DISSERTATION

REPERTOIRES OF LEARNED VOCAL SIGNALS: HOW DO THEY ORIGINATE, AND ARE THEY ADAPTIVE?

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY DAVID E. GAMMON ENTITLED "REPERTOIRES OF LEARNED VOCAL SIGNALS: HOW DO THEY ORIGINATE, AND ARE THEY ADAPTIVE?" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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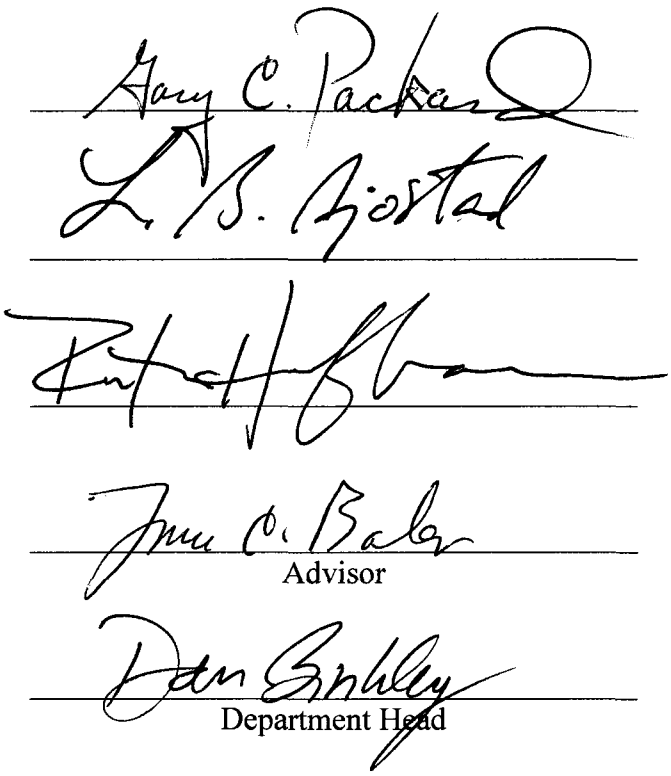

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ABSTRACT OF DISSERTATION

REPERTOIRES OF LEARNED VOCAL SIGNALS: HOW DO THEY ORIGINATE, AND ARE THEY ADAPTIVE?

Two patterns are commonly found in studies of birdsong: individuals sing multiple versions of the song (i.e., they have a 'repertoire' of 'song types') and populations show geographic variation in the acoustic structure of songs (i.e., the songs of different locations sound different). In contrast, most black-capped chickadees (*Poecile atricapillus*) across North America share a simple 'fee-bee' song type that does not vary geographically. Repertoires and small-scale geographic variation have been found in only a few regions across their range such as Fort Collins, Colorado. I described the singing behavior of Fort Collins chickadees for the first time and tested the possible functional significance of the evolved song types.

Fort Collins chickadees sing three song types that are acoustically distinct from the continental song type and from each other. Acoustic structure for each song type varied geographically in the area surrounding Fort Collins, and acoustic variations corresponded to the acoustic variation present in the songs of individual juveniles at the time of natal dispersal, showing that inaccurate learning can account for geographic variation. More acoustic variation existed within an area containing small discontinuous

populations than within an area containing larger continuously distributed population, suggesting that isolation and/or high population turnover may increase the rate of song evolution. Furthermore, the song type most similar to the continental song type varied the least.

I tested four popular hypotheses for possible functional significance associated with the novel repertoire and did not find support for any of them. When neighboring males were involved in a territorial singing contest, use of the same song type did not indicate the likelihood of conflict escalation. Additionally, song types were used in similar proportions when males communicated with females vs. other males. The abundance of song learning errors combined with the effects of isolation on song evolution appeared sufficient to account for the existence of repertoires and geographic variation without invoking an adaptive origin. I hypothesize that repertoires and geographic variation arose in most species as a consequence of the learning process rather than as an adaptation.

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Isaac Newton is attributed to saying, "If I have seen farther than others, it is because I stood on the shoulders of giants." While I am not sure how much farther I have seen than others, I certainly stood on the shoulders of a lot of previous scientists. First of all, I must acknowledge Janine Clemmons who studies recombinant chick-a-dee vocalizations; when I first told her about the funky Fort Collins chickadee songs, her excitement caught me off guard and fueled my ambition to do research. Secondly, I acknowledge Don Kroodsma, who humbly encouraged me as I collected evidence showing that his paper (Kroodsma et al. 1999) did not tell the complete story. Don also introduced me to the wonders of the chickadee dawn chorus when he visited in April

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I recorded chickadees in dozens of locations, and this was possible only with the permission of numerous landowners. I thank each of them for their willingness to allow a young scientist to chase birds on their property: the Belinski, Brinks, Coley, Martin, McGraw, McGrew, Ziebig, and Zimdahl families, Brett Bruyere, Phil Carpenter, Brian Green, Tinka Greenwood, Jan Kollar, Dean Rydholm, and Mel Young.

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OVERVIEW OF CHAPTERS

I investigated a repertoire of multiple song types in black-capped chickadees in the Fort Collins, Colorado area in an attempt to answer two broad questions about the cultural evolution of birdsong: 1) "How does birdsong evolve?" and 2) "Why does birdsong evolve?" The first question deals primarily with changes in the acoustic structure of songs and includes questions such as, "What are the physiological, ecological and social constraints limiting birdsong evolution?" and "What are the ecological and social factors promoting acoustic changes in song?" The second question deals primarily with the functional significance of evolutionary novelties in birdsong and includes questions such as, "Does possession of a repertoire of song types facilitate male-male communication during singing contests between territorial neighbors?" and "Are multiple song types preferred over a single song type by females?" I address the first broad question in Chapter's 1 and 2 and the second broad question in Chapter's 4 and 5.

In Chapter 1, I describe the acoustic structure of the three novel song types found in Fort Collins and compare them to the continental song type found in most black-capped chickadees across North America. I also outline some of the processes of cultural evolution that may have produced this repertoire. In Chapter 2, I describe geographic acoustic variation for each of the song types in the area surrounding Fort Collins. Then I

relate this variation to the geography of the populations and to the acoustic structure of each song type.

Before investigating the possible functional significance of the novel Fort Collins repertoire, I had to distinguish when males direct their songs to other males vs. when they direct their songs to females. Territorial countersinging between neighboring males obviously indicates male-male communication, but it was not so obvious when I began studying chickadees what indicated male-female communication. In Chapter 3, I provide evidence that males direct songs during the dawn chorus primarily to females.

In Chapter's 4 and 5 I investigate the possible functional significance of the Fort Collins repertoire. In Chapter 4 I address two hypotheses for the function of repertoires regarding male-male communication; in Chapter 5 I address two hypotheses for the function of repertoires regarding male-female communication. Hypotheses were taken from the birdsong literature, and all have received empirical support in other species. At the end of Chapter 5 I summarize findings from Chapter's 4 and 5 and discuss their significance.

CHAPTER I

DESCRIPTION OF SONG TYPES

Many behavior patterns are acquired through cultural transmission rather than exclusively through genetic inheritance (Cavalli-Sforza and Feldman 1981). Social learning often results in local behavioral traditions that can persist for several generations in natural and experimental populations (e.g., antipredatory response traditions: Curio et al. 1978; foraging traditions: Laland and Plotkin 1990, Aisner and Terkel 1992, Galef and Allen 1995; vocal traditions in birds and mammals: Grant and Grant 1996, Payne 1996, Yurk et al. 2002). Although we know that maintenance of these traditions involves social learning combined with selective processes, we know very little about the ways in which behavioral innovations are introduced, whether those cultural innovations are normally adaptive, or what evolutionary constraints shape behavioral innovations and allow certain traditions to persist (Galef 1995, 1996, Laland 1996).

Vocal learning is a classic example of social learning (Laland et al. 1993) that has been documented in nearly all songbirds that have been evaluated (Kroodsma and Baylis 1982), but is also found in hummingbirds (Baptista and Schuchmann 1990), parrots (Farabough and Dooling 1996), and four groups of mammals (Janik and Slater 1997). Most of these taxa exhibit geographic variation in the acoustic structure of their learned

vocalization(s), and individuals possess repertoires of multiple vocal types within a functional class of vocalizations (Mundinger 1982, Catchpole and Slater 1995). The term 'repertoire' is also used in the literature to refer to the entire range of functionally distinct vocalization classes that an individual of a species uses (Bradbury and Vehrencamp 1997: p. 416), but I confine my use of it in this paper to multiple vocal types within one particular class of vocalizations. Although repertoires are found in many taxa (Krebs and Kroodsma 1980), researchers have rarely been able to document their origin; a rare exception was the documentation of the origin of a song repertoire in an island population of the western gerygone (*Gerygone fusca*, Baker et al. 2003a). Geographic variation and repertoire patterns are certainly attributable in part to a perpetuation of changes in signal structure accrued as a result of imperfect social learning (Kroodsma and Miller 1982), but there is disagreement over whether these patterns have adaptive value (Wiens 1982, Baker and Cunningham 1985, Hasselquist et al. 1996, Kroodsma 1996, Lynch 1996, Beecher et al. 1997, Lachlan and Slater 1999). In addition we often lack details on how social, physiological, morphological, and habitat constraints shape the variation in vocal signals (Wiley and Richards 1982, Podos 1996, 2001).

Perhaps the most well known exception to the common occurrence of repertoires and geographic song variation in songbirds is the whistled 'fee-bee' song (Fig. 1.1) of the black-capped chickadee (*Poecile atricapillus*, Hailman 1989, Kroodsma et al. 1999). Despite the fee-bee song being a learned vocalization (Shackleton and Ratcliffe 1993, Kroodsma et al. 1995), and despite the presence of copy errors developed in both laboratory experiments (Shackleton and Ratcliffe 1993, Kroodsma et al. 1995) and natural populations (Smith 1991: p.61, Unpublished data), a recent analysis of adult

chickadee songs from nineteen widespread North American locations revealed that, with few exceptions, individual black-capped chickadees from Nova Scotia to British Columbia all share a single invariant song type (Kroodsma et al. 1999), which I refer to as the continental song type. Geographic variation occurred only in the songs of populations located on islands off Massachusetts as well as some 'habitat islands' in Oregon and Washington, and individuals within these isolated populations used multiple song types (Kroodsma et al. 1999). I will refer to 'song type' in this chapter as a discrete category of song possessing a distinct pattern of notes and acoustic structure. Although Kroodsma et al. (1999) did not explicitly define 'song type', their use of the term was similar to mine.

Casual observations of singing behavior in black-capped chickadee in Fort Collins, Colorado, first led me to believe that chickadees in this area sing three song types instead of one, and that these song types could be distinguished according to the presence of introductory notes. I termed these song types: 'chick-a-fee-bee' (containing two introductory notes that resemble, at least superficially, the beginning notes of the 'chick-a-dee' call), 'fa-fee-bee' (containing an introductory note resembling a shortened 'fee' syllable) and 'fee-bee-3' (containing no introductory notes, Fig. 1.1). Here I report on singing behavior of individuals and populations of chickadees in Fort Collins, compare it with singing behavior normally found in black-capped chickadees, and discuss general implications for cultural evolution through social learning.

METHODS

Description of Field Recordings

Field recordings of adult male black-capped chickadees were made in the wild during the springs of 2001 and 2002. Using the 2002 recordings, I describe singing behavior in individual black-capped chickadees within Fort Collins. Using the 2001 recordings, I describe geographic variation in singing behavior within and outside of Fort Collins. Two small samples of songs recorded 9 km northeast of Fort Collins from one male chickadee in 1994 and another in 1997 provide historical perspective on the Fort Collins song types.

Individual Variation

Recordings in 2002 were made of individual birds at five different study sites in Fort Collins during the dawn chorus to determine repertoire sizes for individuals, to establish the acoustic features defining each song type, and to determine if pitch varied over singing bouts as has been shown in black-capped chickadees in other parts of the continent (Horn et al. 1992; Kroodsma et al. 1999). Recordings were made 23 April to 8 May between 0530 and 0630 hours. Most recordings were made with a Sony minidisc recorder (MZ-R700) connected to a Sennheiser microphone (ME62) mounted in a 60 cm Telinga Pro-universal parabola. The remaining recordings were made with a Marantz tape recorder (PMD201) connected to a Sennheiser microphone (ME62) mounted in a 45 cm parabola. I found no differences relevant to my analyses in sonograms made from recordings using the two types of equipment, and a detailed post hoc statistical analysis comparing acoustic measurements made from sonograms of minidisc and audio tape

recordings revealed no significant differences (DEG Unpublished data). True pitch for the recordings was verified using a 440 Hz tuning fork, and no pitch discrepancies were found for either recording system.

When recording an individual, all songs that a male uttered were recorded until he stopped singing for at least three minutes. If neighboring chickadees also sang during the focal male's singing bout, this was noted by verbal narrations onto the tape. To ensure that repertoires were recorded fully, I used only singing bouts that contained at least six switches between song types. Assuming that a bird was equally likely to switch to any of the three types, this rule gave me a 97% chance of detecting all song types for individuals given that the bird actually possessed all three types. Ten birds met this criterion, and each sang between 90 and 517 songs ($\overline{X} = 258$ songs) during its bout. Although birds were unbanded, singers stayed within their territories during the dawn chorus and usually at least 10 m away from the nearest singing neighbor (Ficken et al. 1978). Thus, it was possible to follow an individual during the entire bout. If there was any uncertainty about an individual's identity, the data were not used.

Population Variation

The 2001 recordings were used to establish that chickadee songs from all study sites within Fort Collins segregate into distinct types, whereas outside of Fort Collins and surrounding dispersal areas all songs classify as the single continental 'fee-bee' song type documented by others (Weisman et al. 1990, Horn et al. 1992, Kroodsma et al. 1999). When making recordings in 2001, I tried to record birds from as many widespread study sites as possible. Consequently, all birds were unbanded. Field recordings were made 10

February to 25 April between 0600 and 1200 hours using a Marantz tape recorder (PMD201) connected to a Sennheiser microphone (ME62) mounted in a 45 cm parabola. Recordings were made at various study sites within Fort Collins and at several sites in Colorado and Utah not connected to Fort Collins by dispersal corridors (Fig. 1.2). Using the recordings from each study site, I created a catalogue of songs in which each song came from a different individual. For each bird recorded, I included only the first song in which the signal-to-noise ratio was adequate to allow acoustic measurements. When assembled, this catalogue contained a song recording from 57 individuals in 10 Fort Collins study sites and 30 individuals in 6 study sites outside of Fort Collins (Fig. 1.2).

The 1994 and 1997 recordings were both made using a Sony tape recorder (TCM 5000 EV) connected to a Sennheiser shotgun microphone (ME 80). Each set of recordings was made of a single male black-capped chickadee during its dawn singing bout (approximately 0600 hours).

Description of Acoustic Measurements

For the 2001 song recordings, I made spectrograms of all recordings using Real-Time Spectrogram software (Kay Elemetrics, version 2.3) with a 44.1 kHz sampling rate and 1024-point transform size. For the 1994 and 1997 song recordings, I made spectrograms using Syrinx software (John Burt, http://www.syrinxpc.com/index.html) sampling at 22.05 kHz with 16-bit accuracy and 1024-point transform size. Songs were classified as chick-a-fee-bee, fa-fee-bee or fee-bee-3 songs based on the syllables that appeared on the spectrogram. For the 1994 and 1997 recordings, I did not make further acoustic measurements after classifying the songs into song types. For the 2001

recordings, I took one fee-pitch and one bee-pitch measurement at the midpoint of each fee and bee syllable using the onscreen cursors, which allowed a pitch resolution of 4 Hz. My methods for measuring fee- and bee-pitch differed from those used previously (Weisman and Ratcliffe 1989, Weisman et al. 1990, Horn et al. 1992, Kroodsma et al. 1999), but those previously used methods differed from each other as well. My method provided a consistent and efficient measure of pitch. Fee-pitches were divided by bee-pitches to obtain a 'pitch ratio' for each song.

I measured several acoustic variables on the 2002 dawn chorus songs to quantify variation in each of the three Fort Collins song types with regards to pitch, syllable duration, amplitude breaks within the fee and bee syllables, and the amount of frequency modulation (i.e., 'glissando') within fee and bee syllables. These acoustic variables have all proven useful in previous studies of the black-capped chickadee song (Ficken et al. 1978, Ratcliffe and Weisman 1985, Weisman and Ratcliffe 1989, Weisman et al. 1990, Horn et al. 1992, Shackleton et al. 1992, Kroodsma et al. 1999). Exact details on how acoustic measurements were made will be given below.

Following the examples of Horn et al. (1992) and Kroodsma et al. (1999), I measured the pitches of both the fee and bee syllable in every song given by every bird throughout its dawn singing bout. For detailed acoustic measurements on the 2002 data, I used a subset of the song recordings from the dawn chorus. This subset consisted of the first song uttered for each song type of each bird in which the signal came in at least three times greater amplitude than the background noise. Thus, this subset consisted exclusively of high quality songs in which duration and other acoustic measurements could be made unambiguously. For one bird, the signal amplitude was always too low,

and I excluded his songs from the data subset. The song types of the remaining nine birds were equally represented. For each fee and bee syllable of each song in this subset, I measured syllable duration, the temporal location of amplitude breaks with respect to syllable onset, and the frequency modulation over the syllable.

Dawn chorus songs from 2002 were originally made into wave files using Syrinx software sampling at 22.05 kHz with 16-bit accuracy. Each song was classified into a chick-a-fee-bee, fa-fee-bee, or fee-bee-3 based on the introductory notes (i.e., 'chick-a', 'fa', or none) appearing on the spectrogram. Within a long sequence of chick-a-fee-bees or fa-fee-bees, birds rarely (0.27% of all songs) gave a song lacking the typical introductory note(s). Following the example of previous work on the classification of songs within song bouts into discrete types (Stoddard et al. 1988), I classified these songs as chick-a-fee-bee or fa-fee-bee respectively.

Prior to making pitch and frequency modulation measurements, I reproduced sound spectrograms using a 1024-point transform size, thus maximizing pitch resolution. Fee- and bee-pitch measurements were taken directly from the spectrogram using the syllable midpoint method described previously. As in the 2001 recordings, fee-pitches were divided by bee-pitches to obtain a pitch ratio for each song in the 2002 recordings. Pitch measurements were also taken for the introductory notes of chick-a-fee-bees and fa-fee-bees. Fa syllables were measured using the midpoint method, and chick-a syllables were measured at the maximum frequency. Frequency modulation measurements were taken by measuring the highest and lowest pitches during the fee and bee syllables and dividing the higher pitch by the lower pitch to get a pitch interval (Weisman et al. 1990).

Duration measurements for fee and bee syllables of the dawn chorus subset were taken using an oscillogram display within Syrinx software. I measured the temporal onset and ending of each fee and bee syllable within the subset using the onscreen cursors, which allowed a temporal resolution of 0.004 sec. The same method was used to measure the temporal location of each amplitude break within fee and bee syllables.

Statistical Analysis

All statistical tests were two-tailed. Using the entire 2002 dawn chorus data set, I computed the frequencies with which birds gave each song type within the Fort Collins samples using N = 10 individuals. Using the subset of high quality songs, I performed univariate analyses of variance for each acoustic measurement to see which acoustic variables distinguished song types best. This was accomplished by examining the relative values of F-statistics and looking for natural breaks in their distribution. All acoustic variables were first checked for ANOVA assumptions. For the acoustic measurements that were shown to distinguish song types best, I performed a nested ANOVA nesting bird within song type using a sample of 10 randomly selected songs of each song type for birds possessing all three song types. This partitioned variation in those acoustic variables into variation between song types and variation among birds giving a particular song type. One bird gave only eight fa-fee-bees, providing a slightly decreased sample size for that individual.

To determine whether pitch-shifting (Horn et al. 1992, Kroodsma et al. 1999) occurred within the Fort Collins song types, fee-pitch, bee-pitch, and pitch ratio of each bird's dawn song recordings made in 2002 were graphed against song number to see

whether individual birds varied absolute pitch and pitch ratio over time. Following the example of Weisman et al. (1990), I graphed fee-pitches of the 2001 data set against bee-pitches, using one graph for Fort Collins sites and one graph for all other sites. If song types possessed a characteristic pitch ratio, then each graph should possess a linear cluster of points for each song type represented. I also looked for evidence of geographic variation in the absolute fee- and bee-pitches used for each song type between Fort Collins study sites and between all other study sites using ANOVA.

RESULTS

Birds in the 2002 Fort Collins dawn chorus recordings typically repeated one song type several times before switching to another ('eventual variety', Hartshorne 1956). All songs in the dawn chorus dataset were classified as one of the three Fort Collins song types (chick-a-fee-bee, fa-fee-bee, or fee-bee-3). Of the ten birds, eight sang all three song types, and two sang just chick-a-fee-bee and fa-fee-bee. Generally, chick-a-fee-bee was the most common song type within individuals (20–62% of all songs during an individual's dawn singing bout) followed by fa-fee-bee (7–54%) and then fee-bee-3 (7–33%). For the eight birds possessing all three song types, the average frequencies of usage were: chick-a-fee-bee: 45%, fa-fee-bee: 36%, fee-bee-3: 19%. Countersinging and occasional song type-matching between neighboring birds occurred for each of the three song types, and this will be reported in Chapter 4.

Whenever Fort Collins study sites were intensively sampled in 2001 (i.e., songs from at least 10 males), all three song types were detected. The two song types containing introductory notes were present in the early recordings: 1994 recordings contained 24

songs from one bird including some chick-a-fee-bees and fa-fee-bees, and the 1997 recordings consisted exclusively of 18 chick-a-fee-bees from one bird. None of the 30 songs recorded in 2001 outside of Fort Collins contained introductory notes.

The obvious acoustic differences distinguishing the Fort Collins song types were the presence of chick-a and fa syllables, and the consistent amplitude break in the fee syllable of chick-a-fee-bees (Fig. 1.1). Beyond this, song types varied in other acoustic dimensions (Table 1.1). By comparing coefficients of variation it can be seen that for each song type, temporal variables varied more than pitch variables. Pitch variables (fee-pitch, bee-pitch and pitch ratio) distinguished song types far better than any other acoustic measurement (One-way ANOVA: pitch ratio: $F_{2,24}$=204, P<0.001; fee-pitch: $F_{2,24}$=277, P<0.001; bee-pitch: $F_{2,24}$=91.6, P<0.001; Table 1.1). Two other acoustic variables distinguished song types moderately well although their F-statistics were one to two orders of magnitude lower: the temporal location of the amplitude break in bee (One-way ANOVA: $F_{2,24}$=5.70, P=0.010, Table 1.1) and the amount of frequency modulation in bee (One-way ANOVA: $F_{2,24}$=3.72, P=0.041, Table 1.1). Tukey's pairwise comparisons showed that the temporal location of the amplitude break in the bee syllable was similar in chick-a-fee-bee and fa-fee-bee but lower in fee-bee-3, and that the bee syllable of fa-fee-bee has greater frequency modulation than the bee syllable of chick-a-fee-bee.

Song types did not differ significantly in the durations of fee syllables (One-way ANOVA: $F_{2,24}$=2.77, P=0.085, Table 1.1) and bee syllables (One-way ANOVA: $F_{2,24}$=0.24, P=0.792, Table 1.1). I also found no difference in the amount of frequency modulation between fee and bee syllables within any of the song types (Paired t-tests:

chick-a-fee-bee: t_8=-0.54, P=0.602; fa-fee-bee: t_8=1.82, P=0.105; fee-bee-3: t_6=-0.94, P=0.383; Table 1.1). Because two of the nine birds that contributed songs for the ANOVA analyses gave only two song types, I performed the above analyses excluding songs from these two birds. The only result that changed was that the amount of frequency modulation in the bee syllable failed to distinguish any of the song types (One-way ANOVA: $F_{2,20}$=2.19, P=0.140).

Most of the variation in pitch variables was attributable to differences between song type rather than differences between individuals using a given song type (Nested ANOVA: pitch ratio: variation between song types=96.33%, $F_{2,21}$=249, P<0.001, variation between birds=3.04%, $F_{21,214}$=44.0, P<0.001; fee-pitch: variation between song types=97.15%, $F_{2,21}$=328, P<0.001, variation between birds=2.33%, $F_{21,214}$=45.4, P<0.001; bee-pitch: variation between song types=83.13%, $F_{2,21}$=46.8, P<0.001, variation between birds=14.27%, $F_{21,214}$=55.5, P<0.001). Pitch information for each song type is summarized in Fig. 1.3. Each Fort Collins song type possessed a narrow range of pitch ratios that did not overlap with the pitch ratios of other Fort Collins song types (chick-a-fee-bee median pitch ratio=1.21; fa-fee-bee=1.33; fee-bee-3=1.09). Throughout a male chickadee's dawn singing bout, a distinctive pitch ratio was maintained over a sequence containing a given song type, even while slight shifts occurred in the absolute fee- and bee-pitches used for that song type (Fig. 1.4). Additionally, the pitch ratios for Fort Collins fa-fee-bees were greater than all pitch ratios recorded outside of Fort Collins as well as all pitch ratios reported in the literature (Kroodsma et al. 1999: p. 392). This occurred even though absolute fee- and bee-pitches used in Fort Collins song types fell within the range of fee- and bee-pitches found elsewhere (Fig. 1.5).

Graphs of fee-pitch vs. bee-pitch for each geographic area recorded in the 2001 data set are shown in Fig. 1.5. While songs in Fort Collins clustered into three groups according to pitch as reported above, the songs from all other study sites clustered into a single group. The linear relationship within each of the three Fort Collins clusters suggests that the Fort Collins song types do indeed have a characteristic pitch ratio. The songs recorded outside of Fort Collins also clustered linearly.

To summarize, after considering the presence of introductory notes and the fee amplitude break found in chick-a-fee-bee songs, nearly all of the remaining acoustic variation was in pitch features. These pitch features completely distinguished song types from one another. Using 2001 data, I found that the absolute fee- and bee-pitches used for each song type varied between study sites in Fort Collins (Two-way ANOVA: fee: $F_{9,56}=5.71$, $P<0.001$; bee: $F_{9,56}=3.01$, $P=0.007$), while there was no significant geographic variation in whistled pitches outside of Fort Collins despite the fact that recordings were made at study sites much more widely-scattered (see Fig. 1.2) than Fort Collins sites (One-way ANOVA: fee: $F_{5,29}=1.05$, $P=0.41$; bee: $F_{5,29}=1.12$, $P=0.38$).

DISCUSSION

My song recordings show that black-capped chickadees in Fort Collins, Colorado, currently sing three discrete song types. Given that individual black-capped chickadees across the majority of North America share a single song type (Kroodsma et al. 1999), the singing behavior of Fort Collins chickadees probably represents the evolution of a vocal signal repertoire, with all Fort Collins song types being derived from the single song type found in most continental chickadee populations. The same three song types

were found in individuals of several black-capped chickadee populations throughout Fort Collins. These song types were stereotyped in their acoustic structure, particularly with regard to pitch ratio. The most common song types were chick-a-fee-bee and fa-fee-bee, both of which contained introductory notes differing from previously reported black-capped chickadee songs. Within the immediate Fort Collins area, these two types have existed at least since 1994. Song introductory notes were not found in any of the other study sites reported in this study. Each Fort Collins song type exhibited a characteristic pitch ratio formed from a bounded range of fee- and bee-pitches that varied slightly among study sites. Individual black-capped chickadees in Fort Collins had an average repertoire size of 2.8 and switched between song types using eventual variety. During bouts of singing the same song type, individuals slightly adjusted the absolute fee- and bee-pitches while retaining the song type's characteristic pitch ratio as has been shown for other black-capped chickadee populations (Weisman et al. 1990, Horn et al. 1992, Kroodsma et al. 1999).

All Fort Collins song types were used in territorial countersinging using the same temporal pattern of vocal exchange that has been described by others (Shackleton and Ratcliffe 1994). This suggests that although the number of song types has increased within the Fort Collins black-capped chickadee populations, the territorial function of those song types does not differ from that of the single continental fee-bee song form. Future investigations will examine the frequency of song type-matching within Fort Collins populations and whether matching signals an increased probability of aggression (Krebs et al. 1981, Burt et al. 2001, Molles and Vehrencamp 2001).

Some disagreement has existed over whether continental black-capped chickadee songs should be classified into a single song type (Hailman 1989, Horn et al. 1992, Shackleton and Ratcliffe 1994, Kroodsma et al 1999). When individual chickadees sing long sequences of the continental song type, they occasionally 'pitch-shift' by changing the pitches (i.e., frequencies) of the fee- and bee-syllables. Some have argued that this pitch-shifting functions analogously to song type-switching (Horn et al. 1992, Shackleton and Ratcliffe 1994). Nevertheless, over bouts of several hundred songs individuals will sing all whistled pitches within their frequency range (Weisman et al. 1990, Horn et al. 1992, Kroodsma et al. 1999). Thus, because large samples of songs do not cluster into discrete categories, it is generally concluded that individual black-capped chickadees possess a single song type (Kroodsma et al. 1999).

Cultural Evolution

Although I have not tested for genetic differences separating Fort Collins chickadee populations from other continental populations, the fact that the fee-bee song is learned (Shackleton and Ratcliffe 1993, Kroodsma et al. 1995) suggests that the Fort Collins song types are cultural innovations. The most likely scenario is that the new song types arose within or near Fort Collins some time ago and became established through dispersal of individuals and eventual social conformity among individuals in local populations. Such a process would unfold as cultural mutations originated and spread within and between Fort Collins populations as suggested by the model of Lynch (1996). In a small, geographically isolated population, new song improvisations (i.e., cultural

mutations) can originate and spread rapidly, even within a few decades (Baker et al. 2003a, Unpublished data).

Evolutionary Constraints

The process of cultural evolution is also subject to social (e.g., Nelson and Marler 1994, Nelson 2000), physiological (e.g. Podos 1996, 1997), environmental acoustic (Wiley and Richards 1982), and other selective constraints (Lynch and Baker 1993). My result showing that pitch ratio for a given song type varies little between birds compares to that of Weisman et al. (1990), who also found that pitch ratio in the continental black-capped chickadee song type does not vary significantly between birds. Thus, even in Fort Collins where a song type repertoire has evolved, pitch ratio continues to be a defining feature of song type as it is in other black-capped chickadee populations. Weisman and Ratcliffe (1989) showed that songs containing pitch ratios altered by 0.12 or more from the population average were not as effective at eliciting a territorial response. I suggest that social constraints guided the evolution of each Fort Collins song type and that during the course of evolution, songs of a given song type possessing pitch ratios different from the population average for that same song type were discriminated against. Social discrimination against abnormal pitch ratios has also been shown in white-throated sparrows (*Zonotrichia albicollis*, Hurly et al. 1990) and veeries (*Catharus fuscescens*, Weary et al. 1991), although individuals of other species, such as the closely related Carolina chickadee, do not discriminate against altered pitch ratios (Lohr et al. 1994).

Among animals possessing socially learned traditions, social selection for particular behavioral variations is likely widespread (Galef 1995). For example, although

white-crowned sparrows (*Zonotrichia leucophrys*) learn multiple song types early in life, they retain only the song that most closely matches the song of males with whom they interact during their first spring (Nelson and Marler 1994, Nelson 2000). In a nonbirdsong example, primate tool-use foraging innovations are often subjected to social forces that determine whether the innovations will become established within a population (Van Schaik et al. 1999).

A further constraint that may have guided chickadee song evolution is a physiological limit to the range of whistled fundamental frequencies that black-capped chickadees can produce. Although Fort Collins song types exhibit stereotyped fee- and bee-pitches, the whistled pitches of all three song types lie within the range of continental fee- and bee-pitches reported in the literature (approximately 2.7 to 4.6 kHz). Mountain chickadees (*Poecile gambeli*), the most closely related congener of black-capped chickadees (Gill et al. 1993), exhibit a smaller range of whistled pitches in their songs (3.5 to 4.6 kHz, Lohr 1995: Ch. 4, Wiebe and Lein 1999, Unpublished data). Carolina chickadees alternate between high (5.4 to 7.0 kHz) and low (3.0 to 4.2 kHz) frequencies during their song (Lohr et al. 1991, 1994). It is not known whether the higher pitches used are fundamental frequencies or result instead from the vocal tract suppressing the fundamental and emphasizing the second harmonic.

All laboratory-raised black-capped chickadees, including one male tutored on Carolina chickadee song, developed songs with whistled fundamental frequencies lying between 2.7 and 4.6 kHz (Shackleton and Ratcliffe 1993, Kroodsma et al. 1995); for several wild populations, the range of whistled fundamental frequencies was nearly identical (2.7 – 4.5 kHz, Ficken et al. 1978, Ratcliffe and Weisman 1985, Hill and Lein

1987, Weisman et al. 1990, Horn et al. 1992, Hammond 1993, Kroodsma et al. 1999, Unpublished data). Songbirds increase fundamental frequency through flexion of the large muscle ventral to the syrinx vocal organ (Goller and Suthers 1995, 1996), and production of higher fundamental frequencies probably requires substantial effort (Suthers et al. 1999). I hypothesize that black-capped chickadees are not capable of singing whistled fundamental frequencies higher than 4.6 kHz.

Comparison With Other Chickadee Populations

Kroodsma et al. (1999) also found repertoires and geographic variation in black-capped chickadee song, but only at the extremes of the species' range, particularly in islands off the coast of Massachusetts and in two 'habitat island' populations in the Pacific Northwest. In many ways the Fort Collins area qualifies as a habitat island because habitat available to black-capped chickadees is bounded on the north, east, and south by the less-inhabitable, tree-sparse Great Plains, and on the west by coniferous habitat populated by mountain chickadees.

Although it thus appears that multiple song types appear in several black-capped chickadee habitat islands, the Fort Collins repertoire differs in some respects. First, nearly all variant song types found by Kroodsma et al. (1999) were monotonal (i.e., pitch ratio $\approx$ 1), while Fort Collins song types all contained pitch ratios > 1. Second, the pitch ratios for each Fort Collins song type are different from one another. The median pitch ratios for the three song types were 1.21 (chick-a-fee-bee), 1.33 (fa-fee-bee) and 1.09 (fee-bee-3). Considering that 95% of the pitch ratios found in continental black-capped chickadee songs lie between 1.10 and 1.17 (Weisman et al. 1990) and that the maximum pitch ratio

previously reported is 1.30 (Kroodsma et al. 1999: p. 392), it appears that Fort Collins chickadees extended the limits of pitch ratio values during the evolution of their repertoire.

Finally, Fort Collins chickadee singing behavior is distinguished by the widespread occurrence of song introductory notes. Chickadees from other populations appear to use an introductory syllable analogous to the Fort Collins fa syllable (Kroodsma et al. 1999: fig. 1, Hammond 1993: fig 3, Christie et al. 2004: fig. 4), but investigators classified this introductory note as simply an amplitude break within the fee syllable even though there was around 0.05 sec silence separating the introductory syllable and the fee. Introductory notes are also found in the songs of mountain chickadees (Colver et al. 1999, Wiebe and Lein 1999, Unpublished data).

Conclusion

I find it remarkable that such a well-studied songbird normally exhibiting a lack of geographic song variation across several thousand kilometers of its geographic range (Hailman 1989, Kroodsma et al. 1999) has evolved the song type repertoire that I describe here. Kroodsma et al. (1999) suggested that social pressures exist in black-capped chickadees that favor individual birds that sound similar to other conspecifics. Perhaps in Fort Collins those pressures were attenuated because of less population mixing between isolated Fort Collins and surrounding chickadee populations and because of a more sparse distribution during early colonization. These factors could have decreased the magnitude of social selection, and this relaxed social selection regime may have allowed song mutants to become abundant in local habitat patches. It is also possible that

selection for song conformity was overwhelmed by a higher cultural mutation rate in Fort Collins. Although I do not currently know why the chickadee song repertoire originated, numerous adaptive hypotheses have been proposed to explain why vocal repertoires persist (Catchpole & Slater 1995: Ch. 8). To my knowledge, none of these hypotheses have been tested on a population with a recently evolved repertoire. The Fort Collins song repertoire, with its novel introductory notes and easily quantifiable acoustic variation, will allow testing of hypotheses regarding the origin of song repertoires. The repertoire also provides an opportunity to examine how vocal signal innovations originate and the mechanisms of cultural evolution, which may provide evolutionary principles that generalize to other species possessing socially learned behavioral traditions.

TABLE 1.1: Means, standard deviations (SD), and coefficients of variation (CV) for all acoustic measurements made on each of the three Fort Collins, Colorado song types. Recordings were made during a dawn singing bout from nine separate birds at five study sites in Fort Collins, and acoustic measurements were made for a single high-quality song from each song type of each bird. Two birds did not possess the fee-bee-3 song type. See text for measurement details.

	Chick-a-fee-bee			Fa-fee-bee			Fee-bee-3		
Acoustic Variable	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
C1 peak frequency (Hz)	5601	194	3.5	*	*	*	*	*	*
C2 peak frequency (Hz)	5504	185	3.4	*	*	*	*	*	*
Fa-pitch (Hz)	*	*	*	4276	110	2.6	*	*	*
Fee-pitch (Hz)	4091	71	1.7	4119	87	2.1	3337	53	1.6
Fee frequency modulation	1.020	0.010	1.0	1.023	0.014	1.3	1.032	0.027	2.6
Bee-pitch (Hz)	3382	57	1.7	3091	45	1.5	3051	64	2.1
Bee frequency modulation	1.019	0.009	0.9	1.036	0.020	2.0	1.023	0.009	0.9
Pitch ratio (fee/bee)	1.210	0.023	1.9	1.333	0.026	2.0	1.094	0.020	1.8
Fee-duration (s)	0.325	0.024	7.4	0.358	0.038	10.7	0.319	0.048	14.9
Fee amplitude break (s)	0.119	0.021	17.3	*	*	*	*	*	*
Bee-duration (s)	0.398	0.017	4.2	0.385	0.069	17.9	0.398	0.036	9.1
Bee amplitude break (s)	0.193	0.009	4.5	0.192	0.018	9.5	0.171	0.016	9.4

Fig. 1.1. Oscillograms and matching spectrograms of the three black-capped chickadee song types found in Fort Collins, Colorado (A-C) and the continental song type (D) showing typical within-song type variation. In all song types, the two long whistled syllables are termed 'fee' and 'bee'. Although fee-bee-3 and the continental song type appear similar, fee-bee-3 pitch is more stereotyped.

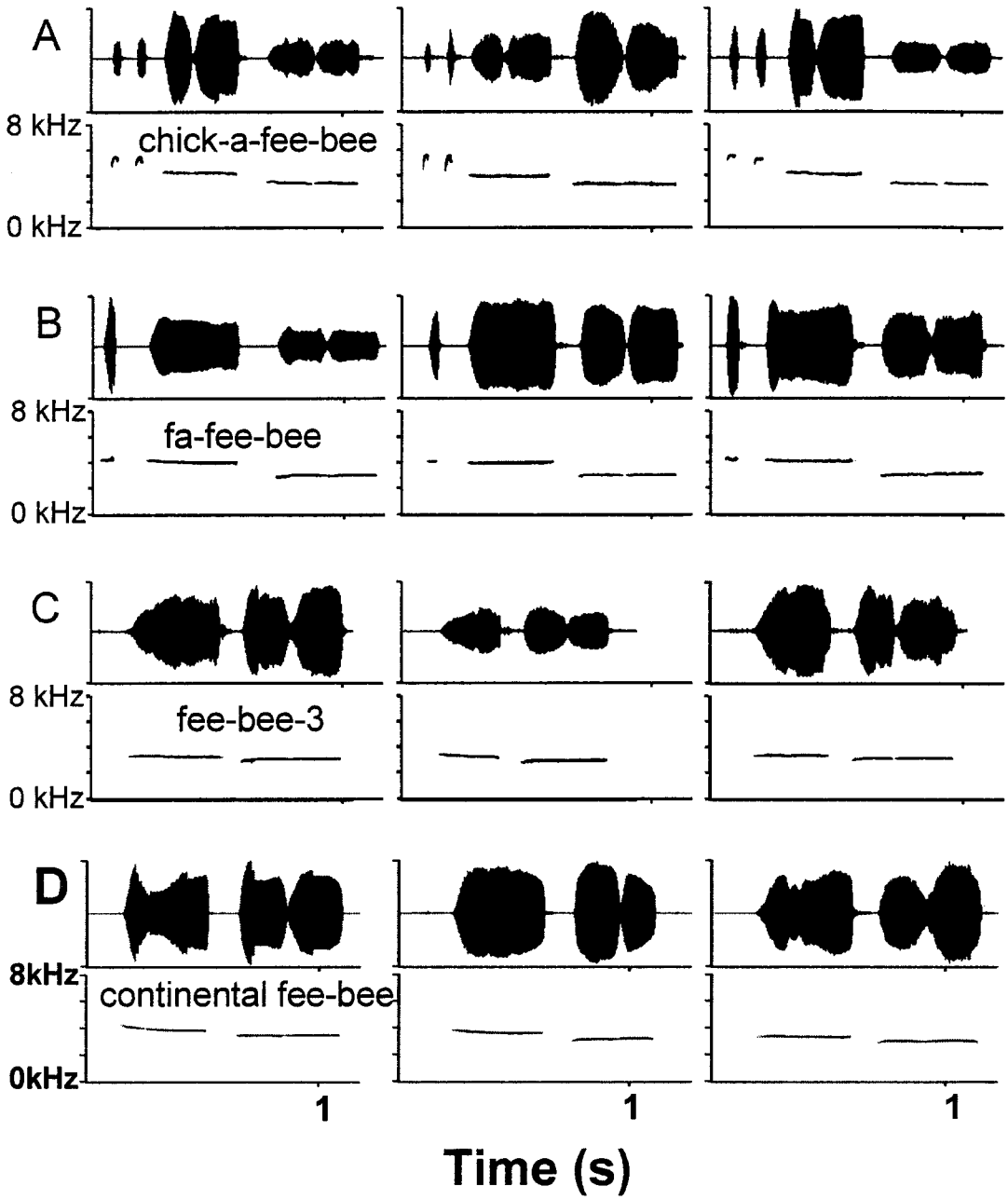


Fig. 1.2. Map of study sites where recordings were made in Spring 2001. The number of birds recorded at each site is included in parentheses. Fort Collins included 57 birds at 10 study sites.

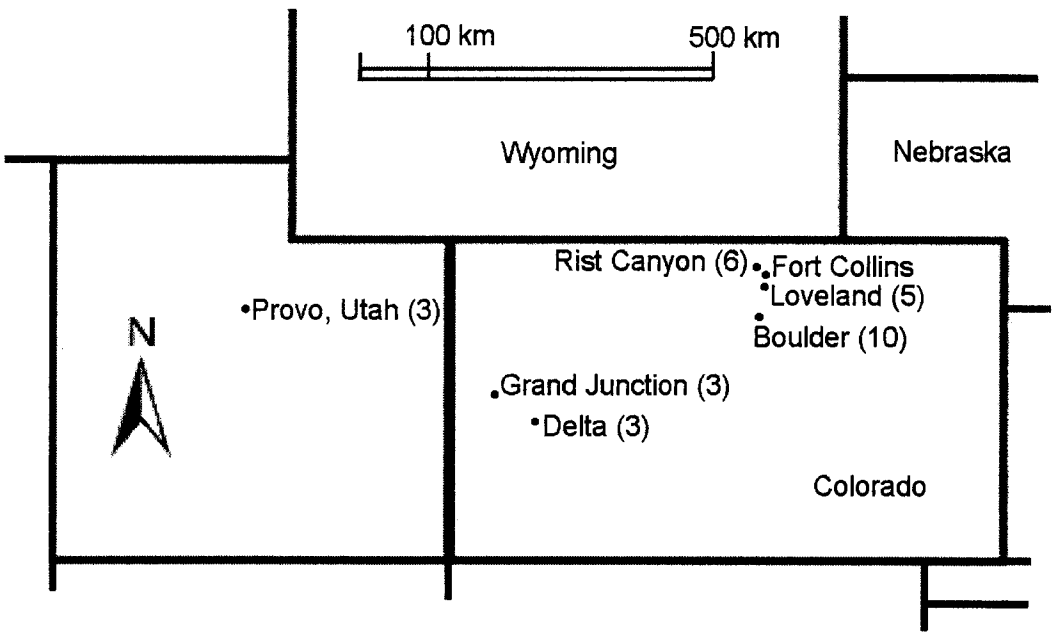
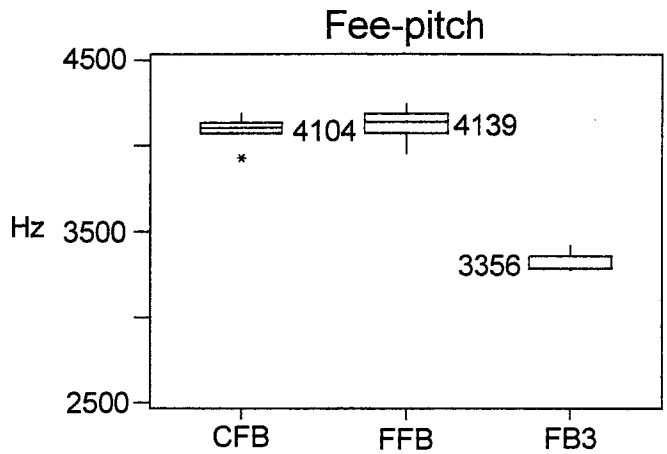
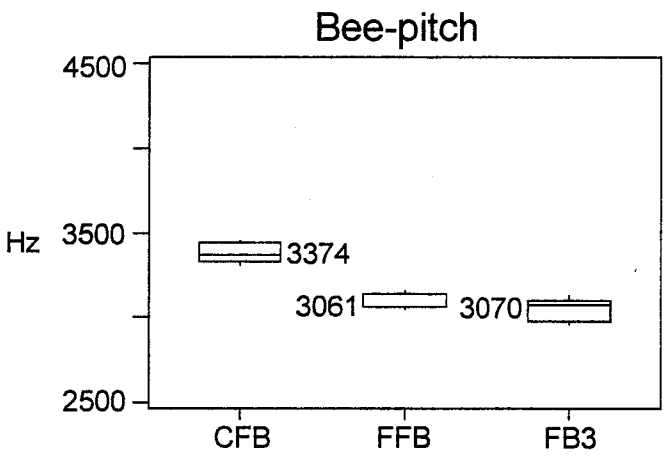


Fig. 1.3 Boxplots showing variation in pitch parameters (fee-pitch, bee-pitch, and pitch ratio) in the three Fort Collins black-capped chickadee song types (CFB = chick-a-fee-bee, FFB = fa-fee-bee, FB3 = fee-bee-3). Medians (horizontal line within the box), quartiles (top and bottom of box), the 0.05 and 0.95 quantiles (tips of vertical whiskers), and extreme data points (asterisks) are shown for each song type. Data were obtained from 2002 dawn chorus subset songs. Each Fort Collins song type possessed a pitch ratio that distinguished it from all other Fort Collins song types.





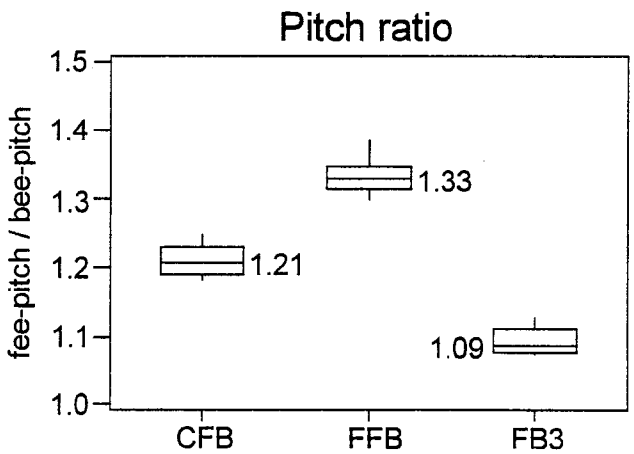


Fig. 1.4. Singing behavior of three individual Fort Collins black-capped chickadees from three separate study sites during their entire dawn singing bouts, showing how each male used his song types over extended song sequences. The x-axis represents the song number in all graphs, and each tick represents 100 songs. In the top graphs, the y-axis shows the fee- and bee-pitches used in each song, while the corresponding graphs below show the pitch ratios (i.e., fee-pitch divided by bee-pitch) for the same songs. The two arrows under "Bird 1" show that during this bird's dawn singing bout, it switched song type at around song 200 from chick-a-fee-bee to fa-fee-bee with a corresponding decrease in bee-pitch of ~300 Hz (upper panel) and an increase in the pitch ratio of ~0.11 (lower panel). The two arrows under "Bird 9" demonstrate how birds practiced limited pitch-shifting (i.e., fee- and bee-pitches both decrease, upper panel) while preserving the pitch ratio (lower panel). Arrows point to pitch-shifting within the fee-bee-3 song type, but pitch-shifting occurred in all song types.

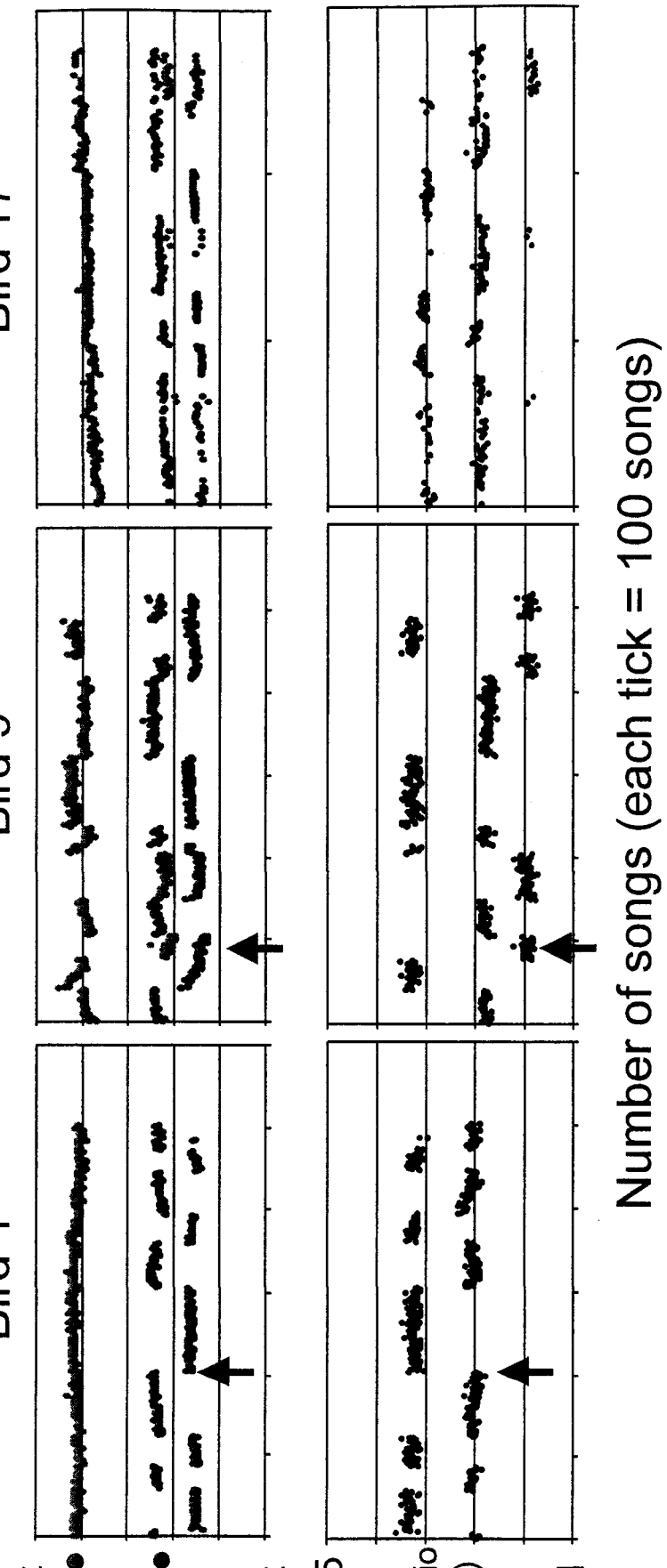
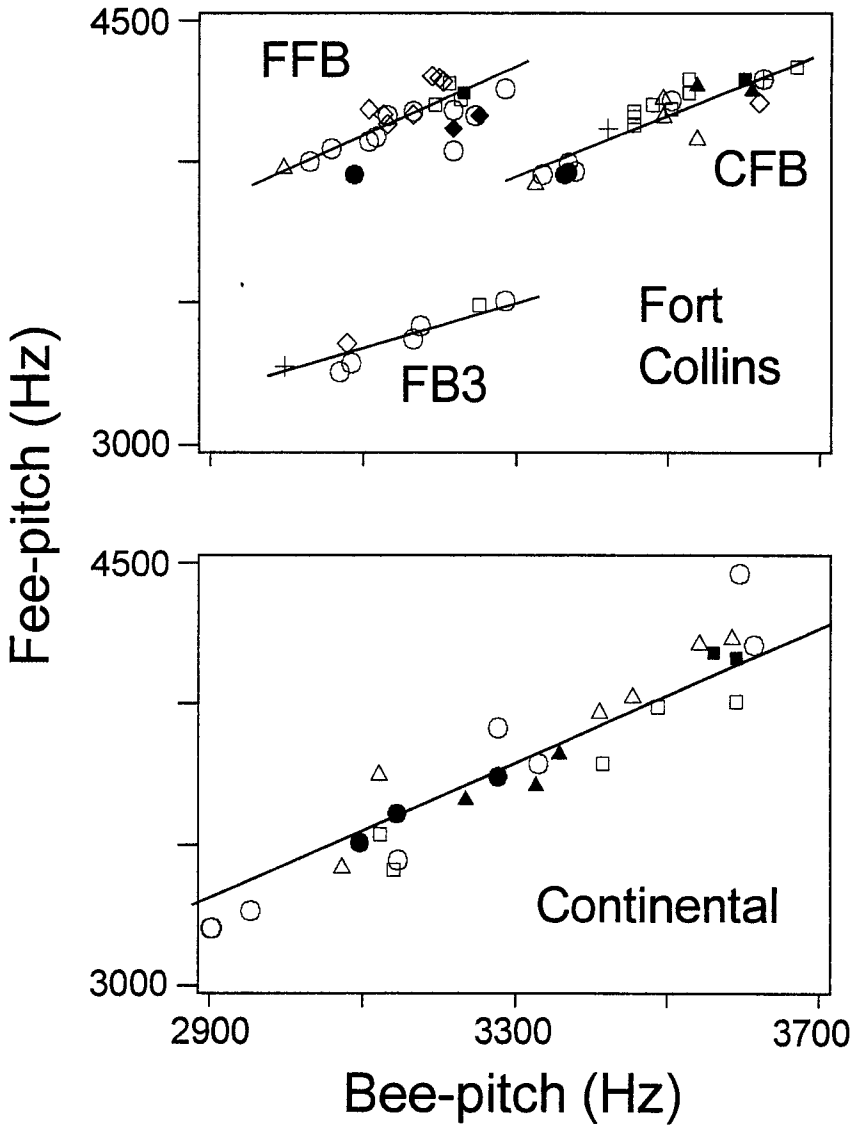


Fig 1.5. Scatter plots showing the fee- and bee-pitches for black-capped chickadee songs recorded at Fort Collins study sites (top) and all other study sites combined (bottom). Clusters identifying each song type within Fort Collins are labeled, and line slopes indicate the pitch ratios for each Fort Collins song type (CFB = chick-a-fee-bee, pitch ratio = 1.21; FFB = fa-fee-bee, pitch ratio = 1.33; FB3 = fee-bee-3, pitch ratio = 1.09). Each study site within a graph is represented by a different symbol. For continental sites, open circle = Boulder, CO, closed circle = Delta CO, open square = Loveland, CO, closed square = Grand Junction, CO, open triangle = Rist Canyon, CO, and closed triangle = Provo, UT. The fee- and bee-pitches used in the three Fort Collins song types all lie within the range of pitches used outside of Fort Collins.



CHAPTER II

DESCRIPTION OF GEOGRAPHIC VARIATION

Geographic variation has been documented in the learned vocalizations of numerous songbirds (Krebs and Kroodsma 1980, Kroodsma and Baylis 1982), parrots (Bradbury et al. 2001), hummingbirds (Ficken et al. 2000), pinnipeds (e.g., seals, Morrice et al. 1994), cetaceans (e.g., whales and dolphins, Winn et al. 1981, Ford 1991, Ding et al. 1995) and humans (Cavalli-Sforza and Wang 1986). In contrast, geographic variation has rarely been found in non-learned vocalizations such as the songs of nonoscine passerines (Trainer and Parsons 2001) and the vocalizations of non-marine mammals (Janik and Slater 1997). When geographic variation in the vocalizations of nonoscine passerines and nonmarine mammals is found, it is often clinal and can be traced to ecological or genetic factors rather than learned differences (Mundinger 1982). Thus in general, there appears to be concordance between social learning of learned vocalizations and geographic variation in learned vocalizations (Krebs and Kroodsma 1980). Indeed, the presence of geographic variation on small spatial scales is often used to infer that individuals acquire their vocalizations through imitation of the vocalizations of conspecifics, i.e., 'vocal learning' (Kroodsma and Baylis 1982).

Additionally, there is ample evidence indicating that animals that learn vocalizations do not always imitate accurately. Laboratory and field studies of song development in a variety of species have demonstrated some of the ways in which novel vocal material might be introduced into a population (Nottebohm 1968, Marler and Peters 1982, Lynch 1996). For example, during early developmental stages individual syllables are often poorly formed, and syllables or syllable groups often undergo recombination (i.e., repetitions, deletions and rearrangements). Therefore, song might diverge through changes in syllable acoustic morphology and through recombination. This has been shown to be the case for songs from different geographic areas (Lynch and Baker 1994, Miyasato and Baker 1999). In only a few instances, however, has geographic variation been compared to the vocal learning 'mistakes' (i.e., cultural mutations) made by individuals in those populations displaying geographic variation (Kroodsma 1974, Jenkins 1978).

We also understand little about how geographic acoustic variation is affected by spatial structure between populations. Humans have fragmented the landscapes for many species, resulting in decreased connectedness between populations and, in some cases, decreasing local population sizes (Vitousek 1994). Many have shown in the field and laboratory that vocal development of juvenile songbirds is influenced by social interactions (Kroodsma 1974, Jenkins 1978, Nelson and Marler 1994, Payne 1996, Nordby et al. 2000), usually to the effect that a juvenile conforms the acoustic features of its song(s) to match the songs of its social models, such as neighboring territory holders. Because juveniles in smaller or disconnected populations are exposed to fewer neighbors

and limited social interaction, one might expect that learned vocalizations in these populations might be more likely to diverge.

In chapter 1, I described novel acoustic divergence in the black-capped chickadee's (*Poecile atricapillus*) 'fee-bee' song. Individual chickadees across most of its North American range normally possess a single song type that does not vary geographically over several thousand kilometers (Kroodsma et al. 1999). In Fort Collins, Colorado, however, most individuals possess three acoustically distinct song types distinguished from the continental song type by novel introductory syllables, stereotyped pitch relationships between the 'fee' and 'bee' syllables of each song type, and an amplitude break in the fee syllable of one of the song types (Fig. 2.1, Chapter 1). In this chapter I describe microgeographic variation between the songs of adult chickadees in the immediate Fort Collins area for each of the three song types and compare this variation to acoustic variation in the songs of individual juveniles around the time of natal dispersal. I also examine acoustic divergence between the songs of continuously distributed populations along a riparian corridor and the songs found in discontinuous, low abundance habitat.

METHODS

Sampling of Juvenile Song

My rationale for recording songs from juveniles was that any novel vocal material within a population would likely be introduced by juveniles following natal dispersal, which occurs two to four weeks after fledging (Baker et al. 2003b). Following dispersal, juveniles remain within the space of a few hectares for the rest of their lives (Smith

1993). My goal was to record fee-bee song from juveniles at approximately the time of natal dispersal to document the types of acoustic variation within each song type that could contribute to geographic divergence in signal structure.

Repeated visits were made to several study sites within Fort Collins during the fledgling period (June and early July) in 2003 to monitor the development of chickadee family groups. I recorded juvenile fee-bee songs using a MiniDisc recorder (Sony MZ-N1) attached to a Sennheiser microphone (ME62) mounted in a Telinga Pro-Universal 60 cm parabola. Songs given by juveniles were easily distinguished from songs given by adults because of their lower amplitude, a lack of pitch control during note production and recombinant syntax (Baker et al. 2003b), all of which rarely occur in adult song. Through observations made daily or every other day, the time within one day of natal dispersal was determined, and only songs recorded within three days of natal dispersal were sampled for analysis. If a solo juvenile appeared at a study site that I had been monitoring and no solo juvenile had been observed previously at that location, I assumed it had left its parents within the previous three days, and I sampled its songs as well.

Description of study sites for sampling adult song

Black-capped chickadees favor deciduous trees for regular breeding and foraging activities (Smith 1993). In the North American Great Plains region chickadee distribution is limited to riparian corridors, urban areas containing deciduous trees, and isolated clumps of trees with nearby water. Fort Collins, Colorado, lies at the western edge of the Great Plains next to mountainous coniferous habitat inhabited by mountain chickadees (*Poecile gambeli*) but not by black-capped chickadees.

Within Fort Collins itself, black-capped chickadees are abundant and distributed relatively continuously. Outside of the city, chickadee distribution is continuous only along the Poudre River riparian vegetation corridor (Unpublished data). Small chickadee populations (≤4 pairs per population) also exist in isolated habitat patches a few dozen kilometers north of Fort Collins with low connectivity between patches. These tree clumps serve as small 'habitat islands' for chickadees, and not all habitat patches are occupied. As one goes farther north, elevation increases and habitat connectivity decreases until eventually there is no available chickadee habitat for approximately 20 km in any direction (Unpublished data). Thus, these habitat islands north of Fort Collins are likely to serve as 'sink' populations (Hanski and Gilpin 1997) that depend on dispersal from Fort Collins juveniles. For this chapter, I divided my study sites into 'River' sites and 'Islands North' sites (Fig. 2.2).

Sampling of adult song

Adult male black-capped chickadees were recorded during the spring of 2003 from multiple study sites throughout the River and Islands North areas in northern Colorado (Fig. 2.2). Many recordings were made during the dawn chorus when each chickadee sings hundreds of songs (Otter and Ratcliffe 1993) and cycles through all song types in its repertoire (Chapter 5). Other recordings were made opportunistically after the dawn chorus or by stimulating chickadees to sing using playback of synthetic song. Recordings were made with the same equipment used in recording juvenile song. Nearly all birds were unbanded, but recordings were made systematically so that individuals were not sampled more than once.

Spectrograms were made of all song recordings using Syrinx software (John Burt, http://www.syrinxpc.com/index.html), sampling at 22.05 kHz with 16-bit accuracy and 1024-point transform size, and all songs were classified into three groupings based upon the three Fort Collins song types described previously (Fig. 2.1, Chapter 1). Although variation occurred within each song type grouping, the presence of introductory notes and the pitch relationships for the whistled 'fee' and 'bee' notes made such classification unambiguous. I will refer to the song type groupings as CFB, FFB and FB3 (Fig. 2.1) and use the longer names ('chick-a-fee-bee', 'fa-fee-bee' and 'fee-bee-3') only to describe the particular variant of each grouping most common in Fort Collins chickadees. I chose the highest quality representative of each song type recorded from each of the males for further analysis. Quality was determined by signal-to-noise ratio. Because individual males possess multiple song types (Chapter 1), several males contributed more than one song representative to this data set.

In Chapter 1, I classified the principal acoustic dimensions distinguishing the three song types found in Fort Collins. These acoustic dimensions were the pitches of the fee and bee syllables, the ratio formed when dividing the fee-pitch by the bee-pitch, the number and type of introductory syllables, the presence or absence of an amplitude break in the fee-syllable, and any syllable recombination (i.e., deleting, repeating or in some way reordering existing syllables or syllable groups). In this chapter I used the same acoustic dimensions to classify acoustic variation within each song type grouping (Fig. 2.3). Pitches were measured at the midpoint of each fee and bee syllable using the onscreen cursors within Syrinx (Chapter 1), which allowed a pitch resolution of 6 Hz.

When recording quality was not sufficiently high to make all acoustic measurements, the songs were excluded from analyses.

Analysis of adult song

After classifying the acoustic characteristics of each song, I compared the amount of acoustic divergence within a song type grouping using divergence measures analogous to measures of song similarity in bioacoustic software (e.g., Charif et al. 1995, Tchernichovski et al. 2000), but I based my measures on the kinds of errors in learning (i.e., cultural mutations) introduced by imperfect imitation. I did not include any of the pitch measurements in my divergence measure because pitch varies within individuals for each song type and overlaps between individuals (Horn et al. 1992, Chapter 1, Unpublished data), and I wanted to focus on more consistent differences among individuals. Because I sampled only one song for each individual, pitch differences between sampled songs might have existed because of within-individual differences rather than between-individual differences. Each song in my data set was compared against all other songs of the same song type grouping within its own study site and the two closest study sites. In simple terms I tried to measure how different each song was from songs of the same song type grouping used by nearby individuals. For all River study sites, the two closest study sites were always River sites, and for Islands North sites, the two closest study sites were always Islands North sites.

To measure the amount of divergence between two songs, I counted the number of differences between songs in all discrete variables. These variables were the presence or absence of an amplitude break in the fee syllable, the number and type of introductory

notes, and any syllable recombination. These characters generally varied little within individuals. I added these values to yield an integer representing the number of discrete differences between the songs (range={0,6}, Fig. 2.4).

Because each song was compared with other songs recorded within its study site and songs recorded at the two closest study sites, several divergence measures were recorded for each song. These divergence values were then averaged to get a single divergence value for each song. Divergence data were not normally distributed, so I applied a log(x+1) transformation. I then constructed a mixed ANOVA model with two fixed effects: "area" (River vs. Islands North) and "song type grouping", and two random effects: "study site" and "bird". Bird was nested within study site, and study site was nested within area. My goals were to find out whether song divergence data were different in the two areas and whether some song types were more likely to diverge than others.

I next wanted to compare the acoustic structure of the introductory syllables of CFB and FFB song types groupings between the Islands North and River areas. I examined introductory syllables from songs only from study sites where both types of introductory notes were present. To make comparisons, I randomly picked five CFBs and five FFBs from both the Islands North and River areas. I isolated the introductory syllables from each wave file and examined their spectrograms visually.

I next looked for patterns of acoustic change moving along the River. Each River study site was assigned a distance measure based on how far downstream it was from the most upstream study site (GRock, Fig. 2.2). River distances were obtained from the USGS National Hydrography Database (http://nhd.usgs.gov/index.html). I made tables

and graphs of the acoustic measurements from songs at each study site vs. River distance and looked for patterns moving upstream or downstream.

Analysis of juvenile song

If acoustic divergence in adult birdsong results from the introduction of novel acoustic material by juveniles following natal dispersal, then juvenile song should vary acoustically at the time of dispersal and this variation should correspond to the acoustic differences between the songs of adults. To see if this was the case, I made spectrograms of all songs. Then I inspected spectrograms visually and searched for correspondence between acoustic variation within individual juveniles and acoustic variation distinguishing the songs of adults from separate study sites.

RESULTS

Songs were recorded from juveniles of two family groups in Fort Collins immediately prior to dispersal of the juveniles and from three individual juveniles in Fort Collins immediately following their natal dispersal. Adult songs were recorded from 88 individuals at 17 River study sites and from 24 individuals at 12 Islands North study sites. I recorded multiple song types from many individuals, so some individuals were represented by more than one song. Of the 210 total adult songs that were counted, 85 were classified into the CFB song type grouping, 71 were classified as FFB, and 52 were classified as FB3. Each song type grouping was distributed throughout all study sites. Although some individuals were represented in more than one of the song type groupings, every song within a song type grouping came from a different individual. I

did not analyze two songs from one individual possessing five song types at the farthest north study site in the Islands North area because these two songs did not classify into any of the three song type groupings. One of these songs combined a whistle with a buzzy note, and the other contained a string of several short, frequency-modulated whistles (Fig. 2.5).

Area, song type grouping, the interaction between area and song type grouping, and study site all had statistically significant effects on divergence data values, but bird did not have an effect (Mixed model: Area: $F_{1,92}=73.30$, $P<0.001$; Song type grouping: $F_{2,92}=17.87$, $P<0.001$; Area X Song type grouping: $F_{2,92}=5.58$, $P=0.005$; Study site(area): $F_{27,92}=3.17$, $P<0.001$; Bird(study site): $F_{83,92}=0.60$, $P=0.992$). Raw divergence scores were generally three to five times higher in the Islands North area than in the River area for all song type groupings, but the difference was least pronounced in the FB3 song type grouping (Fig. 2.6). Additionally, the song type groupings with introductory notes (CFB and FFB) possessed four to five times as much divergence as the song type grouping lacking introductory notes (FB3, Fig. 2.6).

The acoustic morphology of both types of introductory notes varied some geographically (Fig. 2.7) in both the River and Islands North area. Neither area, however, possessed obviously more variation in introductory note morphology.

Gradual transitions in many acoustic dimensions were found moving both upstream and downstream from Fort Collins (Fig. 2.8, Table 2.1). Pitch ratios between each song type grouping were maximally different in Fort Collins (within Fort Collins: FFB average pitch ratio=1.32, N=24; CFB average pitch ratio=1.20, N=26; FB3 average pitch ratio=1.09, N=10), while moving away from Fort Collins both upstream and

downstream, pitch ratios for all song type groupings began to converge upon approximately 1.15 (Fig. 2.8). Introductory notes for CFB were found only in Fort Collins, but introductory notes for FFB were distributed farther upstream and downstream before they also phased out (Table 2.1). The amplitude break in the fee syllable of CFB was also found farther upstream and downstream from Fort Collins, but it also phased out eventually (Table 2.1). At the extreme upstream and downstream study sites, nearly all songs were similar to the continental song type in that they lacked introductory notes and amplitude breaks in fee, and possessed pitch ratios similar to those reported for the continental song type (~1.15, Weisman et al. 1990, Kroodsma et al. 1999). Islands North songs were similar to Fort Collins songs in that they usually possessed introductory notes and used comparable pitch ratios (Fig. 2.8), but there was noticeably more variation within and between Islands North study sites in the amount of recombination, the number of introductory syllables and the presence/absence of the amplitude break in the fee syllable (Table 2.1).

The songs of juvenile black-capped chickadees in Fort Collins were still plastic at the time of natal dispersal and varied in multiple acoustic dimensions including all dimensions I measured to characterize adult song. Furthermore, the acoustic variation between the songs of individual chickadees recorded near the time of natal dispersal was similar to the acoustic variation distinguishing songs recorded from adults at different study sites (Fig. 2.9-2.12).

DISCUSSION

The fee-bee song of the black-capped chickadee has been described as "remarkably invariant across most of their range in North America" (Kroodsma et al. 1999). In contrast, I found a great deal of geographic variation in the songs of black-capped chickadees in an area spanning a few dozen kilometers around Fort Collins, Colorado. I also found variation between the songs of individual juveniles at the time of natal dispersal, which has been observed in other species as well (Marler and Peters 1982, Nelson 2000, Baker et al. 2003b). The acoustic differences distinguishing songs of adult birds from different study sites coincided with the types of vocal learning errors made by Fort Collins juveniles at the time of natal dispersal. Apparently geographic acoustic variation in black-capped chickadees can be attributed to imperfect song imitation. This finding parallels results from studies of other species also showing a direct connection between microgeographic variation and the types of cultural mutations introduced into a population (Kroodsma 1974, Jenkins 1978).

When moving along river habitat away from Fort Collins, both upstream and downstream, I found gradual acoustic transitions leading to songs resembling the continental song type. The geographic locations of these transitions differed depending on the acoustic feature. For example, the introductory notes of FFB were found farther upstream and downstream than the introductory notes of CFB. Gradual acoustic transitions along the Fort Collins River area were also found for another of the chickadee's learned vocalizations, the 'gargle', in an earlier study (Miyasato and Baker 1999, Baker et al. 2000). Gradual acoustic change has been found in the learned vocalizations of many species (e.g., song sparrow, *Melospiza melodia*, Searcy et al. 2002, within human language, *Homo sapiens*, Cavalli-Sforza and Wang 1986), but more abrupt

acoustic changes have been found in other species (e.g., Nuttall's white-crowned sparrows, *Zonotrichia leucophrys nuttalli*, Baptista 1975; corn buntings, *Emberiza calandra*, McGregor 1980, killer whales, *Orcinus orca*, Ford 1991).

Divergence differences between geographic areas

In contrast to the gradual acoustic transitions between chickadee songs along the River area, sharp acoustic transitions were found between many Islands North study sites. Acoustic divergence was generally three to four times as great in the Islands North area vs. River area for all song type groupings. One chickadee in the most extreme northern site within the Islands North area developed two novel song types that did not resemble any black-capped chickadee song ever reported (Fig. 2.5). The difference in divergence within the Islands North area vs. divergence in the River area might be attributable to one or more of several mechanisms since the two areas differed in many ways ecologically and socially. The three most important ecological factors distinguishing the two areas were differences in the amount of habitat connectivity, differences in the sizes of populations, and perhaps whether the population was a source or a sink.

First, habitat connectivity was much lower between Islands North study sites. Reduced connectivity would decrease dispersal between populations, and there may be a relationship between the amount of dispersal between populations and the amount of acoustic divergence between populations. This relationship has never been investigated directly to my knowledge, but songs of island populations are often different from the songs of mainland populations for a variety of species (Lynch and Baker 1994, Baker

1996, Baker et al. 2003c), perhaps because of limited dispersal between island and mainland populations.

The second factor distinguishing Islands North populations was that they were small whereas most River populations were much larger. During the chickadee dawn chorus Islands North males had an average of one audible singing neighbor; River males had an average of seven audible singing neighbors (Unpublished data). In some species, juveniles alter their song structure to match the song structure of their neighbors following dispersal (Kroodsma 1974, Nelson 1992, 2000, Payne 1996, Nordby et al. 2000). In smaller populations there may be decreased social pressure for dispersing juveniles to conform to the local song forms. Decreased selective pressure during juvenile song development would allow song to diverge from the local acoustic pattern.

The third factor between Islands North and River areas is that Islands North populations may have been 'sink' populations (unpublished data). Sink populations often go locally extinct and thus depend on juvenile colonization to start up the population again (Hanski and Gilpin 1997). Thus, the songs used in sink populations likely reflect the songs developed by the founding individual(s). Under this scenario, a 'withdrawal of learning' might take place (Thielcke 1973) in which acoustic material present in the dispersing juvenile's parent population is lost as the juvenile settles elsewhere. Conversely, novel acoustic material might be introduced by the juvenile as shown by the extra acoustic material possessed by the juveniles I recorded at the time of natal dispersal (Fig. 2.9-2.12).

At this time I cannot distinguish between the relative contributions of the three factors listed above. All three may have played a role in generating greater acoustic

divergence between Islands North study sites relative to the River sites. Nevertheless, it would be useful to gain a better understanding of the specific social and ecological factors promoting geographic divergence in song. Individuals of many songbird species are less responsive to song that is dissimilar from their own song (Baker 1983, Baker et al. 1987a, Searcy et al. 2002), and acoustic divergence has frequently been hypothesized to lead to reproductive isolation and eventually speciation (Baker and Cunningham 1985, Slabbekoorn and Smith 2002).

Divergence differences between song type groupings

When comparing divergence for each of the song type groupings, CFB and FFB diverged approximately four to five times as much as FB3, which diverged only slightly in the Islands North area and hardly at all in the River area (Fig. 2.6). Divergence differences between song type groupings are probably related to differences in the acoustic structure of the song type groupings. In Fort Collins, CFB and FFB songs both possess evolutionary novelties normally not found in chickadee song (introductory syllables and the fee-break in CFB); in contrast, FB3 is acoustically similar to continental fee-bee song, differing only in its use of stereotyped fee- and bee-pitches (Chapter 1). In many aspects of biology, evolutionary novelties have opened up new possibilities for further evolutionary divergence. For example, the evolutionary novelty of flight probably facilitated bird ecological diversification and rapid speciation (Gill 1995). I propose that the introductory notes and fee-break in CFB and FFB are cultural evolutionary novelties that facilitated further diversification of song.

In addition to Fort Collins, Colorado, introductory syllables and/or amplitude breaks in the fee syllable have evolved independently in several geographic areas: Washington state (Hammond 1993: fig. 3), a group of islands off the coast of Massachusetts (Kroodsma et al. 1999: fig. 1), Ontario (Christie et al. 2004: fig. 3), Fort Lupton, Colorado (Unpublished data), and Casper, Wyoming (Unpublished data). In all of these areas where song recordings were made at multiple study sites (Washington, Massachusetts, Fort Collins and Fort Lupton), geographic variation existed for the song types possessing these evolutionary novelties (Kroodsma et al. 1999, this chapter, Unpublished data). This suggests that chickadee song types possessing these acoustic novelties are highly prone to cultural mutation. In contrast, all other locations across North America with recordings of chickadee song from multiple study sites show a lack of geographic variation (Kroodsma et al. 1999), just as I found little to no geographic variation within my FB3 song type grouping. I suggest that black-capped chickadee song across most of North America does not vary geographically, at least in part, because the acoustic structure of the continental song type is less prone to cultural mutation during juvenile song imitation than is the acoustic structure of most learned vocalizations.

Table 2.1: Table summarizing discrete acoustic variation for all songs of all study sites. Area column indicates River (R) or Islands North (I). River distance indicates the distance downstream from the topmost site (GRock). Islands North distances indicate the distance from LMP site in Fort Collins. All distances are given in kilometers. 'CFB', 'FFB' and 'FB3' indicate song type groupings (see Fig. 2.1). 'Recom' refers to the presence of recombination (see code at the bottom of this heading). 'Fee-break' refers to the presence/absence of the amplitude break in the fee syllable. Numbers within the table indicate the number of songs sampled at a particular site that fell within that category. For example, of the 7 CFB songs sampled from site ELC, none possessed recombination, 5 possessed two introductory syllables while 2 did not possess introductory syllables, and all 7 possessed an amplitude break in the fee syllable. Recombination code: a = drop 2nd half of bee syllable, b = expand amplitude break in bee so that bee becomes two syllables, c = drop bee syllable, d = replace bee syllable with 'D' syllable from the chick-a-dee call, e = repeat terminal syllable, f = repeat entire song, g = replace fee syllable with a bee syllable, h = replace bee syllable with an additional song, i = append additional song.

			CFB											FFB										FB3									
			Recom		Number of intros							fee-break		Recom		Number of intros							fee-break		Recom		Number of intros							fee-break	
Area	Dist	Site	no	yes	0	1	2	3	4	5	6	no	yes	no	yes	0	1	2	3	4	5	6	no	yes	no	yes	0	1	2	3	4	5	6	no	yes
R	0	GRock	1		1							1		2		2							2		1		1							1	
R	6.1	Gate	2		2								2	1		1							1		1		1							1	
R	7.99	PRock	2		2							1	1	1		1							1		3		3							3	
R	15.1	Ted	3		3								3	2		2							2		2		2							2	
R	19.1	Tam	5		5								5	5		1	3	1					5		4		4							4	
R	21.1	CLP	3		3								3	2		1	1						2		1		1							1	
R	21.9	Lion	6	1de	7								7	1	1df		2						2		1		1							1	
R	25.9	WLMP	3				3						3	5			5						5		3		3							3	
R	28.4	LMP	9	1a			10						10	8			8						8		3	1h,1e	5							5	
R	31.8	ML	6				6						6	8			8						8		0										
R	36.9	ELC	7		2		5						7	4			4						4		2		2							2	
R	51.6	WIN	4		4								4	3			3						3		4		4							4	
R	86	Gre	1		1								1	1		1							1		1		1							1	
R	115	70R	1		1							1		1					1					1	1		1							1	
R	133	MAS	1		1							1		1			1						1		1		1							1	
R	174	FtMo	2		2							2		1		1							1		2		2							2	
R	258	STER	4		4							4		4		4							4		4		4							4	
I	8.52	WavT	2				2					2		2			2						2		2		2							2	
I	9.13	Elco2	2				2					1	1	1			1						1		0										
I	11.7	StMa	2				2					2		2			2						2		2		2							2	
I	14	Well	2				2						2	1					1				1		0										
I	14.5	WEL4	3		3								3		2c		1	1					2		2		2							2	
I	15.5	66E	1				1						1	0											0										
I	15.7	Waver	2		1		1					1	1	2		1		1					2		2		2							2	
I	16.5	Zimd		1c			1					1		2				2					2		2		2							2	
I	18.7	EX281	4		3		1					3	1	3				3					2	1	2		2							2	
I	20.2	Ziggy	1				1					1		1						1			1		1		1							1	
I	24.4	Ivan		1b			1					1			1b			1					1			1gb	1							1	
I	32	McGr	1	1f		2						2		3							1	2	2	1	1	1i	2							2	

Fig 2.1. Spectrograms and oscillograms of the three black-capped chickadee song types found in Fort Collins, Colorado, on which I based my three song type groupings: CFB, FFB and FB3. In Fort Collins, CFB and FFB songs both possessed novel introductory syllables, and CFB also possessed a novel amplitude break in the fee syllable.

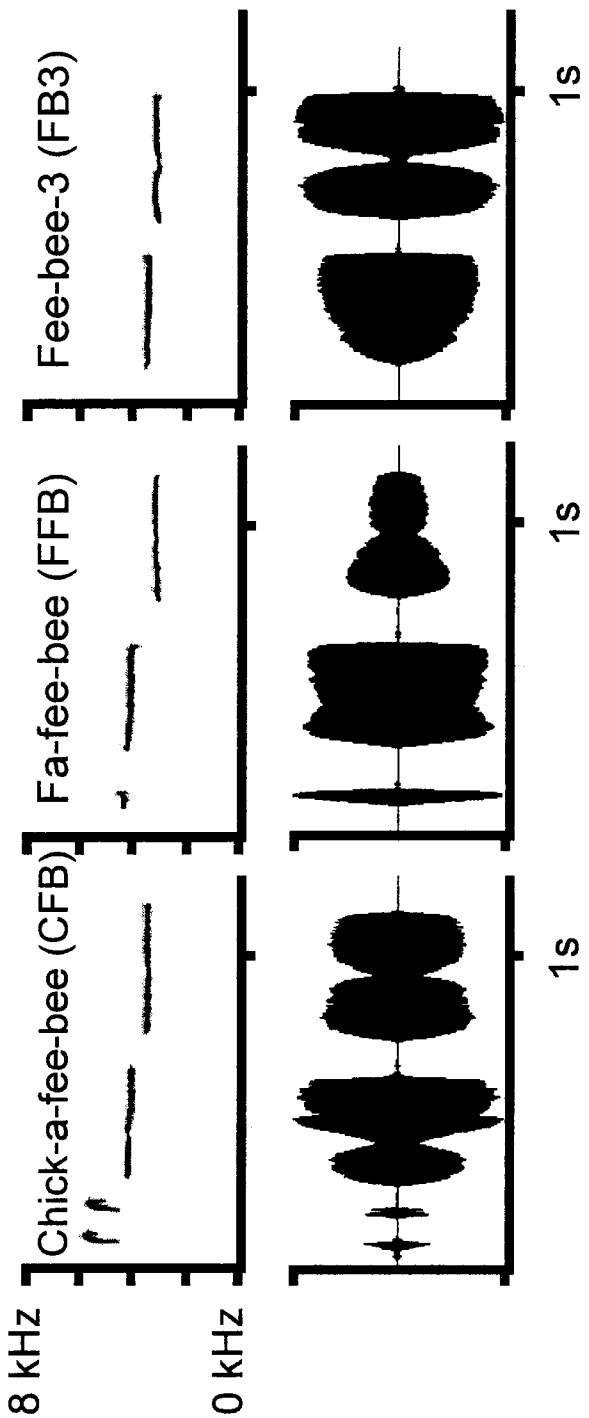


Fig. 2.2. Map of all study sites where I recorded adult chickadee song. I grouped study sites according to whether they were located along the river system passing through Fort Collins ('River' = circles) or in the small habitat islands located north of Fort Collins ('Islands North' = squares). Each study site is labeled with its name and the number of individuals recorded at that site. Four additional River sites were located downstream of the 'Gre' site: '70R' (29km east of Gre), 'Mas' (47km east of Gre), 'FtMo' (88km east of Gre) and 'Ster' (172km east of Gre).

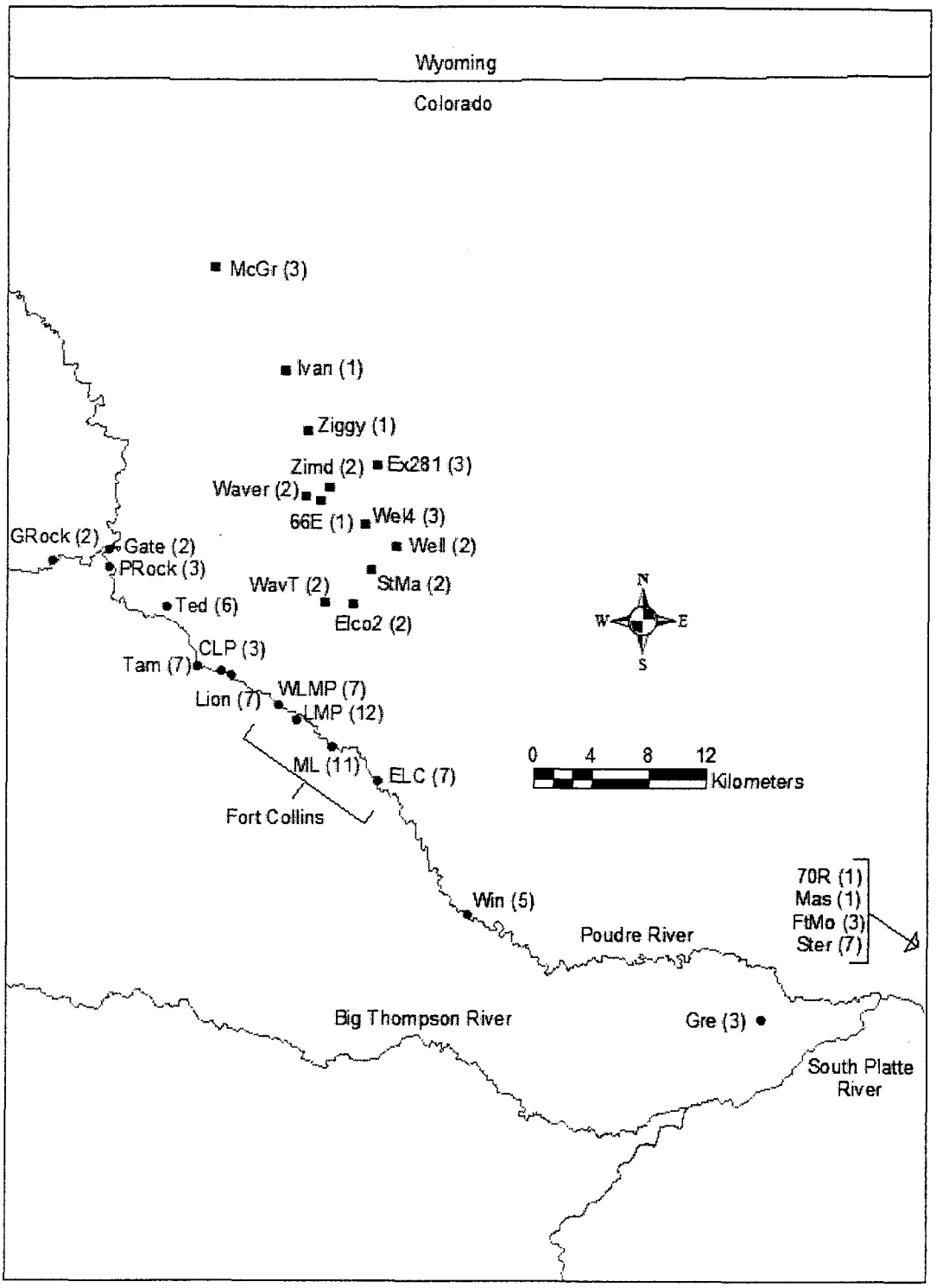


Fig. 2.3. Spectrogram of a CFB from the study site labeled StMa in the Islands North area showing the acoustic measurements I used to quantify each song: A=pitch at midpoint of fee syllable, B=pitch at midpoint of bee syllable, C=pitch ratio (A divided by B), D=type of introductory syllable ('chick-a' or 'fa'), E=number of introductory syllables, F=presence/absence of amplitude break in fee, G=presence/absence of recombination. In the spectrogram illustrated, the values are A=4063Hz, B=3478Hz, C=1.168, D='chick-a', E=2, F=no break, G=no recombination.

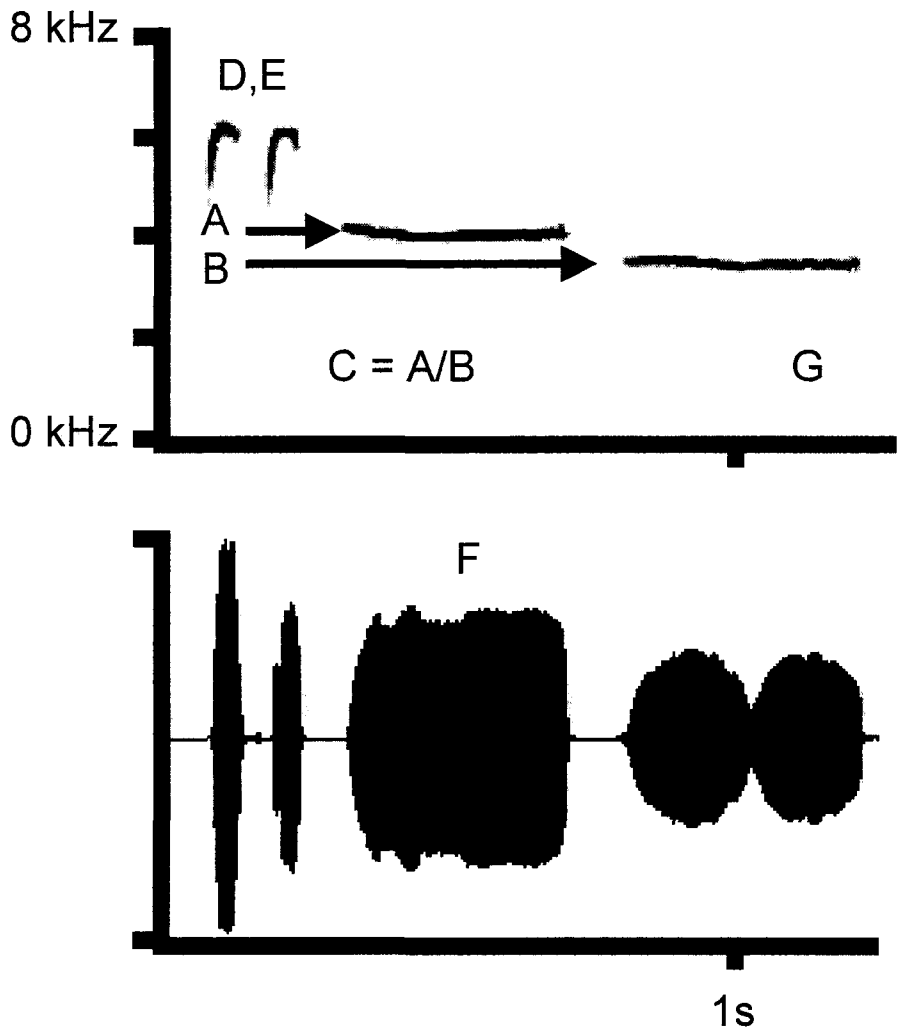


Fig. 2.4. Spectrograms of two FFB songs illustrating how I made pairwise divergence measurements between two songs. The two songs shown possess the same type of introductory note type (0 divergence points) but differ in the number of introductory notes by 2 (2 points). Both songs lack an amplitude break in fee (0 points), and the second FFB song lacks a bee syllable (1 point). Therefore these two songs would receive a divergence value of: $0 + 2 + 0 + 1 = 3$. Pitch measurements were not used in the divergence measure. See text for details.

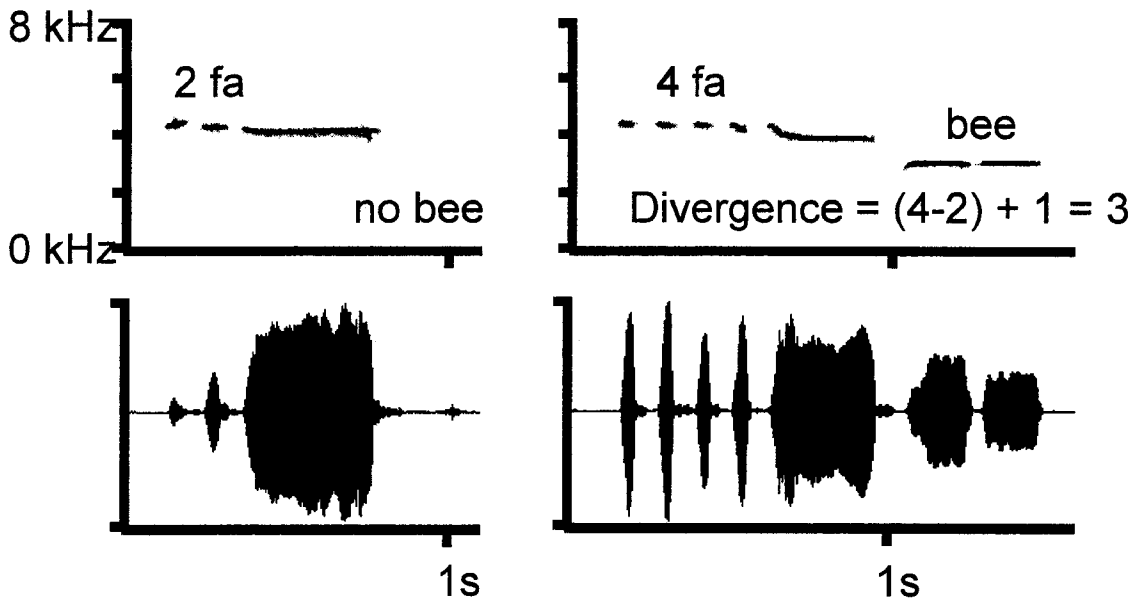


Fig. 2.5. Of the 210 songs sampled, these two song types that did not fit into the CFB, FFB and FB3 song type groupings. An individual at the McGr study site (most extreme northern site in Islands North area) used both of these song types commonly in addition to CFB, FFB and FB3.

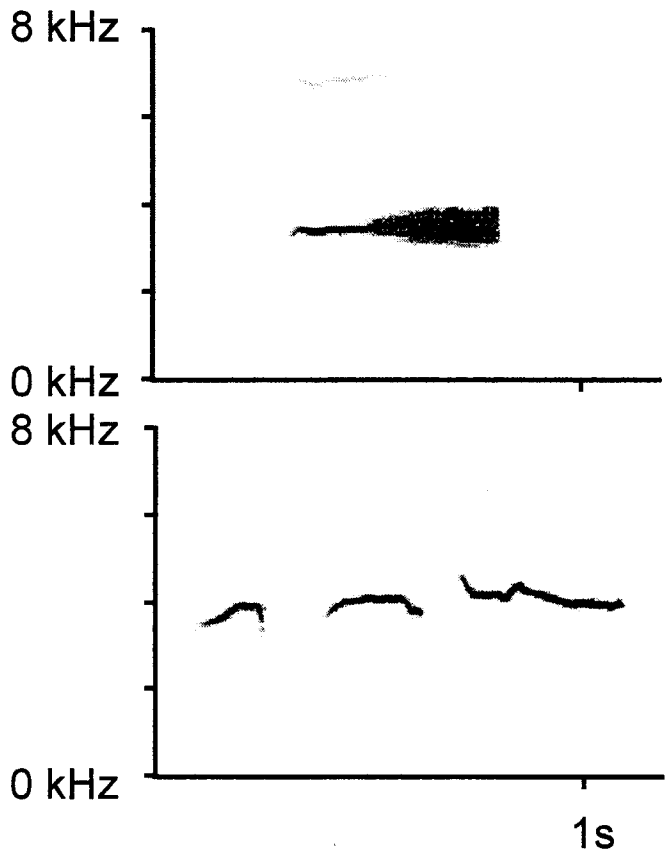


Fig. 2.6. Boxplots comparing raw divergence values between the three song type groupings (CFB, FFB and FB3) in the two geographic areas (R = River, I = Islands North). Medians (horizontal line within the box, labeled), quartiles (top and bottom of box), 0.05 and 0.95 quantiles (tips of vertical whiskers) and extreme data points (asterisks) are shown for each song type. Divergence was higher in the Islands North area, and CFB and FFB song diverged more than FB3 song. See Fig. 2.2 for a map of the geographic areas and Fig. 2.1 for representative spectrograms of the three song type groupings.

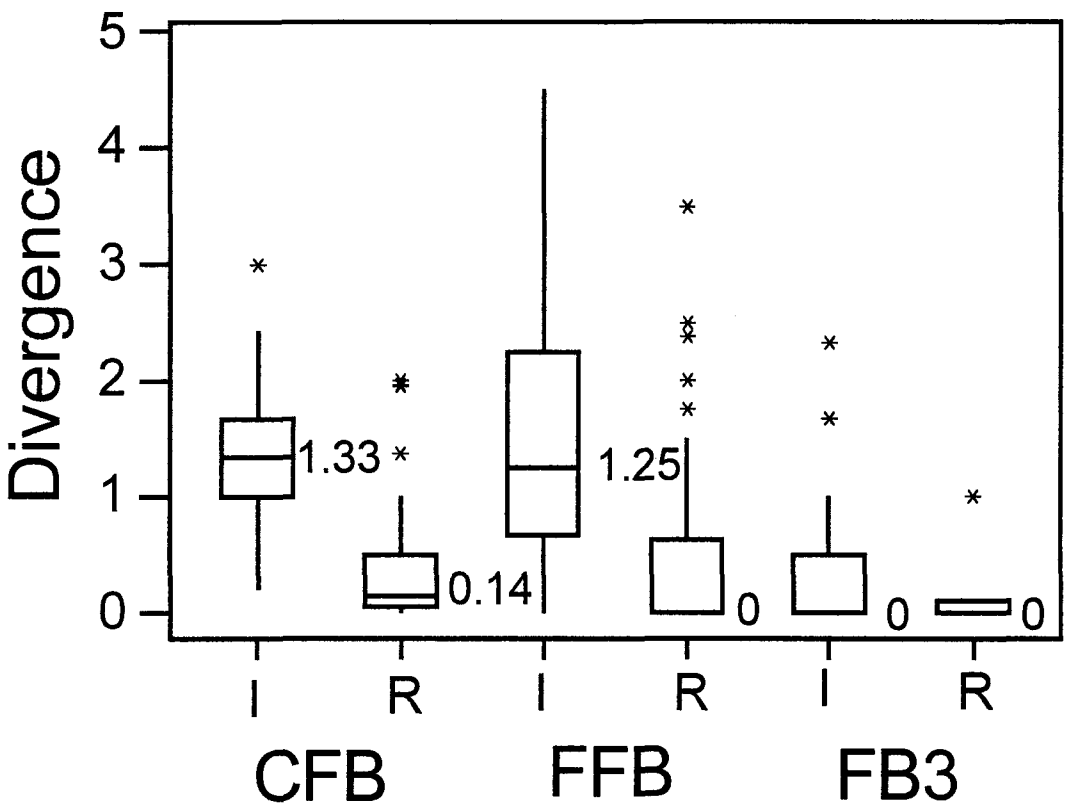


Fig. 2.7. Spectrograms showing variation in the acoustic morphology of the 'chick-a' (a-b) and 'fa' (c-d) introductory syllables. Each syllable or syllable group came from a different individual in Fort Collins (a, c) or the Islands North (b, d) area. Each syllable or syllable group is labeled with the study site where it was recorded. Acoustic morphology varied for both syllable types, but variation was not more extreme in either geographic area.

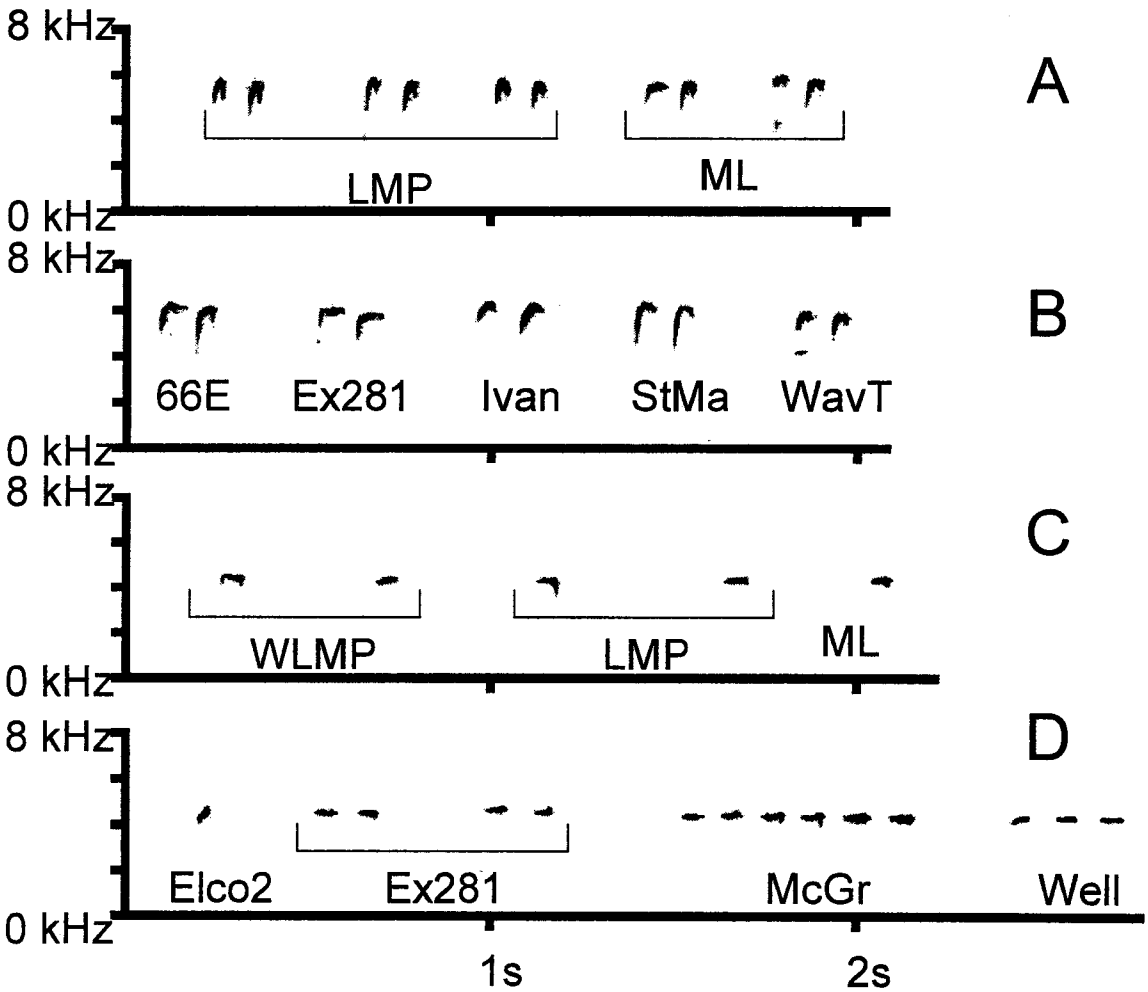
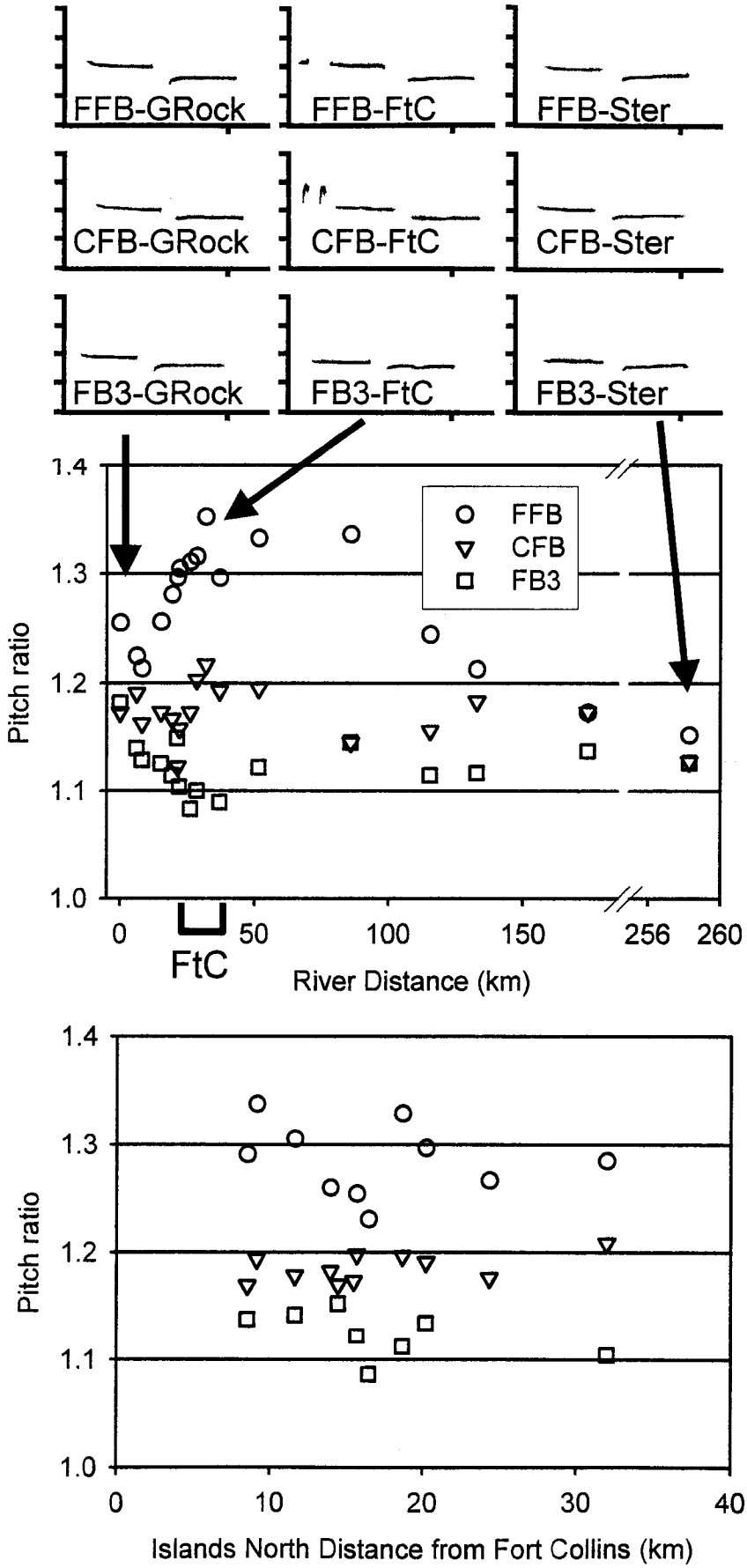


Fig. 2.8. Spectrograms (a) and graphs (b-c) summarizing how pitch ratio varied for each song type grouping along the River area (b) and in the Islands North area (c). The songs of each song type grouping within a study site were averaged for simpler presentation. The location of Fort Collins along the River area is indicated with 'FtC'. The spectrograms (a) show a representative song of each song type grouping recorded from the farthest upstream site (GRock), in Fort Collins itself (FtC), and at the farthest downstream site (Ster). Pitch ratios for song type groupings were maximally different in Fort Collins and converged on 1.15 whether going upstream or downstream. There was no pattern in the Islands North area.



Figs. 2.9-2.12. Comparisons of acoustic variation in juvenile (J) and adult (A) songs. In each figure, a single juvenile uttered the top two songs at the time of its natal dispersal, and adults from separate study sites uttered the bottom two songs. Each spectrogram is labeled according to the age of the individual recorded (J = juvenile, A = adult) and the study site where it was recorded. All juveniles were recorded within Fort Collins, and each adult was recorded from one of the study sites listed in Fig. 2.2. Each figure shows different kinds of acoustic variation distinguishing chickadee song. Juveniles always sang at lower amplitude and with less pitch control than adults. Acoustic differences within the songs of juveniles were similar to the acoustic differences between the songs of adults showing that imperfect song learning can account for geographic acoustic variation in chickadees.

Fig. 2.9: Variation in number of intros

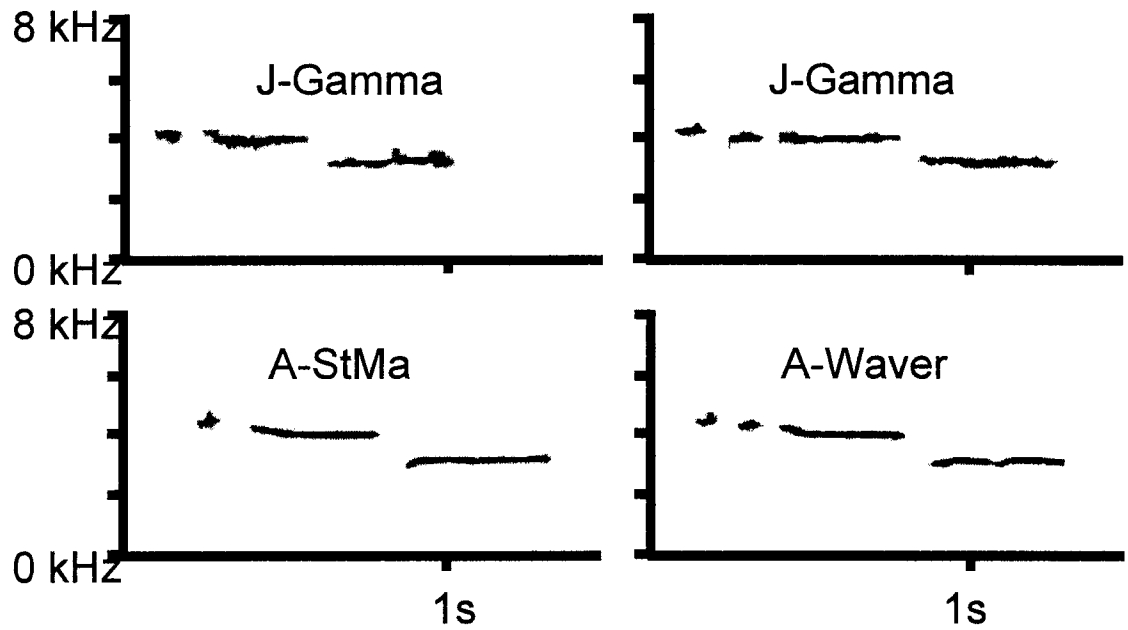


Fig. 2.10: Variation in shape of intros

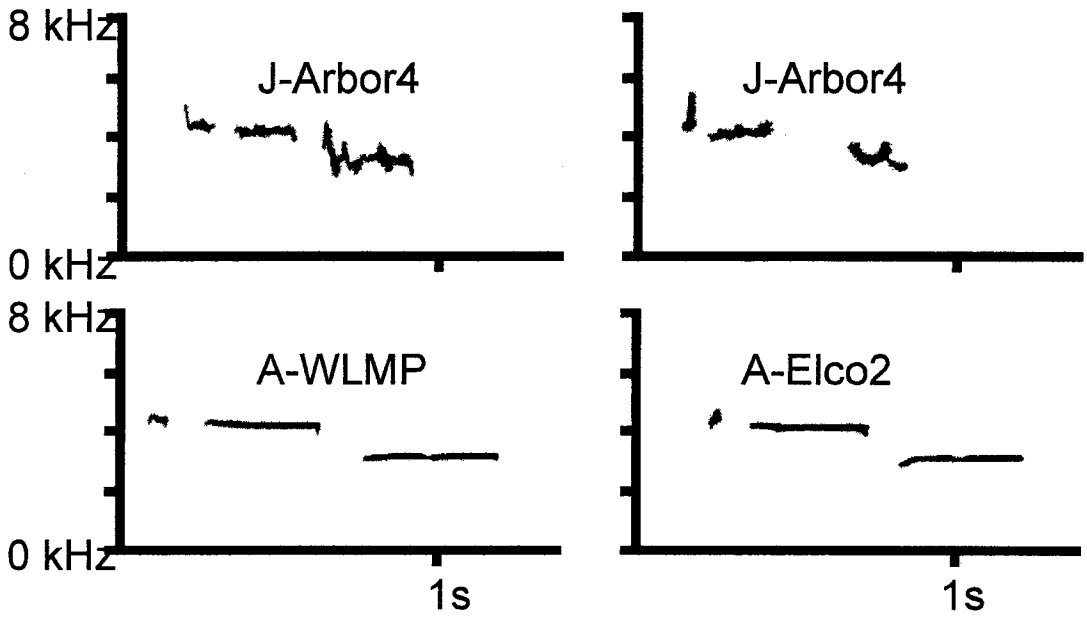


Fig. 2.11: Presence/absence of fee-break

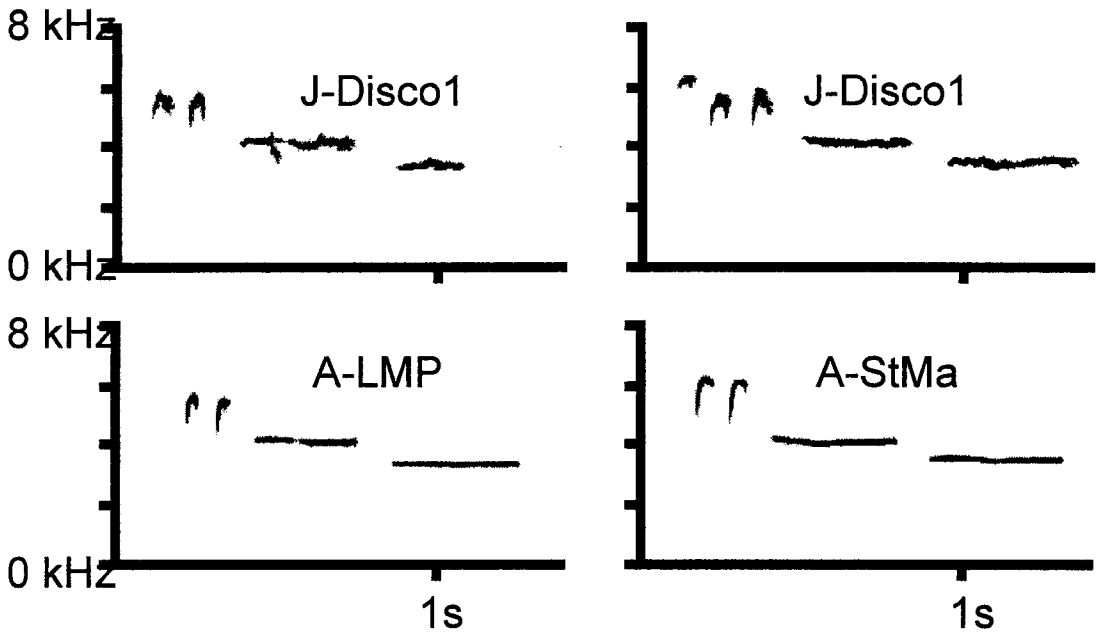
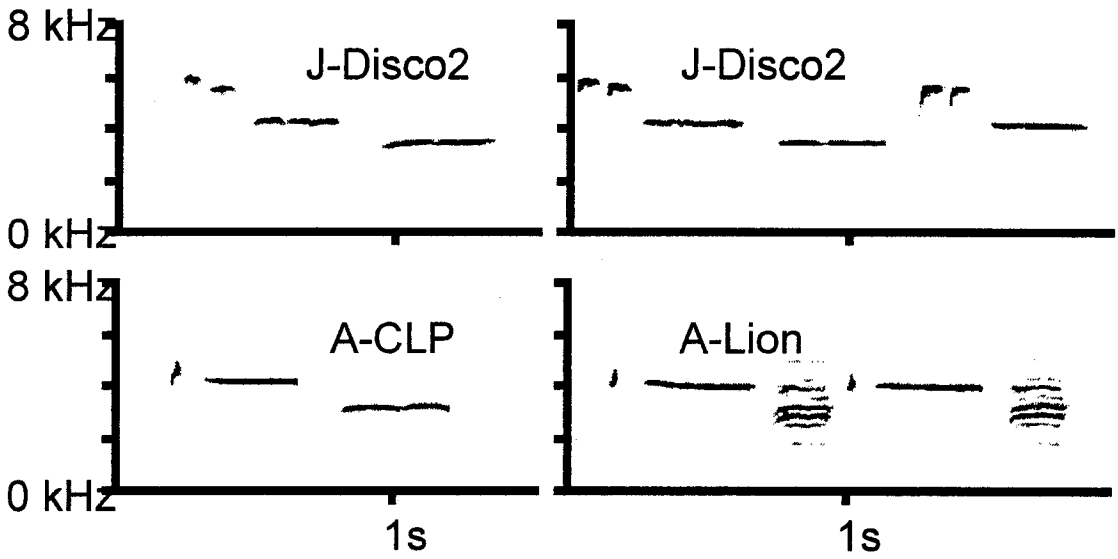


Fig. 2.12: Recombination



CHAPTER III

DAWN CHORUS AND SUBSEQUENT SEXUAL ACTIVITY

The purpose of this chapter is to establish conclusively that the male Black-capped Chickadee (*Poecile atricapillus*) dawn chorus is directed primarily at females and possesses a sexual function. Dixon and coworkers (1970) and Ficken and coworkers (1985) described behavior associated with copulation in Black-capped Chickadees, but they did not mention anything about preceding singing behavior or what time-of-day the copulations occurred. Otter and Ratcliffe (1993) found that male chickadees sing longer following experimental mate removal (presumably for the purpose of attracting mates), and Smith (1988) found that extra-pair copulations in chickadees nearly always occur within one hour of dawn. No one, however, has made detailed observations of individual males following their dawn bouts to look for subsequent sexual behavior. Here I report post-dawn chorus behavior for several chickadee pairs and describes subsequent sexual interactions.

METHODS

I made all observations between 04:00 and 05:30 MST in several study sites in northeastern Colorado in 2002 and 2003. In each case, a single male was observed

throughout his dawn singing bout along with subsequent behavior. All but one male was observed during the typical Black-capped Chickadee breeding season (early April to mid May), and the remaining male was observed in mid-June (probably involved in a second breeding effort). Two field assistants and I made field recordings using a Sony minidisc recorder (either model MZ-R700 or model MZ-N1) connected to a Sennheiser microphone (ME62) mounted in a 60 cm Telinga Pro-universal parabola, and we narrated behavioral observations onto these recordings. When the focal male stopped singing for at least three min, we assumed that the dawn singing bout had finished. Since I was interested primarily in male-female interactions, data were excluded if the focal male did not have a mate or if vegetation density obscured whether a female had arrived or not.

For each dawn singing bout, I recorded the following variables: whether a female arrived, the distance between where she was first detected and the location where the male began his bout, how many songs the focal male sang after her arrival, whether the male attempted copulation, and any male-male agonistic interactions during the bout. A male-male agonistic interaction was defined as an approach by either the focal male or an adjacent male neighbor within 10 m that led to displacement and/or 'gargle' calls (Ficken et al. 1987). For all copulation attempts that immediately followed a dawn bout, I noted whether males used 'variable see' and gargle calls (see Ficken et al. 1985). I often observed wing-quivering in either or both males and females, but I did not consistently take data on this behavior so I do not report it. For many pairs, I determined breeding stage by making additional observations for up to two hours after the end of the dawn bout. Breeding stage is important because pairs tend to cease sexual activity soon after beginning incubation phase (Ficken et al. 1985).

RESULTS

I obtained data for 17 Black-capped Chickadee males from 14 study sites. The greatest distance between two study sites was 160 km. Males always began singing prior to dawn before any female activity, and bouts never ended until female arrival. Eleven focal males stopped singing immediately after female arrival, and five sang fewer than ten additional songs after female arrival. The remaining male stopped singing temporarily after female arrival to copulate with her and then continued singing in her presence for a few minutes before ending his overall dawn bout with another copulation attempt. In contrast to male-female interactions related to the dawn chorus, male-male interactions were very rare. Only 1 of the 17 focal males was ever involved in a male-male agonistic interaction during his dawn singing bout.

Females usually arrived near the location where the male chickadee began singing his dawn bout. For four of the males, I did not know exactly where the male chickadee began its dawn singing bout, so it was impossible to compute the distance. For the remaining 13, however, all females but one arrived less than 25 m from the location where the male began singing ($\overline{X}$ ±SD = 11.5±6.9 m). One male, whose mate's roosting location was known prior to observation, was particularly instructive. Although he took several flights during his dawn bout, he spent approximately 75% of his time within 8 m of her roosting location and approximately 40% of his time within 2 m. While singing, he often approached and/or looked into the nest cavity.

For 14 of the 17 males, termination of the male's dawn singing bout was accompanied by a copulation attempt. Only three males did not attempt copulation

immediately after female arrival; two were in nest-building phase and copulated >30 min following the dawn bout. Interestingly, both of these copulations were followed immediately by the female beginning to gather nesting material. The other male that did not copulate with his mate after her arrival was confirmed to be in incubation phase.

All 14 males that attempted copulation following their dawn bout uttered strings of variable see calls during their copulation attempts, and 13 males terminated strings of variable sees with gargle calls. The terminal gargle syllable was remarkably stereotyped acoustically. All 13 males terminated their gargles using a single low-pitched buzzy syllable (Fig. 3.1a-d; syllable 16 in Baker et al. 2000), although one male used an alternative syllable ending once before switching to several gargles containing the typical terminal syllable, and recordings from one male were too faint to be 100% certain of the presence of the buzzy syllable. The typical gargle terminal syllable varied in its duration, but always possessed the low-pitched buzziness. An examination of variable see/gargle combinations used by five different birds in an agonistic context (see Baker et al. 2000 for methods) revealed that these vocalizations all contained the terminal buzzy syllable (Fig. 3.1e-f). Thus, it appears that all Black-capped Chickadee variable see/gargle combinations terminate using an acoustically similar syllable. This buzzy syllable was also found in many gargle types not preceded by variable sees (Baker et al. 2000).

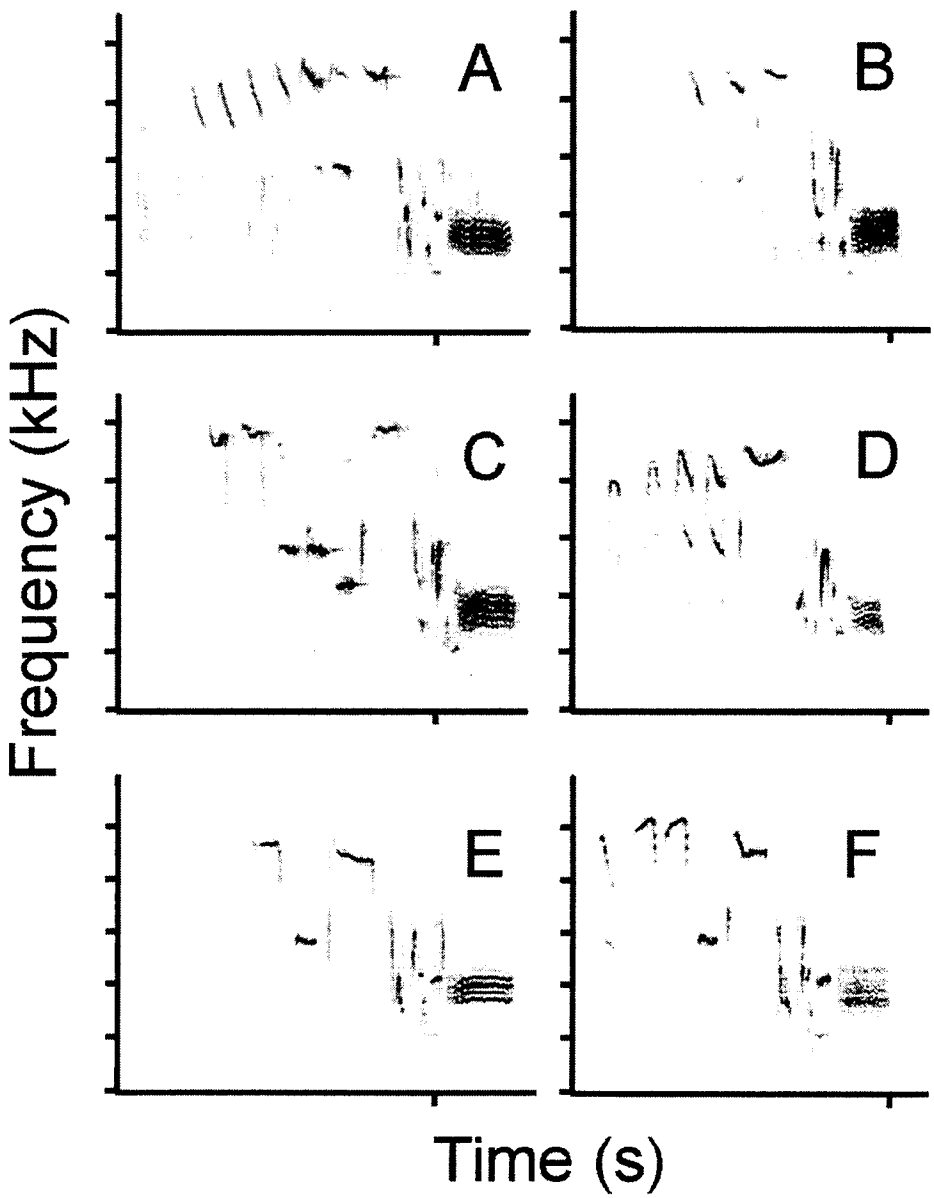
DISCUSSION

From these data, several clear patterns emerge. Male Black-capped Chickadee dawn singing during the breeding season is directed primarily at females rather than rival males, and males focus their singing near the female roosting location. Males continue to

sing until the female arrives, at which time they attempt copulation. These patterns have also been found in Great Tits (*Parus major*, Mace 1986) and Collared Flycatchers (*Ficedula albicollis*, Pärt 1991). When attempting copulation, male chickadees always utter strings of variable sees and nearly always follow these sees with gargles possessing a low-pitched buzzy terminal syllable. This syllable appears acoustically similar to the precopulatory gargle terminal syllables used by chickadees in Wisconsin (see Ficken et al. 1985: fig. 1b) and also terminates many gargles used in agonistic contexts whether or not these gargles are preceded by variable sees (Baker et al. 2000). I hypothesize that this gargle syllable may possess key acoustic features that has an effect on female reproductive physiology similar to the effect that a particular trilled syllable of a male Canary (*Serinus canaria*) has on females (Vallet and Kreutzer 1995).

My results on mating behavior agree with those of Dixon and coworkers (1970) and Ficken and coworkers (1985), who also found that chickadee copulation was preceded by variable see and gargle calls. Male chickadees tend to give these two vocalization categories only when highly aroused, in either sexual or agonistic contexts (Ficken et al. 1978; Personal observations). The high pitch of variable sees (~9 kHz, Ficken et al. 1978) and the acoustic complexity of the gargle (Ficken et al. 1987) combined with recent findings on the physiological production of birdsong (Suthers et al. 1999) suggest that these call categories may be physiologically difficult for chickadees to produce.

FIG. 3.1. Spectrograms showing variable see strings ending in gargles possessing a special terminal syllable. Calls a-d were used in a precopulatory context, and calls e-f were used in an agonistic context. All calls were sampled at 22.05 kHz. Calls a, b, and c were obtained from males at three separate study sites within Fort Collins, Colorado, call d was obtained from a bird at Fort Lupton, Colorado, approximately 60 km from the Fort Collins study sites, and calls e and f were obtained from males at two Fort Collins study sites. Ticks represent 1 sec on the time axis and 3 kHz on the frequency axis.



CHAPTER IV

MALE-MALE VOCAL COMMUNICATION

Individuals of most songbird species possess more than one song type (Krebs and Kroodsma 1980), and much attention in the last several decades has focused on the possible function of such repertoires (reviewed in Catchpole and Slater 1995: Ch. 8). The prevailing view is that song repertoires provide fitness benefits to the individuals possessing them, either through intrasexual or intersexual signaling behavior (Catchpole and Slater 1995). For example, individuals may benefit from possessing the same song types as their territorial neighbors (Beecher et al. 1997). In this explanation, repertoires improve communication between territorial rivals, allowing an individual to signal the likelihood of conflict escalation (as measured by approach distance, time spent within a specified distance of a playback speaker, etc.) through song type-matching or by repertoire-matching the songs of its opponent (Krebs et al. 1981, Beecher et al. 1996; Burt et al. 2002). A second explanation for repertoires suggests that they allow individuals to use different songs in different contexts such as when communicating with males vs. females (Morse 1966, Staicer 1989) or as a graded series of aggressive signals directed to rival males (Lein 1978). A third alternative is that repertoires may arise in

response to female preference, possibly because large repertoires indicate high male quality (Catchpole 1986, Searcy 1988).

Although studies have supported one or more of the above hypotheses, such support demonstrates only the current utility of the song repertoire, not necessarily the selective advantage that promoted its origin (Gould and Lewontin 1979). Tests of the above hypotheses on species in which some populations exhibit repertoires and others possess only a single song type, especially if the multiple song types can be shown to derive from the single song type, will improve our understanding of what advantages are provided by repertoires.

Male black-capped chickadees (*Poecile atricapillus*) normally possess a single territorial 'fee-bee' song type across most of their North American geographic range (Kroodsma et al. 1999). Chickadees in a few areas, however, such as four or more Pacific Northwest populations and three islands off the Massachusetts coast, sing several acoustically distinct song types that vary geographically (Kroodsma et al. 1999). I recently described a repertoire of three song types discovered in another divergent area, Fort Collins, Colorado, and labeled the three song types 'chick-a-fee-bee', 'fa-fee-bee' and 'fee-bee-3' (Chapter 1). These song types differ in the presence or absence of particular introductory notes and in the pitch relationships of the whistled 'fee' and 'bee' syllables found in their songs (Fig. 4.1). The fee-bee-3 song type is acoustically similar to the continental fee-bee song type, but it possesses more stereotyped whistled pitches. Most individual males in the Fort Collins populations possess the entire song repertoire of three types, although a small number of birds apparently lack the fee-bee-3 song type. The repertoire is localized to the Fort Collins area and differs acoustically from the

repertoires of other divergent areas. Because most chickadee populations outside this area share a single song type (Kroodsma et al. 1999, Chapter 1), the three Fort Collins song types probably all derived evolutionarily from the single continental song type. Thus, this novel repertoire allows us to test hypotheses that explain why a species formerly singing a single song type might evolve multiple song types.

Through observations of natural territorial encounters and through experimental song playback, I address two hypotheses for the function of song repertoires in intrasexual communication between territorial males: A) that the song types in a repertoire are used as a graded series of aggressive signals (Lein 1978), and B) that repertoires permit song type-matching, which signals an increased likelihood of escalation in a contest between males (Krebs et al. 1981). The first hypothesis (A) predicts that different song types will be associated with different levels of aggression. The second hypothesis (B) predicts that song type-matching will be associated with higher levels of aggression in territorial interactions.

METHODS

I collected data on natural countersinging between neighboring males and conducted two playback experiments to simulate intrusion of one male into another's territory.

Observational study

The purposes of the observational study were to investigate which song types are used in countersinging between neighboring males, whether song type-matching during

these contests occurs at a level different from chance, and whether matching is associated with conflict escalation. Field recordings in countersinging duos were made during the months of March, April and May in 2001, 2002 and 2003 at twelve locations in the Fort Collins area. All recordings were made after the dawn chorus because males direct their dawn chorus singing primarily at females (Otter and Ratcliffe 1993, Chapter 3). Recordings in 2001 were made using a Marantz tape recorder (PMD201) connected to a Sennheiser microphone (ME62) mounted in a 45 cm parabola. Recordings in 2002 and 2003 were made using a Sony MiniDisc recorder (MZ-R700 or MZ-N1) connected to a Sennheiser microphone (ME62) mounted in a 60 cm Telinga Pro-universal parabola. Although different equipment was used each year, this had no effect on my ability to identify song types used during countersinging. Data were taken each time exactly two countersinging chickadees were encountered. I defined a countersinging event as two neighboring males alternating songs with each other after the dawn chorus. Every countersinging event within a year involved a unique pair of birds. I noted the first song type used by each of the two birds at the initiation of each countersinging event, and during 2003 I noted, when possible, whether or not countersinging escalated. Escalation occurred when one male chased or physically displaced its rival or approached while uttering 'gargle' calls (Shackleton and Ratcliffe 1994). Gargle calls are signals of dominance in agonistic encounters (Baker et al. 1991).

General methods for playback experiments

I used two approaches to simulate territorial invasion through playback. In the first approach ('Matching'), the experimenter responded to a singing bird by matching 10

of its songs and then not matching 10 more of its songs, with treatment order randomized and counterbalanced across all trials. In the second approach ('Single Song Type'), the experimenter broadcast a single song type for four minutes regardless of which song type the subject used in response. All three Fort Collins song types were used equally as stimuli following a stratified random design. The main questions I asked in both experiments were whether the subject matched song type at levels different from chance, whether matching song type was associated with greater territorial aggression, and whether any particular song types were associated with greater territorial aggression.

Stimulus songs were prepared using chickadee recordings from several locations in Fort Collins from 2001 or early 2002. Only high-quality songs with a signal at least five times as loud as background noise were used in making stimuli. Each song used came from either a different individual or a different song type of the same individual, and each song stimulus was easily classified as one of the three song types. To make stimuli, I entered song recordings into Real-Time Spectrogram software (Kay Elemetrics, version 2.3) using a 44.1 kHz sampling rate and 16-bit accuracy. I then performed high-pass filtering of each song to remove background noise lower than the pitches used for that particular song type. The peak amplitude for each song was scaled to a specific magnitude using Multi-Speech software (Kay Elemetrics, version 2.3) so that songs could be broadcast at the same volume. After editing, stimuli were recorded onto a MiniDisc (Sony MZ-R700) using a separate track for each stimulus song.

Trials were conducted between 6:00 and 10:00 h in several locations throughout Fort Collins on unbanded males. The Matching playback experiment lasted from 25 April to 4 May 2002, and the Single Song Type playback experiment lasted from 6 May

to 14 May 2002. Chickadee territorial activity was high during these periods. To begin a trial after identifying a focal bird, an amplified speaker (Miner off Electronics SME-AFS) was placed on the ground facing up. Whenever possible, the speaker was placed within one meter of a branch suitable for perching. Territorial borders were not known prior to experimentation so speaker placement within a territory probably was not consistent across trials. If two males approached within 20 m of the speaker during a trial, however, it was discarded. Therefore all trials represent the approach of a single male.

The speaker was connected to the MiniDisc system by an 8 m cord, and the MiniDisc system was handheld by an experimenter several meters away from the speaker. Broadcasting of stimuli began after the focal male was >10m away from the speaker and had not sung for at least one minute. My protocol allowed me to control which stimulus song was played as well as the timing of each stimulus. Stimuli were chosen for each trial so that the focal male was unfamiliar with the individual that was the source of the song stimulus. Therefore the experiment simulated invasion by a stranger. Throughout a trial, I answered each song given by the focal male with a stimulus song. Songs were broadcast in an interactive manner (Dabelsteen and McGregor 1996) with the onset of a stimulus song following the ending of the subject's song by approximately 1.5 seconds. Thus, I was generally able to avoid overlapping the songs of focal males. If neighboring chickadees began singing, I continued to respond exclusively to the focal male. Stimulus songs were broadcast at 80 dB (A-weighted, slow, Radio Shack 33-2050 sound level meter) measured at 1 m from the speaker, which was approximately equal to the volume of focal birds' songs. During each trial, I noted the song type(s) used by the

subject, song type switches that occurred, the presence of countersinging neighbors, and the closest approach to the speaker during each treatment.

In a prior study I fully recorded the song repertoires of ten male black-capped chickadees at five study sites in Fort Collins (mean # songs per bird = 258, mean # song type switches per bird = 20) and found that all ten possessed chick-a-fee-bees and fa-fee-bees, while eight of the ten (at least one bird from each of the five study sites) also possessed the fee-bee-3 song type (Chapter 1). Thus, Fort Collins chickadees should nearly always be capable of matching chick-a-fee-bees and fa-fee-bees, but a few birds may be unable to match the fee-bee-3 song type. I compensated for this when appropriate, by performing additional statistical tests excluding birds hearing fee-bee-3 stimulus.

I used G-tests (Sokal and Rohlf 1995) to decide whether the song type usage and song type matching were different from chance expectations. Two-tailed, nonparametric statistical tests were used for all other comparisons, with an alpha level of 0.05. My general strategy was to look for possible confounding effects such as the presence of singing neighbors, treatment order, and my use of the same stimulus songs in more than one trial. I then followed these with tests comparing territorial aggression between the different treatment groups.

Matching playback experiment

I began the Matching playback experiment by broadcasting the non-song 'chick-a-dee' call every 3.5 seconds until the focal male approached the speaker and began singing. Chick-a-dee calls have been used successfully in other chickadee playback

studies to elicit approach and a territorial singing response (Shackleton et al. 1992, Otter et al. 2002, Mennill and Ratcliffe In press). If the subject did not approach and sing within 4 minutes, I aborted the trial. Once the subject began singing, the experimenter answered twenty of his songs in the interactive manner described above. Half of the males tested heard ten repetitions of a single stimulus song matching his song type followed by a ten repetitions of one nonmatching song. The other half received the nonmatching treatment first and the matching treatment second. Treatment order followed a stratified random design. Since Fort Collins chickadees possess three song types, there were two choices of song stimuli to use during the nonmatching treatment. I alternated the way I chose nonmatching stimuli so that all song types would be equally represented. If the focal male stopped singing for at least 30 seconds at any point during the trial (occurred in 3 of 23 trials), the experimenter began broadcasting chick-a-dee calls again until the bird began singing again. If the focal male switched song types, I also switched to preserve the matching-nonmatching treatment structure. A total of 31 unique stimulus songs were used during the Matching experiment, and all subjects except two received a unique combination of song stimuli. Trials began with the onset of the first song stimulus and ended with the onset of the subject's song that followed the final stimulus song.

Single Song Type playback experiment

I began the Single Song Type playback experiment by broadcasting a stimulus song within a bird's territory every 4 seconds until the subject began singing. If the male did not sing within 3 minutes, I abandoned the trial. Once the male began singing, the

experimenter played the same stimulus song for four minutes in the same interactive manner as that used in the Matching playback experiment. Unlike that experiment, however, the same stimulus song was used throughout the trial regardless of what song type(s) the subject used. Since I began by broadcasting stimulus songs in the Single Song Type playback experiment rather than chick-a-dee calls, the subject's song response determined whether or not the trial would include song type-matching. If the subject stopped singing for 30 seconds, I broadcast the stimulus song every 4 seconds until the male began singing again. Trials began with the onset of the subject's first song and ended four minutes later. A total of 18 stimulus songs were used during the course of the Single Song Type playback experiment, and five stimuli were used more than once.

RESULTS

Observational study results

Observational data were obtained for 40 countersinging duos, and we noted the first song type used by each of the 80 singing males at the initiation of the countersinging event. Of these 80 songs, 35 were chick-a-fee-bees (44%), 31 were fa-fee-bees (39%) and 14 were fee-bee-3s (18%). Males matched song type approximately twice as often as would be expected by randomly pairing these songs together (Fig. 2, exp = 37%, obs = 65%, G-test, $G=12.6$, $df=1$, $P<0.001$). For countersinging contests in 2003, we observed the progression of the conflict for 14 of the 22 duos, nine of which involved males matching song type. Only three of the nine duos matching song type escalated their conflict, while three of the five non-matching duos escalated (relationship between matching and escalation not statistically significant, Contingency Table G-test, $G=0.9$,

df=1, P=0.33). Thus, we failed to find evidence that matching indicated conflict escalation in natural countersinging bouts.

General results for playback experiments

Male chickadees always responded to song playback by singing and flying towards the speaker and perching nearby where they remained for some time. In 80% of the trials, the subject approached the speaker within 5m. In over 75% of the trials the subject was the only male that responded vocally. In each experiment, there was no effect on the subject's response caused by the presence of singing neighbors, the identity of the stimuli used, or the treatment order (Kruskal-Wallis and Mann-Whitney U-tests, P>0.20 in all tests). Therefore these variables were excluded from further analyses.

Matching playback experiment

Subjects responded to hearing the chick-a-dee stimulus by singing one of the three Fort Collins song types. When considering just the first song elicited by the chick-a-dee stimulus from each of the 23 subjects, song types were used in approximately the same proportions as those in the observational study (52% chick-a-fee-bee, 39% fa-fee-bees and 9% fee-bee-3s, G-test, G=1.6, df=2, P=0.45). During Matching playback trials, subjects did not approach closer when using a particular song type (Fig. 4.3a, Kruskal-Wallis test, H=1.59, df=2, P=0.45). Subjects switched song type in eight instances, but the tendency to switch was not associated with whether the experimenter was matching its song type or not (three switches during match vs. five switches during nonmatch, G-test, G=0.5, df=1, P=0.48). Additionally, subjects did not approach more closely while

their song type was matched (Fig. 4.4, Wilcoxon signed rank test, N=17, W=76.5, P=1.00).

Single Song Type playback experiment

In contrast with the high rate of matching song type in natural countersinging (65%), only 5 of the 24 subjects in the Single Song Type playback experiment matched the song type of the stimulus, which was not different from chance expectations (Fig. 4.5, exp = 33%, obs = 21%, G-test, G=1.8, df=1, P=0.18). Song type-matching was not associated with a closer approach (Fig. 4.6, Mann-Whitney U-test, N_1=19, N_2=5, W=247.0, P=0.52), and this result did not change after excluding trials with fee-bee-3 stimuli (Mann-Whitney U-test, N_1=12, N_2=4, W=115.5, P=0.11). Three subjects switched song types to match or not to match during the trial, but all switches occurred while >5m from the speaker, and none of the switches were obviously associated with escalation (a flight toward the speaker). Finally, birds did not approach more closely when hearing a particular song type (Fig. 4.3b, Kruskal-Wallis test, H=1.6, df=2, P=0.44).

DISCUSSION

Male black-capped chickadees did not preferentially use some song types over others during experimental playback, and they did not approach the speaker closer when singing or hearing a particular stimulus song type. It thus appears unlikely that the song types in the Fort Collins chickadee repertoire form a graded series of aggressive signals. It is possible, however, that song types possess different functions. For example, males

might use different song types when communicating with females than when communicating with males (Morse 1966, Staicer 1989). In Chapter 5 I investigate this possibility.

Song type-matching did not signal an increased likelihood of conflict escalation in natural and simulated male-male countersinging contests. My failure to find evidence of this relationship contrasts with results from many other songbird species possessing repertoires (Burt et al. 2001, Molles and Vehrencamp 2001, Vehrencamp 2001), although not all studies have found a relationship between matching and escalation (*e.g.*, Falls 1985).

Others have studied song-matching in black-capped chickadee populations in Ontario, Canada, although they defined 'song-matching' as matching pitch with the second whistled syllable (Horn et al. 1992, Shackleton and Ratcliffe 1994, Otter et al. 2002, Mennill and Ratcliffe In press). They employed this definition because black-capped chickadees normally possess only a single 'fee-bee' song type that varies continuously in the absolute pitch with which it is given. In these studies, pitch-matching was associated with increased likelihood to escalate the conflict in both natural (Shackleton and Ratcliffe 1994) and playback-simulated (Otter et al. 2002, Mennill and Ratcliffe, In press) countersinging bouts. These playback studies used unfamiliar ('stranger') song stimuli, as I did, and measured conflict escalation using a multivariate response variable weighted heavily for the closest approach measure that I used. Unlike my study, however, territorial boundaries were known prior to experimentation and speakers were consistently placed either 10-20 m inside territory boundaries (Otter et al. 2002) or at the center of a male's territory (Mennill and Ratcliffe In press). Although I

did not know territory boundaries for my subjects, the fact that non-focal neighboring males responded vocally to less than 25% of my trials, combined with the fact that neighbors never approached speakers within 20 m, suggests that my protocol was adequate. In contrast to the Ontario studies, my observational data and my playback experiments indicated that song type-matching did not signal the likelihood of conflict escalation in Fort Collins chickadee populations. I suggest that the Fort Collins repertoire did not evolve as a mechanism to allow chickadees to signal conflict escalation through matching song type.

Matching song type may still be adaptive in some other way for Fort Collins chickadees. Although chickadees did not match song type above chance levels for unfamiliar song stimuli, chickadees in natural countersinging interactions matched song type nearly twice as often as chance expectations. What song type-matching indicated to these chickadees and whether it can be considered adaptive, however, was not clear from my study. Falls (1985) suggested that matching might facilitate communication between males through directing a response at a particular singer. This process might require familiarity with singing opponents. Thus, the natural song type-matching that I observed may indicate that birds recognized the individuals with whom they were countersinging whereas the lack of matching during playback indicates that the birds would require more familiarity with the stimulus song before matching above chance level. An alternative explanation is that chickadees more commonly match songs heard off territory rather than on their territory.

In summary, my results did not support either the hypothesis that song types form a graded signal of aggression or the hypothesis that matching song type signals an

increased likelihood of escalation in a contest between males. The lack of support for either of these hypotheses suggests they do not account for the evolution of the novel chickadee song repertoire found in Fort Collins. Several possibilities remain that may explain why the chickadee song repertoire evolved. Perhaps the repertoire evolved in response to unidentified intrasexual selective forces that do not involve males communicating the likelihood of conflict escalation to other males. Another possibility is that the repertoire evolved in response to intersexual rather than intrasexual selection; I investigated male-female communication in a Chapter 5. Finally, the chickadee repertoire may have differentiated as a nonadaptive consequence of accumulated song learning errors combined with the effects of geographic isolation.

Fig. 4.1. Sound spectrograms of the three black-capped chickadee song types (chick-a-fee-bee, fa-fee-bee and fee-bee-3) found in Fort Collins, Colorado, and the continental fee-bee song found throughout most of the species' North American geographic range outside of Fort Collins. Each song type within Fort Collins possesses a unique combination of introductory syllables and stereotyped fee- and bee-pitches. The fee-bee-3 song type is acoustically similar to the continental song type, but the fee- and bee-pitches are more stereotyped.

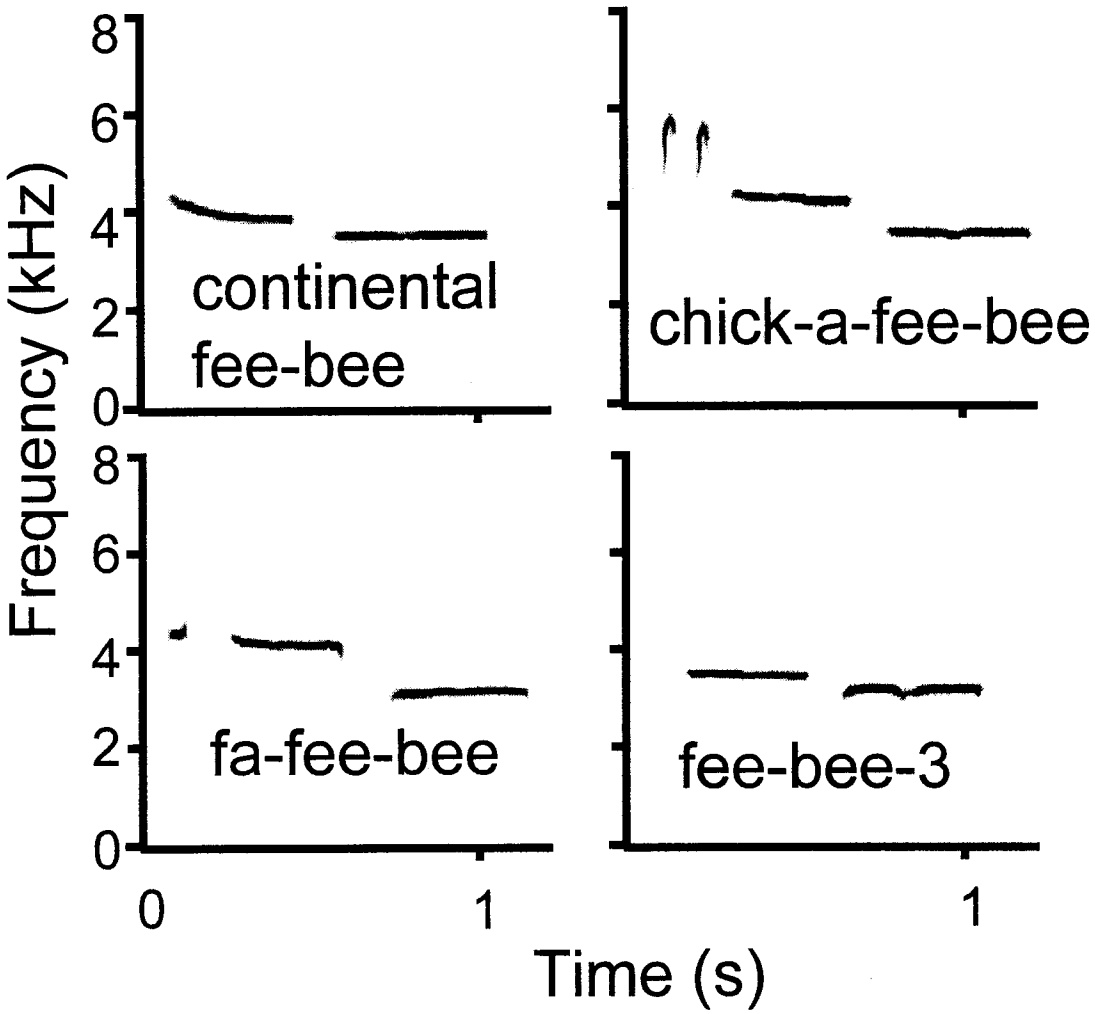
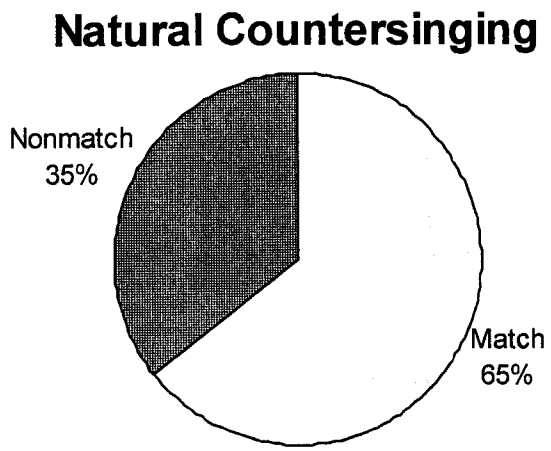


Fig. 4.2. Pie charts showing that black-capped chickadees match song type more often than chance expectations during natural countersinging contests (26 of 40 duos matched, 65%).



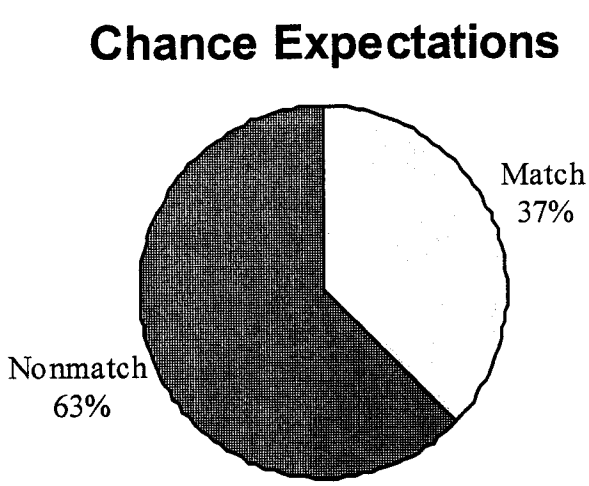


Fig. 4.3. Boxplots showing variation in minimum distance to speaker based upon which song type was sung by the subject in the Matching playback experiment (A, $N = 23$ birds) or which song type was broadcast during the Single Song Type playback experiment (B, $N = 24$ birds, CFB = chick-a-fee-bee, FFB = fa-fee-bee, FB3 = fee-bee-3). Medians (horizontal line within the box), quartiles (top and bottom of box), and the 0.05 and 0.95 quantiles (tips of vertical whiskers) are shown for each song type. The illustrations show that no song type was associated with a closer approach.

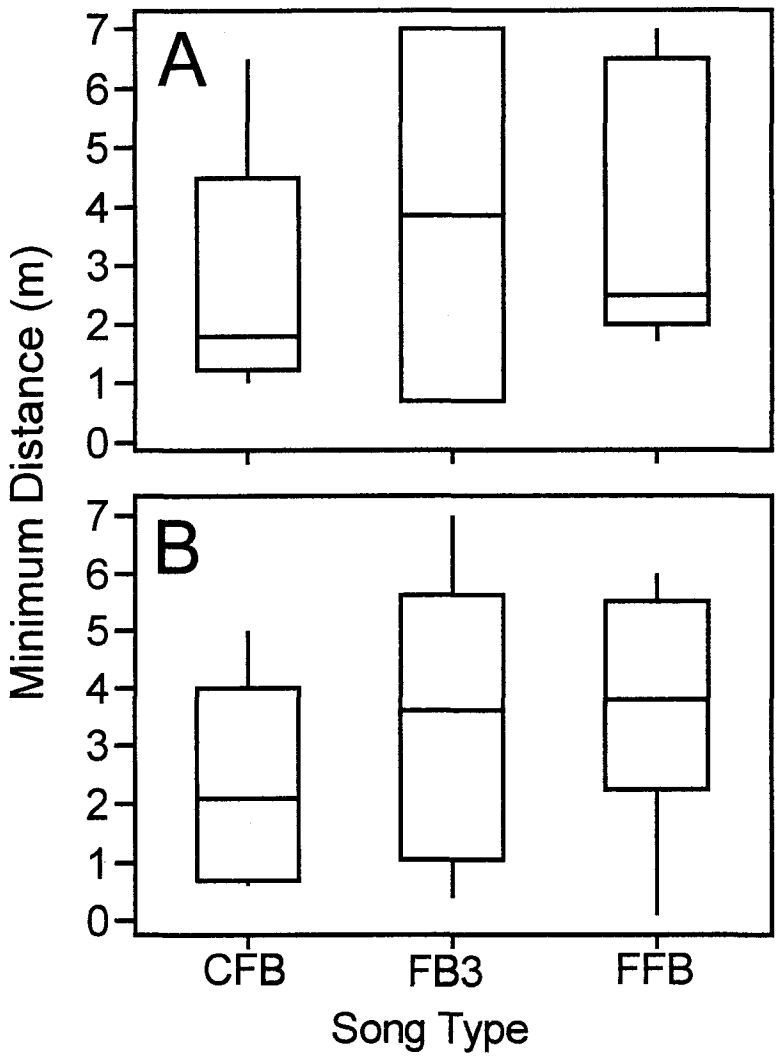


Fig. 4.4. Scatterplot of the minimum distance to the speaker during the match and nonmatch treatments for each bird of the Match playback experiment ($N = 23$ birds). If matching song type were associated with an aggressive response, data would cluster in the top left half of the graph.

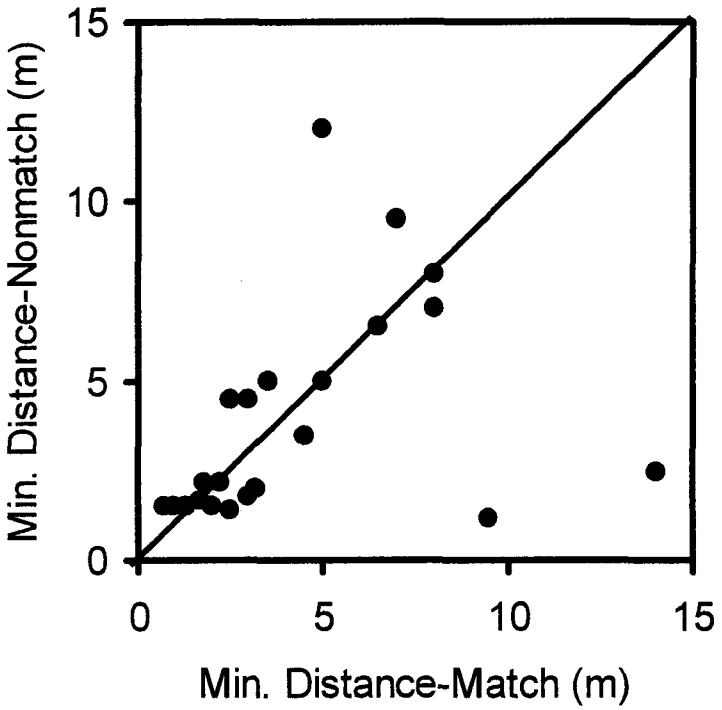
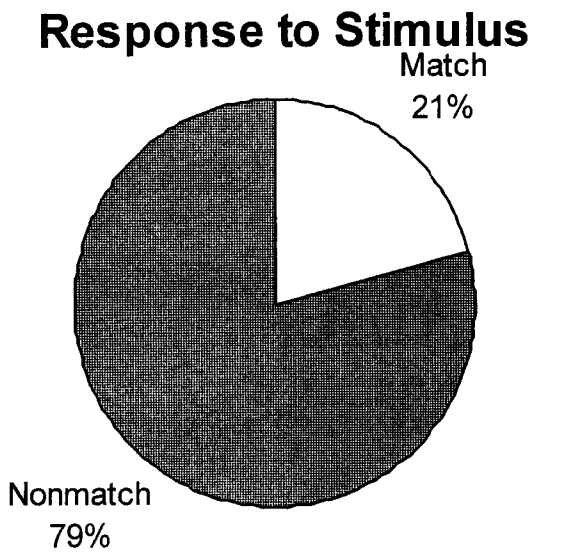


Fig. 4.5. When responding to a stimulus song in the Single Song Type playback experiment ($N = 24$ birds) the observed frequency of song type-matching the stimuli did not differ significantly from chance expectations.



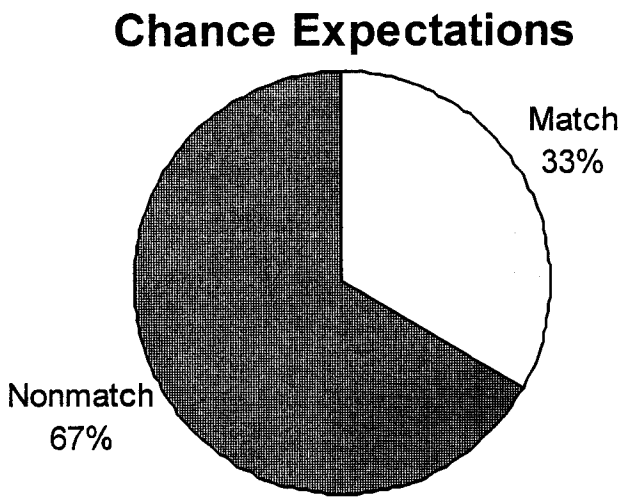
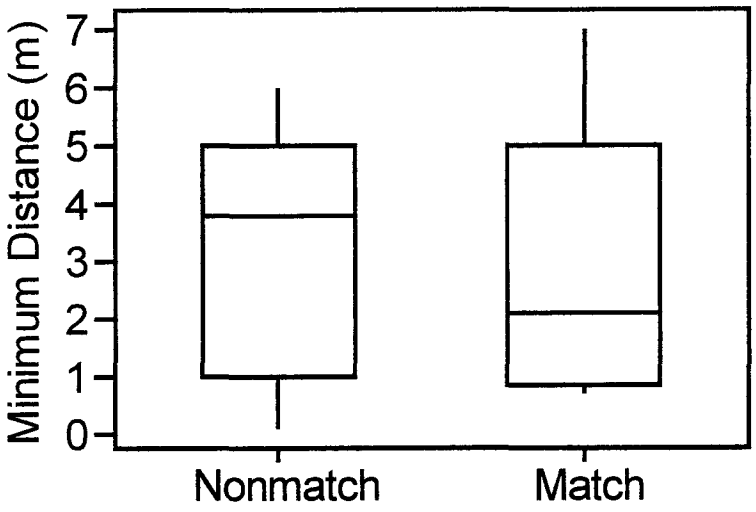


Fig. 4.6. Boxplot showing that subjects' closest approach was similar whether they matched or did not match the stimulus song type during the Single Song Type playback experiment ($N = 24$ birds). See Fig. 4.3 legend for boxplot symbols.



CHAPTER V

MALE-FEMALE VOCAL COMMUNICATION

It is generally thought that learned vocal signal repertoires, such as those possessed by many oscine passerines (i.e., songbirds, Krebs and Kroodsma 1980), provide fitness benefits to the individuals possessing them, either through intrasexual or intersexual communication or both (Catchpole and Slater 1995: Ch. 8). Abundant evidence demonstrates the current utility of learned vocal repertoires in several species (reviewed in Catchpole and Slater 1995: Ch. 8), but this may not explain why repertoires originated (Gould and Lewontin 1979). In Chapter 4 I examined Fort Collins, Colorado, black-capped chickadee (*Poecile atricapillus*) populations in which a 'fee-bee' song repertoire of three distinct types is apparently derived from the single song type found across most of North America (Chapter 1). I found no evidence that possession of a repertoire provided males with an adaptive advantage in male-male singing interactions. This paper extends that study by examining potential intersexual selection judged from observations of male-female interactions. Specifically, I investigated two hypotheses with regard to intersexual selection: 1) Repertoires evolve because of female preference

for multiple song types over single song types, and 2) Repertoires evolve so that males can use different song types when communicating with females vs. males.

A number of authors have invoked intersexual selection to explain the existence of male ornaments. These ornaments might be morphological, such as a peacock's tail (Petrie et al. 1991), or behavioral, such as the construction of elaborate bowers (Diamond 1986). Some have considered birdsong to be a male sexual ornament (e.g., Darwin 1871, Searcy and Andersson 1986) and argued that repertoires exist because of sexual selection. The strongest support for this hypothesis comes in the form of correlations between repertoire size and male reproductive success within a population (e.g., Catchpole 1986, Hasselquist et al. 1996), and from the observation of more female copulation solicitation displays in response to playback containing a greater number of song types (e.g., Searcy 1984, Catchpole et al. 1984, Baker et al. 1986, 1987b).

There is, unfortunately, little natural variation in repertoire size among Fort Collins black-capped chickadee populations (Chapter 1) so testing for correlations between repertoire size and reproductive success becomes problematic. Additionally, I was unable to cause oestradiol-implanted female chickadees to solicit copulations in response to playback of male song (Unpublished data). Thus, two common methods for studying intersexual selection for repertoire size are precluded. Instead I examined repertoire usage during the spring dawn chorus. In Chapter 3 I showed that male chickadee dawn singing bouts are directed predominantly at females and usually lead to copulation attempts. If repertoires evolved in response to intersexual selection, one might expect the dawn chorus singing behavior of a male chickadee to advertise possession of a repertoire. I compared dawn chorus singing behavior between chickadee populations

possessing a repertoire and populations possessing a single song type. Finally, I compared repertoire usage of Fort Collins male chickadees when communicating with males vs. females to see if the repertoire evolved to allow males to use different song types when communicating with females vs. males.

METHODS

Two types of data were collected in the field: dawn chorus data in which male song is directed to females (Otter and Ratcliffe 1993, Chapter 3) and countersinging data in which male song is directed to males. Dawn chorus data were collected from 11 males at 4 study sites with high chickadee abundance in Fort Collins where most individuals possess three song types. Data were also collected from 8 males at 3 study sites in Loveland, Colorado, an area of high chickadee abundance where individuals apparently possess a single song type (Chapter 1). Loveland is 21 km south of Fort Collins, and the cities are separated by several kilometers of treeless plains not inhabited by black-capped chickadees. Countersinging data were collected only from Fort Collins birds, and I analyzed the same data set described in Chapter 4. In brief, this countersinging data set consisted of each song type used by 40 independent pairs of countersinging chickadees (N=80 songs) at 12 study sites in the Fort Collins area.

Dawn chorus recordings were made during 2003 between 05:00 and 06:30 MDT. All but one male was recorded during the typical breeding season (early April to mid May). The remaining male was recorded in mid-June (probably involved in a second breeding effort as his singing led to a copulation attempt). Field recordings were made using a Sony MiniDisc recorder (MZ-N1 or MZ-R700) connected to a Sennheiser

microphone (ME62) mounted in either a 60 cm Telinga Pro-universal parabola or a 45 cm aluminum parabola. Each male was recorded until his mate appeared or until he stopped singing for at least three minutes. Although birds were unbanded, singers stayed within their territories during the dawn chorus and at least 10 m away from the nearest singing neighbor (see also Ficken et al. 1978). Thus, it was possible to follow an individual during the entire bout. If there was any uncertainty about an individual's identity during any portion of the bout, the data were not used.

I made spectrograms of all dawn chorus recordings using Syrinx software (John Burt, http://www.syrinxpc.com/index.html) sampling at 22.05 kHz with 16-bit accuracy and 1024-point transform size. All black-capped chickadee songs, including the three song types found in the Fort Collins repertoire, possess two pure tone whistles ('fee' and 'bee') with the second whistled syllable emitted at a lower frequency than the first (Fig. 5.1). The pitches of the fee and bee syllables easily distinguish the three Fort Collins song types from each other (Chapter 1). I measured pitch at the midpoint of the fee and bee syllables for every song from both Fort Collins and Loveland using the onscreen cursors within Syrinx, which allowed a pitch resolution of 6 Hz.

Some disagreement has existed over whether black-capped chickadee songs from elsewhere in North America should be classified into a single song type (Hailman 1989, Horn et al. 1992, Shackleton and Ratcliffe 1994, Kroodsma et al. 1999). When individual chickadees sing long sequences of the continental song type, successive songs usually possess the same fee- and bee-pitches, but birds occasionally 'pitch-shift' by abruptly changing the fee- and bee-pitches to begin a string of songs containing the new fee- and bee-pitches. Over bouts of several hundred songs, however, individuals will sing all

whistled pitches within their frequency range (Weisman et al. 1990, Horn et al. 1992, Kroodsma et al. 1999). Thus, since large samples of songs do not cluster into discrete categories, it is generally concluded that individuals possess a single, but continuously variable, song type (Kroodsma et al. 1999). To verify whether Loveland chickadees use a single song type, I plotted fee-pitch against bee-pitch for every song within an individual's dawn bout to see if songs from each individual clustered into discrete categories or were distributed fairly continuously.

Fort Collins chickadee songs were classified easily into one of the three song types by visual inspection. Usage frequencies for each song type of each Fort Collins bird during his dawn bout were then computed. The song-switching rate for Fort Collins dawn bouts was defined as the number of song type switches over the entire bout divided by the number of songs in the bout. For Loveland birds, I followed the convention of Horn et al. (1992) and Shackleton and Ratcliffe (1994) by labeling any pitch-shift greater than 80Hz between successive songs as a song-switch, which allowed me to calculate a Loveland switching rate. The original justification for the 80Hz rule was that during the dawn bouts for 23 males, only 5% of pitch-shifts between successive songs were greater than 80 Hz (Horn et al. 1992). I compared song-switching rates between Fort Collins and Loveland birds using a two-tailed t-test.

The other hypothesis I tested was whether Fort Collins males would use some song types more often when communicating with females (dawn bout singing) vs. males (territorial countersinging) as has been found in North American warblers (e.g., Morse 1966, Staicer 1989). For each Fort Collins male's dawn bout, I computed the frequency with which each song type was used and then averaged frequencies across all males to

get population usage frequencies for each song type. I labeled these usage frequencies as 'expected' and used a G-test (Sokal and Rohlf 1995: p. 688) to compare them with the observed usage frequencies of song types delivered during male-male territorial countersinging.

RESULTS

All dawn bout songs from Fort Collins birds clustered by pitch into three distinct song types, as expected (Fig. 5.2a-c, compare to Fig. 1.5). The dawn bout of one Loveland individual showed three song clusters that appeared similar to the three song clusters of the Fort Collins individuals, but none of his songs possessed the introductory notes found in two of the three Fort Collins song types (Chapter 1). I suggest that this Loveland individual used three song types possibly derived from the Fort Collins population, but intermediate between the two population patterns. The distance between Fort Collins and Loveland is probably within the range of maximum chickadee dispersal (Weise and Meyer 1979), suggesting that this individual may have hatched in Fort Collins and then dispersed to Loveland. None of the other Loveland individuals, including 15 individuals not reported in this study and >10 individuals recorded during two previous field seasons, showed a song cluster pattern resembling the Fort Collins cluster pattern (Unpublished data). Because this variant individual used multiple song types not related to the songs of any of his neighbors, I excluded his songs from my analysis.

Dawn songs from all other Loveland chickadees did not cluster into multiple pitch groups (Fig. 5.2d-f). Song pitches for some of these Loveland individuals were

distributed continuously (e.g., Fig. 5.2d-e) while pitches for other Loveland individuals showed gaps in their distribution along a continuous axis (e.g., Fig. 5.2f). In other continental populations, individual black-capped chickadees have been found to use a somewhat limited set of pitches during a given morning bout, but these same individuals sing a continuous range of pitches when sampled over several days (Horn et al. 1992, Kroodsma et al. 1999). This fact, combined with the overall greater dispersion in pitch plots (e.g., Fig. 5.2d-f), led me to believe that Loveland chickadees sing a single song type.

Fort Collins chickadees did not have a higher song-switching rate than Loveland chickadees (Fig. 5.3, t-test, t_{14}=-0.88, P=0.81). The average switching rate was slightly higher in Loveland, although not significantly so (Fig. 5.3).

Fort Collins males used all their song types when communicating with females during the dawn chorus or with males during countersinging exchanges (Fig. 5.4). Furthermore, the usage frequencies for song types did not differ between the two groups (Fig. 5.4, G-test, G=1.07, df=2, P=0.59).

DISCUSSION

Black-capped chickadees in Fort Collins sing a repertoire of three distinct song types whereas chickadees in Loveland sing a single song type in the manner described for other continental chickadee populations (Weisman et al. 1990, Horn et al. 1992, Shackleton and Ratcliffe 1994, Kroodsma et al. 1999). Song-switching rates during the dawn chorus are similar for chickadees from Fort Collins and Loveland, suggesting that the Fort Collins song type repertoire did not evolve as an adaptation to allow males to

stimulate females. Furthermore, Fort Collins male chickadees use their song types in roughly the same proportions when communicating with females vs. males, suggesting that the Fort Collins song type repertoire did not evolve as an adaptation to allow males to use different song types when communicating with females vs. males.

When combined with the results of my earlier study examining how Fort Collins chickadees use their song type repertoire when communicating with other males (Chapter 4), I failed to find support for any of the popular adaptive hypotheses for the existence of song type repertoires (see Catchpole and Slater 1995: Ch. 8). These hypotheses have received considerable support in explaining the current utility of learned song type repertoires in a variety of species (e.g., Morse 1966, Lein 1978, Krebs et al. 1981, Catchpole et al. 1984, Hasselquist et al. 1996, Burt et al. 2001, Vehrencamp 2001), but my studies are the first to examine the utility of a repertoire known to derive from a single song type. Although the Fort Collins song type repertoire allowed natural song type-matching at levels above chance, song type-matching did not indicate conflict escalation in natural or simulated countersinging exchanges (Chapter 4) as the matching-escalation hypothesis predicts (Vehrencamp 2001). I found no evidence that Fort Collins males used different song types for different purposes (Chapter 4 and the present chapter). I also found no evidence that the Fort Collins repertoire evolved in response to a female preference for faster presentation of multiple song types.

I cannot rule out that the Fort Collins song type repertoire evolved as an adaptive means of improving male-female communication. For example, I did not test whether females have a preference for the introductory components in two of the novel Fort Collins song types (see Fig. 5.1a-b). I also did not test whether females have a preference

for the Fort Collins song type possessing novel pitch combinations (i.e., the top left cluster in Fig. 5.2a-c). Nevertheless, my failure to find evidence for improved male-female communication in chickadees with repertoires suggests that an adaptive function for the Fort Collins song type repertoire may not currently exist.

It is well established that songbirds learn their songs (Kroodsma and Baylis 1982), and imperfect song learning is commonly thought to contribute to the formation of repertoires and geographic variation in song (Kroodsma and Miller 1982). The rate at which inaccurate song imitations originate and persist within songbird populations has rarely been measured, but all estimates of this 'cultural mutation rate' have been several orders of magnitude above typical genetic mutation rates (Lynch 1996 Lachlan and Slater 1999, 2003). Thus, enormous potential exists for song divergence within and between populations caused by the song-learning process itself. Furthermore, birdsong often diverges more between isolated populations (Lynch and Baker 1994, Chapter 2). Thus, it is possible that song learning, perhaps combined with the effects of geographic isolation, is sufficient to cause a nonadaptive origin of song repertoires. It is also possible that the Fort Collins song repertoire might become adaptive sometime in the future according to one of the hypotheses tested in my studies (i.e., an 'exaptation', sensu Gould & Vrba, 1982).

Fig. 5.1. Sound spectrograms of the three black-capped chickadee song types found in Fort Collins, Colorado (a-c), and three representatives of the single continental song type found in Loveland, Colorado (d-f). All song types possess a whistled 'fee' and 'bee' syllable. Each song type within Fort Collins possesses a unique combination of introductory syllables and stereotyped fee- and bee-pitches that vary little within and between birds, while the continental song type contains substantial pitch variation.

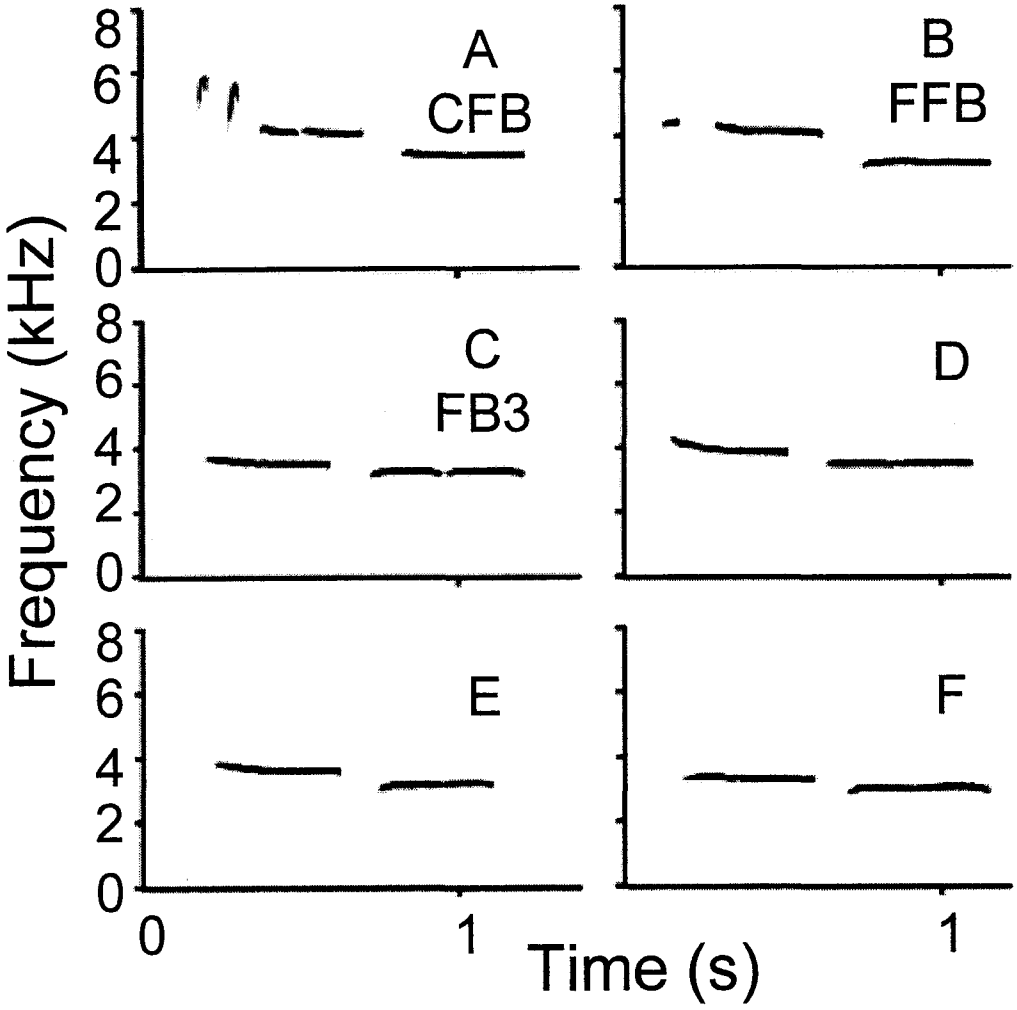


Fig. 5.2. Scatter plots showing the fee- and bee-pitches for black-capped chickadee songs recorded from three Fort Collins (a-c) and three Loveland (d-f) individuals during their dawn singing bouts (N = 90-721 songs per bout). All Fort Collins chickadees sang multiple song types. In contrast, Loveland individuals sang a single song type containing substantial pitch variation within the song type.

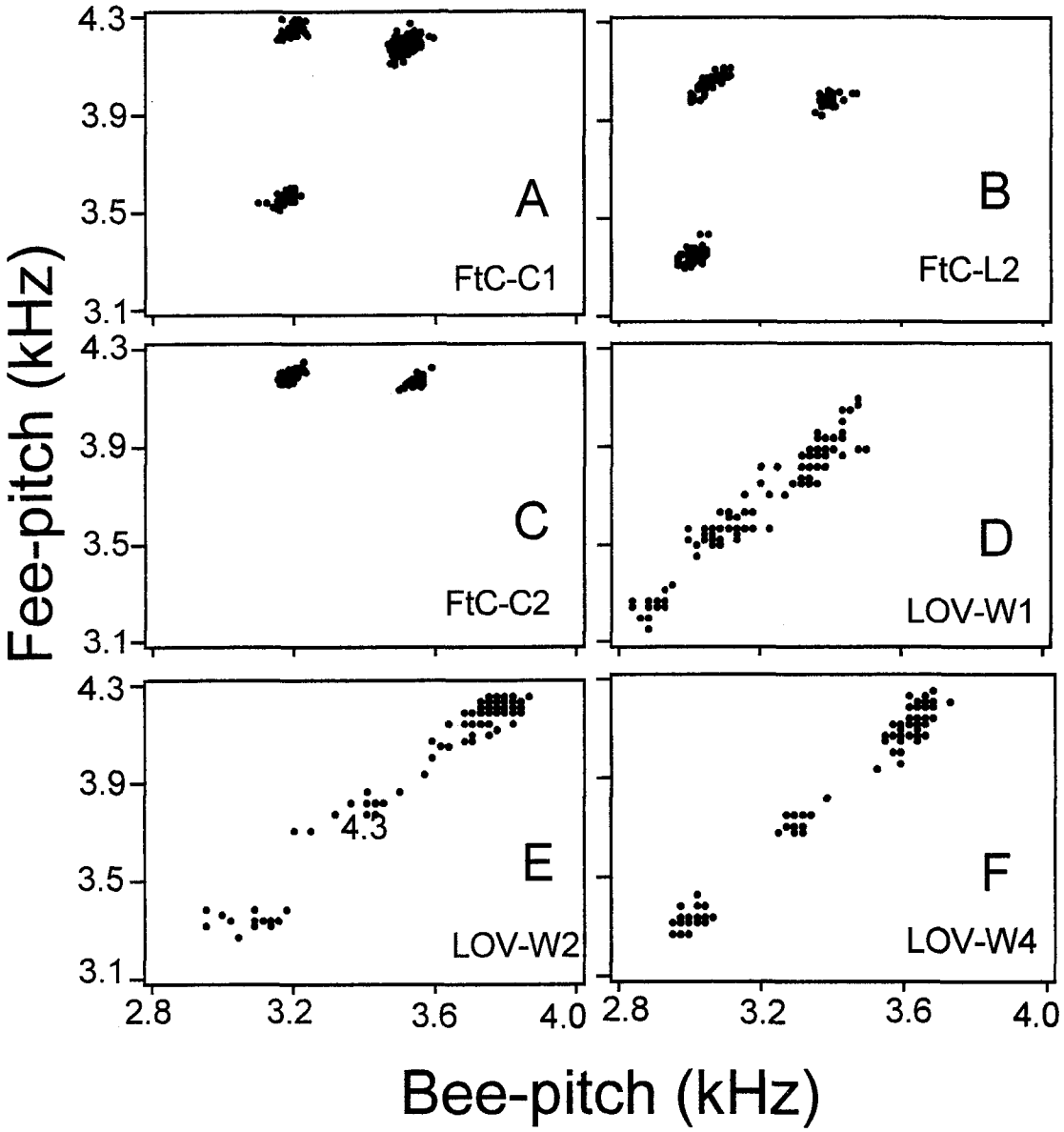


Fig. 5.3. Boxplots showing that chickadee song switching rates during the dawn chorus (number of song switches / total number of songs) are similar in Fort Collins (N = 11 birds) and Loveland (N = 7 birds). Medians (horizontal line within the box), quartiles (top and bottom of box), and the 0.05 and 0.95 quantiles (tips of vertical whiskers) are shown for each song type.

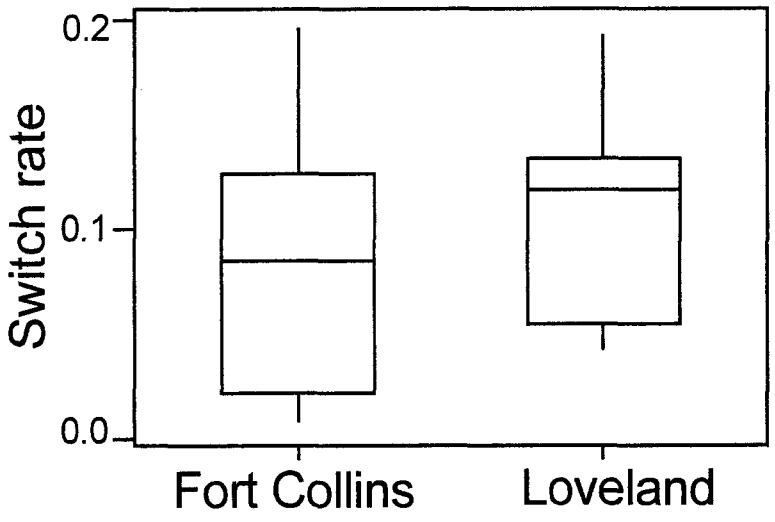
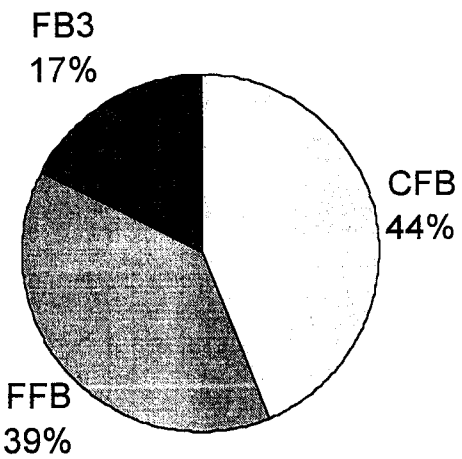


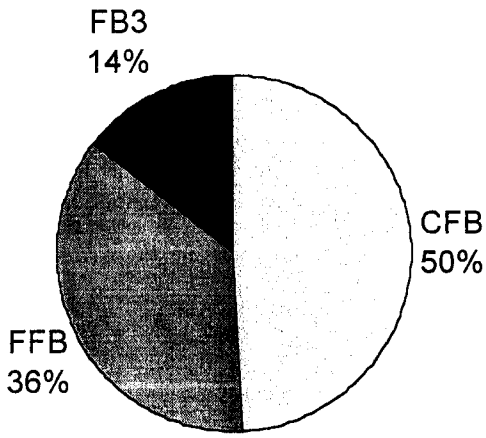
Fig. 5.4. Pie charts show that song type usage frequencies for Fort Collins black-capped chickadees are similar regardless of whether males are directing their songs to other males during countersinging exchanges (a, $N = 80$ birds) or to females during the dawn chorus (b, $N = 11$ birds). 'CFB', 'FFB', and 'FB3' refer to the Fort Collins song types and correspond to the labels in Fig. 1a-c.

Communication with:

A-Males



B-Females



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