Lack of response to conspecific juvenile vocalizations in two avian brood parasites

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Abstract

Juveniles of interspecific avian brood parasites need to locate and recognize conspecifics to continue their life cycle after being reared by individuals of another species. However, little is known about the recognition mechanisms and cues involved in this critical stage of their lives. It has been proposed that adult parasites could show some kind of parental behavior by actively searching and interacting with conspecific young, which in turn could serve the juveniles to learn and/or reinforce the learning of conspecific characteristics. Since acoustic communication is one of the main channels used by birds, if such form of parental behavior by adult avian brood parasites does exist, adult cowbirds should recognize and respond positively to vocalizations of conspecific juveniles. We experimentally tested whether adult shiny *Molothrus bonariensis* and screaming *M. rufoaxillaris* cowbirds respond positively towards acoustic signals of conspecific juveniles using two playback experiments, one conducted in semi-captivity and the other in the field. In both cases, we presented 3 types of playbacks to adults of both cowbird species: vocalizations of screaming cowbird juveniles, and white noise as a control. In the experiment in semi-captivity, no significant differences were found between treatments. In the field experiment, no conspecific sapproached the playback area, but hosts of the species whose playback was played were attracted to the loudspeaker. In conclusion, our results do not support the predictions we tested from the parental behavior hypothesis and indicate that adult shiny and screaming cowbirds do not respond positively to acoustic signals of conspecific juveniles, at least not to the type of signals used in our experiments.

Key words: brood parasitism, fledglings, *Molothrus*, parental behavior hypothesis, vocal recognition.

The juvenile stage represents a critical period in the life of birds during which individuals must develop the flight and feeding abilities that will allow them to survive as adults (Naef-Daenzer and Grüebler 2016). At this stage, young birds also learn the characteristics of their species that are necessary to recognize conspecifics and potential sexual partners in the future (Oetting et al. 1995; Ten Cate and Vos 1999). In most bird species, this learning process occurs at a very young age, usually using their parents or siblings as models (Grant and Grant 1997, 2018; Irwin and Price 1999; Slagsvold and Hansen 2001; Slagsvold 2019). However, in interspecific avian brood parasites, where females lay their eggs in the nests of other species and avoid all parental care, this learning process would lead to misimprinting on the host species. Obviously, a different process must be at play in brood parasites to avoid imprinting on heterospecifics, but little is known about the mechanisms and cues involved in conspecific recognition in these taxa (but see Hauber et al. 2001 and Crudele et al. 2023 for a possible mechanism in brood-parasitic cowbirds). Examining the mechanisms of recognition and association with conspecifics is thus particularly relevant in this group (Göth and Hauber 2004). Despite the importance of early social development for the success of the brood-parasitic strategy, data on interactions of young parasites with conspecifics are lacking for most parasite lineages

(i.e., most Cuculidae species, the South American blackheaded duck Heteronetta atricapilla or the African honeyguides). The scarce available data come mainly from studies on the great spotted cuckoo Clamator glandarius and cowbirds (Kennerley et al. 2022). At first, it was suggested that interactions between young parasites and adult conspecifics began in foraging grounds once independence was achieved (Woodward 1983). However, later studies showed that young parasites can recognize and/or interact with adult conspecifics from earlier stages of development (Soler and Soler 1999; Hauber et al. 2001; Hauber 2002; Crudele et al. 2022). For example, adult great spotted cuckoos may occasionally visit nests of its magpie *Pica pica* host during the last days of the nestling period and maintain contact with conspecific fledglings later, interacting with them vocally or even occasionally feeding them (Soler and Soler 1999). In cross-fostering experiments of cuckoo chicks to host nests outside the cuckoo's breeding range, where visual or acoustic communication with adult parasites was not possible, young cuckoos learned to recognize conspecifics only when reared alongside another parasite chick in the nest, which would indicate that recognition is not innate but must be learned (Soler and Soler 1999). Interestingly, in another cross-fostering experiment in a host population sympatric with the cuckoo but with no records of parasitism, parasitic fledglings aggregated with conspecifics in

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the presence of an adult cuckoo, suggesting the adult facilitated this recognition (Soler and Soler 1999). Furthermore, a great spotted cuckoo female was recorded interacting with her own fledglings, even though interactions between adults and fledglings would not be limited to related individuals (Soler and Soler 1999). These observations have led to the proposal of the parental behavior hypothesis, which suggests that adult parasites could visit nestlings/fledglings as a parental behavior that allows imprinting to occur.

This hypothesis has also been suggested for the brownheaded cowbird Molothrus ater, but the evidence supporting it is indirect (Hahn and Fleischer 1995; Hauber 2002). Early DNA fingerprinting studies revealed that brown-headed cowbird females associate with their own juvenile offspring at feeding sites more often than would be expected by chance (Hahn and Fleischer 1995). Moreover, the probability of detecting female brown-headed cowbirds near host nests was higher during the early fledgling stage than during the incubation and nestling stages and higher near parasitized than non-parasitized nests (Hauber 2002). White et al. (2002) found an effect of the presence of conspecific adults on the social behavior of parasitic juveniles in a set of experiments that showed that young brown-headed cowbirds were sensitive and responded to the presence of conspecific adults. The juveniles aggregated more with other juveniles and sang more often when they were in the presence of adults than when housed without adults. Some of the benefits for juvenile cowbirds of joining conspecifics soon after becoming independent can include lowered risk of predation by foraging in groups, facilitation in locating food sources and communal roosts, settlement of dominance hierarchies at an earlier age, and learning of a greater repertoire of vocal and courtship features (West and King 1988; Freeberg et al. 1995, 1999; O'Loghlen and Rothstein 1995; West et al. 1996; Hauber et al. 2000, 2001; Smith et al. 2002; White et al. 2002).

There are two possible non-mutually exclusive mechanisms for conspecific recognition in brood parasites. Recognition and initiation of socialization could be started by adults approaching juveniles and/or by juveniles recognizing and approaching adults. Most studies on conspecific recognition in brood parasites have focused on the second mechanism (i.e., recognition of adults by juvenile parasites) and have involved cowbird juveniles raised or kept in captivity (King and West 1977; Hauber et al. 2001; White et al. 2002; Miller et al. 2006; Crudele et al. 2023), and showed that the cowbird's "chatter call" might serve as an initial password that unambiguously identifies the chatterer as a conspecific, since cowbird nestlings and fledglings preferentially respond to chatters but not to other conspecific and heterospecific vocalizations. On the other hand, only one previous study (Hauber 2002) has investigated whether there is recognition of juveniles by adults as a possible mechanism to learn conspecific characteristics. Hauber (2002) showed that brown-headed cowbird adults preferentially associate with conspecific juveniles when given a choice between a conspecific or a heterospecific individual. In the present work, we studied the recognition of juvenile vocalizations by adults in another two cowbird species, where this type of recognition has not been studied yet.

In birds, acoustic signals represent the main communication channel. Birds vocalize to communicate with conspecifics, mainly to attract mates or establish a territory, but avian vocalizations can serve other purposes, such as maintaining group contact or signaling about food or danger (Catchpole and Slater 2008; Gil and Llusia 2020). In cowbirds, recognition of certain stereotypical, species-specific vocalizations is innate in juveniles and adults (King and West 1977; Hauber et al. 2001; Hauber 2002; Crudele et al. 2022), so if adult cowbirds actively contact juveniles, it is expected that adult parasites respond positively to conspecific juvenile vocalizations.

In this study, we experimentally evaluated whether adults of shiny M. bonariensis and screaming M. rufoaxillaris cowbirds can recognize conspecific juveniles based on acoustic signals. The shiny cowbird is a generalist brood parasite that lays its eggs in nests of over 250 host species, with at least 102 of them raising them successfully to fledglings (Ortega 1998; Lowther 2023). During the reproductive season, females lay their eggs before sunrise and spend the rest of the day feeding and searching for potential host nests to parasitize (Gloag et al. 2013; Scardamaglia and Reboreda 2014; Scardamaglia et al. 2017). They are socially promiscuous, and females search for host nests without male assistance (Wiley 1988; Kattan 1997; Gloag et al. 2013; Scardamaglia and Reboreda 2014). On the other hand, the screaming cowbird is one of the most specialized brood parasites, using the gravish baywing Agelaioides badius as its primary host throughout most of its distribution (De Mársico et al. 2010). They are socially monogamous, with males and females searching for host nests together (Mason 1987; Scardamaglia and Reboreda 2014; Scardamaglia et al. 2022).

We tested if adult shiny and screaming cowbirds are attracted by acoustic signals from conspecific juveniles through playback experiments. We predicted that adult cowbirds should: 1) spend a higher proportion of time near the loudspeaker when conspecific juvenile calls were broadcast than when heterospecific juvenile calls or a control sound were broadcasted; 2) approach the loudspeaker faster (i.e., shorter latency) when conspecific juvenile calls were broadcasted than when heterospecific juvenile calls or a control sound were broadcasted; and 3) approach the loudspeaker more frequently in response to conspecific juvenile calls than heterospecific juvenile calls or a control sound.

Materials and Methods

Study site

The study was conducted at Reserva El Destino (35°08'S, 57°23'W), near the town of Magdalena, in the province of Buenos Aires, Argentina, from December 2018 to January 2019, during the reproductive season of shiny and screaming cowbirds. At this site, the shiny cowbird uses as its main hosts the chalk-browed mockingbird (*Mimus saturninus*; frequency of parasitism 89%, Gloag et al. 2013) and the house wren (*Troglodytes aedon*; frequency of parasitize baywing nests with a frequency close to 100% (De Mársico et al. 2010).

Experimental design

The study consisted of playback experiments in semi-captivity and in the field. All experiments were video recorded and later analyzed using BORIS (Behavioral Observation Research Interactive Software) version 7.4.7 (Friard and Gamba 2016).

Playback sequences

Calls for broadcasts were obtained from audio recordings of hand-reared fledglings (13–20 days after hatching) performed under standardized conditions during a previous study (De Mársico et al. 2012). All recorded vocalizations consisted of begging calls (i.e., calls uttered by juveniles during food solicitation displays). To avoid pseudoreplication, we used vocalizations of at least 7 different individuals per species (7 screaming cowbirds and 10 shiny cowbirds). Each recording was converted to a spectrogram using default settings in Raven Pro 1.4 (K. Lisa Yang Center for Conservation Bioacoustics 2014). Spectrograms were then visually inspected to extract 10-20 good-quality calls per individual. With the selected calls, we created 3-min broadcast sequences (one per individual) using Raven Pro 1.4 editing tools. The sequences consisted of 18 bouts of 5 calls each, standardized at a call rate of 1 call per second and separated by 5 sec of silence. The amplitude of calls used during playbacks was standardized with Root mean square within and between samples. As a control sound, we used white noise samples created using version 2.2.1 of Audacity® recording and editing software (Audacity Team 2017). We generated 20-msec pulses of white noise (i.e., a random signal of constant power density) with the default settings of the noise generator tool, and then used these pulses to create the playback sequences in the same manner as the experimental call stimuli. White noise playbacks therefore consisted of 18 bouts of 5 pulses of white noise each, broadcast at a rate of 1 pulse per second and separated by a 5-s silence period.

Semi-captivity experiment

We captured 9 shiny cowbirds (4 females and 5 males) and 13 screaming cowbirds (8 females and 5 males) using mist nets and walk-in traps baited with millet. Each bird was banded with a unique color-ring combination and a metal ring with a unique identification number. Blood samples (20–30 l) were drawn from the brachial vein to confirm the sex of screaming cowbird individuals by molecular sexing, since they show low sexual dimorphism (for details, see De Mársico et al. 2010). After capture, birds were transferred to a laboratory in the field site and water ad libitum until the next morning, when experiments were carried out.

Playback experiments were conducted in a 7 m \times 1 m \times 1 m (length \times width \times height) experimental arena constructed with a steel frame and covered by nylon anti-bird garden netting. The experimental arena (Figure 1) was divided into 3

areas: a neutral area located in the center (3 m length, containing 6 equidistant perches) and 2 response areas (right and left, 2 m length each, containing 4 equidistant perches each). A loudspeaker (model Philips BT2500B), from which the stimuli were broadcast using a wav/mp3 audio player (Zoom N4H, Zoom, Hauppage, NY, USA), was placed at the corner of each response area, and food and water were available in the neutral area.

All experiments were carried out between 8:00 AM and 1:00 PM. Each trial began with the release of the bird inside the experimental arena, where it was left to habituate for 40 min. Three different treatments were presented sequentially, each consisting of 3-min broadcast sessions, preceded by a 10-min period of silence. Each treatment was played from one of the two loudspeakers placed in the response areas. Broadcast treatments consisted of 1) vocalizations of shiny cowbird juveniles; 2) vocalizations of screaming cowbird juveniles; and 3) white noise as a control. Each bird was tested only once, using a randomly selected sequence of each treatment. The order of presentation of treatments and the location of the active speaker (right/left) in the experimental arena were counterbalanced between individuals. We quantified the intensity of response to playbacks using the following variables: 1) the time elapsed until the bird entered the active speaker area ("latency"), 2) the number of times the bird approached (i.e., made contact with) the loudspeaker ("approaches"), and 3) the proportion of time that the bird spent in the active speaker area ("duration").

Field experiment

To study if shiny and screaming cowbird adults are attracted by vocalizations of conspecific juveniles, we conducted a playback experiment in the field. We chose 14 random locations within the study site, where we had seen or heard both species of cowbirds immediately before the onset of the experiment. At each site, we presented 3 different treatments sequentially, each consisting of 3-min broadcast sessions, preceded by a 5-min period of silence. Broadcast treatments consisted of 1) vocalizations of shiny cowbird juveniles; 2) vocalizations of screaming cowbird juveniles; and 3) white noise as a control.

Before the start of each trial, a loudspeaker (model Philips BT2500B) was placed in a tree branch. The loudspeaker was connected to a wav/mp3 audio player (Zoom N4H, Zoom,

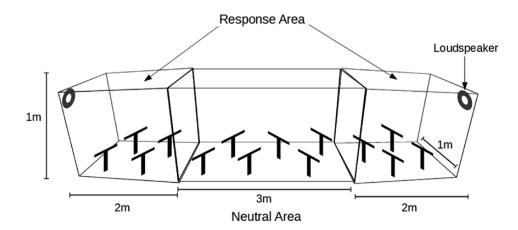


Figure 1 Experimental arena and setup. The arena consisted of two response areas to the sides, each containing 4 perches, and a neutral area in the center, containing 6 perches. Each response area had a loudspeaker from where the stimuli were broadcast.

Hauppage, NY, USA) from which we played the treatments. We broadcast call treatments from a hide placed 10 m away from the loudspeaker, from which we simultaneously monitored bird behavior in real time using 8×42 binoculars. The experiments were carried out in the morning between 10 AM and 12 PM, and in the afternoon between 4 PM and 6 PM. The order of presentation of treatments was counterbalanced between sites. We quantified the response to playbacks using the following variables: 1) species of the first individual to respond (i.e., approaching within a 5-m radius of the speaker), 2) number of individuals of each species that responded, and 3) emission of vocalizations and identity of the individuals that emitted them.

The protocol and procedures employed were ethically reviewed and approved by the Provincial Organism for Sustainable Development (Disp. N° DI-2019-48, OPDS, Buenos Aires, Argentina) and comply with the current laws of Argentina.

Statistical analysis

We tested for differences in latency among treatments for each species using a stratified Cox proportional hazard model, as suggested by Jahn-Eimermacher et al. (2011), using the coxph function from the survival library (Therneau 2015) in R 3.6.0 (R Core Team 2020). The model included treatment and treatment order as fixed factors, and bird identity as a random effect. The frequency to approach the active loudspeaker was not analyzed statistically because approaches were too few to make comparisons (see Results). We used Wilcoxon tests to analyze whether the proportion of time spent by the birds in the response area during each treatment did not differ from that expected by chance (0.29), which would indicate no response to treatments, or whether it was higher or lower, which would indicate attraction or aversion to playbacks, respectively. The proportion of time expected by chance at each of the response areas was 0.29 (= 2/7) since the total length of the experimental arena was 7 m, and each response area was 2 m long. Furthermore, to analyze whether the duration of the response varied across treatments for each species, we used generalized linear mixed models with beta distributions. Models were fitted using the glmmTMB function included in package glmmTMB in R version 3.6.0 (R Core Team 2020). We included treatment (type of playback: conspecific, heterospecific, control), and treatment presentation order as fixed factors, and bird identity as a random effect. Due to the type of data obtained (see below) results from the field experiment were analyzed qualitatively.

Results

Semi-captivity experiment

For shiny cowbirds, the latency to respond to playbacks did not differ either between treatments ($\chi^2 = 2.39$, P = 0.30) or the order of presentation of treatments ($\chi^2 = 0.62$, P = 0.43). Similarly, for screaming cowbirds, the latency to respond to playbacks was not associated with treatment ($\chi^2 = 1.87$, P =0.39) nor the order of presentation of treatments ($\chi^2 = 0.02$, P = 0.88).

Regarding the number of approaches to the loudspeaker, none were recorded for shiny cowbirds, while only two screaming cowbirds approached the loudspeaker 4 and 3 times, respectively during the conspecific vocalization treatment. Duration in the response area during treatments did not differ from that expected by chance in any of the cases (all tests P > 0.05). Furthermore, response duration did not differ between treatments for shiny cowbirds (Figure 2A, Table 1A) or screaming cowbirds (Figure 2B, Table 1B). The order of presentation had no effect on the response in either species.

Field experiment

In 7 out of the 14 trials, playback of juvenile vocalizations attracted birds, yet not conspecific adults. In all cases, the individuals that approached the loudspeaker were of a host species of the cowbird whose playback was being played (Table 2). All these birds vocalized in response to the juvenile vocalizations broadcast.

Discussion

In this paper, we studied if adults are attracted by conspecific begging calls in two avian brood parasites, testing some predictions of the parental behavior hypothesis in both semi-captivity and field playback experiments. Contrary to our predictions, adult cowbirds did not respond differentially to conspecific vocalizations of juveniles in the semi-captivity experiment. Moreover, no conspecifics were attracted during the field experiment, despite being present in the area while the playbacks were being played, reinforcing our result that adult parasites do not respond to conspecific juvenile vocalizations.

One possible explanation for these negative results is that both acoustic and visual cues are necessary to elicit a response. Hauber (2002) performed choice trials with brownheaded cowbird adults in the laboratory to determine if they approached conspecific juveniles preferentially. Unlike the present work, this study used live birds, thus visual and acoustic signals were simultaneously presented. Two sets of stimulus pairs were presented: the first set composed of a juvenileplumaged female brown-headed cowbird and an adult male song sparrow Melospiza melodia, and the second set composed of a juvenile-plumaged male brown-headed cowbird and an adult male red-winged blackbird Agelaius phoeniceus. The results differed from ours since he observed that both brown-headed cowbird females and males associated with conspecific juveniles significantly more than expected by chance. So, it is possible that vocal recognition occurs after visual exposure to the juvenile or that the interaction of both stimuli is necessary for eliciting recognition.

Another possible explanation is that the acoustic signals used in the experiments were not adequate to trigger a response in adult cowbirds. Considering that the recordings used corresponded to begging calls of fledglings between 13 and 20 days of age, it is possible that the stimulus needed to trigger a response is other types of vocalizations or vocalizations of older individuals. Preliminary results of two studies in which juveniles of shiny and screaming cowbirds were radio-tracked after fledging indicate they disperse and leave their host territories at around 38-52 days of age (Crudele et al., unpublished data and De Mársico et al., unpublished data). So, it is possible that fledglings older than 20 days emit other types of vocalizations before the age of dispersion and that those are recognized by adult parasites. Nonetheless, it is important to note that the acoustic signals used in the field experiment were effective in attracting individuals of common hosts of shiny and screaming cowbirds, which indicates that the lack of response was not due to the stimuli being unrealistic or to

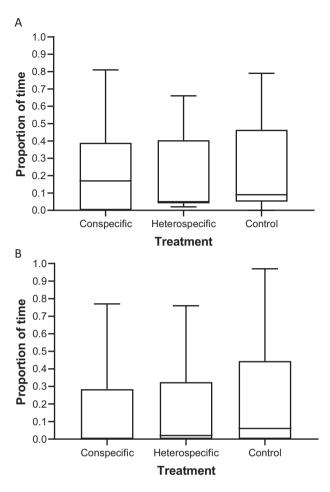


Figure 2 Proportion of time spent in the active loudspeaker area as a function of treatment (playback type: conspecific, heterospecific, and control) for (A) shiny cowbirds and (B) screaming cowbirds.

 Table 1 Results of the generalized linear mixed models for the duration of response that birds spent in the active speaker area as a function of treatment (playback type: conspecific, heteroespecific, and control) and order of presentation of treatments for (A) shiny cowbirds and (B) screaming cowbirds

Parameters	Coefficients	SE	z	Р
(A)Shiny cowbirds				
Intercept (Conspecific)	-0.86	0.60	-1.43	0.15
Treatment (Heterospecific)	-0.18	0.48	-0.38	0.70
Treatment (Control)	0.05	0.48	0.11	0.91
Order	-0.06	0.25	-0.25	0.80
(B) Screaming cowbirds				
Intercept (Conspecific)	-1.18	0.50	-2.33	0.02
Treatment (Heterospecific)	0.50	0.43	1.16	0.25
Treatment (Control)	0.73	0.41	1.78	0.08
Order	-0.35	0.22	-1.60	0.11

the experimental design per se. Similarly to what we found in our field experiment, when a stuffed great spotted cuckoo fledgling was presented simultaneously with a cuckoo fledging playback in the field, adult hosts (i.e., magpies) were attracted to the site (Soler et al. 2014). Furthermore, the response of adult magpies to the playback-stuffed cuckoo depended on

 Table 2 Playback treatment, species, and number of individuals that approached the loudspeaker for experiments where there was a response

Sample #	Treatment (playback)	Recruited species	# recruited birds	
1	Screaming cowbird	Baywing	1	
5	Screaming cowbird	Baywing	3	
6	Shiny cowbird	House wren	2	
7	Shiny cowbird	Chalk-browed mockingbird	2	
8	Screaming cowbird	Baywing	4	
9	Shiny cowbird	Rufous-collared sparrow	3	
12	Shiny cowbird	Chalk-browed mockingbird	2	

The baywing *Agelaioides badius* is the exclusive host to the screaming cowbird in the study area. The house wren *Troglodytes aedon*, the chalkbrowed mockingbird *Mimus saturninus*, and the rufous-collared sparrow *Zonotrichia capensis* are common hosts to the shiny cowbird in the study area.

whether the adults were caring for cuckoo fledglings themselves or caring only for their own magpie fledglings, being willing to feed or attack the model, respectively (Soler et al. 2014). In our case, we could not determine whether the hosts that were attracted to the site were caring for parasite fledglings, and since no stuffed model was presented, we observed neither positive nor negative responses from hosts, but just attraction to the playback site.

Finally, a third explanation for our results is that recognition and initiation of socialization depend exclusively on juveniles and not adults, that is, that juveniles themselves are the ones that respond to vocalizations and/or visual cues of adults. Previous field studies and laboratory choice experiments support an active role of fledglings in their social interactions with conspecific adults. In field observations, juvenile brown-headed cowbirds were seen "chasing" adults soon after becoming independent from their foster parents (Woodward 1983; Hauber et al. 2001), while in a laboratory choice experiment, juvenile cowbirds spent a greater proportion of time in the proximity of a conspecific female adult stimulus (Hauber 2002). Furthermore, Hauber et al. (2001) suggested that cowbirds may use "passwords" for species recognition. Young parasites could innately recognize certain phenotypic traits that are reliable signs of conspecific identity, such as certain vocalizations produced by the adults. This could in turn trigger additional learning of the phenotypic characteristics of the signaler, such as plumage characteristics or certain behaviors. For example, in the brown-headed cowbird, the chatter call of adult males and females (an innate species-specific vocalization) has been proposed as such a password (Hauber et al. 2001), and this mechanism was confirmed in shiny cowbirds, where conclusive evidence shows that imprinting is the mechanism that allows conspecific recognition and is initiated after juveniles listen to the chatter call (Crudele et al. 2023). A similar mechanism could be at play in parasitic male indigobirds (Vidua spp.), which exhibit mimicry songs that are

5

common to conspecific males that share the same host species and non-mimicry songs that are common to conspecific males from the same local neighborhood (Payne et al. 1998; Da Costa and Sorenson, 2014). Young males who disperse outside their local "dialect neighborhood" may use mimicry songs to identify conspecifics (Da Costa and Sorenson 2014).

We acknowledge that a potential issue when carrying out studies with birds captured from the wild is that they may not express their natural behavior in the cage. However, two points can be made, which allow us to trust our results. First, previous studies carried out with caged brown-headed cowbird adults testing whether they approached conspecific or heterospecific juveniles showed differential responses towards the different stimuli (Hauber 2002). Similarly, caged juvenile shiny cowbirds responded to shiny cowbird adults' stimuli (Crudele et al. 2023). Second, the consistency of the results obtained in the semi-captivity experiment with those of the field playback experiment further suggests a true lack of response of the birds tested in cage.

In conclusion, our results are not consistent with the predictions from the parental behavior hypothesis that we tested (Soler and Soler 1999), as they show that adults of shiny and screaming cowbirds do not respond differentially to acoustic signals of conspecific juveniles, at least not to begging calls of fledglings between 13 and 20 days of age. Therefore, begging calls would not be enough stimulus to attract adult cowbirds, or adults' visits to territories where young would not be guided by the presence of nestlings/fledglings but for other reasons (i.e., adults may visit host territories searching for new nests and during these visits, if they find young, they interact with them). The agreement of the results in semicaptivity and field experiments supports this interpretation.

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Conflict of Interest

The authors declare no conflicts of interest.

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