Black-capped chickadees, *Poecile atricapillus*, avoid song overlapping: evidence for the acoustic interference hypothesis

David R. Wilson [a, c, *], Laurene M. Ratcliffe [b], Daniel J. Mennill [c]

[a] Department of Psychology, Memorial University of Newfoundland, St John's, NL, Canada
[b] Department of Biology, Queen's University, Kingston, ON, Canada
[c] Department of Biological Sciences, University of Windsor, Windsor, ON, Canada

Many animals produce sounds that overlap the sounds of others. In some animals, overlapping is thought to be an aggressive signal important in resource defence. Yet, overlapping can also occur by chance, and therefore its function is controversial. In this study, we conducted two experiments to test the function of overlapping in black-capped chickadees, *Poecile atricapillus*. In experiment 1, we simulated territorial intrusions by broadcasting songs inside established chickadee territories. Resident males overlapped the playback-simulated intruders significantly less than expected by chance, as in most species in which overlapping has been described. Chickadees also overlapped more when they were farther from the intruder. This pattern suggests that chickadees avoid overlapping as a mechanism for reducing acoustic interference ('interference avoidance hypothesis'). However, the pattern could also constitute submissive signalling if chickadees signal de-escalation (associated with greater distance between opponents) through increasing rates of overlapping ('submissive signalling hypothesis'). Therefore, in experiment 2, we contrasted these two hypotheses by comparing responses to playback stimuli with low or high interference potential and low or high signal value. We manipulated interference potential by broadcasting stimuli at different amplitudes. We manipulated signal value by broadcasting either song stimuli, which elicit aggression, or white noise stimuli with matching time-amplitude characteristics. If overlapping is a submissive signal, then we predicted that chickadees would avoid overlapping song stimuli, but not white noise stimuli, which lack signal value. Contrary to this prediction, chickadees overlapped song and white noise stimuli equally often, but significantly less often than expected by chance. Furthermore, chickadees overlapped both types of stimuli more often when they were broadcast at lower amplitudes (i.e. lower interference potential). Together, these findings provide compelling evidence that overlapping is not a signal in this species, and that chickadees avoid overlapping both biotic and abiotic sounds as a mechanism for reducing interference.

© 2016 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Animal signals are remarkably variable. For species that communicate acoustically, individuals can vary the structure of their signals (e.g. temporal, frequency or amplitude characteristics), the composition of their signalling sequences (e.g. signalling rate or number of signals produced), and even the relationships between their signals and the signals of others (e.g. song type matching in birds; Bradbury & Vehrencamp, 2011; Todt & Naguib, 2000). Although such variation is widespread, its function is not always clear. It could be random or vestigial with no biological function, or it could arise as a response to the animal's environment. For

example, an animal might vocalize more loudly to overcome the masking effects of abiotic noise (Brumm & Zollinger, 2011). Such variation would improve signal efficacy, but would not constitute a signal per se. Of course, signal variation could also serve a communicative function, as has been demonstrated for signals produced by diverse animals in various contexts in all known signalling modalities (Bradbury & Vehrencamp, 2011).

One important benefit of acoustic signals is that they can transmit over relatively long distances. This expands a signal's active space (i.e. the area over which it can be detected; Marten & Marler, 1977), but also increases the probability that multiple signallers will share that space. In these situations, one individual will often produce a signal while another individual's signal is already being produced. Known as 'overlapping', this phenomenon has

* Correspondence: D. R. Wilson, Department of Psychology, Memorial University of Newfoundland, 232 Elizabeth Avenue, St John's, NL A1B 3X9, Canada.
   E-mail address: dwilson@mun.ca (D. R. Wilson).

been studied in insects, anurans, mammals and birds (Gerhardt & Huber, 2002; Greenfield, 1994; Naguib & Mennill, 2010; Schulz, Whitehead, Gero, & Rendell, 2008). The greatest focus, however, has been in the context of dyadic territorial singing interactions in birds (reviewed in Naguib & Mennill, 2010).

There are several hypotheses for the function of overlapping (reviewed in Helfer & Osiejuk, 2015). The null hypothesis states that song overlapping serves no biological function, and that it occurs only by chance whenever signalling animals share an acoustic space (Searcy & Beecher, 2009, 2011). Under this hypothesis, the probability of overlapping should depend only on the signalling rate of the signaller and the duty cycle of the other individual (i.e. the proportion of time that its signal can be heard; Searcy & Beecher, 2009, 2011). There are also at least five alternative hypotheses. First, overlapping could constitute incidental interference that animals actively avoid (Ficken, Ficken, & Hailman, 1974; Fuller, Warren, & Gaston, 2007; Searcy & Beecher, 2009, 2011; Wasserman, 1977). In this case, overlapping would serve no function and would occur less often than expected by chance, particularly as the degree of interference increases (e.g. when signalling animals are closer to each other). Second, overlapping could be a mechanism for jamming the signals of other animals, and, as such, should occur more often than expected by chance (Corcoran & Conner, 2014; Helfer & Osiejuk, 2015). Third, overlapping could be an aggressive signal (Kunc, Amrhein, & Naguib, 2006; Mennill & Ratcliffe, 2004b), which should occur more often than expected by chance, and more frequently as a signaller escalates towards physical confrontation (e.g. by increasing when the signaller is close to an intruder and decreasing when they are distant; see Searcy & Beecher, 2009, for a review of criteria used to categorize a signal as 'aggressive'). Fourth, overlapping could be a submissive signal, which should occur more frequently as a signaller de-escalates a physical confrontation (e.g. by increasing when the signaller and intruder are farther apart, and decreasing when they are closer together; Searcy & Beecher, 2009). Fifth, overlapping could be a signal of quality, particularly if the ability to detect and rapidly respond to an intruder's signal is governed by neuromuscular performance (Helfer & Osiejuk, 2015). Under this hypothesis, overlapping should occur more often than expected by chance, and variation in overlapping rates should correlate with male quality (Bischoff, Tschirren, & Richner, 2009; Helfer & Osiejuk, 2015). We refer to the null hypothesis as the 'chance occurrence hypothesis' and to the five alternative hypotheses as the 'interference avoidance', 'signal jamming', 'aggressive signalling', 'submissive signalling' and 'signaller quality hypotheses', respectively. These hypotheses are not mutually exclusive and, in some cases, may even generate similar predictions. For example, the signal jamming, aggressive signalling and signaller quality hypotheses all predict that overlapping occurs at levels exceeding chance. Similarly, the signaller quality hypothesis predicts that a signaller's quality is positively correlated with their rate of overlapping, yet the signal jamming and aggressive signalling hypotheses would make the same prediction if high-quality individuals were more aggressive.

The function of song overlapping in birds has become a controversial topic (Helfer & Osiejuk, 2015; Naguib & Mennill, 2010; Searcy & Beecher, 2009, 2011; Todt & Naguib, 2000). Over the last 30 years, most studies have promoted the aggressive signalling hypothesis (Naguib & Mennill, 2010). However, these studies have been challenged recently because they did not demonstrate that overlapping met the criteria for being an aggressive signal, and because they did not account for the number of overlaps that would be expected to occur by chance (Searcy & Beecher, 2009, 2011). As an example, consider two birds that overlap each other more frequently when the distance between them is small. Being close to one's opponent is a necessary

precursor to attack, so the increase in the number of overlaps could be interpreted as a signal of aggression. However, birds often increase their singing rate when they are close to each other (Baker, Wilson, & Mennill, 2012; Benedict, Rose, & Warning, 2012), so the increase in the number of overlaps could simply reflect the concomitant increase in the number of overlaps expected by chance. Studies that have accounted for chance occurrence have shown that birds overlap at or below chance levels (Ficken et al., 1974; Fitzsimmons, Foote, Ratcliffe, & Mennill, 2008; Foote, Fitzsimmons, Mennill, & Ratcliffe, 2008; Gochfeld, 1978; Maynard, Ward, Doucet, & Mennill, 2012; Wasserman, 1977; Yang, Ma, & Slabbekoorn, 2014), which contradicts the signal jamming, aggressive signalling and signaller quality hypotheses. Furthermore, relationships between overlapping rates (after controlling for chance occurrence) and the distance between singing birds have been inconsistent (Searcy & Beecher, 2009), so it has not been possible to distinguish among the chance occurrence, interference avoidance, aggressive signalling and submissive signalling hypotheses. When considering the entire literature of song overlapping, Searcy and Beecher (2009, 2011) suggested that song overlapping may not be a signal in any species in which it has been examined. This view was challenged by Naguib and Mennill (2010).

Black-capped chickadees, *Poecile atricapillus* (hereafter 'chickadees'), are ideal for investigating the function of song overlapping. During the breeding season, territorial males overlap neighbours and territorial intruders at chance levels (Fitzsimmons et al., 2008) or below chance levels (Foote et al., 2008; Masco, Allesina, Mennill, & Pruett-Jones, 2015). Furthermore, interindividual variation in overlapping rates does not correlate with social dominance (Fitzsimmons et al., 2008; Foote et al., 2008), which is a predictor of fitness in this species (Schubert et al., 2007). These findings contradict the signal jamming, aggressive signalling and signaller quality hypotheses. In addition, variation in overlapping rates does not correlate with the distance between singing males (Foote et al., 2008), and fails to predict a signaller's probability of attack (Baker et al., 2012). These findings contradict the aggressive signalling and submissive signalling hypotheses. It is worth noting, however, that several previous studies had promoted the aggressive signalling hypothesis (Fitzsimmons et al., 2008; Mennill & Ratcliffe, 2004a; Mennill, Ratcliffe, & Boag, 2002), although their findings were challenged and debated in three recent reviews (Naguib & Mennill, 2010; Searcy & Beecher, 2009, 2011). Finally, male chickadees that are overlapped tend to truncate their two-note fee bee songs, which is consistent with the interference avoidance hypothesis (Mennill & Ratcliffe, 2004b). However, results from a multispeaker playback experiment showed that high-ranking males are also more likely to approach a simulated intruder that consistently overlaps another simulated intruder (Mennill & Ratcliffe, 2004a), which contradicts the interference avoidance hypothesis. Therefore, the function of song overlapping remains unclear in black-capped chickadees.

In the current study, we conducted three experiments to elucidate the function of song overlapping in chickadees. In experiment 1, we simulated territorial intrusions by broadcasting the chickadee's fee bee song inside the breeding territories of resident males. The resident males overlapped the simulated intruders less often than expected by chance, and were even less likely to overlap when they were close to the intruders (see Experiment 1, Results). This pattern is most consistent with the interference avoidance hypothesis, but could also be explained by the submissive signalling hypothesis if chickadees signal decreasing aggression through increasing rates of overlapping. Therefore, in experiment 2, we contrasted the interference avoidance hypothesis and submissive signalling hypothesis by comparing male responses to playback stimuli with low or high

interference potential and low or high signal value. High-value stimuli were conspecific songs that are known to elicit aggression (Baker et al., 2012), whereas low-value stimuli were white noise stimuli, which lack signal value and which should not elicit aggression. In experiment 3, we tested whether the white noise stimuli from experiment 2 were, in fact, of little value to the chickadees, thereby confirming this important assumption from experiment 2.

GENERAL METHODS

We conducted three experiments on a population of free-living chickadees located near the Queen's University Biological Station in Ontario, Canada (Universal Transverse Mercator coordinates: 18T 394859 m E, 4935524 m N). Some individuals in the population had been colour banded in the context of previous studies and could be identified by leg bands during playback experiments. Others were not colour banded and could not be identified on the basis of bands. To ensure that unbanded males were not sampled repeatedly within a given experiment, we separated trials involving unbanded males by a minimum of 400 m. This distance exceeds the average diameter of a male's territory in this population (mean $\pm$ SD = 152 $\pm$ 35 m; after Mennill, Ramsay, Boag, & Ratcliffe, 2004). The three experiments were conducted in the same general area, so it is possible that individuals were tested in more than one experiment. The dominance status of the birds was not measured in any of the experiments in this study.

Playback trials were conducted during the breeding season when males were associating closely with mates, singing frequently and vigorously defending territories. During playback trials, the subject was often accompanied closely by a second individual, which we assumed was his mate. We identified the male because only males sing regularly in this species. If both individuals sang, if neither individual sang, or if more than two individuals approached, we aborted the trial and repeated it at a new location. All trials were conducted by a single observer (D.R.W.) at times when there was no precipitation and the estimated wind speed was less than 15 km/h.

Wherever possible, we used the same equipment, software and settings in all of our experiments. We used a single recording apparatus, which consisted of a shotgun microphone (Audio-Technica; model AT8015b; frequency response 40–20 000 Hz) and a digital audio recorder (Marantz; model PMD660). Audio recordings and playback stimuli always had the same settings (44 100 Hz sampling rate; 16-bit encoding accuracy; WAVE format), and, whenever we viewed spectrograms of audio recordings, we always applied the same spectrogram settings (512-point fast Fourier transform size; Hamming window; 87.5% overlap; 43 Hz frequency resolution, 2.9 ms temporal resolution). When broadcasting stimuli through a loudspeaker, we measured the peak amplitude of the stimuli in decibels (re 20 µPa) with a digital sound level meter held 1 m from the sound source (RadioShack digital sound level meter; model 33-2055; C weighting; fast response). When conducting statistical analyses, our tests were always two tailed, and we considered our results statistically significant when $P \leq 0.05$.

Songs used as playback stimuli in our three experiments were derived from 41 colour-banded males in the context of a previous study (Wilson & Mennill, 2010). They were recorded during naturally occurring singing bouts in 2009 using the same recording apparatus as described above. From each male, we selected a single song with typical structure and a high signal-to-noise ratio, which we determined by viewing a spectrogram in Raven Pro software (v.1.4; Cornell Lab of Ornithology Bioacoustics Research Program, Ithaca, NY, U.S.A.).

All methods involving animals were approved by the University of Windsor Animal Care Committee (AUPP number 09-06).

EXPERIMENT 1

Methods

In the first playback experiment, our goal was to determine how often male chickadees overlap the songs of a simulated intruder, and whether their probability of overlapping depends on the intruder's proximity.

Procedure

Upon finding a suitable location, we set up the playback apparatus, which consisted of a digital audio player (Apple iPod) connected to an amplified loudspeaker (Califone; model PA285AV; frequency response 200–5000 Hz). We placed the loudspeaker on a tree branch approximately 1.5 m above the ground, oriented it so that it faced upwards, and adjusted its volume so that it broadcast song stimuli at 80 dB SPL, as measured with a sound level meter. We then sat on the ground 10 m away from the speaker, waited silently for 5 min to allow birds in the vicinity to habituate to our presence, and began the playback trial. Although we did not measure the effect of the observer's presence on subjects' responses, subjects did not appear to hesitate or change their behaviour in any way as they passed near to the observer during their approach to the speaker. A possible concern with our experimental design is that a subject's propensity to approach the playback apparatus could depend on the individual's social status, which could also influence their propensity to overlap. However, a previous study involving colour-banded chickadees failed to find any relationship between a male chickadee's social status and his propensity to overlap, pass over or attack a simulated intruder (Baker et al., 2012).

During the playback trial, we broadcast a fee bee song at a rate of 15 songs/min for 15 min. This rate is comparable to natural singing rates reported for this population (dominant males: average singing rate: median = 13 songs/min; interquartile range 11–15 songs/min; maximum singing rate: median = 21 songs/min; interquartile range 20–22 songs/min; subordinate males: average singing rate: median = 11 songs/min; interquartile range 10–11 songs/min; maximum singing rate: median = 18 songs/min; interquartile range 16–20 songs/min; Otter, Chruszcz, & Ratcliffe, 1997). We always broadcast the same song within a trial and a different song recorded from a different male among trials. As recommended by Searcy and Beecher (2011), we used noninteractive playback so that the subject controlled how often it overlapped the playback stimuli. During the playback, we used our recording apparatus to make a continuous audio recording of our surroundings. As soon as a chickadee could be heard singing, he became our subject and we immediately focused our microphone on him for the remainder of the trial. Whenever the subject changed locations, we estimated his distance from the playback speaker and dictated it onto the trial recording.

Our distance estimates ranged between 120 m, which was the maximum distance at which we could hear the subject's songs, and 0 m, which occurred when the subject perched on the playback speaker. In general, we could see the subject when he was within 30 m of the loudspeaker. We acknowledge that the accuracy of our distance estimates probably improved when the subject was closer. We estimate that our accuracy was $\pm$10 cm when the subject was within 1 m of the speaker, $\pm$1 m when he was between 1 and 10 m, $\pm$5 m when he was between 10 and 30 m, and $\pm$10 m when he was between 30 and 120 m. Although the accuracy of our estimates varied with distance, we know of no reason why this should bias our analysis of the relationship between the subject's distance to

the playback speaker and his probability of overlapping the playback stimulus.

In total, we completed 92 trials in which a single male approached and sang. Each trial involved a different subject and, in 33 of these, the subject was accompanied by his mate. All trials were conducted between 13 April and 19 May 2010, between 0627 and 1354 hours.

Stimuli

Songs used as playback stimuli were derived from 20 colour-banded males in the context of a previous experiment (Wilson & Mennill, 2010). As part of another study examining the effect of a song's dominant frequency on receiver responses, we created six different versions of each song stimulus. Using the 'pitch shifter' function in Audition (software, v.2.0; Adobe, San Jose, CA, U.S.A), we transposed each one so that the dominant frequency of its bee note was either 2800, 3000, 3200, 3400, 3600 or 3800 Hz. This allowed us to present realistic acoustic stimuli that spanned the frequency spectrum of normal chickadee songs (Mennill & Otter, 2007). We filtered each song with a 2200–5200 Hz band-pass filter, normalized its peak amplitude to −1 dB, and added enough silence to its beginning and end so that it would play at a rate of 15 songs/min when played on loop. The final 120 song stimuli were transferred to our digital audio player for playback in the field. When beginning a new trial, we selected a song stimulus at random and without replacement, but with the constraint that we used the six stimuli derived from one male before proceeding to the stimuli from another male.

Analysis

We scored each subject's response to the playback by viewing a spectrogram of its trial recording in Raven Pro software. We noted the distance between the subject and speaker whenever the subject sang. Some subjects sang atypical versions of the fee bee song, including 67 males that sang one or more fee songs (473 fee songs in total), and one male that sang 21 bee songs and 77 bee bee songs. We included all of these in our analyses. We also measured the start and end times of each stimulus and subject song. Ideally, these times would be based on recordings obtained at the subject's position, since overlapping is controlled by the subject (Naguib, 2005). As this was not possible, stimulus and subject songs were sometimes recorded when the subject was as much as 120 m away from the speaker. At this distance, the playback stimulus requires approximately 356 ms to travel from the speaker to the subject, and the subject's song requires another 356 ms to travel from the subject back to our microphone. To ensure that these distance-dependent time lags did not confound our analysis of how an intruder's proximity affected overlapping, we corrected the start and end times of each stimulus and subject song. We calculated the speed of sound following the formula presented in Wölfel and McDonough (2009):

$$\text{speed of sound (m/s)} = 331.5 \times ((\text{temperature (°C)} + 273.15)/ 273.15)^{0.5} \quad (1)$$

Temperature had been recorded with a thermometer during each trial (±0.5 °C accuracy). For each stimulus song, we calculated the time required for it to travel from the speaker to the subject (minus the time it would have taken for the sound to travel the 10 m from the speaker to our microphone), and then added this value to the stimulus song's start and end times. For each subject song, we calculated the time required for it to travel from the subject to our microphone, and then subtracted this value from the song's start and end time.

We derived four additional variables from the initial data set. First, we counted the number of times that the subject overlapped a song stimulus. An overlap occurred when the subject's song began while a stimulus song was playing. Second, we calculated the duty cycle of the playback stimulus by dividing the length of one stimulus song by 4 s, which was the time between the beginning of one stimulus and the beginning of the next. Although the song rate was always 15 songs/min, the subtle variation in the length of individual songs created some variation in the duty cycles of the playback stimuli (median = 0.25; interquartile range 0.23–0.26; range 0.20–0.30). Third, we calculated the number of overlaps expected by chance by multiplying the duty cycle of the stimulus by the number of songs that the subject sang during the 15 min playback (Ficken et al., 1974). Fourth, for each of the subject's songs, we calculated his instantaneous singing rate (songs/min) by dividing one by the amount of time between the beginning of the song and the beginning of the subject's next closest song (i.e. either the preceding or subsequent song).

In our first analysis, we compared the number of overlaps observed across an entire trial to the number of overlaps expected by chance using a Wilcoxon signed-ranks test with continuity correction. We used a nonparametric analysis because the data violated the parametric assumption of normality and could not be corrected with a data transformation.

In our second analysis, we used a repeated measures logistic regression to identify the factors affecting moment-to-moment variation in the probability of overlapping. We included whether or not the subject's song overlapped a playback stimulus as the response variable (coded as 0 = no overlap, 1 = overlap), and the distance between the subject and speaker at the time the song was produced as a covariate with fixed effects. We also included the subject's instantaneous singing rate (songs/min) and the duty cycle of the playback stimulus (%) as covariates with fixed effects, since these affect the subject's probability of overlapping the stimulus by chance. Trial number (1–92) was included as a subject variable with random effects to control for repeated measurements from the same individual. We modelled within-subject dependencies using an exchangeable working correlation matrix structure. Model effects were assessed using a type III analysis, and model coefficients were assessed using Wald statistics.

Results

The 92 subjects sang a median of 50 songs during the 15 min trial (interquartile range 31–83 songs; range 2–188 songs). Our duty cycle model predicted that subjects would overlap simulated intruders by chance a median of 12 times (interquartile range 8–20 overlaps; range 0–51 overlaps). Yet subjects overlapped intruders a median of only three times (interquartile range 1–5 overlaps; range 0–25 overlaps), which was significantly less often (i.e. approximately one quarter as often) than was expected by chance (Wilcoxon signed-ranks test with continuity correction: $T_s = 98$, $N = 92$, $P < 0.001$; Fig. 1). Overall, 88 of 92 subjects (i.e. 96%) overlapped the simulated intruder less often than expected by chance.

Subjects produced a total of 5870 songs; 405 of these (i.e. 7%) overlapped the songs of the simulated intruders. Our song-level analysis showed that subjects were less likely to overlap intruders' songs when the distance between them was small, even after controlling for the significant effects of singing rate and stimulus duty cycle (Table 1; Fig. 2).

Of the 92 subjects included in our analysis, 34 (37%) approached to within 1 m of the playback speaker, and 21 (23%) attacked the speaker by landing on it, pecking it or striking it with their feet as they flew past. On average, the 92 subjects approached to within 20 ± 27 m of the playback speaker (mean ± SD; range 0–100 m).
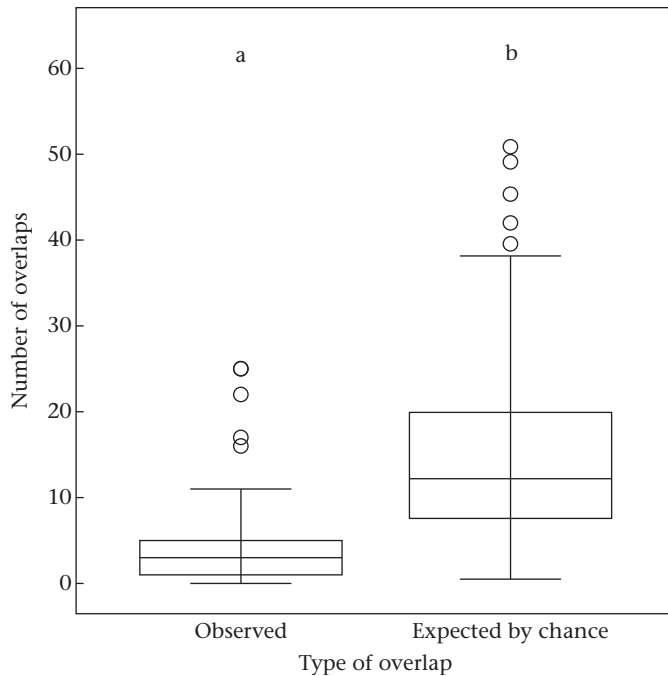
Figure 1. Frequency with which black-capped chickadees overlapped the songs of a playback-simulated intruder in experiment 1. The Y axis shows the number of overlaps observed or expected during an entire 15 min playback trial for 92 males. We calculated chance expectation by multiplying the number of songs sung by the subject during the 15 min playback trial by the duty cycle of the playback stimulus. Boxes show median and interquartile range, and whiskers show the minimum and maximum values within 1.5 times the height of the box. Outliers are shown by open circles. Treatments were compared with a Wilcoxon signed-ranks test with continuity correction. Different letters above bars indicate a significant difference between the number of overlaps observed and expected ($P < 0.001$).

Figure 2. Relationship between song overlapping and the distance to a playback-simulated intruder in black-capped chickadees in experiment 1. The Y axis shows the distance between the playback speaker and the subject whenever the subject produced a song. The X axis divides songs into those that overlapped the intruder ($N = 405$ songs from 74 subjects) and those that did not ($N = 5465$ songs from 92 subjects). Boxes show median and interquartile range, and whiskers show the minimum and maximum values within 1.5 times the height of the box. Outliers are shown by open circles. The relationship between distance and the overlapping response was analysed with a repeated measures logistic regression. Different letters above bars indicate a statistically significant relationship between distance and the probability of overlapping ($P < 0.001$).

Table 1
Analysis of the factors associated with song overlapping in black-capped chickadees

Parameter	Coefficient	SE	Odds ratio	Wald χ^2	P
Intercept	−7.501	1.321	0.001	32.2	1.4×10^{-8}
Distance to speaker (m)	0.016	0.003	1.016	35.1	3.1×10^{-9}
Song rate (songs/min)	0.024	0.012	1.025	4.3	3.8×10^{-2}
Stimulus duty cycle (%)	0.165	0.053	1.179	9.7	1.8×10^{-3}

Data were analysed with a repeated measures logistic regression with an exchangeable working correlation matrix structure. The reference category was songs that did not overlap. Logistic regression coefficients relate their corresponding parameters to the natural logarithm of the odds of overlapping (i.e. the logit). Because they are difficult to interpret, we have also provided odds ratios (i.e. $e^{coefficient}$) for each parameter. For a one-unit increase in a particular parameter, the odds of overlapping increase by a factor equal to that parameter's odds ratio. For example, when a subject is 25 m from the speaker, singing 15 songs/min in response to a simulated intruder with a duty cycle of 25%, the subject's odds of overlapping are 0.073−1 (i.e. $e^{(0.016 \times 25 + 0.024 \times 15 + 0.165 \times 25 - 7.501)}$). These odds correspond to a probability of overlapping of $0.073/(1 + 0.073) = 0.068$. If the intruder's singing rate increases to 16 songs/min while the other parameters remain constant, then the subject's odds of overlapping increase to $0.073 \times 1.025 = 0.075$ to 1. These odds correspond to a probability of overlapping of $0.075/(1 + 0.075) = 0.070$.

EXPERIMENT 2

Methods

We conducted a second playback experiment to determine whether the pattern of overlapping documented in experiment 1 is a submissive signalling strategy or a mechanism for avoiding ac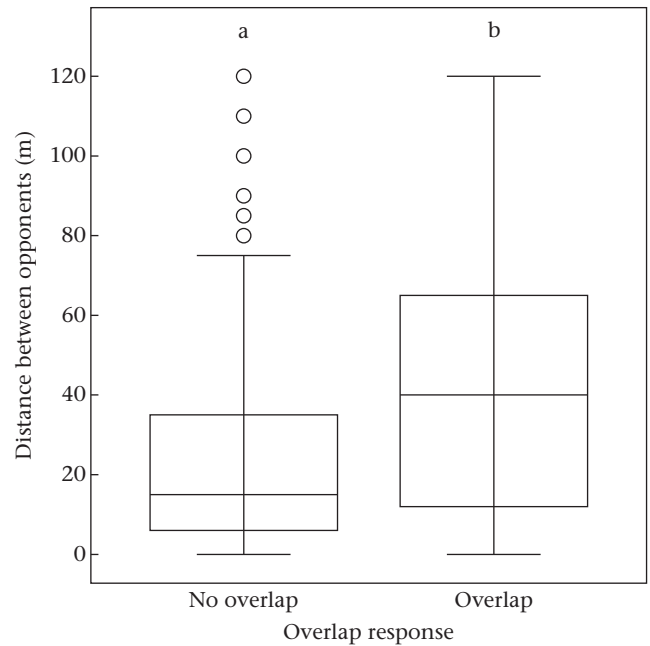oustic interference. We used a two-factor design in which we independently manipulated the signal value and interference potential of playback stimuli (Fig. 3). Signal value was manipulated by broadcasting fee bee song stimuli (high signal value) or corresponding white noise stimuli with matching time-amplitude characteristics (low signal value). Interference potential was manipulated by broadcasting those stimuli at a range of amplitudes: 90 dB SPL (high interference potential); 84, 78, 72, 66 or 60 dB SPL (low interference potential). We predicted that, if overlapping is a submissive signalling strategy, then resident males approaching the loudspeaker should avoid overlapping song stimuli, but not white noise stimuli, which lack signal value. We also predicted that, if overlap avoidance is a mechanism for reducing interference, then chickadees should avoid overlapping song stimuli and white noise stimuli, and should also avoid overlapping louder stimuli more so than quieter stimuli.

Procedure

When a suitable location was found, we mounted a loudspeaker (Anchor-Audio Minivox PB-25; 100–12 000 Hz frequency response) on top of a tripod facing upwards, adjusted it to a height of 1.5 m, and connected it to a digital audio player (Apple iPod) containing the playback stimuli. We then sat on the ground 15 m away and began the playback, which consisted of two phases. During the lure phase, we broadcast pre-recorded chick-a-dee calls on a loop to lure the resident male chickadee to the playback location. If he approached to within 10 m of the speaker, he became our experimental subject, and we began the experimental phase of the trial by broadcasting a preselected stimulus sequence. If a male did not approach to within 10 m of the speaker during the lure phase, the trial was aborted and repeated at a different location.
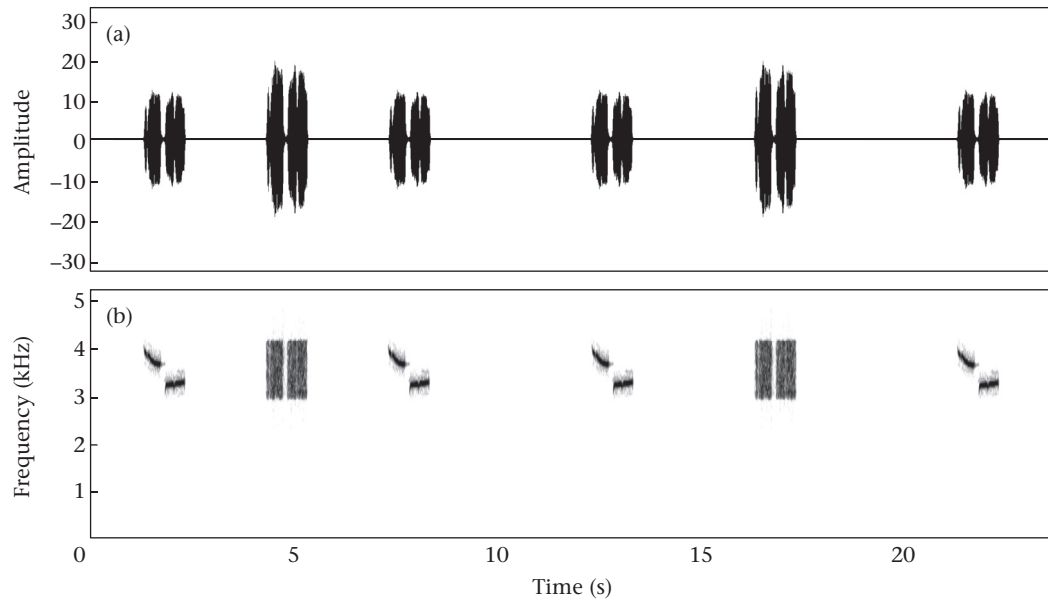
Figure 3. An example of a stimulus sequence used in experiment 2 to test the submissive signalling and acoustic interference hypotheses in black-capped chickadees. The example depicts the sequence as (a) a waveform (units on the *Y* axis are arbitrary) and (b) a spectrogram. It shows only 24 s of the 301 s sequence and includes only four of the 38 song stimuli and two of the 38 white noise stimuli. When constructing stimulus sequences, the order of song and white noise stimuli was randomized, and each stimulus was made to begin 3, 4 or 5 s after the beginning of the last stimulus. The three latencies were selected at random, but with the constraint that each was used 25 times throughout the sequence. Although the song and white noise stimuli had different peak amplitudes, their root-mean-square amplitudes were identical. When broadcast through our playback system, the two stimulus types were 90 dB SPL when measured with a sound level meter held 1 m away. The spectrogram has a frequency resolution of 43 Hz, a temporal resolution of 2.9 ms and an amplitude range of 35 dB (depicted by the grey scale).

Unlike in experiment 1, it was important to lure the subjects to a standard distance from the speaker so that the subject–speaker distance did not have a strong influence on the amplitude of the playback stimuli at the subject's location.

Throughout the playback trial, we recorded the subject's vocal response with our recording apparatus. Whenever the subject changed perches, we estimated his distance from the speaker and dictated it onto the first channel of the recording. We also recorded the lure and the stimulus sequence directly onto the second channel of the recording by connecting the second input channel of the audio recorder to a secondary output from the audio player. This was important because the microphone could not always detect the low-amplitude stimuli being broadcast from the speaker, particularly when the microphone was pointed away from the speaker, at the subject.

In total, we completed 61 trials in which a single male approached and sang. In 43 of these, the subject was accompanied by his mate. We attempted an additional 12 trials, but we excluded these because the responding male did not approach to within 10 m of the playback speaker. All trials were conducted between 15 and 25 April 2012, between 0644 and 1345 hours.

Stimuli

We used a single lure to attract all subjects to their playback locations. It consisted of two chick-a-dee calls recorded in 2009 from a single male located approximately 10 km away from our study site. Using Audition software, we filtered the calls with a 1–8 kHz band-pass filter, normalized each one to a peak amplitude of −6 dB, and placed them into a WAVE-format digital file for playback. We added enough silence to the beginning and end of each call so that it played at a constant rate of 15 calls/min when played on loop. At the beginning of each trial, we adjusted the volume of the playback speaker so that it broadcast the lure at a natural amplitude of 85 dB SPL, as measured with a sound pressure level meter.

Experimental stimuli were constructed according to a two-factor design, where signal value was a within-subject factor (i.e. song or white noise) and stimulus amplitude was a between-subject factor (i.e. 90, 84, 78, 72, 66 or 60 dB SPL). Each subject received a single stimulus sequence containing 38 repetitions of a given song stimulus and 38 repetitions of the matching white noise stimulus. The order of song stimuli and white noise stimuli was randomized within the sequence so that subjects could not predict which type of stimulus they would hear next. We also randomized the amount of silence following each stimulus so that subjects could not predict when the next stimulus would occur. Specifically, we adjusted the silent intervals so that each stimulus began either 3, 4 or 5 s after the beginning of the previous stimulus. We selected these intervals at random, but with the constraint that each one was used 25 times throughout the sequence. Consequently, each stimulus sequence had a duration of 301 s, contained 38 song stimuli and 38 white noise stimuli, and had an average stimulus presentation rate of 15 stimuli/min (Fig. 3). We included both song stimuli and white noise stimuli in each playback sequence because a sequence containing only white noise stimuli would probably have failed to maintain a singing response from subjects.

All stimuli within a given playback sequence had the same root-mean-square (RMS) amplitude. We used RMS amplitude instead of peak amplitude because RMS amplitude provides a better match between the perceived loudness of song stimuli and their corresponding white noise stimuli. When broadcast through our playback system, the amplitudes of the song stimuli and their matching white noise stimuli could not be distinguished by ear or with a sound level meter. In contrast, when matched according to peak amplitude, song stimuli sounded significantly louder than their corresponding white noise stimuli, as assessed by ear and with a sound level meter.

The songs used for constructing playback sequences were derived from 11 colour-banded males in the context of a previous study (Wilson & Mennill, 2010). We filtered the songs with a band-pass filter (2700–4550 Hz), and then measured their minimum and

maximum peak frequencies (defined as the frequency of maximum amplitude). For each song stimulus, we created its corresponding white noise stimulus by generating white noise in Audition software. We matched the length of the noise to the length of the song, and used a band-pass filter to match the minimum and maximum frequencies of the noise to the minimum and maximum peak frequencies of the corresponding song. Using Syrinx-PC software (v.2.6 h; J. Burt, Seattle, WA, U.S.A.), we copied the amplitude envelope of the song stimulus and applied it to the white noise stimulus (4 ms resolution) so that the stimuli shared the same time–amplitude characteristics. After constructing a matching white noise stimulus for each song stimulus, we used Sample Manager software (v.3; Audiofile Engineering, St Paul, MN, U.S.A.) to normalize the 11 song stimuli and 11 white noise stimuli to an RMS amplitude of −15.1 dB. This was the maximum amplification that could be applied to all stimuli without clipping any of them. From this original stimulus set, we created five duplicate sets that spanned a 30 dB range in 6 dB increments. These were used to create the final playback sequences.

When conducting playback trials, we selected stimulus sequences at random and without replacement, but with the constraint that we used all six sequences derived from one male before proceeding to the sequences from another. The amplitude of the stimulus sequences when broadcast through our playback system was established automatically when we calibrated the playback speaker at the beginning of the lure phase. The six amplitude treatments were broadcast at 90, 84, 78, 72, 66 and 60 dB SPL. We confirmed these amplitudes in the field with a sound level meter held 1 m away.

Analysis

For each trial, we used Raven Pro software to view a spectrogram of the trial recording. We measured the beginning and end of each stimulus from the second audio channel, and the beginning and end of each of the subject's songs from the first audio channel. We used these measurements to count the number of times that the subject overlapped a song stimulus or a white noise stimulus during the experimental phase of the trial. Our counts excluded any songs that were produced while the subject was more than 10 m from the speaker because we wanted to minimize the effect of subject–speaker distance on the subject's perceived amplitude of the playback stimuli. This was important because it allowed us to distinguish the effects of perceived stimulus amplitude from the effects of stimulus distance on the subject's overlapping rate. We also note that, in 44 of the 61 trials, subjects produced at least one song that included only the fee note of the fee bee song. We included these in our counts of songs and song overlaps.

We used a duty cycle model to calculate the number of times that each subject was expected to overlap a song or a white noise stimulus by chance. Duty cycle was defined as the duration of one stimulus divided by 8 s, which was the average amount of time between the beginning of one stimulus and the beginning of the next stimulus of the same type (i.e. white noise or song). Within a sequence, the song and white noise stimuli had the same duty cycle, since both stimuli were of identical duration. We calculated the number of overlaps expected by chance by multiplying the stimulus duty cycle by the number of songs produced by the subject during the experimental phase of the playback (excluding any songs produced while the male was more than 10 m from the speaker).

We tested for an effect of signal value by using a nonparametric Friedman test to compare the number of overlaps expected by chance, the number of times that subjects overlapped song stimuli and the number of times that subjects overlapped white noise stimuli. We used a nonparametric analysis because our data violated the parametric assumption of normality and could not be

corrected with a transformation. Since the overall model was significant (see Results, below), we conducted three post hoc pairwise comparisons following the nonparametric procedure described by Dunn (1964). We tested for an effect of interference potential by using a Spearman rank correlation to compare stimulus amplitude (i.e. 90, 84, 78, 72, 66 and 60 dB SPL) to the number of times that subjects overlapped the stimuli. In this analysis, the numbers of times that subjects overlapped song and white noise stimuli were combined, since we were testing for the main effect of stimulus amplitude. We used a correlation analysis because, despite having discrete values, stimulus amplitude was measured on a continuous scale (Sokal & Rohlf, 1995).

Results

Subjects sang a median of 23 songs (interquartile range 15–36; range 1–75) while within 10 m of the loudspeaker during the experimental phase of playback. Our duty cycle model predicted that subjects would overlap the 38 song stimuli and the 38 white noise stimuli a median of 3 times each (interquartile range 2–6 overlaps; range 0–14 overlaps). Yet subjects overlapped a median of only 0 song stimuli (interquartile range 0–1; range 0–4) and 0 white noise stimuli (interquartile range 0–1; range 0–5), which was significantly less than expected by chance (Friedman test: $\chi^2_2 = 90.2$, $N = 61$, $P < 0.001$; nonparametric post hoc test comparing overlaps of song stimuli to overlaps expected by chance: $T = 8.0$, $N = 61$, $P < 0.001$; nonparametric post hoc test comparing overlaps of white noise stimuli to overlaps expected by chance: $T = 7.5$, $N = 61$, $P < 0.001$; Fig. 4). Overall, 59 of 61 subjects (i.e. 97%)
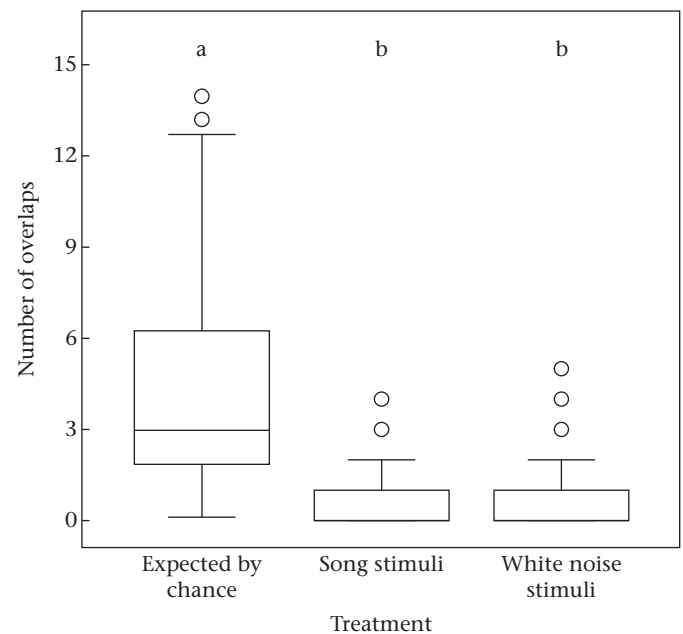
Figure 4. Relationship between signal value and the frequency of overlapping by black-capped chickadees in experiment 2. We broadcast stimulus sequences containing 38 song stimuli and 38 matching white noise stimuli to each of 61 males. The figure shows the number of times that subjects overlapped each stimulus type, as well as the number of overlaps that were expected by chance. We calculated chance expectation by multiplying the number of songs sung by the subject during the 5 min stimulus sequence by the duty cycle of either stimulus type (duty cycle was identical for song and white noise stimuli). Boxes show median and interquartile range, and whiskers show the minimum and maximum values within 1.5 times the height of the box. Outliers are shown with open circles. Treatments were compared with a Friedman test and three post hoc pairwise comparisons. Different letters above bars indicate that the corresponding treatments were significantly different from each other (post hoc tests: $P < 0.001$).

overlapped song stimuli less often than expected by chance. Similarly, 59 of 61 subjects (i.e. 97%) overlapped white noise stimuli less often than expected by chance. Signal value did not influence how often subjects overlapped acoustic stimuli, since subjects overlapped song stimuli and white noise stimuli at approximately the same rate (nonparametric post hoc test: $T = -0.5$, $N = 61$, $P = 0.587$; Fig. 4).

Interference potential, which we assume was determined by the amplitude of our playback stimuli, had a small but significant effect on the subjects' probability of overlapping. Specifically, subjects overlapped quieter stimuli more often than louder stimuli (Spearman rank correlation: $r_S = -0.31$, $N = 61$, $P = 0.015$; Fig. 5).

EXPERIMENT 3

Methods

Procedure

We conducted a playback experiment to verify our assumption that the white noise stimuli used in experiment 2 were of little biological value to male black-capped chickadees. Following the same general methods described in experiment 2, we conducted 30 playback trials. For each one, we set up our playback apparatus, broadcast the lure stimulus on loop until the resident male approached to within 10 m of the playback speaker, and then broadcast one of three experimental treatments: silence (10 trials), song stimuli (10 trials), or white noise stimuli (10 trials). Unlike in experiment 2, this was a between-subjects design in which each male received only one of the three experimental treatments. Throughout the playback trial, we recorded the subject's vocal response and his distance from the playback speaker. We then
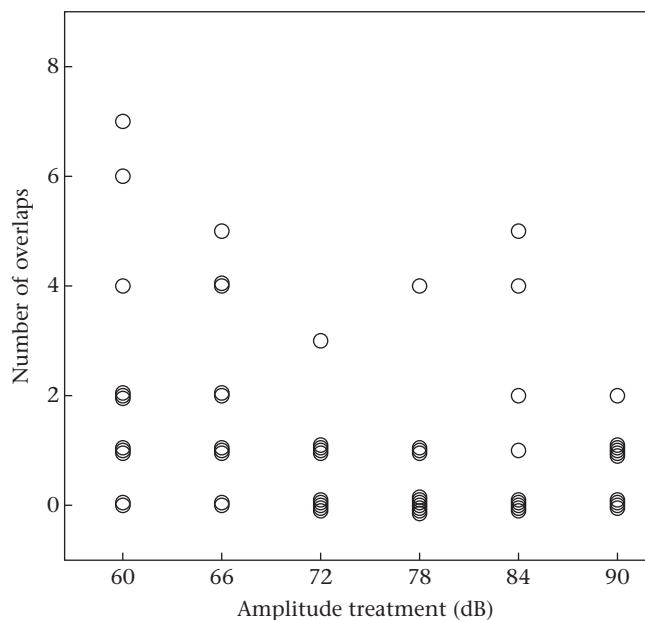
viewed the trial recordings and counted the number of songs that the subject produced while the experimental stimulus was playing, excluding any songs that were produced while the subject was more than 10 m away from the speaker (as in experiment 2). If white noise stimuli lack signal value, then chickadees should sing fewer songs in response to white noise stimuli versus song stimuli, and approximately the same number of songs in response to white noise stimuli versus silence.

Stimuli

For subjects receiving the 'silence' treatment, we broadcast 301 s of silence. We used this treatment as a control to determine the subject's singing response after the lure could no longer be heard. For subjects receiving the 'song stimulus' treatment, we broadcast a stimulus sequence containing 76 repetitions of a fee bee song. For subjects receiving the 'white noise stimulus' treatment, we broadcast a stimulus sequence containing 76 repetitions of a matching white noise stimulus (see the second and fifth sounds in Fig. 3, for example). The song and white noise stimuli were created following the same methods used to create the initial stimulus set in experiment 2, except that the songs were derived from a different set of 10 males. For both treatments, the stimuli were broadcast at a constant rate of 15 stimuli/min, which resulted in sequences that were 301 s in duration. Song and white noise stimuli were broadcast at 90 dB SPL, as measured with a sound level meter held 1 m away.

During the playback trials, we selected treatments at random, but with the constraint that each treatment was used to test 10 males. The 30 trials were conducted between 26 and 29 April 2012.

Analysis

We evaluated the biological significance of white noise stimuli by comparing the number of songs produced by subjects during the

Figure 5. Relationship between the interference potential of acoustic stimuli and the frequency of overlapping by black-capped chickadees in experiment 2. We broadcast stimulus sequences containing 38 song stimuli and 38 matching white noise stimuli to each of 61 males. The figure shows the number of times that subjects overlapped the 76 stimuli in relation to stimulus amplitude. To minimize the effect of attenuation on interference potential, we limited our analysis to songs that the subject sang while within 10 m of the playback speaker. Amplitude was measured in decibels (re 20 μPa) with a sound level meter. We assumed that stimuli had low interference potential when broadcast at low amplitude, and high interference potential when broadcast at high amplitude. Overlapping data points have been offset on the graph by ±0.05 overlaps so that individual data points can be resolved.
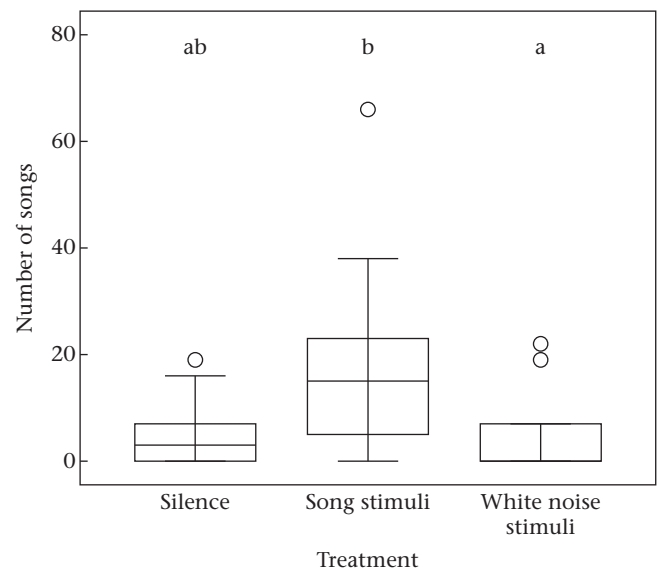

Figure 6. Signal value of the three playback treatments used in experiment 3. We lured male black-capped chickadees to a distance of 10 m from the playback speaker, and then broadcast silence ($N = 10$), song stimuli ($N = 10$), or white noise stimuli ($N = 10$) for a period of 5 min. The Y axis shows the number of songs produced by the subject during the 5 min period, and is assumed to reflect the subject's perceived signal value. Boxes show median and interquartile range, and whiskers show the minimum and maximum values within 1.5 times the height of the box. Outliers are shown with open circles. Treatments were compared with a Kruskal–Wallis test and three post hoc pairwise comparisons. Different letters above bars indicate that the corresponding treatments were significantly different from each other ($P < 0.05$).

three experimental treatments. We predicted that subjects would produce more songs in response to biologically relevant song stimuli, since these indicate the presence of an intruding conspecific. In contrast, we predicted that subjects would produce fewer songs in response to silence and white noise stimuli, since these treatments do not indicate the presence of a conspecific. Treatments were compared using a nonparametric Kruskal–Wallis test. Pairwise comparisons were conducted following the nonparametric procedure described by Dunn (1964).

Results

Our experimental treatments evoked significantly different singing responses from male chickadees (Kruskal–Wallis test: $\chi^2_2 = 6.5$, $N = 30$, $P = 0.039$; Fig. 6). Post hoc tests showed that chickadees produced significantly more songs in response to song stimuli than they did in response to white noise stimuli ($T = 2.5$, $N = 20$, $P = 0.014$). They also tended to produce more songs in response to song stimuli than they did in response to silence, although this difference was not statistically significant ($T = -1.8$, $N = 20$, $P = 0.070$; Fig. 6). The number of songs produced in response to white noise stimuli and silence were statistically indistinguishable ($T = 0.6$, $N = 20$, $P = 0.517$; Fig. 6).

DISCUSSION

We conducted three experiments to elucidate the function of song overlapping in black-capped chickadees. In experiment 1, we simulated territorial intrusions by broadcasting songs inside established territories. In response, resident males sang vigorously, approached the speaker, and, in many cases, attacked the speaker. However, they overlapped the speaker significantly less often than expected by chance, and were even less likely to overlap when they were close to the speaker. Being close to the speaker increases the amplitude of the intruder's songs at the subject's location and thus creates greater interference potential for the subject. Therefore, our finding that chickadees were more likely to avoid overlapping when close to the speaker suggests that overlap avoidance is a mechanism for reducing acoustic interference. However, this pattern of overlapping could also constitute a submissive signal, since overlapping was rarely associated with the close approaches that are a necessary precursor to attack in this species (see Experiment 1, Results; Baker et al., 2012). In experiment 2, we distinguished between the interference avoidance hypothesis and the submissive signalling hypothesis. We lured resident males to a standard distance from our loudspeaker and then broadcast song stimuli and white noise stimuli at a variety of amplitudes. Resident males avoided overlapping both types of stimuli and were significantly more likely to avoid overlapping louder stimuli. These findings again support the interference avoidance hypothesis because subjects avoided overlapping white noise stimuli, which do not have signal value, as often as they avoided overlapping song stimuli, which do have signal value. They are also inconsistent with the submissive signalling hypothesis, which predicts that subjects should avoid overlapping nearby song stimuli and ignore nearby white noise stimuli. In experiment 3, we confirmed that white noise stimuli did not have signal value to chickadees by showing that chickadees sang a similar number of songs in response to white noise stimuli and silence, but significantly more songs in response to song stimuli.

Our first major finding was that territorial male chickadees overlapped the songs of intruders significantly less often than expected by chance (Figs. 1 and 4). This finding is consistent with two previous studies on chickadees. First, Foote et al. (2008) showed that chickadees overlap neighbours during the dawn chorus at rates significantly below chance (17.8% versus 19.6% of songs), although the difference was much less pronounced than in our study (Figs. 1 and 4). We suggest that overlapping was less frequent in our study because the distance between males was smaller. In Foote et al. (2008), the males involved in singing interactions were probably in separate territories; the average distance ± SD between the males was 109 ± 40 m (range 25–215 m), and the average minimum distance ± SD was 59 ± 35 m (range 3–134). In our study, the simulated intruder was most likely inside the subject's territory; the average distance ± SD between the intruder and the subject was 25 ± 26 m (range 0–120 m), and the average minimum distance ± SD was 20 ± 27 m (range 0–100 m). Therefore, males in our study were closer to each other, which would have increased the interference that they imposed on each other. Second, using data from Baker et al. (2012), Masco et al. (2015) showed that chickadees overlap the songs of a simulated intruder significantly less often than expected by chance, although the authors did not report the magnitude of the difference.

Our finding that chickadees overlapped less often than chance is inconsistent with a third study from this population. Fitzsimmons et al. (2008) recorded natural diurnal singing contests and found that 16% of males' songs overlapped the songs of their neighbours. This rate of overlapping was not significantly different from chance. A possible explanation for the discrepancy is that Fitzsimmons et al. (2008) did not account for the amount of time required for songs to travel between males, whereas our study and the study by Foote et al. (2008) did. When considered collectively, the studies on black-capped chickadees (Baker et al., 2012; Fitzsimmons et al., 2008; Foote et al., 2008; Masco et al., 2015) refute the signal jamming, aggressive signalling and signaller quality hypotheses, and, with the exception of Fitzsimmons et al. (2008), also contradict the chance occurrence hypothesis. The studies also contribute to a growing consensus that song overlapping in birds rarely occurs at levels exceeding chance (Masco et al., 2015; Naguib & Mennill, 2010; Searcy & Beecher, 2009, 2011). Indeed, overlapping during male–male song contests has been documented in at least 15 avian species, and none have been shown to overlap at levels exceeding chance (Helfer & Osiejuk, 2015; Masco et al., 2015; Naguib & Mennill, 2010; Searcy & Beecher, 2009, 2011).

Our second major finding was that resident male chickadees decreased their rates of overlapping when they were close to simulated intruders. This finding refutes the chance occurrence hypothesis. It also provides additional support for the interference avoidance hypothesis because the amplitude and interference potential of the intruder's songs would have been greater at the resident male's location when the resident was closer to the intruder. Another study did not find a relationship between distance and the probability of overlapping in chickadees, but, as noted above, the minimum and average distances between males were significantly greater in that study (Foote et al., 2008).

The relationship between distance and overlapping observed in experiment 1 could also constitute a submissive signalling strategy if chickadees signal de-escalation (indicated by a greater distance to their opponent) through increasing rates of overlapping (as suggested for male banded wrens, *Thryothorus pleurostictus*, by Searcy & Beecher, 2009). We suggest that this is unlikely because Baker et al. (2012) showed that a male's overall rate of overlapping does not predict his probability of subsequently attacking or not attacking a simulated intruder. However, Baker et al. (2012) did not compare changes in overlapping rates to changes in the distance between males, so it is possible that increasing rates of overlapping signal de-escalation, as revealed by greater and greater distances between opponents. In other species, the relationship between overlapping and aggression is also unclear. For example, in European robins, *Erithacus rubecula*, and golden whistlers, *Pachycephala*

pectoralis, males were reported to overlap rivals more often in aggressive situations (Brindley, 1991; van Dongen, 2006). However, in their review of aggressive signalling, Searcy and Beecher (2009) argued that both of these results could be artefacts created by uncontrolled changes in singing rate. Furthermore, in banded wrens, males that were more likely to overlap a simulated intruder were more likely to retreat early, which suggests that overlapping in that species is a signal of de-escalation, not aggression (Searcy & Beecher, 2009; Vehrencamp, Hall, Bohman, Depeine, & Dalziell, 2007).

Our third major finding was that chickadees avoided overlapping intruders' songs at the same rate that they avoided overlapping abiotic sounds with equal interference potential, and that they were more likely to avoid overlapping either type of sound when it was broadcast at a higher amplitude. This finding is inconsistent with the submissive signalling hypothesis. It also provides compelling support for the interference avoidance hypothesis because it explains overlap avoidance and variation in overlap avoidance without any reference to conspecifics. For example, it explains the relationship between overlapping and the distance to an intruder because it shows that overlapping rates are controlled by the amplitude of the intruder's songs when they reach the resident, and not by the resident's distance from the intruding male. Our finding complements a recent study by Goodwin and Podos (2013), which showed that chickadees avoid acoustic interference from masking tones by shifting the frequency of their songs away from the tone's frequency. It also supports a growing number of studies showing that animals reduce the masking effects of anthropogenic or abiotic noise by adjusting the temporal, amplitude or frequency characteristics of their acoustic signals (Brumm & Zollinger, 2011; Nemeth et al., 2013; Radford, Kerridge, & Simpson, 2014).

Our study provides new insight into previous chickadee research that examined song overlapping from the perspective of receivers and eavesdroppers. Using interactive playback, Mennill and Ratcliffe (2004b) showed that chickadees that are overlapped respond with increased variation in song timing and song length, as compared to chickadees that are not overlapped. Our findings suggest that this is not a signalling response, but, rather, a mechanism that chickadees use to avoid the interference associated with being overlapped. Other chickadee studies have focused on how eavesdroppers respond to signalling interactions that involve overlapping. For example, two studies found that female chickadees respond differently when they hear interactions in which their partner is both overlapped and frequency-matched by another male (Mennill, Boag, & Ratcliffe, 2003; Mennill et al., 2002). Similarly, Toth, Mennill, and Ratcliffe (2012) found that male chickadees responded differently to two simulated males that differed in their overlapping and frequency-matching behaviour. Our findings suggest that frequency matching may have been more important than overlapping for female and male eavesdroppers in these previous studies. Finally, in a multispeaker playback experiment, Mennill and Ratcliffe (2004a) found that resident male chickadees were more likely to approach a simulated male that was overlapping his opponent than a simulated male that was being overlapped. Our study does not easily explain this finding because the resident male approached the speaker that posed the greatest risk of interference. We suggest that resident males might have approached the overlapping speaker because that speaker was easier to detect or localize, or because it was the last location where a simulated intruder could be heard (Mennill & Ratcliffe, 2004a).

In conclusion, our study provides new insight into the function of song overlapping. In two independent experiments, we show that chickadees overlap simulated territorial intruders significantly less often than expected by chance, and that their rate of overlapping is even lower when they are closer to the intruder. These findings are inconsistent with the chance occurrence, signal jamming, aggressive signalling and signaller quality hypotheses. We then show that chickadees avoid overlapping the songs of intruders at the same rate that they avoid overlapping abiotic noise, and that they are more likely to avoid overlapping either type of sound when it is broadcast at a higher amplitude. These findings are inconsistent with the submissive signalling hypothesis. We conclude that song overlapping is probably not a signal in this species, and that chickadees avoid overlapping songs and other noises as a mechanism for avoiding acoustic interference.

References

Baker, T. M., Wilson, D. R., & Mennill, D. J. (2012). Vocal signals predict attack during aggressive interactions in black-capped chickadees. *Animal Behaviour, 84*, 965–974. http://dx.doi.org/10.1016/j.anbehav.2012.07.022.

Benedict, L., Rose, A., & Warning, N. (2012). Canyon wrens alter their songs in response to territorial challenges. *Animal Behaviour, 84*, 1463–1467. http://dx.doi.org/10.1016/j.anbehav.2012.09.017.

Bischoff, L. L., Tschirren, B., & Richner, H. (2009). Long-term effects of early parasite exposure on song duration and singing strategy in great tits. *Behavioral Ecology, 20*, 265–270. http://dx.doi.org/10.1093/beheco/arp012.

Bradbury, J. W., & Vehrencamp, S. L. (2011). *Principles of animal communication*. Sunderland, MA: Sinauer.

Brindley, E. L. (1991). Response of European robins to playback of song: neighbor recognition and overlapping. *Animal Behaviour, 41*, 503–512. http://dx.doi.org/10.1016/S0003-3472(05)80853-X.

Brumm, H., & Zollinger, S. A. (2011). The evolution of the Lombard effect: 100 years of psychoacoustic research. *Behaviour, 148*, 1173–1198. http://dx.doi.org/10.1163/000579511x605759.

Corcoran, A. J., & Conner, W. E. (2014). Bats jamming bats: food competition through sonar interference. *Science, 346*, 745–747. http://dx.doi.org/10.1126/science.1259512.

van Dongen, W. (2006). Variation in singing behaviour reveals possible functions of song in male golden whistlers. *Behaviour, 143*, 57–82. http://dx.doi.org/10.1163/156853906775133588.

Dunn, O. J. (1964). Multiple comparisons using rank sums. *Technometrics, 6*, 241–252. http://dx.doi.org/10.2307/1266041.

Ficken, R. W., Ficken, M. S., & Hailman, J. P. (1974). Temporal pattern shifts to avoid acoustic interference in singing birds. *Science, 183*, 762–763. http://dx.doi.org/10.1126/science.183.4126.762.

Fitzsimmons, L. P., Foote, J. R., Ratcliffe, L. M., & Mennill, D. J. (2008). Frequency matching, overlapping and movement behaviour in diurnal countersinging interactions of black-capped chickadees. *Animal Behaviour, 75*, 1913–1920. http://dx.doi.org/10.1016/j.anbehav.2007.11.006.

Foote, J. R., Fitzsimmons, L. P., Mennill, D. J., & Ratcliffe, L. M. (2008). Male chickadees match neighbors interactively at dawn: support for the social dynamics hypothesis. *Behavioral Ecology, 19*, 1192–1199. http://dx.doi.org/10.1093/beheco/arn087.

Fuller, R. A., Warren, P. H., & Gaston, K. J. (2007). Daytime noise predicts nocturnal singing in urban robins. *Biology Letters, 3*, 368–370. http://dx.doi.org/10.1098/rsbl.2007.0134.

Gerhardt, H. C., & Huber, F. (2002). *Acoustic communication in insects and anurans: Common problems and diverse solutions*. Chicago, IL: University of Chicago Press.

Gochfeld, M. (1978). Intraspecific social stimulation and temporal displacement of songs of the lesser skylark, *Alauda gulgula. Journal of Comparative Ethology, 48*, 337–344.

Goodwin, S. E., & Podos, J. (2013). Shift of song frequencies in response to masking tones. *Animal Behaviour, 85*, 435–440. http://dx.doi.org/10.1016/j.anbehav.2012.12.003.

Greenfield, M. D. (1994). Synchronous and alternating choruses in insects and anurans: common mechanisms and diverse functions. *American Zoologist, 34*, 605–615.

Helfer, B., & Osiejuk, T. S. (2015). It takes all kinds in acoustic communication: a new perspective on the song overlapping phenomenon. *Ethology, 121*, 315–326. http://dx.doi.org/10.1111/eth.12356.

Kunc, H. P., Amrhein, V., & Naguib, M. (2006). Vocal interactions in nightingales, *Luscinia megarhynchos*: more aggressive males have higher pairing success. *Animal Behaviour, 72*, 25–30. http://dx.doi.org/10.1016/j.anbehav.2005.08.014.

Marten, K., & Marler, P. (1977). Sound transmission and its significance for animal vocalization. *Behavioral Ecology and Sociobiology, 2*, 271–290. http://dx.doi.org/10.1007/bf00299740.

Masco, C., Allesina, S., Mennill, D. J., & Pruett-Jones, S. (2015). The song overlap null model generator (SONG): a new tool for distinguishing between random and non-random song overlap. *Bioacoustics, 25*, 29–40.

Maynard, D. F., Ward, K.-A. A., Doucet, S. M., & Mennill, D. J. (2012). Calling in an acoustically competitive environment: duetting male long-tailed manakins avoid overlapping neighbours but not playback-simulated rivals. *Animal Behaviour, 84*, 563–573. http://dx.doi.org/10.1016/j.anbehav.2012.06.008.

Mennill, D. J., Boag, P. T., & Ratcliffe, L. M. (2003). The reproductive choices of eavesdropping female black-capped chickadees, *Poecile atricapillus*. *Naturwissenschaften, 90*, 577–582. http://dx.doi.org/10.1007/s00114-003-0479-3.

Mennill, D. J., & Otter, K. A. (2007). Status signaling and communication networks in chickadees: complex communication with a simple song. In K. A. Otter (Ed.), *Ecology and behavior of chickadees and titmice* (pp. 215–234). New York, NY: Oxford University Press.

Mennill, D. J., Ramsay, S. M., Boag, P. T., & Ratcliffe, L. M. (2004). Patterns of extrapair mating in relation to male dominance status and female nest placement in black-capped chickadees. *Behavioral Ecology, 15*, 757–765. http://dx.doi.org/10.1093/beheco/arh076.

Mennill, D. J., & Ratcliffe, L. (2004a). Do male black-capped chickadees eavesdrop on song contests? A multi-speaker playback experiment. *Behaviour, 141*, 125–139. http://dx.doi.org/10.1163/156853904772746637.

Mennill, D. J., & Ratcliffe, L. M. (2004b). Overlapping and matching in the song contests of black-capped chickadees. *Animal Behaviour, 67*, 441–450. http://dx.doi.org/10.1016/j.anbehav.2003.04.010.

Mennill, D. J., Ratcliffe, L. M., & Boag, P. T. (2002). Female eavesdropping on male song contests in songbirds. *Science, 296*, 873. http://dx.doi.org/10.1126/science.296.5569.873.

Naguib, M. (2005). Singing interactions in songbirds: implications for social relations and territorial settlement. In P. K. McGregor (Ed.), *Animal communication networks* (pp. 300–319). Cambridge, U.K.: Cambridge University Press.

Naguib, M., & Mennill, D. J. (2010). The signal value of birdsong: empirical evidence suggests song overlapping is a signal. *Animal Behaviour, 80*(3), e11–e15. http://dx.doi.org/10.1016/j.anbehav.2010.06.001.

Nemeth, E., Pieretti, N., Zollinger, S. A., Geberzahn, N., Partecke, J., Miranda, A. C., et al. (2013). Bird song and anthropogenic noise: vocal constraints may explain why birds sing higher-frequency songs in cities. *Proceedings of the Royal Society B: Biological Sciences, 280*, 20122798. http://dx.doi.org/10.1098/rspb.2012.2798.

Otter, K., Chruszcz, B., & Ratcliffe, L. (1997). Honest advertisement and song output during the dawn chorus of black-capped chickadees. *Behavioral Ecology, 8*, 167–173. http://dx.doi.org/10.1093/beheco/8.2.167.

Radford, A. N., Kerridge, E., & Simpson, S. D. (2014). Acoustic communication in a noisy world: can fish compete with anthropogenic noise? *Behavioral Ecology, 25*, 1022–1030. http://dx.doi.org/10.1093/beheco/aru029.

Schubert, K. A., Mennill, D. J., Ramsay, S. M., Otter, K. A., Boag, P. T., & Ratcliffe, L. M. (2007). Variation in social rank acquisition influences lifetime reproductive success in black-capped chickadees. *Biological Journal of the Linnean Society, 90*, 85–95. http://dx.doi.org/10.1111/j.1095-8312.2007.00713.x.

Schulz, T. M., Whitehead, H., Gero, S., & Rendell, L. (2008). Overlapping and matching of codas in vocal interactions between sperm whales: insights into communication function. *Animal Behaviour, 76*, 1977–1988. http://dx.doi.org/10.1016/j.anbehav.2008.07.032.

Searcy, W. A., & Beecher, M. D. (2009). Song as an aggressive signal in songbirds. *Animal Behaviour, 78*, 1281–1292. http://dx.doi.org/10.1016/j.anbehav.2009.08.011.

Searcy, W. A., & Beecher, M. D. (2011). Continued scepticism that song overlapping is a signal. *Animal Behaviour, 81*(1), e1–e4. http://dx.doi.org/10.1016/j.anbehav.2010.10.013.

Sokal, R. R., & Rohlf, F. J. (1995). *Biometry*. New York, NY: W. H. Freeman.

Todt, D., & Naguib, M. (2000). Vocal interactions in birds: the use of song as a model in communication. *Advances in the Study of Behavior, 29*, 247–296. http://dx.doi.org/10.1016/S0065-3454(08)60107-2.

Toth, C. A., Mennill, D. J., & Ratcliffe, L. M. (2012). Evidence for multicontest eavesdropping in chickadees. *Behavioral Ecology, 23*, 836–842. http://dx.doi.org/10.1093/beheco/ars038.

Vehrencamp, S. L., Hall, M. L., Bohman, E. R., Depeine, C. D., & Dalziell, A. H. (2007). Song matching, overlapping, and switching in the banded wren: the sender's perspective. *Behavioral Ecology, 18*, 849–859. http://dx.doi.org/10.1093/beheco/arm054.

Wasserman, F. E. (1977). Intraspecific acoustical interference in the white-throated sparrow (*Zonotrichia albicollis*). *Animal Behaviour, 25*, 949–952. http://dx.doi.org/10.1016/0003-3472(77)90045-8.

Wilson, D. R., & Mennill, D. J. (2010). Black-capped chickadees, *Poecile atricapillus*, can use individually distinctive songs to discriminate among conspecifics. *Animal Behaviour, 79*, 1267–1275. http://dx.doi.org/10.1016/j.anbehav.2010.02.028.

Wölfel, M., & McDonough, J. (2009). *Distant speech recognition*. West Sussex, U.K.: Wiley.

Yang, X. J., Ma, X. R., & Slabbekoorn, H. (2014). Timing vocal behaviour: experimental evidence for song overlap avoidance in Eurasian wrens. *Behavioural Processes, 103*, 84–90. http://dx.doi.org/10.1016/j.beproc.2013.11.011.