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Black-capped chickadees, *Poecile atricapillus*, can use individually distinctive songs to discriminate among conspecifics

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The ability to discriminate among signallers and to respond to them on an individual basis provides receivers with substantial benefits. For example, discriminating among signallers allows receivers to ignore unreliable individuals or to focus their territorial defence on unfamiliar intruders. Such discrimination requires signals to be individually distinctive; that is, signals must vary more among than within individuals. Furthermore, receivers must be able to discriminate among the signals of different individuals. In this study, we used fine structural analysis to show that the simple songs of male black-capped chickadees are individually distinctive, but that substantial variation exists both within and among recordings of the same individual. This finding emphasizes the need for multiple recordings of each individual in studies of individual distinctiveness, since failing to measure variation across recordings of the same individual can make it difficult to determine whether signals vary among individuals or whether they simply vary among different recording sessions. To test whether chickadees discriminate among the signals of different individuals, we used a playback experiment in which we broadcast priming and discrimination stimuli to 45 territorial males. When individuals heard the playback of two different males, they produced more songs and remained near the loudspeaker for a longer period than when they heard two different exemplars from the same male. Chickadees can therefore discriminate among singers based exclusively on their songs, which may help to explain how chickadees eavesdrop on singing contests and subsequently select extrapair mates on the basis of song contest performance.

Keywords:

black-capped chickadee

communication network

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eavesdropping
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individually distinctive signal

Many animal species produce signals that influence the behaviour of receivers. Important examples include signals that coordinate group movements, warn others of danger, signal aggressive intent, identify food sources, or attract potential mates (Bradbury & Vehrencamp 1998). Within a given signal class, variation in signal use or in signal structure can further influence the receiver's response. For example, such variation can encode referential information (e.g. food location, von Frisch 1967; predator type, Cheney & Seyfarth 1988) or the signaller's motivational state (Morton 1977). It may also allow receivers to discriminate between broad classes of signallers, such as neighbours and strangers, males and females, familiar and unfamiliar, mature and immature, or dominant and subordinate (e.g. Ryan 1980; Stoddard 1996; Sherman et al. 1997; Blumstein & Munos 2005; Gherardi et al. 2005). Finally, if receivers can identify individual signallers, then they may even be capable of tailoring their responses according to the signaller's reliability (Cheney & Seyfarth 1988; Hare & Atkins 2001; Blumstein et al. 2004) or to their relationships with specific individuals (e.g. mates, kin, competitors, neighbours; Caldwell 1992; Stoddard 1996; Sherman et al. 1997; Bergman et al. 2003).

Receivers can use a variety of mechanisms to identify signallers. If they are close to each other, then the receiver might identify the signaller using visual cues (e.g. Dale et al. 2001; Tibbetts 2002). Alternatively, if signallers tend to signal consistently from the same location, then receivers might intuit their identity by localizing their signals (Lovell & Lein 2005). A more flexible method of recognition, however, would be to identify signallers based exclusively on their signals (Sherman et al. 1997; Tibbetts & Dale 2007). This form of recognition could expand the range over which receivers recognize signallers, particularly when animals communicate over long distances, through visual obstruction, or across temporal gaps.

For signallers to be recognized by their signals, they must have individually distinctive signals (Falls 1982; Weary et al. 1990). This pattern is widespread among taxa and signalling modalities; for example, it has been documented in the acoustic signals of birds (e.g. McDonald et al. 2007), the visual signals of lizards (e.g. Martins 1991), the chemical signals of rodents (e.g. Johnston et al. 1993), and the electrical signals of fish (e.g. McGregor & Westby 1992). The pattern may even be ubiquitous, as any morphological or physiological idiosyncrasies in signal production mechanisms would tend to create individually distinctive signals. Of course, individual recognition also requires receivers to discriminate among the signals of different individuals (Sherman et al. 1997; Tibbetts & Dale 2007). Although individual discrimination is less studied than individual distinctiveness, it has been documented in several of the systems in which individually distinctive signals have been described, including the acoustic signals of rodents and birds (e.g. Godard 1991; Hare 1998; Blumstein & Daniel 2004), the visual signals of lizards (e.g. van Dyk & Evans 2007), the chemical signals of rodents (e.g. Johnston 2003) and the electrical signals of fish (e.g. Graff & Kramer 1992).

Individual distinctiveness is measured by comparing within-individual to among-individual variation in signal structure (Falls 1982). In some studies, however, the within-individual variance estimate is derived from a single sampling session of each individual (e.g. Naguib et al. 2001; Sousa-Lima 2002; Fenton et al. 2004; Fitzsimmons et al. 2008a; Kennedy et al. 2009). This method of sampling potentially confounds the comparison, as the among-individual estimate also includes variance generated by differences among sampling sessions (reviewed in Ellis 2008). Variance owing to differences among sampling sessions can arise from changes in the way that the animal produces the signal, which might reflect changes in the animal's motivation, diet, or the time of day, and also from changes in the way that the signal is sampled, which might reflect changes in topography, precipitation, masking noise, recording distance, wind speed, temperature and humidity at the time the signal was sampled (Morton 1977; Ferkin et al. 1997; Larom et al. 1997; Lengagne & Slater 2002). More commonly, however, studies simply fail to report the number of sampling sessions per individual, so the sources of signal variation in those studies remain unclear (reviewed in Ellis 2008). In either case, concluding that a species has individually distinctive signals when the number of sampling sessions per individual is one or unknown may be incorrect; a simple alternative interpretation may be that signals vary more among than within sampling sessions.

An analogous problem exists in many perceptual studies that use a habituation/discrimination paradigm to show individual discrimination. Here, each subject is habituated to a series of signals that are derived from the same individual. Following habituation, each subject is then presented with either a control stimulus, which is a different signal from the same individual, or an experimental stimulus, which is a different signal from a different individual (Halpin 1974; Johnston & Jernigan 1994). Individual discrimination is inferred if subjects respond more strongly to the experimental stimulus than to the control stimulus (Halpin 1974; Johnston & Jernigan 1994). Results may be confounded, however, if the control stimulus and its corresponding habituation series are acquired from the same sampling session, as the experimental stimulus and its habituation series are necessarily derived from different sessions (e.g. Hare 1998; Mendl et al. 2002; Kazial et al. 2008; Tang-Martínez & Bixler 2009). Again, concluding individual discrimination in this context may be incorrect; a simple alternative may be that subjects during the discrimination phase respond more strongly when the habituation and discrimination stimuli are derived from different sessions, as opposed to different individuals.

Black-capped chickadees are ideal for studying individual recognition. During the breeding season, males compete in singing contests that function in territory maintenance and mate attraction (Mennill & Otter 2007). Both females and neighbouring males eavesdrop on these singing contests, and the outcomes influence reproductive behaviour (Mennill et al. 2002; Mennill & Ratcliffe 2004). For example, a male with high-ranking dominance status will seldom lose paternity, but, if his songs are contested by an aggressive opponent, his female may switch from a monogamous to a polygamous mating strategy (Mennill et al. 2002). This could occur because the female, unaccustomed to hearing her dominant male lose his singing interactions, seeks extrapair matings with neighbouring males. Alternatively, neighbouring males that normally avoid the dominant male might perceive his defeat as a unique opportunity to invade his territory and solicit copulations from his female. Both of these mechanisms require individuals to eavesdrop on singing interactions and to identify winning and losing contestants. How eavesdropping chickadees recognize individual contestants, however, remains unknown. They probably cannot view multiple contestants during singing interactions, as contestants are often separated by thick vegetation ($\overline{X}$ ± SE distance between contestants during naturally occurring contests: 57.6 ± 3.6 m; Fitzsimmons et al. 2008b). Males also sing from multiple locations, so singing location might be a poor proxy for singer identity (Fitzsimmons et al. 2008b). Recognizing individuals by their songs, however, could allow receivers to evaluate extrapair mating opportunities over a broad geographical range (songs transmit at least 80 m, across multiple territories; Christie et al. 2004a). Two studies provide tentative support for this mechanism. First, Christie et al. (2004a) showed that wild male chickadees have individually distinctive songs. However, they did not account for the confounding effects of multiple recording sessions, so it remains unclear whether songs differ among males or simply among recording sessions. Second, Phillmore et al. (2002) used operant go/no-go discrimination to train captive chickadees to discriminate among eight vocalizations recorded from eight different individuals. They did not include multiple vocalizations from each individual, however, so it remains unknown whether chickadees discriminated among individuals or simply among different vocalizations.

In the current study, we recorded individuals over multiple sessions, and tested whether male black-capped chickadee songs are individually distinctive. Using playback, we then tested whether chickadees discriminate among the songs of different individuals. In both tests, we account for the potentially confounding effects of multiple recording sessions.

METHODS

General

We studied a free-living population of black-capped chickadees at the Queen’s University Biological Station (44°34’N, 76°19’W) between 10 January and 21 May 2009. During January, we captured 200 chickadees in Potter traps baited with sunflower seeds. We attached an aluminium Canadian Wildlife Service band and a unique combination of three coloured leg bands to their legs for identification. We estimated sex using the formula in Desrochers (1990), which incorporates measures of body mass, wing length and outer rectrix length (males are slightly larger than females). We confirmed the sex of birds in spring by observing reproductive behaviour. All research complied with the ASAB/ABS *Guidelines for the Use of Animals in Research* and was approved by the Animal Care Committee at the University of Windsor (AUPP 09-06).

Individual Distinctiveness

Male black-capped chickadees produce a simple two-note song that is referred to onomatopoetically as a fee-bee. The fee note has descending frequency modulation, whereas the bee note has a nearly constant frequency that is lower than the minimum frequency of the fee note (see Figure 1 in Mennill & Otter 2007). Although the song is simple, individual males can vary their songs by transposing the two-note phrase along a continuous frequency range of approximately 860 Hz. The song is approximately 1 s in duration and is repeated many times during the dawn chorus and throughout the day.

We recorded songs from chickadees during naturally occurring song bouts during the breeding season between 22 April and 13 May 2009 on mornings (0530–0915 hours) when wind speed did not exceed 5 km/h. When a male was heard singing, we approached him to within 5.7 ± 3.3 m ($\overline{X}$ ± SE), identified him, and remained as still as possible. Singing was recorded with a Marantz recorder (model PMD660; sampling rate 44 100 Hz; accuracy 16 bits; format WAVE) and a shotgun microphone that was pointed directly at the singing male (Audio-Technica AT8015; frequency response 40–20 000 Hz). We ended recording when the subject stopped singing or flew away, or when we had recorded a minimum of 30 songs. We noted the time, described the recording location, and measured the approximate distance between the microphone and subject.

We reviewed sound spectrograms of all recordings using Syrinx-PC (v. 2.6h; J. Burt, Seattle, WA, U.S.A.; settings: FFT: 1024, Hanning window) and retained for analysis all recordings that satisfied three criteria: (1) the singer's identity was unambiguous, (2) the singer was recorded on 2 or more days and (3) at least 10 songs from each recording session were not distorted or masked by other sounds. A total of 55 recordings from 23 males satisfied these criteria.

We analysed song structure using SASLab Pro (v. 4.40; Avisoft Bioacoustics, Berlin) following the methods outlined in Christie et al. (2004a). From each recording, we selected the first 10 songs that were not distorted or overlapped by other sounds. Each song was filtered using a 2.5–5.0 kHz bandpass filter and then normalized to -1 dB. For each of the 550 songs selected, we generated a spectrogram (1024 points, 87.5% overlap, Hanning window, time resolution 2.9 ms, frequency resolution 43 Hz) and measured six structural features (see Figure 1 in Christie et al. 2004a), including (1) song length (s), (2) fee length (length of the fee note relative to song length), (3) fee amplitude (root mean square (RMS) amplitude of the fee note relative to that of the entire song, calculated as $20\log(RMS_{fee}/RMS_{song})$), (4) glissando ratio (frequency at fee_{start}/freqency at fee_{end}), (5) interval ratio (frequency at fee_{end}/frequency at bee_{start}) and (6) bee frequency (frequency at middle of bee note). All measurements were made using the 'automatic parameter measurements' feature of SASLab Pro to eliminate human bias in the measurement of fine structural details (settings: -20 dB re. maximum amplitude, hold time 170 ms for measurements of the entire song, hold time 70 ms for measurements of the fee or bee element).

For each male, we estimated three levels of variability for each of the above six structural features. First, we estimated variability within recording sessions of the same male by calculating the average absolute difference of a structural feature among all possible pairwise comparisons of the 10 songs selected from each recording session (45(*k*) comparisons per male, where *k* is the number of recording sessions for that male). Second, we estimated variability among different recording sessions of the same male by calculating the average absolute difference of a structural feature among all possible pairwise comparisons of songs from different recording sessions of the same male (100(k(k - 1)/2) comparisons per male, where k is the number of recording sessions for that male). Finally, we estimated variability among different recording sessions of different males by calculating the average absolute difference of a structural feature among all possible pairwise comparisons of the male's first recording session with the first recording session of each of the remaining 22 males (2200 comparisons per male). Only the first recording session was used when comparing variability among males because the number of recording sessions differed among different males. In total, this method produced three variability estimates for each of the six structural features for each of the 23 males.

Individual Discrimination

We conducted a discrimination playback study on 45 territorial males during the breeding season between 8 May and 21 May 2009. Immediately before starting a trial, we set up the playback apparatus in the centre of a male’s territory. We chose the centre of the territory to reduce the probability of attracting multiple males and to ensure that our playback stimuli simulated a territorial intrusion. We defined a male’s territory as the region occupied exclusively by him and his mate, as observed by us during the previous month. The playback apparatus consisted of a digital audio player (an Apple iPod) connected in stereo to two active speakers (Califone, model PA285AV, frequency response 200–5000 Hz) that were placed 10 m apart atop 1.8 m poles. The volume of each speaker was set such that stimuli were broadcast at 85 dB SPL at a distance of 1 m (measured with a RadioShack sound level meter, model 33-4050, C weighting, fast response), which we determined to be a natural sound pressure level by comparison to chickadees in the field.

We began each trial by broadcasting chick-a-dee calls repeatedly through one of the two speakers (selected at random) to lure the resident male to the playback location (Fig. 1; these calls are structurally distinct from the chickadee's song). When the subject approached the speaker to within 5 m, we terminated the chick-a-dee calls and began the priming phase. The priming phase consisted of broadcasting one of 15 priming stimuli repeatedly for 2 min from the second speaker. Immediately following the priming phase we began the discrimination phase. We selected one of three discrimination stimuli that was appropriate for the preceding priming stimulus (see below) and broadcast it repeatedly for 2 min through the original speaker. Priming and discrimination stimuli were selected at random and without replacement, but with the condition that they were derived from males residing at least five territories away from the playback location, to consistently simulate an unfamiliar individual. A postplayback observation period followed the discrimination phase and ended when the subject was no longer visible (Fig. 1). D.R.W. and an assistant conducted all trials while sitting quietly beside the audio player, 15 m from both playback loudspeakers. The assistant controlled the audio player and selected the playback stimuli, leaving D.R.W. blind to the discrimination treatment being broadcast. D.R.W. identified the subject with binoculars, recorded the subject's vocalizations throughout the trial, and noted when the subject disappeared from view. Chickadees were easily observed during the playback trial, and we considered the individual's disappearance from view to be their departure from the playback area. Males in adjacent territories were tested on different days, and trials were aborted if a second male appeared at any time during the trial. Trials continued, however, if the subject's mate appeared.

The lure stimulus consisted of two chick-a-dee calls that were recorded during a single recording session from an individual that was located more than 10 km away from the study site. The calls were acquired using the same recording apparatus and procedure as described above. Using Audition (v. 2.0; Adobe, San Jose, CA, U.S.A.), we filtered the two calls using a 1.0–8.0 kHz bandpass filter, normalized each call to -1 dB, and then separated the two calls with 4 s of silence. We used this single stimulus as a standardized lure during all playback trials.

Priming and discrimination stimuli were derived from the final songs (see Individual Distinctiveness, above). Stimuli were created in 15 blocks, in which each block contained one priming stimulus and three discrimination stimuli corresponding to three experimental treatments. Each of the 15 priming stimuli was derived from a different male and contained five of the 10 songs from a given recording session (songs and session selected at random). We then separated the five songs from each other with 4 s periods of silence, such that each five-song stimulus would be repeated five times during its corresponding 2 min priming phase (i.e. 25 songs over 2 min). Discrimination stimuli were constructed following the same procedure, except that the source of the five songs varied according to treatment. Songs were either from the same recording session of the same male that was used in the priming phase (i.e. the five songs not used in the priming stimulus), from a different recording session of the same male (songs and session selected at random), or from a different recording session of a different male (songs, session and male were selected at random, but with the constraints that the session had not been used to create a priming stimulus and the male had not been used to create another discrimination stimulus).

We added 2 s of silence to the beginning and end of each stimulus (1 lure stimulus, 15 priming stimuli and 45 discrimination stimuli) so that vocalizations would always play after 4 s of silence when stimuli were repeated during playback. The lure, priming and discrimination stimuli were then saved as stereo WAVE files (sampling rate 44.1 kHz, accuracy 16 bits). The 15 priming stimuli, however, were always saved in the first channel, whereas the lure stimulus and the 45 discrimination stimuli were always saved in the second channel. This allowed us to broadcast the three phases of the trial alternately through the two playback speakers, which enabled us to standardize the subject's position relative to the active speaker at the beginning of both the priming and discrimination phases. In addition, when the priming and discrimination stimuli were derived from the same male, the use of two loudspeakers allowed us to simulate one territorial intruder moving between two song perches 10 m apart; when the stimuli were derived from different males, the use of two loudspeakers allowed us to simulate two different territorial intruders singing from perches 10 m apart.

Subjects' responses were scored from sound spectrograms of the trial recordings using Syrinx-PC. For each trial, an observer who was blind to the experimental treatment measured three response variables: (1) the total time in which the subject was visible following the onset of the discrimination phase, (2) the total number of songs produced during the 2 min discrimination phase and (3) the total number of songs produced during the variable-length postplayback observation period. Singing and approaching are both territorial responses of male black-capped chickadees (Mennill & Otter 2007; Fitzsimmons et al. 2008b).

Statistical Analysis

We tested for individual distinctiveness by using a nonparametric Friedman test to compare the three variability estimates (same male, same session; same male, different session; different male, different session) of a given structural feature (song length, fee length, fee amplitude, glissando ratio, interval ratio, bee frequency). Nonparametric analyses were used because the variability estimates consistently violated the parametric assumptions of normality and homoscedasticity. Where an overall model was significant, we conducted three post hoc comparisons using a nonparametric Tukey procedure (Zar 1999). A separate analysis was conducted for each of the six structural features.

In addition to our direct measures of structural feature variation, we conducted a discriminant function analysis, which predicts singer identity using functions derived from linear combinations of the six structural features. Initially, we included only those songs that were derived from each male's first recording session (i.e. 230 songs from 23 males), but, to examine the effect of multiple recording sessions on the model's predictive utility, we reran the analysis using all of the recording sessions from each male (i.e. 550 songs from 55 recording sessions of 23 males). In both analyses, we tested the predictor variables for possible multicollinearity by examining variance inflation factors. Variance inflation factors exceeding 10 indicate possible multicollinearity (Chatterjee et al. 2000); our greatest variance inflation factor was 1.89 in the analysis of the first recording session and 1.58 in the analysis of all recording sessions. Finally, we used simple linear regression to test whether the number of recording sessions for a given male predicted the percentage of correct assignment for that same male. For all analyses, we report only the percentage of cross-validated songs that were correctly assigned to individual (i.e. each song was classified using functions derived from all songs other than that song).

To test for individual discrimination, we compared each response variable across the three experimental treatments using a single factor ANOVA. Where an overall model was significant, we conducted two post hoc comparisons using unpaired *t* tests and corrected for multiple comparisons using the Bonferroni method (i.e. $\alpha = 0.025$). The two post hoc tests compared the different male–different session treatment to each of the two same-male treatments. A separate analysis was conducted for each response variable. Note that subjects were not evenly or unimodally distributed as a function of the number of songs that they produced; rather, the distribution was distinctly bimodal during each phase of the experiment, with one group of birds producing no songs and another group producing many songs. Given that singing and not singing represented two distinct categories of response, we excluded nonsingers from the analysis of song production. Also, the total number of songs produced during the postplayback phase was log transformed prior to analysis to achieve homoscedasticity. Following transformation, all data complied with the parametric assumptions of normality and homoscedasticity. All statistical analyses were two tailed and were conducted using SPSS for Mac (version 17.0, Chicago, IL, U.S.A.).

RESULTS

Individual Distinctiveness

Songs varied more among males than within males, even after accounting for differences among recording sessions. For each of the six structural features, there were one or more significant differences among the three variability estimates (Friedman tests: all $\chi^2 \geq 10.78$, all $N = 23$, all $P \leq 0.005$; Fig. 2), and, in every case, the different male–different session estimate was significantly greater than the same male–same session estimate (nonparametric Tukey procedure: all $q_{0.05,\infty,3} \geq$

4.56, $q_{\text{critical}} = 3.31$; Fig. 2). More importantly, however, the different male–different session estimate was also significantly greater than the same male–different session estimate for three of the six structural features (nonparametric Tukey procedure: all $q_{0.05,\infty,3} \geq 3.55$, $q_{\text{critical}} = 3.31$; Fig. 2a, b, d); the remaining three structural features showed similar nonsignificant trends (all $q_{0.05,\infty,3} \geq 2.73$, $q_{\text{critical}} = 3.31$; Fig. 2c, e, f). Finally, three of the six same male–different session estimates were significantly larger than their corresponding same male–same session estimates (nonparametric Tukey procedure: all $q_{0.05,\infty,3} \geq 3.55$, $q_{\text{critical}} = 3.31$; Fig. 2a, d, f), despite the fact that they originated from the same male; the remaining three structural features showed similar nonsignificant trends (all $q_{0.05,\infty,3} \geq 1.49$, $q_{\text{critical}} = 3.31$; Fig. 2b, c, e). In other words, songs were more variable among than within recording sessions of the same individual, which reveals that significant structural variation is generated by differences among recording sessions.

Discriminant function analysis assigned songs to the correct males on the basis of fine structural measurements at levels significantly exceeding chance (Table 1). When we included only the first recording session of each male, the analysis assigned 75.2% of the 230 songs to the correct male, which exceeds the 4.3% correct assignment expected by chance. When we included multiple recording sessions from each male, the analysis assigned 51.6% of the 550 songs to the correct male, which also exceeds the 4.3% correct assignment expected by chance. Finally, the number of recording sessions from a particular male did not predict the percentage of songs that the discriminant function analysis correctly assigned to that male (simple linear regression: $F_{1,21} = 0.04$, $P = 0.839$, $R^2 = 0.002$).

Individual Discrimination

The experimental treatment did not affect the rate of singing, which was reflected by the total number of songs produced during the 2 min discrimination phase (ANOVA: $F_{2,35} = 0.44$, $P = 0.649$; Fig. 3). In contrast, treatment had a significant effect on the duration of subjects' responses (ANOVA: $F_{2,42} = 4.91$, $P = 0.012$). Consistent with individual discrimination, subjects that received the different male–different session treatment remained in the area for longer than subjects that received either the same male–same session treatment (post hoc unpaired *t* test: $t_{28} = 2.54$, $P = 0.017$, $\alpha = 0.025$) or the same male–different session treatment ($t_{28} = 2.47$, $P = 0.020$, $\alpha = 0.025$). Experimental treatment also had a significant effect on the total number of songs produced by subjects during the postplayback observation period (ANOVA: $F_{2,27} = 7.96$, $P = 0.002$; Fig. 3). Specifically, the birds that received the different male–different session treatment produced significantly more songs than the birds that received the same male–different session treatment (post hoc unpaired *t* test: $t_{20} = 3.72$, $P = 0.001$, $\alpha = 0.025$; 11 of 15 males in each group sang during the postplayback observation period), although birds in the former treatment group also remained in the playback area for longer. Surprisingly, the birds that received the different male–different session treatment did not produce more songs than the birds that received the same male–same session treatment (post hoc unpaired *t* test: $t_{17} = 0.66$, $P = 0.521$, $\alpha = 0.025$; 11 of 15 males that received the former treatment sang; 8 males that received the latter treatment sang), although this may simply reflect the smaller sample size in this comparison. Finally, the number of songs produced (ANOVA: $F_{2,36} = 1.48$, $P = 0.242$) and the time spent in the playback area (ANOVA: $F_{2,42} = 0.00$, $P > 0.999$) did not vary during the priming phase as a function of the subsequent experimental treatment.

DISCUSSION

Male black-capped chickadees showed individually distinctive structural variation in their simple two-note songs. Furthermore, in a discrimination playback experiment, chickadees showed the strongest response when the priming and discrimination stimuli were derived from different males. Black-capped chickadees can therefore discriminate among the songs of different individuals.

Variation in song structure originated from three distinct sources. First, songs varied considerably within recording sessions of the same male, which may reflect behavioural or physiological variation in song production, in situ variation in either sound transmission or recording fidelity, or both (Morton 1977; Larom et al. 1997). Second, significant variation in song structure was also associated with differences between recording sessions of the same individual. This probably reflects the fact that a single recording session undersamples the variation contained in an individual's song repertoire, as is the case in white-throated magpie-jays, *Calocitta formosa*, humpback whales, *Megaptera novaeangliae*, common loons, *Gavia immer*, and Weid's marmosets, *Callithrix kuhli* (reviewed in Ellis 2008). For example, bee frequency was consistent within, but not between, recording sessions of the same male (Fig. 3f). This pattern of frequency shifting is consistent with that described for natural singing bouts, where males only change their singing frequency after producing an average of 30 to 41 songs (Christie et al. 2004b; Horn et al. 1992). In addition, several extraneous factors, such as weather and recording distance, vary more among than within recording sessions, and each of these factors can affect measures of signal variation (Morton 1977; Ferkin et al. 1997; Larom et al. 1997; Lengagne & Slater 2002). It is important to note that the variation associated with recording session was not due to males altering the absolute frequency of their songs, as five of the six structural features were independent of absolute frequency (see also Christie et al. 2004a). Finally, substantial variation could also be attributed to differences among individuals, which means that chickadees have individually distinctive songs (Falls 1982; Ellis 2008).

Structural variation enabled the discriminant function analysis to correctly assign the majority of songs to their corresponding males. Note, however, that this analysis was challenged with discriminating among the songs of 23 different males, which exceeds the number of males that would typically be heard by an individual chickadee in the wild (Christie et al. 2004a; Fitzsimmons et al. 2008b). Consistent with previous studies in other taxa (reviewed in Ellis 2008) and with our own direct measures of structural feature variation, assignment accuracies declined when more than one recording session from each individual was included in the analysis (75.2% to 51.6%). Surprisingly, however, the decline in assignment accuracy was unrelated to the number of additional recording sessions included. This contrasts with previous work on white-throated magpie jays, in which assignment accuracy declined as the number of additional recording sessions and the time over which they were obtained increased (Ellis 2008). Our failure to detect such a relationship probably reflects the fact that all of our recording sessions were obtained over a relatively short period (3 weeks), as compared to the multiyear study conducted by Ellis (2008).

We documented three distinct levels of variation in song structure, but, in our playback study, males responded to these three levels of variation with only two distinct levels of response (see Fig. 3). Surprisingly, the magnitude of a subject’s response did not vary as a linear function of the structural dissimilarity between the priming and discrimination stimuli, but, rather, increased only when the priming and discrimination stimuli were derived from different males. This result suggests that subjects escalate their response either when the structural dissimilarity of priming and discrimination stimuli exceeds a certain threshold, or when they detect a novel signature, voice, or other individual-specific attribute encoded in the discrimination stimuli. The possibility that subjects discriminated among individuals based upon signatures encoded in their signals, as opposed to the degree of structural dissimilarity among their signals, is an exciting avenue for future research, and would provide important insight into the precise mechanism underlying individual discrimination.

Although necessary for individual recognition, the combination of individual distinctiveness and individual discrimination does not necessarily imply that chickadees can recognize individuals by their songs. To demonstrate true individual recognition, it would also be necessary to show that subjects associate an individual's signals with an individual-specific aspect of the signaller that is not communicated directly by the signal itself (Tibbetts & Dale 2007). Hare & Atkins (2001) provide a particularly clear example of this. They made one group of Richardson's ground squirrels, *Spermophilus richardsonii*, reliable by repeatedly pairing the playback of their alarm calls with the presentation of a predator model. They also made a second group of squirrels unreliable by repeatedly broadcasting their alarm calls in the absence of a predator model. During subsequent probe trials, in which the calls of both groups were played in the absence of a predator model, receivers showed reduced responsiveness only to the unreliable callers, thereby confirming that receivers recognize individuals based exclusively on their alarm signals. Using a similar experimental approach, it may be possible for future studies to test for true individual recognition in chickadees.

Individual discrimination, independent of individual recognition, has several potential benefits for black-capped chickadees. For example, most males sing during the dawn chorus, so, by simply listening to the dawn chorus, a male could survey his potential competitors and adjust his territory defence and mate-guarding strategies accordingly. Similarly, an eavesdropping female could survey potential extrapair mates and adjust her mating strategy accordingly. Both of these seem possible given previous work in other taxa on signaller enumeration (McComb et al. 1994; Wich & de Vries 2006; Sloan & Hare 2008), and the fact that both sexes of chickadees are known to eavesdrop on singing males (Mennill et al. 2002; Mennill & Ratcliffe 2004). Individual discrimination can also form the basis for discriminating between broader groups of individuals that are defined by functionally important factors, such as familiarity and social status (e.g. Bergman et al. 2003). Discriminating neighbours from strangers, for example, has been shown to be important in a variety of species (e.g. Stoddard et al. 1990; Lovell & Lein 2004). In chickadees, dominant males sire the majority of extrapair offspring (Otter et al. 1998), so discriminating among the songs of dominant and subordinate males could be especially important. For example, the songs of a nearby dominant male might elicit increased mate-guarding behaviour by resident males or cause resident females to begin prospecting for extrapair mates. Future work should therefore explore whether chickadees are capable of discriminating among broader groups of individuals, such as dominants and subordinates, neighbours and strangers, and mates and nonmates. Of course, individual discrimination could also provide the basis for individual recognition, in which case individuals could further adjust their territorial, mating and mate-guarding behaviour according to signaller identity.

Our analysis of six structural features revealed substantial variation both within and among recordings of the same individual (see Fig. 2). Given these results, it is critical that future studies examining individually distinctive signals incorporate and account for both of these sources of variation. This is particularly relevant when recording sessions are short, as longer recording sessions will tend to sample a greater range of each factor that is responsible for signal variation. It is equally important that individual discrimination studies incorporate these sources of variation so that the effects of signaller and recording session on subjects' responses can be differentiated. Finally, it may also be necessary to revisit the conclusions of previous studies that did not account for the potentially confounding effects of recording session.

In conclusion, we showed that the songs of male black-capped chickadees vary more among males than within males, and that this effect persists even after accounting for the considerable variance resulting from differences among multiple recording sessions of the same individual. We therefore conclude that male black-capped chickadees have individually distinctive songs. In addition, we showed that male chickadees respond more to the playback of a second male than to a second playback of a single male, which shows that chickadees can also discriminate among individuals based exclusively on their songs. Male chickadees therefore satisfy two important criteria that are necessary for individual recognition.

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Table 1

Discriminant function analysis of songs from single and multiple recording sessions of 23 male black-capped chickadees

Function	Eigenvalue	% Variance explained	Song length	Fee length	Fee amplitude	Glissando ratio	Interval ratio	Bee frequency
Single recording session per male								
1	10.5	50.0	0.52	0.02	0.04	0.31	0.30	-0.86
2	4.9	23.5	0.67	-0.16	0.15	0.39	0.40	0.57
3	2.6	12.7	-0.63	0.11	0.01	0.86	1.02	-0.15
4	1.7	8.1	-0.40	0.61	0.20	0.50	-0.43	-0.09
5	1.1	5.3	-0.07	0.94	0.16	-0.63	0.06	-0.03
6	0.1	0.4	0.09	-0.19	0.97	-0.09	-0.01	0.09
Multiple recording sessions per male								
1	2.2	38.0	0.96	-0.25	0.00	0.21	0.24	-0.14
2	1.1	19.5	0.20	0.36	0.06	0.09	-0.77	0.59
3	1.0	17.4	-0.29	0.77	0.17	0.25	0.55	0.17
4	0.9	16.3	0.56	-0.33	0.07	-0.58	0.20	0.84
5	0.4	7.8	-0.11	-0.58	0.03	0.83	0.29	0.25
6	0.1	1.1	0.06	-0.13	1.00	-0.10	-0.07	0.08

Shown for each function are the eigenvalue, the percentage of variance in each structural feature that is explained by the function, and the standardized coefficients that relate each structural feature to the function.

Figure 1

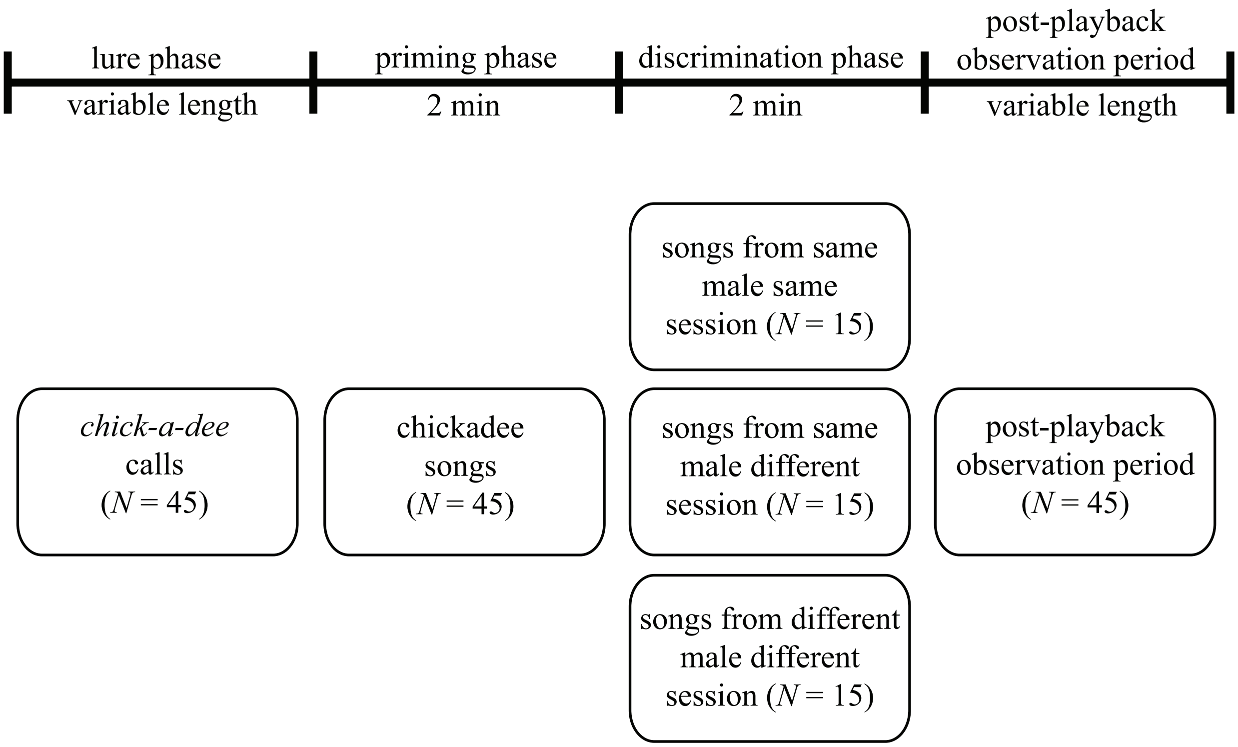

Figure 1. Timeline and schematic of the discrimination experiment used to test for individual discrimination in 45 male black-capped chickadees.

Figure 2

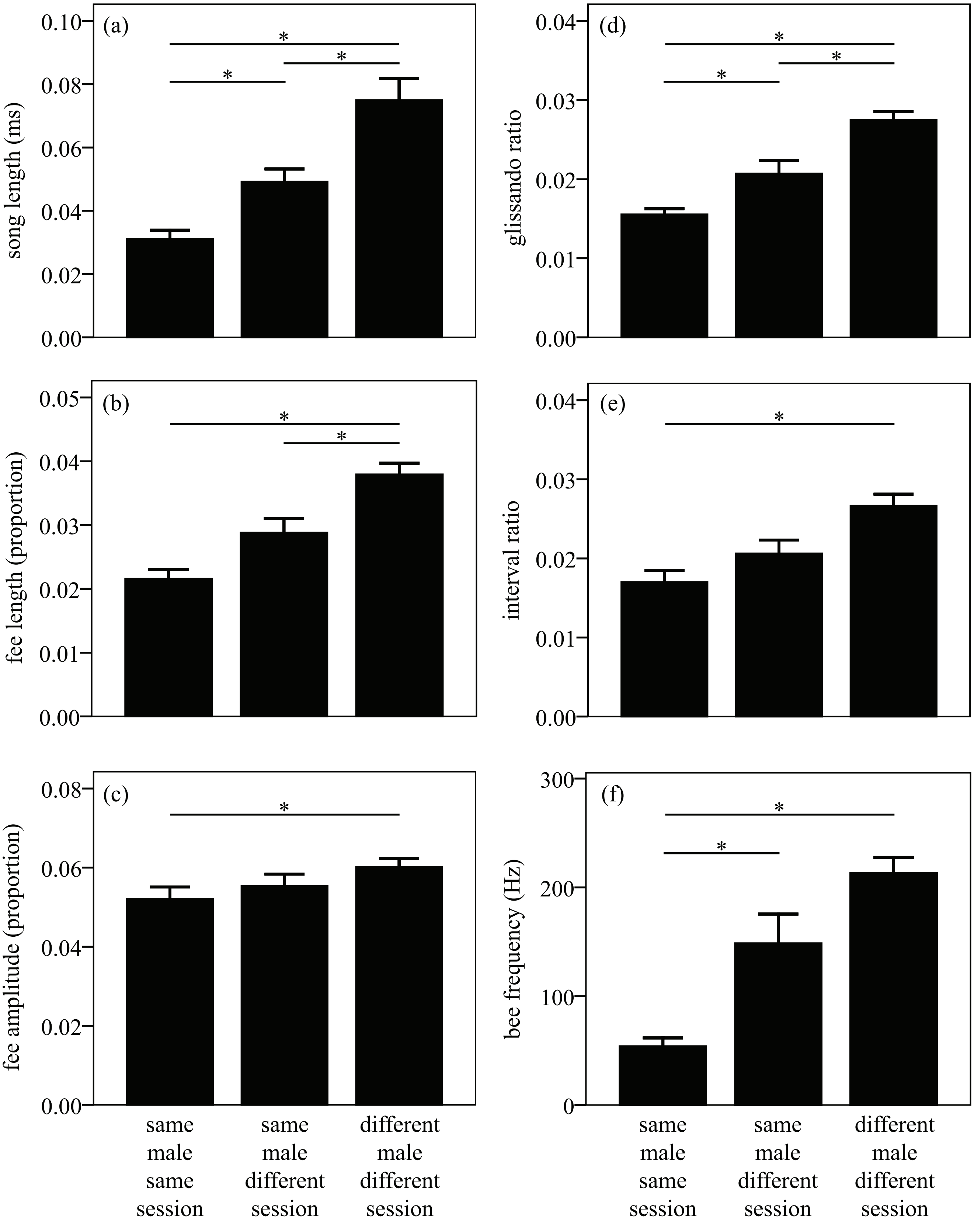
(a)
song length (ms)
0.10
0.08
0.06
0.04
0.02
0.00
*
*
*
(b)
fee length (proportion)
0.05
0.04
0.03
0.02
0.01
0.00
*
*
(c)
fee amplitude (proportion)
0.08
0.06
0.04
0.02
0.00
*
(d)
glissando ratio
0.04
0.03
0.02
0.01
0.00
*
*
*
(e)
interval ratio
0.04
0.03
0.02
0.01
0.00
*
(f)
bee frequency (Hz)
300
200
100
0
*
*
same male same session
same male different session
different male different session
same male same session
same male different session
different male different session

Figure 2. Male black-capped chickadees have individually distinctive structural variation in their simple two-note songs. For each of 23 males, we calculated three estimates of song structure variation, which were based on variation within recording sessions of the same male, variation across recording sessions of the same male, and variation across recording sessions of different males. We then repeated this process for each of the six structural features. Shown for each structural feature is the mean ± SE of each variability estimate. Statistically significant differences between variability estimates are denoted by an asterisk.

Figure 3

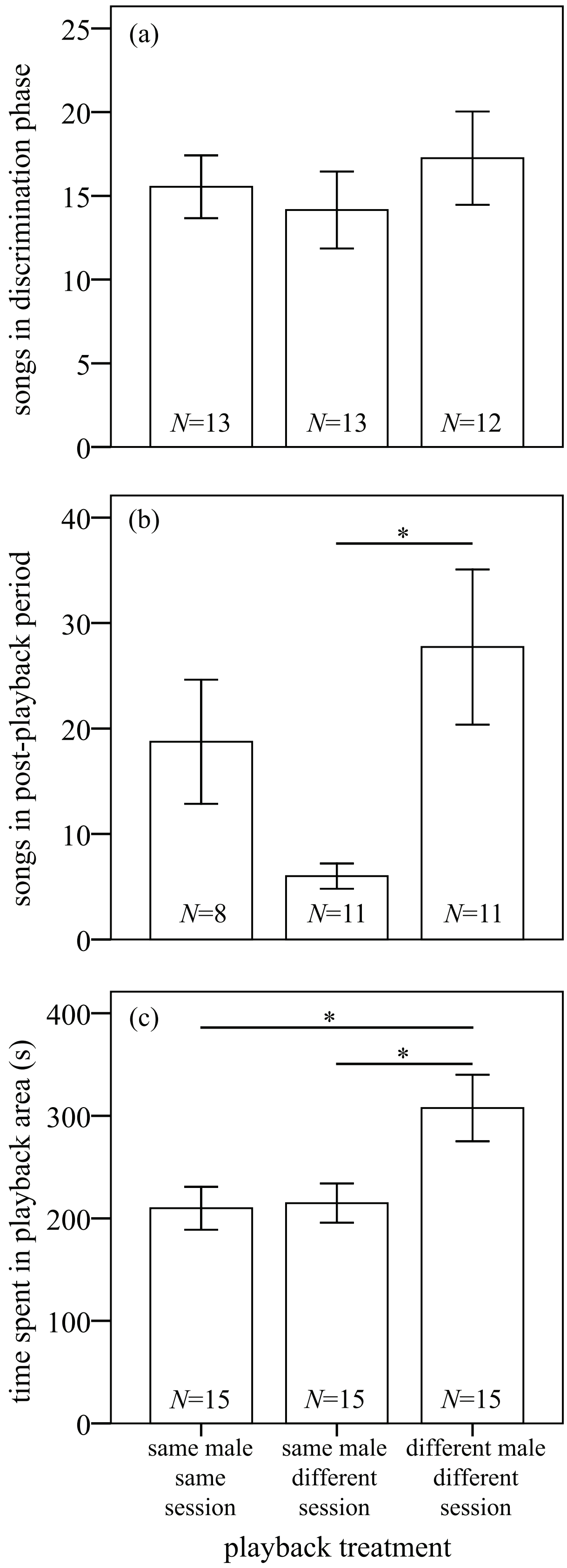
(a)
songs in discrimination phase
25
20
15
10
5
0
N=13
N=13
N=12
(b)
songs in post-playback period
40
30
20
10
0
*
N=8
N=11
N=11
(c)
time spent in playback area (s)
400
300
200
100
0
*
*
N=15
N=15
N=15
same male same session
same male different session
different male different session
playback treatment

Figure 3. Responses of male black-capped chickadees to three discrimination treatments that differed in their similarity to a preceding priming stimulus. Priming and discrimination stimuli were derived from the same recording session of the same male, from different recording sessions of the same male, or from different recording sessions of different males. Behavioural responses include (a) the number of songs produced during the 2 min discrimination phase, (b) the number of songs produced during the variable-length postplayback observation period and (c) the total time elapsed between the start of the discrimination phase and the subject's disappearance from the playback area. Although 15 individuals received each playback treatment, not all individuals sang during each phase, giving rise to the variable sample sizes. Shown for each response variable are the mean ± SE. Statistically significant differences between treatments are denoted by an asterisk.