

Innate development of acoustic signals for host parent–offspring recognition in the brood-parasitic Screaming Cowbird *Molothrus rufoaxillaris*

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Young birds communicate their need to parents through complex begging displays that include visual and acoustic cues. Nestlings of interspecific brood parasites must ‘tune’ into these communication channels to secure parental care from their hosts. Various studies show that parasitic nestlings can effectively manipulate host parental behaviour through their begging calls, but how these manipulative acoustic signals develop in growing parasites remains poorly understood. We investigated the influence of social experience on begging call development in a host-specialist brood parasite, the Screaming Cowbird *Molothrus rufoaxillaris*. Screaming Cowbird nestlings look and sound similar to those of the primary host, the Greyish Baywing *Agelaioides badius*. This resemblance is likely to be adaptive because Baywings discriminate against fledglings unlike their own and provision nests at higher rates in response to Baywing-like begging calls than to non-mimetic begging calls. By means of cross-fostering and playback experiments, we tested whether the acoustic cues that elicit recognition by Baywings develop innately in Screaming Cowbird nestlings or are acquired through social experience with host parents or nest mates. Our results suggest that begging call structure was partially modulated by experience because Baywing-reared Screaming Cowbird and host nestlings were acoustically more similar as age increased, whereas acoustic similarity between cross-fostered and Baywing-reared Screaming Cowbird nestlings decreased from 4–5 to 8–10 days of age. Cross-fostered Screaming Cowbirds developed begging calls of lower minimum frequency and broader bandwidth than those of Baywing-reared Screaming Cowbirds by the age of 8–10 days. Despite the observed differences in begging call structure, however, adult Baywings responded similarly to begging calls of 8- to 10-day-old cross-fostered and Baywing-reared Screaming Cowbirds, suggesting that these were functionally equivalent from the host’s perspective. These findings support the idea that, although rearing environment can influence certain begging call parameters, the acoustic cues that serve for offspring recognition by Baywings develop in young Screaming Cowbirds independently of social experience.

Keywords: begging call, brood parasitism, vocal development, vocal mimicry.

Avian obligate brood parasites must obtain adequate parental care from heterospecific hosts to survive to independence. In altricial birds, dependent young stimulate parental provisioning through begging displays that combine complex

visual and acoustic traits such as gape coloration, body postures and vocalizations (Wright & Leonard 2002). The acoustic component of begging displays (i.e. begging calls) plays a major role in signalling offspring identity and need, and can be used by parents to adjust their provisioning effort and allocate food within the brood (Beecher *et al.* 1981, Burford *et al.* 1998, Leonard & Horn 2001, Glassey & Forbes 2002). Parasite nestlings are thus

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expected to be under selection to ‘tune’ into the acoustic communication system of their hosts to secure a sufficient food supply.

It is well known that young brood parasites can manipulate host parental behaviour effectively via their begging calls, for example by calling faster and louder than host young or by including in their begging calls acoustic features that better stimulate host provisioning (Kilner *et al.* 1999, Madden & Davies 2006, Gloag & Kacelnik 2013, Rivers *et al.* 2014). In addition to call rate and amplitude, the acoustic structure of parasite begging calls itself may influence host behaviour. This is the case for some host-specialist parasites that trick their hosts into accepting them by vocally resembling host young (Langmore *et al.* 2003, 2008). However, how begging call structure develops in growing parasites remains poorly understood.

Experimental studies involving cross-fostering of parasitic nestlings to alternative hosts suggest that begging call structure is not genetically fixed but can be learned or shaped by early social experience, at least to some extent (Madden & Davies 2006, Langmore *et al.* 2008, Roldán *et al.* 2013). In the host-specialist Horsfield’s Bronze Cuckoo *Chrysococcyx basalis*, newly hatched nestlings innately develop begging calls that closely match those of its primary host, the Superb Fairywren *Malurus cyaneus*, but they modify their begging call structure when reared in nests of the Buff-rumped Thornbill *Acanthiza reguloides* to resemble this secondary host vocally (Langmore *et al.* 2008). Likewise, vocal differences among Common Cuckoo *Cuculus canorus* nestlings from different hosts are not innate but are presumably acquired through a ‘trial and error’ process that secures the profitability of begging calls (Madden & Davies 2006). Contrary to Bronze Cuckoos, Great Spotted Cuckoo *Clamator glandarius* and Shiny Cowbird *Molothrus bonariensis* nestlings do not acoustically resemble any particular host and show a similar overall begging call structure across host species, although call rate and amplitude can vary according to host-related environmental conditions (Roldán *et al.* 2013, Tuero *et al.* 2016). This diversity in the degree of vocal mimicry and plasticity across parasite species suggests multiple evolutionary solutions to the common problem of producing manipulative signals to exploit the host’s parental behaviour. To explain such diversity, an understanding is required of how genetic

and environmental factors influence the development of the appropriate acoustic cues for different parasite species.

The Screaming Cowbird *Molothrus rufoaxillaris* is a specialist parasite that has the Grayish Baywing *Agelaioides badius*, hereafter Baywing, as its primary host. Screaming Cowbird nestlings and fledglings closely resemble host young in visual appearance and begging calls (Fraga 1979, De Mársico *et al.* 2012). This resemblance cannot be attributed to common descent, as these taxa do not have a recent common ancestor (Lanyon 1992); rather, it seems to be a counter-adaptation evolved in the parasite in response to host discrimination against juveniles unlike their own (Fraga 1998, De Mársico *et al.* 2012). A previous study showed that playback of Screaming Cowbird begging calls elicited increases in nest provisioning rates by adult Baywings equivalent to those of playback of Baywing begging calls, whereas non-mimetic begging calls of the closely related Shiny Cowbird failed to induce a parental response (Ursino *et al.* 2017). The question then arises of how the acoustic cues that serve as a signal for offspring recognition to Baywing hosts develop in Screaming Cowbird nestlings.

Our aim in this study was to test the effect of social experience on vocal development in the Screaming Cowbird by combining cross-fostering and playback experiments. We predicted that if host-specific recognition cues are acquired through social experience, Screaming Cowbird nestlings cross-fostered to a different host species will be acoustically less similar to Baywing nestlings than Baywing-reared Screaming Cowbirds, and their begging calls will elicit a lower response from Baywing adults. Alternatively, if host-specific recognition cues develop innately (i.e. independently of the parasite’s social experience), begging calls of Screaming Cowbird nestlings cross-fostered to an alternative host will be structurally similar to those of host and Screaming Cowbird nestlings from Baywing nests, and will elicit similar responses from Baywing adults.

METHODS

Study area and species

Fieldwork was conducted in the private reserve ‘El Destino’ near the town of Magdalena, in the Province of Buenos Aires, Argentina (35°08’S,

57°25'W), during the southern breeding seasons 2010–2011, 2011–2012, 2012–2013 and 2013–2014. The area comprises native forest patches dominated by *Celtis ehrenbergiana* and *Scutia buxifolia* embedded within a matrix of humid grasslands and pastures. Baywings and Screaming Cowbirds are year-round residents in the area. Baywings breed from late November to mid-February in nests built by other species (mainly furnariids), secondary cavities and nestboxes. They are facultative cooperative breeders, with one to four helpers at the nest that typically join the breeding pair after hatching and participate in brood provisioning and nest defence (Fraga 1991, Ursino *et al.* 2011). Nearly all Baywing nests are parasitized by Screaming Cowbirds, typically more than once (De Mársico *et al.* 2010). The modal clutch size of Baywings is four eggs; incubated clutches have mostly four host eggs plus two parasite eggs (De Mársico *et al.* 2010). Baywing and Screaming Cowbird eggs hatch after 13 and 12 days of incubation, respectively, and the nestling period lasts for 12–16 days (De Mársico *et al.* 2010). After fledging, host and Screaming Cowbird young remain near the natal territory for at least 2–3 weeks in the company of Baywing adults (Ursino *et al.* 2011).

Data collection and analysis

Effect of social experience on begging call structure of Screaming Cowbird nestlings

To investigate the effect of social experience on vocal development in Screaming Cowbirds we recorded begging calls from nestlings that were reared by Baywings or cross-fostered to a non-host species, the Chalk-browed Mockingbird *Mimus saturninus* (hereafter, Mockingbird). The latter is a primary host of the Shiny Cowbird in the study area and suitable to rear nestlings of the closely related Screaming Cowbird. Begging calls of Screaming Cowbird nestlings in Mockingbird nests were recorded during breeding seasons in 2010–2011 and 2011–2012 in the course of another study (M. C. De Mársico, R. Gloag, V. D. Fiorini & J. C. Reboreda unpubl. data.). For that study, Mockingbird nests were artificially parasitized before the onset of incubation with a single fresh Screaming Cowbird egg obtained from nearby Baywing nests. Using fresh eggs allowed us to exclude the possibility that cross-fostered Screaming Cowbirds had already acquired Baywing-

specific vocal signals through embryonic learning when transferred to Mockingbird nests (see Colombelli-Négrel *et al.* 2012). Under these conditions, Screaming Cowbird eggs hatch a day before or on the same day as the eggs of the Mockingbird. All except one Mockingbird egg in experimental nests were removed soon after clutch completion under permit from the local authority on protected areas (OPDS, Res 202/12). This was necessary to secure the survival of Screaming Cowbird nestlings because they are often outcompeted by larger Mockingbird nest-mates in broods having two or more host young (De Mársico & Reboreda 2008). We recorded the begging calls of Screaming Cowbird nestlings at the age of 4–5 and 8–10 days post-hatching ($n = 11$ and 7 nests, respectively) by attaching a lapel microphone on the nest rim, connected by a cable to a digital handy audio recorder (M-Audio Microtrack II, Hampshire, UK). Begging calls of Screaming Cowbird and Mockingbird nestlings are clearly distinct from each other, and thus misidentifications from these recordings were unlikely (Fig. S1). Recordings were made between 07:00 and 11:00 h, and lasted for 1.5–3 h, after which we removed the recording equipment from the nest. Differences in sample sizes between age classes here and elsewhere were due to nest predation.

For Screaming Cowbird nestlings reared by Baywings we could not reliably assign individual begging calls to each species from recordings made directly at Baywing nests due to the vocal similarity between parasitic and host nestlings (Fig. S2). Instead, during the 2012–2013 and 2013–2014 breeding seasons, we recorded Screaming Cowbird begging calls by temporarily removing the focal nestling from the nest at 4–5 and 8–9 days of age ($n = 14$ and 11, respectively). At multiply parasitized nests, we recorded only one Screaming Cowbird nestling to avoid pseudo-replication. Nestlings were placed in a small Styrofoam container lined with cotton and equipped with a lapel microphone connected by a cable to a digital handy audio recorder (Zoom H4N, Zoom, Hauppage, NY, USA). Recording distance and volume were held constant within and among recording sessions. Screaming Cowbird nestlings rarely vocalized spontaneously outside the nest, and thus we stimulated them to beg by broadcasting short (2-s) sequences of contact calls of adult Baywings every 10 min while simultaneously holding food (commercial mealworms *Tenebrio molitor*) with tweezers

above their heads. Only vocalizations produced during begging postural displays were recorded to ensure that these were begging calls. Recording sessions were conducted between 08:00 and 16:00 h and lasted until nestlings produced at least 10 distinctive begging calls, up to a maximum duration of 2 h. Once the recording session was finished, we fed the nestling with mealworms until satiation and immediately returned it to the nest. In most cases, nestlings spent no more than 30–40 min outside the nest. In those cases in which nestlings did not beg within 2 h ($n = 5$ of 14 nests), we repeated the procedure on the following day. No nestling was harmed or died because of this manipulation. Baywings readily accepted temporarily removed nestlings back into their broods and resumed their normal parental activity as soon as we left the vicinity of the nest.

To assess whether begging call structure of cross-fostered and Baywing-reared Screaming Cowbird nestlings matches that of Baywing nestlings, we used a sample of begging calls recorded at a set of unparasitized Baywing nests during the breeding seasons in 2009–2010, 2010–2011, 2011–2012, 2012–2013 and 2013–2014 ($n = 14$ and 8 at 4–5 and 8–10 days post-hatching, respectively). Recordings were conducted during the morning (07:00–11:00 h) using the same equipment and procedures as described above for Screaming Cowbird nestlings cross-fostered to Mockingbird nests.

All audio files were saved in wav format with 44.1-kHz sampling rate and 16-bit resolution. We converted digital audio recordings to spectrograms using RAVEN PRO 1.4 (Cornell Bioacoustics Research Program, Ithaca, NY, USA) using default settings (Hann window of 256 samples and 248-Hz filter bandwidth, time grid with hop size of 128 samples and 50% overlap, and frequency grid with discrete Fourier transform size of 256 samples and grid spacing of 172 Hz). We defined begging calls as discrete syllable bouts in the spectrograms. To characterize the begging call structure of nestlings for each age class, we first chose 10 good-quality calls per individual nestling from which we extracted the following acoustic parameters, excluding harmonics: minimum frequency (kHz), maximum frequency (kHz), bandwidth (i.e. the difference between maximum and minimum frequency), peak frequency (kHz, the frequency for which amplitude is greatest) and syllable duration (s). We chose these variables as standard measurements that allowed us to quantify the acoustic

structure of begging calls while avoiding as much as possible redundancy and overparameterization of the models used for data analyses. The variables chosen have previously been used to analyse begging call structure of brood-parasitic nestlings (Madden & Davies 2006, Langmore *et al.* 2008, De Mársico *et al.* 2012, Gloag & Kacelnik 2013). We did not analyse amplitude-related variables because differences in recording procedures between host species preclude proper comparisons. We measured these parameters using on-screen cursors or automatic measurements in RAVEN PRO 1.4. For polysyllabic calls, we calculated mean syllable duration by averaging the total call length over the number of syllables. Begging call measurements were averaged to obtain one value per nestling and age class (i.e. 4–5 and 8–10 days of age) for each of the five acoustic parameters.

To test for differences in begging call structure among the three groups of nestlings (i.e. cross-fostered Mockingbird-reared Screaming Cowbirds, Baywing-reared Screaming Cowbirds and Baywings), we performed a permutational multivariate analysis of variance (PERMANOVA; Anderson 2001), with nestling group and sampling season as additive explanatory variables. PERMANOVA is a non-parametric test that computes pseudo- F statistics by permuting a distance matrix under the null hypothesis of no differences among groups. We used Euclidean distance matrices that were computed for each age class from standardized measures of the five call parameters. We performed PERMANOVA tests on the Euclidean distance matrixes with 9999 permutations using the function *adonis* of the *vegan* library (Oksanen *et al.* 2016) in R 3.4.2 (R Core Team 2017). Following significant multivariate tests, *post-hoc* pairwise comparisons with sequential Bonferroni correction were performed using the function *pairwise.perm.manova* of *RVAideMemoire* library (Hervé 2016). As PERMANOVA is sensitive to heterogeneity of dispersion for unbalanced designs (Anderson & Walsh 2013), we examined the assumption of multivariate dispersion homogeneity using the *permutest* and *betadisper* functions of the *vegan* library before doing the tests. This assumption was met for both age classes (Fig. S3). We further examined how the original call variables contribute to the observed variability in begging call structure by performing a principal component analysis (PCA) for each age class on a correlation matrix of the standardized begging call

measures. PCAs were done, and respective loadings extracted, using the *princomp* function in R 3.4.2 (R Core Team 2017). PCAs produced ordination patterns equivalent to those derived from PERMANOVA (i.e. principal coordinates plots derived from Euclidean distance matrices).

We are aware that our study involved different sampling methods, which might obscure the results. To check whether the recording method could have biased parameter estimations, we compared the begging call structure of Baywing nestlings recorded at unparasitized nests with that of Baywing nestlings recorded individually using exactly the same protocol as described above for Baywing-reared Screaming Cowbird nestlings. A PERMANOVA test on these two groups showed no significant differences in overall begging call structure for each age class (4–5 days: $P = 0.77$; 8–10 days: $P = 0.33$; Tables S1 and S2, and Fig. S4). Hence, we are confident that the sampling method itself did not introduce any systematic bias in call parameter estimation.

The effect of social experience on vocal development of Screaming Cowbird nestlings could also be confounded by differences in nestling physical condition between host species. Nestling condition may influence the rate and intensity of begging displays and, as a result, may affect the acoustic structure of begging calls (e.g. Sacchi *et al.* 2002). To assess whether Baywing-reared and cross-fostered Screaming Cowbird nestlings differ in physical condition, we compared nestling body mass (M) at 4 and 8 days post-hatching. Sample sizes for this analysis were 10 Baywing-reared and six cross-fostered Screaming Cowbirds (one nestling from each group was excluded due to insufficient growth data). Nestling body mass was estimated by adjusting the observed mass of individual nestlings to the logistic growth curve $M = A/(1 + \exp(-K*(T - T_0)))$, where A is the asymptotic mass, K is the growth constant, T is nestling age (in days) and T_0 is the age of maximum growth. Growth curves were fitted by least-squares estimation using the *nls* function in R 3.4.2 (R Core Team 2017). Starting estimates for model parameters were the values reported previously for Screaming Cowbird nestlings in Baywing nests (De Marsico *et al.* 2010). Comparisons were done using *t*-tests.

Effect of social experience on the development of vocal cues for offspring recognition by Baywings

To test whether Baywings respond similarly to begging calls of Baywing-reared and cross-fostered

Screaming Cowbird nestlings, we conducted a playback experiment during the 2013–2014 breeding season at 18 Baywing nests that had nestlings of 10–14 days of age. Pilot experiments showed that, at this stage, adult Baywings respond to playback of nestling calls in the absence of visual stimuli and there is little interference between the experimental manipulation and the host's own brood. We used a repeated-measures design to account for variation across nests in helper number and other nest-related variables that could affect the response to playbacks. Each nest was presented sequentially with three, 3-min broadcast sessions, each preceded by a 10-min period of silence, of begging calls of: (1) Baywing-reared Screaming Cowbird nestlings, (2) Mockingbird-reared Screaming Cowbird nestlings and (3) Baywing nestlings from unparasitized nests (=control). We used recordings of begging calls of 8- to 10-day-old nestlings for all broadcast treatments. To avoid pseudoreplication we generated 6–10 unique call samples for each treatment by editing spectrograms of field recordings using RAVEN PRO 1.4. Each call sample consisted of a 30-s sequence of begging calls (repetitions of 10 distinct begging calls) of one individual chick followed by 30 s of silence. Root mean square (RMS) amplitude and call rate were standardized within and among treatments. We fixed the call rate at 1 call/s, which approximates the mean call rate observed in field recordings of Baywing broods (0.98 calls/s). Treatment order was rotated among nests.

To conduct the playback experiment we placed a loudspeaker (Ipok P-55, China) at approximately 2 m from the focal nest, attached to a tree branch with duct tape and connected through a cable to a wav/mp3 audio player (Zoom N4H). A camcorder (HDR CX110 Sony, Tokyo, Japan) was mounted on a tripod near the nest to record Baywing behaviour during broadcast treatments. Baywings always resumed their normal parental activity as soon as we walked away from the nest, but we allowed them to acclimatize further to the experimental setup for 20 min before initiating the experiment. We broadcast call treatments from a hide placed 10 m from the nest, from which we monitored adult Baywing behaviour in real time using 8 × 42 binoculars.

We quantified the intensity of host response to playbacks using the following variables: (1) time

elapsed until an adult Baywing (parent or helper) perched at less than 0.5 m from the loudspeaker for the first time ('latency'); (2) total amount of time spent by at least one adult Baywing at less than 0.5 m from the loudspeaker ('duration'); (3) number of times an adult Baywing perched within 0.5 m from the speaker ('approaches'); and (4) maximum number of adults responding simultaneously ('recruitment'). We tested for differences in latency among treatments using a stratified Cox proportional hazard model, as suggested by Jahn-Eimermacher *et al.* (2011), using the *coxph* function from *survival* library (Therneau 2015) in R 3.4.2 (R Core Team 2017). The model included treatment and treatment order as fixed factors, and nest identity as a random effect. To assess the effect of fixed factors on the latency to respond we conducted a likelihood ratio (LR) test against the null model (i.e. a model including only an intercept and the random effect). Differences among broadcast treatments in the remaining response variables were tested using non-parametric Friedman tests.

RESULTS

Effect of social experience on begging call structure of Screaming Cowbird nestlings

At the age of 4–5 days, Screaming Cowbird and Baywing nestlings had simple and stereotyped begging calls characterized by a single 'peep' note with a peak frequency of ~ 6.4–6.8 kHz (Fig. 1a–c). Despite overall similarity, PERMANOVA showed significant differences in begging call structure between Screaming Cowbird and Baywing nestlings ($pseudo-F_{2,32} = 2.89$, $P = 0.017$, *post-hoc* comparisons: $P < 0.05$), but not between Baywing-reared and cross-fostered Screaming Cowbird nestlings (*post-hoc* comparison: $P = 0.18$). The ordination plot derived from Euclidean distances shows extensive overlap in begging call structure between Screaming Cowbird and Baywing nestlings at this age (Fig. 2a). Baywing-reared and cross-fostered Screaming Cowbird nestlings separated from Baywings only on the second principal coordinate, which accounted for 26% of the variance in begging call structure. According to PCA, Screaming Cowbird begging calls were associated with lower minimum frequency and broader bandwidth (Fig. 2a, Table 1). Sampling

season had no significant effect on the observed variability in begging call structure at 4–5 days of age (PERMANOVA: $pseudo-F_{4,32} = 0.84$, $P = 0.83$).

By days 8–10, begging calls of parasitic and host nestlings became polysyllabic and more complex in frequency modulation (Fig. 1d–f; Table 1). Begging call structure differed between cross-fostered Screaming Cowbird and the other two groups (PERMANOVA: $pseudo-F_{2,23} = 3.06$, $P = 0.0101$; *post-hoc* comparisons: $P < 0.05$) but not between Baywing-reared Screaming Cowbird and Baywing nestlings (*post-hoc* comparison: $P = 0.26$; Fig. 2b). Cross-fostered Screaming Cowbird nestlings separated from the other two groups on the second principal coordinate, which accounted for 30% of the variance in begging call structure (Fig. 2b). Begging calls of cross-fostered Screaming Cowbird nestlings were associated with lower minimum frequency and broader bandwidth (Fig. 2b, Table 1). Sampling season had no significant effect on the observed variability in begging call structure (PERMANOVA: $pseudo-F_{4,23} = 0.19$, $P = 0.98$).

We did not find differences between Baywing-reared and Mockingbird-reared Screaming Cowbird nestlings in estimated mass at either 4 days (mean \pm sd: 20.1 \pm 2.8 g and 20.5 \pm 2.8 g, respectively; $t_{14} = -0.27$, $P = 0.79$) or 8 days of age (mean \pm sd: 36.9 \pm 3.6 g and 35.8 \pm 4.0 g, respectively; $t_{14} = 0.56$, $P = 0.59$).

Effect of social experience on the function of Screaming Cowbird begging calls

Adult Baywings responded to at least one playback treatment in 14 of 18 experimental nests. The remaining four nests were excluded from statistical analysis. We did not find a significant effect of playback treatment and treatment order on the latency to respond to playbacks (LR test: treatment, $\chi^2_2 = 0.54$, $P = 0.76$; order, $\chi^2_2 = 1.06$, $P = 0.59$; Fig. 3). Likewise, the predictor variables had no significant effect on response duration (Friedman test: treatment, $\chi^2_2 = 2.38$, $P = 0.30$; order, $\chi^2_2 = 3.17$, $P = 0.20$; Fig. 4a), the number of approaches to the loudspeaker (treatment, $\chi^2_2 = 3.12$, $P = 0.21$; order, $\chi^2_2 = 0.98$, $P = 0.61$; Fig. 4b) or recruitment (treatment, $\chi^2_2 = 4.04$, $P = 0.13$; order, $\chi^2_2 = 0.84$, $P = 0.66$; Fig. 4c).

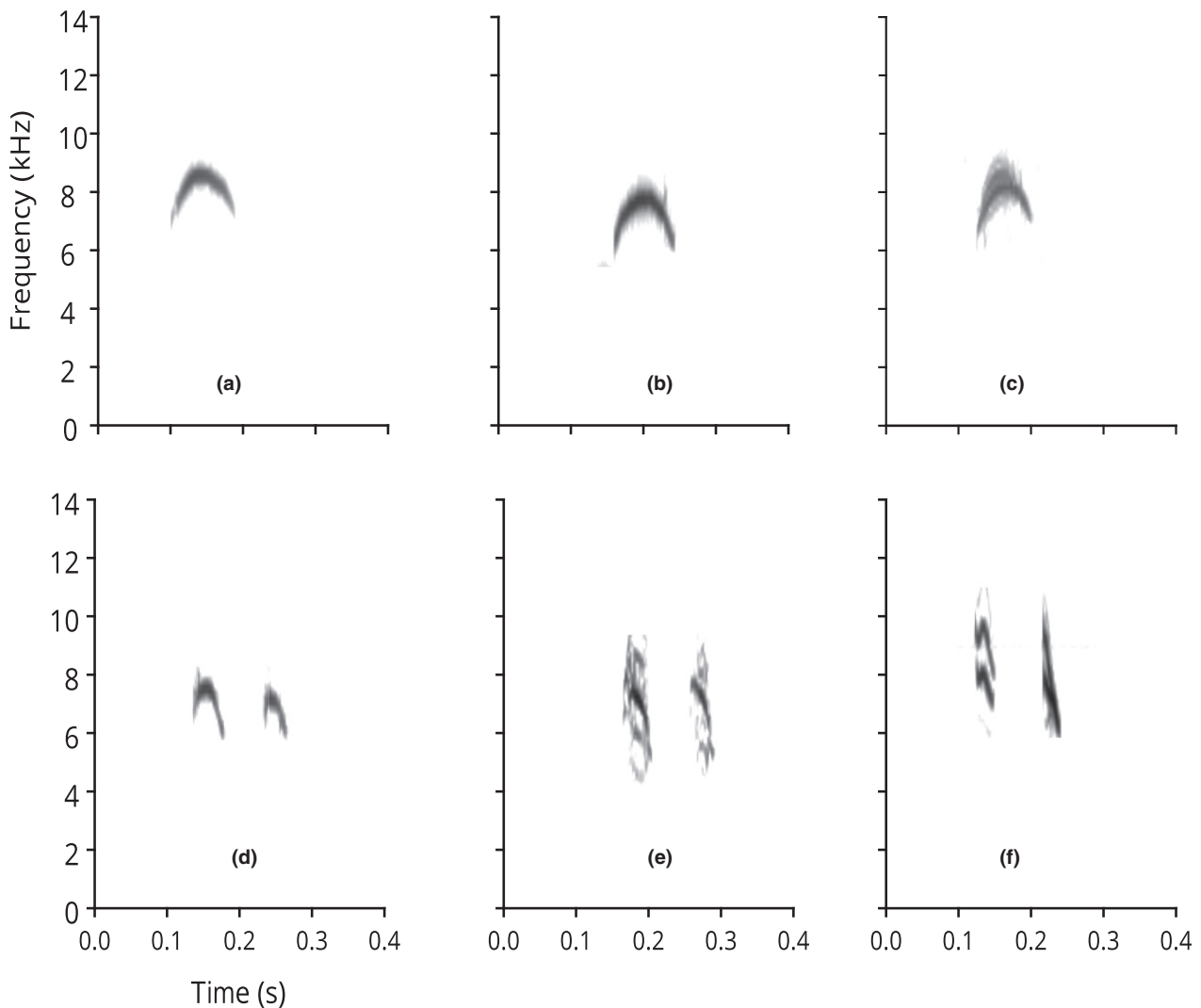


Figure 1. Representative spectrograms of begging calls of Screaming Cowbird and Baywing nestlings at 4–5 (a–c) and 8–10 (d–f) days of age. (a,d) Baywing-reared Screaming Cowbird. (b,e) Mockingbird-reared Screaming Cowbird. (c,f) Baywing.

DISCUSSION

The results of the cross-fostering and playback experiments presented here suggest that the acoustic structure of Screaming Cowbird begging calls involved both innate and environmentally modulated components. Begging calls of cross-fostered and Baywing-reared Screaming Cowbird nestlings were initially similar but acoustic similarity decreased as nestlings aged. By 8–10 days of age, Baywing-reared Screaming Cowbirds were acoustically more like Baywing than cross-fostered Screaming Cowbird nestlings. Despite the observed acoustic differences, adult Baywings responded similarly to playbacks of begging calls of 8-day-old

cross-fostered Screaming Cowbird, Baywing-reared Screaming Cowbird and Baywing nestlings, suggesting that these calls were functionally equivalent from the host's perspective. These findings support the idea that, although the rearing environment can influence certain begging call parameters, the acoustic cues that serve for offspring recognition to adult Baywings develop independently of social experience in young Screaming Cowbirds.

The observed acoustic differences between Baywing-reared and Mockingbird-reared Screaming Cowbird nestlings at 8–10 days of age were unlikely to be confounded with nestlings' physical condition, as they grew similarly in both host species. However, differences could be at least partly due

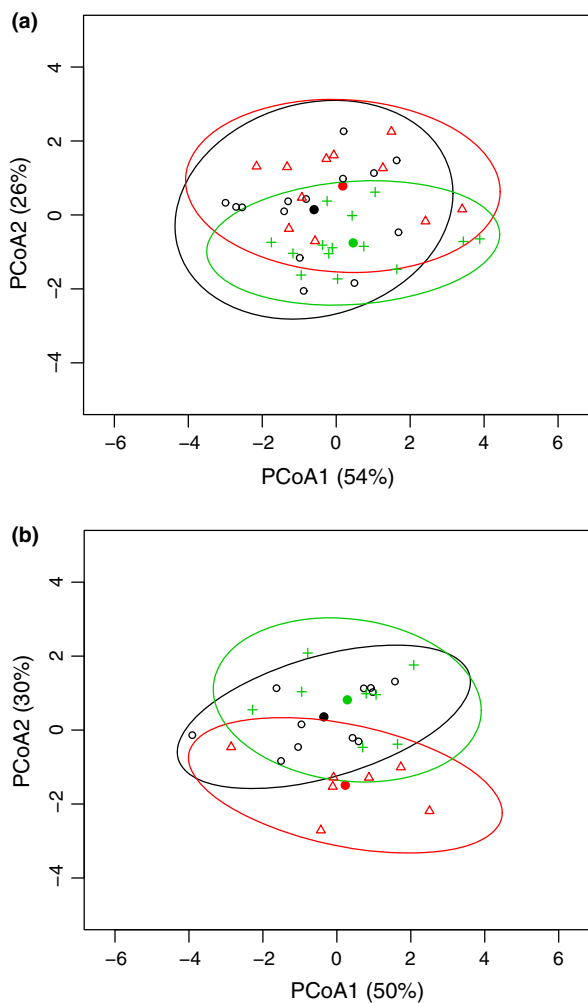


Figure 2. Principal coordinates plot derived from permutational multivariate analysis of variance (PERMANOVA) on begging calls of (a) 4- to 5-day-old and (b) 8- to 10-day-old nestlings. Black circles = Baywing-reared Screaming Cowbird; red triangles = cross-fostered Screaming Cowbird; green crosses = Baywing. Solid circles represent the centroids of each group. Ellipses depict 95% confidence interval for mean distances to the corresponding centroids.

to variation between host species in the motivation to beg in parasitic nestlings. In Mockingbird nests, Screaming Cowbird nestlings are rapidly outgrown by their larger host nest-mates and are thus expected to be hungrier than in Baywing nests and to compete more strongly to secure sufficient provisioning (De Marsico & Reboreda 2008). We observed that begging displays of Screaming Cowbird nestlings were consistently more intense at Mockingbird than at Baywing nests, as expected if

Table 1. Loadings of the original begging call variables on the two first principal components extracted from the principal component analysis (PCA) for each age class.

Call parameters	4-5 days		8-10 days	
	PC 1	PC 2	PC 1	PC 2
Minimum frequency	0.328	-0.722	0.354	0.640
Maximum frequency	0.586	0.158	0.618	-0.104
Peak frequency	0.557	-0.187	0.580	0.216
Bandwidth	0.409	0.645	0.353	-0.634
Syllable duration	0.268	-0.02	-0.177	-0.362

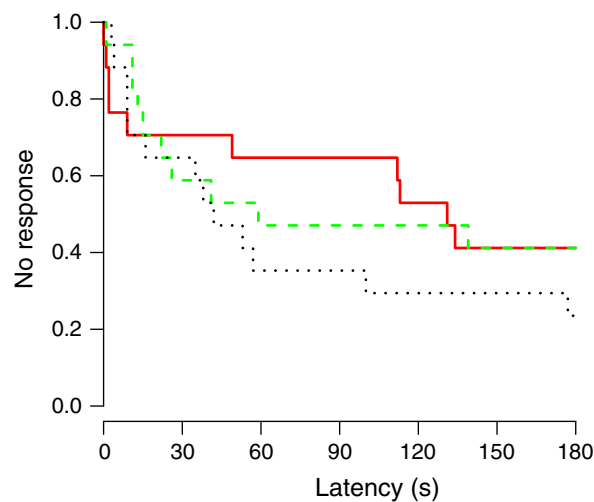


Figure 3. Kaplan-Meier estimates of the latency to respond to playbacks of begging calls of 8- to 10-day-old nestlings (Baywing-reared Screaming Cowbird = dashed red line; cross-fostered Screaming Cowbird = dotted blue line; Baywing = solid black line). Each line represents the proportion of nests ($n = 14$) at which Baywing adults did not respond to the playback treatment over treatment duration (s).

parasitic nestlings adjust their signalling in each host species according to hunger level or the perceived level of within-brood competition. These observations accord with prior experimental work showing that Screaming Cowbird nestlings deprived of food increase the intensity of their begging displays as deprivation time increases (Lichtenstein 2001) and are more consistent with begging effort by Screaming Cowbirds as a signal of hunger rather than need (Mock *et al.* 2011). Such overall increase in begging intensity in cross-fostered Screaming Cowbird nestlings could help explain the differences in minimum frequency and

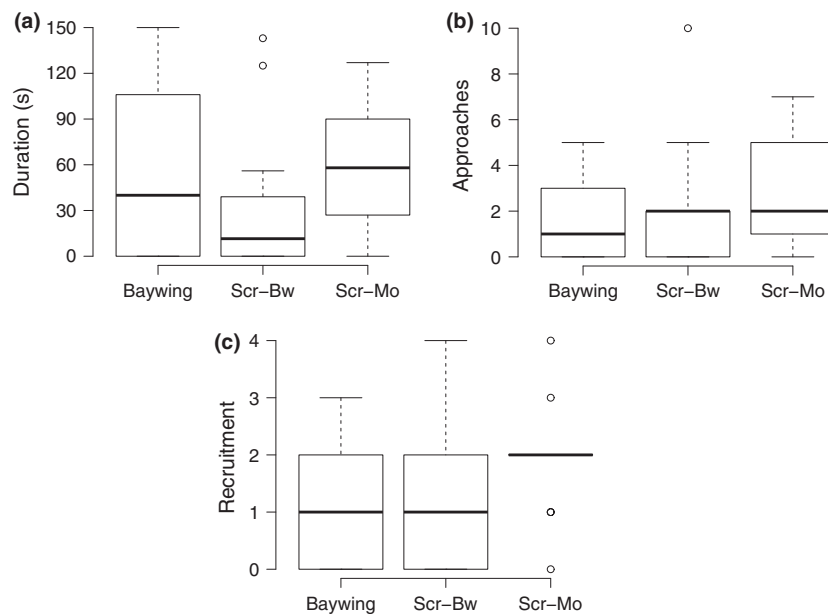


Figure 4. Boxplots of (a) total time spent by at least one adult Baywing near the loudspeaker ('duration'), (b) number of times an adult Baywing approached the loudspeaker ('approaches') and (c) number of responding individuals ('recruitment') as a function of playback treatments. Treatments consisted of begging calls of 8- to 10-day-old nestlings. 'Scr-Bw' = Baywing-reared Screaming Cowbird, 'Scr-Mo' = Mockingbird-reared Screaming Cowbird, and 'Bw' = Baywing nestlings. Sample size was 14 nests.

bandwidth between them and Baywing-reared nestlings by the age of 8–10 days. In other passerine species, deprived nestlings increase call duration, bandwidth or frequency modulation as their hunger level increases (Leonard & Horn 2006, Marques *et al.* 2008, Reers & Jacot 2011, but see Anderson *et al.* 2010). Interestingly, however, adult Baywings responded similarly to begging calls of cross-fostered and Baywing-reared Screaming Cowbird nestlings, suggesting that the acoustic cues for offspring recognition remained unaffected despite the observed differences in call structure.

Studies in other cowbird species suggest an effect of nest environment on certain aspects of begging behaviour independently of short-term need (Rivers *et al.* 2014, Tuero *et al.* 2016). Nest environment may influence begging call structure if nestlings adjust their begging calls to better exploit their host's sensory preferences (Madden & Davies 2006) or in response to within-brood competition (Roulin *et al.* 2009, Rivers *et al.* 2014, Tuero *et al.* 2016). Further studies comparing the effect of begging calls of Mockingbird-reared vs. Baywing-reared Screaming Cowbird nestlings on Mockingbird provisioning behaviour would be useful to evaluate whether parasite nestlings vary their begging calls to make them more profitable in any

given host. Likewise, assessing the effect of nest mate size on begging behaviour of parasitic nestlings while controlling for short-term need would test whether Screaming Cowbirds are able to modify their begging calls solely in response to their perceived level of competition. Conversely, it would be interesting to examine whether host nestlings modify their begging calls in the presence of Screaming Cowbird nestlings. In the Song Sparrow *Melospiza melodia*, a common host of the Brown-headed Cowbird *Molothrus ater*, nestlings increased the frequency and amplitude of their begging calls at parasitized nests, which would allow them to better cope with competition with cowbird nestlings for parental feedings (Pagnucco *et al.* 2008, Boncoraglio *et al.* 2009).

Differences in begging call structure between parasite nestlings reared by different host species could also arise if they learn to match the begging calls of host nestlings (Langmore *et al.* 2008). Such a learning process is unlikely in the Screaming Cowbird because begging calls of Mockingbird-reared Screaming Cowbird nestlings were clearly distinct from those of Mockingbird nestlings regardless of age. However, in Baywing nests, begging call similarity between Screaming Cowbird and host nestlings increased as they aged. This

pattern suggests that parasitic nestlings could refine some host-specific acoustic cues during vocal development, for example if host parents reinforce mimetic calls by increasing food provisioning (Langmore *et al.* 2008). Consistent with this idea, Baywing-reared Screaming Cowbird nestlings showed reduced variability in call parameters from 4–5 to 8–10 days of age (Fig. S3). Such apparent refinement of vocal mimicry in older Screaming Cowbird nestlings had no detectable effects on host responsiveness to playbacks, but we cannot dismiss the possibility that it becomes more important after fledging, when exhibiting Baywing-like acoustic signals seems to be critical to elicit parental care and avoid host discrimination (De Marsico *et al.* 2012). A recent study in the Brown-Headed Cowbird shows that begging calls of parasite fledglings, but not nestlings, consistently match the peak frequency of host's begging calls, suggesting that social experience may shape some vocal modifications that help parasites to procure resources after fledging (Liu *et al.* 2016). Nevertheless, it is worthwhile to note that we have observed Screaming Cowbird young recently fledged from Mockingbird nests being called and escorted by two or more adult Baywings from neighbouring territories (De Marsico & Reboreda 2008). Although anecdotal, these observations further suggest that Screaming Cowbird nestlings acquire host-specific recognition cues in the complete absence of early social interactions with Baywing parents and nest mates.

Our results are in agreement with previous studies in other non-evictor parasite species showing that begging calls of parasitic young can include both genetically fixed and plastic components (Roldan *et al.* 2013, Tuero *et al.* 2016). A difference between these other brood parasites and the Screaming Cowbird is that in the former, nestlings do not match the begging call structure of any particular host (Gloag & Kacelnik 2013, Roldan *et al.* 2013). Despite the observed differences in acoustic structure, begging calls of both Baywing-reared and cross-fostered Screaming Cowbird nestlings showed overall similarity to each other and to Baywing begging calls and seemed to be functionally equivalent from the host's perspective. This idea is further supported by the fact that Baywings provisioned unparasitized and parasitized broods at similar rates (Ursino 2016) and increased feeding rates equally in response to playbacks of Screaming Cowbird and conspecific begging calls

(Ursino *et al.* 2017). Our findings add evidence to the idea that vocal development of Screaming Cowbird nestlings is mainly driven by host discrimination against non-mimetic young (Fraga 1998, De Marsico *et al.* 2012), although the specific begging call parameters involved in offspring recognition by Baywings have yet to be determined. We point to frequency-related variables (i.e. minimum frequency and bandwidth) as possible candidates, as Screaming Cowbird and Baywing nestlings overlapped extensively in those call parameters as they aged. Future playback experiments that assess the response of Baywing hosts to changes in specific call parameters would help to determine which aspects of begging call structure are critical for offspring recognition and which encode information on nestling need or condition.

The degree of mimicry of host-specific signals by young Screaming Cowbirds seems to be the outcome of a long-term coevolutionary history with its primary host (De Marsico *et al.* 2012). From the Baywing perspective, acceptance of Screaming Cowbird nestlings and fledglings seems maladaptive, but it would be favoured if the costs of rejecting their own young by mistake outweigh the benefits of further discrimination. From the parasite's perspective, the striking resemblance to Baywing nestlings allows them to manipulate the host's parental behaviours efficiently. However, the innate development of Baywing-like vocal cues might also limit the ability of Screaming Cowbirds to colonize new host species if parasitic young fail to tune into the sensory preferences of other potential hosts.

Finally, the results presented here emphasize the importance of combining spectrogram analyses with playback experiments to better assess the relative influence of genetic and environmental factors on vocal development in brood parasites. In this study, using only one of these approaches would have led us to incorrect or incomplete conclusions about how social experience affects the acoustic structure and function of Screaming Cowbird begging calls.

CONCLUSIONS

Nestling begging calls are complex signals central to parent–offspring communication. Hence, it is expected that parasite nestlings produce acoustic cues that allow them to effectively ‘tune’ into those communication channels and manipulate host parental behaviour in their favour. When

hosts can acoustically discriminate between their own and foreign nestlings, begging calls of parasite nestlings must communicate the appropriate signals to trick hosts into feeding them (Langmore *et al.* 2003). This requires integrating signals of identity that should be relatively fixed within individuals to avoid recognition errors, and signals of need that should be plastic enough to reflect reliably short-term changes in hunger level (Reers & Jacot 2011). Our results are consistent with this scenario: we found that Screaming Cowbird nestlings developed begging calls more similar to Bay-wing nestlings when reared by this host than when cross-fostered to Mockingbird nests, but those acoustic differences had no detectable effect on host response to begging calls. These findings suggest that begging call structure of Screaming Cowbirds is adapted to match the acoustic preferences of its primary host, which may have constrained the developmental plasticity of vocal signals in this parasite species.

The mechanisms underlying begging call development and the resulting level of vocal plasticity in parasitic nestlings may have evolutionary implications for host–parasite interactions (Liu *et al.* 2016). For instance, the ability to learn and exploit their host's sensory preferences may provide brood parasites with the potential to colonize new hosts successfully (Agrawal 2001, Roldán *et al.* 2013). Conversely, relatively fixed developmental pathways are expected to be favoured over phenotypic plasticity if the fitness costs to parasite nestlings of mismatching host preferences are predictably high (Agrawal 2001). Such specialization may limit the opportunities for host shifts relative to more generalist brood parasites (Payne & Payne 2002). Further studies on vocal development in other specialist and generalist brood parasites combined with playback experiments will greatly contribute to our understanding of the ecology and evolution of manipulative signals to exploit parental care.

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REFERENCES

- Agrawal, A.A. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science* **294**: 321–326.
- Anderson, M.J. 2001. A new method for non parametric multivariate analysis of variance. *Austral. Ecol.* **26**: 32–46.
- Anderson, M.J. & Walsh, D. 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? *Ecol. Monogr.* **83**: 557–574.
- Anderson, M.G., Brunton, D.H. & Hauber, M.E. 2010. Reliable information content and ontogenetic shift in begging calls in Grey Warbler nestlings. *Ethology* **116**: 357–365.
- Beecher, M.D., Beecher, I.M. & Hahn, S. 1981. Parent-offspring recognition in Bank Swallows (*Riparia riparia*): II Development and acoustic basis. *Anim. Behav.* **29**: 95–101.
- Boncoraglio, G., Saino, N. & Garamszegi, L.Z. 2009. Begging and cowbirds: brood parasites make hosts scream louder. *Behav. Ecol.* **20**: 215–221.
- Burford, J.E., Friedrich, T.J. & Yasukawa, E. 1998. Response to playback of nestling begging in the Red-winged Blackbird (*Agelaius phoeniceus*). *Anim. Behav.* **56**: 555–561.
- Colombelli-Négrel, D., Hauber, M.E., Robertson, J., Sulloway, F.J., Hoi, H., Griggio, M. & Kleindorfer, S. 2012. Embryonic learning of vocal passwords in superb fairy-wrens reveals intruder cuckoo nestlings. *Current Biol.* **22**: 2155–2160.
- De Mársico, M.C. & Reboreda, J.C. 2008. Differential reproductive success favours strong host preference in a highly specialized brood parasite. *Proc. R. Soc. B* **275**: 2499–2506.
- De Mársico, M.C., Mahler, B. & Reboreda, J.C. 2010. Reproductive success and nestling growth of the Baywing parasitized by Screaming and Shiny Cowbirds. *Wilson. J. Ornithol.* **122**: 417–431.
- De Mársico, M.C., Gantchoff, M.G. & Reboreda, J.C. 2012. Host-parasite coevolution beyond the nestling stage? Mimicry of host fledglings by the specialist Screaming Cowbird. *Proc. R. Soc. B* **279**: 3401–3408.
- Fraga, R.M. 1979. Differences between nestlings and fledglings of Screaming and Bay-winged Cowbirds. *Wilson Bull.* **91**: 151–154.
- Fraga, R.M. 1991. The social system of a communal breeder, the Bay-winged Cowbird *Molothrus badius*. *Ethology* **89**: 195–210.
- Fraga, R.M. 1998. Interactions of the parasitic Screaming and Shiny Cowbirds (*Molothrus rufoaxillaris* and *M. bonariensis*) with a shared host, the Bay-winged Cowbird (*M. badius*). In Rothstein, S.I. & Robinson, S.K. (eds) *Parasitic Birds and Their Hosts: Studies in Coevolution*: 173–193. New York, NY: Oxford University Press.
- Glassey, B. & Forbes, S. 2002. Muting individual nestlings reduces parental foraging for the brood. *Anim. Behav.* **63**: 779–786.
- Gloag, R. & Kacelnik, A. 2013. Host manipulation via begging call structure in the brood-parasitic Shiny Cowbird. *Anim. Behav.* **86**: 101–109.
- Hervé, M. 2016. *RVAideMemoire: Diverse Basic Statistical and Graphical Functions. R package version 0.9-60*. Available at <https://CRAN.R-project.org/package=RVAideMemoire> (accessed November, 2017)

- Jahn-Eimermacher, A., Lasarzik, I. & Raber, J. 2011. Statistical analysis of latency outcomes in behavioral experiments. *Behav. Brain Res.* **221**: 271–275.
- Kilner, R.M., Noble, D.G. & Davies, N.B. 1999. Signal of need in parent-offspring communication and their exploitation by the Common Cuckoo. *Nature* **397**: 667–672.
- Langmore, N.E., Hunt, S. & Kilner, R.M. 2003. Escalation of a coevolutionary arms race through host rejection of brood parasitic young. *Nature* **422**: 157–160.
- Langmore, N.E., Maurer, G., Adcock, G.J. & Kilner, R.M. 2008. Socially acquired host-specific mimicry and the evolution of host races in Horsfield's Bronze-cuckoo *Chalcites basalis*. *Evolution* **62**: 1689–1699.
- Lanyon, S.M. 1992. Interspecific brood parasitism in blackbirds (Icterinae): a phylogenetic perspective. *Science* **255**: 77–79.
- Leonard, M.L. & Horn, A.G. 2001. Begging calls and parental feeding decisions in Tree Swallows (*Tachycineta bicolor*). *Behav. Ecol. Sociobiol.* **49**: 170–175.
- Leonard, M.L. & Horn, A.G. 2006. Age-related changes in signalling of need by nestling Tree Swallows (*Tachycineta bicolor*). *Ethology* **112**: 1020–1026.
- Lichtenstein, G. 2001. Selfish begging by screaming cowbirds, a mimetic brood parasite of the bay-winged cowbird. *Anim. Behav.* **61**: 1151–1158.
- Liu, W.-C., Rivers, J.W. & White, D.J. 2016. Vocal matching and intensity of begging calls are associated with a forebrain song circuit in a generalist brood parasite. *Devel. Neurobiol.* **76**: 615–625.
- Madden, J.R. & Davies, N.B. 2006. A host-race difference in begging calls of nestling cuckoos *Cuculus canorus* develops through experience and increases host provisioning. *Proc. R. Soc. B* **273**: 2343–2351.
- Marques, P.A.M., Vicente, L. & Márquez, R. 2008. Iberian Azure-winged Magpie *Cyanopica (Cyana) cooki* nestlings begging calls: call characterization and hunger signalling. *Bioacoustics* **18**: 133–149.
- Mock, D.W., Dugas, M.B. & Strickler, S.A. 2011. Honest begging: expanding from signal of need. *Behav. Ecol.* **22**: 909–917.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. & Wagner, H. 2016. *vegan: Community Ecology Package*. R package v 2.4-0. Available at <https://CRAN.R-project.org/package=vegan> (accessed November, 2017).
- Pagnucco, K., Zanette, L., Clinchy, M. & Leonard, M.L. 2008. Sheep in wolf's clothing: host nestling vocalizations resemble their cowbird competitor's. *Proc. R. Soc. B* **275**: 1061–1065.
- Payne, R.B. & Payne, L.L. 2002. Begging for parental care from another species: specialization and generalization in brood-parasitic finches. In Wright, J. & Leonard, M.L. (eds) *The Evolution of Begging. Competition, Cooperation and Communication*: 429–449. Dordrecht: Kluwer Academic Publishers.
- R Core Team. 2017. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Reers, H. & Jacot, A. 2011. The effect of hunger on the acoustic individuality in begging calls of a colonially breeding weaver bird. *BMC Ecol.* **11**: 3.
- Rivers, J.W. 2007. Nest mate size, but not short-term need, influences begging behavior of a generalist brood parasite. *Behav. Ecol.* **18**: 222–230.
- Rivers, J.W., Blundell, M.A. & Rothstein, S.I. 2014. Mismatched begging displays between foreign and host offspring reduce brood parasite fitness. *Behav. Ecol.* **25**: 785–793.
- Roldán, M., Soler, M., Márquez, R. & Soler, J.J. 2013. The vocal begging display of Great Spotted Cuckoo *Clamator glandarius* nestlings in nests of its two main host species: genetic differences or developmental plasticity? *Ibis* **155**: 867–876.
- Roulin, A., Dreiss, A., Fioravanti, C. & Bize, P. 2009. Vocal sib-sib interactions: how siblings adjust signalling level to each other. *Anim. Behav.* **77**: 717–725.
- Sacchi, R., Saino, N. & Galeotti, P. 2002. Features of begging calls reveal general condition and need of food of Barn Swallow (*Hirundo rustica*) nestlings. *Behav. Ecol.* **13**: 268–273.
- Therneau, T. 2015. *A Package for Survival Analysis in S.* v. 2.38. Available at <http://CRAN.R-project.org/package=survival> (accessed November, 2017).
- Tuero, D.T., Gloag, R. & Reboreda, J.C. 2016. Nest environment modulates begging behavior of a generalist brood parasite. *Behav. Ecol.* **27**: 204–210.
- Ursino, C.A. 2016. *Efecto de dos parásitos de cría sobre el cuidado parental de una especie con cría cooperativa, el Músico (Agelaioides badius)*. Doctoral dissertation, Facultad de Ciencias Exactas y Naturales. Universidad e Buenos Aires, Central Library Dr. Luis Federico Leloir.
- Ursino, C.A., De Mársico, M.C., Sued, M., Farall, A. & Reboreda, J.C. 2011. Brood parasitism disproportionately increases nest provisioning and helper recruitment in a cooperatively breeding bird. *Behav. Ecol. Sociobiol.* **65**: 2279–2286.
- Ursino, C.A., Gloag, R., Reboreda, J.C. & De Mársico, M.C. 2017. Host provisioning behaviour favours mimetic begging calls in a brood parasitic cowbird. *Behav. Ecol.* **29**: 328–332.
- Wright, J. & Leonard, M.L. 2002. *The Evolution of Begging. Competition, Cooperation and Communication*. Dordrecht: Kluwer Academic Publishers.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Spectrograms of begging calls of cross-fostered Screaming Cowbird (A–C) and Chalk-browed Mockingbird nestlings (B–D) at 4–5 (top) and 8–10 days post-hatching (bottom) to illustrate the clear differences in acoustic structure between these species. Mockingbird begging calls were characterized by a single flat note of longer duration, lower frequency and narrower bandwidth than those of Screaming Cowbird nestlings.

Figure S2. Spectrograms of begging calls of mixed broods of host and Screaming Cowbird nestlings recorded at naturally parasitized Baywing nests.

Figure S3. Boxplots of distance to centroids from PERMANOVA of begging calls at (A) 4–5 and (B) 8–10 days of age. Bw-Scr = Baywing-reared Screaming Cowbirds, Mo-Scr = Mockingbird-reared Screaming Cowbirds, Baywing = Baywing nestlings. The assumption of homogeneity of multivariate dispersion was met for both age classes (4–5 days: $P = 0.37$; 8–10 days: $P = 0.90$).

Table S1. Begging call parameters (mean \pm sd) at 4–5 days of age for Baywing nestlings recorded at unparasitized nests ($n = 14$) or individually as described for Baywing-reared Screaming Cowbird nestlings ($n = 13$).

Table S2. Begging call parameters (mean \pm sd) at 8–10 days of age for Baywing nestlings recorded at unparasitized nests ($n = 8$) or individually as described for Baywing-reared Screaming Cowbird nestlings ($n = 11$).

Figure S4. Principal coordinates plot derived from permutational multivariate analysis of variance (PERMANOVA) on begging calls of Baywing nestlings recorded within unparasitized nests or individually at (A) 4–5 days and (B) 8–10 days post-hatching. Black circles = nestlings recorded individually; red triangles = nestlings recorded within unparasitized nests. Solid circles represent the centroids of each group and ellipses depict the 95% confidence interval for mean distances to the corresponding centroids.