, & Roberts, 2004). In the current design, when the probability of reinforcement is decreased, the 15% of rewarded song trials that do not result in reward are presented under extinction (i.e., the response is made but no reinforcement is provided). Once these extinction trials are experienced, birds may start responding more variably by responding to all songs; however, this effect of variability in responding depends on the bird's preference strength for the rewarded song category. This suggest that a bird's underlying preference for an acoustic signal can impact its performance during an instrumental learning task, especially when the stimuli used during the instrumental learning task are biological salient vocalizations, such as conspecific songs.

In other Parids, exploration style correlates with dominance status measured in captive individuals (mountain chickadees, Fox, Ladage, Roth, & Pravosudov, 2009; great tits, *Parus major*, Verbeek, Boon, & Drent, 1996; Verbeek, de Goede, Drent, & Wiepkema, 1999) and wild populations (great tits, Dingemanse & de Goede, 2004). Taken together, previous studies examining individual differences in dominance status, acoustic preference, learning speed and performance accuracy suggest that there may be a complex relationship between all of these measures that may affect different cognitive aspects of the individual, including how they respond during operant tasks similar to the tasks used in the current study. We did not know the relative dominance rank of each subject; however, each subject's relative dominance rank may have influenced their acoustic preference and their performance during the instrumental learning task. For example, a low-ranking male tested with songs produced by high-ranking and low-ranking males (similar to the current study) may preform differently compared to a high-ranking male tested with the same stimuli. The possible effects of individual characteristics should be considered in future tasks examining the perception of biologically relevant signals.

Conclusions

In the current study, we used two operant conditioning tasks to examine acoustic preference, ability to discriminate, and discrimination mechanisms of conspecific songs that varied with male dominance rank. Our results from the acoustic preference task suggest that preference for dominant or subordinate songs varies depending on the singer's geographic origin and varies depending on the sex of the receiver. Results from the test sessions during the instrumental learning task suggest that songs may belong to open-ended categories based on dominance rank; however, results from discrimination training did not demonstrate a benefit of using open-ended categorization over rote memorization, possibly due to the salience of the signal. In examining the relationship between the two tasks, we found that a bird's acoustic preference correlates with its performance accuracy when the reinforcement probability is decreased. Given the increasing number of studies reporting individual differences in various cognitive processes, including, acoustic preferences, learning speed, and performance accuracy, the results of the current study suggest that the relationship between all three of these measures need careful consideration in future work.

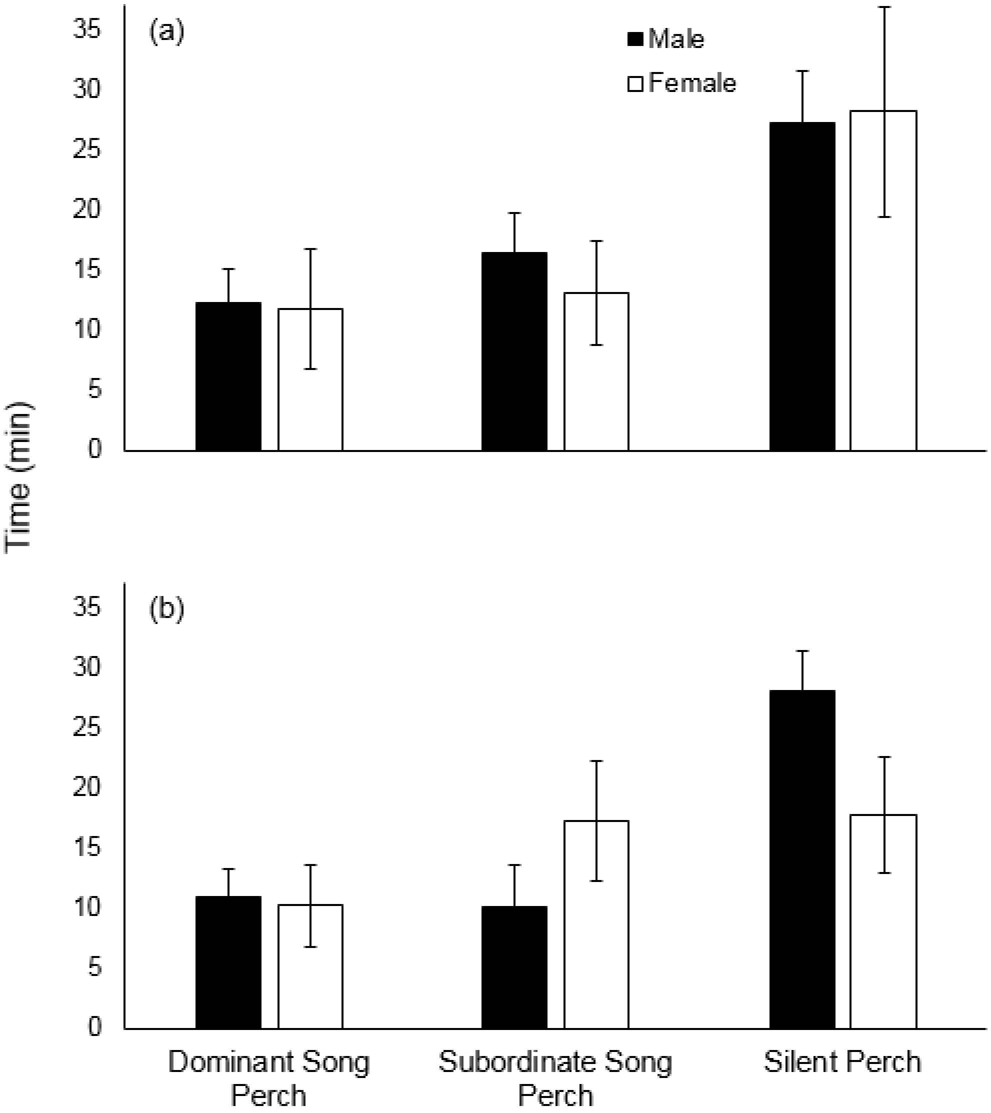

Figure 3-1. The average ± SE amount of time spent on each perch by male (black bars) and female (white bars) black-capped chickadees for (a) British Columbia songs and (b) Ontario songs during Experiment 1.

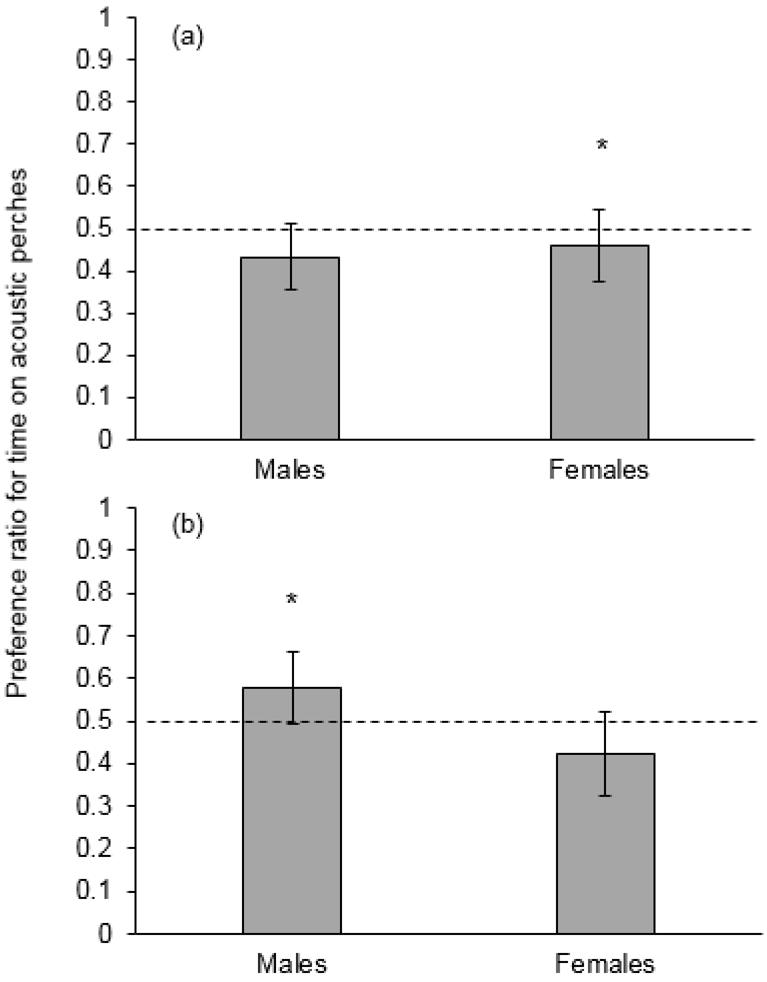

Figure 3-2. Preference ratios for (a) British Columbia songs and (b) Ontario songs. Preference ratios were calculated by taking the time spent on the dominant song perch divided by the total amount of time on both acoustic perches. (*) indicates that the average preference ratio for that group was significantly different from chance ($p \leq 0.05$). Error bars represent standard error of the mean.

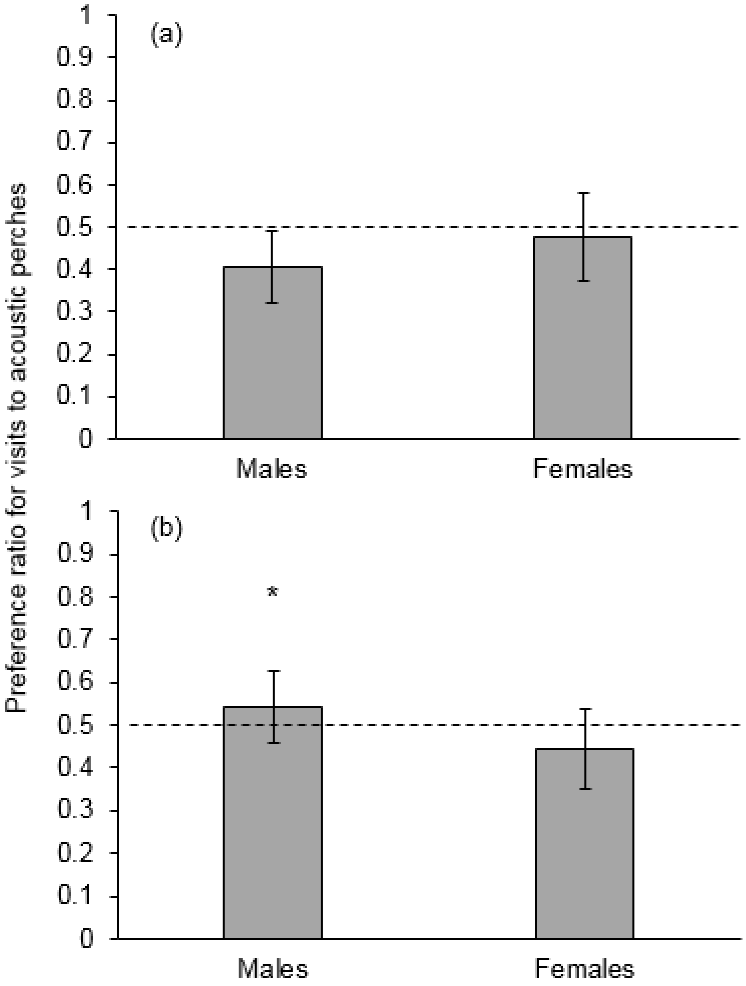

Figure 3-3. Preference ratios for (a) British Columbia songs and (b) Ontario songs. Preference ratios were calculated by taking the number of visits to the dominant song perch divided by the total number of visits to both acoustic perches. (*) indicates that the average preference ratio for that group was significantly different from chance ($p \leq 0.05$). Error bars represent standard error of the mean.

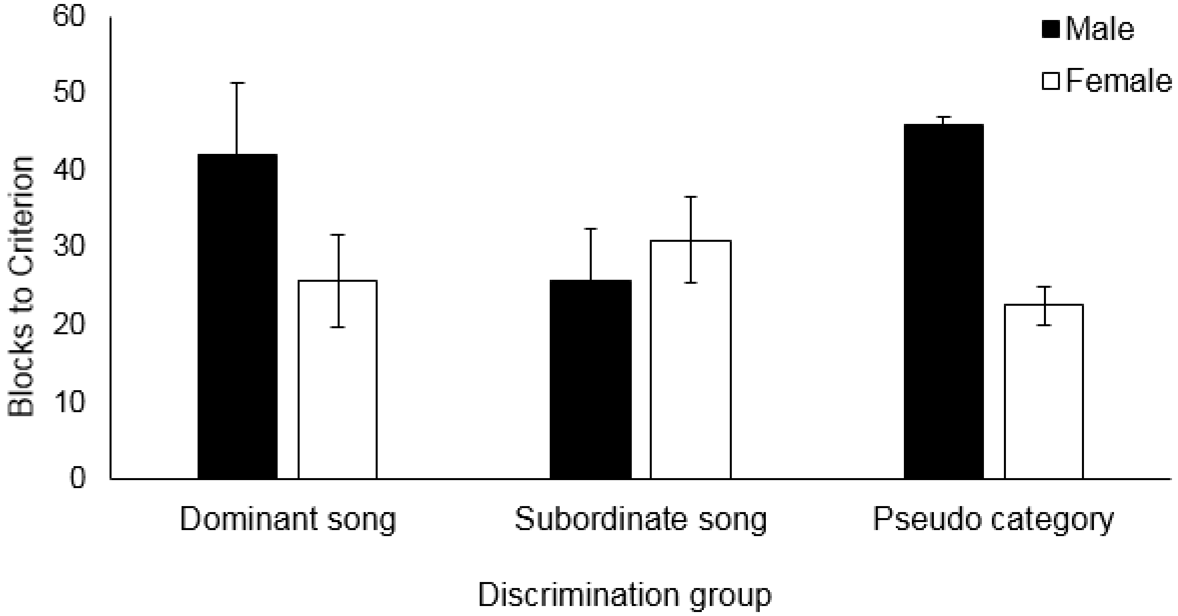

Figure 3-4. The average ± SE number of 200-trial blocks to complete discrimination training for male (black bars) and female (white bars) subjects in each discrimination group.

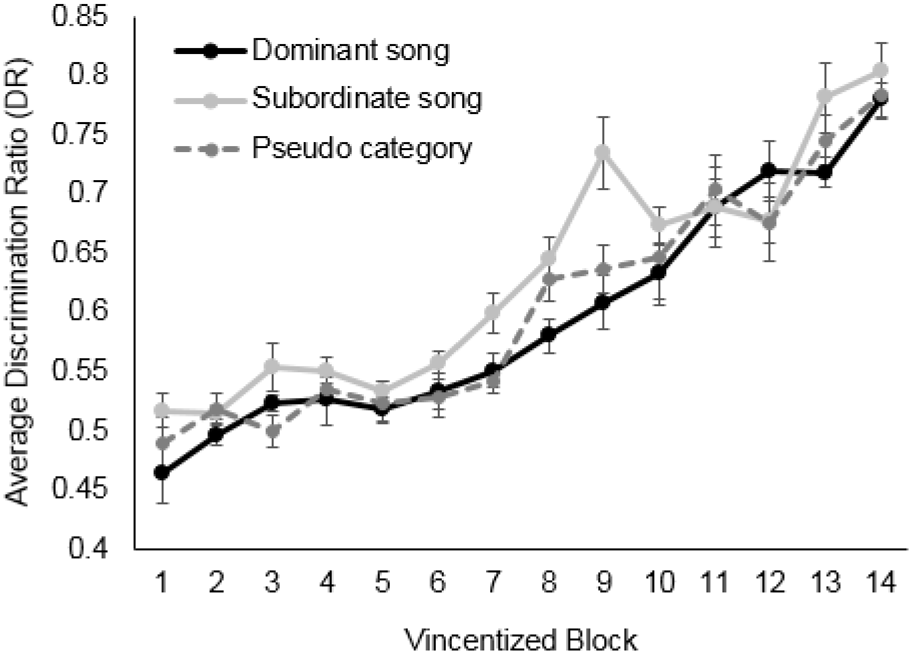

Figure 3-5. Vincentized acquisition curves for birds in each group during discrimination training. Error bars represent standard error of the mean.

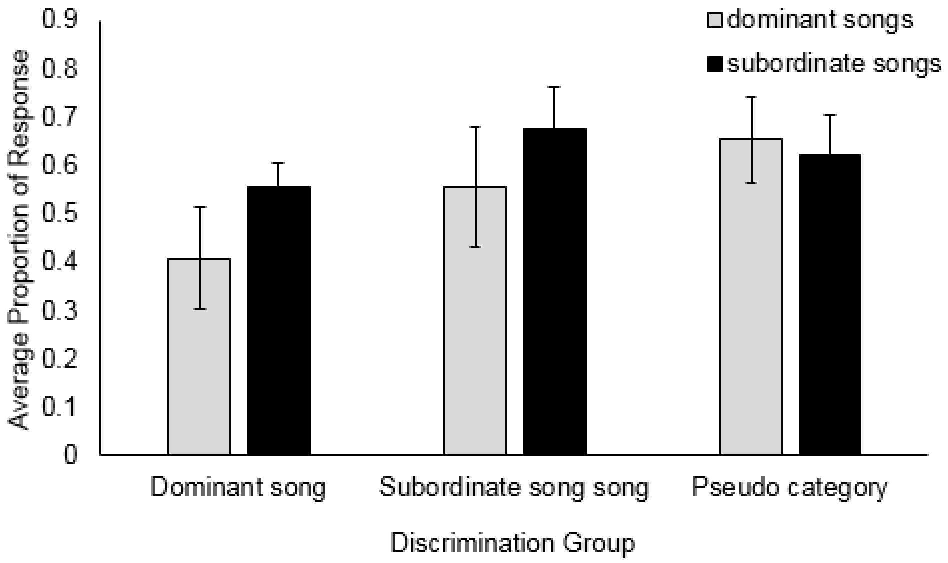

Figure 3-6. Average ± SE proportion of response by birds in each discrimination group to songs produced by dominant (gray bars) and subordinate (black bars) males from the University of Northern British Columbia.

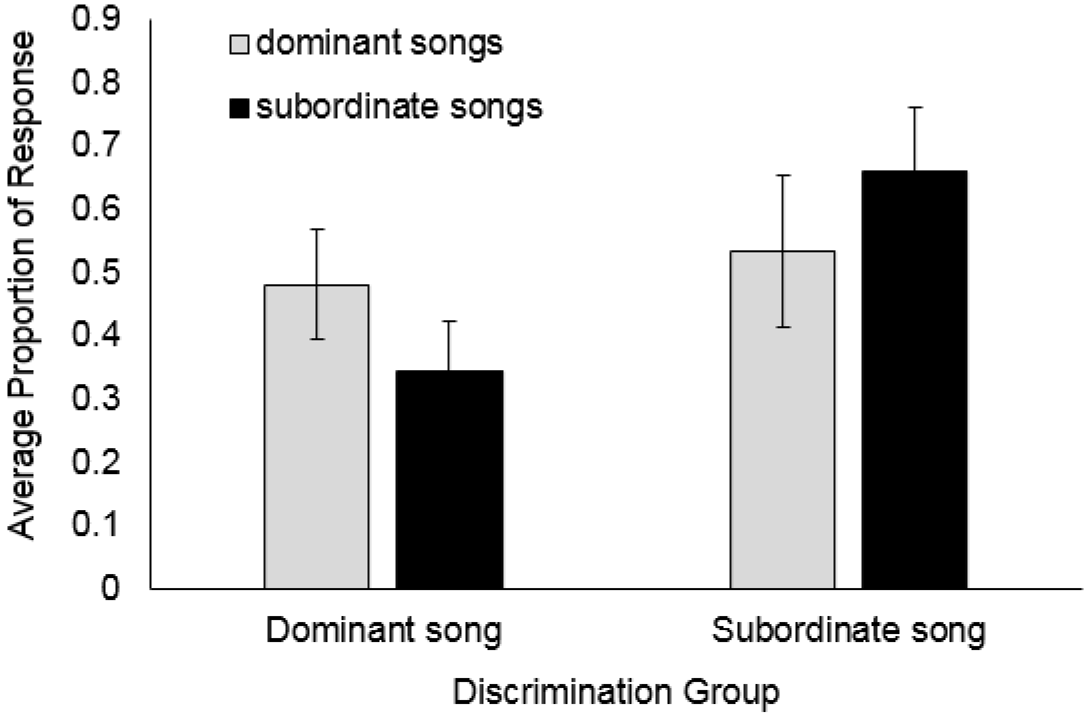

Figure 3-7. Average ± SE proportion of response by birds in each discrimination group to songs produced by dominant (gray bars) and subordinate (black bars) males from Ontario.

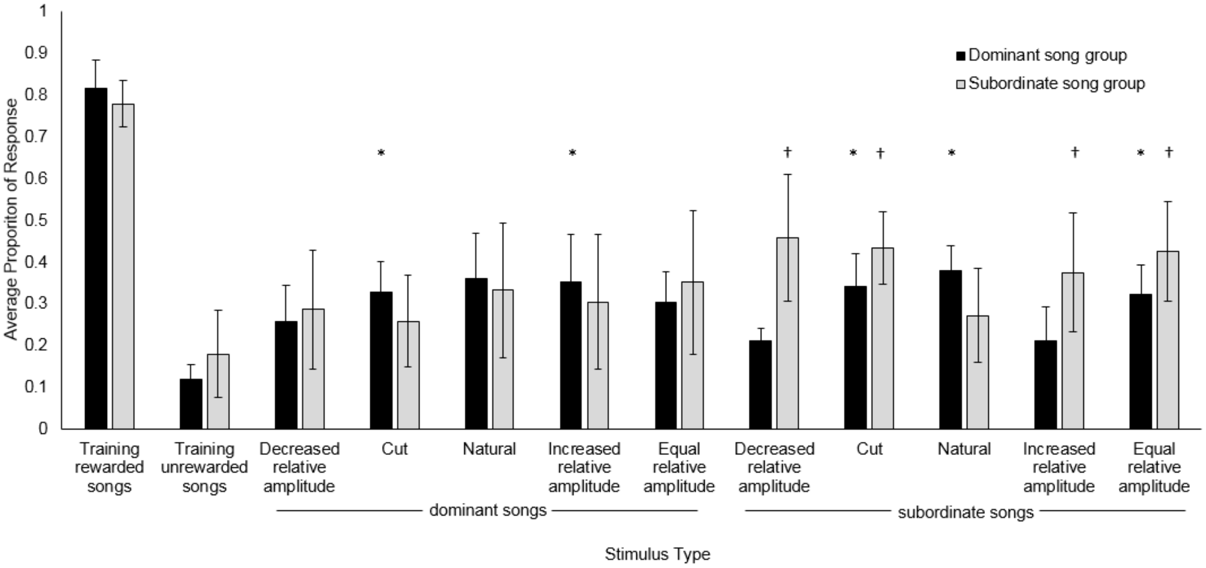

Figure 3-8. Mean ± SE proportion of response to manipulated test stimuli. Black bars represent the responding by birds in the dominant song discrimination group and gray bars represent the responding by birds in the subordinate songs group. Test stimuli were presented in five ways (relative amplitude decreased, song note cut apart and put back together with no manipulation, natural (unmanipulated) songs, relative amplitude increased, and relative amplitude made equal). (*) indicates a significant difference ($p \leq 0.05$) compared to the training unrewarded songs for birds in the dominant song discrimination group. (†) indicates a significant difference ($p \leq 0.05$) compared to the training unrewarded songs for birds in the subordinate song discrimination group. All test stimuli were responded to significantly less compared to the training rewarded songs ($p \leq 0.05$; see text for details).

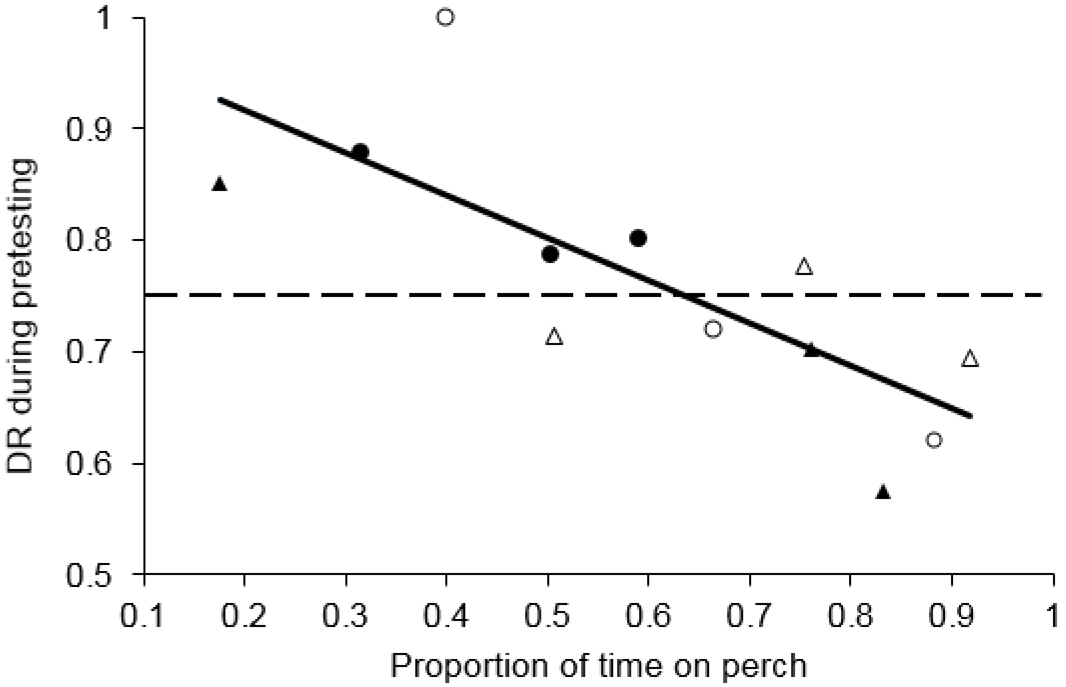

Figure 3-9. Proportion of time spent on the perch associated with the rewarded category (x-axis) and the discrimination ratio (DR) during the first 200-trial bin of pretesting (y-axis). Filled shapes represent male subjects and open shapes represent female subjects. Circles represent subjects in the dominant song group and triangles represent individuals in the subordinate song group. The dashed line represents the DR (i.e., 0.75) that birds were required to reach during pretesting.

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Chapter 4 Black-capped chickadees categorize songs based on geographic information

Introduction

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

When variations in vocal signals exist, animals may benefit by the ability to detect the acoustic differences in vocalizations. In fact, the results of playback studies suggest that birds can perceive geographic variation in acoustic signals. Males have stronger behavioural responses (e.g., approach and singing) to local songs compared to songs produced by males from more distant populations (e.g., white-crowned sparrow, Milligan & 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, exhibit preferences for male songs based on geographic information (e.g., white-crowed sparrow, Baker, Spitler-Nabors, & Bradley, 1981; rufous-collared sparrow, *Zonotrichia capensis,* Danner et al., 2011). In order for songbirds to respond differently to different vocal signals, there must be perceptible acoustic variation within the vocalizations being compared. If there are perceptual differences, there are several mechanisms the birds could employ to discriminate the vocalizations.

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

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

In contrast to the geographic variation that is found in the songs produced by other songbird species (e.g., swamp sparrow, *Melospiza georgiana,* Marler & Pickert, 1984 song sparrows, *M. 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, 2013b) 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 geographic variation has been described in this song (although, variation has been found in geographically-isolated populations of chickadees, where some animals produce unusual songs; see Gammon & Baker, 2004; Kroodsma et al. 1999). Recently, we described subtle variation within *fee-bee* songs produced by black-capped chickadees from different populations (Hahn et al. 2013a); 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, Mennill, & Ratcliffe, 2004b;

variation in the consistency of the relative amplitude in songs from British Columbia, Hahn et al. 2013a; Hoeschele et al. 2010).

In the current study, we used an operant go/no-go task to address two questions: (1) Do black-capped chickadees perceive acoustic differences and categorize songs based on geographic location? (2) If so, what are the acoustic mechanism(s) that chickadees use to perform this discrimination? To compare chickadees' abilities using open-ended categorization versus rote memorization, we employed a true category/pseudo category paradigm (similar to Bloomfield, 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 geographic locations (i.e., British Columbia or Ontario). If songs produced by birds within each geographic region are perceptually similar to one another, birds could learn a general category rule (e.g., respond to any song from British Columbia) and use this category rule when making a response. Birds in the pseudo category group discriminated between the same songs as the true category group, but the songs were assigned to random "categories." In other words, responses made to half of the British Columbia and half of the Ontario songs were reinforced, while the other half were non-reinforced. Therefore birds in the pseudo category group could not use a category rule when responding, and had to rely on rote memorization to remember each reinforced and non-reinforced song. Following discrimination training, we presented all birds with novel songs from each location. If birds in the true category group continued to respond to novel songs based on the initial training contingencies (i.e., based on geographic location), this would be further evidence that birds were using open-ended categorization. In contrast, we expected birds in the pseudo category discrimination group to respond non-differentially to the novel songs because birds in this group will not have learned to respond based on a category rule during discrimination.

In Experiments 2 and 3, we presented birds with songs that we experimentally manipulated to understand the specific acoustic mechanisms that birds used when performing these discriminations. Specifically, we wanted to examine whether birds used the overall song duration (i.e., the acoustic feature that shows the most variation between these populations; Hahn et al. 2013a) as a cue when discriminating. We also examined if birds used acoustic features within either the first (i.e., *fee*) or second (i.e., *bee*) note to discriminate between British Columbia and Ontario songs, by presenting birds in the true category groups with songs that we edited to include one note from each of the two locations, or single notes (*fee* only or *bee* only) presented by themselves. Black-capped chickadees occasionally produce single *fee* notes, but rarely produce *bee* notes by themselves. However, by testing both single *fee* notes and single *bee* notes, we could examine whether birds are attending to one of the two notes more than the other when discriminating. For example, birds may use information within the *fee* note when discriminating because this note contains acoustic features that they hear when a song is first initiated. In contrast, birds may use information with the *bee* note when discriminating because information within this note is more recent in memory. These manipulations of acoustic features and structure allowed us to examine if birds used certain features within the song to discriminate between the geographic locations.

Experiment 1

Methods

Subjects

Eleven black-capped chickadees (six males and five females, identified by DNA analysis; Griffiths, Double, Orr, & Dawson, 1998) were captured in Edmonton (North Saskatchewan River Valley, 53.53˚N, 113.53˚W; Mill Creek Ravine, 53.52˚N, 113.47˚W) or Stony Plain (53.46˚N, 114.01˚W), Alberta, Canada. At time of capture, birds were identified as at least one year of age by plumage (Pyle, 1997). 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.

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

Apparatus

Birds were tested between October 2011 and February 2012. 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 cm × 16 cm) on one side of the cage. The position of the bird was monitored via infrared beams in the feeder and the perch closest to the feeder (i.e., request perch; see Sturdy & 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, England) or an NAD310 Integrated Amplifier (NAD Electronics, London, England) and a speaker to the side of the feeder (Fostex FE108 Σ or Fostex FE108E Σ full-range speaker; Fostex Corp., Japan; frequency response range 80-18,000 Hz). In each cage there were three perches, a water bottle, cuttlebone, and grit cup. During testing, birds were kept on the natural light cycle and were provided one superworm twice a day; however, during the operant discrimination task, Mazuri was only available as a reward for correct responding.

Acoustic stimuli

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 (see Hahn et al. 2013a for details regarding song recordings). Songs were of high quality (i.e., no audible interference and low background noise when viewed on a spectrogram with amplitude cutoffs of -35 to 0 dB relative to song peak amplitude). Stimuli were resampled from 22,050 to 44,100 Hz using SIGNAL 5.10.29 software (Engineering Design, Berkeley, CA, USA) and bandpass filtered outside the range of the songs to remove background noise using GoldWave version 5.58 (GoldWave, Inc., St. John's, NL, Canada). Using SIGNAL, songs were edited from longer audio files to contain 5 ms before and after each song, the stimuli were tapered to remove transients, and amplitude was equalized. During the experiment, stimuli were presented at ~75 dB as measured by a Radio Shack Sound Level Meter (Radio Shack, Fort Worth, TX, USA) or Brüel & Kjær Type 2239 (Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark; A weighting, slow response) at the approximate height and position of a bird's head when on the request perch. Only one song produced by a given individual was used as a stimulus during Experiment 1.

Procedure

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

Discrimination training. During this phase the procedure from Pretraining was maintained; however, only ten British Columbia songs and ten Ontario songs were presented. Half of the songs were now assigned to be S+ and half as S-. 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 ITI with the houselight off. Responses to S+ songs resulted in 1 s access to food. *Discrimination training* continued until birds completed three 200-trial blocks with a discrimination ratio (DR) ≥ 0.75 with the last two blocks being consecutive. See Response measures for DR calculations.

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

Transfer training. This phase was identical to Discrimination training, except that an additional ten British Columbia songs and ten Ontario songs were presented. Responses to these transfer songs continued to be reinforced based on the same contingencies as in Discrimination training (i.e., based on geographic location or pseudorandomized). *Transfer training* continued until birds completed three 200-trial blocks with a DR ≥ 0.75 with the last two blocks being consecutive. One bird (a male in pseudo category group) died during this phase; in our analysis we examined responding during the first block of Transfer training, so we included this bird in the analysis.

Response measures

To determine how well birds were discriminating S+ from S- stimuli, we calculated a discrimination ratio (DR). First, we calculated the percent response for each stimulus exemplar using the following formula: $R+/(N-I) \times 100$, where R+ is the number of trials that the bird visited the feeder, N is the total number of trials, and I is the number of trials that the bird left the perch before the song finished playing (i.e., interrupted trials). The DR was calculated by dividing the average percent response to S+ stimuli by the average percent response to all 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.

Statistical analyses

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

We conducted parallel analyses using the arcsine square root transformed (to correct for non-normality) proportion of response using the following formula: arcsin[sqrt(x)], where x equals the untransformed proportion of responding. In most instances, the transformed data produced the same pattern of results, so we only report the untransformed data below. We specify any differences between the analyses of the transformed and untransformed data. All statistics were conducted in Statistica v12 (StatSoft, Inc).

Ethical note

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

Results

Trials to criterion

Data from three birds (one female in the Ontario S+ group and two males in the pseudo category group) had to be removed from the analysis due to equipment failure during Discrimination training, resulting in data from seven subjects ($N = 3$ for British Columbia S+ group; $N = 2$ for Ontario S+ group; $N = 3$ for pseudo group). Independent samples *t*-tests on the number of 200-trial blocks to complete Discrimination training revealed no significant difference between the two true category (i.e., British Columbia S+; Ontario S+) groups ($t = 3.00$, $P = 0.058$), but found a significant difference between the true and pseudo category groups ($t = 7.36$, $P = 0.0003$), with the pseudo category group requiring more than twice as many trial blocks to complete the discrimination (average number of trial blocks = 8.0 and 19.33, for true and pseudo category groups, respectively). See Figure 4-1.

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 (Training S+ songs, Training S- songs, Testing S+ songs, Testing 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 Testing song). For this analysis, the Training S+ and Training S- songs refer to the rewarded and unrewarded (respectively) songs presented during Discrimination training and the Testing S+ and Testing 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 ($F_{3,21} = 191.32$, $P < 0.001$). There was also a significant Stimulus Type × Discrimination Group interaction ($F_{6,21} = 19.00$, $P < 0.001$). When we conducted the analyses on the arcsine square root transformed data the main effect of Discrimination group was also significant ($F_{2,7} = 5.49$, $P = 0.037$). We conducted planned comparisons to assess the significant interaction. For each group, we compared the Training S+ songs to the Training S- songs and the Testing S+ songs to the Testing S- songs.

Birds in all groups responded significantly more to the Training S+ songs compared to the Training S- songs (British Columbia S+ group, $P < 0.001$; Ontario S+ group, $P = 0.007$; Pseudo category group, $P = 0.013$). Birds in the true category groups responded significantly more to the Testing S+ songs compared to the Testing S- songs (British Columbia S+ group, $P < 0.001$; Ontario S+ group, $P < 0.001$), while there was no significant difference in the response by birds in the pseudo category group ($P = 0.388$). See Figure 4-2.

Experiment 2

Results from Experiment 1 suggested that birds could discriminate between songs produced by birds from different geographic locations. Because birds in the true category group continued to respond to the novel songs presented during Transfer training based on the contingencies from Discrimination training, it suggests that birds in the true category group were not simply memorizing individual songs in order to complete the discrimination. In Experiment 2, we tested a new group of black-capped chickadees on geographically-based song discrimination using stimuli from the same two locations as Experiment 1. However, in Experiment 2 we made three important changes from Experiment 1: (1) we included two pseudo category groups (S+ songs for one group were the S- songs for the second group, and vice versa), (2) we presented novel songs without reinforcement to the true category and pseudo category groups, and (3) we included songs that we experimentally manipulated in order to examine the perceptual mechanisms for the discrimination (true category group only).

Methods

Subjects

Sixteen black-capped chickadees (eight male, eight female) were tested between July 2012 and January 2013. Birds were captured in the same locations as Experiment 1 as well as Kananaskis Country (51.02˚N, 115.03˚W), Alberta, Canada. 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. The conditions prior to the experiment and the apparatus remained the same as Experiment 1.

Acoustic stimuli

Natural stimuli. Ten British Columbia songs and ten Ontario songs were used as stimuli during Discrimination training. An additional seven songs from each location were used as natural stimuli during Generalization and Transfer training. Ten songs recorded at John Prince Research Forest, British Columbia were used as natural, unmanipulated stimuli during test sessions. Only one song recorded from a given individual was used during Discrimination training and Generalization. Songs were prepared and presented in the same way as in Experiment 1. Songs manipulated and used during additional test sessions were further prepared as described below.

Spliced songs. We created spliced song stimuli to test if birds were preferentially using information in one of the two notes to discriminate songs produced by birds from the two geographic locations. Songs (*N* = 16) were bandpass filtered in Goldwave and the *fee* and *bee* notes from eight British Columbia and eight Ontario songs were edited into individual WAV files using SIGNAL. Four types of spliced songs were created: British Columbia-British Columbia (*fee* and *bee* notes from two songs from British Columbia), Ontario-Ontario (*fee* and *bee* notes from two songs from Ontario), British Columbia-Ontario (*fee* note from a British Columbia song and *bee* note from an Ontario song), and Ontario-British Columbia (*fee* note from an Ontario song and *bee* note from a British Columbia song). When creating the spliced songs, we made the internote interval constant for all stimuli at 100 ms, which is similar to the internote interval in natural songs (e.g., $X_{\text{internote}} = 135$ ms, Ficken et al. 1978) and the internote interval used by other studies manipulating song features (e.g., $X_{\text{internote}} = 100$ ms, Hahn et al., 2015; Hoeschele, Guillette, & Sturdy, 2012). Because songs are sung over a range of absolute frequencies (Weisman & Ratcliffe, 1989; Weisman et al., 1990; Horn et al., 1992; Mennill & Otter, 2007) and we wanted to ensure we were not creating songs with species-atypical internote interval ratios which may affect responding (see Hoeschele et al., 2012), we changed the start frequency of the *fee* note to match the start frequency of the *fee* note that was being replaced in the song using Audacity 1.3.12.

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

Ontario songs unmanipulated, and British Columbia and Ontario songs with a total duration increased.

Procedure

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

Discrimination training. The procedure and criterion for Discrimination training remained the same as Experiment 1. As in Experiment 1, birds were randomly assigned to either a true category discrimination group (*N* = 12; British Columbia S+ Group: three males, three females; Ontario S+ Group: three males, three females) or pseudo category discrimination group (*N* = 4; two males, two females). Birds in the pseudo category discrimination group were divided into two subgroups (one male and one female in each subgroup). Each subgroup discriminated five randomly-selected S+ British Columbia songs and five randomly-selected S+ Ontario songs from five different S- British Columbia songs and five different S- Ontario songs. The S+ songs for one subgroup were the S- songs for the other subgroup, and vice-versa.

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

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

Transfer training. Following Generalization, all birds in the true category groups (British Columbia S+ and Ontario S+) continued onto Transfer training. During this phase, the 14 songs used during Generalization were now included as S+ or S- stimuli (contingencies based on their location of origin). Each stimulus was 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., 34 songs from Transfer training were each presented six times), in addition, two or three John Prince Research Forest songs, four spliced songs, and six total duration manipulated songs were each presented once, resulting in a 216- or 217-trial block. For each test session, a minimum of three trial blocks were completed and these were included in the analysis. After each test session, birds completed one block of Transfer training with a DR ≥

0.75 before moving onto the next test session. The order of the test sessions was pseudorandomized between discrimination group and sex. An individual song was manipulated in multiple ways (i.e., duration increased, duration decreased, and unmanipulated); however, only one of these manipulations was included in a single test session, 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. We also calculated a modified two-tailed 95% confidence interval (CI) using the mean (*M*) and standard deviation (SD) of the percent response for all S- stimuli on the final day of Discrimination training, using the following formula: CI = $M \pm 1.96 \times \text{SD}$. If birds significantly discriminated a particular S+ stimulus from the S- stimuli, the percent response to that S+ stimulus would be greater than the 95% CI.

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

Statistical analyses

We conducted an ANOVA on the number of trials to criterion to determine if birds in the two true category groups differed in their speed of acquisition. We also conducted similar analyses to determine if birds in the true and pseudo category groups differed in their speed of acquisition. In order to compare acquisition during Discrimination training across birds, we

Vincentized the acquisition curves to 13 blocks of data (the minimum number of blocks it took a bird to reach criterion) in order to compare across birds. We calculated the Vincentized blocks by taking the total number of trial blocks it took an individual bird to reach criterion (e.g., 30) and dividing by 13 (e.g., 2 with a remainder of 4). We computed the Vincentized blocks by putting the number of actual trial blocks that a bird did into groups the size of the whole-number quotient (in this example, 26 of the actual trial blocks were placed into 13 Vincentized blocks- each Vincentized block contained 2 actual trial blocks). The 7th Vincentized block would contain additional trial blocks based on how large the remainder was (in this example, the remainder equaled 4, so the 7th Vincentized block would contain 2 + 4 = 6 actual trial blocks). The DRs for all blocks grouped into a Vincentized block were averaged (see Kling & Riggs, 1971; Hahn et al. 2015; Hoeschele et al. 2012).

We also examined the number of S+ stimuli that were responded to ≥ 95% CI in order to determine if the number of stimulus exemplars responded to varied among groups. We conducted additional ANOVA and Tukey's planned comparisons on the proportion of response to the test stimuli using both the untransformed and arcsine square root transformed data. In most instances, analysis of untransformed and transformed data yielded a similar pattern of results and levels of significance, so we only report the untransformed data. We specify any differences between the transformed and untransformed data. All statistics were conducted in Statistica v12 (StatSoft, Inc).

Results

Trials to criterion

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

A Sex × 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 (Fs ≤ 1.22, Ps ≥ 0.31), indicating that there was no significant difference in the number of trials to reach criterion for birds rewarded for responding to British Columbia versus Ontario songs.

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

We conducted a Sex × Discrimination Group (True, Pseudo) ANOVA on the number of 200-trial blocks to complete Discrimination training. There were no significant main effects or interactions (Fs ≤ 0.685, Ps ≥ 0.43), indicating no significant difference in discrimination performance between the true and pseudo category groups. See Figure 4-1.

Acquisition

To examine task acquisition across blocks, we conducted repeated measures ANOVA with Sex and Discrimination Group (British Columbia S+, Ontario S+, Pseudo) as categorical predictors and the DR for each Vincentized block (1-13) as dependent variables. There was a significant main effect for Discrimination Group ($F_{2,8} = 5.5$, $P = 0.032$). To examine this significant main effect, we conducted a Tukey's post hoc test. Birds in the British Columbia S+ group had significantly higher DRs compared to birds in the Ontario S+ group ($P = 0.032$) and the pseudo category group ($P = 0.022$; see Figure 4-3). There was also a significant main effect for Vincentized blocks ($F_{12, 96} = 67.4$, $P < 0.001$; as Vincentized blocks increased, the DRs increased. There were no other significant main effects or interactions (Fs ≤ 1.6, Ps ≥ 0.052).

Number of S+ songs responded to ≥ 95% confidence interval (CI)

Birds could obtain a high DR by responding to a few S+ stimuli, while responding less to all S- and the remaining S+ stimuli. We examined how many of the S+ stimuli were well-discriminated by determining how many S+ stimuli were responded to ≥ 95% CI during the final block of Discrimination training. We conducted a Sex × Discrimination Group (British Columbia S+, Ontario S+, Pseudo) ANOVA to determine if the true category and pseudo category groups differed in the number of exemplars responded to greater than the 95% CI. There were no significant main effects or interactions (Fs ≤ 0.45, Ps ≥ 0.65). Out of 10 S+ stimuli, the means ± SE for the number of stimuli responded to greater than the 95% CI were: British Columbia S+ group: 8 ± 2.0 and 8.67 ± 0.88, for male and female subjects, respectively; Ontario S+ group: 8 ± 1.0 and 8 ± 1.15, for male and female subjects, respectively; and pseudo category group were: 9 ± 0 and 9.5 ± 0.5, for male and female subjects, respectively.

Generalization

We conducted a Sex × Discrimination Group (British Columbia S+, Ontario S+, Pseudo) × 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 ($F_{1,10} = 5.24$, $P = 0.045$), a significant Discrimination Group × Stimulus Type interaction ($F_{2,10} = 33.81$, $P < 0.001$), and a significant Sex × Discrimination Group × Stimulus Type interaction ($F_{2,10} = 7.29$, $P = 0.011$). 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 ($t = 3.24$, $P = 0.009$), birds in the Ontario S+ group responded significantly more to Ontario songs ($t = 8.02$, $P < 0.001$), while birds in the pseudo category group did not respond significantly differently to the two song types ($t = 0.41$, $P = 0.688$; see Figure 4-4a).

Additional test sessions

John Prince Research Forest songs. We conducted a *t*-test to examine the percentage of response to the two true category groups (British Columbia S+, Ontario S+) to songs from John Prince Research Forest, British Columbia. There was no significant difference between the two groups ($t = 0.57$, $P = 0.58$), indicating that when tested with songs from a third location, birds responded similarly to the new songs regardless of which geographic location was the S+ category during Discrimination training (i.e., British Columbia or Ontario).

Spliced songs. We conducted a Sex × Discrimination Group (British Columbia S+, Ontario S+) × 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 ($F_{3,24} = 8.51$, $P < 0.001$). There were no other significant main effects or interactions ($F\text{s} \leq 0.48$, $P\text{s} \geq 0.51$). We conducted planned comparisons on the scaled proportion of response to the different types of spliced songs by birds in the British Columbia S+ group. For this analysis, we compared the spliced control (British Columbia-British Columbia) stimuli to the other three types. Birds responded significantly more to British Columbia-British Columbia songs compared to the Ontario-Ontario songs ($t = 2.73$, $P = 0.026$) and the Ontario-British Columbia songs ($t = 2.76$, $P = 0.025$). When we conducted the same analysis on the arcsine square root transformed data, there was no significant difference in responding to British

Columbia-British Columbia and Ontario-Ontario songs ($t = 2.25$, $P = 0.055$). 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 ($t = 2.57$, $P = 0.033$). See Figure 4-5a.

Total duration manipulated songs. We conducted a Sex × Discrimination Group (British Columbia S+, Ontario S+) × Stimulus Type (British Columbia and Ontario songs with a total duration decreased, British Columbia and Ontario songs unmanipulated, and British Columbia and Ontario songs with a total duration increased) ANOVA on the scaled proportion of response to the songs in which the song duration was manipulated. This analysis revealed a significant Stimulus Type × Discrimination Group interaction ($F_{5,40} = 17.16$, $P < 0.001$). There were no other significant main effects or interactions (Fs ≤ 1.36, Ps ≥ 0.26). We conducted planned comparisons on the scaled proportion of response to the different total duration-manipulated songs by birds in the British Columbia S+ group. For this analysis, we compared the control stimuli (British Columbia unmanipulated songs) to the other manipulated song types. Birds responded significantly more to British Columbia unmanipulated songs compared to all Ontario song stimuli (decreased total duration, $t = 5.08$, $P < 0.001$; unmanipulated, $t = 4.37$, $P = 0.002$; increased total duration, $t = 3.06$, $P = 0.016$). We conducted similar planned comparisons for the responding by birds in the Ontario S+ group, by comparing the control stimuli (Ontario unmanipulated songs) to the other song types. Birds responded significantly more to Ontario unmanipulated songs than to British Columbia unmanipulated songs ($t = 3.31$, $P = 0.011$) and British Columbia songs increased in total duration ($t = 3.71$, $P = 0.006$). See Figure 4-6.

Experiment 3

Both Experiments 1 and 2 revealed that when presented with novel songs, birds in the true category groups continued to respond to the songs based on the contingencies learned during Discrimination, while birds in the pseudo category group responded non-differentially. This suggests that birds in the true category group were using a category-based perceptual mechanism when discriminating among songs. Results from Experiment 1 suggest birds in the true category groups learned the discrimination in fewer trials compared to birds in the pseudo category group; however, this result was not replicated in Experiment 2. Some of the birds (8 of 11 subjects) from Experiment 1 had previous operant experience discriminating *fee-bee* songs, while none of the birds in Experiment 2 had experience discriminating *fee-bee* songs. In addition, we used different song exemplars as discrimination stimuli in Experiments 1 and 2. In Experiment 3, we used the same stimuli that we used in Experiment 1, but the chickadees used as subjects had no previous operant experience discriminating *fee-bee* songs (subjects had previous operant discrimination experience, see below). In addition, we tested birds with manipulated songs (spliced songs and total duration manipulated songs, as in Experiment 2, and single note songs).

Methods

Subjects

Eighteen black-capped chickadees (nine males, nine females) were tested between September 2013 and June 2014. Birds were captured in the same locations as Experiment 1. Birds had previous operant experience discriminating *chick-a-dee* call notes (Guillette, Hahn, Hoeschele, Przyslupski, & Sturdy, 2015).The conditions prior to the experiment and the experimental apparatus remained the same as Experiments 1 and 2.

Acoustic stimuli

Natural stimuli. The same songs used as stimuli during *Discrimination training* in Experiment 1 were used as stimuli in Experiment 3. In addition, the same songs used during Transfer training in Experiment 1 were used during Generalization in Experiment 3; however, during Transfer training in Experiment 1 the songs were reinforced (based on the Discrimination contingencies). In contrast, during Generalization in Experiment 3, responses to songs were neither reinforced nor punished. Manipulated stimuli were prepared further as described below.

Spliced songs. Songs ($N = 18$) were bandpass filtered in Goldwave and the *fee* and *bee* notes from nine British Columbia and nine Ontario songs were edited into individual WAV files using SIGNAL. The same four types of spliced songs were created as Experiment 2 (British Columbia-British Columbia, Ontario-Ontario, British Columbia-Ontario, Ontario-British Columbia). When creating the spliced songs, we standardized the internote interval to 100 ms, as in Experiment 2. The average ± SD interval ratio of the songs used during the spliced song test (1.15 ± 0.03, range = 1.09-1.19) approximated the interval ratio of songs reported in previous studies (1.13 ± 0.01, British Columbia songs; 1.14 ± 0.02, Ontario songs, Hahn et al. 2013a) and the songs presented during Discrimination training (1.13 ± 0.02, range = 1.10-1.18).

Single note songs. Four British Columbia and four Ontario songs were used during single note tests. To create single note stimuli, the two notes from each song were separated and each note was saved as an individual WAV file using SIGNAL. The notes were further processed the same as the other acoustic stimuli.

Total duration manipulated songs. We manipulated the total duration of five British Columbia and five Ontario songs. We selected songs from each location that fell within one SD of the average for the stimuli used during Discrimination training (1100.61 ms ± 73.6 ms for

British Columbia songs; 1044.84 ms ± 38.3 ms for Ontario songs). We then increased and decreased the length of these songs by 1.5 and 2.5 SD of all songs presented during Discrimination training using Audacity 1.3.12 in the same manner as Experiment 2. We included more manipulations of the total duration in Experiment 3 to examine how birds would respond to smaller incremental changes to the song duration. This resulted in ten types of total duration manipulated songs: British Columbia and Ontario songs with a total duration decreased by 2.5 SD, decreased by 1.5 SD, unmanipulated, increased by 1.5 SD, and increased by 2.5 SD.

Procedure

Pretraining. The procedure for *Pretraining* remained the same as in Experiments 1 and 2. Birds remained on *Pretraining* until they had consistently high response rates for future Discrimination training stimuli (either responding to ≥ 60% of trials for six blocks or responding to ≥ 30% of trials for twelve blocks) and test stimuli (either responding to ≥ 60% of trials for four blocks or responding to ≥ 30% of trials for eight blocks) and four blocks with ≤ 3% difference in responding to future S+ and S- stimuli.

Discrimination training. The procedure and criterion for Discrimination training was the same as Experiments 1 and 2. Birds were randomly assigned to either a true category discrimination group ($N = 12$; British Columbia S+ group: three males, three females; Ontario S+ group: three males, three females) or pseudo category discrimination group ($N = 6$; Subgroup 1: one male, two females; Subgroup 2: two males, one female).

Pretesting. The procedure and criterion for Pretesting remained the same as Experiment 2.

Generalization. During Generalization, the stimuli and reinforcement contingencies from Pretesting were maintained. In addition, 20 songs not heard during Discrimination training (ten British Columbia and ten Ontario songs) were introduced. The procedure and criterion was the same as Experiment 2. Following Generalization, birds completed one bin of Pretesting with a DR ≥ 0.75 before moving onto the next test session.

Additional test sessions. The remaining test stimuli (i.e., 18 spliced songs, 16 single note songs, and 50 total duration manipulated songs) were divided into seven test sessions. The stimuli and contingencies from Pretesting were maintained, in addition, two or three spliced, two or three single note, and seven or eight total duration manipulated songs were each presented once, resulting in 212-trial blocks. For each test session, a minimum of three blocks were completed, followed by one block of Pretesting with a DR ≥ 0.75. As in Experiment 2, although an individual song was manipulated in multiple ways, only one of these manipulations from a particular song was presented during a single test session and the order in which a bird completed these probe sessions was pseudorandomized (between discrimination group and sex).

Response measures

DRs, 95% CIs, and scaled proportion of response to test stimuli were calculated as in Experiment 2.

Statistical analyses

We conducted the same analyses as in Experiment 2. However, to compare task acquisition during Discrimination training across groups we Vincentized the learning curves from Discrimination training to 10 blocks of data (the minimum number of blocks it took a bird to reach criterion) following the same methods as Experiment 2.

Results

Trials to criterion

We conducted a Sex × 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. There were no significant main effects or an interaction (Fs ≤ 1.38, Ps ≥ 0.28), indicating that there was no significant difference in the number of trials to reach criterion for birds rewarded for responding to British Columbia versus Ontario songs.

We conducted a *t*-test on the number of 200-trial blocks to complete Discrimination training for the two pseudo category groups. There was no significant difference in the speed of acquisition between the two pseudo category groups ($t = 0.32$, $P = 0.77$), so we combined the two groups in the remaining analyses.

We conducted a Sex × Discrimination Group (True, Pseudo) ANOVA on the number of 200-trial blocks to complete Discrimination training. There was a significant main effect for Discrimination Group ($F = 14.77$, $P = 0.002$), with the true category group completing the discrimination in significantly fewer trials compared to the pseudo category groups ($X = 19.18$ and 39.83, for true and pseudo category groups, respectively). See Figure 4-1.

Acquisition

To examine task acquisition across blocks, we conducted a repeated measures ANOVA with Sex and Discrimination Group (British Columbia S+, Ontario S+, Pseudo) as categorical predictors and the DR for each Vincentized block (1-10) as dependent variables. There was a significant main effect for Vincentized blocks ($F_{9,\,99} = 73.5$, $P < 0.001$); as Vincentized blocks increased, the DRs increased. There were no other significant main effects or interactions (Fs ≤ 1.61, Ps ≥ 0.072).

Number of S+ songs responded to ≥ 95% confidence interval (CI)

We examined how many of the S+ stimuli were well-discriminated by determining how many S+ stimuli were responded to ≥ 95% CI during the final block of Discrimination training. We conducted a Sex × Discrimination Group (British Columbia S+, Ontario S+, Pseudo) ANOVA to determine if the true category and pseudo category groups differed in the number of exemplars responded to greater than the 95% CI. There were no significant main effects or interactions (Fs ≤ 2.95, Ps ≥ 0.09). Out of 10 S+ stimuli, the means ± SE for the number of stimuli responded to greater than the 95% CI were: British Columbia S+ group: 8.67 ± 0.88 and 10 ± 0, for male and female subjects, respectively; Ontario S+ group: 9.67 ± 0.33 and 9.67 ± 0.33, for male and female subjects, respectively; pseudo category: 9.33 ± 0.67 and 6.67 ± 1.45, for male and female subjects, respectively.

Generalization

We conducted a Sex × Discrimination Group (British Columbia S+, Ontario S+, Pseudo) × Stimulus Type (British Columbia song, Ontario song) ANOVA on the scaled proportion of response to examine the response to untrained British Columbia and Ontario songs during *Generalization*. This analysis revealed a significant Discrimination Group × Stimulus Type interaction ($F_{2,12} = 57.44$, $P < 0.001$). There were no other significant main effects or interactions (Fs ≤ 2.79, Ps ≥ 0.10). To examine the significant interaction we conducted planned comparisons. Birds in the British Columbia S+ group responded significantly more to British Columbia songs compared to Ontario songs ($t = 6.94$, $P < 0.001$). Birds in the Ontario S+ group responded significantly more to Ontario songs compared to British Columbia songs ($t = 8.21$, P

< 0.001). Birds in the pseudo category group did not respond significantly differently to British Columbia and Ontario songs ($t = 0.89$, $P = 0.39$). See Figure 4-4b.

Additional test sessions

Spliced songs. We conducted a Sex × Discrimination Group (British Columbia S+, Ontario S+) × 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 main effect of Stimulus Type ($F_{3,24} = 3.65$, $P = 0.027$). There were no other significant main effects or interactions (Fs ≤ 2.2, Ps ≥ 0.11). See Figure 4-5b.

Single note songs. We conducted a Sex × Discrimination Group (British Columbia S+, Ontario S+) × Stimulus Type (British Columbia *fee* note, British Columbia *bee* note, Ontario *fee* note, Ontario *bee* note) ANOVA on the scaled proportion of response to the single note songs. This analysis revealed a significant Discrimination Group × Stimulus Type interaction ($F_{3,24} = 6.36$, $P = 0.003$). There were no other significant main effects or interactions (Fs ≤ 2.37, Ps ≥ 0.16). We conducted planned comparisons by examining the proportion of responding by birds in the British Columbia S+ group. For this analysis, we compared the responding to British Columbia *fee* notes and British Columbia *bee* notes to the other single note stimuli. Birds responded significantly more to British Columbia *fee* notes compared to Ontario *bee* notes ($t = 2.42$, $P = 0.042$), but when we conducted the analysis on the arcsine square root transformed data, this was not significant ($t = 2.28$, $P = 0.052$). We conducted similar planned comparisons for birds in the Ontario S+ group by comparing the proportion of responding to Ontario *fee* notes and Ontario *bee* notes to the other single note stimuli. Birds responded significantly more to

Ontario *bee* notes compared to Ontario *fee* notes ($t = 3.04$, $P = 0.016$), and British Columbia *fee* notes ($t = 3.48$, $P = 0.008$). See Figure 4-7.

Total duration manipulated songs. We conducted a Sex × Discrimination Group (British Columbia S+, Ontario S+) × Stimulus Type (British Columbia and Ontario songs with the total duration decreased by 2.5 SD, decreased by 1.5 SD, unmanipulated, increased by 1.5 SD, increased by 2.5 SD) 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 ($F_{9,72} = 4.51$, $P = 0.001$). There were no other significant main effects or interactions (Fs ≤ 5.22, Ps ≥ 0.052). We conducted planned comparisons on the scaled proportion of response to the different total duration manipulated songs by birds in the British Columbia S+ group. For this analysis, we compared the control stimuli (British Columbia unmanipulated songs) to the other song types. There was significantly less responding to Ontario songs decreased by 2.5SD ($t = 2.46$, $P = 0.039$) and Ontario songs decreased by 1.5SD ($t = 3.21$, $P = 0.012$). We conducted similar planned comparisons for the responding by birds in the Ontario S+ group, by comparing the control stimuli (Ontario unmanipulated song) to the other song types. There was significantly less responding to British Columbia songs increased by 1.5SD ($t = 2.64$, $P = 0.03$) and significantly more responding to Ontario songs increased by1.5SD ($t = 2.66$, $P = 0.029$). When the same analysis was conducted on the arcsine square root transformed data, birds responded significantly more to unmanipulated Ontario songs compared to unmanipulated British Columbia songs ($t = 2.54$, $P = 0.034$) and Ontario songs increased by 2.5SD ($t = 2.43$, $P = 0.041$). See Figure 4-8.

Discussion

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

Perceptual categorization

In Experiments 1 and 3, we found that birds in the true category group learned the discrimination in fewer trials compared to birds in the pseudo category group, suggesting that birds in the true category group perceived songs as belonging to separate perceptual categories while pseudo birds did not. However, in Experiment 2, there was no significant difference in the number of trials to complete Discrimination training for the true and pseudo category groups. It is possible that, in Experiment 2, the pseudo category S+ songs happened to be more acoustically similar to one another simply by chance than to the S- songs and vice versa, thus creating "categories" of songs that the birds could use when discriminating (i.e., birds in pseudo category group used open-ended categorization when discriminating). It is also possible that the song stimuli used in Experiment 2 were not ideal exemplars of geographic origin, making it difficult for birds in the true category group to detect a categorical difference; therefore, birds in both the true and pseudo category groups were using rote memorization, resulting in no difference in how long it took the groups to learn the task.

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* (Experiments 2 and 3) suggest that birds in the true category group were using open-ended categorization, as birds in the true category groups continued to respond to novel songs based on the contingencies from Discrimination training, while birds in the pseudo category group responded to the novel songs similarly regardless of geographic location. Interestingly, in Experiment 2, when we tested birds with novel songs from a third geographic location (i.e., 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 acoustically distinct from the songs produced by birds in the other two locations. It is possible that birds were categorizing by microgeographic differences in songs. Microgeographic differences exist in other vocalizations produced by black-capped chickadees (i.e., gargle calls) in locations that are separated by distances of only 5.7 km (Ficken & Weise, 1984). The distances between the locations that we tested were much greater: 133 km separated the two British Columbia sites and over 3,460 km separated the Ontario site from the British Columbia sites. Similarly, white-crowned sparrows produce one song type that varies geographically and geographic variation is evident for locations that are relatively close (3.2 km) and locations that are further apart (160 km; Marler & Tamura, 1962).

While it is less likely that non-migratory birds would encounter individuals from the extremes of their geographic range (as we tested in the current study), black-capped chickadees disperse (e.g., up to 11 km for juvenile dispersal reported in Weise & Meyer, 1979) and movements of longer distances have also been reported (e.g., 50-2,000 km; Brewer, Diamond, Woodsworth, Collins, & Dunn, 2006) so birds may encounter individuals originating from different geographic 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 habitat elevations (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, Ficken, & Witkin, 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 non-members (Nowicki, 1983). Geographic differences in *fee-bee* songs would allow chickadees to determine the geographic origin of conspecifics and distinguish a local bird from a bird that originated from a more distant geographic region.

Acoustic mechanisms

In Experiments 2 and 3, 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. In both experiments, we presented spliced songs and songs in which we altered the total duration; in Experiment 3, we also presented *fee* and *bee* notes individually.

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 also suggest that birds were using other acoustic cues within the songs (besides only total duration) to discriminate. 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 only one of the two song notes. To examine this possibility, we presented birds with spliced songs that contained one song note from each population (Experiments 2 and 3) and we presented birds with individual notes (Experiment 3).

Results from the single note tests suggest that birds in each discrimination group (i.e., British Columbia S+ and Ontario S+) were using a different mechanism when discriminating. For example, when presented with spliced songs in Experiment 2, birds in the British Columbia S+ group responded significantly less when an Ontario note was presented first in the song, providing evidence that birds rewarded for responding to British Columbia songs were relying on acoustic information in the *fee* note. A different pattern of responding emerged for birds in the Ontario S+ group. The results from the single note tests suggest that birds in this group relied on information within the *bee* note when responding; birds responded significantly more to Ontario *bee* notes compared to *fee* notes from either location.

Birds demonstrated some transfer of responding to single note songs, providing evidence that they attend and respond to acoustic features within individual notes; however, responding to the single note songs also suggest that the individual note types are acoustically similar between regions, because there was no significant difference when comparing the responding to *fee* notes from different locations and there was no significant difference when comparing the responding to *bee* notes from different locations. In addition, in Experiment 3, birds responded similarly to the different spliced songs (see Figure 4-5), supporting the idea that there were similarities between the notes from different locations. If the individual note types from each location are acoustically similar, birds likely used a combination of acoustic features when multiple features were available (i.e., when discriminating among natural songs).

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

In other songbird species, birds may rely on acoustic features in only one portion of the song in order to perceive geographic differences. For example, in one subspecies of white-crowned sparrow (*Zonotrichia leuophrys pugetensis*) males use the terminal trill portion of the song as an acoustic cue for geographic variation more than the introductory components of the song (Nelson & Soha, 2004); however in another subspecies of white-crowned sparrow (*Z. l. nuttalli*) males rely on the introductory components of the song when distinguishing between local and foreign dialects (Thompson & 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, & Cummingham, 1987), demonstrating that the mechanisms used to perceive geographic differences in songs can also vary within a single species. These differences in perception may be related to biological relevance. In the current study, we presented chickadees with songs from two distant geographic regions, but chickadees were not tested with local songs. Acoustic similarities or differences between the songs produced in the subjects' location of origin (i.e., central Alberta) and the songs used as stimuli (i.e., songs from northern British Columbia and eastern Ontario) may influence the biological salience of the songs. European starlings discriminate between variation in geographic dialects and show stronger responses (i.e., vocalize more often and with a shorter latency) to a familiar song dialect (Adret-Hausberger, 1982), suggesting that local songs are a more salient acoustic signal. Further work is needed to 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. In all three experiments, when chickadees were presented with novel song stimuli from the two locations, only birds in the true category groups continued to respond to songs based on the contingencies learned during Discrimination training, which suggests that these birds were using open-ended categorization. In contrast, chickadees in the pseudo category groups responded to the novel songs non-differentially, which suggests that birds in these groups were relying on rote memorization to learn the task during the initial Discrimination training. Tests with manipulated song stimuli (spliced songs, single note songs, and total duration manipulated songs) revealed that birds were likely using multiple acoustic features when discriminating, and the specific features used by birds to discriminate among the songs may be influenced by the particular songs initially discriminated during acquisition (i.e., different song stimuli were used in Experiments 2 and 3), as well as which songs are reinforced during acquisition training (i.e., whether British Columbia or Ontario songs were the S+ stimuli).

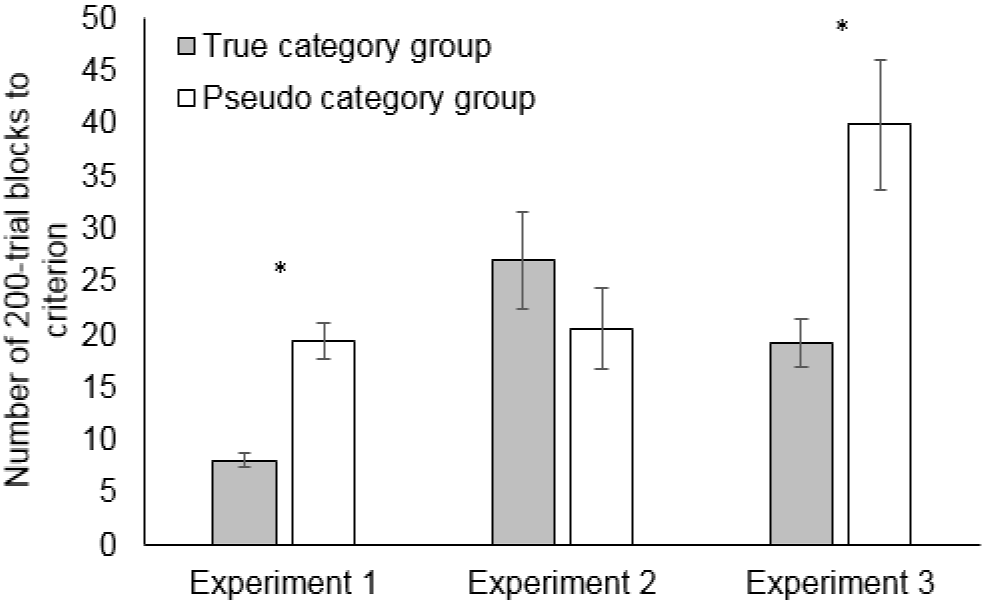

Figure 4-1. The average ± SE number of 200-trial blocks to reach the Discrimination training criterion for birds in the true category group (gray bars) and pseudo category group (white bars) in each experiment. (*) indicates a significant difference in the number of trial blocks to reach criterion between the true and pseudo category groups ($p \leq 0.05$).

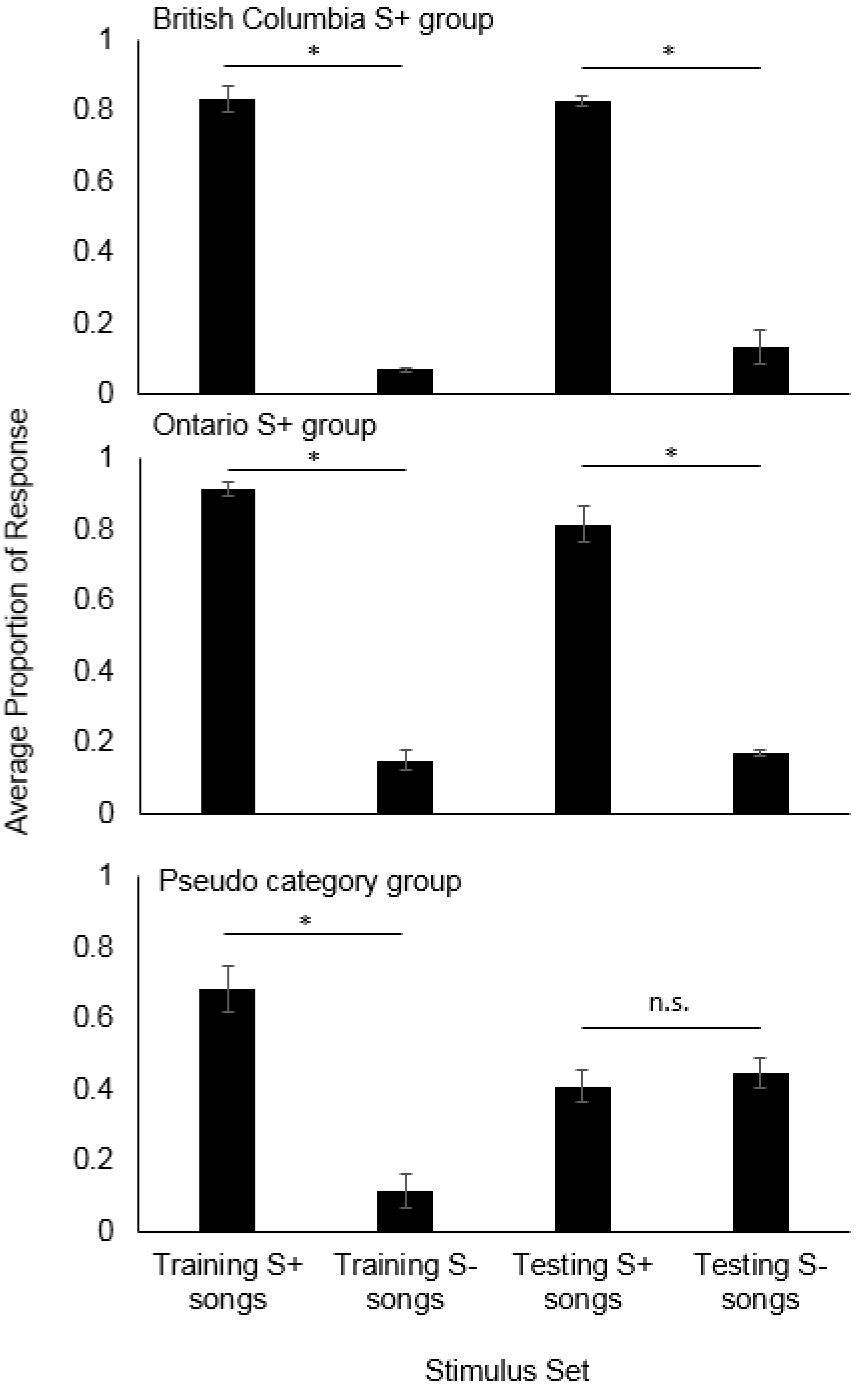

Figure 4-2. Average ± SE proportion of response to Training S+ songs, Training S- songs, Testing S+ songs, and Testing S- songs during the first 200-trial block of Transfer training for each group in Experiment 1. Lines indicate the comparisons that were made (*) indicates a significant difference in responding by that discrimination group; n.s. indicates no significant difference; $p \leq 0.05$).

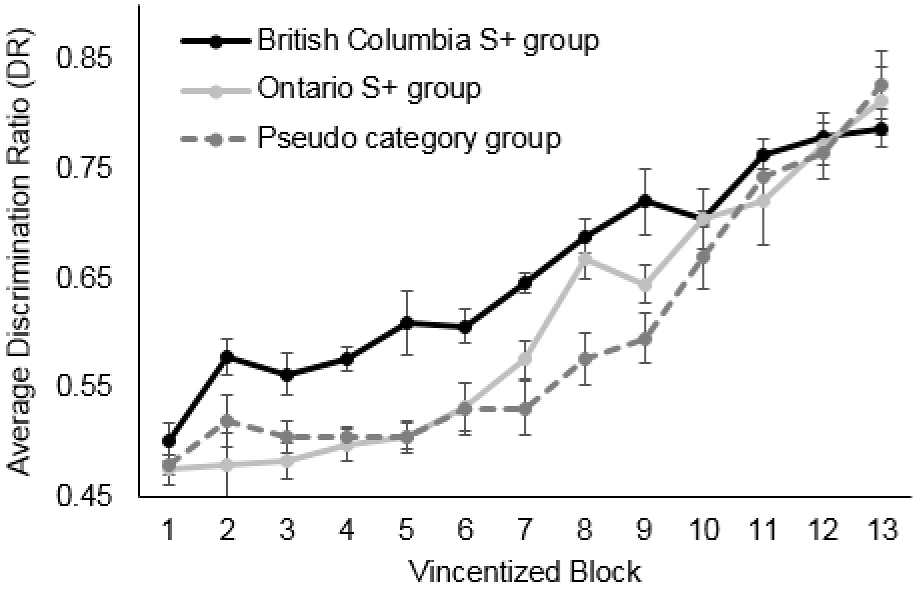

Figure 4-3. Vincentized acquisition curves for birds in each group during Discrimination training in Experiment 2. Error bars represent standard error of the mean.

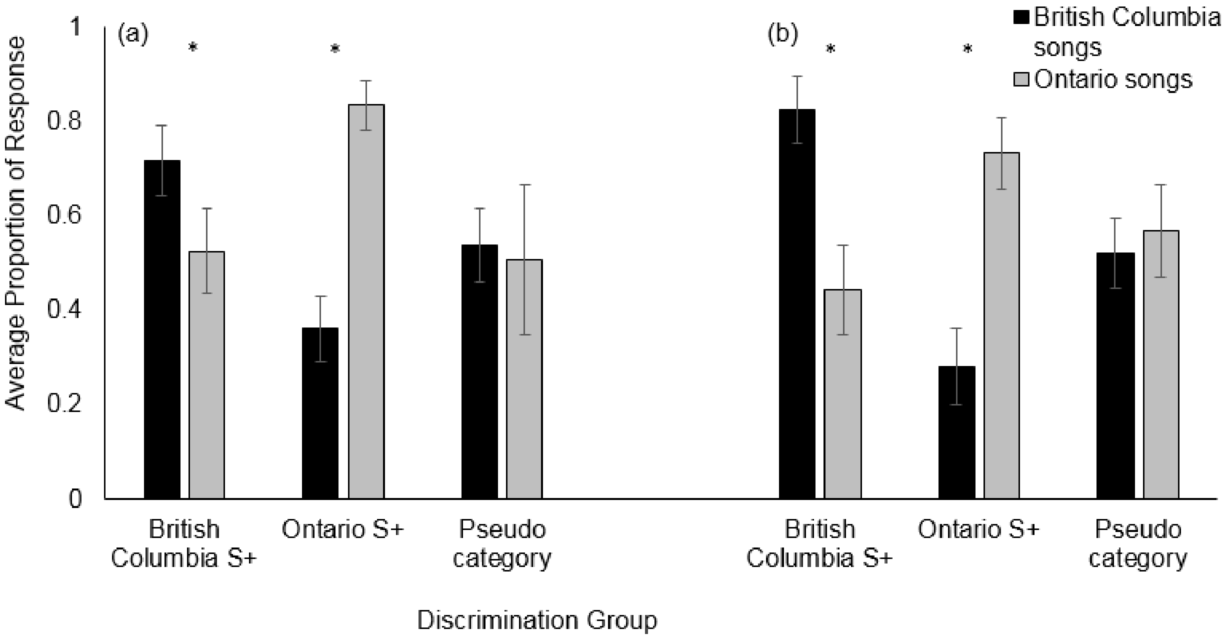

Figure 4-4. Average ± SE proportion of response for each discrimination group during Generalization for (a) Experiment 2 and (b) Experiment 3. (*) indicates a significant difference in response to British Columbia and Ontario songs by that discrimination group ($p \leq 0.05$).

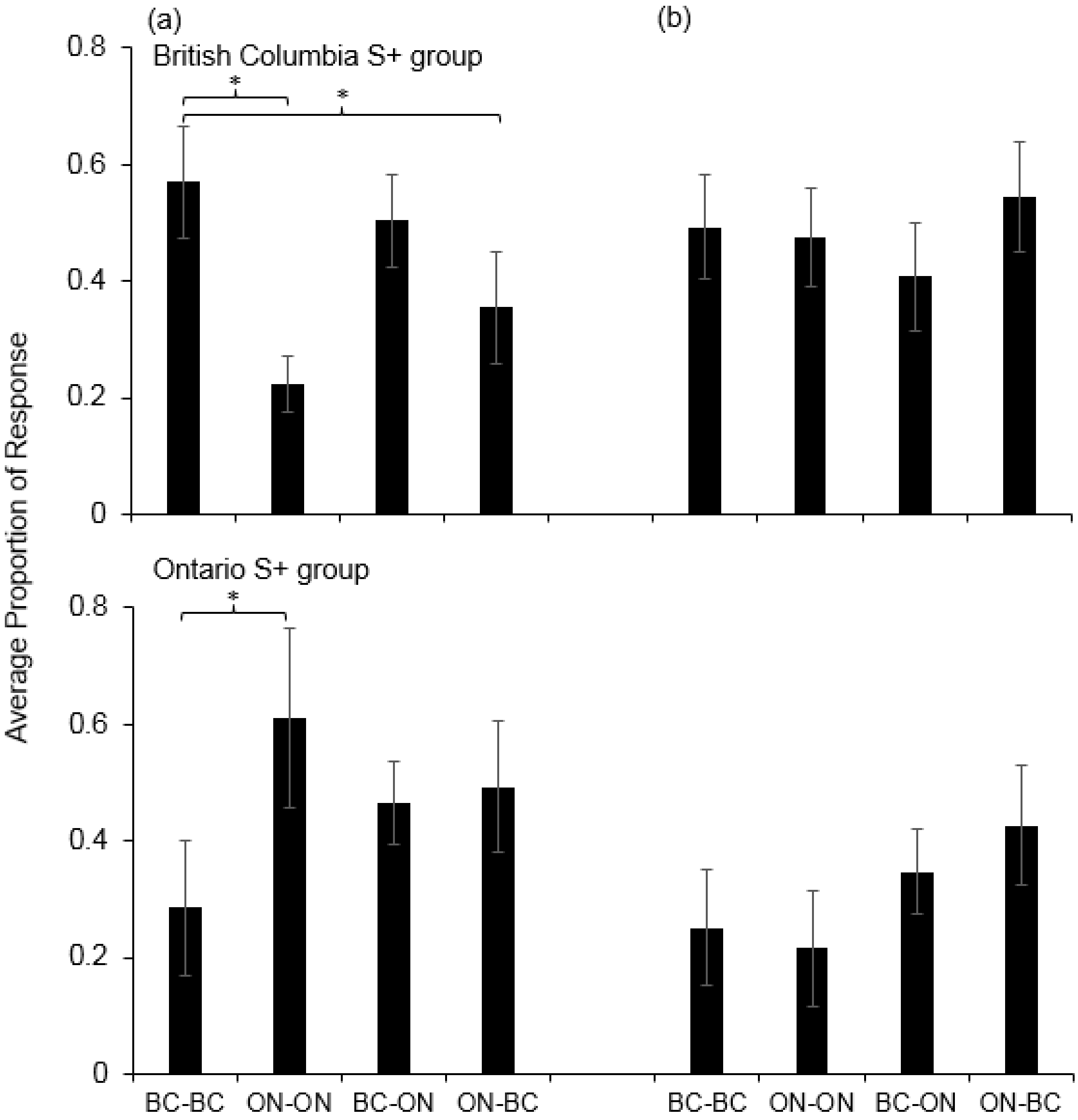

Figure 4-5. Average ± SE proportion of response to spliced songs for (a) Experiment 2 and (b) Experiment 3. The different stimulus types were: British Columbia *fee* and *bee* notes (BC-BC), Ontario *fee* and *bee* notes (ON-ON), British Columbia *fee* note and Ontario *bee* note (BC-ON), and Ontario *fee* note and British Columbia *bee* note (ON-BC). The top panel displays the response by birds in the British Columbia S+ discrimination group; the bottom panel displays the response by birds in the Ontario S+ discrimination group. (*) indicates a significant difference in response ($p \leq 0.05$).

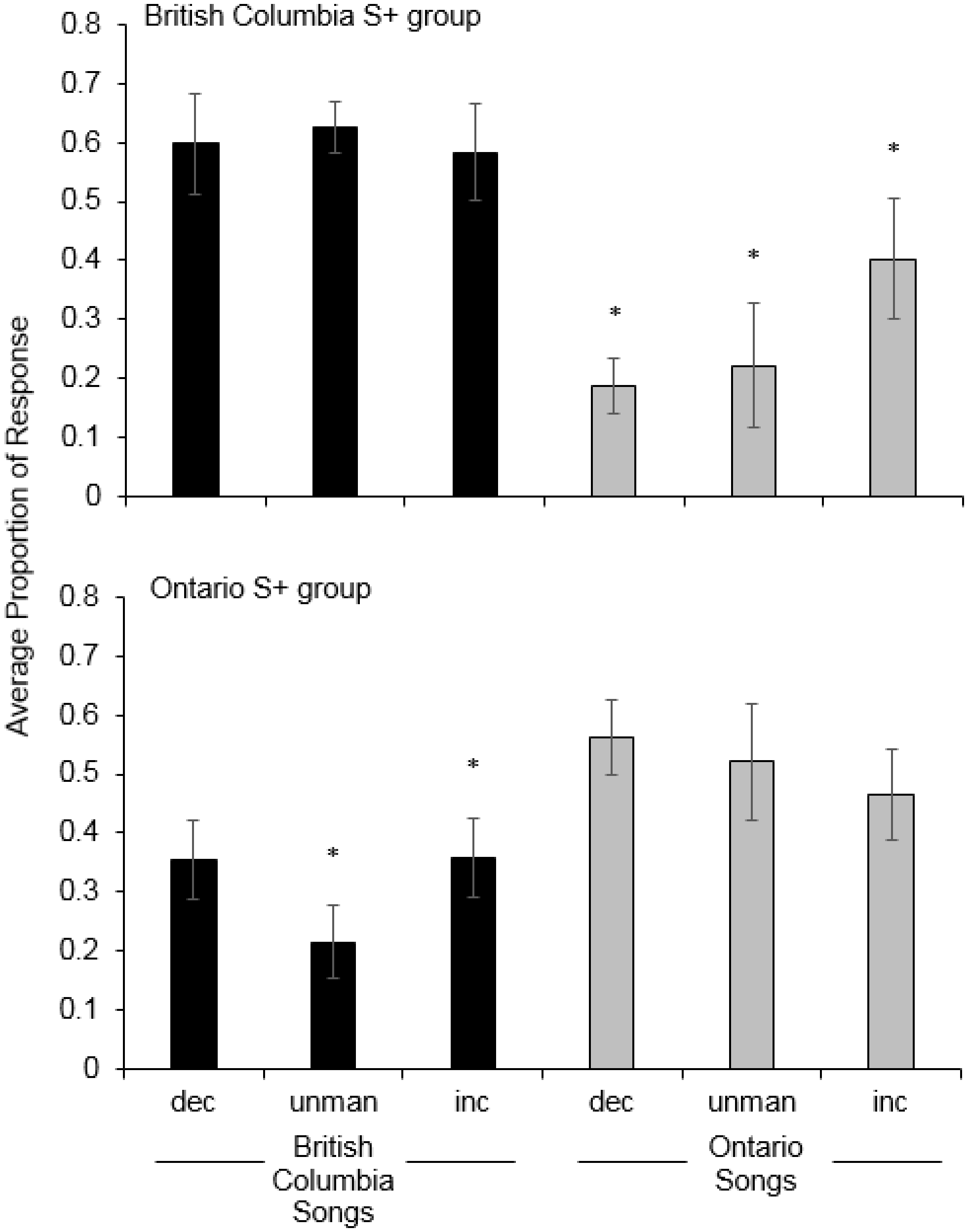

Figure 4-6. Average ± SE proportion of response during Experiment 2 to total duration manipulated songs. Black bars are British Columbia songs and gray bars are Ontario songs. Stimuli were presented in three ways: decreased in duration (dec), unmanipulated (unman) and increased in duration (inc). (*) indicates a significant difference in response compared to the unmanipulated control songs (unman British Columbia songs for birds in British Columbia S+ discrimination group; unman Ontario songs for birds in Ontario S+ discrimination group; $p \leq 0.05$).

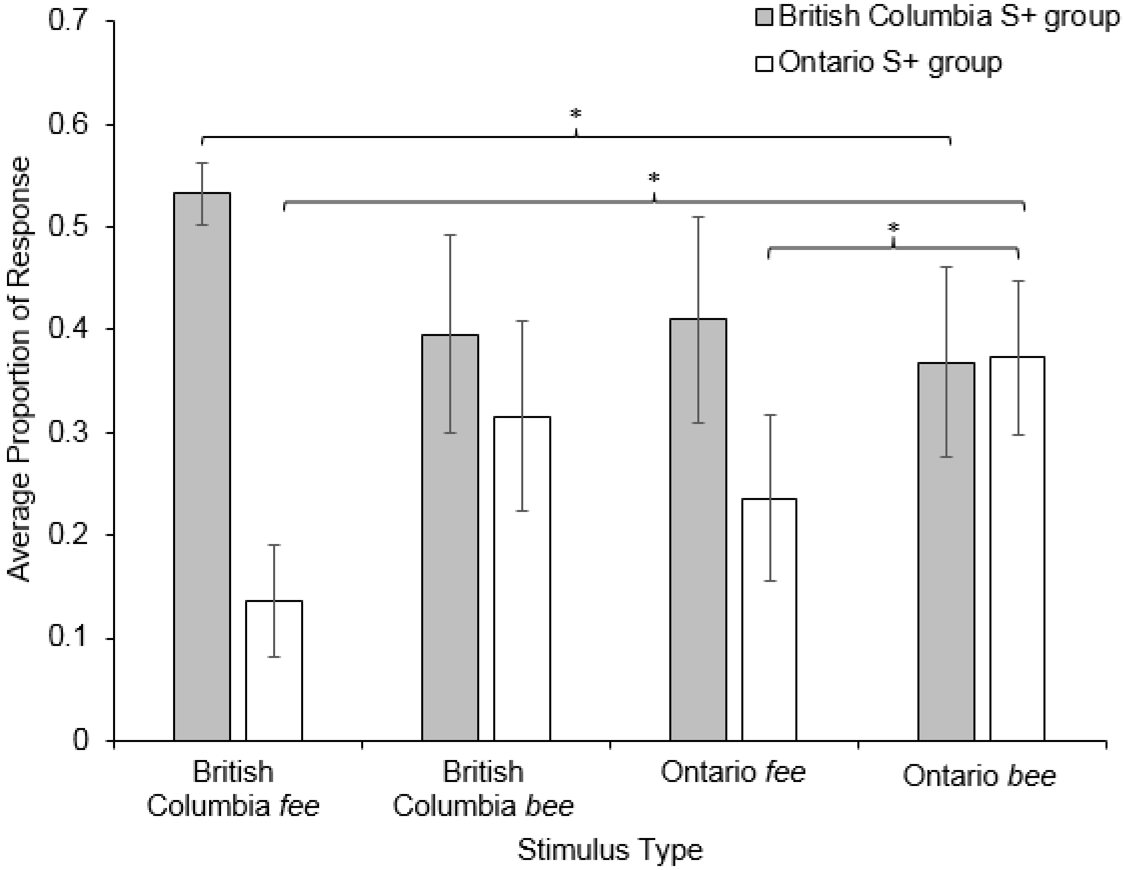

Figure 4-7. Average ± SE proportion of response to single note songs during Experiment 3. (*) indicates a significant difference in response ($p \leq 0.05$).

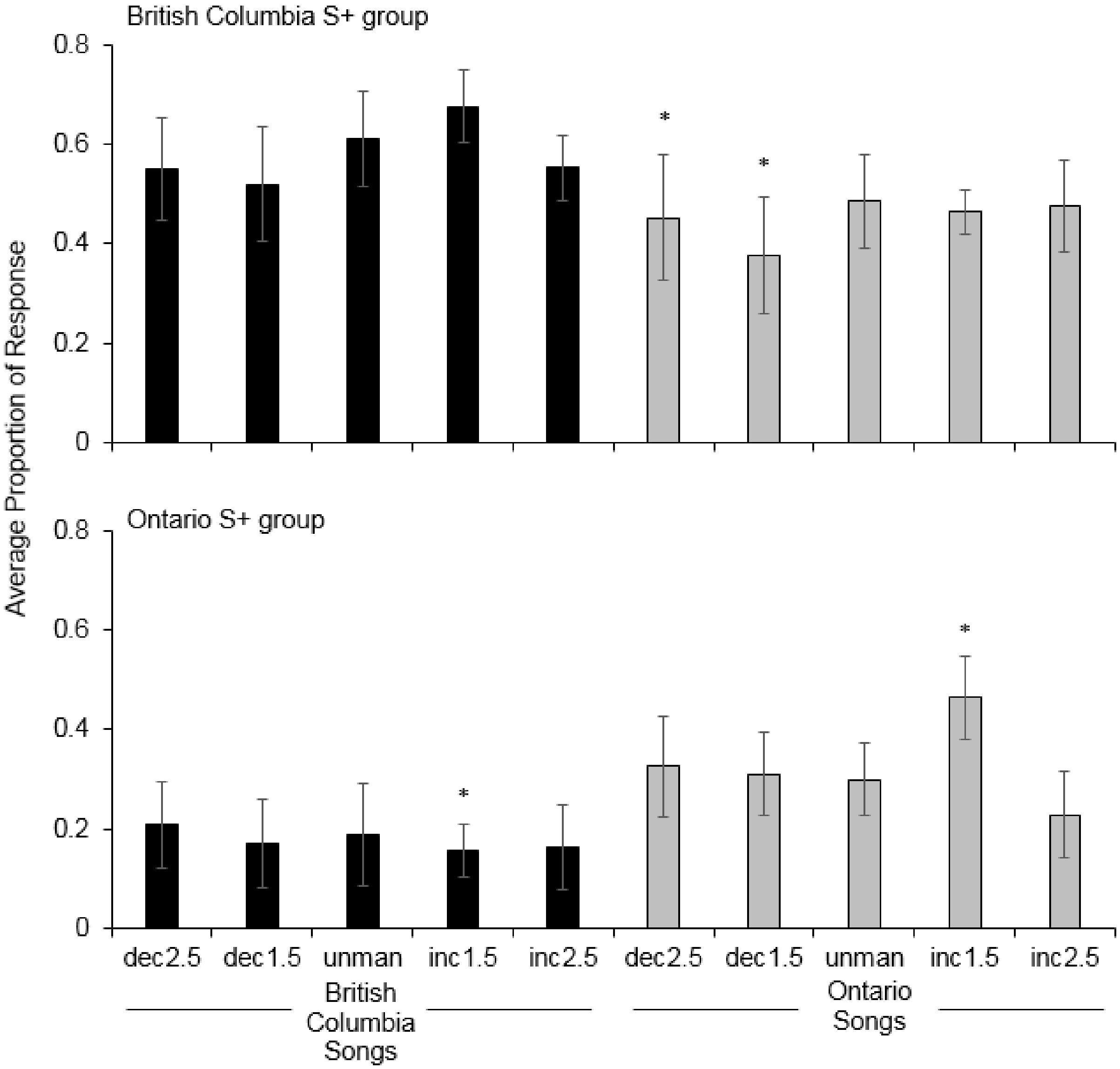

Figure 4-8. Average ± SE proportion of response during Experiment 3 to total duration manipulated songs. Black bars are British Columbia songs and gray bars are Ontario songs. Stimuli were presented in five ways: decreased by 2.5 SD (dec2.5), decreased by 1.5 (dec1.5), unmanipulated (unman), increased by 1.5 (inc1.5), and increased by 2.5 (inc2.5). (*) indicates a significant difference in response compared to the unmanipulated control songs (unman British Columbia songs for birds in British Columbia S+ discrimination group; unman Ontario songs for birds in Ontario S+ discrimination group; $p \leq 0.05$).

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Chapter 5 Female song in black-capped chickadees (*Poecile atricapillus*): Acoustic song features that contain individual identify information and sex differences[2]

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Introduction

In the oscine songbirds, song is considered a sexually-selected signal (Catchpole & Slater, 2008; Searcy & Yasukawa, 1996), produced by males and serving two main functions: (1) territoriality and (2) mate attraction (Catchpole & Slater, 2008). Most studies examining songbird vocalizations have concentrated on temperate species, in which males have long, complex songs and females are thought to lack song (Langmore, 1998; Riebel, 2003). In contrast, females of tropical species often sing solo song or duets (Slater & Mann, 2004) and the contrast between how common female-produced song is in the tropics compared to the relatively uncommon songs produced by females in the North Temperate Zone may be one reason songs produced by the latter are often overlooked (Catchpole & Slater, 2008). However, there are an increasing number of accounts of female song in temperate songbirds, and in many species, it is now recognized that both males and females can produce song (for reviews see Langmore, 1998; Riebel, 2003). For example, in a literature review of 233 European species, Garamszegi et al. (2007) reported descriptions of female song in over 100 species, and only eight species in which it was specified that females do not sing. In the remaining species, there was no indication of the presence or absence of female song.

In many temperate species in which female song has been described, the description has been limited to accounts of rare or atypical female song in the field (song sparrow, *Melospiza melodia*, Arcese et al., 1988; hooded warbler, *Wilsonia citrina*, Evans Ogden et al., 2003; common yellowthroat, *Geothlypis trichas*, Taff et al., 2012). In general, few studies examining North Temperate Zone species have quantitatively examined female song and structurally compared male and female songs (e.g., Baptista et al., 1993; Hoelzel, 1986; Pavlova et al., 2005). Because females sing infrequently in many temperate songbird species, it is difficult to make comparisons between male and female song. For example, an instance of rare female song in a hooded warbler was found to be structurally similar to male song, and playback response by males to this song was similar to a typical male song (Evans Ogden et al., 2003); however, these results cannot be generalized to the population, due to its singular occurrence. In the current study, we recorded captive-housed male and female black-capped chickadees (*Poecile atricapillus*), a North American songbird, that were wild-caught as adults. We obtained multiple songs from both males and females and conducted a bioacoustic analysis of these songs. In this species, song produced by males has been well-studied, but female song has not.

Compared to the songs of most songbirds, black-capped chickadee *fee-bee* song appears to be relatively simple. While male song has been the focus of many studies, there are only a few accounts of females singing (Dwight, 1897; Hill & Lein, 1978) and female song is often described as softer than male song (Dixon, 1970; Odum, 1942). This lower amplitude song is termed *faint fee-bee* (Ficken et al., 1978), thereby distinguishing it from the typical *fee-bee* song. Both males and females can produce *faint fee-bees*, which are a close-range vocalization, usually used near the nest. In contrast, normal *fee-bee* song is typically described as a male-produced signal used in mate attraction and territory defense (Ficken et al., 1978; Smith, 1991). There are accounts of females singing in the field; for example, Hill and Lein (1987) reported a female singing normal *fee-bee* songs while away from her mate.

In temperate species, the description of female song may be limited because females sing different or less-complex songs, females spend less time singing compared to males, and in many species females and males are monomorphic, so singing females may be incorrectly identified as male (Arcese et al., 1988; Langmore, 1998). The first possibility, that female song is qualitatively different than male song, is unlikely for black-capped chickadees, as males produce relatively simple song, and the few descriptions of female song have described it as similar to male song. However, the second and third possibilities may be true of black-capped chickadees: Females may spend less time singing and black-capped chickadees are monomorphic, so females may be mistaken for males (especially if wild birds are not banded and sexed). Our wild-caught females sing loud *fee-bee* songs in captivity (pers. obs.) and these observations led us to record females producing song.

In the current study, our aim is twofold: (1) to provide a quantitative description of the acoustic structure of female black-capped chickadee *fee-bee* songs and (2) to make a direct comparison to male *fee-bee* songs. We recorded both males and females under the same conditions and, using bioacoustic analyses, measured seven acoustic features within the songs. To our knowledge, this study is the first to provide a quantitative description of the acoustic structure of female black-capped chickadee song and make a comparison between male and female song.

Materials and methods

Subjects

Vocalizations from eleven female and ten male chickadees, at least one year of age (determined by the shape and color of their outer tail retrices at capture, Pyle, 1997), were used in the current analysis. Birds were captured between 29 February 2008 and 19 March 2012 from several areas around Edmonton, Alberta (53˚53N. 113˚53W; 53˚52N, 113˚47W) and Stony Plain, Alberta (53˚45N, 114˚01W). Sex was determined by DNA analysis (Griffiths et al., 1998). Birds were housed individually in colony rooms in Jupiter Parakeet cages (0.3 × 0.4 × 0.4 m; Rolf C. Hagen, Inc., Montreal, Canada). Birds had food (Mazuri Small Bird Maintenance Diet; Mazuri, St. Louis, MO), water (supplemented with vitamins three times per week; Hagen, Rolf

C. Hagen, Inc., Montreal, Canada), grit, and cuttle bone *ad libitum*. Birds were given three to five sunflower seeds daily, one super worm three times per week, and an egg and greens (spinach or parsley) mixture twice per week. Birds were maintained on a light-dark cycle that approximated the natural light cycle for Edmonton, Alberta and was adjusted according to the time of year.

Recordings

Birds were recorded between 20 March and 14 June 2012. A recording session for an individual bird lasted approximately 30 min and all recordings took place between the hours of 9:00 (when colony lights turned on) and 13:20. Birds were recorded individually in sound-attenuating chambers (1.7 × 0.84 × 0.58m; Industrial Acoustics Company, Bronx, NY) using a AKG C 1000S (AKG Acoustics, Vienna, Austria) microphone connected to a Marantz PMD670 (Marantz America, Mahwah, NJ) digital recorder (16 bit, 44,100 Hz sampling rate). Birds were transported in their home cages from the colony room to a room containing the sound chamber. All birds remained in their home cage during the recording session. During the recording sessions we monitored and noted the sound pressure level (A weighting, slow response) of the *fee-bee* songs with a Brüel & Kjær Type 2239A (Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark) integrating sound level meter. A microphone extension cable allowed us to locate the body of the sound level metre outside the chamber and to monitor the amplitude of the vocalizations. The amplitude was recorded for nineteen of the twenty-one birds (90%; n= 578 songs) whose *fee-bee* songs were included in the analysis. The microphone of the sound level meter was positioned 0.1m above and slightly behind the cage. A vocalizing bird could be between 0.1 and 0.55m from the microphone of the sound meter, depending on its location in the cage. Following a recording session, audio files were downloaded and saved onto PC and analyzed using SIGNAL 5.10.29 software (Engineering Design, Berkley, CA).

The number of days that a bird was recorded varied between individuals. For an individual bird, songs included in the analysis were recorded over 1-4 days (average = 1.43 days). In our comparison of male and female songs, we were careful to avoid pseudoreplication, which can occur when including more than one vocalization per individual or per recording session, and controlled for the confounding effects of pseudoreplication by conducting permuted discriminant function analyses (see "Statistical Analysis" below).

Bioacoustic analysis

Nine females and nine males produced more than twenty *fee-bee* songs across recording sessions. From these individuals, twenty high quality songs (i.e., not distorted from cage noise) were randomly selected to include in the analysis. Two females and one additional male produced 12-16 songs and these were also included in the analysis.

We made a total of seven acoustic measurements on each song. These acoustic measurements were chosen because they have been measured previously in studies examining acoustic features within the *fee-bee* song (e.g., Christie et al., 2004a; 2004b; Hoeschele et al., 2010) and because these measures provide a comprehensive acoustic description of the *fee-bee* song. Temporal and spectral measures were taken from sound spectrograms with amplitude cutoffs of -35 to 0 dB relative to song peak amplitude. We examined two temporal measurements: (1) total duration of song and (2) the proportion of song occupied by the *fee* note (*fee* note duration divided by the total duration of the song), three spectral measurements: (1) *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 (2) the interval ratio between the notes (calculated by dividing the end frequency of the *fee* note by the start frequency of the *bee* note), (3) absolute frequency (measured as the start frequency of the *bee* note; Christie et al., 2004a; Otter & Ratcliffe,, 1993) and two amplitude measurements: (1) relative amplitude of the *fee* and *bee* note (calculated by dividing the maximum amplitude for the *bee* note by the maximum amplitude for the *fee* note) and (2) the root mean squared (RMS) amplitude ratio, or the relative loudness of the *fee* note to the entire song (calculated as the RMS amplitude of the *fee* note divided by the RMS ratio of the entire song; see Figure 5-1 for measurements). Temporal measurements were made with a spectrogram window size of 256 points and time resolution of 5.8 ms, and the spectral measurements were made with a spectrogram window size of 1,024 points and frequency resolution of 43.1 Hz.

Statistical analysis

To examine if acoustic features in songs vary by individual, we calculated the potential for individual coding (PIC) for each of the acoustic features. We calculated the coefficients of variation between individuals (CV_b) using the following formula: $CV_b = (SD/mean)\times100$, where SD is the standard deviation and mean is the average for the total sample, and the coefficient of variation within an individual (CV_w) using the formula: $CV_w = (SD/mean)\times100$, where the SD and mean are calculated from each individual's songs (Charrier et al., 2004; Sokal and Rohlf, 1995). For each acoustic feature, the PIC value is the ratio CV_b/mean CV_w, where mean CV_w is the average CV_w calculated for all individuals (Charrier et al., 2004). Acoustic features with a PIC value greater than 1 may be used for individual identification.

To further examine song in terms of individual differences, we conducted three stepwise discriminant function analyses using SPSS (version 19.0.0, SPSS, Inc, Chicago, IL) to classify songs in terms of what individual produced the vocalization. Discriminant function analyses are commonly used in behavioural and bioacoustic research to determine if, for example, vocalizations differ by individual or group. In addition, discriminant function analyses are used to determine which features within the vocalizations could be used to discriminate between the individuals or groups (Mundry & Sommer, 2007). If one or more of the acoustic features we measured in the *fee-bee* song varies among individuals, discriminant function analyses should be able to use the features to accurately classify the songs (Tabachnick & Fidell, 2007). We report both the original and cross-validated percentage of correct classifications, as well as the standardized and structure coefficients for the first discriminant function derived from each of these analyses. We used the leave-one-out method of cross-validation, in which one case is withheld at a time and the discriminant function is derived from the remaining cases. Using the discriminant function that was derived, the withheld case is then classified. These steps are repeated until all cases have been classified in this manner (Betz, 1987). This method of cross-validation can provide a useful estimate for how well the derived discriminant function can predict group membership with a new sample (i.e., a set of cases not used to derive the discriminant function). From the standardized coefficients, we can examine the relative importance of each variable to the discriminant score; a greater contribution is associated with a standardized coefficient with a larger magnitude. One limitation of standardized coefficients is that when two variables are strongly correlated with one another, each variable's importance to the discriminant score may not be accurately represented. In contrast, structure coefficients are correlation coefficients that are not affected by other variables. As the structure coefficient's magnitude increases, it represents a closer relationship between the variable and the discriminant function (Klecka, 1980).

To analyze songs in terms of the sex of the producer, we conducted discriminant function analyses using the leave-one-out method of cross-validation 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 first randomly selected ten songs from each individual and conducted a stepwise discriminant function analysis to classify songs based on the sex of the producer. Within an individual, song features can vary each time the song is produced, so the specific songs used within the discriminant function will affect the classification results. For this reason, we repeated this randomization process 100 times and calculated the average percentage of correct classifications. We conducted binomial tests on the results from these stepwise discriminant function analyses to determine which acoustic features were used to classify the songs significantly more than would be expected by chance, and using only these acoustic features, we conducted permuted discriminant function analyses as suggested by Mundry and Sommer (2007), because our data set contained more than one song per individual which can lead to issues of pseudoreplication. As before, we randomly selected ten songs per individual, repeated this 100 times, and calculated an average percentage of correct classifications. Next, we conducted 1,000 permuted discriminant function analyses, in which we randomly selected ten songs per individual and randomly assigned the songs produced by an individual to one of two groups (i.e., male or female). Following Mundry and Sommer (2007), we calculated a *p*-value by finding the proportion of permuted discriminant function analyses that resulted in a percentage of correct classifications equal to or greater than the average percentage of correct classifications from the original data set. With this procedure, there are two levels of randomization within the permutation: (1) randomizing which group an individual belongs to prior to the classification by the discriminant function (i.e., male or female) and (2) randomly selecting which songs produced by each individual will be included in the discriminate function analysis. Although we included more than one song per individual across recording sessions in our discriminant function analyses, by using these randomization procedures and testing our results against the true null hypothesis distribution (i.e., the resultant distribution from the randomized data set), we eliminate the problem of pseudoreplication that can occur with a traditional discriminant function analysis. In addition, an equal number of songs from each individual were included in each discriminant function (i.e., ten songs) and the analyses accounted for the different number of males versus females (i.e., songs from eleven females and ten males were included), by indicating that the discriminant functions should use 'prior probabilities' when classifying songs.

In addition to the discriminant function analyses, we conducted a corresponding multivariate analysis of variance (MANOVA) in SPSS. Using the seven features we measured, we calculated an average song for each of the 21 birds and compared songs produced by males and females using a MANOVA. Similar to the permuted discriminant function, by using the average song parameters for each individual in the MANOVA, we avoid the pseudoreplication that can occur when using multiple songs from an individual.

Results

We first wanted to determine if there were amplitude differences in the songs produced by males and females. During the recording sessions, we recorded the amplitude of songs produced by nine males (275 songs) and ten females (303 songs). We used these amplitude measurements to compare the amplitude of male- and female-produced songs. First, we calculated the coefficient of variation for each sex. Coefficients of variation are useful to compare the relative amount of variation in two groups with different means (Sokal & Rohlf, 1995). The coefficient of variation (CV) was found using the following formula: CV = (SD/mean)×100. For the 275 male songs, CV = 17.63% (mean±SD = 68.91±12.15). For the 303 female songs, CV = 10.08% (mean±SD = 70.11±7.069). These results indicate that overall the amplitude of male songs are more variable compared to female songs. However, to determine if the amplitdue of male and female songs varied significantly from each other we conducted a *t* test. Because the amplitude for more than one song per individual was recorded, we took the average amplitude of the songs produced by each individual. There was no significant difference between the sexes $t(17) = 0.38$, $p = 0.71$ (female mean±SD = 67.08±6.74 dB; male mean±SD = 68.62±10.74 dB). These results suggest that while males produce songs with more variable amplitude compared to females, there is no difference between the absolute amplitude of male-produced songs compared to female-produced songs. In other words, females were not just producing very soft songs and males were not only producing loud songs and this suggests that males and females were producing similar songs. The overall structural similarity of male- and female-produced song is illustrated in Figure 5-2.

Acoustic differences by individual

Table 5-1 shows the means, standard deviations, coefficients of variation between and within individuals, and the potential for individual coding value for each acoustic feature. For each sex and for both sexes combined, all seven acoustic features have PIC values greater than 1, indicating that they may contain cues of individual identity. For both sexes combined and for males only, total duration has the highest PIC value. For female birds only, *bee* start frequency has the highest PIC value.

We conducted three discriminant function analyses to classify the songs based on individual. The first discriminant function analysis classified songs produced by birds of both sexes. This analysis correctly assigned 48.0% of all cross-validated cases to the correct individual (chance = 1/21 = 4.8%). Two additional discriminant function analyses conducted with only male or only female songs correctly classified 59.2% and 62.0%, respectively, of all cross-validated cases in terms of the individual. Table 5-2 gives the eigenvalue, squared canonical correlation coefficient, standardized and structure coefficients for the first discriminant function for each of the three analyses conducted. Examining the standardized coefficients and the correlation coefficients reveals that total duration shows the strongest relationship with the discriminant functions for each analysis. Table 5-2 also lists the feature that had the second largest standardized and structure coefficient for the first discriminant function, as well as the feature that had the second largest PIC value. These features differed between sexes (for males only relative amplitude varies with individual, while for females only *bee* start frequency varies), suggesting that there may be differences in what acoustic features account for individual recognition in male compared to female songs.

Acoustic differences by sex

The percentage of correct classifications from the discriminant function analyses are reported as mean±SD and the range of percent correct classifications for the 100 iterations is reported. The stepwise discriminant function analyses conducted to classify songs based on sex revealed a mean percentage of correct classifications of 65.39±2.24%; range: 60.48-70.48%. Binomial tests found that total duration ($z = 3.0$, $p = 0.0013$), *fee* proportional duration ($z = 2.2$, $p = 0.139$), *fee* glissando ($z = 10.0$, $p \leq 0.0001$), interval ratio ($z = 3.8$, $p \leq 0.0001$), and *bee* start frequency ($z = 5.0$, $p \leq 0.0001$) were used in significantly more discriminant function analyses than would be expected by chance (chance = 0.50). Using only these features (i.e., total duration, *fee* proportional duration, *fee* glissando, interval ratio, and *bee* start frequency) we conducted permuted discriminant function analyses. Results revealed that there was no difference in the acoustic features between male and female songs (average percentage of correct classifications for the original data set = 64.76±2.20%; range: 60.0-70.48%; 277/1000 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; $p = 0.277$). We also conducted permuted discriminant function analyses using only *fee* glissando, because this feature was used by all discriminant function analyses (100/100) conducted on the original data set (compared to 65/100, 61/100, 69/100, 75/100, for total duration, *fee* proportional duration, interval ratio, and *bee* start frequency, respectively). Results from this permuted discriminant function analysis revealed a significant difference in the *fee* glissando between male and female songs. (average percentage of correct classifications for the original data = 66.09±2.02%; range: 60.48-70.95%; 9/1000 of the permuted data sets had a percentage of correct classification equal to or greater than the average percentage of correct classifications of the original data set; $p = 0.009$).

Results from the MANOVA were in agreement with the results of the discriminant function analyses, revealing that *fee* glissando was significantly different between the male and female songs ($F(1,19) = 7.68$, $p = 0.012$, partial $\eta^2 = 0.29$, observed power = 0.75). The other five features did not differ significantly ($F(1,19) \leq 1.24$, $p \geq 0.28$, partial $\eta^2 \leq 0.06$, observed power ≤ 0.184). See Figure 5-3 for comparison of representative male and female songs with varying *fee* glissando ratios.

Discussion

Our study provides the first detailed description of the structure of female *fee-bee* song in black-capped chickadees. By recording both male- and female-produced song and conducting parallel bioacoustic analyses, we can make direct statistical comparisons between the acoustic features in male and female songs in our study population. In many North Temperate Zone species, females are considered to rarely produce song (Langmore, 1998; Riebel, 2003). However, this study provides evidence that in the black-capped chickadee (a North Temperate Zone species), females can produce song with overall structural similarities to male song (see Figure 5-2). In addition, our results indicate that (1) *fee-bee* songs are individually distinctive for both sexes, and (2) at least one of the measured acoustic features varies between the songs produced by male and female birds.

For this study, we recorded birds held in captivity. These recordings are a first step in identifying a behaviour that has been rarely reported in this species. Recording females in the wild is a logical next step, and will help us to better understand the ecological context and potential function of female song. However, there is no indication that our captive females were only singing because they were being housed in the lab. All birds were captured from the wild as adults, and presumably had normal vocal development prior to being brought into captivity. Our birds are housed singly within colony rooms, but have visual and auditory contact with conspecifics. Avey et al. (2008b) examined the effects of captive housing on black-capped chickadees compared to wild birds and found that the vocal behaviour of wild-caught chickadees housed in captivity mimicked the vocal production of wild birds across both time of day and season. Specifically, *fee-bee* song production in captive black-capped chickadees increased during the spring at dawn, and this result did not differ from *fee-bee* song production in the wild. The captive housing conditions examined in Avey et al. (2008b) are identical to the conditions that the birds in the current study were housed under. It is important to note that Avey et al. did not identify the sex of the birds singing in the field or the laboratory, but females have been heard singing loud songs while housed under these conditions (pers. obs.).

Previous studies have demonstrated that male black-capped chickadee song contains cues regarding individual identity (Christie et al., 2004a; Wilson & Mennill, 2010). Examining songs produced by birds in eastern Ontario, Christie et al. (2004a) found that total duration was the most individually distinctive acoustic feature in male *fee-bee* song. Our results are in agreement with this finding, for both male and female songs. In numerous species, discriminating between individuals via acoustic signals can be used to identify a territory neighbor versus stranger (e.g., alder flycatcher, *Empidonax alnorum*, Lovell & Lein, 2004; song sparrow, Stoddard et al., 1990) or a mate (e.g., great tits, *Parus major*, Lind et al., 1996; zebra finch, *Taeniogpygia guttata*, Miller, 1979; dunnock, *Prunella modularis*, Wiley et al., 1991). Black-capped chickadee males and females eavesdrop on male singing contests (Mennill et al., 2002; Mennill & Ratcliffe, 2004), and operant go/no-go discrimination tasks (Phillmore et al., 2002) and playback tests (Wilson & Mennill, 2010) have demonstrated that male black-capped chickadees can discriminate between *fee-bee* songs produced by individual males. Further studies are needed to determine if females can discriminate between individuals based on their songs, and how the sex of the signaler affects discrimination. In other words, can males and females discriminate between individual females based exclusively on their songs?

In addition to song containing information regarding the individual, results from our discriminant function analyses and MANOVA suggest that at least one acoustic feature, *fee* glissando, varies significantly between the sexes, and may serve as a cue to identify the sex of the signal producer. Playback studies that manipulate the *fee* glissando in male song suggest that it is a species-typical cue. When the frequency change within the *fee* note is removed (i.e., *fee* glissando flattened), males respond less aggressively (Shackleton et al., 1992) and females give fewer copulation-solicitation displays (Ratcliffe & Otter, 1996) in response to playback. The *fee* glissando has been found to remain constant at different absolute pitches within male *fee-bee* song (Christie et al., 2004b), further implicating its importance as a species-typical acoustic feature; however, only the *fee* glissando within male songs was examined. The *fee* glissando may not only be important in species recognition, but our results suggest the *fee* glissando may also be used as a sex cue. Our discriminant function analyses were able to accurately classify birds based on sex by using the *fee* glissando, and this classification was significantly greater when using the original data compared to when permuted data was classified. These discriminant function results indicate that sufficient differences exist to discriminate between male and female songs, but further examination is needed to determine if birds can perceive the difference between male and female songs, and whether the *fee* glissando is important for this discrimination. By systematically decreasing and increasing the frequency change within the *fee* note in male and female songs and presenting these manipulated songs during playback or operant discrimination tasks we can gain a better understanding of this feature's role as a sex identifier.

This study provides a description of female-produced *fee-bee* songs that are acoustically similar to male-produced song, which provides the groundwork for future studies that are needed to examine female song development, perception, and function, in addition to the song system in female black-capped chickadees. First, there have been few studies examining male song learning in black-capped chickadees under laboratory conditions (Kroodsma et al., 1995; Shackleton & Ratcliffe, 1993). Baker et al. (2003) examined the vocal development of *fee-bee* songs in the field, and found that the structure of songs produced by fledglings was similar to adult song, although songs of juveniles often contained more than the typical two notes. Baker et al. (2003) did not identify the sex of the juvenile birds, so it is unknown whether the fledglings producing song were males or females. Kroodsma et al. (1995) found that young female birds tape-tutored in the lab produced subsong (described as “long, continuous, subdued ‘warbling’”; Kroodsma et al., 1995), but as adults these females did not produce songs. In the current study, all birds were wild-caught as adults before they were brought into the lab, and presumably had normal vocal development in the wild. Most male songbirds must learn their songs from a tutor (Catchpole & Slater, 2008), but there may be differences between male and female songbirds in terms of how or when song is learned. For example, male and female northern cardinals (*Cardinalis cardinalis*) commonly sing, but females, which learn their songs faster compared to males, require exposure to conspecific songs in order for song to develop, while males do not (Yamaguchi, 2001). Further studies are needed to determine when female black-capped chickadees learn to sing and from whom. Second, our results suggest that there are sufficient acoustic differences in *fee-bee* songs that birds could use to perceive an individual’s sex. Behavioural tests can provide insight into the perception of male versus female songs, and whether behavioural response varies based on the sex of the listening chickadee. In other songbird species, male and females differ in their song discrimination abilities. For example, red-wing blackbird (*Agelaius phoeniceus*) males are able to discriminate between their mate’s songs and other females’ songs (Beletsky, 1983b), while females cannot discriminate one individual female from another (Beletsky, 1983a). Third, studies examining the function of female song in this species are needed. From our analysis, we cannot make any conclusions regarding the function of female *fee-bee* song. One possibility is that female song is used for mate recognition. In order for a signal to be used for mate recognition, it needs to contain information regarding the individual’s identity and sex. Our results suggest that both of the above assumptions are met with female *fee-bee* song, and as such, it may be a signal used for communication between mates. To best understand the function of female song, female song needs to be observed in the field. A field study would allow researchers to access the social and/or ecological contexts under which females produce song, and this type of observational study is the first step in determining the song's function.

With the demonstration that female black-capped chickadees can produce songs, further studies into the female song system in this species are needed. Songbirds are commonly used as a model for human language development, because of the many parallels between birdsong and human speech, including analogous neural pathways important for vocalization and language learning (Doupe & Kuhl, 1999). In the songbird brain, the song system, which is important for song learning, production, and discrimination (Doupe & Kuhl, 1999), is generally smaller in females, especially in females of species that do not sing (Ball & MacDougall-Shackleton, 2001). Often, only male songbirds are used as neurobiological models, because in many species, only males learn their songs. In black-capped chickadees, males must learn their songs (Shackleton & Ratcliffe, 1993) and both sexes learn aspects of their calls (Hughes et al., 1998), additionally, both the behaviour and vocalizations of this species have been extensively studied, making it a useful model species. When birds hear song, neural changes are initiated by immediate early gene expression. In chickadees, the sex of the signal receiver and producer affects the expression of an immediate early gene (i.e., ZENK); presentation of male song results in more immediate early gene expression than female song in some areas of the brain, while there is no difference in gene expression when females are presented with male versus female song (Avey et al., 2008a). It may be the case that for listening females, there was no observed difference in the levels of immediate early gene expression either because females do not discriminate between the sexes based on song or because females normally only respond to male songs in the wild, and as such, their brain activation is high for all similar signals. However, before we can make these conclusions, more research is needed to examine the function of female-produced song (i.e., its context specificity) and to examine the similarities and differences in the song system (including song learning and development) of males and females black-capped chickadees.

Conclusion

Our results demonstrate that male and female black-capped chickadees produce individually distinctive song, and at least one acoustic feature (i.e., *fee* glissando) varies between songs produced by the different sexes. Descriptions of female song in this species have been limited (Dwight, 1897; Hill & Lein, 1987), and we encourage researchers to examine the presence of female song in other temperate songbird species. Future research should focus on the development, perception, and function of female black-capped chickadee song. In addition, the female song system in this species needs to be examined further in order to gain a better understanding of song in this species.

Table 5-1. Means, standard deviations, coefficients of variation between individuals (CVb), coefficients of variation within an individual (CVw), and potential for individual coding value (PIC) for each feature measured in *fee-bee* songs.

	Total Duration (ms)	*Fee* Proportional Duration	*Fee* Glissando	Interval Ratio	Relative Amplitude	RMS Ratio	*Bee* Start Frequency (Hz)
Both Sexes							
mean	963.85	0.42	1.11	1.12	1.02	1.26	3279.97
SD	86.36	0.04	0.04	0.04	0.17	0.58	184.18
CV_b	8.96	8.88	4.04	4.00	16.28	46.38	5.62
CV_w	5.56	6.88	3.27	2.78	12.91	38.44	3.62
PIC	1.61	1.29	1.23	1.44	1.26	1.21	1.55
Females							
mean	966.31	0.42	1.12	1.13	1.00	1.24	3250.94
SD	100.61	0.05	0.05	0.05	0.15	0.63	201.55
CV_b	10.41	10.84	4.32	4.71	14.76	50.68	6.20
CV_w	6.72	8.73	3.70	3.37	13.19	41.44	3.67
PIC	1.55	1.24	1.17	1.40	1.12	1.22	1.69
Males							
mean	961.25	0.42	1.10	1.11	1.04	1.28	3310.77
SD	68.24	0.03	0.04	0.03	0.18	0.53	158.52
CV_b	7.10	6.20	3.31	2.92	17.41	41.73	4.79
CV_w	4.30	4.84	2.80	2.12	12.59	35.14	3.56
PIC	1.65	1.28	1.18	1.37	1.38	1.19	1.34

Table 5-2. The original and cross-validated percentage of correct classifications by individual using a stepwise discriminant function analysis, for all birds (i.e., both sexes), females only, and males only. For each discriminant function analysis conducted, the eigenvalue, squared canonical correlation coefficient (R^2_c), and the acoustic features with the two largest (respectively) standardized (Std coeff) and structure coefficients (Str coeff) for the first discriminant function is listed. The two acoustic features with the largest potential for individual coding values (PIC) for each group are also given. TD: total duration; IR: interval ratio; RA: relative amplitude; BSF: *bee* start frequency.

Group	Original	Cross-Validated	Eigenvalue	R_c^2	Std coeff	Str coeff	PIC
Both Sexes	55.7	48.0	1.47	0.59	TD, IR	TD, IR	TD, BSF
Female	70.7	62.0	1.50	0.59	TD, BSF	TD, BSF	BSF, TD
Male	65.3	59.2	1.98	0.67	TD, RA	TD, RA	TD, RA

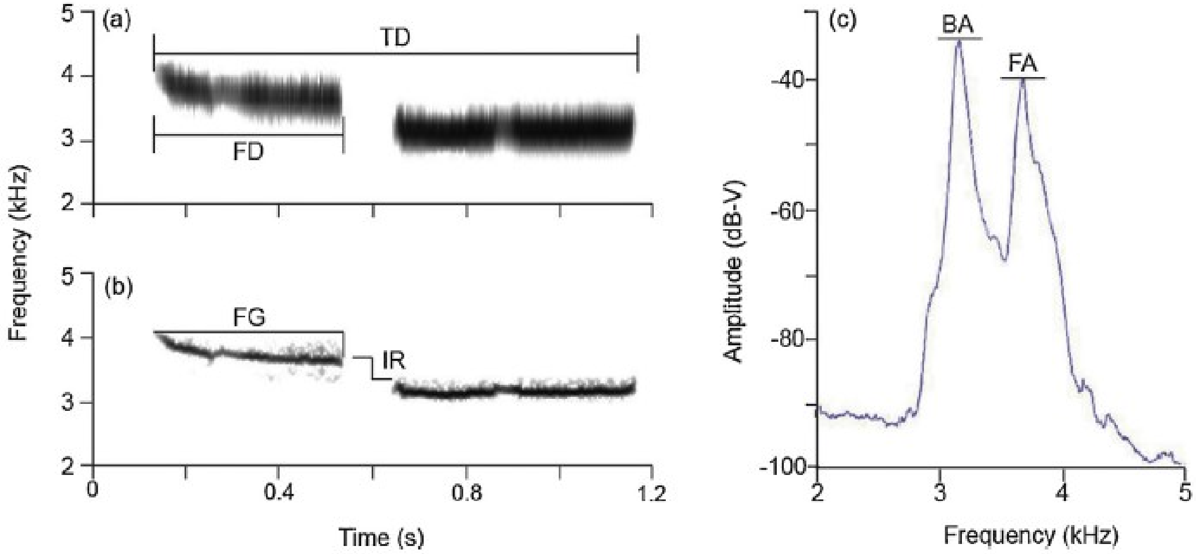

Figure 5-1. Sound spectrogram and power spectrum depicting acoustic measurements made in *fee-bee* songs. (a) Sound spectrogram (time resolution = 5.8 ms); measurements included: TD: total duration of song and FD: *fee* note duration (measured as a proportion of *fee* duration to total song duration). (b) Sound spectrogram (frequency resolution = 43.1 Hz); measurements included: FG: *fee* glissando (ratio of frequency decrease within *fee* note) and IR: interval ratio (frequency ratio between the notes). (c) Power spectrum (FFT window = 65,536 points; 88 Hz smoothing); measurements included: BA: *bee* note amplitude and FA: *fee* note amplitude.

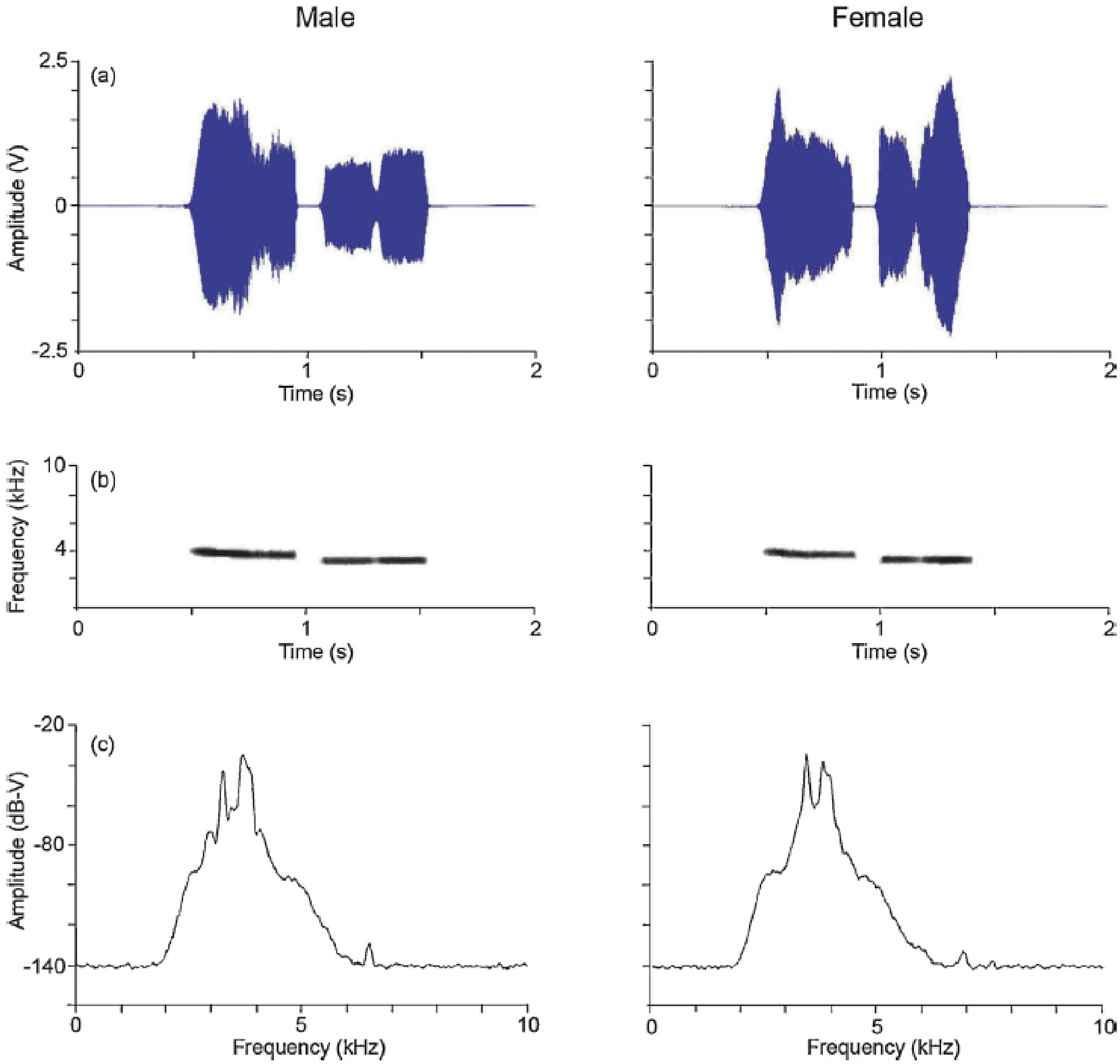

Figure 5-2. Comparison of male and female black-capped chickadee *fee-bee* songs, showing the overall structural similarity of the songs produced by each sex. (a) Oscillogram of male and female song. (b) Hanning sound spectrogram (FFT window = 256 points; −35 to 0 dB relative to song peak amplitude) of male and female song. (c) Power spectrum (FFT window = 65,536 points; 88 Hz smoothing) of male and female song.

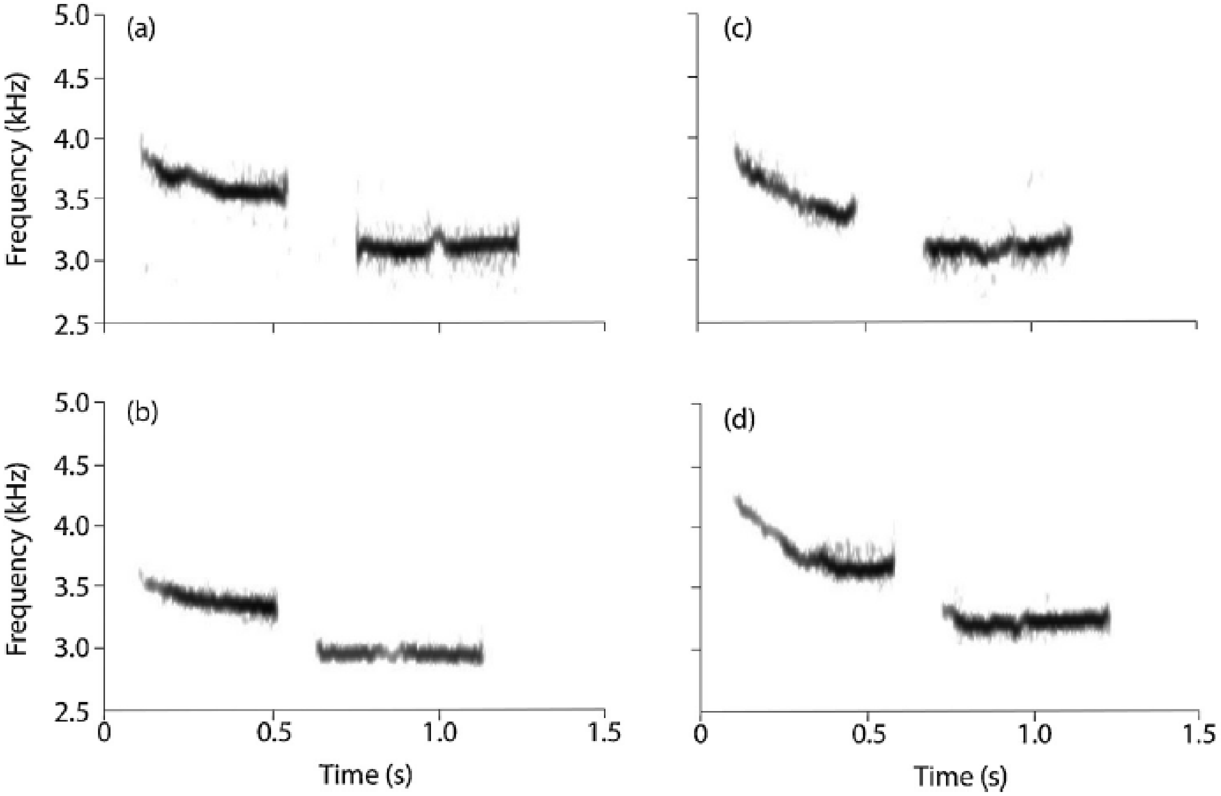

Figure 5-3. Sound spectrograms (FFT window = 1024 points; −35 to 0 dB relative to song peak amplitude) of representative fee-bee songs showing frequency (kHz, y-axis) by time (seconds, x-axis). Songs produced by males (a and b) and songs produced by females (c and d).

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Chapter 6 Biological salience influences performance and acoustic mechanisms for discrimination of male and female songs[3]

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Introduction

Darwin (1859) proposed the theory of sexual selection, describing males as the sex that produces elaborate traits for male-male competition or mate attraction. Male bird song offers an example of elaborate traits arising through a process of sexual selection: males produce songs to advertise territory to rival males and attract females (for review see Catchpole & Slater, 2008). However, male songbirds are not the only sex that produces songs. In the tropics, female song production is common (Slater & Mann, 2004), and while it is frequently stated that only male songbirds produce songs in North Temperate Zone species (for reviews see Langmore, 1998; Riebel, 2003), recent evidence suggests that female song is common (Garamszegi, Pavlova, Eens, & Møller, 2007; Odom, Hall, Riebel, Omland, & Langmore, 2014).

The ability to discriminate between male and female songs is important for songbirds, as the information contained within songs varies with the producer of the acoustic signal and the intended receiver (e.g., mate attraction versus territoriality). For songs to be effective as communication signals, the receiver needs to recognize and respond appropriately to the acoustic signals (e.g., mate recognition, Lind, Dabelsteen, & McGregor, 1996; recognition of territory neighbours, Brooks & Falls, 1975). In order for birds to discriminate between male and female songs, the songs must contain discriminable acoustic features. Differences in the singing behaviour and song structure of male and female songs is evident in various species (e.g., white-crowned sparrows, *Zonotrichia leucophrys*, Baptista, Trail, DeWolfe, & Morton, 1993; bellbirds, *Anthornis melanura,* Brunton & Li, 2006; European robins, *Erithacus rubecula,* Hoelzel, 1986; European starlings, *Sturnus vulgaris,* Pavlova, Pinxten, & Eens, 2005). Even for species in which males and females produce acoustically similar vocalizations, it would be advantageous for birds to quickly assess the sex of a singing individual.

Open-ended categorization (as described by Herrnstein, 1990) is a strategy that allows individuals to classify signals based on perceptual similarity. With open-ended categorization, animals can learn a general 'category' rule for all signals containing perceptually similar features. For example, *chick-a-dee* calls have been shown to belong to open-ended categories based on the species producing the call (e.g., mountain chickadee calls versus black-capped chickadee calls, Bloomfield, Farrell, & Sturdy, 2008). With open-ended categorization, birds can discriminate between species based solely on vocalizations and without prior experience with the calling individual. Open-ended categorization would also allow birds to rapidly evaluate the sex of a singing individual, classify the signal according to sex of the singer, and to generalize unfamiliar songs. The rationale for using open-ended categorization rather than rote memorization for successful discrimination is that if signals are more perceptually similar to other signals within the same group or category, an individual can form a general rule to classify signals into that category. In contrast, to discriminate via rote memorization, an individual must have experience with and memorize all exemplars. If the signals to be discriminated do not contain a discernible feature that allows them to be categorized, rote memorization must be used. Because rote memorization requires memorization of each signal, while open-ended categorization does not, open-ended categorization is a more flexible cognitive process.

In order for open-ended categorization to be an effective strategy, songs must contain at least one feature that is more similar within the category than between categories. Operant discrimination tasks have provided evidence that songbirds use open-ended perceptual category mechanisms to discriminate biologically important acoustic signals, such as conspecific vocalizations (e.g., European starlings, Braaten, 2000; Gentner & Hulse, 1998; zebra finches, *Taeniopygia guttata*, Sturdy, Phillmore, Price, & Weisman, 1999; black-capped chickadees, *Poecile atricapillus,* and mountain chickadees, *P. gambeli,* Bloomfield & Sturdy, 2008). However, to our knowledge, no studies have used this type of task to examine the perceptual categorization of songs based on sex of the singer.

Recently it was reported that both male and female black-capped chickadees produce songs (Hahn, Krysler, & Sturdy, 2013b) and that these songs induce different amounts of immediate early gene expression, depending on the sex of the bird producing the song and the sex of the bird hearing the song (Avey, Kanyo, Irwin, & Sturdy, 2008). Black-capped chickadee songs are a relatively simple two note, tonal signal called the *fee-bee* song and the songs produced by males and females contain overall structural similarity (i.e., songs of each sex are tonal and contain two notes; Hahn et al., 2013b). The first note in the song (i.e., *fee* note) is produced at a higher frequency relative to the second note (i.e., *bee* note), and, across song renditions, males are able to maintain a consistent relative frequency between the two notes (Horn, Leonard, Ratcliffe, Shackleton, & Weisman, 1992; Weisman, Ratcliffe, Johnsrude, & Hurly, 1990). Although the songs produced by males and females are acoustically similar overall, there is at least one acoustic feature that differs between the sexes, called the *fee* glissando (Hahn et al., 2013b). The *fee* glissando is a frequency decrease within the *fee* note and is highly stereotypic in male songs (Christie, Mennill, & Ratcliffe, 2004). The *fee* glissando is an important acoustic cue for the perception of song; flattening the *fee* glissando in male songs results in less aggressive responses from rival males (Shackleton, Ratcliffe, & Weary, 1992) and fewer copulation-solicitation displays from females (Ratcliffe & Otter, 1996). Male *fee-bee* songs are used to attract mates and repel rival males, so the ability to quickly identify the sex of a singing individual would be beneficial, thus saving time and energy trying to mate with the wrong sex or repelling a potential mate. A category perception-like mechanism would allow birds to determine the sex of a vocalizing bird without previous experience with that individual and before visual contact is available.

In the current study, we use an operant go/no-go paradigm to examine (a) if male and female songs belong to acoustic categories that black-capped chickadees can perceive, and (b) the underlying proximate mechanisms of this sex-based discrimination. In experiments 1 and 2, we trained black-capped chickadees on an operant discrimination task, and tested birds using songs in which we manipulated acoustic features. We were interested in examining if birds use acoustic features in one or both notes within the song, and specifically if birds use the *fee* glissando when discriminating between the sexes. In experiment 3, we trained artificial neural networks (ANNs) using the same stimuli that the birds discriminated in experiment 2. We trained ANNs in order to clarify the results from the first two experiments, using a model that does not have any biological confounds. Ultimately, this work will provide valuable insights into the perceptual mechanisms behind a sex-based discrimination of songs in this species and these results will also add to a growing body of literature on female song in a temperate songbird species.

Experiment 1

Methods

Subjects

Seventeen black-capped chickadees (eight males and nine females, identified by DNA analysis; Griffiths, Double, Orr & Dawson, 1998) were tested between December 2012 and April 2013. Birds at least one year of age (determined by examining the color and shape of their outer tail retrices; Pyle, 1997) 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 between January 2010 and February 2012.

Prior to the experiment, birds were individually housed in Jupiter Parakeet cages (30 × 40 × 40 cm, Rolf C. Hagen, Inc., Montreal, QB, Canada) in colony rooms containing other black-capped chickadees. Birds had visual and auditory, but not physical, contact with one another. Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St Louis, MO, USA), water (vitamin supplemented on alternating days; Prime vitamin supplement; Hagen, Inc.), grit, and cuttlebone. Birds were given three to five sunflower seeds daily, one superworm (*Zophobas morio*) three times a week, and a mixture of greens (spinach or parsley) and eggs twice a week. Birds were maintained on a light:dark cycle that mimicked the natural light cycle for Edmonton, Alberta, Canada.

Throughout the experiment, birds were housed individually in operant chambers (see apparatus below), maintained on the natural light cycle, and had *ad libitum* access to water (vitamin supplemented on alternate days), grit, and cuttlebone. Birds were given two superworms daily (one in the morning and one in the afternoon). Food (i.e., Mazuri) was only available as a reward for correct responding during the operant discrimination task. Birds had previous experience discriminating synthetic tones or musical chords and other *fee-bee* song stimuli (Hoeschele, Weisman, Guillette, Hahn, & Sturdy, 2013; Hoeschele, Cook, Guillette, Hahn, & Sturdy, 2014; Hahn, unpublished data), but no operant experience with the stimuli used in this experiment. Birds serving as subjects did not produce any of the vocalizations used as stimuli. In experiment 1, some birds were housed in the same colony room as the birds that produced the songs used as stimuli (seven birds were housed in the same colony room as 13 birds whose vocalizations were used as stimuli: three in 'male song' group, two in the 'female song’ group, two in ‘pseudo’ group; 10 birds were housed in the same colony room as 16 birds whose vocalizations were used as stimuli: three in ‘male song’ group; four in ‘female song’ group; three in ‘pseudo’ group)]. However, subject birds did not previously hear the particular songs that were used as stimuli during the experiment, because all songs used as stimuli were recorded in the laboratory by placing an individual bird in a sound attenuating chamber and recording spontaneous vocalizations (see Hahn et al., 2013b for details). In experiment 2, the birds used as subjects were housed in a separate colony room from the birds whose songs were used as stimuli. Some of our song stimuli were recorded from birds captured in the same location as the experimental birds. However, all of the experimental birds were captured at least one year after the recorded birds, so it is unlikely that the experimental birds had prior experience with birds whose vocalizations we used, and they did not have experience with the specific song stimuli recorded in the laboratory.

Apparatus

During the experiment, birds were housed individually in modified colony room cages (30 × 40 × 40 cm) placed inside a ventilated, sound-attenuating chamber. The chambers were illuminated by a 9-W, full spectrum fluorescent bulb. Each cage contained three perches, a water bottle, and a grit cup. An opening on the side of the cage (11 × 16 cm) gave each bird access to a motor-driven feeder (see Njegovan, Hilhorst, Ferguson, & Weisman, 1994). Infrared cells in the feeder and the request perch (perch closest to the feeder) monitored the position of the bird. A personal computer connected to a single-board computer (Palya & Walter, 2001) scheduled trials and recorded responses to stimuli. Stimuli were played from a CD through either a Cambridge A300 Integrated Amplifier, Cambridge Azur 640A Integrated Amplifier (Cambridge Audio, London, England), or an NAD310 Integrated Amplifier (NAD Electronics, London, England)

and through a Fostex FE108 Σ or Fostex FE108E Σ full-range speaker (Fostex Corp., Japan; frequency response range 80-18 000 Hz) located beside the feeder. See Sturdy and Weisman (2006) for a detailed description of the apparatus.

Acoustic stimuli

A total of 64 (32 male; 32 female) *fee-bee* songs recorded in our laboratory were used as stimuli in experiment 1. During the experiment, stimuli were presented at approximately 75 dB as measured by a Brüel & Kjær Type 2239 (Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark) decibel meter (A weighting, slow response) at the approximate height and position of a bird's head when on the request perch. See below for more specific details regarding stimuli preparation.

Natural stimuli. Sixteen male songs and 16 female songs were used as stimuli during discrimination training. We included one vocalization per individual as stimuli during discrimination training. An additional five songs from each sex were used during transfer (see Procedure below). Songs used as stimuli during Transfer were produced by individuals not heard during discrimination training. Vocalizations were of high quality (i.e., no audible interference and low background noise when viewed on a spectrogram with amplitude cutoffs of -35 to 0 dB relative to song peak amplitude). Songs were bandpass filtered (2000-5000 Hz; outside the frequency range of songs) using GoldWave version 5.58 (GoldWave, Inc., St. John's, NL, Canada). For each stimulus, 5 ms of silence was added to the leading and trailing portion of the song and tapered to remove transients, and amplitude was equalized using SIGNAL 5.10.29 software (Engineering Design, Berkeley, CA, USA). Stimuli used during test sessions were further prepared as described below.

Spliced songs. Spliced song stimuli were created to test if birds were using information in one of the two song notes (i.e., *fee* or *bee* note) when discriminating between male and female songs. Songs ($N = 14$) were bandpass filtered in Goldwave (see *Natural Stimuli* above) and the *fee* and *bee* notes of seven male and seven female songs were edited into individual WAV files using SIGNAL. Four types of spliced songs were created: M-M (*fee* and *bee* notes from male songs); F-F (*fee* and *bee* notes from female songs); M-F (*fee* note from a male song and *bee* note from a female song); F-M (*fee* note from a female song and *bee* note from a male song). For each pair of songs to be used as spliced stimuli, the length of the *fee* and the *bee* note were roughly equal (range for difference between the two *fee* notes and two *bee* notes were: 0.23-14.31 ms and 11.14-36.59 ms, respectively; these measurements were within one standard deviation of all male and female songs measured and analyzed in Hahn et al. 2013b; $X_{\textit{fee}\text{ note}} = 53.34$ ms, $X_{\textit{bee}\text{ note}} = 53.08$ ms). When creating the spliced songs, we held the internote interval constant for all stimuli at 100 ms, which is similar to the internote interval found in natural songs ($X_{\text{internote}} = 135$ ms, Ficken, Ficken, & Witkin, 1978; $X_{\text{internote}} = 114$ ms, based on measurements by Hahn et al., 2013b) and the internote interval used by other studies manipulating song features (e.g., $X_{\text{internote}} = 100$ ms, Hoeschele, Guillette, & Sturdy, 2012).

Fee glissando manipulated songs. We created stimuli with a manipulated *fee* glissando to determine if birds were using the frequency decrease within the *fee* note (i.e., *fee* glissando) to discriminate between male and female songs. To create the *fee* glissando manipulated stimuli, we used four male and four female songs that had a natural *fee* glissando that approximated the average *fee* glissando from all songs presented during discrimination training (mean ± SD for *fee* glissando from training stimuli = 1.088 ± 0.036; range for *fee* glissando of songs used as *fee* glissando manipulated stimuli = 1.082-1.095). Each song was manipulated in four ways:

increased to a *fee* glissando frequency ratio of 1.17 (2 SD greater than natural), increased to a frequency ratio of 1.13 (1 SD greater than natural), decreased to a frequency ratio of 1.05 (1 SD less than natural), and decreased to a frequency ratio of 1.01 (2 SD less than natural). The natural song was also presented (i.e., unmanipulated), in order to compare the proportion of responding to the *fee* glissando manipulated stimuli to the unmanipulated song. This resulted in ten different types of *fee* glissando manipulated songs: female and male songs with a *fee* glissando decreased by 2 SD (F-d2; M-d2), decreased by 1 SD (F-d1; M-d1), unmanipulated (F-unman; M-unman), increased by 1 SD (F-in1; M-in1), and increased by 2 SD (F-in2; M-in2). See Figure 6-1 for sample spectrograms of *fee* glissando manipulated stimuli.

Procedure

A summary of the procedural design is provided in Figure 6-2. Below we describe the details about each experimental stage.

Pretraining. Pretraining began once the bird learned to use the request perch and feeder to obtain food. During pretraining, birds received food for responding to all stimuli that would be presented during the experiment. A trial began when the bird landed on the request perch and remained for between 900-1100 ms. A randomly-selected stimulus played without replacement until all 96 stimuli had been heard. If the bird left the request perch before a stimulus finished playing, the trial was considered interrupted, and the houselight turned off for 30 s. If the bird entered the feeder within 1 s after the entire stimulus played, it was given 1 s access to food, followed by a 30-s intertrial interval (period during which the bird could not trigger a new stimulus) with the houselight on. If a bird remained on the request perch during the stimulus presentation and the 1 s following the completion of the stimulus it received a 60-s intertrial interval with the houselight on, but this intertrial interval was terminated if the bird left the request perch. This was to encourage a high level of responding on all trials. Birds continued on pretraining until they completed six 192-trial blocks of ≥ 60% responding on average to all stimuli, at least four 192-trial blocks ≤ 3% difference in responding to future rewarded and unrewarded stimuli, and at least four 192-trial blocks in which the bird had ≥ 60% feeding to spliced and *fee* glissando manipulated songs to ensure birds were also responding to these stimuli. Pretraining was used to ensure birds were responding to all songs that would be used as stimuli during the experiment and that birds were responding equally to future discrimination stimuli.

Discrimination training. The procedure was the same as during pretraining; however, only 16 male and 16 female songs were presented and birds were differentially reinforced for responding to certain songs. Responses to rewarded songs resulted in 1 s access to food, as during pretraining. Responses to unrewarded songs, resulted in a 30-s intertrial interval with the houselight off. Discrimination training continued until birds completed three 192-trial blocks with a discrimination ratio (DR) ≥ 0.75 with the last two blocks being consecutive. For DR calculations see response measures, below.

Birds were randomly assigned to either a true category discrimination group ($N = 12$) or pseudo category discrimination group ($N = 5$). Black-capped chickadees in the true category discrimination group were divided into two subgroups: one subgroup discriminated rewarded male songs from unrewarded female songs (male song discrimination Group; three male and three female subjects), while the other subgroup discriminated rewarded female songs from unrewarded male songs (female song discrimination group; three male and three female subjects).

The pseudo category discrimination group was also divided into two subgroups. Each subgroup discriminated 16 randomly-selected rewarded songs (eight male songs; eight female songs) from 16 unrewarded songs (eight male songs; eight female songs; subgroup 1: one male and two female subjects; subgroup 2: one male and one female subject).

Discrimination-85. This phase was identical to discrimination training, except that rewarded songs were reinforced with a reduced probability (i.e., $P = 0.85$). On 15% of trials, when a discrimination stimulus in the rewarded song category played entering the feeder resulted in a 30-s intertrial interval, during which the houselight remained on, but there was no access to food. This was employed to prepare birds for transfer and manipulated test trials in which stimuli were neither rewarded nor punished. Discrimination-85 training continued until birds completed two consecutive 192-trial blocks with a DR ≥ 0.75.

Transfer. During transfer, the stimuli and reinforcement contingencies from discrimination-85 were maintained. In addition, ten untrained songs (five male and five female songs not heard during discrimination) were introduced. If birds in the true category groups are using open-ended categorization, birds should continue to respond to songs produced by the sex that were associated with reward during discrimination training (e.g., birds reinforced for responding to male songs should continue to respond to novel male songs during transfer). Birds in the pseudo category group should respond nondifferentially to male and female songs during transfer. These new (i.e., transfer) stimuli were each presented once during a 202-trial block (songs from discrimination-85 were presented six times each; randomly-selected without replacement). Responses to transfer stimuli resulted in a 30-s intertrial interval with the houselight on, but no access to food (i.e., trials were neither rewarded nor punished). All birds completed a minimum of 3 blocks of transfer and these were all included for analysis. Following

Transfer, birds completed one block of discrimination-85 training with a DR ≥ 0.75 before moving onto the remaining test sessions. Data from two birds (one male in the male song group and one male in the female song group) had to be eliminated from the analysis of this experimental stage due to errors during the experimental program.

Spliced and fee glissando manipulated songs. The remaining test stimuli (i.e., 14 spliced songs and 40 *fee* glissando manipulated songs) were divided into five test sessions. Similar to transfer, these sessions contained: the 32 stimuli from discrimination-85, each presented six times; two or three spliced songs, each presented once; and eight *fee* glissando manipulated songs, each presented once. This resulted in a 202- or 203-trial block. During a given test session, each bird completed three blocks. After each session, birds completed one block with a DR ≥ 0.75 of discrimination-85 training before starting the next test session. Although an individual song used as a stimulus during these test sessions was manipulated in multiple ways (e.g., the *fee* glissando from an individual song was increased and decreased to produce more than one test stimulus), only one of these manipulated songs produced by a given individual was presented during each test session. Thus there were numerous stimuli presented between birds hearing various manipulations of a given song. The order each bird completed these test sessions was pseudo-randomized (between discrimination group and sex). One bird (a female in the female song group) did not have data from a test session due to a program error; in the analyses, we only included data for the spliced and *fee* glissando manipulated songs from the other five sessions.

Response measures

For each stimulus exemplar, a percent response was calculated by the following formula: R+/(N-I), where R+ is the number of trials in which the bird went to the feeder, N is the total number of trials, and *I* is the number of interrupted trials in which the bird left the perch before the entire stimulus played. For discrimination and discrimination-85 training, we calculated a discrimination ratio (DR), by dividing the mean percent response to all rewarded stimuli by the mean percent response of rewarded stimuli plus the mean percent response of unrewarded stimuli. A DR of 0.50 indicates equal responding to rewarded and unrewarded stimuli, whereas a DR of 1.00 indicates perfect discrimination.

To examine how many of the rewarded stimuli were well-discriminated, we adapted the two-tailed 95% confidence interval (CI) using the mean (*M*) and standard deviation (SD) of the percent response for all unrewarded stimuli on the final day of discrimination training, using the following formula: $\mathrm{CI} = M \pm 1.96 \times \mathrm{SD}$. If birds are significantly discriminating an individual rewarded stimulus from unrewarded stimuli, the percent response to that rewarded stimulus would thus be greater than the 95% CI.

To compare responding to manipulated stimuli in a manner that accounted for individual differences in response levels among subjects, we scaled the proportion of response for each individual subject. The highest proportion of response to a manipulated stimulus was rescaled to 1.0 and the proportion of response to all other stimuli were rescaled as a ratio of the highest proportion of responding. This rescaling was conducted separately for transfer stimuli, spliced stimuli and *fee* glissando manipulated stimuli.

Statistical analyses

We conducted analyses of variance (ANOVAs) on the number of trials to criterion and DRs for the true and pseudo category groups. The number of trials required to reach criterion during discrimination training differed among birds. In order to compare the discrimination data across birds, we Vincentized the learning curves from discrimination to ten blocks of data (the minimum number of blocks it took a bird to reach criterion). We calculated the Vincentized blocks by taking the total number of trial blocks until an individual bird reached criterion (e.g., 26)and divided that number by ten (in this example, 2 with a remainder of 6). We calculated the Vincentized blocks by placing the total number of trial blocks a bird completed into groups the size of the whole-number quotient (in this example, 2). For this example, the 1st and 2nd trial blocks the bird completed were placed into the first Vincentized block, the 3rd and 4th trial blocks the bird completed were placed into the second Vincentized block, etc. The 5th and 6th Vincentized blocks would contain additional trial blocks based on how large the remainder was after the total number of trial blocks was divided by 10 (in this example the remainder equaled 6, and this remainder is evenly placed into the 5th and 6th Vincentized blocks, so the 5th and 6th Vincentized blocks would each contain 2 + 3 = 5 actual trial blocks grouped into it). The DRs for all blocks grouped into a Vincentized block were then averaged (see Kling & Riggs, 1971; Hoeschele et al., 2012). By using Vincentized blocks, we could compare a bird that took relatively few trials to reach criterion to a bird that took twice as many trials to reach criterion. We examined the number of rewarded stimuli responded to greater than the 95% CI to determine if the number of exemplars responded to differed among groups. We conducted additional ANOVAs and Tukey's planned comparisons on the proportion of responding to the manipulated stimuli. We conducted parallel analyses using the arcsine square root transformation of the proportion of responses to correct for non-normality, using the following formula: arcsin[sqrt(x)], where x equals the untransformed proportion of responding. In most cases, analysis of transformed and untransformed data generated a similar pattern of results and levels of significance, so only the untransformed data is reported. We indicate any differences between the transformed and untransformed data in the sections below. All statistics were conducted in Statistica v10 (StatSoft, Inc)

Ethical note

Throughout experiments 1 and 2, we monitored the birds daily, provided *ad libitum* water, grit, and cuttlebone, and provided each bird with two superworms. Birds remained in the testing apparatus throughout testing, minimizing the transport and handling of each bird. Following the experiment, birds were returned to the colony room for use in future experiments. One bird in experiment 2 was removed from the experiment and returned to the colony room because it injured its leg while in the operant chamber; the bird subsequently returned to good health. All other birds remained healthy during the experiment. All procedures were conducted in accordance with the Canadian Council on Animal Care (CCAC) Guidelines and Policies with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta (AUP 108), which is consistent with the ABS Guidelines for the Use of Animals in Research. Birds were captured and research was conducted under an Environment Canada Canadian Wildlife Service Scientific permit, Alberta Fish and Wildlife Capture and Research permits, and City of Edmonton Parks Permit.

Results

Comparison of true and pseudo category discriminations

Trials to criterion. For each discrimination group, the average number of 192-trial blocks to reach criterion were: male song group, $X = 26.67$; female song group, $X = 29.33$; pseudo category group, $X = 50.2$. We conducted a sex × discrimination group (male song, female song) ANOVA on the number of 192-trial blocks to reach criterion. There was a significant main effect of sex ($F_{1,8} = 7.66$, $P = 0.024$) with males ($X = 19.83$) reaching criterion faster compared to females ($X = 36.17$). There was no significant main effect of discrimination group and there was no significant interaction (Fs ≤ 1.69, Ps ≥ 0.23), indicating that there was no significant difference in the number of trials to reach criterion for birds in the two true category groups.

We conducted a one-way ANOVA on the number of 192-trial blocks for the two pseudo category discrimination groups (pseudo group 1, pseudo group 2). There was no significant difference ($F_{3,13} = 1.08$, $P = 0.39$) in the number of trials to reach criterion for birds in the two pseudo category groups, so we combined the two groups in the remaining analyses.

We conducted an independent-samples *t* test on the number of 192-trial blocks to reach criterion for the true category and pseudo category groups. There were no significant difference in the discrimination performance between the true and pseudo category groups ($t_{15} = 1.51$, $P = 0.15$).

Acquisition (DR comparison). To examine task acquisition across blocks, we conducted a repeated measures ANOVA with sex and discrimination group (male song, female song, pseudo) as categorical predictors and the DR calculated for each Vincentized block (1-10) as dependent variables. There was a significant main effect for Vincentized block ($F_{9,\ 99} = 106.0$, $P < 0.001$). As Vincentized blocks increased, the DRs increased. There were no other significant main effects or interactions (Fs ≤ 2.8, Ps ≥ 0.07), indicating no significant difference in task acquisition for birds learning a true category discrimination compared to pseudo category discrimination.

Number of rewarded songs >95% confidence interval (CI). Individuals can obtain a high DR by responding to only a few rewarded stimuli, while responding less to all unrewarded and the remaining rewarded stimuli. To examine how many of the rewarded stimuli were well-discriminated, we determined how many rewarded stimuli were responded to > 95% CI during the final block of discrimination training. We conducted a sex × discrimination group (male song, female song) ANOVA to determine if the two true category groups differed in the number of exemplars responded to greater than the 95% CI. There were no significant main effects or interactions (Fs ≤ 5.26, Ps ≥ 0.051). Out of 16 rewarded stimuli, the mean ± SE number of stimuli responded to > 95% CI by male and female subjects, respectively, was 12.0 ± 1.53 and 14.67 ± 0.33 in the male song group, and 9.0 ± 2.08 and 13.67 ± 1.86, in the female song group.

We also examined the number of rewarded songs that were significantly (> 95% CI) discriminated by comparing the two true category groups to the pseudo category group. For this analysis, we conducted a sex × discrimination group (male song, female song, pseudo) ANOVA to determine if the pseudo category group differed in the number of exemplars responded to >95% CI compared to the true category groups. There was a significant main effect of sex ($F_{1,11} = 6.02$, $P = 0.032$), but no other main effects or interactions. Overall, female birds significantly discriminated a mean ± SE of 14.56 ± 0.63 songs out of 16 songs, while male birds significantly discriminated a mean of 10.88 ± 1.26 songs out of 16 songs. There was no difference in the number of rewarded songs well discriminated between the true category or pseudo category groups.

Test sessions

Transfer. Following discrimination-85 training, birds in both the true category and pseudo category conditions were intermittently presented with male and female songs not presented during discrimination training. We conducted a sex × discrimination group (male song, female song, pseudo) × stimulus type (male song, female song) ANOVA on the scaled proportion of response. There were no significant main effects or interactions (Fs ≤ 2.53, Ps ≥ 0.13). See Figure 6-3.

Spliced songs. We conducted a sex × discrimination group (male song, female song) × stimulus type (F-F, M-M, M-F, F-M) ANOVA on the scaled proportion of response to the spliced songs. This analysis revealed a significant stimulus type × discrimination group interaction ($F_{3,24} = 5.10$, $P = 0.007$) and a significant stimulus type × discrimination group × sex interaction ($F_{3,24} = 3.83$, $P = 0.023$). We conducted planned comparisons on the scaled percentage of responses to the different spliced stimuli by birds in the male song group. For this analysis, we compared the spliced control (i.e., M-M) stimuli to the other three spliced song types. Birds responded significantly more to M-M spliced songs compared to F-F spliced songs ($t = 3.41$, $P = 0.009$). We also conducted planned comparisons for birds in the male song group by examining the responding of each sex separately and found that females responded significantly more to M-M spliced songs compared to M-F spliced songs ($t = 3.17$, $P = 0.013$) and to F-F spliced songs ($t = 3.004$, $P = 0.017$). When the same analysis was conducted on the arcsine transformed data, M-M spliced songs were significantly different compared to M-F spliced songs ($t = 2.53$, $P = 0.035$), but not F-F spliced songs ($t = 2.29$, $P = 0.052$). Male birds in the male song group responded significantly more to M-F spliced songs compared to F-F spliced songs ($t = 2.63$, $P = 0.030$).

We conducted similar planned comparisons on the scaled proportion of responses to the different spliced songs by birds in the female song group. For this analysis we compared the spliced control (i.e., F-F) stimuli to the other three spliced song types. Birds responded significantly more to F-F spliced songs compared to M-M spliced songs ($t = 2.32$, $P = 0.048$). We also conducted planned comparisons for birds in the female song group by examining the responding of each sex separately. For the analysis with the transformed data, female birds in the female song group responded significantly more to M-F spliced songs compared to M-M spliced songs ($t = 2.39$, $P = 0.044$), this significant difference was not found with the untransformed data ($t = 2.20$, $P = 0.059$). There were no significant differences for male birds in the female song group. Overall, these results show that birds responded significantly more to spliced control songs associated with the reinforced category (i.e., M-M for male song group; F-F for female song group) compared to spliced control songs associated with the unreinforced category (i.e., F-F for male song group; M-M for female song group), and responding to the spliced songs varied for male and female subjects. See Figure 6-4.

Fee glissando manipulated songs. We conducted a sex × discrimination group (male song, female song) × stimulus type (F-d2, F-d1, F-unman, F-in1, F-in2, M-d2, M-d1, M-unman, M-in1, M-in2) ANOVA on the scaled proportion of response to the songs in which the *fee* glissando was manipulated. This analysis revealed a main effect of discrimination group ($F_{1,8} = 7.81$, $p = 0.023$) and a significant stimulus type × discrimination group interaction ($F_{9,72} = 5.24$, $P < 0.001$). We conducted planned comparisons on the scaled proportion of responses to the different *fee* glissando manipulated songs by birds in the male song group. For this analysis, we compared the control stimuli (i.e., unmanipulated male songs) to the other manipulated song types. There was a significant difference in responding to all female song stimuli, with birds responding more to the unmanipulated male songs: *fee* glissando decreased by two SD ($t = 3.61$, $P = 0.007$), *fee* glissando decreased by one SD ($t = 2.85$, $P = 0.021$), unmanipulated *fee* glissando ($t = 3.93$, $P = 0.004$), *fee* glissando increased by one SD ($t = 3.81$, $P = 0.005$), and *fee* glissando increased by two SD ($t = 4.98$, $P < 0.001$). In addition, responding to male songs with the *fee* glissando increased by one SD ($t = 2.80$, $P = 0.023$) and two SD ($t = 4.18$, $P = 0.003$) was significantly lower compared to the unmanipulated male songs. We conducted similar planned comparisons for birds in the female song group. For this analysis, we compared the control stimuli (i.e., unmanipulated female songs) to the other manipulated song types. The analysis revealed that responding to male songs with a *fee* glissando increased by two SD was significantly lower compared to the responding to unmanipulated female song stimuli ($t = 2.57$, $P = 0.028$). See Figure 6-5.

Experiment 2

Results from experiment 1 suggested that birds in the pseudo category group learned the task as fast as birds in the true category group; however, the low number of training stimuli used during experiment 1 may have led to this result. In order to better evaluate the discrimination of male and female songs while using a true/pseudo category paradigm, we increased the number of stimuli used during discrimination training in experiment 2. We expected that, with more songs to discriminate, birds in the pseudo discrimination group (i.e., relying on rote memorization) would require more trials to learn the discrimination compared to birds using a categorization strategy in the true category group. In addition, there were two minor differences between the test sessions in experiments 1 and 2. In experiment 2, we presented birds within single-note songs to observe how birds would respond to each note individually (i.e., without acoustic information from the other note). For example, would birds respond to acoustic information within a *fee* note when it is not presented in combination with a *bee* note? We also included additional incremental modifications of the *fee* glissando manipulated songs in experiment 2. We increased and decreased the *fee* glissando up to three standard deviations away from the average, compared to the two standard deviations that we manipulated in experiment 1.

Methods

Subjects

Twenty-four black-capped chickadees (12 males and 12 females, identified by DNA analysis; Griffiths et al., 1998) were tested between September 2013 and June 2014. Birds at least one year of age were captured from the same locations as experiment 1 in January 2013, February 2013 and January 2014. Birds were naïve to the operant discrimination apparatus. The housing conditions prior to testing and the experimental apparatus were the same as experiment 1.

Acoustic stimuli

A total of 122 (61 male; 61 female) *fee-bee* songs recorded in our laboratory were used as stimuli in the current experiment. Stimuli presentation remained the same as in experiment 1. See below for more specific details regarding stimuli preparation.

Natural stimuli. Thirty male songs and 30 female songs were used as stimuli during discrimination training. An additional 15 songs from each sex were used as natural stimuli during transfer. During discrimination training, we included songs produced by eight males and eight females (four songs each from seven birds of each sex and two songs from one bird of each sex). During transfer, we presented songs produced by ten males and ten females (two songs each from five birds of each sex and one song from an additional five birds of each sex). The songs used during transfer were produced by birds not heard during discrimination training. Stimuli were processed in the same manner as in experiment 1. Stimuli used during additional test sessions were further prepared as described below.

Spliced songs. Songs ($N = 16$) were bandpass filtered in Goldwave (see *Natural Stimuli,* experiment 1) and the *fee* and *bee* notes of eight male and eight female songs were edited into individual WAV files using SIGNAL. We created the spliced songs following the same procedure as experiment 1; however, the individual songs used for the spliced songs were different between the two experiments.

Fee glissando manipulated songs. We manipulated the *fee* glissando of four male and four female songs. The songs used as stimuli had a natural *fee* glissando that was within 0.5 SD of the average *fee* glissando from all songs presented during discrimination training (mean ± SD for *fee* glissando from training stimuli = 1.11 ± 0.028; range for *fee* glissando of songs used as *fee* glissando manipulated stimuli = 1.096-1.124). Each song was manipulated six ways: increased to a *fee* glissando frequency ratio of 1.19 (3 SD), increased to a frequency ratio of 1.17 (2 SD), increased to a frequency ratio of 1.14 (1 SD), decreased to a frequency ratio of 1.08 (1 SD), decreased to a frequency ratio of 1.05 (2 SD), and decreased to a frequency ratio of 1.03 (3 SD). We also presented the birds with the natural (i.e., unmanipulated) songs in order to compare the responding to manipulated and unmanipulated stimuli. This resulted in 14 different types of *fee* glissando manipulated songs: female and male songs with a *fee* glissando decreased by 3 SD (F-d3; M-d3), decreased by 2 SD (F-d2; M-d2), decreased by 1 SD (F-d1; M-d1), unmanipulated (F-unman; M-unman), increased by 1 SD (F-in1; M-in1), increased by 2 SD (F-in2; M-in2), and increased by 3 SD (F-in3; M-in3).

Procedure

Pretraining. Once the bird learned to use the request perch and feeder to obtain food, pretraining began. As in experiment 1, during pretraining, birds received food for responding to all future stimuli. Birds remained on pretraining until they had consistently high response rates for future discrimination stimuli (either responding to ≥ 60% of trials for six blocks or responding to ≥ 30% of trials for twelve blocks) and manipulated stimuli (either responding to ≥

60% of trials for four blocks or responding to ≥ 30% of trials for eight blocks), and approximately equal responding (≤ 3% difference) to future discrimination stimuli.

Discrimination training. As in experiment 1, birds were trained on a sex-based discrimination; however, in experiment 2, 30 male and 30 female songs were presented (half of these songs were used as rewarded stimuli and half were used as unrewarded stimuli). Discrimination training continued until birds completed three 300-trial blocks with a DR ≥ 0.75 with the last two blocks occurring consecutively. The same discrimination groups used in experiment 1 were used in experiment 2. Sixteen birds were randomly assigned to a true category group (male song discrimination group: four males, three females; female song discrimination group: four males, five females). Eight birds were randomly assigned to a pseudo category discrimination group (four males, four females). One bird (a male in female song group) was removed from the experiment because it did not reach criterion after one hundred 300-trial blocks (30 000 trials). Two birds (one male in male song group; one male in pseudo category group) were removed from the experiment because of equipment failure early in discrimination training. One bird (a female in female song group) was removed from the experiment because of a procedural error (moved onto discrimination without meeting the pretraining criteria). One bird (female in female song group) injured its leg while in the operant chamber and was removed from the experiment (see Ethical note above). This resulted in six birds in the male song group (three females; three males), six birds in the female song group (three females; three males), and seven birds in the pseudo category group (four females; three males). Discrimination data for one bird (a female in pseudo category group) was lost due to equipment failure and is not included in the analysis of discrimination training.

Discrimination-85. As in experiment 1, this phase was identical to discrimination training except that rewarded songs were reinforced with a reduced probability (i.e., $P = 0.85$). The procedure for this phase was identical to experiment 1 and birds remained on discrimination-85 until completing two consecutive 300-trial blocks with a DR ≥ 0.75.

Transfer. The procedures during transfer were identical to experiment 1 with the exception that 30 songs not heard during discrimination (15 male and 15 female songs) were each presented once during a 330-trial block (discrimination stimuli were presented five times each; randomly-selected without replacement). All birds completed a minimum of three blocks of transfer and these were included for analysis. Following transfer, birds completed one block of discrimination-85 training with a DR ≥ 0.75 before moving onto the next test session.

Spliced, single note, and fee glissando manipulated songs. The remaining test stimuli (i.e., 16 spliced songs, 16 single note songs, and 56 *fee* glissando manipulated songs) were divided into eight test sessions. Similar to transfer, these sessions contained the 60 stimuli from discrimination-85, each presented five times, two or three spliced songs, two or three single note songs, and eight *fee* glissando manipulated songs, each presented once. This resulted a 312- or 313-trial block. All other procedures during these test sessions remained the same as in experiment 1. One bird (a female in the male song group) did not have data from a test session due to a program error; in the analyses, we only included data for the spliced, single note, and *fee* glissando manipulated stimuli from the other seven sessions.

Response measures

Percent response, DRs and the 95% CIs were calculated as in experiment 1. To compare responding to manipulated stimuli, we scaled the proportion of response for each individual subject, as we did in experiment 1. This rescaling was conducted separately for transfer, spliced, single note, and *fee* glissando manipulated stimuli.

Statistical analyses

To compare the number of trials to reach criterion across birds during discrimination training, we Vincentized the learning curves from discrimination to 15 blocks of data (the minimum number of blocks it took a bird to reach criterion) following the same methods as experiment 1. The remaining analyses were the same as experiment 1.

Results

Comparison of true category and pseudo category discriminations

Trials to criterion. For each discrimination group, the average number of 300-trial blocks to reach criterion were: male song group, $X = 26.83$; female song group, $X = 32.67$; pseudo category group, $X = 34.83$. We conducted a sex × discrimination group (male song group, female song group) ANOVA on the number of 300-trial blocks to criterion. There were no significant main effects or interactions (Fs ≤ 0.81, Ps ≥ 0.39), indicating that birds rewarded for responding to male songs discriminated as quickly as birds rewarded for responding to female songs.

We conducted a sex × discrimination group (pseudo group 1, pseudo group 2) ANOVA on the number of 300-trial blocks to reach criterion for the two pseudo category discrimination groups. There were no significant main effects or interactions (Fs ≤ 0.38, Ps ≥ 0.60). Because there were no differences between the two pseudo category groups, we combined the two groups in the remaining analyses.

To compare the performance of birds in the true and pseudo category groups, we conducted a sex × discrimination group (male song, female song, pseudo) ANOVA on the number of 300-trial blocks to reach criterion. There were no significant main effects or interactions (*F*s ≤ 0.74, *P*s ≥ 0.41), indicating that birds in the pseudo category group discriminated as quickly as birds in the true category group.

Acquisition (DR comparison). To examine task acquisition across blocks, we conducted a repeated measures ANOVA with sex and discrimination group (male song, female song, pseudo) as categorical predictors and the DR calculated for each Vincentized block (1-15) as dependent variables. There was a significant main effect for Vincentized block ($F_{14, 168} = 101.0$, $P < 0.001$). As Vincentized blocks increased, the DRs increased. There were no other significant main effects or interactions (*F*s ≤ 1.82, *P*s ≥ 0.20), indicating no difference in task acquisition for birds learning a true category discrimination compared to pseudo category discrimination.

Number of rewarded songs >95% confidence interval (CI). In order to examine how many of the rewarded stimuli were well discriminated, we determined how many rewarded stimuli were responded to > 95% CI during the final block of discrimination training, as we did in experiment 1. We conducted a sex × discrimination group (male song, female song) ANOVA to determine if the two true groups differed in the number of exemplars responded to >95% CI. There were no significant main effects or interactions (*F*s ≤ 2.56, *P*s ≥ 0.15). Out of 30 rewarded stimuli, the means ± SE number of stimuli responded to > 95% CI by male and female subjects, respectively, was 19.0 ± 5.29 and 26.33 ± 2.19 in the male song group, and 28.0 ± 1.15 and 23.0 ± 5.03 in female song group.

We also examined the number of rewarded songs that were significantly (>95% CI) discriminated by comparing the two true category groups to the pseudo category group. For this analysis, we conducted a sex × discrimination group (male song, female song, pseudo) ANOVA to determine whether the pseudo category group differed from the true category groups in the number of exemplars responded >95% CI. There were no significant main effects or interactions (Fs ≤ 2.72, Ps ≥ 0.07). Out of 30 rewarded stimuli, the means ± SE number of stimuli responded to >95% CI was 22.67 ± 3.04 for the male song group, 25.5 ± 2.57 for the female song group, and 24.33 ± 2.076 for the pseudo category group.

Test sessions

Transfer. Once birds in the true category and pseudo category discrimination groups had reached discrimination criterion, we presented the birds with novel male and female songs. We conducted a sex × discrimination group (male song, female song, pseudo) × stimulus type (male song, female song) ANOVA on the scaled proportion of response. There was a significant main effect of discrimination group ($F_{2,\,12} = 13.5$, $P < 0.001$), a significant stimulus type × discrimination group interaction ($F_{2,\,12} = 25.7$, $P < 0.001$), and a significant stimulus type × sex interaction ($F_{1,\,12} = 16.6$, $P = 0.002$). To examine the discrimination group × stimulus type interaction further, we conducted Tukey's post hoc analyses. Birds in the male song group responded significantly more to male songs ($X = 0.76$) compared to female songs ($X = 0.56$), $P = 0.003$. Birds in the female song group responded significantly more to female songs ($X = 0.36$) compared to male songs ($X = 0.19$), $P = 0.01$. Birds in the pseudo category group did not respond significantly differently to male ($X = 0.39$) or female ($X = 0.46$) songs, $P = 0.12$ (Figure 6-3). To examine the stimulus type × sex interaction we conducted a Tukey's post hoc analysis: male birds responded significantly more to female songs ($X = 0.48$) compared to male songs ($X = 0.39$), $P = 0.014$.

Spliced songs. We conducted a sex × discrimination group (male song, female song) × stimulus type (F-F, M-M, M-F, F-M) ANOVA on the scaled proportion of response to the spliced songs. The analysis revealed a significant discrimination group main effect ($F_{1,8} = 17.65$,

$P = 0.003$) and a significant discrimination group × stimulus type interaction ($F_{3,24} = 3.37$, $P = 0.035$). We conducted planned comparisons on the scaled proportion of responses to the different spliced song types by birds in the male song group. Birds responded significantly more to M-M spliced songs compared to F-M spliced songs ($t = 2.83$, $P = 0.022$). We conducted similar planned comparisons for birds in the female song group, but there were no significant differences. When we conducted the analysis with the arcsine square root transformed data there was a significant discrimination group main effect ($F_{1,8} = 17.03$, $P = 0.003$), but there were no significant interactions or other main effects (all Ps ≥ 0.078). See Figure 6-4.

Single notes. We conducted a sex × discrimination group (male song, female song) × stimulus type (male *fee*, male *bee*, female *fee*, female *bee*) ANOVA on the scaled proportion of response to the single note songs. This analysis revealed a significant discrimination group × stimulus type interaction ($F_{3,24} = 7.66$, $P < 0.001$). We conducted planned comparisons to examine the proportion of responding by birds in the male song group. Birds responded significantly more to male *fee* notes compared to female *fee* notes ($t = 4.44$, $P = 0.002$). We conducted similar planned comparisons for birds in the female song group. There was a significantly higher proportion of responding to female *fee* notes compared to male *fee* notes ($t = 4.71$, $P = 0.002$) and female *bee* notes compared to male *fee* notes ($t = 2.39$, $P = 0.044$). See Figure 6-6.

Fee glissando manipulated songs. We conducted a sex × discrimination group (male song, female song) × stimulus type (F- d3, F-d2, F-d1, F-unman, F-in1, F-in2, F-in3, M-d3, M-d2, M-d1, M-unman, M-in1, M-in2, M-in3) ANOVA on the scaled proportion of response to the songs in which the *fee* glissando was manipulated. This analysis revealed a significant discrimination group main effect ($F_{1,8} = 14.1$, $P = 0.006$). There was also a significant discrimination group × stimulus type interaction ($F_{13,104} = 6.66$, $P < 0.001$). We conducted planned comparisons to examine the proportion of responding by birds in the male song group. For this analysis, we compared the control stimuli (i.e., unmanipulated male songs) to the other manipulated song types. Birds responded significantly more to male unmanipulated songs compared to female songs decreased by three SD ($t = 4.20$, $P = 0.003$) and female songs decreased by one SD ($t = 2.32$, $P = 0.049$); however, this last result was not significant when the analysis was carried out with the arcsine square root transformed data ($t = 1.91$, $P = 0.092$).

We conducted similar planned comparisons to examine the proportion of responding by birds in the female song group. For this analysis, we compared the control stimuli (i.e., unmanipulated female songs) to the other manipulated song types. Birds responded significantly more to female unmanipulated songs compared to male unmanipulated songs ($t = 2.74$, $P = 0.026$). There was also significantly more responding to female unmanipulated songs compared to all male manipulated songs (male songs decreased by three SD: $t = 7.42$, $P < 0.001$, decreased by two SD: $t = 4.14$, $P = 0.003$, decreased by one SD: $t = 3.67$, $P = 0.006$, increased by one SD: $t = 2.89$, $P = 0.020$, increased by two SD: $t = 3.47$, $P = 0.008$, increased by three SD: $t = 2.35$, $P = 0.046$). See Figure 6-7.

Experiment 3: Artificial neural network

The results from experiment 2 led to some unexpected findings. First, during the spliced song tests, birds in the male song discrimination group responded similarly to spliced M-M and spliced F-F songs. Conversely, birds in the female song discrimination group responded more to spliced F-F songs compared to spliced M-M songs. This finding is interesting because the songs that the birds discriminated during discrimination training and the songs presented during the test sessions were the same for each group; only the reinforcement contingencies were different during discrimination training. It is unclear why birds in the male song group did not respond differentially between the M-M and F-F spliced songs while birds in the female song group did. Additionally, birds in the pseudo category group learned the discrimination in a similar number of trials as birds in the true category groups. There are a few possibilities that can explain this responding. It is possible that the songs used as discrimination stimuli were not sufficiently acoustically distinct, and all birds needed to rely on rote memorization to learn the discrimination. It is also possible that the songs are so salient that regardless of whether or not a bird could use categorization or needed to rely on rote memorization, the birds could learn the task with either strategy in a similar amount of trials (i.e., there was no benefit to relying on categorization because the task was easily solved with rote memorization). Also, we cannot rule out the possibility that hormone levels of the birds or the season affected the birds' discrimination abilities. To help clarify the results of experiment 2, we trained ANNs using the same stimuli as experiment 2. By using ANNs, we could train the neural networks in a manner analogous to the chickadees' training. This allowed us to: (1) examine how many training trials were required for ANNs to learn a true category discrimination compared to a pseudo category discrimination, and (2) examine how ANNs would respond when presented with novel stimuli. Each ANN's response to a novel stimulus returned a value between 0 and 1, similar to the proportion of response measure that we calculated for the chickadees.

Network architecture

The networks used in the current study used similar settings as those described in Nickerson, Bloomfield, Dawson, and Sturdy (2006) and Guillette et al. (2010). Each network had nine input units that were connected to one output unit and we trained the network using the Rosenblatt program (Dawson, 2004). Each of the nine input units corresponded to one acoustic feature within the *fee-bee* song (total duration, *fee* proportional duration, *fee* glissando, interval ratio, *fee* start frequency, *fee* end frequency, *bee* start frequency, relative amplitude, and root mean square (RMS) ratio; see Hahn et al. 2013a; 2013b for more information regarding these acoustic measurements). The output unit used a sigmoid-shaped logistic equation to transform the sum of the weighted signals from each input into an activity value that ranged between 0 and 1. The learning rate was set at 0.5 and we continued training until the output unit produced a "hit" (defined as an activity level of 0.9 or higher when the correct response was to turn "on" (i.e., rewarded stimulus) or an activity level of 0.1 or lower when the correct response was to turn "off" (i.e., unrewarded stimulus)). Prior to training, the connection weights for each network were set to a random weight between -0.1 and 0.1, so each network served as one "subject." Network responses were averaged over 10 subjects for each discrimination group (male song, female song, pseudo, with five each for pseudo category group 1 and pseudo category group 2). Pilot testing with the pseudo category groups indicated that the network never learned to classify the training stimuli with 100% accuracy, so we could not use perfect performance as the criterion to stop training. Therefore, we stopped training the network after 20,000 training sweeps, which was approximately the number of sweeps that the network reached its maximum number of hits (X = 22.8). ANNs trained to respond to male songs took an average ± SD of 695.9 ± 5.04 sweeps to reach criterion, and ANNs trained to respond to female songs took an average ± SD of 696.5 ± 4.43 sweeps.

Results

Transfer

To examine how the networks responded to the transfer stimuli, we conducted a discrimination group (male song, female song, pseudo) × stimulus type (male song, female song)

ANOVA on the proportion of response. There was a significant main effect of discrimination group ($F_{2,\,27} = 3.90$, $P = 0.033$), and a significant discrimination group × stimulus type interaction ($F_{2,\,27} = 325.86$, $P < 0.001$). To examine the discrimination group × stimulus type interaction further, we conducted Tukey's post hoc analyses. Networks in the male song group responded significantly more to male songs ($X = 0.84$) compared to female songs ($X = 0.09$), $P < 0.001$. Networks in the female song group responded significantly more to female songs ($X = 0.91$) compared to male songs ($X = 0.16$), $P < 0.001$. Networks in the pseudo category group did not respond significantly differently to male ($X = 0.50$) or female ($X = 0.50$) songs, $P = 1.00$. See Figure 6-3.

Spliced and *fee* glissando manipulated songs

To compare the response of networks trained in the male song discrimination and female song discrimination groups to the different spliced stimuli types, we conducted a discrimination group (male song, female song) × stimulus type (F-F, M-M, M-F, F-M) ANOVA on the proportion of response to the spliced songs. The analysis revealed a significant discrimination group main effect ($F_{1,18} = 2\ 605\ 678$, $P < 0.001$) and a significant discrimination group × stimulus type interaction ($F_{3,54} = 575\ 191\ 839$, $P < 0.001$; Figure 6-4).

To examine the network response to the different *fee* glissando manipulated songs, we conducted a discrimination group (male song, female song) × stimulus type (F-d3, F-d2, F-d1, F-unman, F-in1, F-in2, F-in3, M-d3, M-d2, M-d1, M-unman, M-in1, M-in2, M-in3) ANOVA on the proportion of response to the songs in which the *fee* glissando was manipulated. This analysis revealed a significant discrimination group main effect ($F_{1,18} = 5\ 175\ 011$, $P < 0.001$) and a significant discrimination group × stimulus type interaction ($F_{13,234} = 2\ 454\ 875\ 000$, $P < 0.001$). ANNs in the male song group responded near 0 when the *fee* glissando was increased, and near

1.0 when the *fee* glissando was decreased. The opposite responding was found for ANNs in the female song group: when the *fee* glissando was increased, the responding was near 1.0, and when the *fee* glissando was decreased, the responding was near 0.

Discussion

Previous studies have described differences in song structure and singing behavior between male and female songbirds (e.g., Baptista et al., 1993; Brunton & Li, 2006; Hoelzel, 1986; Pavlova et al., 2005), including black-capped chickadees (Hahn et al., 2013b). In the current study, we report on the results of sex-based discriminations of black-capped chickadee songs in which we had two primary aims: (1) to determine if *fee-bee* songs belong to categories defined by the sex of the singer, and (2) to uncover the underlying mechanism for discriminating male and female songs. We tested ANNs in an analogous manner, because unlike chickadees, songs are not a biologically relevant signal to ANNs and ANNs have no experiential factors influencing their response. Overall, our results suggest that male and female songs belong to separate perceptual categories. Results from the ANNs suggest that acoustic differences in the *fee* glissando of male and female songs is a sufficient mechanism for a sex-based discrimination; however, results from chickadees suggest that birds may also use other features within one or both of the notes when discriminating between the sexes.

Experiments 1 and 2: True category and pseudo category discrimination task

The rationale for using a true category/pseudo category discrimination task is that if songs belong to open-ended categories, then birds completing a true category discrimination should be able to learn the task in fewer trials compared to birds completing a pseudo category discrimination, the latter of which requires rote memorization to learn the task. In experiment 1, we found no difference in the speed of acquisition between the true category and pseudo category groups. In experiment 2, although we used almost double the number of training stimuli, we again found no difference in the speed of acquisition between the true category and pseudo category groups. This suggests that either: (1) all birds were using rote memorization to complete the task, or (2) there was no benefit (in terms of how long it took to learn the discrimination) to use open-ended categorization over rote memorization. The response by birds presented with untrained (i.e., transfer or manipulated) stimuli provides evidence for the latter. Our results provide evidence that birds in the true category group were using open-ended categorization. For example, in experiment 2, birds in the true category group demonstrated generalization when presented with the untrained transfer stimuli (e.g., birds reinforced for responding to males songs during discrimination continued to respond significantly more to untrained male songs compared to untrained female songs during transfer), while birds in the pseudo category group responded equally to the untrained male and female songs. In experiment 1, we found a similar but non-significant trend suggesting that birds were capable of generalizing to untrained exemplars during transfer. See Figure 6-3. Additionally, in experiment 1, when birds were presented with the spliced songs, they responded significantly more to the spliced songs that contained both *fee* and *bee* notes that were produced by the sex whose songs were rewarded during discrimination compared to spliced songs that contained two notes produced by the sex whose songs were unrewarded (e.g., birds reinforced for responding to male songs responded significantly more to M-M songs compared to F-F songs).

Experiments 1 and 2: Acoustic mechanisms

Following acquisition and transfer, we presented birds in the true category group with songs that we manipulated in order to determine the mechanism underlying the sex-based discrimination of songs. In experiments 1 and 2, we presented spliced songs and songs in which we altered the *fee* glissando; in experiment 2, we also presented individual *fee* and *bee* notes.

Responses during the test sessions suggest that birds learned a sex-based rule during discrimination training. During experiment 1, birds responded significantly more to the spliced control songs associated with the rewarded category of songs (e.g., M-M songs for birds rewarded for responding to male songs) compared to the spliced control songs associated with the unrewarded category. During experiment 2, there was a similar, but non-significant, trend in responding for birds rewarded for responding to female songs (see Figure 6-4). Birds rewarded for responding to male songs responded more to M-M spliced songs compared to F-M spliced songs, suggesting that they may have been using features in the *fee* note when responding.

To further examine if birds could use acoustic information in only one note, in the absence of information from the other note, we presented birds with *fee* and *bee* notes individually. Birds rewarded for responding to male songs during discrimination training, responded the most to male *fee* notes and the least to female *fee* notes, while responses to both male and female *bee* notes was intermediate. This suggests that there was sufficient information in the *fee* notes for birds to identify sex, which is in line with our previous bioacoustic analyses (Hahn et al., 2013b). There are two possibilities for why birds responded equally to male and female *bee* notes: (1) there is no acoustic difference between male and female *bee* notes (i.e., *bee* notes contain no sex information), or (2) there are acoustic differences, but birds were not attending to these features when discriminating between male and female songs. Results from birds rewarded for responding to female songs provide evidence for the latter explanation. Birds in this discrimination group responded the most to female *fee* notes and female *bee* notes and responded the least to male *fee* notes while responses to male *bee* notes at intermediate levels.

Birds responded more to both female notes presented singly compared to male *fee* notes, suggesting that there are acoustic features within *bee* notes that do in fact contain sex cues (Figure 6-6); however, because birds in this group did not respond differentially between male and female *bee* notes, it suggests that either there are less salient acoustic differences in *bee* notes compared to *fee* notes, or that the birds relied on information in the *bee* notes less than in *fee* notes when discriminating.

It is possible that the acoustic differences in *fee* notes are so salient that during discrimination training, birds mostly used only differences within the *fee* note, and during the single note test sessions when birds were presented with single *bee* notes, they responded nondifferentially. It is also possible that because *bee* notes are usually produced following a *fee* note, hearing a *bee* note individually is so unlikely that it is a less salient signal. A previous playback study found that while chickadees distinguish between *fee* and *bee* notes, when presented with each note-type singly, chickadees respond more slowly to single *bee* notes compared to single *fee* notes (Ratcliffe & Weisman, 1986). This is in line with our results suggesting that birds rely less on *bee* notes for sex discrimination. Although *bee* notes may be less used for discrimination than *fee* notes, the response by birds rewarded for responding to female songs suggests that birds did learn something about the *bee* notes. This provides evidence for the presence of acoustic features within the *bee* note that contain sex information.

Overall, the responses to the single note stimuli suggests that birds were mostly attending to acoustic features within the *fee* note. The use of features within the *fee* note is in line with our previous bioacoustic analyses, which found that the *fee* glissando was statistically different between male and female songs (Hahn et al., 2013b). However, communication signals contain many potential cues (more than was measured in the previous bioacoustic analysis) that could be used by birds when discriminating. Observed patterns of responding during the spliced or single note songs cannot provide evidence that birds were only using one feature (e.g., *fee* glissando) when performing this task. In order to better understand if birds were specifically using the *fee* glissando (i.e., the feature that the bioacoustic analysis suggests contains the most sex information) during the discrimination task, we presented birds with songs in which we experimentally manipulated the *fee* glissando.

In a previous study with black-capped chickadees, experimentally manipulating the frequency of notes (i.e., A, B, C, D notes) within the *chick-a-dee* call influenced how birds responded to the notes, suggesting a change in the perception of the notes (Charrier, Lee, Bloomfield, & Sturdy, 2005). For example, A notes were made to sound more “B-like” by decreasing the absolute frequency, while B notes were made to sound more “A-like” by increasing the absolute frequency. We used a similar rationale when manipulating the *fee* glissando of songs in the current study, with the expectation that increasing the *fee* glissando of male songs would make the male songs sound more “female-like.” During the manipulated *fee* glissando tests, there was some indication that birds were attending to the frequency decrease within the *fee* notes. In experiment 1, when birds that were rewarded for responding to male songs were presented with male songs in which the *fee* glissando was increased, birds responded less (Figure 6-5), suggesting that male songs can be made to sound “less male” by artificially increasing the *fee* glissando. In experiment 2, this pattern of responding by birds rewarded for responding to female songs appeared to occur incrementally: as the *fee* glissando increased in male songs, responding increased, and as the *fee* glissando was flattened in female songs, responding decreased (Figure 6-7); however, increasing the *fee* glissando in female songs did not lead to more responding compared to unmanipulated female songs. This suggests that there might be a ceiling effect, or a point of manipulation at which the songs cannot be made to sound more "female." Based on our results we cannot conclude that birds use only the *fee* glissando to discriminate between male and female songs, since birds could also use information within the *bee* note or other cues within the *fee* note when differentiating between the sexes. For example, a female song whose *fee* glissando is decreased still contains acoustic cues (either in the *bee* note, or other cues within the *fee* note) indicating that it is a female song. In order to examine if birds can use acoustic cues within the *fee* glissando, in the absence of information in the *bee* note, we could present birds with individual *fee* notes in which we manipulate the *fee* glissando. This would be similar to the manipulations of the *chick-a-dee* call conducted by Charrier et al. (2005) in which individual note-types were manipulated and presented. Results from the present study suggest that birds will respond to single *fee* notes; if birds are using the *fee* glissando to discriminate between male and female songs we would expect to see differential responding to manipulated *fee* notes. For example, birds rewarded for responding to female songs would respond more to either male or female *fee* notes in which the *fee* glissando is increased, compared to male or female *fee* notes in which the *fee* glissando is decreased.

Sex differences

Previous operant discrimination tasks with black-capped chickadees have found no difference in responding between the sexes (e.g., Bloomfield et al. 2008; Charrier et al. 2005; Guillette, Reddon, Hurd, & Sturdy, 2009; but see Hoeschele et al. 2012 for a significant sex difference); however, we found a few notable sex differences in the current study. First, during transfer in experiment 2, males responded significantly more to female songs compared to male songs (regardless of which songs were rewarded during discrimination). The function of female song in this species is unknown, but it may function as a signal directed at mates. This may explain why males responded more to female songs compared to male songs, while there was no difference for female subjects, as female songs may not be directed toward or usually heard by other females. In red-winged blackbirds (*Agelaius phoeniceus*), females do not discriminate between the songs of other females (Beletsky, 1983a), while males do discriminate between their mate's song and another female's song (Beletsky, 1983b). In black-capped chickadees, a previous neurobiological study found more immediate early gene expression in male birds presented with male songs compared to female songs, but no difference in gene expression for female birds presented with male or female songs (Avey et al. 2008), indicating a sex difference in the perception of male and female songs.

In experiment 1, when we examined the number of rewarded stimuli that were significantly discriminated (>95% CI), we found that females discriminated significantly more rewarded stimuli compared to males. However, this result was most likely driven by the birds in the pseudo category group, because there was no sex difference when we only compared the two true category groups. This result suggests that females are more adept at memorizing songs. In the wild, females use acoustic features in songs or singing bouts to evaluate a male's quality or dominance (see Ratcliffe, Mennill, & Schubert, 2007; Mennill, Ratcliffe, & Boag, 2002), so it is logical that females attend to and subsequently memorize this salient signal. In line with this, Hoeschele et al. (2012) found that females performed a song discrimination with a higher performance accuracy (defined as higher DRs) compared to males. Results from the current study suggest that females discriminated more accurately by significantly discriminating more of the rewarded songs.

Our results also revealed a significant sex difference in the responding to the spliced song stimuli in experiment 1. These results suggest that females respond more to the *bee* note, while male birds respond more to the *fee* note. In the wild, males need to respond quickly to a singing male, because it may be a signal of a male intruding on its territory. However, for female chickadees it would be important listen to the entire song before making a response. If a female is responding to a male song, she would want to obtain information regarding the male's quality before deciding to mate. In black-capped chickadees, male dominance cues are relative features between the two notes (Hoeschele et al. 2010; Hahn et al. 2013a) or between renditions of songs (Christie et al. 2004), which requires that females be attentive to both notes within the song.

Group differences

During the test sessions, birds in the male song discrimination group had higher overall responding compared to birds in the female song discrimination group. Whatever drove the lower responding by the female song group to the untrained stimuli was consistent between experiments 1 and 2. The two true category groups learned the same discrimination, but with opposite contingencies (i.e., the same songs were presented to both groups, but the rewarded songs for one group were the unrewarded songs for the other group, and vice versa). In experiments 1 and 2, we compared the number of rewarded stimuli that were significantly discriminated (>95% CI) and found no differences between the two true category groups. This suggests that both true category groups learned the same amount of information regarding songs to respond to during discrimination training (in other words, birds in the female song and male song groups responded to a similar number of rewarded songs). However, during the test sessions, birds in the female song group may have been demonstrating a greater degree of stimulus control by responding to rewarded songs, while responding less to any untrained songs, while birds in the male song group were more likely to generalize and respond to the untrained songs. It is possible that female songs are encountered so infrequently in the wild that birds in the female song group memorized the rewarded songs and anything else presented was considered more similar to an unrewarded song. This explanation seems likely, as the rarity of female song production by temperate songbirds is one reason that female song production is often overlooked (Langmore 1998). This bias toward the study of male songs is also true for black-capped chickadees.

The salience of the stimuli may have influenced discrimination performance and subsequent responding during the test sessions; high salience may also be a reason that we did not see a difference in performance between the true category and pseudo category groups. The *fee-bee* songs used as stimuli could be salient to the chickadees for two non-mutually exclusive reasons: (1) songs are so acoustically simple that memorization of spectral features is easy, and (2) because songs are a biologically relevant signal, the biological salience of the stimuli interacted with the rewarding salience of the stimuli during the learning task. Black-capped chickadees are able to memorize individual songs, and this memorization transfers as the songs are presented from varying distances (Phillmore, Sturdy, Turyk, & Weisman, 2002). Additionally, conspecific vocalizations are naturally salient signals, and interactions between the naturally high salience of song, along with the salience that occurs through learning paradigms (e.g., operant go/no-go task) can influence the performance of birds during the learning task (Maney, 2013). Season and corresponding hormone levels may affect the incentive salience of songbird vocalizations (e.g., Phillmore, Veysey, & Roach, 2011). In order to examine the responding to stimuli in the absence of these factors that affect salience, we tested ANNs. The benefit of using computer models, such as ANNs, is that they have no experiential or biological factors, yet can be tested on comparable operant discrimination tasks.

Experiment 3: Artificial neural network

We trained ANNs to sort the songs in an attempt to help clarify the operant discrimination results from the birds. The benefit of using ANNs is that the computer model has no biological predispositions, including experience with *fee-bee* songs, fluctuating hormone levels, or biological motivation to respond. ANNs respond solely based on the measured acoustic features within the songs. Following ANN acquisition training, we presented the ANNs with novel stimuli and examined how they responded; this was analogous to the task with chickadees. We found that ANNs could learn the true category-based discrimination, but never met criterion on the pseudo category discrimination. The ANNs performed in a manner consistent with the sex of the stimuli by forming an open-ended category, as we expected birds to do. In addition, as predicted, the true category ANNs transferred the discrimination to the untrained (i.e., transfer) exemplars, while the pseudo category ANNs did not respond differentially to male and female untrained songs. We also presented the ANNs with the spliced and *fee* glissando manipulated songs. Results revealed that features within the *fee* note, and in particular the *fee* glissando, were important acoustic features and likely a primary mechanism underlying the sex-based discrimination, corresponding with our previous bioacoustic analyses (Hahn et al., 2013b).

Conclusions

Our results provide evidence that black-capped chickadees can discriminate among songs based on the sex of the singer. Evidence from testing with untrained songs further demonstrated that the songs belong to open-ended categories which may be used by birds when discriminating among singers in the wild. The results from ANNs suggest that the *fee* glissando is an important acoustic mechanism for this sex-based discrimination; however, the results with the birds do not identify a specific mechanism. Our results suggest birds rely on information within the *fee* note the most when discriminating among male and female songs, although *bee* notes may also contain sex-based cues. We found no advantage in performance speed for birds performing a true category discrimination compared to a pseudo category discrimination, although the responses to the untrained stimuli suggests that birds in the latter group were learning a category rule. The results of this study suggest that the biological salience of the stimuli influences the discrimination abilities of the birds. When biological and experiential factors were removed by testing ANNs, patterns of responding paralleled what was predicted based on our previous statistical classification.

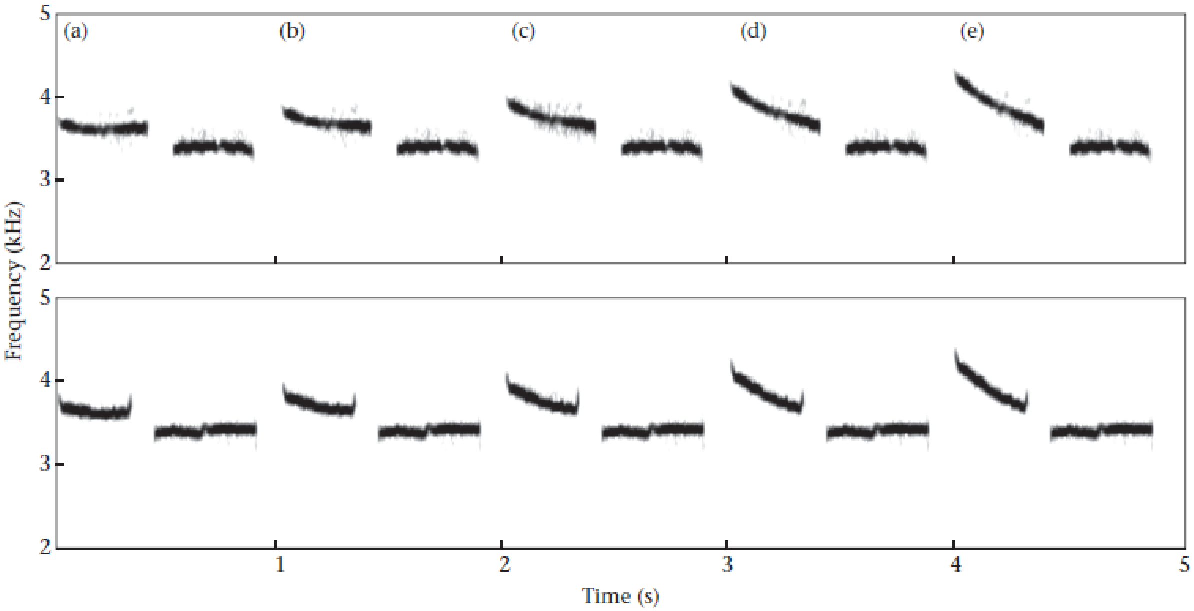

Figure 6-1. Sample sound spectrograms of male (top panel) and female (bottom panel) fee-bee songs with manipulated fee glissando. (a) Decreased by 2 SDs, (b) decreased by 1 SD, (c) unmanipulated, (d) increased by 1 SD and (e) increased by 2 SDs (transform length = 1024 points; -35 to 0 dB relative to peak amplitude; frequency resolution = 43.1 Hz).

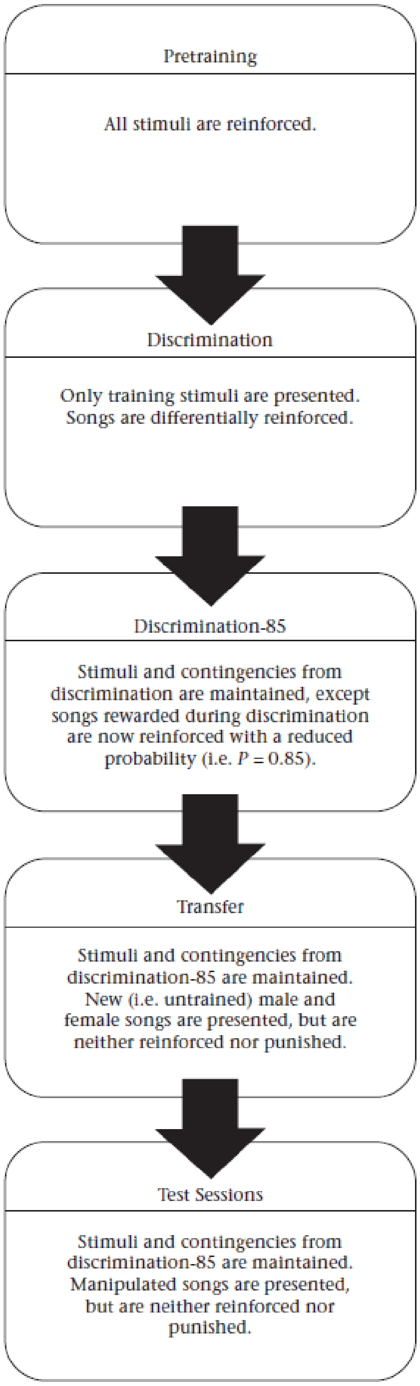

Figure 6-2. Flowchart depicting the order of the experimental procedure. The name of each stage is given in bold, followed by a short description (see text for details).

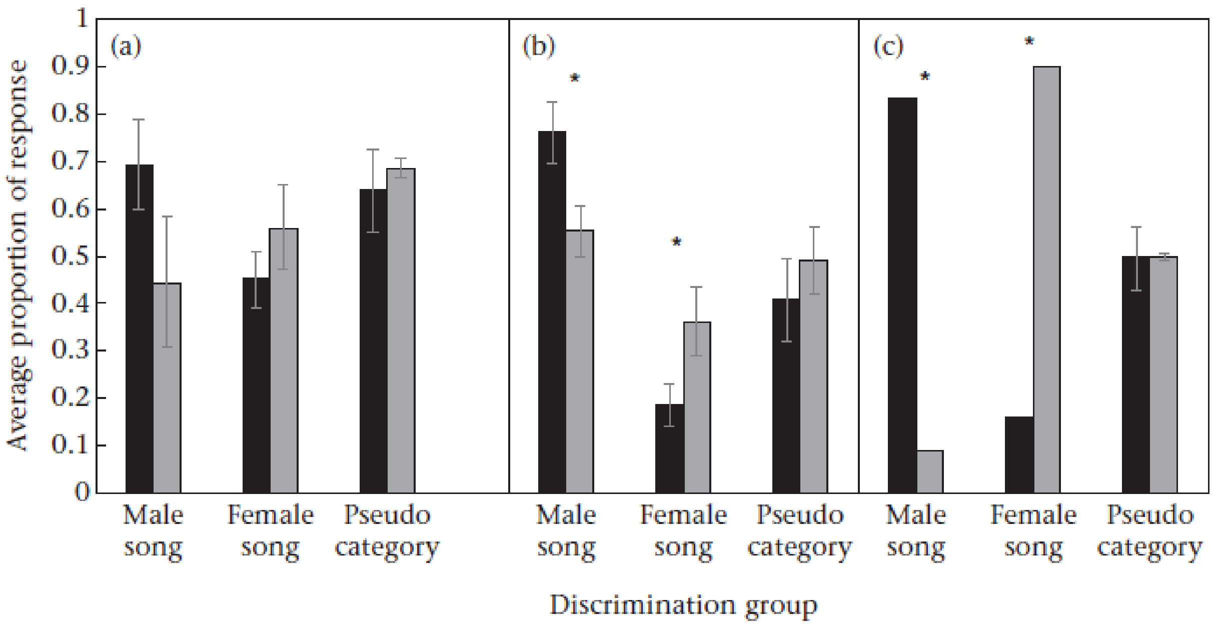

Figure 6-3. Mean ± SE proportion of response during transfer in (a) experiment 1, (b) experiment 2 and (c) experiment 3. ■ : male songs; □ : female songs. *Indicates a significant difference in responding to male and female songs by that discrimination group ($P \leq 0.05$).

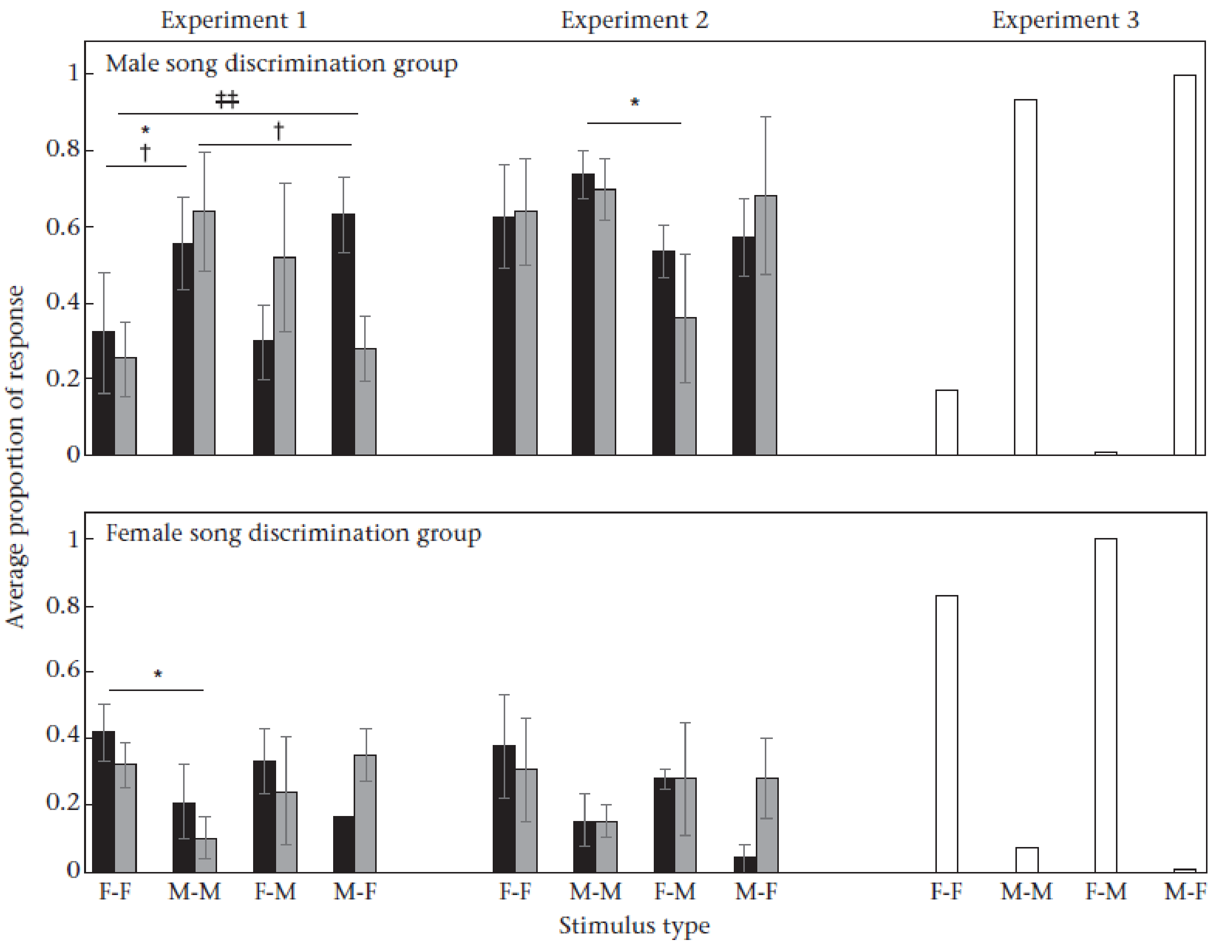

Figure 6-4. Mean ± SE proportion of response to spliced songs in experiments 1-3, ■ : male subjects; : female subjects; ▨ : ANNs. F-F: female fee note and female bee note; M-M: male fee note and male bee note; F-M: female fee note and male bee note; M-F: male fee note and female bee note. *Indicates a significant difference in responding when data from male and female subjects were combined. †Indicates a significant difference in response for female subjects. ‡‡Indicates a significant difference in response for male subjects (all *P*s ≤ 0.05). Responses by ANNs indicated a significant discrimination group) stimulus type interaction and significant discrimination group main effect (*P*s < 0.001).

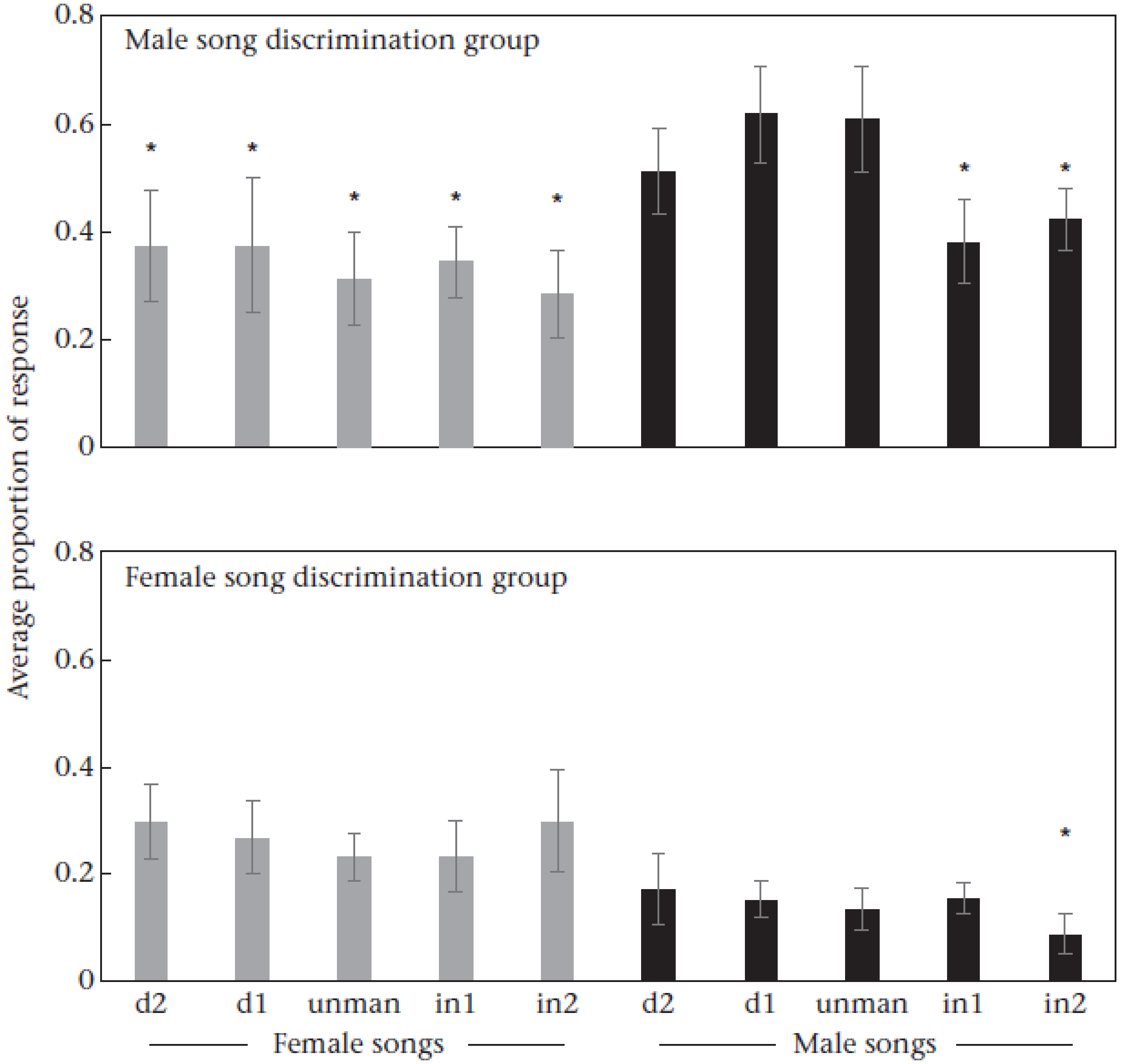

Figure 6-5. Mean ± SE proportion of response to fee glissando manipulated songs in experiment 1. ▨ : female songs; ■ : male songs. Stimuli were presented in five ways: decreased by 2 SDs (d2), decreased by 1 SD (d1), unmanipulated (unman), increased by 1 SD (in1) and increased by 2 SDs (in2). *Indicates a significant difference ($P \leq 0.05$) in response compared to the unmanipulated control songs (i.e. unmanipulated male songs for birds in the male song discrimination group; unmanipulated female songs for birds in the female song discrimination group). Figure 6-5.

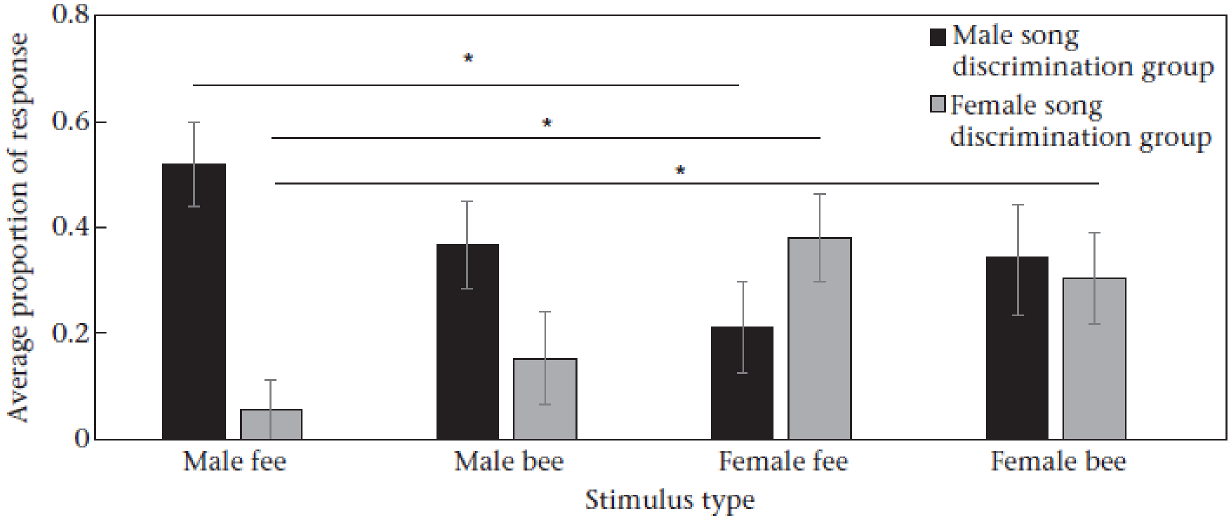

Figure 6-6. Mean ± SE proportion of response to single-note songs in experiment 2. $*P \leq 0.05$.

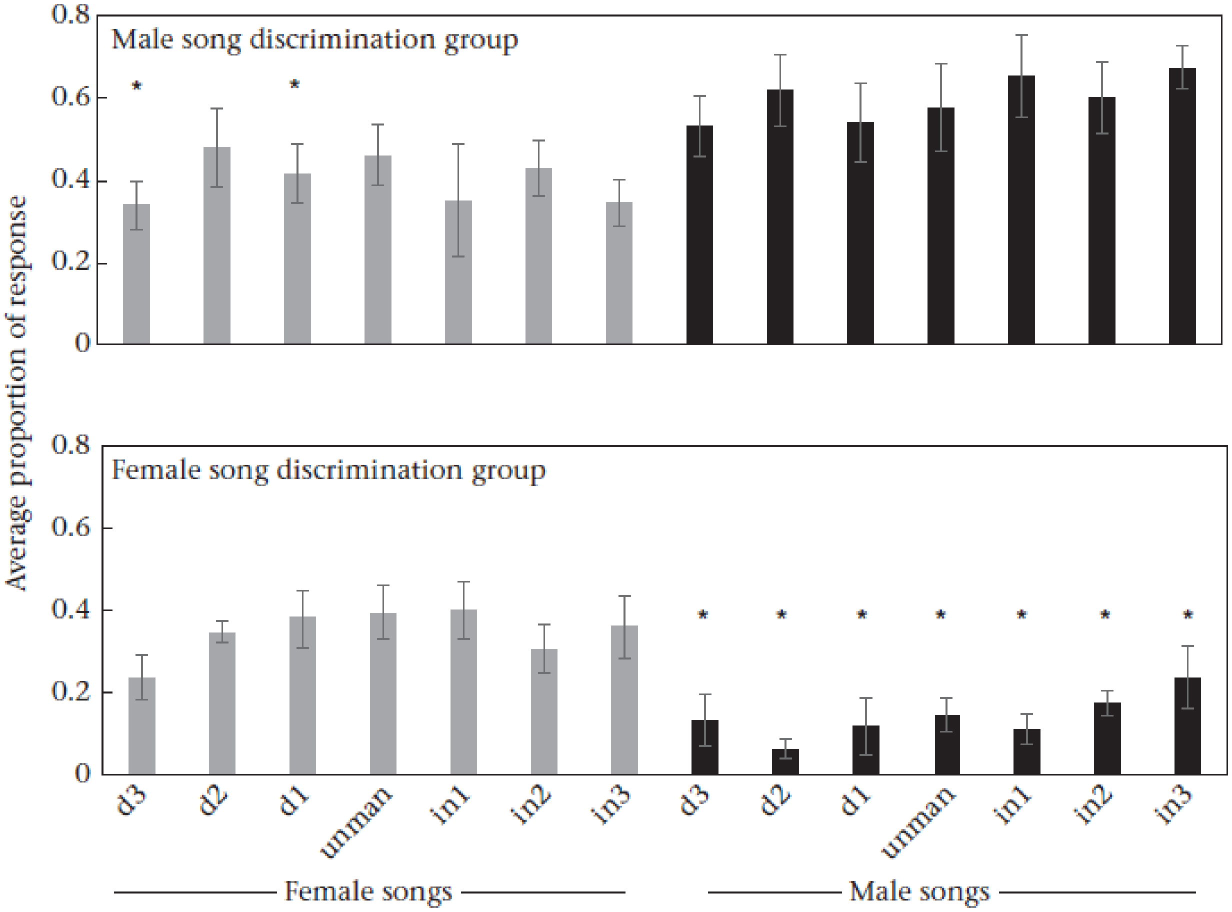

Figure 6-7. Mean ± SE proportion of response to fee glissando manipulated songs in experiment 2. ▨: female songs; ■: male songs. Stimuli were presented in seven ways: decreased by 3 SDs (d3), decreased by 2 SDs (d2), decreased by 1 SD (d1), unmanipulated (unman), increased by 1 SD (in1), increased by 2 SDs (in2), and increased by 3 SDs (in3). *Indicates a significant difference ($P \leq 0.05$) in response compared to the unmanipulated control songs (i.e. unmanipulated male songs for birds in the male song discrimination group; unmanipulated female songs for birds in the female song discrimination group).

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Chapter 7 General discussion

Summary of data chapters

This thesis examined the production and perception of various acoustic cues within black-capped chickadee *fee-bee* songs. Chapters 2, 3, and 4 focused on male-produced song, a vocalization that has been studied in both the field and laboratory (see Mennill & Otter, 2007; Sturdy, Bloomfield, Charrier, & Lee, 2007). Chapters 5 and 6 focused on a vocalization that has not been widely studied – female-produced *fee-bee* song. First, in Chapter 2, I conducted a bioacoustic analysis in order to examine dominance cues within the male song and how these vary depending on geographic location. Next, in Chapter 3, I used two different operant conditioning techniques (i.e., choice preference task and a go/no-go discrimination task) to examine the perception of dominance cues in male *fee-bee* songs. Following the bioacoustic analyses that identified geographic variation in male *fee-bee* songs (Chapter 2), in Chapter 4, I used a go/no-go operant conditioning task to examine the perception of geography-based acoustic cues in male *fee-bee* songs. In Chapter 5, I conducted bioacoustic analyses in order to provide a comparison of male and female songs and identified acoustic features that varied between male and female songs. In Chapter 6, I again employed a go/no-go operant conditioning paradigm to examine the acoustic mechanisms for the perception of male and female songs. Taken together, the results of this research demonstrate a variety of acoustic cues that are contained within a signal that is, at least on the surface, seemingly simple. The studies within this thesis expand on previous research examining the perceptual abilities in chickadees including the discrimination of acoustic variation in conspecific vocalizations.

Open-ended categorization of *fee-bee* songs

Open-ended categorization is a mechanism that allows animals (including humans) to classify stimuli into groups based on perceptual similarity. By using open-ended categorization, an individual can reduce memory load and quickly respond to new (i.e., not previously encountered) stimuli based on past experiences. Animals encounter numerous environmental stimuli daily and numerous species perceive visual (e.g., Guinea baboons, *Papio papio,* Fagot, Bonté, & Parron, 2009; pigeons, *Columba livia*, Herrnstein, Loveland, & Cable, 1976; domestic dogs, *Canis familiaris,* Range, Aust, Steurer, & Huber, 2008) and auditory (e.g., European starlings, *Sturnus vulgaris,* Braaten, 2000; Gentner & Hulse, 1998; zebra finches, *Taeniopygia guttata*, Sturdy, Phillmore, Price & Wesisman, 1999) stimuli as belonging to distinct open-ended categories. In Chapters 3, 4, and 6, I used go/no-go operant conditioning paradigms to examine if chickadees perceive conspecific songs as belonging to open-ended categories based on dominance (Chapter 3), geographic location (Chapter 4) or sex (Chapter 6).

While chickadees may use open-ended categorization to classify songs, birds may also use rote memorization when discriminating. To categorize using rote memorization an individual needs to have experience with and memorize individual songs. While black-capped chickadees can discriminate among individuals based on their songs (Phillmore, Sturdy, Turyk, & Weisman, 2002; Wilson & Mennill, 2010), relying on rote memorization would require increased memory load compared to using open-ended categorization. Therefore, using open-ended categorization should be a faster mechanism to discriminate stimuli and is a more flexible cognitive strategy. To experimentally examine the use of open-ended categorization versus rote memorization, I used a true category/pseudo category paradigm. With this methodology, if songs belong to different categories, the "true category" group could learn a general category-based rule and use open-ended categorization, while the "pseudo category" group would need to rely on rote memorization to learn the task.

Experiments in all three chapters found no difference in the number of trials to reach criterion for chickadees completing a true category versus pseudo category task (the exception was experiments 1 and 3 in Chapter 4). The finding that there was no difference in how long it took chickadees to learn a true category compared to a pseudo category discrimination suggests that either: (1) the songs do not belong to different categories, so birds in both the true category and the pseudo category groups used rote memorization, or (2) there was no advantage (in terms of how many trials it took to learn the task) in using open-ended categorization over rote memorization. However, the responding to novel song exemplars provides evidence that songs do in fact belong to open-ended categories (based on dominance, geography, and sex), supporting the second possibility.

Acoustic mechanisms: Dominance cues

Examining different populations of chickadees, two previous studies identified acoustic cues within *fee-bee* songs that identify a male's dominance rank (i.e., interval frequency ratio in eastern Ontario songs, Christie, Mennill, & Ratcliffe, 2004; amplitude ratio in northern British Columbia songs, Hoeschele et al., 2010). The purpose of Chapter 2 was to examine if there was a dominance cue that was consistent between these two locations. The results confirmed that the relative amplitude varied in dominant and subordinate songs produced by males in British Columbia, but this acoustic feature did not vary in the songs produced by males in Ontario, suggesting that dominance-related cues within songs vary with geographic location.

Next, in Chapter 3, I conducted a series of operant conditioning experiments: a choice preference task and an operant conditioning go/no-go task. The results from the choice preference task suggest that preference for dominant or subordinate songs varies depending on the location-of-origin of the singer and the sex of the signal receiver. Results from the operant conditioning go/no-go task suggest that acquisition performance varies with sex and can vary depending on which stimulus class is rewarded (i.e., birds rewarded for responding to subordinate songs achieved higher DRs during acquisition compared to birds rewarded for responding to dominant songs).

A previous playback study found that female black-capped chickadees from central Alberta (i.e., birds from the same geographic population as birds used in this thesis) responded differentially to playback of dominant and subordinate songs produced by birds in northern British Columbia (Hoeschele et al., 2010). However, the advantage of the current methodology over this previous study is that the current study used an active choice paradigm (i.e., birds chose what songs they heard), while the previous study used a passive playback paradigm. The results from the current choice preference task suggest that preference may vary depending on the singer's geographic location of origin. Further research is needed to examine how local songs (i.e., songs from central Alberta) vary with dominant rank and how variation in local songs compares with the variation reported in other populations (e.g., Christie et al., 2004; Hoeschele et al., 2010). For example, does the relative amplitude of local songs vary with dominance rank? Grava, Grava, and Otter (2012) found that songs produced by socially dominant males varied depending on the habitat-of-origin of the singer, in addition, the habitat of the singer and the song receiver influenced how the songs were perceived by males (Grava, Grava, & Otter, 2013).

While the production of male song varies with dominance rank and geographic location, and these acoustic variations can influence the perception of song, further research is required to understand whether male song changes with rank changes. In black-capped chickadees, dominance hierarchies are generally stable; however, changes in rank do occur (e.g., birds can increase in social rank; see Ratcliffe, Mennill, & Schubert, 2007). It is possible that low-ranking birds are not capable of producing songs that are similar to high-ranking male songs, so even with a change in social rank, the male's song does not change. Alternatively, low-ranking males may be capable of producing songs similar to the songs produced by high-ranking males, but low-ranking males may not produce these songs because of their subordinate status. For example, subordinate brown-headed cowbirds (*Molothrus ater*) are capable of producing the songs that are preferred by females; however, subordinate males do not sing these songs in order to avoid attack from the dominant male (West, King, & Eastzer, 1981).

Acoustic mechanisms: Geographic cues

While the *fee-bee* song has been characterized as invariant (e.g., Hailman, 1989), this is likely because the two-note structure is highly conserved across the species range (however, exceptions to the two-note structure have been reported in geographically-isolated populations of chickadees; see Gammon & Baker, 2004; Kroodsma et al., 1999). More recent work examining chickadee songs (including the results of this thesis) provides evidence that *fee-bee* songs do exhibit acoustic variation. For example, there is acoustic variation in the songs produced by black-capped chickadees from different habitat types (Grava et al., 2012) or habitats with varying levels of anthropogenic noise (Proppe et al., 2012). In the closely-related mountain chickadee (*Poecile gambeli*), there is acoustic variation in the songs produced by individuals from different habitat elevations (Branch & Pravosudov, 2015).

The results of Chapter 4 provide compelling evidence that birds perceive songs as belonging to open-ended, geography-based categories. In experiments 1 and 3, chickadees in the true category discrimination group completed acquisition in significantly fewer trials compared to birds in the pseudo category discrimination group. However, in experiment 2, there was no significant difference in the speed of acquisition for birds in the true category and pseudo category groups. The particular song exemplars that were used during discrimination training may have influenced these differing results and emphasizes the importance of the specific exemplars that are presented during discrimination training and how these exemplars may influence acquisition performance. However, even when the exemplars used during discrimination training contain significant acoustic differences (see results from artificial neural networks in experiment 3 of Chapter 6), there may not be a difference in the acquisition performance of birds (see experiment 2 of Chapter 6).

While the results of Chapter 4 clearly demonstrate that the songs belong to distinct geography-based categories (i.e., birds in all three experiments continued to respond to novel songs based on the contingencies during acquisition), in general, the mechanism that birds were using for the discrimination was less apparent. There was some evidence that birds were using the total duration of the songs (which corresponds to the results of the bioacoustic analyses). For example, in experiment 2, birds rewarded for responding to Ontario songs responded similarly to British Columbia songs that were decreased in duration and Ontario songs, but responded significantly less to unmanipulated British Columbia songs and British Columbia songs increased in duration (bioacoustic analyses indicated that Ontario songs are shorter in duration compared to British Columbia songs). However, these birds responded to Ontario songs increased and decreased in total duration in a manner similar to unmanipulated Ontario songs (see Figure 4-6). This suggests that birds were using acoustic features in addition to the total duration during the discrimination.

Studies with other songbirds examining the acoustic mechanisms underlying vocal discriminations have found that some songbird species rely on one portion of the song (i.e., introductory or terminal portion) more than other portions when discriminating (e.g., Horning, Beecher, Stoddard, & Campbell, 1993; Nelson & Soha, 2004; Thompson & Baker, 1993), this includes research examining other chickadee vocalizations (e.g., *chick-a-dee* calls, Bloomfield, Farrell, & Sturdy, 2008). In the current study, the results from the spliced song and the single note tests did not reveal that birds were relying solely on acoustic features within one of the two notes. By presenting stimuli containing notes spliced together from two different songs, or by presenting single notes, I may have altered other important components of the song. This includes features that were not analyzed in the bioacoustic analyses, such as the interval duration. When I created the spliced song stimuli, I created songs with a consistent interval duration (i.e., 100 ms, based on an interval duration used by previous experiments, Hoeschele, Guillette, & Sturdy, 2012 and Chapter 6 of this thesis). I kept the interval duration consistent because I was specifically interested in examining if birds used acoustic features in a particular song note; however, in retrospect, the interval duration could be an important cue used by birds for discriminating. For example, in corn buntings (*Emberiza calandra*), both the acoustic song elements and the silent portion between notes is used by birds to discriminate among different geographic dialects (Pellerin, 1982). In black-capped chickadee *fee-bee* songs, the relationship between the two notes contains information regarding male dominance rank (e.g., Christie et al., 2004; Hoeschele et al., 2010; Chapter 2 of this thesis), supporting the idea that the silent internote interval of the song is an important component of the song. However, more research is needed to determine if the duration of the internote interval varies with dominance rank or geographic location of origin.

The current work begins to explore the discrimination of songs produced by males from widely-separated locations across the species' range. However, further work is needed to examine how the acoustic structure of local songs (i.e., songs from central Alberta) compares to the songs examined in the current studies (songs from northern British Columbia and eastern Ontario). In addition, future studies can examine how discriminating local songs from songs produced by more distant populations would influence the acquisition performance during a discrimination task (e.g., do birds discriminate among local and distant songs faster compared to birds discriminating among different populations of distant songs?). It is also possible that birds have a preference for local songs over distant songs. In mountain chickadees, females have a preference for local males, although more work is needed to determine if female preference is based on acoustic, visual, or a combination of cues (Branch, Kozlovsky, & Pravosudov, 2015). Results from this study with mountain chickadees, along with the results from Chapter 3 suggest that if birds do have a preference for local songs, this preference may also influence performance during an operant discrimination go/no-go task, and this relationship between acoustic preference and discrimination abilities is something that needs careful consideration in future work.

Acoustic mechanisms: Female song and sex-specific cues

The focus of Chapters 5 and 6 moved from solely examining male-produced songs and instead examined song produced by both males and females. In describing the vocal repertoire of chickadees, Ficken, Ficken, and Witkin (1978) stated that *fee-bee* songs are "produced as far as we know only by males" (p. 35). However, our captive female black-capped chickadees produce loud *fee-bee* songs, which sound similar to male-produced loud songs (pers. obs.) and females producing loud songs in the field has been noted anecdotally (e.g., Dwight, 1897; Hill & Lein, 1987). Langmore (1998) states that female songbird song may be overlooked because (1) a singing female may be wrongly identified as male (especially in monomorphic species), (2) females sing less complex songs, and (3) females sing less frequently compared to males. Black-capped chickadees are sexually monomorphic (to human vision), so it is likely that singing females are thought to be males (e.g., see Dwight, 1897). The second possibility, that females produce less complex song, is not the case for black-capped chickadees, as I demonstrated in Chapter 5 of this thesis; however, more work is needed to determine when and how often female black-capped chickadees produce songs in the field.

By recording both male and female chickadees producing songs and comparing the songs produced by the two sexes using bioacoustics analyses (Chapter 5), I provided the first quantitative description of female-produced song in this species. Bioacoustic analyses are a first step in describing a vocal behaviour. This analysis revealed that the frequency decrease in the first note (i.e., *fee* glissando) is significantly different in male and female songs. Following the description of possible acoustic cues that identify a singing bird's sex (Chapter 5), I conducted subsequent experiments (Chapter 6) to determine if black-capped chickadees can discriminate male and female songs, and to examine if birds use the acoustic cues identified in the bioacoustic analyses when discriminating. Results from the operant discrimination tasks provide evidence that male and female songs belong to open-ended, sex-based categories. Specifically, when presented with novel male and female songs, birds in the true category groups continued to respond to the novel songs based on the contingencies from discrimination training, while birds in the pseudo category group responded to the male and female songs similarly.

In the second experiment of Chapter 6, I nearly doubled the number of discrimination stimuli (60 stimuli were used during discrimination training in experiment 2; 32 stimuli were used in experiment 1). The rationale for increasing the number of discrimination stimuli was that by increasing the number of song exemplars, the cognitive load for birds relying on rote memorization (i.e., the pseudo category group) would increase, and birds completing the pseudo category discrimination would require more trials to reach criterion compared to birds learning the true category group. However, even with this increase in the number of stimuli, there was still no significant difference in how many trials it took birds to learn the true category compared to the pseudo category discrimination. However, the responding by birds to the novel songs and the results from the artificial neural networks (ANNs) clearly indicates that male and female songs contain sex-based acoustic differences. Results from the ANNs suggested that the *fee* glissando is an important acoustic mechanism for the discrimination of songs based on sex. In contrast, results from the birds did not identify a specific acoustic mechanism that birds used to discriminate. Taken together, the results from these experiments suggest that birds use multiple cues when discriminating among songs and the biological salience of songs influence the discrimination abilities of chickadees.

Operant conditioning and biological salience

One strength to the current methodology is that we are examining the perception of biologically-relevant stimuli using controlled laboratory techniques (i.e., operant conditioning). The signal (i.e., *fee-bee* songs) and the acoustic cues that I examined are ecologically-relevant to the specific animal species that I tested. In operant conditioning, the biological relevance and salience of the stimuli can influence an animal's response. For example, in a classic experiment demonstrating biological relevance using rats, Garcia and Koelling (1966) paired either shock or illness with flavored water and an audiovisual stimulus. While rats could learn to associate the shock or illness with both the flavored water and the audiovisual stimulus, results from test sessions revealed that rats associated illness with the flavored water and shock with the audiovisual stimulus. These results demonstrate how biological relevance can influence responding during conditioning, as rats are more likely to get ill after eating something poisonous, while rats are more likely to feel peripheral pain following an encounter with a predator and animals are likely to see or hear a predator coming, essentially as a live audiovisual stimulus).

In the studies in this thesis, I examined the types of acoustic cues black-capped chickadees use when perceiving a biologically-relevant stimulus - conspecific songs. Male songs are considered a sexually-selected trait, used to attract mates and deter rival males. Although, female songbird songs have been examined less often, recent evidence suggests that male and female songs may have evolved under similar selection pressures (Odom, Hall, Riebel, Omland, & Langmore, 2014). The function of song varies depending on the sex of the birds producing and receiving the song (i.e., a male song produced towards a male bird means something different compared to a male song produced towards a female bird). Sex differences in how males and females discriminate songs during an operant discrimination task may occur because the relevance of song changes when song is produced for intrasexual compared to intersexual communication.

Previous studies examining the responding by chickadees on operant discrimination tasks have reported no difference in the discrimination abilities of males and females when *chick-a-dee* calls or call notes were used as stimuli during discrimination training (e.g., Bloomfield et al., 2008; Charrier, Lee, Bloomfield, & Sturdy, 2005; Guillette, Reddon, Hurd, & Sturdy, 2009). While *chick-a-dee* calls are a biologically-relevant vocalization, they are not a vocalization used to attract mates or repel rivals (i.e., a sexually-selected signal), in the same manner as *fee-bee* songs. A recent operant discrimination task with black-capped chickadees that found sex differences in responding also used *fee-bee* songs as stimuli (Hoeschele, Guillette, & Sturdy, 2012). Hoeschele et al. (2012) found that females performed the song discrimination more accurately (i.e., obtained higher DRs) compared to males. The results of Chapter 3 revealed that females learned the discrimination faster compared to males and in Chapter 6, females discriminated significantly more of the rewarded songs compared to males. The significant sex differences that I found in Chapters 3 and 6 appear to be driven mostly by the responding by birds in the pseudo category groups, suggesting that females have enhanced abilities at memorizing conspecific songs. Taken together, these results suggest that sex differences in responding during operant conditioning tasks is likely when sexual signals, such as songs, are used as the discriminative stimuli. The function of song varies depending on if the receiver is a male or a female, in addition, the salience of the signal may vary between the sexes. Females may be more selective in their responses to songs, including more adept at discriminating among songs, because the cost of mistakenly responding to a song is higher for a female (e.g., mating with a low-quality male rather than a high-quality male) compared to a male (e.g., wasting time and energy responding to a non-rival; Ratcliffe & Otter, 1996).

In addition to a bird's sex influencing its responding during a learning task, other aspects of the individual may influence performance. The results from Chapter 3 suggest that how accurately a bird performs during the operant discrimination task can significantly predict the bird's acoustic preference strength. Previous work with black-capped chickadees have found relationships between a bird's performance on operant discrimination tasks and a bird's exploration style (Guillette, Hahn, Hoeschele, Przyslupski, & Sturdy, 2015; Guillette, Reddon, Hoeschele, & Sturdy, 2011; Guillette et al., 2009); however, the current results suggest that a bird's underlying preference for conspecific songs can also influence its abilities on an operant discrimination task. Studies with other Parids have found that dominance status correlates with exploration style (mountain chickadees, Fox, Ladage, Roth, & Pravosudov, 2009; great tits, *Parus major*, Dingemanse & de Goede, 2004; Verbeek, Boon, & Drent, 1996; Verbeek, de

Goede, Drent, & Wiepkema, 1999); however, further work is needed to examine how an individual's social status influences acoustic preference and performance in an operant conditioning task.

For example, a male that is socially subordinate may require more trials in order to reach criterion on an operant discrimination task if the bird is required to respond to dominant male songs. In fact, in the operant discrimination task in Chapter 3, we found that birds rewarded for subordinate songs obtained significantly higher DRs, demonstrating that birds rewarded for responding to subordinate songs were performing more accurately across trials compared to birds rewarded for responding to dominant songs. Again, the biological relevance of the stimuli may have influenced the responding during the task. In the wild, dominant males will chase lower ranking males away from a food source (Smith, 1991). It is possible that the required response during the discrimination task (i.e., fly into the feeder following certain songs) is affected when the rewarded signal is a song produced by a dominant male. Future research needs to consider the biological relevance of the stimuli used during operant conditioning tasks, including the function or meaning of the signals, and how these factors may vary depending on the individual subject (or signal receiver) and the type of task.

Conclusions

In the five studies in this thesis, my aim was to increase the understanding of the production and perception of black-capped chickadee songs. Taken together, these studies demonstrate that a signal that seems relatively simple can contain a variety of acoustic cues. By focusing on one species of songbird, the studies in this thesis have added to our knowledge of black-capped chickadee *fee-bee* songs. Specifically, the findings of these studies indicate additional cues that are contained within male-produced songs, and, additionally, provide the first description of the production and perception of female-produced songs. Further work is needed to compare the developmental processes of song learning in male and female black-capped chickadees, including how early developmental experiences influence the production of these acoustic cues in song. In addition, the findings from these studies suggest that the biological relevance of the signals used as discriminative stimuli, along with individual characteristics of the subjects (e.g., the subject’s sex or acoustic preference), may influence performance during an operant discrimination task. Future work should take into consideration how biological relevance or qualities related to the individual subjects may influence the results of learning tasks.

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