

Original Article

Duty cycle, not signal structure, explains conspecific and heterospecific responses to the calls of Black-capped Chickadees (*Poecile atricapillus*)

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Animals can encode information into signals using at least 2 basic mechanisms. First, signalers can repeat their signals, encoding information into sequence-level parameters, such as signaling rate. Second, signalers can encode information into the fine structural variation of individual signals. This mechanism requires sophisticated encoding and decoding but potentially affords more rapid or efficient information transfer. The chick-a-dee call of Parid birds is a structurally complex signal that conveys food- and predator-related information to both conspecific and heterospecific receivers. However, the basic mechanism by which it communicates information is unclear. Previous research suggests that variation in the number of terminal notes is important, but this structural trait has not been manipulated independently from other structural traits or from sequence-level parameters, such as total duty cycle. We independently manipulated the fine structure and duty cycle of the calls of Black-capped Chickadees (*Poecile atricapillus*) and then broadcast them to potential receivers. Both conspecific and heterospecific receivers ignored manipulations to the fine structure of individual calls when the duty cycle of the signaling sequences was held constant. In marked contrast, receivers exhibited significantly stronger responses when the duty cycle was experimentally increased, and the fine structure of individual calls was held constant. Specifically, signaling sequences with a high duty cycle attracted more conspecific and heterospecific receivers and caused those receivers to approach the speaker more quickly, to approach the speaker more closely, and to remain within 10 m of the speaker for longer. These findings show that receivers respond to a simple sequence-level trait in a structurally complex avian signal. *Key words*: alarm signal, animal communication, chickadee, information encoding, signal. [*Behav Ecol* 22:784–790 (2011)]

INTRODUCTION

The information content of animal signals varies widely among species. In the simplest systems, signals provide binary information, such as the sex of a signaler or the presence or absence of an environmental feature (Bradbury and Vehrencamp 1998). In more sophisticated systems, signals also encode subtle details about the signaler or its environment. For example, signals can encode the identity or motivational state of the signaler, the proximity or type of a nearby predator, or the location and quality of a newly discovered food source (Macedonia and Evans 1993; Bradbury and Vehrencamp 1998).

Signalers can encode detailed information using at least 2 mechanisms. First, by repeating one type of signal, they can encode information within sequence-level parameters, such as signaling rate, total number of signals produced, or consistency in the timing of signal production (Marler et al. 1986). Second, signalers can encode information within the fine structure of individual signals. Here, salient structural parameters might include signal duration or the presence or absence of certain signal components (Sloan et al. 2005).

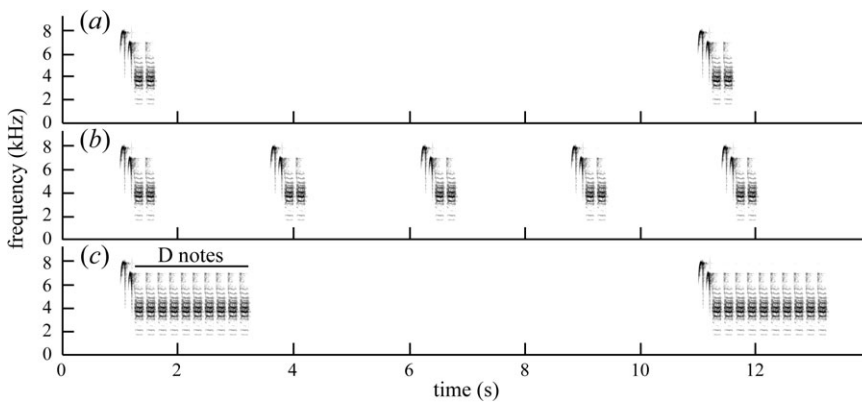
Information encoded in signals can only have communicative value if it is decoded by receivers (Bradbury and Vehrencamp 1998). This is normally demonstrated by showing that receivers respond appropriately to different signal variants. In many systems, encoded information may even be decoded by heterospecific receivers. Yellow-casqued Hornbills (*Ceratogymma elata*), for example, respond appropriately to the “eagle” and “leopard” alarm calls of Diana Monkeys (*Cercopithecus diana*) (Rainey et al. 2004). In some systems, however, encoded information is not decoded by receivers and hence lacks a communicative function (Schibler and Manser 2007).

The chick-a-dee call of birds in the family Paridae provides an excellent model for exploring how information is encoded in acoustic signals. The call consists of up to 4 basic note types (A, B, C, and D notes) that follow rudimentary syntactical rules (Hailman et al. 1985; Hailman 1989). It is produced repeatedly in a variety of important contexts, including territory defense, foraging, and predator confrontation (Smith 1991; Lucas and Freeberg 2007). Furthermore, the chick-a-dee call encodes species information, signaler identity, and group affiliation (Freeberg et al. 2003; Charrier et al. 2004; Charrier and Sturdy 2005; Lucas and Freeberg 2007) as well as information about the eliciting stimulus, such as predator threat or the discovery of food (Baker and Becker 2002; Templeton et al. 2005; Mahurin and Freeberg 2009; Bartmess-LeVasseur et al. 2010).

Recent work has identified variation in the number of D notes in chick-a-dee calls as a potentially informative structural

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**Figure 1**

Sound spectrograms of playback stimuli used to test receiver responses to chick-a-dee calls. Three experimental treatments are shown, including (a) 2-D calls broadcast with a low duty cycle, (b) 2-D calls broadcast with a high duty cycle, and (c) 10-D calls broadcast with a high duty cycle. The silent control treatment is not shown. Note that the stimuli depicted here show only one 14-s portion of each 1-min playback sequence.

feature (the D note is the harmonically rich terminal note type; see Figure 1). Black-capped Chickadees (*Poecile atricapillus*), Carolina Chickadees (*P. carolinensis*), and Tufted Titmice (*Baeolophus bicolor*), for example, all produce more D notes per call as predation risk increases (Templeton et al. 2005; Bartmess-LeVasseur et al. 2010; Courter and Ritchison 2010). Similarly, Carolina Chickadees produce more D notes per call when they first discover a food source, thereby providing a mechanism for recruiting flockmates to food (Mahurin and Freeberg 2009).

Complementing the work on signal production, playback experiments examining receiver responses to chick-a-dee calls have shown that variation in these signals has important communicative value for both conspecific and heterospecific receivers. In a predator context, for example, Black-capped Chickadees, Tufted Titmice, and Red-breasted Nuthatches (*Sitta canadensis*) all exhibit stronger antipredator behavior when they hear chick-a-dee calls produced in response to small raptors that specialize on small songbirds versus large raptors that rarely prey on songbirds (Templeton et al. 2005; Templeton and Greene 2007; Courter and Ritchison 2010). Similarly, in a winter foraging context, Carolina Chickadees exhibit stronger foraging behavior when they hear calls produced in response to a newly discovered food source versus a food source where flockmates are already foraging (Mahurin and Freeberg 2009).

In each of the playback studies described above, subjects responded more strongly to chick-a-dee calls with a greater number of D notes, which has been interpreted as evidence for communication via subtle structural variations of a single call type (Templeton et al. 2005; Templeton and Greene 2007; Mahurin and Freeberg 2009). Although the results are certainly consistent with this idea, they do not exclude a more parsimonious explanation. In each of these previous studies, calls with few D notes were broadcast at the same rate (Templeton et al. 2005; Templeton and Greene 2007; Mahurin and Freeberg 2009) or at a lower rate (Courter and Ritchison 2010) than calls with many D notes, so the total proportion of the playback sequence in which a signal could be heard (i.e., duty cycle) differed dramatically between experimental treatments. Consequently, the duty cycle of the playback sequence, as opposed to the fine structure of individual calls, could readily explain differential responses to treatments by receivers. Therefore, the basic mechanism by which chick-a-dee calls communicate information remains unclear.

The goal of the present study was to determine whether receivers respond to variation in the fine structure of individual chick-a-dee calls, or, alternatively, whether they respond to variation in the duty cycle of overall signaling sequences. To test this, we manipulated the fine structure of Black-capped Chick-

adee calls by experimentally adding or removing D notes. In addition, we independently manipulated the duty cycle of playback sequences by adjusting the playback rate. Stimuli were then broadcast in a winter foraging context, where we observed the effects of fine structure and duty cycle on the responses of conspecific and heterospecific animals. This experimental design is the first to permit differentiation between information encoded in the structural and sequence-level parameters of these complex avian signals.

MATERIALS AND METHODS

Study site and species

Research was conducted between 8 and 28 January 2010 in the vicinity of the Queen's University Biological Station north of Kingston, Ontario, Canada (lat 44°34'35"N–44°36'53"N; long 76°32'17"W–76°15'51"W). The site was predominately mature mixed forest and was snow-covered throughout the study.

Subjects were any Black-capped Chickadees or any heterospecific birds or mammals that responded to our playback stimuli (see definition of "responded" below). Due to the size of the study area, the diversity of species, and the abundance of individuals, it was not possible to individually mark subjects. Instead, we reduced the risk of testing the same individuals in multiple trials by separating playback locations by a minimum distance of 500 m, as in Bartmess-LeVasseur et al. (2010). The average winter home-range diameters of the 6 species that responded in multiple trials, as well as the average distances between trials in which the same species responded, were: Black-capped Chickadee (home range: 431 m, Odum 1942; intertrial distance in our experiment: 9796 m), Brown Creeper (*Certhia americana*; home range: unavailable, but 151 m for closely related Eurasian Treecreeper, *C. familiaris*, Hogstad 1990; intertrial distance in our experiment: 2575 m), Downy Woodpecker (*Picoides pubescens*; home range: 462 m, Kellam et al. 2006; intertrial distance in our experiment: 4337 m), Hairy Woodpecker (*P. villosus*; home range: 1365 m, Covert-Bratland et al. 2006; intertrial distance in our experiment: 1826 m), North American Red Squirrel (*Tamiasciurus hudsonicus*; home range: 98 m, Steury and Murray 2003; intertrial distance in our experiment: 19 839 m), and White-breasted Nuthatch (*S. carolinensis*; home range: 359 m, Butts 1931; intertrial distance in our experiment: 9886 m). We calculated home-range diameters assuming that published home-range areas were circular in shape.

Playback stimuli

Chick-a-dee calls used to create playback stimuli were obtained in the context of a previous study (131 recordings from 17

different individuals; recorded in 2009 by D.R.W. in Ontario, Canada using a Marantz PMD660 digital recorder, Audio-Technica AT8015 shotgun microphone, 44.1 kHz sampling rate, 16-bit accuracy, and WAVE format; Wilson and Mennill 2010), or from the Macaulay Library at the Cornell Lab of Ornithology (30 recordings from 30 different individuals; recorded between 1955 and 1998, from various locations in Canada and the United States). From each individual, we selected one call that had a high signal-to-noise ratio, typical call structure, and no interference from heterospecific sounds or background noise, as determined aurally and by visual inspection of spectrograms. From this initial set of 47 calls, we used the 20 calls with the highest signal-to-noise ratio to create playback stimuli (7 from Wilson and Mennill 2010; 13 from Macaulay Library).

Prior to creating playback sequences, we used Raven Interactive Sound Analysis Software (version 1.4 Pro, Cornell Lab of Ornithology Bioacoustics Research Program, Ithaca, NY) to minimize structural variation in the 20 chick-a-dee calls so that they would differ predominately as a function of our experimental treatments. First, we removed low-frequency noise artifacts with a 1-kHz high-pass filter. To ensure that all calls shared the same basic note composition, we removed all but the first D note and all but the last 2 introductory notes (i.e., A and B notes; see Lucas and Freeberg 2007). To minimize internote amplitude variation, we normalized the introductory notes of each call to a peak amplitude of -1 dB (note that we preserved the natural amplitude difference between the 2 introductory notes) and the D note to -7.6 dB. The 6.6 dB difference between introductory and D notes was based on the mean natural amplitude difference between these note types among the initial set of 47 calls. Finally, we made 2 versions of each call by repeating the remaining D note at a natural rate, which was based on the rate observed in the original call. In the first version, we repeated the D note once, resulting in a 2-D call. In the second version, we repeated the D note 9 times, resulting in a 10-D call. Both of these call structures (i.e., 2 introductory notes and either 2 or 10 D notes) are within the normal range of structural variation observed among the initial set of 47 calls and are typical of chick-a-dee calls generally (Hailman et al. 1985; Hailman 1989).

We used 4 experimental playback treatments: 1) a silent control treatment, 2) a treatment consisting of 2-D chick-a-dee calls broadcast at a low rate ("2-D low duty cycle"; Figure 1a), 3) a treatment consisting of 2-D chick-a-dee calls broadcast at a high rate ("2-D high duty cycle"; Figure 1b), and 4) a treatment consisting of 10-D chick-a-dee calls broadcast at a low rate ("10-D high duty cycle"; Figure 1c). From each of the original 20 stimuli, we used Audition software (version 2.0; Adobe, San Jose, CA) to create 1-min playback sequences for each of these treatments. We included the silent control treatment to assess whether animals would respond simply to the presence of the observer and playback apparatus and to measure the spontaneous arrival of subjects in the playback vicinity independent of chick-a-dee playback. The 2-D low duty cycle and 10-D high duty cycle included the 2-D and 10-D versions of the call, respectively, repeated at a rate of 1 call every 10 s. This rate of repetition is within the natural range observed among the original set of recordings that we used to construct our stimuli (mean \pm standard error = 4.3 ± 0.26 s/call; range = 0.40–22.89 s/call). Although the 2-D low duty cycle and 10-D high duty cycle treatments had structural differences in terms of the number of D notes, they also differed dramatically in terms of duty cycle; the 10-D stimulus had significantly more vocalization per unit time (Figure 1). This potential confound necessitated the 2-D high duty cycle treatment that controlled for variation in duty cycle. This treatment included the 2-D version of the call repeated at

a higher rate of 1 call every 2.6 s, which is also within the natural range of chick-a-dee call production. This treatment featured calls with the identical structure to the 2-D low duty cycle treatment, but with the same duty cycle as the 10-D high duty cycle treatment.

The 80 playback stimuli representing 4 treatments and 20 chickadees were saved as digital sound files (WAV format, 16-bit accuracy, 44.1 kHz sampling rate) to the internal flash memory of a wireless speaker (model Scorpion X1A; Foxpro, Inc, Lewistown, PA) that was used to broadcast stimuli during playbacks.

Playback method

We conducted 4 trials per day for 20 days, resulting in a total of 80 trials. Each trial received a different stimulus to avoid pseudoreplication, and each treatment was used only once in a random order on each day to ensure a balanced design. Trials lasted for 65 min, which was based on the experimental design used by Mahurin and Freeberg (2009). Trials were conducted between 08:40 and 15:45 h. The same person conducted all trials to eliminate potential interobserver variability that might otherwise influence our results.

Prior to commencing a trial, we used a GPS to ensure that the playback site was at least 500 m from all previous playback locations. We chose specific locations for playback where the density of vegetation allowed us to view subjects for at least 10 m in all directions while simultaneously providing subjects with perches at a variety of distances from the loudspeaker. After selecting a site, we hung the wireless playback speaker on a tree limb 1 m above the surface of the snow. Immediately below the speaker, we placed a wooden board ($30 \times 30 \times 0.5$ cm) flat on the surface of the snow and spread one handful of sunflower seeds over its surface to simulate a feeding context. The observer sat 15 m away from the wireless speaker and commenced the trial by using a radio transmitter (model TX-200; Foxpro, Inc) to signal the wireless speaker to broadcast the appropriate stimulus.

Trials began by broadcasting the selected 1-min stimulus at 80 dB sound pressure level, as measured with a sound level meter (a RadioShack model 33-4050 sound meter placed 1 m from the playback speaker; "C" weighting, "fast" response). The 1-min stimulus, followed by 4 min of silence, was repeated every 5 min for 65 min, resulting in a total of 13 5-min playback blocks. This design was based on Mahurin and Freeberg (2009). Whenever a subject appeared for the first time, or whenever a subject changed position, we noted on a dictaphone the time (1-s resolution), the species of each subject, and the distance of each subject to the playback speaker (estimated 0.5-m resolution). Although our measures of distance were estimated, any inaccuracies resulting from this method would not differ systematically as a function of experimental treatment and would therefore produce a conservative estimate of treatment effects.

Analysis

We analyzed chickadee behavior using 2 response variables. The first was the maximum number of individuals detected simultaneously within 10 m of the playback speaker during the 65-min trial. This variable is not artificially inflated by repeated visits from unbanded individuals and has been shown in a previous study to be a reliable estimate of the actual number of respondents (see Bartmess-LeVasseur et al. 2010). The second response variable reflected the intensity of response and was derived by reducing a set of 3 correlated measures into a single factor using principal components analysis (Table 1). These measures included: 1) the latency

Table 1
Details of the principal components analysis used to summarize the intensity of animals' responses during 80 playback trials

Variable	Factor 1 for conspecific responses	Factor 1 for heterospecific responses
Latency to respond	0.93	0.93
Minimum distance	0.94	0.89
Duration of response	-0.88	-0.80
Eigenvalue	2.53	2.28
Variance explained (%)	84.3	76.1

Unrotated factor loading scores are provided for the single factor extracted separately for conspecific animals (Black-capped Chickadees) and heterospecific animals. Analyses were based on the correlation matrix, and factors were extracted when eigenvalues exceeded 1. Factor scores were generated using the regression method.

of the first animal to approach to within 10 m of the playback speaker, 2) the minimum distance to the speaker of the closest animal, and 3) the duration of time in which at least 1 animal was within 10 m of the playback speaker. When no animals came within 10 m of the playback speaker throughout the trial, we assigned values of 10 m for minimum distance, 65 min for latency, and 0 min for duration. The maximum number of individuals detected and the intensity of response were calculated separately for Black-capped Chickadees and heterospecific subjects, resulting in a total of 4 response variables.

Parametric analyses were not used because data violated the parametric assumption of normality and could not be corrected using data transformations. Instead, we tested for the effects of experimental treatment using nonparametric Kruskal–Wallis tests. Where an overall model was significant ($\alpha = 0.05$), we conducted 6 post hoc pairwise comparisons using the nonparametric procedure for multiple comparisons described by Dunn (1964). We maintained the overall type I error rate using the sequential Bonferroni method (Rice 1989). Statistical analyses were conducted using PASW Statistics for Mac (version 18), and 2-tailed tests were used throughout.

RESULTS

Responses of 114 Black-capped Chickadees revealed significant effects of experimental treatment on both the maximum number of individuals detected (Kruskal–Wallis test: adjusted $H = 24.71$, degrees of freedom [df] = 3, $N = 80$, $P < 0.001$; Figure 2a) and the intensity of response (adjusted $H = 23.43$, df = 3, $N = 80$, $P < 0.001$; Figure 2b). For both variables, responses were greater in the 2 treatments with high duty cycles than in the silent control treatment (maximum number of individuals detected: silent control vs. 2-D high duty cycle, $T = 23.25$, $N = 40$, $P_{\text{adj}} = 0.005$; silent control vs. 10-D high duty cycle, $T = 26.68$, $N = 40$, $P_{\text{adj}} = 0.006$; intensity of response: silent control vs. 2-D high duty cycle, $T = 23.15$, $N = 40$, $P_{\text{adj}} = 0.005$; silent control vs. 10-D high duty cycle, $T = 26.58$, $N = 40$, $P_{\text{adj}} = 0.006$) or low duty cycle treatments (maximum number of individuals detected: 2-D low duty cycle vs. 2-D high duty cycle, $T = 18.18$, $N = 40$, $P_{\text{adj}} = 0.015$; 2-D low duty cycle vs. 10-D high duty cycle, $T = 21.60$, $N = 40$, $P_{\text{adj}} = 0.004$; intensity of response: 2-D low duty cycle vs. 2-D high duty cycle, $T = 16.78$, $N = 40$, $P_{\text{adj}} = 0.030$; 2-D low duty cycle vs. 10-D high duty cycle, $T = 20.20$, $N = 40$, $P_{\text{adj}} = 0.008$). Furthermore, responses did not differ as a function of the number of D notes. Although responses were greater in the 10-D high duty

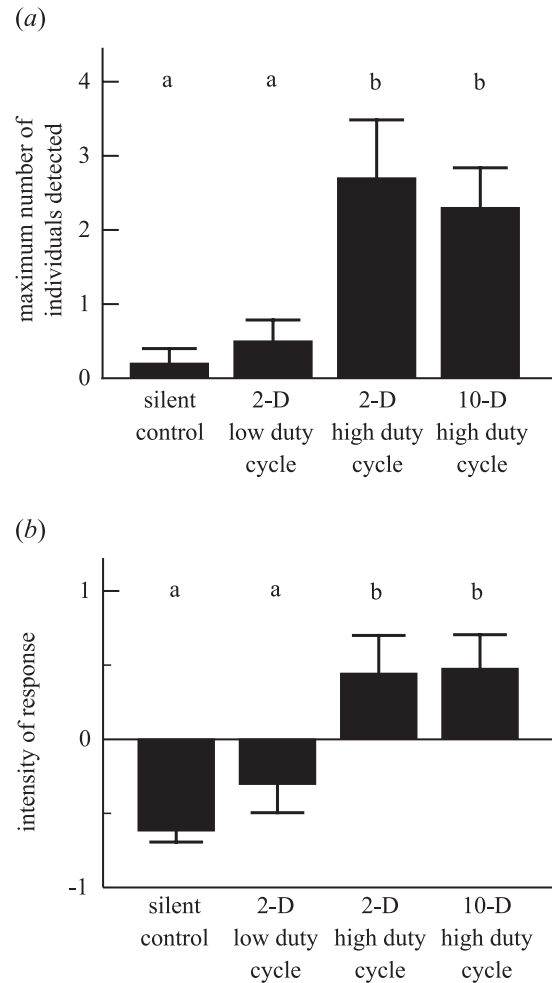


Figure 2

Responses of Black-capped Chickadees to playback of chick-a-dee calls during 80 trials. Shown for each experimental treatment are (a) the maximum number of individuals detected within 10 m of the playback speaker (mean \pm standard error [SE]) and (b) the intensity of response (mean \pm SE), calculated as a principal component incorporating latency to respond, minimum approach distance, and response duration. Treatments included a silent control ($N = 20$ trials) and 3 experimental treatments ($N = 20$ trials per treatment) that differed in terms of the number of D notes per call and the duty cycle of the overall playback sequence. We tested for the effects of experimental treatment using a Kruskal–Wallis test. Where an overall model was significant ($\alpha = 0.05$), we conducted 6 post hoc pairwise comparisons, maintaining the overall type I error rate using the sequential Bonferroni method. Different letters above bars indicate that the corresponding treatments were significantly different from each other (post hoc test: $P_{\text{adj}} \leq 0.05$).

cycle treatment than in the 2-D low duty cycle treatment, the apparent effect of the number of D notes disappeared when duty cycle was held constant (maximum number of individuals detected: 2-D high duty cycle vs. 10-D high duty cycle, $T = 3.43$, $N = 40$, $P_{\text{adj}} = 0.598$; intensity of response: 2-D high duty cycle vs. 10-D high duty cycle, $T = 3.43$, $N = 40$, $P_{\text{adj}} = 0.600$). Finally, responses to the 2-D low duty cycle treatment were statistically indistinguishable from those observed in response to the “silent control” (maximum number of individuals detected: silent control vs. 2-D low duty cycle, $T = 5.08$, $N = 40$, $P_{\text{adj}} = 0.870$; intensity of response: silent control vs. 2-D low duty cycle, $T = 6.38$, $N = 40$, $P_{\text{adj}} = 0.654$), although the means were higher for the 2-D low duty cycle treatment (Figure 2).

Our analysis of heterospecific behavior revealed precisely the same pattern that we observed among chickadees (Figure 3). We observed a significant effect of experimental treatment on both the maximum number of individuals detected (Kruskal–Wallis test: adjusted $H = 20.55$, $df = 3$, $N = 80$, $P < 0.001$; Figure 3a) and the intensity of response (adjusted $H = 20.231$, $df = 3$, $N = 80$, $P < 0.001$; Figure 3b). For both variables, responses were greater in the 2 treatments with high duty cycles than in the silent control (maximum number of individuals detected: silent control vs. 2-D high duty cycle, $T = 20.60$, $N = 40$, $P_{\text{adj}} = 0.006$; silent control vs. 10-D high duty cycle, $T = 17.00$, $N = 40$, $P_{\text{adj}} = 0.008$; intensity of response: silent control vs. 2-D high duty cycle, $T = 19.90$, $N = 40$, $P_{\text{adj}} = 0.006$; silent

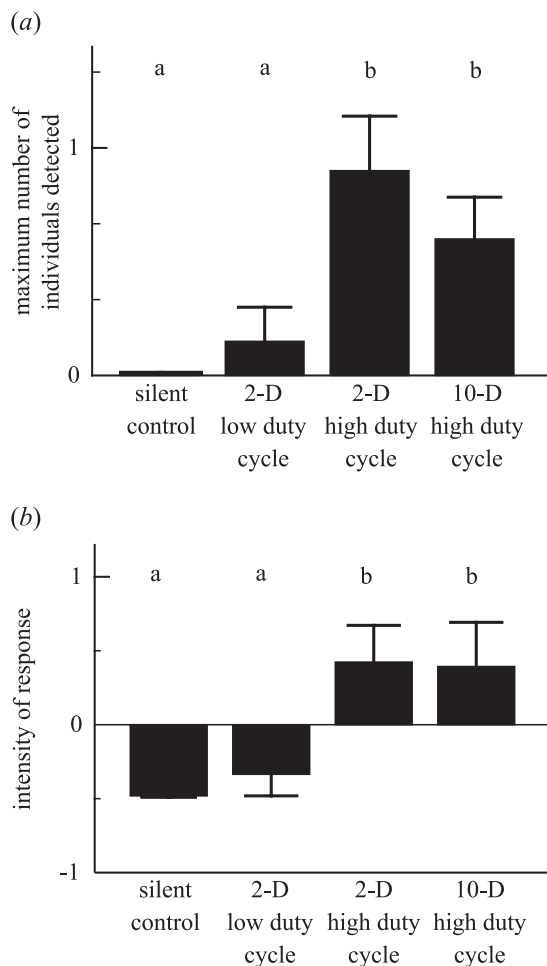


Figure 3 Heterospecific responses to playback of chick-a-dee calls during 80 trials. Shown for each experimental treatment are (a) the maximum number of individuals detected within 10 m of the playback speaker (mean \pm standard error [SE]) and (b) the intensity of response (mean \pm SE), calculated as a principal component incorporating latency to respond, minimum approach distance, and response duration. Treatments included a silent control ($N = 20$ trials) and 3 experimental treatments ($N = 20$ trials per treatment) that differed in terms of the number of D notes per call and the duty cycle of the overall playback sequence. We tested for the effects of experimental treatment using a Kruskal–Wallis test. Where an overall model was significant ($\alpha = 0.05$), we conducted 6 post hoc pairwise comparisons, maintaining the overall type I error rate using the sequential Bonferroni method. Different letters above bars indicate that the corresponding treatments were significantly different from each other (post hoc test: $P_{\text{adj}} \leq 0.05$).

control vs. 10-D high duty cycle, $T = 17.73$, $N = 40$, $P_{\text{adj}} = 0.010$) or low duty cycle treatments (maximum number of individuals detected: 2-D low duty cycle vs. 2-D high duty cycle, $T = 18.20$, $N = 40$, $P_{\text{adj}} = 0.005$; 2-D low duty cycle vs. 10-D high duty cycle, $T = 14.60$, $N = 40$, $P_{\text{adj}} = 0.027$; intensity of response: 2-D low duty cycle vs. 2-D high duty cycle, $T = 17.53$, $N = 40$, $P_{\text{adj}} = 0.008$; 2-D low duty cycle vs. 10-D high duty cycle, $T = 15.35$, $N = 40$, $P_{\text{adj}} = 0.018$). In contrast, there were no differences in the responses of heterospecific animals between 2-D and 10-D treatments when duty cycle was held constant (maximum number of individuals detected: 2-D high duty cycle vs. 10-D high duty cycle, $T = 3.50$, $N = 40$, $P_{\text{adj}} = 1.000$; intensity of response: 2-D high duty cycle vs. 10-D high duty cycle, $T = 2.18$, $N = 40$, $P_{\text{adj}} = 0.697$). Once again, responses to the 2-D low duty cycle treatment were statistically indistinguishable from those observed in response to the silent control (maximum number of individuals detected: silent control vs. 2-D low duty cycle, $T = 2.40$, $N = 40$, $P_{\text{adj}} = 0.667$; intensity of response: silent control vs. 2-D low duty cycle, $T = 2.38$, $N = 40$, $P_{\text{adj}} = 1.000$; Figure 3).

Heterospecific animals observed responding to playback in this study consisted of the following species: Blue Jay (*Cyanocitta cristata*; 1 trial), Brown Creeper (3 trials), Northern Cardinal (*Cardinalis cardinalis*; 1 trial), Downy Woodpecker (3 trials), Eastern Gray Squirrel (*Sciurus carolinensis*; 1 trial), Hairy Woodpecker (2 trials), North American Red Squirrel (2 trials), Red-breasted Nuthatch (1 trial), Ruffed Grouse (*Bonasa umbellus*; 1 trial), Sharp-shinned Hawk (*Accipiter striatus*; 1 trial), and White-breasted Nuthatch (11 trials). Heterospecific behavior was considered collectively across all responding species, so our analyses apply to heterospecifics in the broad sense.

DISCUSSION

Conspecific and heterospecific receivers responded to variation in the duty cycle of call sequences and not to variation in the fine structure of individual calls. Previous playback studies have suggested that urgency-based information is encoded in the fine structure of this referential signal (Templeton et al. 2005; Templeton and Greene 2007). These studies, however, have consistently manipulated the number of D notes in the chick-a-dee call and the duty cycle of the playback sequence together, so it has not been possible to evaluate the independent effects of either trait (Templeton et al. 2005; Templeton and Greene 2007; Mahurin and Freeberg 2009; Courter and Ritchison 2010). When we manipulated these traits independently, we found that receivers responded only to increased duty cycle. Furthermore, receiver responses did not differ when duty cycle was held constant and the number of D notes was varied, suggesting that there was no additive effect of the 2 features on receiver responses. These results provide strong evidence that receivers respond predominately to variation in the duty cycle of the overall signaling sequence and not to structural differences in the call itself.

Our study provides new insight into the information content of chick-a-dee calls. In some species, signals communicate functionally referential information; that is, they encode specific information about an environmental feature, which allows receivers to respond appropriately in the absence of contextual cues (Macedonia and Evans 1993). Chick-a-dee calls, however, are used in a variety of contexts. For example, Templeton et al. (2005) showed that birds emit chick-a-dee calls with more D notes per call in response to more dangerous raptors, whereas Mahurin and Freeberg (2009) showed that birds emit chick-a-dee calls with more D notes per call in response to newly discovered food. It seems unlikely that continuous variation in the number of D notes per call or in the duty cycle would

communicate specific information about such disparate external stimuli (Macedonia and Evans 1993). A more likely explanation is that variation in duty cycle communicates the signaler's motivational state, which would be relatively high when it encounters a dangerous predator, a new food source, or even an aggressive territorial intruder (Morton 1977; Macedonia and Evans 1993; Owings and Morton 1998). From the receiver's perspective, the signaler's motivational state would provide an excellent indication of how urgently the receiver should respond, as the appropriate response in each context is to approach the eliciting stimulus (Smith 1991; Templeton et al. 2005; Mahurin and Freeberg 2009). Once the signaler has approached to within visual range of the stimulus, it could assess the situation using contextual cues and either mob a potential predator, forage on a newly discovered food source, or evict a territorial intruder (as suggested by Mahurin and Freeberg 2009). Low duty cycle, and hence a low motivational state on the part of the signaler, may therefore explain why receiver responses did not differ between the silent control and the 2-D low duty cycle treatments; the urgency associated with the 2-D low duty cycle treatment may simply have been too low to warrant an approach response.

Why chickadees vary the number of D notes in their chick-a-dee calls remains unclear. Aside from affecting duty cycle, which can be achieved readily by adjusting repetition rate (Templeton et al. 2005; Bartmess-LeVasseur et al. 2010; Courter and Ritchison 2010), one possibility is that variation reflects motivation-structural rules. These rules predict that animals will produce harsh, low-frequency sounds in situations where they are highly motivated, such as when they discover a new food source or are attacked by a high-risk predator (Morton 1977; Owings and Morton 1998). Relative to other components of the chick-a-dee call and to other components of the chickadee vocal repertoire, the D note has structural characteristics that most closely match this prediction. Because these rules apply exclusively to the signaler, the variation they produce does not necessarily evoke an adaptive response by receivers and therefore does not necessarily have a communicative function.

Variation in the number of D notes could also be an uninformative correlate of a more informative structural trait, perhaps created by physical constraints on the vocal apparatus (Podos 2001). Previous descriptions of chick-a-dee calls suggest that the number of D notes is indeed correlated with other structural features (Freeberg et al. 2003; Templeton et al. 2005). Furthermore, previous playback studies examining receiver responses to chick-a-dee calls have used unmanipulated stimuli that varied naturally in note composition, so conclusions about the importance of the number of D notes in those studies may have been influenced by a correlated structural trait (Templeton et al. 2005; Templeton and Greene 2007; Mahurin and Freeberg 2009; Courter and Ritchison 2010). In our study, we manipulated the number of D notes independent of other structural features, which allowed us to disentangle the subtle communication effects of signal structure and the absolute number of D notes. It is possible that subjects perceived our manipulations as artificial, but this seems unlikely because subjects' responses were qualitatively similar to those observed in response to unmanipulated chick-a-dee calls (Templeton et al. 2005; Templeton and Greene 2007; Mahurin and Freeberg 2009; Courter and Ritchison 2010).

The relationship between duty cycle and receiver responsiveness can be explained, in part, by general signal design features. For example, increasing duty cycle, either by increasing the calling rate, increasing the number of D notes per call or both, can increase signal detectability in at least 2 ways

(Bradbury and Vehrencamp 1998; Wiley 2006). First, for species that move in and out of the signal's active space, increased duty cycle can increase the probability that receivers move into the active space when the signal is being produced. Second, in environments with fluctuating ambient noise, increased duty cycle can increase the probability that the signal will be produced during a moment of low background noise, effectively increasing the signal's active space (Bradbury and Vehrencamp 1998; Wiley 2006). Regardless of the underlying mechanism, increased signal detectability could be especially important for chickadees and heterospecific receivers that reside in large and noisy habitats (Brumm and Slabbekoorn 2006). Once a signal has been detected, increased duty cycle can also increase signal locatability by providing receivers with more opportunities to sample the signal, which can, in turn, help receivers locate the food or predator that initially evoked the signal (Bradbury and Vehrencamp 1998). If receivers then produce their own signals on locating the original stimulus, then these additional signals may provide yet another mechanism by which new receivers can indirectly detect and locate the original stimulus. In the current study, such indirect effects may have contributed to the large differences observed between experimental treatments. Furthermore, the lack of indirect effects, combined with low detectability and low locatability, may explain why receiver responses to the 2-D low duty cycle treatment did not differ from the silent control. Indeed, potential receivers may have been unable to detect or locate stimuli with such a low duty cycle.

Heterospecific and conspecific receivers exhibited similar responses to variation in duty cycle, suggesting that a common mechanism for extracting information exists among species. Associative learning could provide such a mechanism, as many species can learn to associate species-atypical stimuli with important environmental events (Thorndike 1901). Coevolution of signal variation and heterospecific response could also provide the necessary mechanism (Darwin 1862), particularly because chickadees and many of the heterospecific species observed in this study are sympatric year-round and often form mixed species flocks (Smith 1991). Finally, just as motivation-structural rules lead to similar patterns of signal production across species (Morton 1977; Owings and Morton 1998), a simple physiological mechanism could lead to similar patterns of receiver responses. Because signalers from a wide range of species encode motivational information by increasing duty cycle (Owings and Morton 1998), it seems logical that an equally wide range of species might respond differentially to changes in duty cycle. In other words, signals with a greater duty cycle may stimulate a basic physiological mechanism that increases receiver responsiveness.

In the current study, we show that, although chickadees vary the number of D notes in their chickadee calls in relation to environmental stimuli, it is the increased duty cycle of signaling sequences that affects receiver responses.

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