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Referential signaling in a communally breeding bird

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Referential signaling, a complex form of communication in which specific signals are associated with external referents, was once thought to be limited to primates. Recent research has documented referential signaling in several other cooperative taxa, predominantly in kin-based societies. Here, we show that greater anis, communally nesting birds that breed in nonkin groups, give one type of alarm call in response to aerial threats (flying raptors) and another to more general threats (nonaerial predators). Observational data show that anis give these calls in response to different classes of threats, and playback experiments in the field confirmed that the alarm calls alone are sufficient to elicit appropriate behavioral responses even in the absence of an actual threat. Genetic data on a subset of groups confirmed that breeding groups are composed of nonkin, suggesting that referential alarm calls are often given in situations when no genetic relatives are present. These results suggest that complex referential communication can occur in social groups composed of nonrelatives, despite the absence of kin-selected fitness benefits.

behavior | communication | cooperation | referential signaling | communal

Referential signals are reliably associated with specific objects or events in the environment and can convey information to receivers about these referents (1). In nonhuman animals, most examples concern predator-specific alarm calls, typically given in situations in which it is adaptive for listeners to infer the location or identity of the predator and to react accordingly (2). From an evolutionary perspective, referential alarm calls–like nonreferential alarm calls–can be cooperative, since signalers may risk their own safety in order to warn others (3, 4). Referential alarm calls were first documented in social primates that live in family groups (5), leading researchers to link referential signaling with cognitive complexity and to posit that the inclusive fitness benefits of family living might favor its evolution (6, 7).

Recent studies have documented referential alarm calling in a broader array of taxa, including ~20 birds, but the selective pressures favoring it remain understudied (2). In most cases, referential signals are given in contexts in which the signaler and receivers are likely to be genetic relatives (8). Playback experiments have recently confirmed referential alarm calling in two bird species that live in large groups with mixed kin and nonkin (9, 10); however, in these cases, the intended receivers are unknown (e.g., offspring, related group members, or unrelated group members). Therefore, we currently lack strong evidence for referential alarm calling among nonkin, since no studies have yet combined experimental tests on the signals themselves with data on the social contexts in which the signals are naturally given.

Here, we investigate referential alarm calling in the greater ani (*Crotophaga major*), a Neotropical bird that nests in groups composed of 2 to 3 breeding pairs which all contribute offspring to a single nest. All group members participate in parental care and defense of the mixed clutch. Collective defense against nest predators is an important benefit of communal nesting, and adult group members are almost always unrelated. Despite the lack of kin structure, membership of social groups is stable across years (11, 12).

Eggs and nestlings are preyed on by several predators, including snakes, mammals, and yellow-headed caracaras (*Daptrius chimachima*). By contrast, the only recorded predation attempts on adults are by birds of prey, and, rarely, by crocodiles (*Crocodylus acutus*). Field observations in 2017 by A.G.S. suggested that anis give different types of alarm calls in response to different classes of threats, including 1) a high-frequency pulsed call given in the presence of aerial predators, typically raptors flying overhead (the "high cackle;" Fig. 1*A* and Audio S1), and 2) a harsh, low-frequency, broadband call given to a variety of nonaerial threats, including terrestrial predators and perched raptors (the "scold" call; Fig. 1*B* and Audio S2).

We first describe instances of these alarm calls given in natural circumstances during four years of opportunistic field observations and test whether specific calls are associated with specific classes of predators. Next, we describe the social contexts in which alarm calls are given and confirm that referential alarm calls were frequently given in the absence

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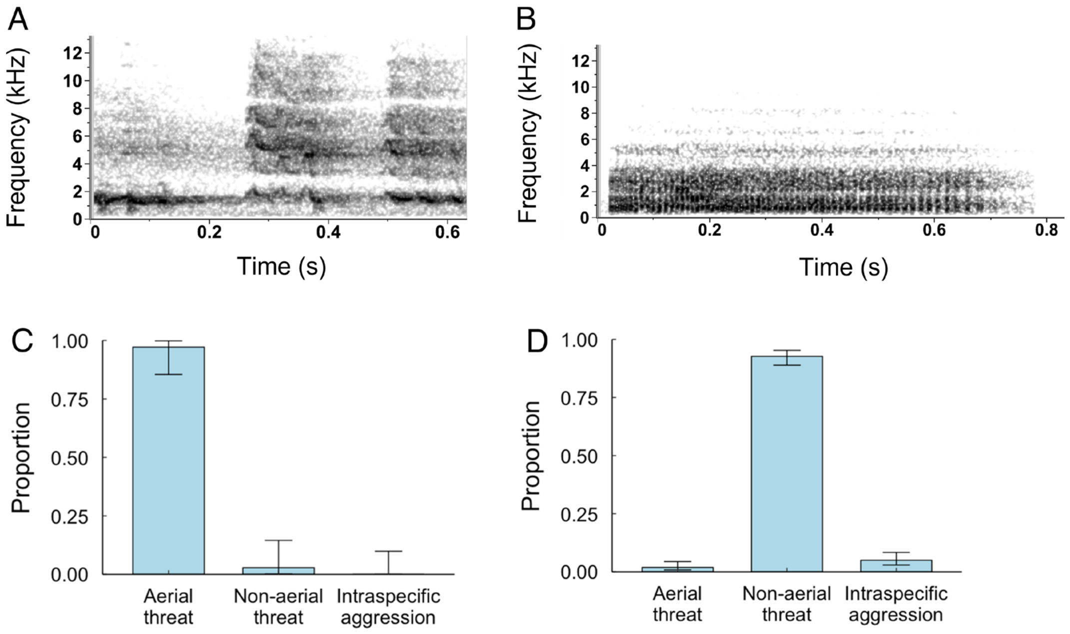

Fig. 1. (*A*) High cackle spectrogram. (*B*) Scold spectrogram. (*C*) Proportions (±95% CI) of high cackle calls associated with different classes of threats. (*D*) Proportions (±95% CI) of scold calls associated with different classes of threats.

of kin. Finally, we use playback experiments to test whether these stimuli elicited appropriate behavioral responses even in the absence of an actual predator.

Methods and Results

Natural Context of Alarm Calls. We opportunistically documented two types of alarm calls (high cackles and scolds) over four y (Dataset S1 and *SI Appendix, Extended Methods*). Overall, 97% of high cackles occurred in the presence of aerial threats, whereas 93% of scolds occurred in the presence of nonaerial threats (Wald $\chi^2 = 7.63$, $P = 0.006$; Fig. 1 *C* and *D*; $N = 35$ high cackles and 260 scolds with identified stimuli). High cackles were almost always associated with the presence of flying raptors ($N = 26$ of 35), including hawks, caracaras, and falcons (*Buteogallus anthracinus*, *B. urubitinga*, *Buteo nitidus*, *Pseudastur albicollis*, *Daptrius chimachima*, and *Falco rufigularis*) or large birds that do not prey on greater anis ($N = 8$ of 35), primarily turkey vultures (*Cathartes aura*). By contrast, scold calls were associated with nonaerial threats ($N = 242$ of 260), including humans, monkeys (*Cebus capucinus, Saguinus geoffroyi*), snakes (*Phrynonax poecilonotus*), and crocodiles.

The high cackle call was given in the presence of conspecifics ($N = 44$ of 45 observations). Of 29 observations with known group composition, offspring (nestlings, fledglings, or related helpers) were present in 21% ($N = 6$), whereas only adult group members were present in 79% ($N = 23$). Although we could not measure relatedness between specific signalers and receivers, genotyping data from a subset of 27 groups in the study population, including 5 in which the high cackle call was observed, confirmed that mean genetic relatedness among adult group members did not differ significantly from zero (Dataset S2 and *SI Appendix, Extended Methods*). Nestlings produced by different pairs within the same communal nest were no more closely related to each other than to nestlings in other nests, confirming that adult breeders were unrelated (mean $r = 0.004 \pm$ SE $= 0.01$ and $r = -0.02 \pm 0.01$, respectively; $Z = 0.80$, $P = 0.43$; *SI Appendix, Extended Methods*).

Field Playback Experiments. We exposed free-living anis to three stimuli: the high cackle, scold, and a control (pale-vented pigeon song, *Patagioenas cayennensis*). We video-recorded focal anis during trials and scored their behaviors (Dataset S3 and *SI Appendix, Extended Methods*). Behavioral responses differed significantly across treatments ($N = 76$ trials; Fig. 2 *A–C*). Anis were more likely to dive downward or move to cover in response to the high cackle (treatment, Wald test: $\chi^2 = 4.52$, $P = 0.03$; Movie S1), and more likely to look around in response to the scold call (treatment, Wald test: $\chi^2 = 14.51$, $P < 0.001$; Movie S2). Response latencies validated this categorical analysis: focal individuals moved their heads more quickly in response to either alarm call than to the control and their whole bodies more quickly in response to the high cackle alarm than to the scold or the control (Fig. 2 *D–F*, Dataset S3, and *SI Appendix, Extended Methods*).

Discussion

Our results demonstrate that greater anis produce different vocalizations in response to aerial and nonaerial threats (the high cackle and the scold), and playback experiments confirmed that these alarm calls elicit appropriate behavioral responses in wild anis. Interestingly, the high cackle call appears to be given only to raptor-like birds in flight, whereas the scold call is given to a variety of nonaerial threats. The combination of a specific aerial alarm with a general nonaerial alarm is common in primates and has been interpreted as evidence for an ancestral origin of referential signaling in that clade (13), but its independent origin in greater anis (and several other birds; ref. 2) instead suggests convergence across multiple lineages. Functionally, a specific aerial alarm is likely to be adaptive in many systems since the position of aerial predators (i.e., above the signaler) is more predictable than that of terrestrial predators (2).

Our observations also suggest that anis can discriminate between different types of raptors, and that the aerial alarm is primarily used for threats to adults. For example, the high cackle alarm was rarely elicited by yellow-headed caracaras (a common predator of eggs and nestlings) and only once by a snail kite (an abundant nonpredator). By contrast, most observations of the high cackle alarm were elicited by black hawks, which are less common in the study area but attack adult anis. This suggests that adult group members, not nestlings, are the most likely receivers of these signals.

Genetic analysis of greater ani nestlings confirmed that adults in breeding groups are unrelated (12). This suggests that referential alarm calling is favored by selective pressures other than kin selection. Since all adult group members reproduce and collectively provide care for the clutch, unrelated coparents may have a direct fitness interest in each other's survival, just as social mates do in species with biparental care. Alarm calling has been

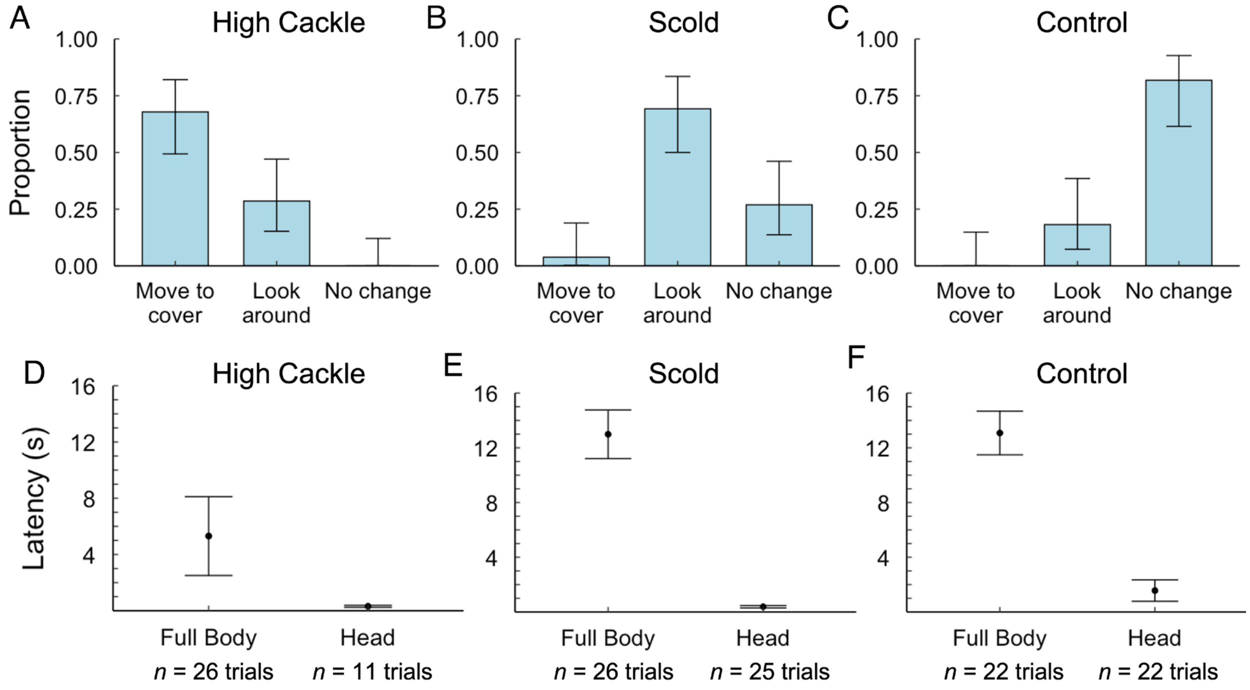

Fig. 2. Behavioral responses to playbacks, showing proportions of trials (±95% CI) resulting in each response type for (*A*) high cackles, (*B*) scolds, and (*C*) control (pale-vented pigeon song); and latency (mean ± 95% CI) to head or full body movement following the (*D*) high cackle, (*E*) scold, and (*F*) control.

hypothesized to warn social mates in several species of socially monogamous birds (14), including at least one referential alarm (15). It is therefore possible that referential alarm calling in greater ani groups represents a form of cooperation among unrelated coparents whose fitness interests are aligned by their communal breeding system.

Data, Materials, and Software Availability. All study data are included in the article and/or *SI Appendix*.

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1. J. M. Macedonia, C. S. Evans, Essay on contemporary issues in ethology: Variation among mammalian alarm call systems and the problem of meaning in animal signals. *Ethology* **93**, 177–197 (1993).
2. S. A. Gill, A. M. K. Bierema, On the meaning of alarm calls: A review of functional reference in avian alarm calling. *Ethology* **119**, 449–461 (2013).
3. J. M. Smith, The evolution of alarm calls. *Am. Naturalist* **99**, 59–63 (1965).
4. P. W. Sherman, Nepotism and the evolution of alarm calls: Alarm calls of belding's ground squirrels warn relatives, and thus are expressions of nepotism. *Science* **197**, 1246–1253 (1977).
5. S. W. Townsend, M. B. Manser, Functionally referential communication in mammals: The past, present and the future. *Ethology* **119**, 1–11 (2013).
6. K. Zuberbühler, "Referential signaling in non-human primates: Cognitive precursors and limitations for the evolution of language" in *Advances in the Study of Behavior*, P. J. B. Slater, J. S. Rosenblatt, C. T. Snowdon, T. J. Roper, (Eds.) & M. Naguib (Collaborator) (Elsevier Academic Press, 2003), **vol. 33**, pp. 265–307.
7. M. Griesser, Referential calls signal predator behavior in a group-living bird species. *Curr. Biol.* **18**, 69–73 (2008).
8. C. L. Smith, Referential signalling in birds: The past, present and future. *Animal Behav.* **124**, 315–323 (2017).
9. L. F. Farrow, S. J. Doohan, P. G. McDonald, Alarm calls of a cooperative bird are referential and elicit context-specific antipredator behavior. *Behav. Ecol.* **28**, 724–731 (2017).
10. L. A. Grieves, D. M. Logue, J. S. Quinn, Joint-nesting smooth-billed anis, *Crotophaga ani*, use a functionally referential alarm call system. *Animal Behav.* **89**, 215–221 (2014).
11. C. Riehl, Z. F. Smart, Climate fluctuations influence variation in group size in a cooperative bird. *Curr. Biol.* **32**, 4264–4269 (2022).
12. C. Riehl, Living with strangers: Direct benefits favour non-kin cooperation in a communally nesting bird. *Proc. Biol. Sci.* **278**, 1728–1735 (2011).
13. C. Cäsar, K. Zuberbühler, Referential alarm calling behaviour in new world primates. *Curr. Zool.* **58**, 680–697 (2012).
14. I. Krams, T. Krama, K. Igaune, Alarm calls of wintering great tits *parus major*: Warning of mate, reciprocal altruism or a message to the predator? *J. Avian Biol.* **37**, 131–136 (2006).
15. S. A. Gill, S. G. Sealy, Functional reference in an alarm signal given during nest defence: Seet calls of yellow warblers denote brood-parasitic brown-headed cowbirds. *Behav. Ecol. Sociobiol.* **56**, 71–80 (2004).