Supporting Online Material

Allometry of Alarm Calls: Black-capped Chickadees Encode Information about Predator Risk in their Mobbing Calls

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Materials and Methods

Three replicate flocks of 6 black-capped chickadees (*Poecile atricapilla*) were captured near Missoula, MT (46o 50' N, 114o, 02' W) during October 2000-March 2001 and October 2001-March 2002. Each flock was held captive for 4-6 weeks. All flocks were captured locally, and each experimental flock consisted of members of the same wild flock. After the experiments, the flocks were released where they had been captured. Chickadees were marked with uniquely colored leg bands for individual identification and housed in a 16m x16m x 4m, outdoor, experimental aviary at the University of Montana's Field Research Station at Fort Missoula (Animal Care # ACC 022-01). The aviary contained numerous live trees and snags to provide a semi-natural habitat. Each flock was allowed to habituate to the aviary for at least one week before the start of the predator presentation experiments. The chickadees quickly habituated to humans: within a week they would land close with little sign of disturbance, and did not give alarm calls in response to us.

Predator Presentations

We presented 17 different treatments (Table 1), including a control (no presentation), a procedural control (a live bobwhite quail, *Colinus virginianus*), two live mammalian predators, and 13 species of live raptors. We used a small ferret (*Mustela putorius*), which was similar in size and shape to weasels (*M. frenata* and *M. erminea*) that occur in the area. Domestic cats (*Felis domesticus*) are also common in the study area. The raptors were from Raptors of the Rockies, a non-profit rehabilitation and education organization (Possession Permit # MB732828). Although most of the birds sustained injuries from cars or were shot so they could not be released back into the wild, all of the birds used in the experiments were otherwise extremely healthy, active and alert. For most of the birds, it is not possible to tell that they were injured or can not fly when they are perched. Pictures of most of the individuals used in these experiments (and descriptions of the nature of their injuries) can be found at www.raptorsoftherockies.org (under "Team").

The order of treatments was randomized for each flock and experiments were separated by at least two days. Prior to each trial, two observers entered the aviary with recording equipment and binoculars and remained stationary for 5 minutes to ensure that any mobbing calls were elicited by the predator stimulus and not the human observers. The aviary abuts a research building, and two windows open into the aviary. A platform extended from one of the windows 1-meter out into the aviary, and a black, opaque curtain was hung on a track that surrounded the platform. Predators were tethered to a perch and quietly placed on the platform through the window. The curtain was closed so that the chickadees could not see the person inside the building, or the placing of the raptor on the platform. The raptors were quiet during this time, so the chickadees did not hear calls before the presentation. The curtain was then slowly drawn open to reveal the perched predator to the chickadees. A curtain hung across the window so the chickadees could not see the person inside the building.

Two observers recorded chickadee vocalizations, noting the calling individual, with Sennheiser shotgun microphones (ME66), Mineroff pre-amplifiers (SME-BA6), and Sony TCM-5000 ("modified bird version") tape recorders. Each observer recorded calls from birds in half of the aviary; to avoid observer bias, we each recorded from the same side for all of the presentations. We were able to identify individual callers most of the time; if we were unsure of the caller's identity, the call was classified as "unknown" for the analyses.

Acoustic Analyses

Spectrographic analyses of 5,440 "chick-a-dee" mobbing calls were conducted with Avisoft-SASLab Pro 3.93. We measured the average calling rate, number of A, B, C, D, and total syllables in each call (S*1*, *S2*) (Fig. 1A). For each predator treatment, we averaged the number of each syllable per call for each individual chickadee then we obtained a flock average from these values. In this manner, the flock was considered the sampling unit but each individual bird was given equal weight in the analyses. This was done to guard against the possibility of biasing the results if some individuals called more than other individuals, or if there were systematic differences between individuals in the acoustic structure of their alarm calls. We then used a univariate ANOVA with Tukey's post-hoc test to conduct pair-wise comparisons among the treatments. We measured each predator to obtain morphometric data, including body length, mass, wingspan, and wing chord and compared these with the number of D syllables using linear regression.

We also conducted more detailed analyses of the acoustic structure of the D notes. We measured fine-scaled features of the D notes (Fig. 1B) randomly selected from 6 individuals (2 per flock) for which we had very high quality recordings. For each individual, we analyzed 5 calls produced in response to the northern pygmy-owl and 5 calls produced in response to the great horned owl. For each call, we measured the duration of the "chick" section, the duration of the "dee" section, the interval between the "chick" and "dee" sections, and the duration of the first D note (Fig. 1A). Using a power spectrum analysis (FFT=512) taken from the center of the first D note of each call, we measured several acoustic features similar to those described by Nowicki (S*3*). These features were the lowest frequency peak above –30dB relative to the peak, the highest frequency peak above –30dB, the frequency of the first two peaks above –30dB (frequency 1&2; used to determine the distance between overtones), the number of peaks above –10dB, the peak frequency, the highest frequency peak above –10dB, and the lowest frequency peak above –10dB. We calculated the interval between overtones by subtracting frequency 1 from frequency 2 and also calculated the bandwidth at –10dB and –30dB. We compared each of the variables using a two-way ANOVA; calling individual was included in the model as a random factor to account for any variation in acoustic features among birds. For all parametric tests used, all variables met the assumptions of the tests.

The acoustic variables were originally measured by Chris Templeton (CT). The coding of these data was not done in a strictly blind fashion: the sound files were identified by individual bird and flock ID, but not predator treatment. We subsequently selected a random sample of the original vocalizations from different predator treatments. One of us (EG) renamed these files with numbers, so there was no reference to flock, bird ID, or predator treatment. Each file was then duplicated twice, so there were three identical copies of each sound file. The copies were assigned random numbers, and the order of all the files was scrambled. CT then recoded these vocalizations in a blind fashion. For some of the variables (# of A, B, C, D syllables, total # of syllables) the blind recoding was identical to the original data. For the other variables dealing with timing and frequency aspects of the calls, the repeatabilities of the measurements (S*4*) were all extremely high (repeatability, $r > 0.98$ for all measurements). Furthermore, the original data were virtually identical to the data recoded by CT in a blind fashion ($r > 0.98$ for all variables).

We conducted another analysis to estimate potential observer bias measuring the acoustic variables. Three students were shown how to measure all the variables from sound files. The students then independently coded the variables from the set of randomly-selected sound files described above (which contained three copies of each vocalization). This was a double-blind procedure, since they did not know anything else about the experiments, the treatments or hypotheses. All individuals were extremely consistent in their scoring of the variables (repeatabilities for all variables comparing the scoring of the three copies of each vocalization were $r > 0.98$ within all observers). Furthermore, the values were extremely consistent between observers ($r > 0.98$ for all variables compared between observers). Thus, the measurements of acoustic variables are highly repeatable and consistent between observers.

Playback Experiments

We tested the behavioral response of chickadees to mobbing calls produced in response to presentations of a great horned owl and a northern pygmy-owl, and control calls of a pine siskin (*Carduelis pinus*; a small sympatric passerine). We only used clean calls recorded from members of the same flock that would experience the playback stimuli because chickadees encode information about flock membership in their “chick-a-dee” calls (S*5*, *S6*). To control for any differences in the call structures of individual birds or the likelihood that other birds would differentially respond to certain individuals (e.g., dominant vs. subordinate birds), we constructed playback libraries from calls recorded from known individuals, and a single bird’s great horned owl and pygmy-owl calls were used as paired playback stimuli. We conducted three replicates of each treatment per flock using three different bird’s calls. Each playback stimulus consisted of 15 seconds of “chick-a-dee” calls from a single individual. Each stimulus contained a sequence of calls produced by one individual chickadee. To avoid pseudoreplication (S*7*, *S8*), we used only unique exemplars. Because the calling rate and the length of “chick-a-dee” calls vary in response to the two different predators, we standardized for the total length of the stimulus (15 sec) instead of the absolute number of calls. A typical pygmy-owl stimulus tape contained approximately seven separate “chick-a-dee” calls, whereas a typical great horned owl tape contained approximately four calls during the 15 seconds; these averages are similar to those observed during the predator presentation experiment. In addition to calling rates, these playback stimuli varied in the number of D notes and other fine-scale acoustic features, as described in the results of the predator presentation experiments.

To assure that any differences in response were not due to habituation, we blocked the three treatments by the time of day and the calendar day relative to the start of the playback experiments. On each day of experiments, three total playbacks (one of each treatment) were conducted. Each playback was separated by at least three hours and we did not conduct playback experiments on subsequent days. Three days of experiments (9 total playbacks) were conducted for each flock. On each day a different individual bird’s calls were used for the pygmy-owl and great horned owl treatments (e.g., "red" on day 1, "blue" on day 2, and "green" on day 3).

Playback vocalizations were broadcast from tape recordings inside the research building to a playback speaker hidden in vegetation in the aviary. The speaker had a built-in amplifier. The sound levels had been adjusted so that the different playback stimuli were the same amplitude. For each trial, the speaker was hidden in one of three different places in the aviary to reduce habituation. Each treatment was broadcast from each speaker location an equal number of times to ensure that speaker placement did not influence the behavioral response of the chickadees.

The chickadees’ responses were characterized by the following behavioral variables: (i) the closest distance that any bird approached the speaker; (ii) the number of birds that came within 3 meters of the speaker; (iii) the number of birds that came within 1 meter of the speaker; (iv) the latency for the birds to return to non-mobbing behavior (i.e., they moved away from the speaker and resumed foraging); and (v) the number of “chick-a-dee” calls that were produced by the flock during the first 90 seconds after the playback began. We taped the playback experiments, and these variables give a good representation of the general intensity of the mobbing responses of the flock.

We used a Kruskal-Wallis non-parametric test for all comparisons because the variances of some variables did not meet the homogeneity assumptions of parametric tests. We used one-tailed, post-hoc Mann-Whitney U tests for pairwise comparisons because we predicted *a priori* that chickadees would respond more strongly to the pygmy-owl treatment than the great horned owl treatment.

References for Supplementary Information

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