

Neotropical Birds Respond Innately to Unfamiliar Acoustic Signals

Luis Sandoval^{1,*} and David R. Wilson²

1. Escuela de Biología, Universidad de Costa Rica, San Pedro, San José, Costa Rica 11501-2060; 2. Department of Psychology, Memorial University of Newfoundland and Labrador, 232 Elizabeth Avenue, St. John's, Newfoundland and Labrador A1B 3X9, Canada

Submitted August 31, 2021; Accepted February 22, 2022; Electronically published July 11, 2022

Online enhancements: supplemental PDF.

ABSTRACT: Many animals respond to heterospecific signals that indicate the presence of food or predators. Although the benefits of responding are clear, the behavioral and cognitive mechanisms underlying responses are not. Whether responses are learned, innate, or an epiphenomenon created by following other species as they respond to signals remains unknown because most studies have involved respondents that are sympatric with their heterospecific signalers and that have therefore had opportunities to learn their signals. In this study, we tested the mechanisms underlying avian responses to heterospecific chick-a-dee calls. All North American parids produce chick-a-dee calls in response to arousing stimuli, such as food and predators, and diverse species respond by approaching the caller and consuming the food or mobbing the predator. We broadcast chick-a-dee calls plus two control stimuli in Costa Rica, Colombia, and Brazil, where no parids ever occur. We conducted our trials in the winter, when Neotropical migrants that might be familiar with chick-a-dee calls were present, and in the temperate breeding season, when migrants were absent. Across 138 trials, 38 resident species from 14 families and four orders responded to chick-a-dee calls by approaching to within 5 m of the playback speaker. A phylogenetic logistic regression showed that whether a species responded was not significantly associated with the species' mean body mass or the structural similarity between its calls and chick-a-dee calls. Residents were significantly more likely to approach chick-a-dee calls than either control stimulus. This pattern was unaffected by the presence of migrants, thus demonstrating that the observed responses are innate. Our study shows that learning cannot fully explain responses to heterospecific chick-a-dee calls and that structural features distinguishing these calls from other vocalizations are important.

Keywords: chickadee, communication network, eavesdropping, food call, mobbing, paridae.

Introduction

Many animals respond to cues and signals produced by other species (Bradbury and Vehrencamp 2011). In some cases, the costs of responding are severe (Dawkins and Krebs 1978). Examples include predators being thwarted by the startle (e.g., butterflies; Vallin et al. 2005) or decoy displays (e.g., birds; Humphreys and Ruxton 2020) of prey, birds starving their offspring by prioritizing brood parasites (Soler 2017), and prey such as fireflies (Lloyd 1965), fish (Pietsch and Grobecker 1978), lizards (Chiszar et al. 1990), and spiders (Wignall and Taylor 2011) being lured to their death by their predators' deceptive signals. Responding to heterospecific cues and signals can also be beneficial, even if the cues or signals are not intended for the eavesdropping individual (Bradbury and Vehrencamp 2011). Many predators and parasites, for example, acquire their prey and hosts by localizing their scents and sounds (Zuk and Kolluru 1998), and many prey find food and avoid predation by responding to heterospecific food calls (e.g., Japanese sika deer [*Cervus nippon*] responding to calls of Japanese macaques [*Macaca fuscata yakui*]; Koda 2012) and alarm calls (Magrath et al. 2015). Given the significant costs of responding to some heterospecific signals and the obvious benefits of responding to others, selection should favor receivers and sensory systems that discriminate among heterospecific signals (Guilford and Dawkins 1991).

Although the costs and benefits of responding to heterospecific signals have been well studied, the behavioral and cognitive mechanisms underlying the responses have not (Magrath et al. 2015). One hypothesis is that animals appear to respond to heterospecific signals only because they follow other species that are themselves responding to signals (Mönkkönen and Forsman 2002; Goodale et al. 2010). A second hypothesis is that animals learn to associate heterospecific signals with adaptive behavioral

* Corresponding author; email: biosandoval@hotmail.com.

ORCID: Sandoval, <https://orcid.org/0000-0002-0793-6747>; Wilson, <https://orcid.org/0000-0002-6558-6415>.

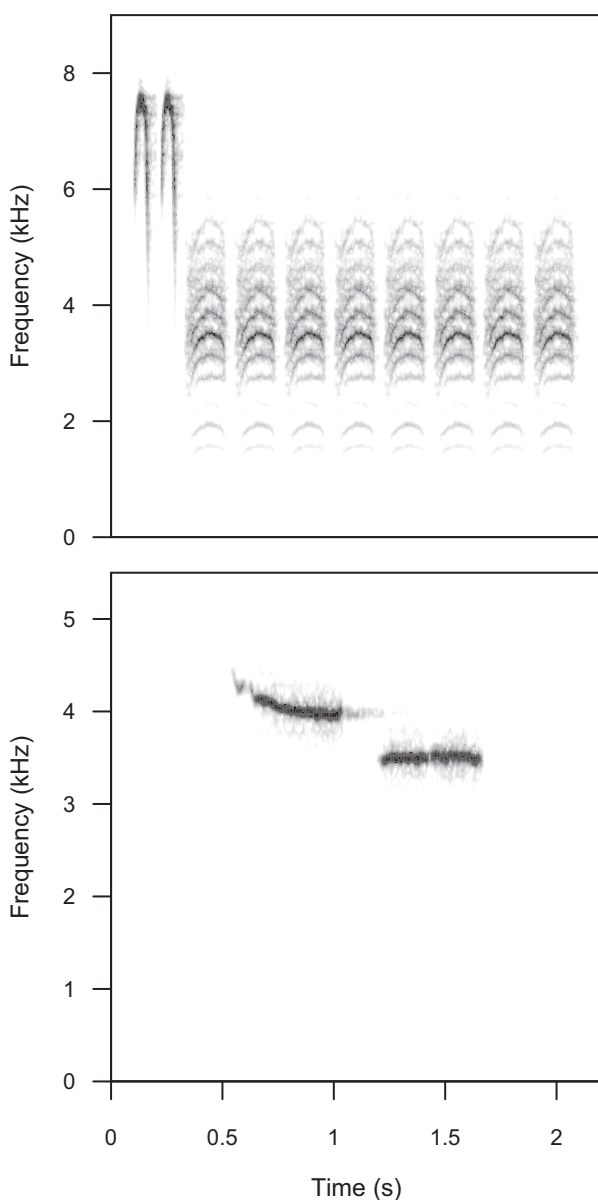


Figure 1: Spectrograms of chick-a-dee call (*top*) and fee bee song (*bottom*) of black-capped chickadee. Chick-a-dee calls begin with a series of introductory notes (A, B, and C notes) that are short, tonal, relatively high frequency, and strongly frequency modulated, followed by a series of dee notes that are relatively long and low frequency, with harsh, harmonic-like structure and little frequency modulation. The presence and number of each note type varies, but the note types are always produced in the same order (A, B, C, dee; Lucas and Freeberg 2007). The example shown here has been standardized for use as a stimulus call (see “Methods”) and includes two introductory notes and eight dee notes. Fee bee songs contain two tonal notes, including an initial high-frequency fee note that descends in frequency, followed after a short silence by a relatively low-frequency bee note of similar duration and little frequency modulation. During a playback, the selected stimulus was broadcast at a rate of six repetitions per minute for 2 min. Spectrograms were generated with a 512-point fast

responses, such as freezing or approaching (Griffin 2004), or with salient environmental features, such as food and predators (Magrath et al. 2015). Such associative learning requires an animal to experience the signal (Griffin 2004; Magrath et al. 2015). Golden-mantled ground squirrels (*Spermophilus lateralis*) provide a clear example because they learn to respond with antipredator behavior to previously unfamiliar sounds that became reliably associated with the appearance of predators (Shriner 1999). A third hypothesis is that responses are innate (Magrath et al. 2015). In this case, even unfamiliar heterospecific signals can elicit responses if they contain characteristics that are familiar because of phylogenetic conservation or evolutionary convergence (Marler 1955; Morton 1977; Jurisevic and Sanderson 1998; Johnson et al. 2003; Fallow et al. 2011) or characteristics that broadly stimulate diverse sensory systems (Endler and Basolo 1998; Owings and Morton 1998; Fitch et al. 2002; Rendall et al. 2009). Of course, these mechanisms are not mutually exclusive. For example, a species might show an innate response to an unfamiliar signal but then show increased responsiveness to the signal after learning that it predicts the presence of food.

All species of Paridae in North America (chickadees: *Poecile atricapillus*, *Poecile carolinensis*, *Poecile cinctus*, *Poecile gambeli*, *Poecile hudsonicus*, *Poecile rufescens*, *Poecile sclateri*; titmice: *Baeolophus atricristatus*, *Baeolophus bicolor*, *Baeolophus inornatus*, *Baeolophus ridgwayi*, *Baeolophus wollweberi*) produce chick-a-dee calls (fig. 1) in response to arousing stimuli, such as predators, food, and territorial intruders (Dixon 1949; Smith 1972; McLaren 1976; Gaddis 1985; Hailman 1989; Charrier et al. 2004; Bloomfield et al. 2005; Hailman and Haftorn 2005; Lucas and Freeberg 2007; Owens and Freeberg 2007; Hoeschele et al. 2009; Moscicki et al. 2010; Cicero et al. 2020; Nosedal and Ficken 2020). Conspecifics and diverse heterospecifics approach chick-a-dee calls, where, upon arriving, they often engage in antipredator, foraging, or aggressive behaviors appropriate to the stimulus eliciting the calls (Hurd 1996; Templeton et al. 2005; Langham et al. 2006; Templeton and Greene 2007; Schmidt et al. 2008; Soard and Ritchison 2009; Mahurin and Freeberg 2009; Courter and Ritchison 2010; Wilson and Mennill 2011; Grava et al. 2012; Hetrick and Sieving 2012; Randler 2012; Dutour et al. 2017, 2020; Landsborough et al. 2020). Heterospecifics responding to chick-a-dee calls potentially benefit by participating in multispecies mobbing events that repel predators and deter their return (Pettifor 1990; Flasskamp 1994; Pavey and Smyth 1998; Consla and Mumme 2012) or by consuming food they might otherwise fail to discover (Dolby and Grubb 1998; Wilson and Mennill 2011).

Fourier transform with Hamming window and 87.5% overlap. Temporal resolution is 2.9 ms, frequency resolution is 43 Hz, and the grayscale represents an amplitude range of 45 dB.

The potential benefits of responding to chick-a-dee calls may thus serve as reinforcement that would motivate heterospecifics to learn to respond to calls directly or to follow other birds as they respond to calls (Griffin 2004). Further evidence that heterospecific responses to chick-a-dee calls are learned is that heterospecifics respond less strongly or not at all to other parid vocalizations, such as songs and contact calls, which are not reliably associated with predators, food, or other reinforcing stimuli (Hurd 1996; Schmidt et al. 2008; Randler 2012). Yet chick-a-dee calls also include harsh (i.e., spanning broad frequency range at each moment in time), low-frequency elements (Hailman 1989; Lucas and Freeberg 2007) that, according to motivation-structural rules, should universally reflect high levels of signaler arousal and hostility (Morton 1977; Owings and Morton 1998) and that might therefore stimulate diverse sensory systems and elicit innate responses from receivers. Consistent with the idea of responses being innate, many birds approach the harsh, low-frequency, and unfamiliar pishing sounds made by ornithologists and birdwatchers (Langham et al. 2006). It is therefore possible that heterospecific responses to chick-a-dee calls are innate instead of learned or that they are both.

The behavioral and cognitive mechanisms underlying heterospecific responses to chick-a-dee calls remain unclear because many of the relevant studies involved respondents that are sympatric with the species producing the calls (Hurd 1996; Templeton and Greene 2007; Schmidt et al. 2008; Wilson and Mennill 2011; Grava et al. 2012; Hetrick and Sieving 2012; Landsborough et al. 2020), thus conflating the potential mechanisms involved. A few studies showed that European birds, including parid and nonparid species, respond to the unfamiliar chick-a-dee calls of allopatric chickadees and titmice from North America (Randler 2012; Dutour et al. 2017, 2020) and that diverse birds in California respond to the chick-a-dee calls of allopatric parids from other parts of North America and Europe (Langham et al. 2006). These studies suggest that heterospecific responses are innate because prior experience with a species' chick-a-dee call is not necessary for the call to elicit a response. However, respondents in these studies were sympatric with local parids and were probably familiar with their calls. It is therefore possible that respondents learned to respond to the chick-a-dee calls of local parids and then generalized that response to the acoustically similar but unfamiliar calls of allopatric parids (Langham et al. 2006; Randler 2012; Dutour et al. 2017, 2020). A critical test of whether heterospecific responses to chick-a-dee calls are innate would be to show that birds that are permanently allopatric to all Paridae respond to their calls. Yet we know of only one such study. Nocera et al. (2008) broadcast chick-a-dee calls from black-capped chickadees (*Poecile atricapillus*) at sites in Belize during spring migration and found that migrants—but not residents—responded (Nocera et al. 2008). The lack

of response by Neotropical residents suggests that chick-a-dee calls elicit responses only from species that are sympatric with parids, though the study cautions that its findings should be replicated at other locations in the tropics and at other times of the year before generalizing its conclusions (Nocera et al. 2008).

In this study, we use an acoustic playback experiment to test whether heterospecific responses to chick-a-dee calls are learned, innate, or an epiphenomenon created by following other species. We tested these three hypotheses by broadcasting chick-a-dee calls of black-capped chickadees plus two control stimuli in Costa Rica, Colombia, and Brazil, where no members of Paridae ever occur. We conducted our trials in the winter, when Neotropical migrants that might be familiar with chick-a-dee calls were present, and in the temperate breeding season, when migrants were absent. If heterospecific responses to chick-a-dee calls are innate, then Neotropical residents should respond more strongly to chick-a-dee calls than to control stimuli, regardless of whether migrants are present. If heterospecifics respond to chick-a-dee calls by following other species that may themselves be familiar with the calls, then resident species should respond more strongly to chick-a-dee calls only when migrants are present. If heterospecifics learn to respond directly to chick-a-dee calls through experience and associative learning, then resident species should never respond to unfamiliar chick-a-dee calls. The study is one of the few to test the cognitive or behavioral mechanisms underlying heterospecific communication in animals and thus provides insight into the development, ecology, and evolution of this behavior.

Methods

General

We conducted playback trials in Costa Rica, Colombia, and Brazil. In Costa Rica, we conducted trials during the temperate breeding season when migrants were away on their breeding grounds (July 8–13, 2013; $N = 30$ trials) and during the preceding (January 7–12, 2013; $N = 30$ trials) and following winters (December 14–19, 2013; $N = 30$ trials) when migrants were present. Trials were conducted at 30 sites that were located at North Heredia (10°01'N, 84°05'W, 1,200–1,500-m elevation; $N = 60$ trials at 20 sites) and the Lankester Botanical Garden (09°50'N, 83°53'W, 1,400-m elevation; $N = 30$ trials at 10 sites) and that were the same among seasons. In Colombia and Brazil, we conducted all trials during the temperate breeding season, when migrants were away (Colombia: August 11–14, 2013; Brazil: September 11–16, 2013). In Colombia, we conducted 27 trials at 27 sites in Medellín (06°14'N, 75°34'W, 1,550-m elevation; $N = 12$ trials) and the Río Claro Natural Reserve

(05°50'N, 74°52'W, 415-m elevation; $N = 15$ trials). In Brazil, we conducted 21 trials at 21 sites in Pousada dos Pirineos (15°50'S, 46°57'W, 895-m elevation; $N = 11$ trials) and Alto Paraiso (14°07'S, 47°31'W, 1,200-m elevation; $N = 10$ trials).

Following a randomized complete block design, we broadcast three playback treatments in random order during each trial before moving to the next site and beginning the next trial. Our study therefore included 138 trials and three treatments, or 414 trial treatments. Playback sites at each general location were selected haphazardly and were located within secondary forest edges, green areas with trees and bushes, cerrado vegetation, or coffee plantations with live fences. Birds in our study were not color banded and could not be identified as individuals. We therefore separated playback sites by at least 100 m to reduce the probability of the same individuals responding at multiple sites. All trials were conducted between 0600 and 1000 hours when diurnal birds are active.

Procedure

After choosing a playback site, we selected a tree that was devoid of fruits and flowers, attached a loudspeaker (Panasonic, model RP-SP48; frequency range: 140–20,000 Hz) to a branch 1.5–2.5 m above the ground, oriented the speaker upward, and connected it to a digital playback device (iPod Nano Touch) containing our playback stimuli. Four flags were placed at 90° angles around the speaker at a distance of 5 m to assist in estimating the distances between the speaker and approaching birds. The observer sat on the ground 8.6 m from the speaker, waited until no birds were detected within 10 m of the speaker for at least 5 min, and began the first of three playback treatments for that trial.

We broadcast three treatments during each trial (see details of stimulus construction below). Our experimental treatment was the chick-a-dee call of the black-capped chickadee, which was repeated at a natural rate of 6 calls min^{-1} for 2 min (fig. 1). Our positive control was the fee bee song of the black-capped chickadee (Ficken et al. 1978), which was also repeated at 6 songs min^{-1} for 2 min (fig. 1). We chose the fee bee song because its production is not associated with external stimuli such as predators or food that might be salient to heterospecifics (Ficken et al. 1978). It thus controlled for heterospecific responses to unfamiliar and functionally irrelevant biological sounds. Our negative control was a 2-min period of silence, which controlled for spontaneous arrivals at our playback apparatus. The order of treatments was randomized but with the constraint that it was balanced among treatments across trials. Following each treatment, we waited until no birds had been seen within 10 m of the loudspeaker for at least 5 min before proceeding to the next treatment. We broadcasted the chick-a-

dee call and fee bee song treatments at a sound pressure level of 80 dB (measured 1 m from the loudspeaker with a Sper Scientific mini sound level meter, model 840014; 32–130-dB response range; fast response; C-weighting).

We did not include a familiar positive control, such as the mobbing call of a local species (as in Nocera et al. 2008), for three reasons. First, no one species that we know of produces high-arousal calls that would be familiar to resident birds at all our playback sites. Second, we were concerned that broadcasting two high-arousal calls in our repeated measures design could either be too disruptive to birds or cause them to habituate to playback stimuli. Finally, interpreting a potential difference in responses to a familiar control stimulus and to our unfamiliar experimental stimulus would be difficult because calls of different species, even when uniformly familiar or unfamiliar to respondents, may be unequally evocative. If responses to the familiar control were stronger than responses to the unfamiliar chick-a-dee call, it would be impossible to know whether the difference was due to the difference in familiarity and thus learning or to differences in the evocativeness of the two stimuli.

Following Sandoval and Wilson (2012), we measured four response variables in situ during each 2-min treatment: (1) number of species observed within 5 m of the speaker; (2) maximum number of birds, which was the sum of the maximum number of individuals of each species that could be observed simultaneously within 5 m of the speaker; (3) latency of the first bird to approach within 5 m of the speaker; and (4) minimum distance of any bird from the speaker. We selected these measures because they do not depend on having color-banded individuals. Times were recorded to the nearest second with a stopwatch, and distances were estimated along the horizontal plane to the nearest 0.1 m. As in Sandoval and Wilson (2012), we used 5 m as the threshold for inclusion because dense vegetation at our sites made it difficult to detect and monitor birds beyond this distance. If no birds approached to within 5 m of the speaker during a 2-min treatment, we reported zeros for number of species and maximum number of birds but did not assign values for latency or minimum distance. In addition to quantitative measures, we noted the species of all responding birds and the species of the first bird to respond. All trials were conducted by just one of the authors, who was familiar with the avian communities at the trial locations. Species names follow the checklists established by the American Ornithological Society for North, Middle, and South American birds (Chesser et al. 2020; Remsen et al. 2021).

Stimuli

We created 30 playback stimuli for the chick-a-dee call treatment and 30 playback stimuli for the positive control

to minimize problems associated with pseudoreplication (Hurlbert 1984). Each stimulus was created in Raven Interactive Sound Analysis Software (ver. 1.4 Pro; Cornell Lab of Ornithology Bioacoustics Research Program, Ithaca, NY) by repeating a single vocalization at a rate of 6 vocalizations min^{-1} for 2 min (fig. 1). Recordings were from the authors' personal collections or the Macaulay Library at the Cornell Lab of Ornithology. Each stimulus individual contributed a maximum of one song and one call. Five individuals contributed both a song and a call, though song and call stimuli from the same individual were never presented to the same subject. Vocalizations were selected on the basis of high signal-to-noise ratios, typical structure, and no overlapping sounds and were filtered with a high-pass filter to remove background noise (chick-a-dee calls at 1 kHz; fee bee songs at 2.7 kHz). As in previous research (Wilson and Mennill 2011; Scully et al. 2019; Landsborough et al. 2020), we standardized the note composition of chick-a-dee calls before constructing the final playback stimuli to minimize potential effects of note syntax on receiver responses (Templeton and Greene 2007; Mahurin and Freeberg 2009; Soard and Ritchison 2009; Courter and Ritchison 2010). For each call, we removed all but the final two introductory notes and all but the first D note, and we then repeated the remaining D note seven times at a natural rate based on the original call to create a call with eight D notes. The amplitude of the D notes was adjusted to -7.6 dB, and the peak amplitude of the two introductory notes was adjusted to -1 dB. This difference reflects the natural amplitude difference observed among note types (Wilson and Mennill 2011). The fee bee songs were normalized to a peak amplitude of -1 dB.

Migratory Status and Sympatry with Paridae

It was important that subjects were unfamiliar with chickadee vocalizations, since prior experience would make it difficult to determine whether responses to calls were learned or innate. We ensured that subjects had no prior experience in two ways. First, we classified each respondent species as a Neotropical resident or Neotropical migrant using the classifications provided in *Birds of the World* (Billerman et al. 2020; table S1). Migrants likely overlap with black-capped chickadees on their temperate breeding grounds or migration routes and were therefore excluded from subsequent statistical analysis. Second, we determined whether each responding species was sympatric with the black-capped chickadee or any other parid, since all North American parids produce some form of the chick-a-dee call. We obtained digital species distribution maps from BirdLife International and Handbook of the Birds of the World (2020; datum: World Geodetic System 1984 [National Imagery

and Mapping Agency 1997]) and projected them using the Lambert Azimuthal Equal Area projection (latitude at projection center, 45° ; longitude at projection center, -100° ; false northing, 0 m; false easting, 0 m) in the R package *rgdal* (Bivand et al. 2020). Using the R package *rgeos* (Bivand and Rundel 2020), we calculated the proportion of each responding species' distribution that is sympatric with the black-capped chickadee and the Paridae (table S1).

As expected, the black-capped chickadee and the Paridae are sympatric with all migrants observed in our study (table S1). The black-capped chickadee is also sympatric with one resident species (house wren [*Troglodytes aedon*]) and the Paridae with 16 resident species (table S1). Although the area of overlap between Paridae and these 16 resident species is typically very small (table S1), we nevertheless investigated whether individuals from the overlap regions could have traveled to our playback locations. For each of the 16 resident species, we determined their movement behavior from their species account in *Birds of the World* (Billerman et al. 2020). Eleven of the 16 are described as sedentary or as wandering locally only along altitudinal gradients. It is therefore unlikely that individuals from these species would have experienced chickadee vocalizations in the region of sympatry and then traveled the minimum 1,500 km to our northernmost playback sites in Costa Rica. Streaked flycatcher (*Myiodynastes maculatus*), vermilion flycatcher (*Pyrocephalus rubinus*), orange-billed nightingale thrush (*Catharus aurantiirostris*), and house wren (*Troglodytes aedon*) are considered resident species, but some of their northernmost populations are known to migrate southward in winter. However, the nightingale thrush and flycatchers were detected only during summer, and house wrens migrate south only as far as central Mexico. Respondents from these species therefore could not have been from the migratory populations. Finally, boat-billed flycatcher (*Megarynchus pitangua*) is known to wander broadly; however, only 1% of its distribution is sympatric with Paridae, and it was detected in only one trial. It is therefore unlikely that this one individual was familiar with chick-a-dee calls. Overall, we are confident that all resident birds responding in our study were unfamiliar with chickadee vocalizations.

We updated our response variables such that number of species and maximum number of birds were exclusively based on Neotropical resident species. Unfortunately, this was not possible for latency or minimum distance because we could not collect those data separately for each species in the field.

Phylogenetic Context

To understand the taxonomic distribution of respondents, we identified all avian species that are sympatric with our

playback locations and thus available to respond. Using the species maps and spatial analysis techniques described above, we identified 751 extant species with breeding ranges overlapping at least one of our six playback locations. We reviewed their habitat descriptions in *Birds of the World* (Billerman et al. 2020) and retained the 692 species that inhabit primarily terrestrial environments, where our playbacks were conducted (tables 1, S2). Species excluded are all members of Anatidae, Laridae, Scolopacidae, Ciconiidae, Alcedinidae, Aramidae, Ralidae (except ocellated crane [*Micropygia schomburgkii*]), Cinclidae, Donacobiidae, Ardeidae, Threskiornithidae, Podicipedidae, Anhingidae, and Phalacrocoracidae. The phylogenetic relationships of the 692 retained species are shown in figure 2.

We also tested whether any species traits predicted whether the species responded to chick-a-dee calls. On

Table 1: Taxonomic distribution of Neotropical residents at our study sites

Order	No. sympatric families	No. sympatric species	No. responding species
All species	58	692	38
Accipitriformes	1	28 (4.0)	0 (.0)
Apodiformes	2	64 (9.2)	4 (10.5)
Caprimulgiformes	1	12 (1.7)	0 (.0)
Cariamiformes	1	1 (.1)	0 (.0)
Cathartiformes	1	4 (.6)	0 (.0)
Charadriiformes	1	3 (.4)	0 (.0)
Columbiformes	1	24 (3.5)	0 (.0)
Coraciiformes	1	4 (.6)	1 (2.6)
Cuculiformes	1	12 (1.7)	0 (.0)
Falconiformes	1	9 (1.3)	0 (.0)
Galbuliformes	2	8 (1.2)	0 (.0)
Galliformes	2	10 (1.4)	0 (.0)
Gruiformes	1	1 (.1)	0 (.0)
Nyctibiiformes	1	2 (.3)	0 (.0)
Passeriformes	30	422 (61.1)	32 (84.2)
Piciformes	4	31 (4.5)	1 (2.6)
Psittaciformes	1	26 (3.8)	0 (.0)
Rheiformes	1	1 (.1)	0 (.0)
Steatornithiformes	1	1 (.1)	0 (.0)
Strigiformes	2	14 (2.0)	0 (.0)
Tinamiformes	1	8 (1.2)	0 (.0)
Trogoniformes	1	6 (.9)	0 (.0)

Note: Shown for 22 orders (and for all species combined) are the number of sympatric families and species plus the number of species that approached to within 5 m of the playback speaker during the chick-a-dee call treatment of at least one trial. Values in parentheses show the percent of all sympatric or responding species. Species were considered sympatric if they inhabited a primarily terrestrial environment and if their breeding range overlapped at least one of our six playback sites, as determined by species distribution maps from BirdLife International and Handbook of the Birds of the World (2020). Taxonomy follows the American Ornithological Society's checklists for North, Middle, and South American birds (Chesser et al. 2020; Remsen et al. 2021). Information about individual species is provided in table S2.

the basis of previous research, we assumed that smaller species (Da Cunha et al. 2017) and species that produce calls that are structurally similar to chick-a-dee calls (Jurisevic and Sanderson 1998; Johnson et al. 2003) would be more likely to respond. For each species that responded to chick-a-dee calls in at least one trial, we identified a closely related species that was sympatric with our study sites but did not respond (see fig. 2). Mean body mass values for species that responded and for the matching species that did not were obtained from *Birds of the World* (Billerman et al. 2020) or *vertnet.org*. For acoustic similarity, we obtained recordings of vocalizations from five individuals per species from *xeno-canto.org*. We used the library's metadata to select high-quality recordings of calls obtained at different locations or in different years, thus minimizing the probability of sampling the same individual twice. We reviewed each recording and identified one call from each with a high signal-to-noise ratio and typical structure. The call (plus 20 ms of silence before and after the call) was normalized to a peak amplitude of 0 dB and exported as a standalone sound clip (WAVE format, 16-bit amplitude encoding, 22.05-kHz sampling rate). We compared the acoustic structure of each extracted call with the structure of each of our 30 chick-a-dee call playback stimuli using spectrogram cross-correlation in Raven (settings: 256-point fast Fourier transform, Hamming window, 87.5% overlap, 0–1-kHz bandstop filter). This technique compares the overall similarity of two sounds by sliding them past each other in time and calculating a correlation coefficient (a value between 0 and 1) at each time offset. The peak correlation coefficient indicates the overall similarity of the two sounds; a correlation of 0 indicates that the sounds do not match at all, whereas a value of 1 indicates that the sounds are identical. For each species, we calculated the median peak correlation between its five vocalizations and the 30 chick-a-dee calls. We note that other variables—including local predation pressure, whether a species forages on the ground, and whether a species resides in stable social groups—might also affect their responses (Sandoval and Wilson 2012; Da Cunha et al. 2017), but our small sample of respondents and the limited information available for many Neotropical residents precluded the inclusion of these variables.

Statistical Analysis

All analyses were conducted in R (R Development Core Team 2020), and all data and R code underlying the analyses and figures are in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.vdncjxwqj>; Sandoval and Wilson 2021). Preliminary analyses showed that the number of resident species and the maximum number of resident birds responding were highly correlated (Spearman

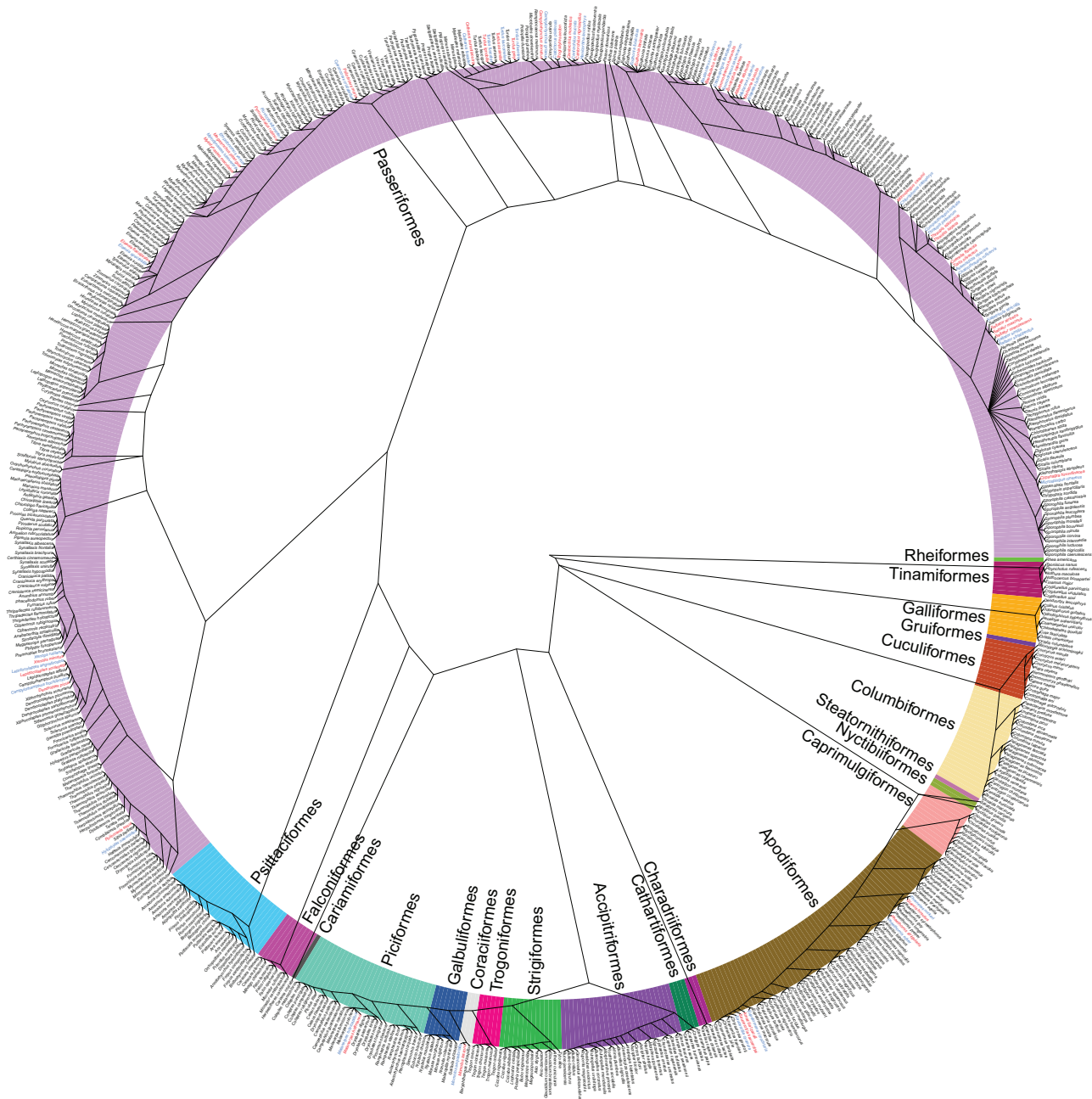


Figure 2: Phylogenetic distribution of extant Neotropical avian species with breeding ranges that overlap at least one of our six playback locations. The tree is a consensus of 10,000 trees provided by birdtree.org, which provides distributions of trimmed phylogenies based on the methodology of Jetz et al. (2012), the updated taxonomy of Jetz et al. (2014), and the backbone tree of Hackett et al. (2008). Source trees were combined in the R package ape (Paradis and Schliep 2019) using the consensus function and a proportion of 0.5 for consensus. Species in red responded to the chick-a-dee call treatment of at least one trial, whereas species in black or blue never responded. Species in blue were selected as nonrespondents in our phylogenetic logistic regression. Species in the same taxonomic order are indicated by shading of the same color. Taxonomy follows the American Ornithological Society’s checklists for South, Central, and North American birds (Chesser et al. 2020; Remsen et al. 2021). Although 692 species were sympatric with our playback locations, only 689 are shown on the tree because three nonrespondent species recognized by the American Ornithological Society’s checklists (*Arremon atricapillus*, *Buteo plagiatus*, *Momotus subrufescens*) were not included in the phylogenies provided by birdtree.org.

correlation: $\rho = 0.99$, $N = 414$, $P < .0001$), even when considering only those trial treatments where at least one resident species responded ($\rho = 0.82$, $N = 121$, $P < .0001$). We assume that this was because most resident species hold year-round territories (Billerman et al. 2020) and that for each species, only the individual or breeding pair that held the territory in which the trial was conducted would have approached the speaker. To avoid redundancy, we eliminated the maximum number of birds from further consideration. The remaining response variables (number of resident species, latency, minimum distance) were either uncorrelated or weakly correlated (all $\rho \leq 0.30$).

We tested our hypotheses using generalized linear mixed models implemented in the R package *glmmTMB* (Brooks et al. 2017). For our first analysis, we tested whether the number of resident species that approached to within 5 m of the speaker was greater in response to unfamiliar chick-a-dee calls than in response to unfamiliar songs or silence and whether the pattern of response was affected by the presence of migrants that might have been familiar with chickadee vocalizations. Playback treatment (chick-a-dee call, fee bee song, silence), the status of migrants (present vs. absent, as determined by season), and their two-way interaction were included as fixed factors. We included country (Costa Rica, Colombia, Brazil) as a fixed factor to account for unmeasured differences among regions that might have affected responses, such as differences in climate, vegetation, and the composition of local avian communities. We included trial number (1–138) nested within playback site (1–78) as a random effect to account for possible dependencies among responses observed at the same site or during the same trial. The response was modeled using a Poisson distribution with log link, which is appropriate for zero-bounded, positively skewed variables that are based on count data (Mun 2008). We included all trial treatments ($N = 414$ treatments from 138 trials) because trial treatments in which species respond and those in which species do not respond are both informative.

Our first analysis could not explicitly consider species identity as a predictor variable, so it is possible that results would have been based on the same few resident species responding in all trials. In our second analysis, we addressed this concern by testing whether a given species' probability of approaching to within 5 m of the speaker differed among treatments and as a function of the presence of migrants. For this analysis, it was necessary to estimate which resident species were present in the vicinity of the playback site at the time of the trial and thus available to respond during a given trial treatment. For each treatment within a trial, we therefore included separate observations for each resident species that ultimately responded during any of the three treatments of the trial. After excluding 42 trials in which no species responded and eight trials

in which only migrants responded, the analysis included 42 resident species observed among 88 trials. The response variable was whether the species approached to within 5 m of the speaker during the trial treatment and was modeled using a binomial distribution and logit link. The binomial distribution is appropriate for variables that include only two outcomes (Mun 2008). Treatment, the status of migrants, their two-way interaction, and country were again included as fixed factors, and species identity and trial number nested within playback site were included as random effects.

For our third analysis, we tested whether the latency of the first resident species to respond differed among treatments and as a function of the presence of migrants. We excluded 293 trial treatments in which no residents responded and 10 trial treatments in which a migrant responded before a resident. Our final sample therefore included 34 different species and 111 trial treatments. Treatment, the status of migrants, their two-way interaction, and country were included as fixed factors, and species identity and trial number nested within playback site were included as random effects. Latency to respond was rescaled to between 0 and 1 and then modeled using a beta distribution and logit link (Migliorati et al. 2018). The beta distribution is appropriate for asymmetric doubly bounded continuous variables, such as latency in this case (Mun 2008; Migliorati et al. 2018).

For our fourth analysis, we tested whether the minimum distance between the speaker and a resident species differed among treatments and as a function of the presence of migrants. We included only trial treatments in which a single resident species responded, thereby allowing us to ascertain species identity and include it in the model. Our final sample included 25 species and 81 trial treatments. Preliminary analyses showed that minimum distance was skewed to the left and bimodal, which made it difficult to analyze statistically. We therefore transformed minimum distance into a dichotomous variable and modeled it using a binomial distribution and logit link. Respondents that approached to a minimum distance from the speaker of 0–4 m were assigned to one category, and those that approached to a minimum distance of 4.1–5 m were assigned to another. We chose a cutoff of 4 because it separated the two modes of the distribution and split the sample into groups of similar size (0–4 m, $N = 43$; 4.1–5 m, $N = 38$). Treatment, the status of migrants, their two-way interaction, and country were included as fixed factors, and species identity and trial number nested within playback site were included as random effects.

We used the *DHARMA* package (Hartig 2020) in R to visualize scaled residuals and assess model fit. Its diagnostic tests and our inspection of residual plots did not identify any problems associated with the overall distribution of

residuals, over- or underdispersion, frequency of outliers, or zero inflation, suggesting that all models were adequately fit. For each model, we used the Anova function in the car package (Fox and Weisberg 2019) to test the statistical significance of each fixed factor. Because we used four separate models to test the same general hypotheses, we controlled experimentwise type I error by applying a Bonferroni correction to alpha. Fixed factors were therefore considered statistically significant when $P \leq .0125$. Where a fixed factor was significant, we conducted multiple pairwise comparisons among its levels using the glht function in the multcomp package (Hothorn et al. 2008). The P values from the multiple comparisons of a given factor were adjusted with the sequential Bonferroni method to control familywise type I error (Holm 1979).

Finally, we used phylogenetic logistic regression, implemented with the binaryPGLMM function (default settings) in the R package ape (ver. 5.5; Paradis and Schliep 2019), to test whether phenotypic differences among species predicted their responses to chick-a-dee calls. For each species, body mass (log transformed) and the median peak correlation between its calls and chick-a-dee calls (square root transformed) were standardized (mean = 0; SD = 1) and included as predictor variables. Whether the species approached to within 5 m of the speaker during the chick-a-dee call treatment of at least one trial was included as a binary dependent variable (0 = no response; 1 = response). The phylogeny for the subset of species included in the analysis was provided by birdtree.org (see details in fig. 2). To account for phylogenetic uncertainty, we repeated the analysis using 10,000 different trees and report mean coefficients and statistics in the results.

Results

Neotropical species near our playback sites are taxonomically diverse; 692 species from 58 families and 22 orders have breeding ranges that overlap at least one of our six playback sites (tables 1, S2; fig. 2). The species are unevenly distributed among orders, with 422 (61.1%) in Passeriformes, 64 (9.2%) in Apodiformes, and the remaining 206 (29.7%) in 20 other orders. Within Passeriformes, the most speciose families are the tyrant flycatchers (Tyrannidae, $N = 93$ species), tanagers and allies (Thraupidae, $N = 84$), ovenbirds and woodcreepers (Furnariidae, $N = 42$), typical antbirds (Thamnophilidae, $N = 28$), and troupials and allies (Icteridae, $N = 27$; tables 1, S2; fig. 2).

Across the 138 trials, 38 resident species from 14 avian families and four orders responded to the chick-a-dee call treatment of at least one trial by approaching to within 5 m of the playback speaker (tables 1, S1, S2; fig. 2). Our phylogenetic logistic regression showed that whether a species responded to the chick-a-dee call treatment was not sig-

nificantly associated with the species' mean body mass (estimate \pm SE = 0.12 ± 0.25 , $Z = 0.47$, $P = .6354$) or the structural similarity between its calls and chick-a-dee calls (estimate \pm SE = 0.10 ± 0.25 , $Z = 0.39$, $P = .6993$). A maximum of three resident species responded during any given trial treatment, and no resident species responded in 71% of trial treatments (293 of 414). Among the subset of 60 trials conducted in the winter, 12 migratory species from four families also responded. Here, a maximum of six migratory species responded during a given trial treatment, and no migrants responded in 88% (158 of 180) of trial treatments (table S1).

Experimental treatment affected the number of resident species approaching the loudspeaker, with significantly more species approaching chick-a-dee calls than silence or fee bee songs and significantly more species approaching fee bee songs than silence (table 2; fig. 3a). The number of resident species approaching the loudspeaker did not differ significantly between the temperate breeding season when migrants were absent and the winter when migrants were present (table 2; fig. 3a). Furthermore, the pattern of responses to experimental treatments was not influenced by the presence or absence of migrants, as revealed by a nonsignificant interaction between treatment and migrant status (table 2; fig. 3a). The number of resident species approaching the speaker was related to the country in which the playbacks were conducted, with significantly fewer species approaching the speaker in Brazil than in Colombia or Costa Rica (table 2).

Experimental treatment also affected the probability that a given resident species would approach to within 5 m of the speaker (table 2; fig. 3b). The 42 resident species that responded across the three treatments were, on average, significantly more likely to respond to chick-a-dee calls than to fee bee songs or silence and significantly more likely to respond to fee bee songs than to silence (table 2; fig. 3b). Their probability of responding was not related to the status of migrants (present/absent), the interaction between treatment and the status of migrants, or the country in which the playbacks were conducted (table 2; fig. 3b).

Experimental treatment, the presence of migrants, their two-way interaction, and country were not significantly associated with residents' latency to approach the speaker ($N = 111$ trial treatments in which a resident species was first to approach) or with their minimum distance from the speaker ($N = 81$ trial treatments in which only a single resident species responded; table 2).

Discussion

Neotropical resident birds observed in our study exhibited innate responses to unfamiliar heterospecific chick-a-dee calls. Results are based on a diverse group of 38 resident

Table 2: Behavioral responses of resident birds during acoustic playback trials

Response and predictor (pairwise comparison)	Estimate	SE	Z	df	χ^2	P
No. resident species:						
Intercept				1	48.0	<.0001
Treatment				2	48.0	<.0001
Call vs. song	1.18	.25	4.7			<.0001
Call vs. silence	2.27	.40	5.7			<.0001
Song vs. silence	1.10	.44	2.5			.0118
Migrants				1	1.8	.1842
Treatment × migrants				2	1.0	.5967
Country				2	13.6	.0011
Colombia vs. Brazil	.97	.37	2.7			.0161
Costa Rica vs. Brazil	1.29	.35	3.7			.0007
Colombia vs. Costa Rica	−.32	.23	1.4			.1650
Whether a species responded (yes/no):						
Intercept				1	11.3	.0008
Treatment				2	76.7	<.0001
Call vs. song	2.98	.43	7.0			<.0001
Call vs. silence	4.23	.52	8.1			<.0001
Song vs. silence	1.25	.47	2.6			.0082
Migrants				1	.6	.4229
Treatment × migrants				2	4.2	.1253
Country				2	.0	.9846
Latency to respond (s):						
Intercept				1	1.3	.2564
Treatment				2	.1	.9378
Migrants				1	.6	.4479
Treatment × migrants				2	.0	.9893
Country				2	8.3	.0157
Minimum distance (0–4 or 4.1–5 m):						
Intercept				1	1.5	.2165
Treatment				2	3.4	.1844
Migrants				1	.0	.8370
Treatment × migrants				2	2.8	.2466
Country				2	2.3	.3092

Note: Responses include the number of resident species approaching to within 5 m of the loudspeaker, whether a given resident species approached to within 5 m, the latency of the first resident species to approach to within 5 m, and their minimum approach distance. We broadcast three treatments per trial, including silence and the fee bee songs and chick-a-dee calls of black-capped chickadees. Trials were conducted in Costa Rica ($N = 30$), Colombia ($N = 27$), and Brazil ($N = 21$) in the temperate breeding season, when migrants were absent, and in Costa Rica ($N = 60$) during winter, when migrants were present. Statistically significant results are in bold. Separate generalized linear mixed models were used to model number of resident species (Poisson distribution, log link), whether a given resident species responded (binomial distribution, logit link), latency of the first resident species to respond (beta distribution, logit link, overdispersion parameter = 3.28), and minimum approach distance (binomial distribution, logit link). For each model, analysis of deviance was conducted on the predictor variables using type III Wald χ^2 tests. Where a predictor was statistically significant ($\alpha = 0.0125$), we conducted multiple pairwise comparisons among its levels (shown in parentheses); estimates and standard errors (SEs) of the differences (natural log scale for number of resident species, logit scale for whether a given species responded, latency to respond, and minimum approach distance), Z statistics, and adjusted P values ($\alpha = 0.05$; P values adjusted using the sequential Bonferroni method) are reported. Random effect for number of resident species: $N = 414$ trial treatments across 138 trials nested within sites, trial:site variance \pm SD = 0.06 ± 0.25 . Random effects for whether a given species responded: $N = 411$ observations from 42 species during 88 trials nested within sites, species variance $< 0.01 \pm < 0.01$, trial:site variance $< 0.01 \pm < 0.01$. Random effects for latency to respond: $N = 111$ trial treatments involving 34 species and 82 trials nested within sites, species variance $< 0.01 \pm < 0.01$, trial:site variance = 0.04 ± 0.20 . Random effects for minimum approach distance: $N = 81$ trial treatments involving 25 species and 64 trials nested within sites, species variance = 0.01 ± 0.11 , trial:site variance = 0.28 ± 0.53 .

species representing 14 avian families and four orders and thus add to the growing list of animals that respond to heterospecific signals (Magrath et al. 2015). More generally, our study shows that diverse animal species have a mechanism with which they can respond immediately to unfamiliar and potentially salient heterospecific signals.

Such a mechanism has broad implications for heterospecific communication. For example, it could facilitate adaptive behavioral responses by juveniles that have yet to learn the sounds of their environment or by invaders that are leading range expansions into unfamiliar soundscapes. It also provides a simple mechanism that can help explain

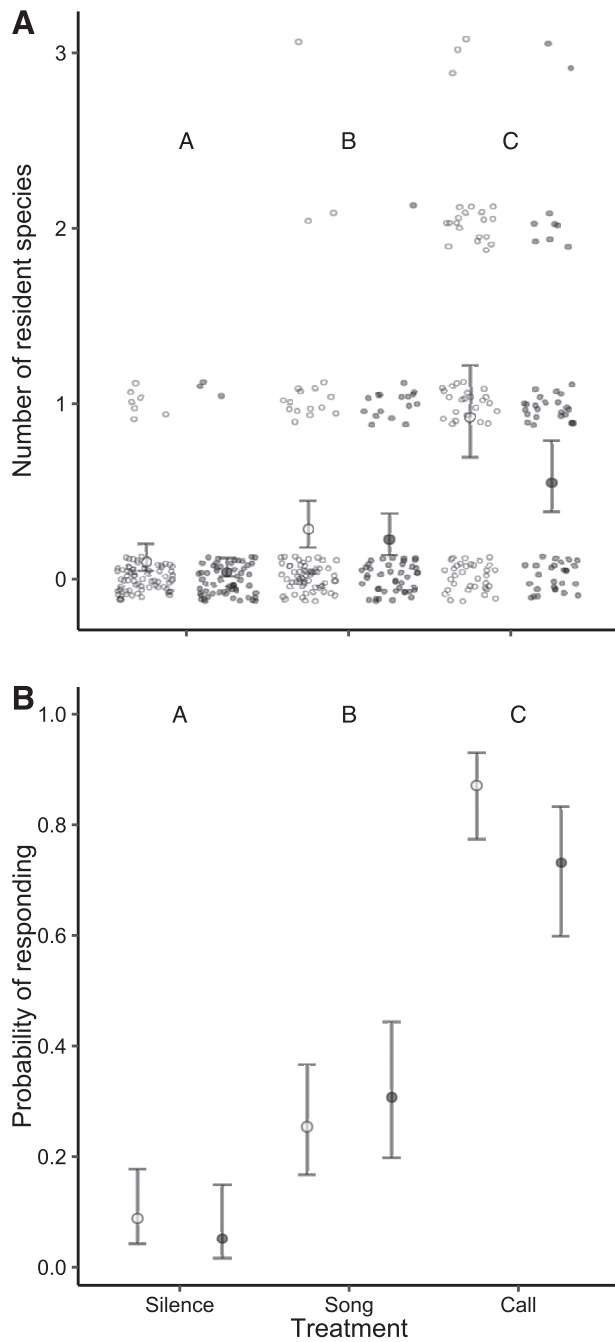


Figure 3: Number of resident species approaching to within 5 m of loudspeaker ($N = 414$ trial treatments; A) and probability of any given resident species approaching to within 5 m of loudspeaker ($N = 411$ observations from 42 species during 88 trials; B). Playback treatments included silence and the fee bee songs and chick-a-dee calls of black-capped chickadees. Trials were conducted in the breeding season, when migrants were absent (open circles), and in the winter, when migrants were present (closed circles). Large circles and error bars show estimated marginal means and their 95% confidence intervals for each combination of treatment and season; estimates are derived from generalized linear mixed models (see table 2 and text

how diverse animal species interact in large and complex multispecies communication networks, since participants would not necessarily need to learn diverse signals and the environmental features they predict.

Several cognitive mechanisms potentially explain the innate responses of Neotropical residents to unfamiliar heterospecific chick-a-dee calls (Magrath et al. 2015; Dutour et al. 2017). One possibility is that certain acoustic characteristics stimulated diverse sensory systems by exploiting deeply rooted sensory biases (Endler and Basolo 1998; Ryan 1998; Rendall et al. 2009). A loud bang, for example, does not necessarily communicate information but nevertheless causes startle effects in many animals (Davis 1984). Harsh sounds, such as the dee notes of chick-a-dee calls, may have similarly widespread effects on arousal and attraction (Fitch et al. 2002). Information-based explanations are also possible. For example, unfamiliar chick-a-dee calls may have characteristics in common with the vocalizations of some Neotropical residents due to phylogenetic signal or convergent evolution (Marler 1955; Morton 1977; Jurisevic and Sanderson 1998; Johnson et al. 2003; Dutour et al. 2017). If such characteristics encode information about arousal or salient stimuli, as the harsh and low-frequency characteristics of chick-a-dee calls and other diverse predator mobbing calls are known to do (Marler 1955; Morton 1977; Jurisevic and Sanderson 1998; Johnson et al. 2003; Templeton et al. 2005; Mahurin and Freeberg 2009; Dutour et al. 2017), then it may be adaptive for resident species to respond to them. It is important to note that although the structure of chick-a-dee calls is highly conserved throughout the Paridae (Hailman 1989; Lucas and Freeberg 2007), the respondent species in our study are largely allopatric to the Paridae (table S1), thus limiting the likelihood that responses evolved in response to chick-a-dee calls specifically. Furthermore, we do not know of any resident species near our study sites that produce signals with characteristics approximating the harsh dee notes of chick-a-dee calls, but it is possible that responses to such characteristics remain conserved in resident species even if the characteristics themselves are no longer produced by local birds (Endler and Basolo 1998; Ryan 1998; Rendall et al. 2009; Dutour et al. 2017). Future playback experiments could use natural variants of chick-a-dee calls to identify candidate structural characteristics that elicit heterospecific responses in Neotropical residents, and additional playbacks

for details) in which the effect of country is averaged among the three countries in which trials were conducted. Raw data are shown as small circles in A only ($N = 234$ trial treatments when migrants were absent, $N = 180$ trial treatments when migrants were present) and are jittered vertically and horizontally to reduce overlap and facilitate viewing. All values are shown untransformed. Treatments with different letters are statistically different from each other.

involving manipulated chick-a-dee calls or synthetic stimuli could then test the causal effect of those traits on receiver responses. Once salient traits are identified, researchers could test whether they are present and phylogenetically conserved among local birds. Variation in the structure (e.g., bandwidth, duration) and number of dee notes predicts responses to chick-a-dee calls by some parids and some sympatric nonparids in the temperate zone (Templeton et al. 2005; Templeton and Greene 2007; Mahurin and Freeberg 2009; Soard and Ritchison 2009; Courter and Ritchison 2010; Randler 2012), so we suggest that future research begin by focusing on these characteristics.

The taxonomic distribution of the Neotropical residents that responded to chick-a-dee calls in our study loosely mirrors the taxonomic distribution of Neotropical residents that are sympatric with our study sites. Most respondents were Passeriformes (84.2%) and Apodiformes (10.5%), which comprise most Neotropical species at our study sites (Passeriformes: 61.1%; Apodiformes: 9.2%; tables 1, S2; fig. 2). Within Apodiformes, only the hummingbirds responded to chick-a-dee calls (family Trochilidae, $N = 4$), whereas in Passeriformes, respondents were distributed widely among Fringillidae ($N = 1$), Furnariidae ($N = 3$), Mitrospingidae ($N = 1$), Parulidae ($N = 1$), Passerellidae ($N = 4$), Thamnophilidae ($N = 1$), Thraupidae ($N = 8$), Troglodytidae ($N = 4$), Turdidae ($N = 4$), and Tyrannidae ($N = 4$; tables S1, S2; fig. 2). These results suggest that respondents are taxonomically widespread and that any sensory biases or shared signal characteristics driving responses are deeply rooted. Our phylogenetic logistic regression found no relationship between whether a species responded and either their body mass or the similarity between their calls and chick-a-dee calls, though the small sample of responding species ($N = 38$) and the limited information available for many Neotropical residents precluded a more comprehensive analysis.

Our results do not support the hypothesis that residents were simply following migrants that were familiar with chick-a-dee calls, since residents' responses were as strong in the temperate breeding season when migrants were absent as they were in the winter when migrants were present. In fact, we were surprised that so few migrants responded to our playbacks during winter, since many are sympatric with the Paridae in summer and have been shown in previous studies to respond to their chick-a-dee calls (table S1; Hurd 1996; Nocera et al. 2008). We know that migrants were present near our study sites because we consistently observed them there. Whether migrants responded to our playbacks may have been related to whether the migrants overwinter at our playback sites or whether they were migrating through (Nocera et al. 2008). Using playbacks of chick-a-dee calls in Belize during the spring migration, Nocera et al. (2008) showed that migrants passing

through respond strongly to chick-a-dee calls, whereas migrants overwintering in Belize do not. It is therefore possible that many of the migrants we saw near our study sites were overwintering there as opposed to migrating through, though a detailed survey of migratory species at the time and location of our playbacks would be needed to establish which migratory species were present but not responding (Nocera et al. 2008). Another possibility is that migrants were present and did respond to our playbacks but did not approach to within the 5-m radius required by our protocol for them to be considered a respondent. In the only other study that we know of that broadcast chick-a-dee calls in the tropics, Nocera et al. (2008) purposefully chose open playback sites that allowed researchers to observe birds within a 20-m radius of the playback speaker. During 48 trials in which chick-a-dee calls were broadcast in that study, 48 individuals from 10 migratory species (out of 24 confirmed to be present at the time and location of the study) approached to within 20 m of the speaker, though it is unclear how many of those approached to within 5 m of the speaker (Nocera et al. 2008). Whatever the reason for the general lack of response by migrants in our study, the residents responding to unfamiliar chick-a-dee calls in our study did not simply follow migrants that were familiar with the calls.

Our findings also do not support the learning hypothesis, which predicts that resident species should never respond to chick-a-dee calls because they have not had the experience necessary to associate the calls with salient stimuli, such as food or predators (Griffin 2004). Consistent with the learning hypothesis, Nocera et al. (2008) showed that resident birds in Belize do not respond to chick-a-dee calls. However, the authors attribute the lack of response to the birds' breeding status, suggesting that active breeders are more likely than inactive breeders to respond to mobbing calls (Nocera et al. 2008). In our study, residents responded strongly to chick-a-dee calls, though we do not know whether respondents were actively breeding at the time of our trials. That residents in our study responded to unfamiliar chick-a-dee calls does not mean that associative learning is unimportant in governing heterospecific responses, only that it was not necessary among the residents observed in our study. Indeed, there is evidence that birds can and do learn to respond to heterospecific vocalizations when they are paired with salient stimuli (Vieth et al. 1980; Griffin 2004; Wheatcroft and Price 2013; Magrath et al. 2015). In many avian species, for example, individuals respond to heterospecific mobbing calls more strongly in regions where signalers and receivers are sympatric versus allopatric (Wheatcroft and Price 2013; Magrath et al. 2015). In other species, naive nestlings do not respond initially to heterospecific alarm calls, despite responding to conspecific alarm calls, yet do respond to

heterospecific alarm calls later in life after gaining experience (Haff and Magrath 2012; Magrath et al. 2015; Dutour et al. 2019; Carlson et al. 2020). A future study could test for additive effects of learning by pairing predator stimuli, such as taxidermic mounts or vocalizations of local predators, with unfamiliar chick-a-dee calls in one group and unfamiliar control calls in another group during a series of learning trials. Afterward, a novel exemplar of a chick-a-dee call would be broadcast to both groups during a test trial. If responses to chick-a-dee calls are exclusively innate, then both groups should respond similarly during the test trial. If learning also contributes, then birds that experienced chick-a-dee calls paired with predator stimuli during the learning trials should respond more strongly.

Fewer resident species responded to playbacks conducted in Brazil than to playbacks conducted in Colombia or Costa Rica, though the probability of any given species responding was the same among countries. The intensity of response among respondents, as reflected by their latency to approach and their minimum distance from the speaker, also did not differ among countries. The difference in the number of species responding cannot be attributed to differences in species richness, since our sympatry analysis indicates that species richness is greater at our two study locations in Brazil (mean = 298 species) than at our two study locations in either Colombia (mean = 242 species) or Costa Rica (mean = 155 species). If structural characteristics of chick-a-dee calls—or the responses of receivers to those characteristics—are phylogenetically conserved, then the weaker response in Brazil could be explained by differences in the phylogenetic relatedness between local avifauna and the Paridae. Future research could test this possibility by broadcasting chick-a-dee calls to diverse tropical species and comparing their phylogenetic relatedness with the Paridae to whether they respond to their calls. Unfortunately, we could not test this relationship because we could not ascertain which species heard our playbacks but chose not to respond. A different playback approach in which species were first confirmed to be present before commencing the playbacks would be needed to test this hypothesis.

Species that are sympatric with black-capped chickadees benefit when responding to chick-a-dee calls by detecting and locating predators earlier than they would without the calls (Pettifor 1990; Flasskamp 1994; Hurd 1996; Pavey and Smyth 1998; Consla and Mumme 2012; Landsborough et al. 2020) and by accessing food they might otherwise fail to discover (Mahurin and Freeberg 2009; Wilson and Mennill 2011). If responses to heterospecific chick-a-dee calls are at least partially innate, as our results based on Neotropical residents suggest, then these foraging and anti-predator benefits could be further enhanced by allowing birds to respond adaptively to chick-a-dee calls the first

time they hear them and thus earlier in life than if they had to learn to associate calls with predators or food through experience (Griffin 2004; Hollén and Radford 2009; Magrath et al. 2015). Although the resident species in our study will not normally hear chick-a-dee calls, our results based on 38 species and 14 avian families suggest that the observed innate response to chick-a-dee calls is taxonomically widespread. Furthermore, nine of the 14 families that responded to chick-a-dee calls in our study (table S1; excluding Furnariidae, Mitrospingidae, Momotidae, Thamnophilidae, Thraupidae) include at least some species that are sympatric with the Paridae throughout much of their range (Billerman et al. 2020) and that therefore could enjoy the benefits of responding innately to chick-a-dee calls.

Acknowledgments

We thank Vicerrectoría de Investigación, Universidad de Costa Rica, for supporting this investigation (project B9-123). We also thank two anonymous reviewers, associate editor Tony Williams, data editor Bob Montgomerie, and coeditor Erol Akçay for providing constructive and insightful feedback that improved the manuscript.

Statement of Authorship

L.S. and D.R.W. conceptualized the experiment, developed the methods, validated the data, and edited the manuscript. L.S. collected the data and acquired the funds for the experiment. D.R.W. conducted data visualization and statistical analysis and wrote the initial draft.

Data and Code Availability

The data and R code to reproduce the figures and analyses have been deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.vdncjxwq>; Sandoval and Wilson 2021).

Literature Cited

- Billerman, S. M., B. K. Keeney, P. G. Rodewald, and T. S. Schulenberg. 2020. *Birds of the world*. Cornell Laboratory of Ornithology, Ithaca, NY.
- BirdLife International and Handbook of the Birds of the World. 2020. Bird species distribution maps of the world. Version 2020.1.
- Bivand, R., R. Keitt, and B. Rowlingson. 2020. rgdal: bindings for the “Geospatial” Data Abstraction Library.
- Bivand, R., and C. Rundel. 2020. rgeos: interface to Geometry Engine–Open Source (‘GEOS’).
- Bloomfield, L. L., L. S. Phillmore, R. G. Weisman, and C. B. Sturdy. 2005. Note types and coding in parid vocalizations. III. The chick-a-dee call of the Carolina chickadee (*Poecile carolinensis*). *Canadian Journal of Zoology* 83:820–833.

- Bradbury, J. W., and S. L. Vehrencamp. 2011. Principles of animal communication. 2nd ed. Sinauer, Sunderland, MA.
- Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, et al. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R Journal* 9:378–400.
- Carlson, N. V., S. D. Healy, and C. N. Templeton. 2020. Wild fledgling tits do not mob in response to conspecific or heterospecific mobbing calls. *Ibis* 162:1024–1032.
- Charrier, I., L. L. Bloomfield, and C. I. Sturdy. 2004. Note types and coding in parid vocalizations. I. The chick-a-dee call of the black-capped chickadee (*Poecile atricapillus*). *Canadian Journal of Zoology* 82:769–779.
- Chesser, R. T., S. M. Billerman, K. J. Burns, C. Cicero, J. L. Dunn, A. W. Kratter, I. J. Lovette, et al. 2020. Check-list of North American birds (online). American Ornithological Society, Chicago.
- Chiszar, D., D. Boyer, R. Lee, J. B. Murphy, and C. W. Radcliffe. 1990. Caudal luring in the southern death adder, *Acanthophis antarcticus*. *Journal of Herpetology* 24:253–260.
- Cicero, C., P. Pyle, and M. A. Patten. 2020. Juniper titmouse (*Baeolophus ridgwayi*). Version 1.0. In P. G. Rodewald, ed. *Birds of the world*. Cornell Lab of Ornithology, Ithaca, NY.
- Consla, D. J., and R. L. Mumme. 2012. Response of captive raptors to avian mobbing calls: the roles of mobber size and raptor experience. *Ethology* 118:1063–1071.
- Courter, J. R., and G. Ritchison. 2010. Alarm calls of tufted titmice convey information about predator size and threat. *Behavioral Ecology* 21:936–942.
- Da Cunha, F. C. R., J. C. R. Fontenelle, and M. Griessera. 2017. Predation risk drives the expression of mobbing across bird species. *Behavioral Ecology* 28:1517–1523.
- Davis, M. 1984. The mammalian startle response. Pages 287–351 in R. C. Eaton, ed. *Neural mechanisms of startle behavior*. Plenum, New York.
- Dawkins, R., and J. R. Krebs. 1978. Animal signals: information or manipulation? Pages 282–309 in N. B. Davies, ed. *Behavioural ecology: an evolutionary approach*. Blackwell, Oxford.
- Dixon, K. L. 1949. Behavior of the plain titmouse. *Condor* 51:110–136.
- Dolby, A. S., and T. C. Grubb. 1998. Benefits to satellite members in mixed-species foraging groups: an experimental analysis. *Animal Behaviour* 56:501–509.
- Dutour, M., J. P. Léna, A. Dumet, V. Gardette, N. Mondy, and T. Lengagne. 2019. The role of associative learning process on the response of fledgling great tits (*Parus major*) to mobbing calls. *Animal Cognition* 22:1095–1103.
- Dutour, M., J. P. Léna, and T. Lengagne. 2017. Mobbing calls: a signal transcending species boundaries. *Animal Behaviour* 131:3–11.
- Dutour, M., T. N. Suzuki, and D. Wheatcroft. 2020. Great tit responses to the calls of an unfamiliar species suggest conserved perception of call ordering. *Behavioral Ecology and Sociobiology* 74:37.
- Endler, J. A., and A. L. Basolo. 1998. Sensory ecology, receiver biases and sexual selection. *Trends in Ecology and Evolution* 13:415–420.
- Fallow, P. M., J. L. Gardner, and R. D. Magrath. 2011. Sound familiar? acoustic similarity provokes responses to unfamiliar heterospecific alarm calls. *Behavioral Ecology* 22:401–410.
- Ficken, M. S., R. W. Ficken, and S. R. Witkin. 1978. Vocal repertoire of the black-capped chickadee. *Auk* 95:34–48.
- Fitch, W. T., J. Neubauer, and H. Herzl. 2002. Calls out of chaos: the adaptive significance of nonlinear phenomena in mammalian vocal production. *Animal Behaviour* 63:407–418.
- Flasskamp, A. 1994. The adaptive significance of avian mobbing. V. An experimental test of the “move on” hypothesis. *Ethology* 96:322–333.
- Fox, J., and S. Weisberg. 2019. An R companion to applied regression. 3rd ed. Sage, Thousand Oaks, CA.
- Gaddis, P. 1985. Structure and variability in the vocal repertoire of the mountain chickadee. *Wilson Bulletin* 97:30–46.
- Goodale, E., G. Beauchamp, R. D. Magrath, J. C. Nieh, and G. D. Ruxton. 2010. Interspecific information transfer influences animal community structure. *Trends in Ecology and Evolution* 25:354–361.
- Grava, A., T. Grava, and K. A. Otter. 2012. Differential response to interspecific and intraspecific signals amongst chickadees. *Ethology* 118:711–720.
- Griffin, A. S. 2004. Social learning about predators: a review and prospectus. *Learning and Behavior* 32:131–140.
- Guilford, T., and M. S. Dawkins. 1991. Receiver psychology and the evolution of animal signals. *Animal Behaviour* 42:1–14.
- Hackett, S. J., R. T. Kimball, S. Reddy, R. C. K. Bowie, E. L. Braun, M. J. Braun, J. L. Chojnowski, et al. 2008. A phylogenomic study of birds reveals their evolutionary history. *Science* 320:1763–1768.
- Haff, T. M., and R. D. Magrath. 2012. Learning to listen? nestling response to heterospecific alarm calls. *Animal Behaviour* 84:1401–1410.
- Hailman, J. P. 1989. The organization of major vocalizations in the paridae. *Wilson Bulletin* 101:305–343.
- Hailman, J. P., and S. Haftorn. 2005. Gray-headed chickadee (*Poecile cinctus*). *Birds of North America Online*. Cornell Lab of Ornithology, Ithaca, NY.
- Hartig, F. 2020. DHARMA: residual diagnostics for hierarchical (multi-level/mixed) regression models.
- Hetrick, S. A., and K. E. Sieving. 2012. Antipredator calls of tufted titmice and interspecific transfer of encoded threat information. *Behavioral Ecology* 23:83–92.
- Hoeschele, M., D. E. Gammon, M. K. Moscicki, and C. B. Sturdy. 2009. Note types and coding in parid vocalizations: the chick-a-dee call of the chestnut-backed chickadee (*Poecile rufescens*). *Journal of the Acoustical Society of America* 126:2088.
- Hollén, L. I., and A. N. Radford. 2009. The development of alarm call behaviour in mammals and birds. *Animal Behaviour* 78:791–800.
- Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* 6:65–70.
- Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50:346–363.
- Humphreys, R. K., and G. D. Ruxton. 2020. Avian distraction displays: a review. *Ibis* 162:1125–1145.
- Hurd, C. R. 1996. Interspecific attraction to the mobbing calls of black-capped chickadees (*Parus atricapillus*). *Behavioral Ecology and Sociobiology* 38:287–292.
- Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* 54:187–211.
- Jetz, W., G. H. Thomas, J. B. Joy, K. Hartmann, and A. O. Mooers. 2012. The global diversity of birds in space and time. *Nature* 491:444–448.
- Jetz, W., G. H. Thomas, J. B. Joy, D. W. Redding, K. Hartmann, and A. O. Mooers. 2014. Global distribution and conservation of evolutionary distinctness in birds. *Current Biology* 24:919–930.
- Johnson, F. R., E. J. McNaughton, C. D. Shelley, and D. T. Blumstein. 2003. Mechanisms of heterospecific recognition in avian mobbing calls. *Australian Journal of Zoology* 51:577–585.

- Jurisevic, M. A., and K. J. Sanderson. 1998. A comparative analysis of distress call structure in Australian passerine and non-passerine species: influence of size and phylogeny. *Journal of Avian Biology* 29:61–71.
- Koda, H. 2012. Possible use of heterospecific food-associated calls of macaques by sika deer for foraging efficiency. *Behavioural Processes* 91:30–34.
- Landsborough, B., D. R. Wilson, and D. J. Mennill. 2020. Variation in chick-a-dee call sequences, not in the fine structure of chick-a-dee calls, influences mobbing behaviour in mixed-species flocks. *Behavioral Ecology* 31:54–62.
- Langham, G. M., T. A. Contreras, and K. E. Sieving. 2006. Why pishing works: titmouse (*Paridae*) scolds elicit a generalized response in bird communities. *Ecoscience* 13:485–496.
- Lloyd, J. E. 1965. Aggressive mimicry in photuris: firefly femmes fatales. *Science* 149:653–654.
- Lucas, J. R., and T. M. Freeberg. 2007. “Information” and the chick-a-dee call: communicating with a complex vocal system. Pages 199–213 in K. A. Otter, ed. *Ecology and behavior of chickadees and titmice: an integrated approach*. Oxford University Press, New York.
- Magrath, R. D., T. M. Haff, P. M. Fallow, and A. N. Radford. 2015. Eavesdropping on heterospecific alarm calls: from mechanisms to consequences. *Biological Reviews* 90:560–586.
- Mahurin, E. J., and T. M. Freeberg. 2009. Chick-a-dee call variation in Carolina chickadees and recruiting flockmates to food. *Behavioral Ecology* 20:111–116.
- Marler, P. 1955. Characteristics of some animal calls. *Nature* 176:6–8.
- McLaren, M. 1976. Vocalizations of the boreal chickadee. *Auk: Ornithological Advances* 93:451–463.
- Migliorati, S., A. M. Di Brisco, and A. Ongaro. 2018. A new regression model for bounded responses. *Bayesian Analysis* 13:845–872.
- Mönkkönen, M., and J. T. Forsman. 2002. Heterospecific attraction among forest birds: a review. *Ornithological Science* 1:41–51.
- Morton, E. S. 1977. On the occurrence and significance of motivation-structural rules in some bird and mammal sounds. *American Naturalist* 111:855–869.
- Moscicki, M. K., M. Hoeschele, and C. B. Sturdy. 2010. Note types and coding in parid vocalizations: the chick-a-dee call of the Mexican chickadee *Poecile sclateri*. *Acta Ornithologica* 45:147–160.
- Mun, J. 2008. Understanding and choosing the right probability distributions. Pages 899–917 in J. Mun, ed. *Advanced analytical models: over 800 models and 300 applications from the Basel II Accord to Wall Street and beyond*. Wiley, Hoboken, NJ.
- National Imagery and Mapping Agency. 1997. Department of Defense World Geodetic System 1984: its definition and relationships with local geodetic systems. National Geospatial-Intelligence Agency, Springfield, VA.
- Nocedal, J., and M. S. Ficken. 2020. Bridled titmouse (*Baeolophus wollweberi*). In A. F. Poole and F. B. Gill, eds. *Birds of the world*. Cornell Lab of Ornithology, Ithaca, NY.
- Nocera, J. J., P. D. Taylor, and L. M. Ratcliffe. 2008. Inspection of mob-calls as sources of predator information: response of migrant and resident birds in the Neotropics. *Behavioral Ecology and Sociobiology* 62:1769–1777.
- Owens, J. L., and T. M. Freeberg. 2007. Variation in chick-a-dee calls of tufted titmice, *Baeolophus bicolor*: note type and individual distinctiveness. *Journal of the Acoustical Society of America* 122:1216–1226.
- Owings, D. H., and E. S. Morton. 1998. *Animal vocal communication: a new approach*. 1st ed. Cambridge University Press, Cambridge.
- Paradis, E., and K. Schliep. 2019. Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35:526–528.
- Pavey, C. R., and A. K. Smyth. 1998. Effects of avian mobbing on roost use and diet of powerful owls, *Ninox strenua*. *Animal Behaviour* 55:313–318.
- Pettifor, R. A. 1990. The effects of avian mobbing on a potential predator, the European kestrel, *Falco tinnunculus*. *Animal Behaviour* 39:821–827.
- Pietsch, T. W., and D. B. Grobecker. 1978. The compleat angler: aggressive mimicry in an antennariid anglerfish. *Science* 201:369–370.
- Randler, C. 2012. A possible phylogenetically conserved urgency response of great tits (*Parus major*) towards allopatric mobbing calls. *Behavioral Ecology and Sociobiology* 66:675–681.
- R Development Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Remsen, J. V. J., J. I. Areta, E. Bonaccorso, S. Claramunt, A. Jaramillo, D. F. Lane, J. F. Pacheco, et al. 2021. A classification of the bird species of South America. American Ornithological Society, Chicago.
- Rendall, D., M. J. Owren, and M. J. Ryan. 2009. What do animal signals mean? *Animal Behaviour* 78:233–240.
- Ryan, M. J. 1998. Sexual selection, receiver biases, and the evolution of sex differences. *Science* 281:1999–2003.
- Sandoval, L., and D. R. Wilson. 2012. Local predation pressure predicts the strength of mobbing responses in tropical birds. *Current Zoology* 58:781–790.
- . 2021. Data from: Neotropical birds respond innately to unfamiliar acoustic signals. *American Naturalist*, Dryad Digital Repository, <https://doi.org/10.5061/dryad.vdncjxswq>.
- Schmidt, K. A., E. Lee, R. S. Ostfeld, and K. Sieving. 2008. Eastern chipmunks increase their perception of predation risk in response to titmouse alarm calls. *Behavioral Ecology* 19:759–763.
- Scully, E. N., B. C. Schuldhaus, J. V. Congdon, A. H. Hahn, K. A. Campbell, D. R. Wilson, and C. B. Sturdy. 2019. ZENK expression in the auditory pathway of black-capped chickadees (*Poecile atricapillus*) as a function of D note number and duty cycle of chick-a-dee calls. *Behavioural Brain Research* 356:490–494.
- Shriner, W. M. K. 1999. Antipredator responses to a previously neutral sound by free-living adult golden-mantled ground squirrels, *Spermophilus lateralis* (Sciuridae). *Ethology* 105:747–757.
- Smith, S. T. 1972. *Communication and other social behavior in Parus carolinensis*. 1st ed. Nuttall Ornithological Club, Cambridge, MA.
- Soard, C. M., and G. Ritchison. 2009. “Chick-a-dee” calls of Carolina chickadees convey information about degree of threat posed by avian predators. *Animal Behaviour* 78:1447–1453.
- Soler, M. 2017. *Avian brood parasitism: behaviour, ecology, evolution and coevolution*. Sinauer, Sunderland, MA.
- Templeton, C. N., and E. Greene. 2007. Nuthatches eavesdrop on variations in heterospecific chickadee mobbing alarm calls. *Proceedings of the National Academy of Sciences of the USA* 104:5479–5482.
- Templeton, C. N., E. Greene, and K. Davis. 2005. Behavior: allometry of alarm calls: black-capped chickadees encode information about predator size. *Science* 308:1934–1937.
- Vallin, A., S. Jakobsson, J. Lind, and C. Wiklund. 2005. Prey survival by predator intimidation: an experimental study of peacock butterfly defence against blue tits. *Proceedings of the Royal Society B* 272:1203–1207.

Vieth, W., E. Curio, and U. Ernst. 1980. The adaptive significance of avian mobbing. III. Cultural transmission of enemy recognition in blackbirds: cross-species tutoring and properties of learning. *Animal Behaviour* 28:1217–1229.

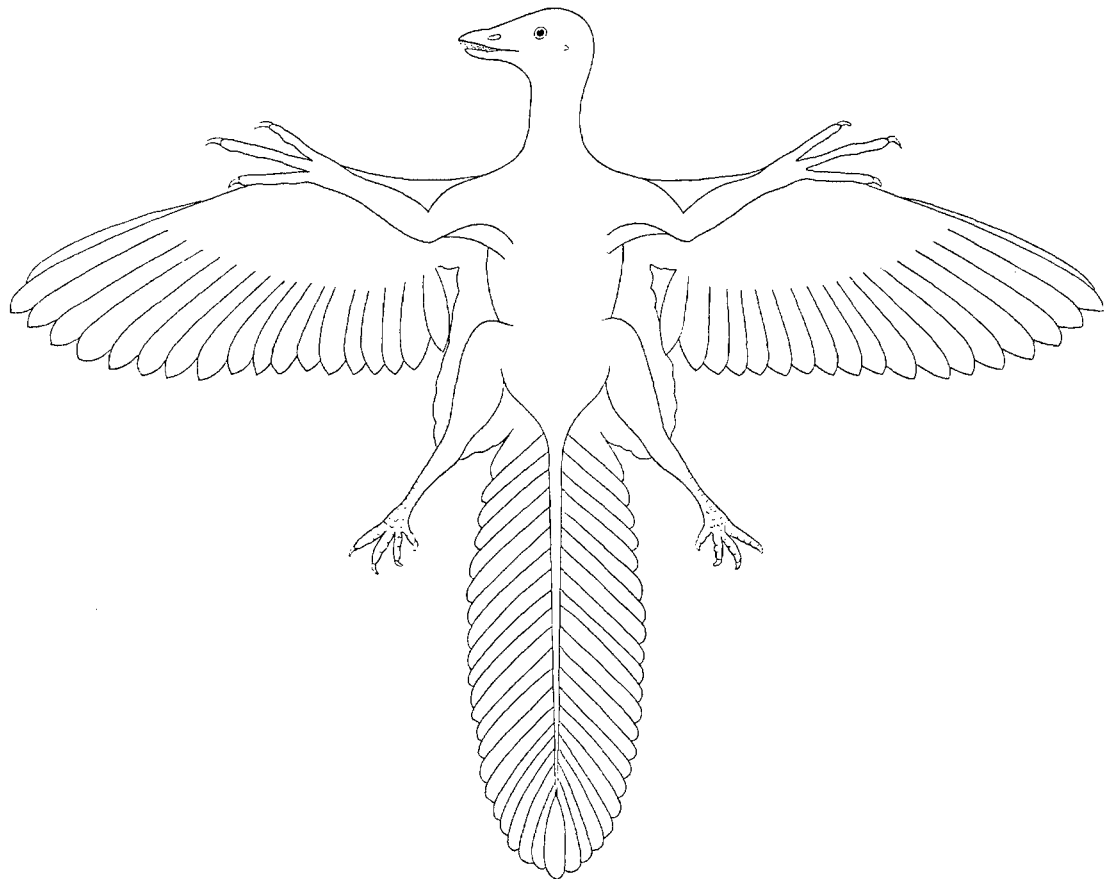
Wheatcroft, D., and T. D. Price. 2013. Learning and signal copying facilitate communication among bird species. *Proceedings of the Royal Society B* 280:20123070.

Wignall, A. E., and P. W. Taylor. 2011. Assassin bug uses aggressive mimicry to lure spider prey. *Proceedings of the Royal Society B* 278:1427–1433.

Wilson, D. R., and D. J. Mennill. 2011. Duty cycle, not signal structure, explains conspecific and heterospecific responses to the calls of black-capped chickadees (*Poecile atricapillus*). *Behavioral Ecology* 22:784–790.

Zuk, M., and G. R. Kolluru. 1998. Exploitation of sexual signals by predators and parasitoids. *Quarterly Review of Biology* 73:415–43/8.

Associate Editor: Tony D. Williams
Editor: Erol Akçay



“Archæopteryx exhibits naturally a more primitive condition of structure than do modern birds, but even here the features are truly bird-like.” Figured: “Archæopteryx macroura.” From “Volant Adaptation in Vertebrates” by Richard S. Lull (*The American Naturalist*, 1906, 40:537–566).