

Article

Imperfect mimicry of host begging calls by a brood parasitic cuckoo: a cue for nestling rejection by hosts?

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Abstract

Coevolutionary interactions between avian brood parasites and their hosts often lead to the evolution of discrimination and rejection of parasite eggs or chicks by hosts based on visual cues, and the evolution of visual mimicry of host eggs or chicks by brood parasites. Hosts may also base rejection of brood parasite nestlings on vocal cues, which would in turn select for mimicry of host begging calls in brood parasite chicks. In cuckoos that exploit multiple hosts with different begging calls, call structure may be plastic, allowing nestlings to modify their calls to match those of their various hosts, or fixed, in which case we would predict either imperfect mimicry or divergence of the species into host-specific lineages. In our study of the little bronze-cuckoo (LBC) *Chalcites minutillus* and its primary host, the large-billed gerygone *Gerygone magnirostris*, we tested whether: (1) hosts use nestling vocalizations as a cue to discriminate cuckoo chicks; (2) cuckoo nestlings mimic the host begging calls throughout the nestling period; and (3) the cuckoo begging calls are plastic, thereby facilitating mimicry of the calls of different hosts. We found that the begging calls of LBCs are most similar to their gerygone hosts shortly after hatching (when rejection by hosts typically occurs) but become less similar as cuckoo chicks get older. Begging call structure may be used as a cue for rejection by hosts, and these results are consistent with gerygone defenses selecting for age-specific vocal mimicry in cuckoo chicks. We found no evidence that LBC begging calls were plastic.

Key words: begging call, bronze-cuckoo, brood parasitism, coevolution, gerygone.

Obligate avian brood parasites such as cuckoos lay their eggs in the nests of other species, relying on the hosts to rear their offspring (Rothstein 1990). Hosts may evolve ways to recognise and reject parasites from their nest (Davies 2000). In turn, such defences select for brood parasites that mimic host young and thus evade detection (Soler 2014). For example, many hosts have evolved the ability to detect and remove eggs from their clutch that differ in appearance from their own eggs, and many brood parasites have evolved highly refined, host-specific egg mimicry in response (Brooke M de and Davies 1988).

Likewise, some hosts reject parasitic nestlings (Langmore et al. 2003; Grim 2006; Sato et al. 2010; Tokue and Ueda 2010), selecting for brood parasites that mimic 1 or more aspects of host chick appearance (e.g. skin color or plumage; Langmore et al. 2011; De Mársico et al. 2012; Noh et al. 2018).

In systems where chick rejection by hosts occurs, nestling vocalizations are a candidate cue by which host parents may detect a parasitic chick. The begging vocalizations of young chicks vary greatly in acoustic structure between species (e.g., call duration,

frequency, and syllable number per call; Marler 2002), and can show species-specific qualities even in young nestlings (Gloag and Kacelnik 2013). Brood parasitism would provide strong selection on hosts to recognize their own-species begging calls, which would, in turn, select for parasite mimicry of host begging calls. Consistent with this idea, superb fairy-wrens *Malurus cyaneus* targeted by 2 species of bronze-cuckoo are less likely to reject the parasite whose call resembles their own young's (Horsfield's bronze-cuckoo, *Chalcites basalis*) than the parasite whose call does not (shining bronze-cuckoo, *Chalcites lucidus*). However, parasites might also evolve call types similar to those of their primary host for reasons unrelated to a host's anti-parasite defenses. A shared habitat, nest design, and predation risk may lead to call convergence in host and parasites (Briskie et al. 1999; Leonard and Horn 2005). Parasites may also benefit from host-like call types if they facilitate greater provisioning by host parents, regardless of whether hosts engage in nestling rejection (Ursino et al. 2018). Indeed, several brood parasitic species are reported to have calls similar to hosts, despite hosts in these systems having no known ability to reject parasite young (Redondo and Arias de Reyna 1988; Redondo 1993; Anderson et al. 2009a, 2009b). Thus whether nestling vocalizations are part of the arms race between host defense and parasite counter-defense remains unclear.

Visual chick mimicry by parasites in response to host defense has important implications for host specialization. That is, visual mimicry is likely to evolve in concert with increased host specialization, because different hosts will often have different appearances and parasites can match only one. Unlike visual traits, however, vocal traits of young parasites may exhibit a degree of plasticity (Jamie and Kilner 2017). The extent to which vocal mimicry requires host specialization is therefore uncertain. For example, whereas Horsfield's bronze-cuckoo chicks are good matches for their primary host, the superb fairy-wren, they will develop different call types if reared in a secondary host, the buff-rumped thornbill *Acanthiza reguloides* (Langmore et al. 2008). They initially produce variable notes in the thornbill nest. However, the calls are rapidly refined to resemble the begging calls of the thornbill through a trial and error process in response to provisioning by host parents. Common cuckoo *Cuculus canorus* nestlings also modify their begging calls when they are cross-fostered to alternative hosts, which suggests that begging call structure can be modified through learning and experience (Madden and Davies 2006). In these cases, phenotypic plasticity allows at least some degree of host-matching, without the need for host specialization (Langmore et al. 2008). Animals subject to variable environments should be under selection for phenotypic flexibility and modify their behavior accordingly; they may be capable of altering the acoustic characteristics of their vocalizations (Catchpole and Leisler 1989; Casar et al. 2013). Many studies have shown that birds can modify their calls in response to changing circumstances and environments (Bermudez-Cuamatzin et al. 2010; Rose 2020).

The little bronze-cuckoo (LBC) *Chalcites minutillus* is an obligate brood parasite that specializes on hosts of the genus *Gerygone* (Higgins 1999). The gerygone hosts of the LBC have the most sophisticated discrimination of brood parasite nestlings yet described for a cuckoo host, and are capable of recognizing and rejecting the nestling cuckoo within hours of its hatching (Sato et al. 2010). Such chick rejection occurs despite the high visual similarity of newly hatched cuckoos and gerygones (Langmore et al. 2011). In a previous study, we demonstrated that large-billed gerygones (LBGs) *Gerygone magnirostris* identify the cuckoo hatchling in part

using the visual cue of down feathers, because gerygones rejected cuckoos, and even sometimes their own young, if down feathers were artificially trimmed (Noh et al. 2018). However, gerygone parents in that study rejected trimmed cuckoos far more often than trimmed gerygones, indicating that additional, as yet unidentified cues, are also involved in their chick recognition. The begging call of newly hatched chicks is one plausible cue that hosts may use to recognize cuckoo chicks. Here, we record the begging calls of LBCs and their hosts to better understand the evolution of chick vocalizations in this system. In particular, we tested whether: (1) the hatching calls of rejected LBC chicks differ from those that are not-rejected, which would support call structure as a cue for rejection; (2) nestling LBCs mimic the begging calls of the LBG throughout the nestling period; and (3) the begging calls of LBCs are plastic (i.e., vary depending on the host environment) or fixed. We investigated the vocal plasticity of LBC nestlings via a cross-fostering experiment, in which we placed cuckoos into the nests of a naïve local host (the lovely fairy-wren [LFW], *Malurus amabilis*) whose nestling call structure differs from that of the cuckoo's natural gerygone hosts.

Materials and Methods

Study species, site, and field methods

The study was conducted during the breeding season of the LBC in Cairns (August–December 2016–2019) and Lockhart River (July 2018), Queensland, Australia. In these areas, LBGs are a common host and their defense have been well studied (Noh et al. 2018). The LBC lays dark brown or olive-green eggs that are cryptic inside their dark nest (Brooker and Brooker 1989; Langmore et al. 2009a, 2009b); gerygones rarely reject cuckoo eggs (Gloag et al. 2014). The female cuckoo usually removes 1 host egg at the time of her own egg-laying (Langmore et al. 2009a, 2009b; Gloag et al. 2014). The cuckoo chick hatches after ~15–17 days of incubation, typically 1–2 days before the host chicks, and then ejects the host eggs or chicks from the nest within 24 h (Noh et al. 2018). Gerygones may reject the cuckoo chick, however, within few hours of hatching, thereby saving their own young and proceeding with the brood (Sato et al. 2010; Tokue and Ueda 2010). At our study site, 69% of cuckoo chicks are rejected (Noh et al. 2018). If the cuckoo is instead accepted, it fledges after 16–18 days in the nest.

Fairy gerygones *Gerygone palpebrosa* are known as a secondary host of LBCs in northern Australia. However, the defense behavior against cuckoos has been poorly studied. We searched fairy gerygone nests to record the begging calls of cuckoo chicks in their nests, and to compare the begging calls of cuckoo chicks in fairy gerygone nests and those of cuckoo chicks in LBG nests. Insufficient fairy gerygone nests were found to include them in statistical analyses, but we discuss recordings that were made at 2 fairy gerygone nests.

LFWs were used in this study as a “naïve host” in which to test the plasticity of cuckoo begging calls. LFWs are an insectivorous passerine of similar size to LBGs that are endemic to Cape York Peninsula, Queensland, Australia. They are rare hosts of the brush cuckoo *Cacomantis variolosus* (De Geest and Leitão 2017), but no parasitism by the LBC has been reported. They lay clutches of 2–3 eggs, typically between August and November. Females incubate for a period of 12–16 days, and nestling period lasts for 12–14 days (Leitão et al. 2019).

The nests of gerygones and LFWs were located by daily searching and monitored subsequently with nest checks at least every 3–

4 days (gerygones: 16°55' S, 145°46' E and 12°37'S 143°25'E, and LFWs: 16°55' S, 145°46' E). We searched different habitats to locate each species. Large-LBGs build their nests along tidal or fresh water creeks. Fairy gerygones build nests in dense mangrove and at forest edges (Higgins 1999). LFWs inhabit rainforest edge, woodland, and mangroves (Leitão et al. 2019).

Begging call recordings

We recorded the begging calls of large-LBGs and LBCs on Day 0 (hatching day), Day 3 ± 1, Day 7 ± 2 and Day 13 ± 3 during 3–5 provisioning visits in 2016–2019. We also recorded the begging calls of fairy gerygones. Calls were recorded using a Sony tie-clip miniature microphone (ECM T6) and a recorder (TASCAM DR-05). The microphone was clipped to the back of the nests. All the nests were monitored from a hide or filmed using video cameras (Panasonic VX870M) to confirm that recordings did not disrupt provisioning by parents and to capture instances of chick rejection by host parents. Video cameras or hide were placed approximately 5–7 m from the nests.

Cross-fostering of cuckoos to a naïve host

Cross-fostering experiments were conducted in 2017 and 2018, during the incubation period. Because the incubation period of cuckoo eggs is longer than that of LFW eggs, cuckoo eggs were pre-incubated in LBG nests where they had been originally laid, and then transferred to LFW nests so that the cuckoo chicks hatched 1–3 days before the LFW chicks were due to hatch (as typically happens in the nests of their biological hosts). In total, 24 cuckoo eggs (18 in 2017 and 6 in 2018) were moved to the nests of LFWs, and all were accepted by their hosts. The cuckoo chicks when hatched evicted the host eggs from the nest, thus nest owners had only one chick. Ten nests (9 in 2017 and 1 in 2018) were depredated at the egg stage, and 14 nests survived to hatch (9 in 2017 and 5 in 2018). Of the 14 cuckoo nestlings, 12 (8 in 2017 and 4 in 2018) were depredated in the nest, and only 2 fledged (1 in 2017 and 1 in 2018). Nest predation rates were high but similar to what has been recorded for LFWs when not manipulated (83%; Leitão et al., 2019). For comparison, we also monitored 28 LBG nests that were naturally parasitized by LBCs in 2017.

To assess whether LBC calls varied depending on host of rearing, we recorded the calls of both cuckoos reared in LFW nests ($n = 12$) and LFWs from unparasitized nests ($n = 6$) on days 3 ± 1, Day 7 ± 2, and Day 13 ± 3 in 2016–2018. To determine whether begging calls produced by cross-fostered LBCs in the nests of LFWs were sufficient to stimulate adequate provisioning by the foster parents, we also compared growth rates of cross-fostered cuckoos to those of cuckoos reared by their natural host, the LBG. We measured the weight of all chicks at 4 developmental stages (Day 0 = hatching day, Day 3 ± 2, Day 7 ± 2, and Day 13 ± 2).

Analysis

From each call recording, we analyzed 5 begging calls recorded from nestlings whereas parents were brooding or when parents visited for provisioning. Six acoustic features for each call (a note) were measured; call duration (second), high frequency (kHz), low frequency (kHz), peak frequency (kHz), frequency bandwidth (kHz), and the difference in frequency between the beginning and the end of the call. All measurements of begging calls were conducted using RavenPro sound analysis software (version 1.5, Cornell Laboratory of Ornithology, Ithaca, NY).

To compare the begging calls of cuckoos and same-age LBG nestlings, we first used linear mixed models (function `lmer` in R, library `lmerTest`; Bates and Maechler, 2009) with fixed effects of species, age, and the interaction of group (classified based on chick species and species of foster parents) and age to look for evidence of mimicry by cuckoos. To compare the variation in call parameters between LBG nestlings and LBC chicks, we calculated the coefficient of variation for each series of 5 calls from each chick, and this was regressed against nestling age for each species separately following the method of Langmore et al. (2008). To assess differences in the calls of accepted cuckoos, rejected cuckoos, and hosts on hatching day (when most chick rejection occurs), we performed a discriminant function analysis using a stepwise procedure (JMP, version 6.0). Discriminant function analysis is used to determine whether a set of variables is effective in distributing things of the same type into groups, classes, or categories (Poulsen and French 2008). Therefore, it is useful in determining the set of characteristics that allows for the best discrimination between the groups (Poulsen and French 2008). We also used pairwise comparisons to test the same dataset for multiple comparisons between group levels for each call variable, and using R.

To assess the plasticity of LBC begging calls, we used a discriminant function analysis to test whether the model could distinguish between the begging calls of 4 categories of chick (cuckoo chicks reared by LBGs, cuckoo chicks reared by LFWs, LBG chicks, and LFW chicks). We also used linear mixed models with fixed effects of species, age, and the interaction of group and age to compare the begging calls of cuckoos reared by the 2 hosts. A nest identifier was included as a random effect because calls from multiple chicks in a nest were recorded.

Finally, to test whether cuckoo chicks suffered a growth cost when they were reared by the naïve host (LFW), we used a linear mixed model, with cuckoo weight as the response variable. A previous study showed that a third-order polynomial provides a good fit to the growth patterns of bronze-cuckoos from hatching day to 13 days old (Medina et al., 2019), so we fitted a third-order polynomial for the growth model. We included in the model chick age, host species, year, the number of times the chick was handled, and the interaction between age and host species as fixed effects. Each cuckoo chick was measured multiple times, so a nest identifier was included as a random effect. In addition, to complement this analysis, we calculated the residuals of each data point from the average growth curve (Anderson et al., 2009a, 2009b). Positive residual values designate better growth performance of an individual chick compared with the average and vice versa. We used the residuals in a linear mixed model to explore whether host species could explain variation in the residuals. We used the residuals as the response variable and we included host species, year, and the number of times the chick was handled as fixed variables, and nest ID as a random effect. These analyses were conducted using R version 3.4.4 (R Development Core Team, 2018) and the `lmerTest` and `emmeans` package.

Results

Do LBC nestling calls resemble those of their gerygone host?

The begging calls of LBCs broadly resembled those of LBGs on hatching day (no difference in max frequency [kHz], min frequency [kHz], peak frequency [kHz], frequency bandwidth [kHz], or delta frequency between cuckoo and host nestlings, all $P > 0.05$).

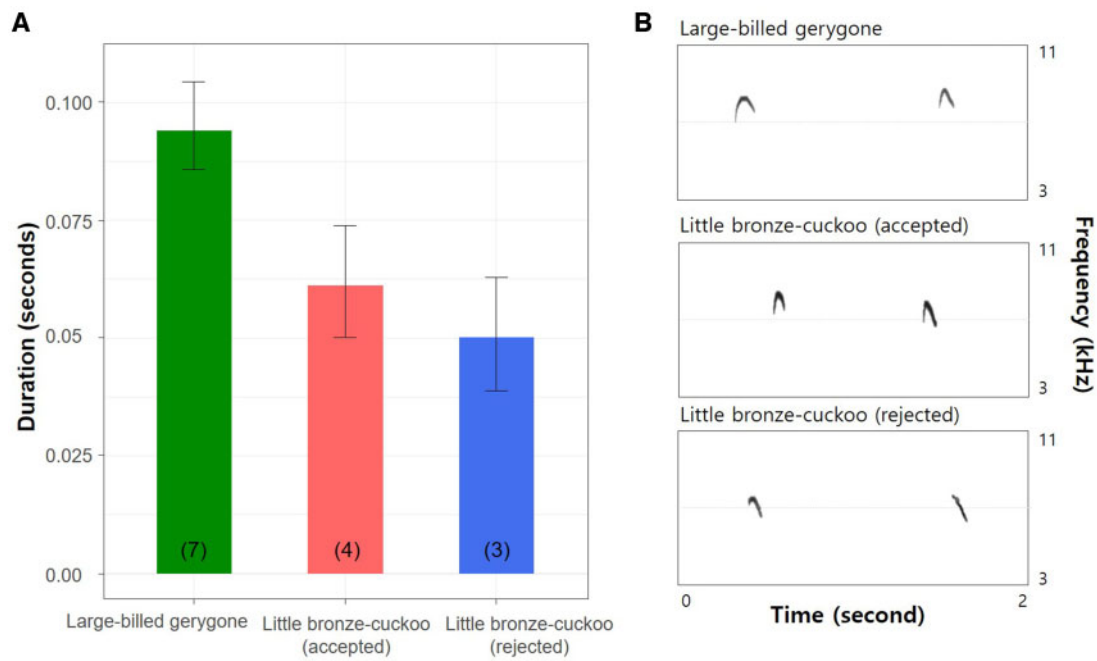


Figure 1. (A) Mean \pm SE duration (seconds) of begging calls produced by LBG chicks ($n=7$), accepted cuckoo chicks ($n=4$), and rejected cuckoo chicks ($n=3$) on hatching day. (B) Sonograms of nesting begging calls for LBGs chicks, accepted cuckoo chicks, and rejected cuckoo chicks.

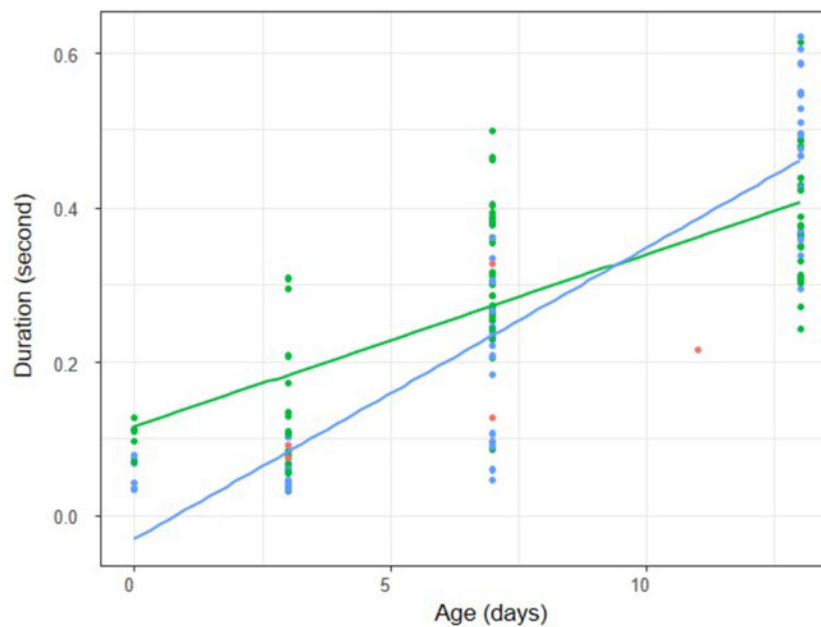


Figure 2. Duration (seconds) of begging calls in relation to age of LBGs (green, $n=36$ chicks), LBCs (blue, $n=38$), and fairy gerygones (red, $n=2$).

However, there was one notable exception; the duration of calls made by cuckoo chicks was significantly shorter than those made by host chicks ($P < 0.05$). We found similar results among rejected cuckoos, accepted cuckoos, and hosts nestlings; no difference in max frequency (kHz), min frequency (kHz), peak frequency (kHz), frequency bandwidth (kHz), or delta frequency between cuckoo and host nestlings (all $P > 0.05$; [Figure 1](#)). The calls of accepted cuckoo chicks were intermediate in duration and did not differ significantly in this trait from rejected cuckoos ($P=0.79$; [Figure 1](#)), or host

chicks ($P=0.08$; [Figure 1](#)). The presence of the host chick in the nest did not affect chick rejection ($\chi^2_1=0.17$, $P=0.68$); 41% of cuckoos (13 out of 32) were rejected when the cuckoo chick was the only chick in the nest, and 75% of cuckoo chicks (6 out of 8) were accepted when host chicks were also in the nest (observational data in 2017).

The begging calls of LBG become increasingly different to those of their host's young as they aged ([Figure 3](#)). A discriminant function analysis failed to discriminate between LBC and LBG begging calls

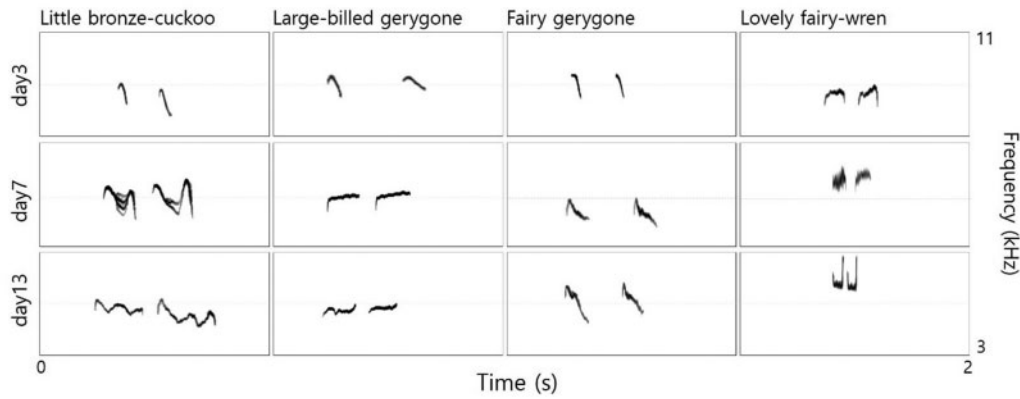


Figure 3. Sonograms of nestling begging calls for LBCs, LBGs (primary host), fairy gerygones (secondary host), and LFWs (nonhost) at 3 different ages (Days 3, 7, and 13) during the nestling period.

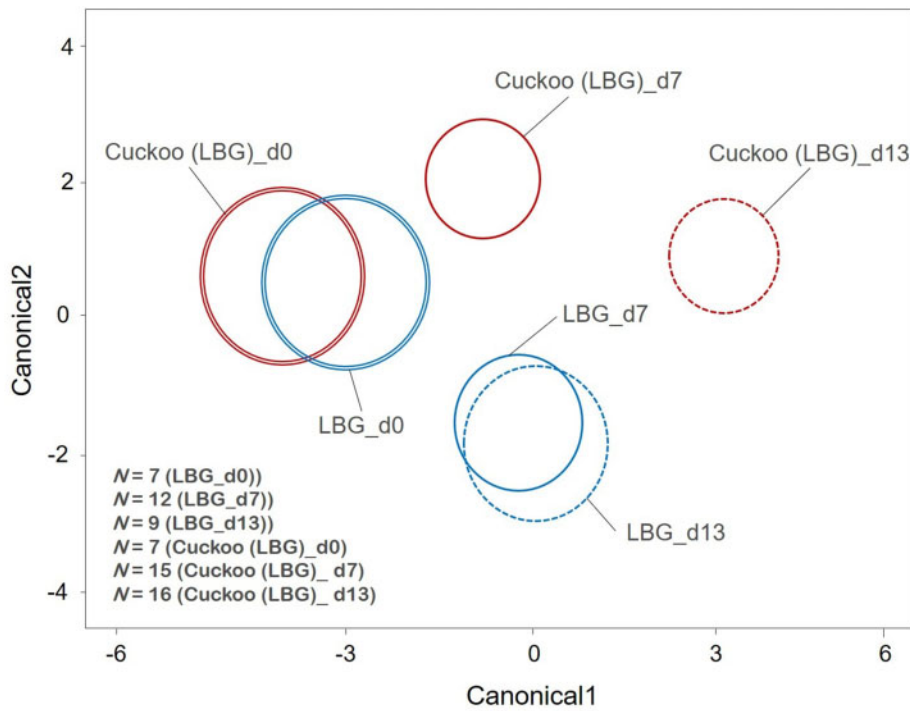


Figure 4. Canonical plots from discriminant function analysis based on the 6 begging call measurements (call duration [sec], high frequency [kHz], low frequency [kHz], peak frequency [kHz], frequency bandwidth [kHz], and the difference in frequency between the beginning and the end of the call) from LBG chicks (blue), and LBC chicks reared by LBGs (red) at 3 different nestling stages, Day 0 (double solid line) and Day 7 (single solid line), and Day 13 (dotted line). Discriminant function analysis labels each multivariate mean with a circle. The size of the circle corresponds to a 95% confidence limit for the mean. Groups that are significantly different have nonintersecting circles.

on hatching day, but was able to distinguish between their calls with high accuracy at later ages, based on all call variables except frequency bandwidth (Days 7 and 13, Figure 4 and Table 1). We found significant change in the coefficient of variation with nestling age for both species. LBG chicks showed a significant decrease for minimum frequency ($F_{1,28} = 8.14, P = 0.01$), maximum frequency ($F_{1,28} = 6.93, P = 0.01$), and peak frequency ($F_{1,28} = 5.66, P = 0.02$). LBC chicks showed a significant decrease for maximum frequency ($F_{1,44} = 6.49, P = 0.01$) and significant increase for frequency

bandwidth ($F_{1,44} = 13.43, P = 0.00$). When coefficient of variation data from LBC and LBG were combined in a single analysis of variance for each call parameter, there was no significant effect of “age” or “species” for the call parameters except frequency bandwidth (Figure 7 and Table 1). In general, call variability stayed relatively constant in LBC young, but decreased with age in gerygone young (Figure 7 and Table 2). We also found a significant interaction between host species and nestling age for call duration, maximum frequency, and differences in frequency (cuckoo nestlings reared by

LBGs (LBG_cuckoo) versus LBG nestlings in Table 3), indicating that the changes in call structure with age differ between LBGs and LBCs.

Table 1. Discriminant function analysis of the begging calls of cuckoo chicks reared by LBG and cuckoo chicks cross-fostered LFW, LBG chicks, and LFW chicks. Significant *P*-values are shown in bold

Nestling age	Significant variables	Wilk's λ	Exact <i>F</i>	<i>P</i> -value
Day 3	<ul style="list-style-type: none"> Call duration Differences in frequency 	< 0.0001	3.30	< 0.0001
Day 7	<ul style="list-style-type: none"> Call duration Maximum frequency Minimum frequency Peak frequency 	< 0.0001	7.57	< 0.0001
Day 13	<ul style="list-style-type: none"> Call duration Maximum frequency Minimum frequency Differences in frequency 	< 0.0001	12.53	< 0.0001

Recordings from 2 nests of an alternative gerygone host, the fairy gerygone, indicated that calls are similar to those of LBGs in most traits, though shorter in duration than the average of LBG calls (Figure 2).

Do LBC chicks modify their begging calls when cross-fostered to a naïve host?

The begging calls of LFWs were significantly different from those of LBGs and LBC at all ages (Figure 5 and Table 1). However, the begging calls of cuckoos reared by LFWs did not differ significantly from those reared by LBGs at any of the 3 stages of the nestling period (Figure 5). Consistent with these results, we found no significant effect of host species and no interaction between host species and nestling age for any call variable (cuckoos reared by LBG versus cuckoo nestlings reared by a nonhost, the LFW (LFW_cuckoo) in Table 3).

The failure of cuckoos to match the begging call of their naïve LFW hosts did not cause them detectable harm. Cuckoo nestling growth neither differ significantly according to the host of rearing (gerygone or fairy-wren; $F_{1,38} = 1.32$, $P = 0.26$, Figure 6A), nor did it differ between years (2017 versus 2018; $F_{1,39} = 2.38$, $P = 0.13$), or

Table 2. Comparison of changes in call variability with age for LBC and LBG. Significant *P*-values are shown in bold

Call variable	Parameter estimates							
	Analysis of variance		Age		Species		Age \times Species	
	$F_{3,72}$	<i>P</i> -value	<i>t</i>	<i>P</i> -value	<i>t</i>	<i>P</i> -value	<i>t</i>	<i>P</i> -value
Call duration	0.25	0.86	-0.61	0.54	0.11	0.91	0.14	0.89
Frequency bandwidth	6.27	< 0.0008	3.30	0.002	3.73	0.0004	-2.82	0.006
Maximum frequency	6.16	< 0.0009	-2.76	0.01	-1.05	0.30	-0.12	0.91
Minimum frequency	5.516	0.002	1.00	0.32	0.55	0.59	-2.33	0.02
Peak frequency	3.689	0.01	0.131	0.90	0.21	0.834	-1.644	0.11
Difference in frequency	2	0.12	0.51	0.62	1.19	0.24	-1.96	0.053

Significant results are in bold.

Table 3. Comparison of age-matched begging calls between cuckoo nestlings reared by LBGs (LBG_cuckoo) versus LBG nestlings and cuckoos reared by LBG versus cuckoo nestlings reared by a nonhost, the LFW (LFW_cuckoo)

Call variable	Group		Age		Group \times age	
	Estimate (Standard Error)	<i>P</i> -value	Estimate (s.e.)	<i>P</i> -value	Estimate (s.e.)	<i>P</i> -value
Cuckoo (LBG_cuckoo) versus host (LBG)						
Call duration	0.13 (0.03)	< 0.001	0.04 (0.002)	< 0.001	-0.02 (0.004)	< 0.001
Frequency bandwidth	-256.69 (78.65)	0.002	-18.62 (6.08)	0.003	3.91 (9.76)	0.69
Maximum frequency	470.32 (228.48)	0.04	44.52 (16.75)	0.01	-54.69 (27.22)	0.04
Minimum frequency	731.74 (196.60)	< 0.001	2.20 (14.81)	0.88	26.18 (23.96)	0.28
Peak frequency	533.31 (208.17)	0.01	9.90 (15.56)	0.53	-27.25 (25.215)	0.28
Differences in frequency	-743.39 (252.56)	< 0.001	2.96 (19.44)	0.88	-82.78 (31.24)	< 0.001
Cuckoo in primary host (LBG_cuckoo) versus cuckoo in naïve host (LFW_cuckoo)						
Call duration	0.01 (0.03)	0.71	0.04 (0.002)	< 0.001	-0.0006 (0.004)	0.15
Frequency bandwidth	93.87 (136.86)	0.49	-18.62 (8.80)	0.04	4.86 (18.74)	0.80
Maximum frequency	92.63 (300.30)	0.76	43.54 (18.40)	0.02	-14.91 (38.33)	0.70
Minimum frequency	-47.89 (266.74)	0.86	-1.17 (17.15)	0.95	-44.97 (36.52)	0.22
Peak frequency	69.48 (267.76)	0.80	8.32 (16.91)	0.63	-9.96 (35.80)	0.78
Differences in frequency	89.98 (359.49)	0.80	2.85 (22.78)	0.90	31.89 (48.29)	0.51

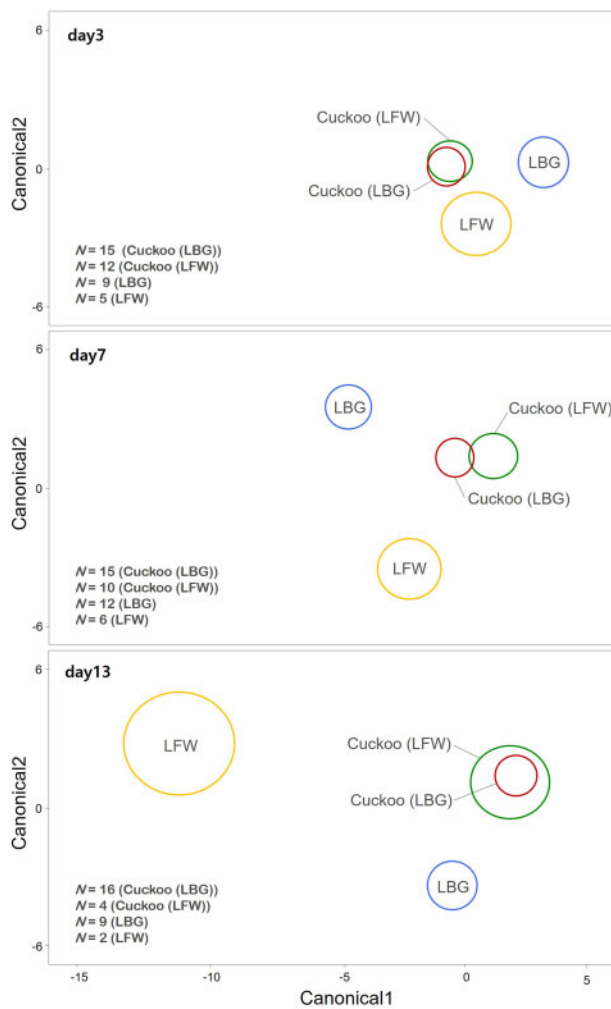


Figure 5. Canonical plots from discriminant function analysis based on the 6 begging call measurements from LBG (blue), LFW chicks (yellow), and LBC chicks reared by LBG (LBG_cuckoo, red) and LFWs (LFW_cuckoo, green) at 3 different ages (Days 3, 7, and 13) during the nestling period. Discriminant function analysis labels each multivariate mean with a circle. The size of the circle corresponds to a 95% confidence limit for the mean. Groups that are significantly different have nonintersecting circles.

in relation to the number of times the chick was handled ($F_{1,48} = 1.48$, $P = 0.23$). We found the same results when using residuals from the average curve ($F_{1,38} = 0.89$, $P = 0.35$, Figure 6B). Again, neither year ($F_{1,40} = 2.20$, $P = 0.15$) nor the number of times the chick was handled ($F_{1,42} = 0.976$, $P = 0.33$) had a significant effect on chick growth.

Discussion

We find that LBCs produce a begging call that resembles that of their LBG host on hatching day, but becomes increasingly dissimilar as chicks age throughout the nestling period (Figure 4). The period of closest cuckoo call resemblance to that of their gerygone hosts corresponds to the period during which selection for call mimicry should be strongest in cuckoos, as gerygonids typically reject cuckoo chicks within 2 days of hatching (Sato et al. 2010; Noh et al. 2018). Cuckoos that survive beyond 2 days of hatching are thereafter accepted by hosts (Noh et al. 2018), thus selection for call mimicry

beyond 2 days of age may be weak. At older nestling ages, mimetic cuckoo calls could be favored, even in the absence of chick rejection by hosts, if they stimulate greater provisioning by the foster parents; that is, if they better “tune into” host parental provisioning rules (Davies 2011; Ursino et al. 2018). However, parasite begging calls in some species have been shown to induce adequate provisioning by resembling host begging calls in some, but not all, features of call structure (Madden and Davies 2006), or via per-unit structures that differ considerably from those of host young’s calls (Davies et al. 1996; Gloag and Kacelnik 2013). Thus, the imperfect call mimicry of LBC nestlings at older ages, as observed in our study, may reflect selection for resemblance of some call features but not others.

We also found that, on hatching day (the day when gerygonids typically reject LBC chicks), the begging calls of LBC nestlings that were subsequently rejected differed from those that were accepted. Specifically, the duration of the begging calls of rejected cuckoo chicks was shorter than the duration of host begging calls, whereas the duration of the begging calls of accepted cuckoo chicks did not differ significantly from the duration of host begging calls (Figure 1). This result is consistent with call duration on hatching day being a cue for gerygonids when discriminating between own and parasite chicks, provided that gerygonid parents have a finely attuned ability to detect differences in call duration (< 0.25 s). Likewise, it suggests that begging call mimicry of hatchling LBCs has evolved in response to host discrimination of acoustic cues. If so, small differences in nestling vocalizations can explain how gerygonids discriminate LBC chicks from their own young, despite the cuckoo’s striking visual mimicry of host nestlings (Langmore et al. 2011). In addition, because many rejections of the cuckoo chick occur before any host young have hatched, recognition by LBG must involve a comparison between the cuckoo chick phenotype and an internal template. There may yet be other cues that also play a role in gerygonid discrimination of newly-hatched chicks. Recognition systems that rely on multiple cues are likely to be more robust and less error-prone than those based on single cues (Langmore et al. 2009a, 2009b). Future studies may consider multivariate analyses of multiple cues (e.g., both nestling appearance and begging calls) to help tease apart the relative importance of each cue type in host rejection decisions.

If gerygonid hosts use short call duration as a cue to detect foreign chicks, why did we observe some LBCs to produce short begging calls? One explanation is that secondary hosts of LBCs, such as mangrove gerygonids *Gerygone laevigaster* (parasitism rate 43% of nests, Tokue and Ueda 2010) or fairy gerygonids *G. palpebrosa* (parasitism rate unknown), produce calls for shorter duration, selecting for calls of intermediate duration in cuckoo chicks to facilitate exploitation of other species in the *Gerygone* genus. Imperfect mimicry for this purpose has been identified in the egg color (Feeney et al. 2014) and the nestling skin color (Langmore et al. 2011) of another species of bronze-cuckoo. Whereas we currently lack sufficient data from the various other gerygonid hosts of LBCs to test this idea, our preliminary data show that fairy gerygonid chicks do produce shorter begging calls than those of LBG chicks (Figure 2).

Our cross-fostering experiments demonstrated that LBCs retained the same begging call structure when they were cross-fostered to a novel host with a different begging call, the LFW, as to when they were reared in gerygonids (Table 3 and Figure 5). In this respect, LBC chicks differ from the chicks of the congeneric Horsfield’s bronze-cuckoo *C. basalis*, which was found to alter its begging call when cross-fostered to a different host (Langmore et al. 2008). A possible explanation for this result relates to the different strategies of parasitism of the 2 species; the LBC is a specialist that exploits *Gerygone* hosts only, whereas the Horsfield’s bronze-

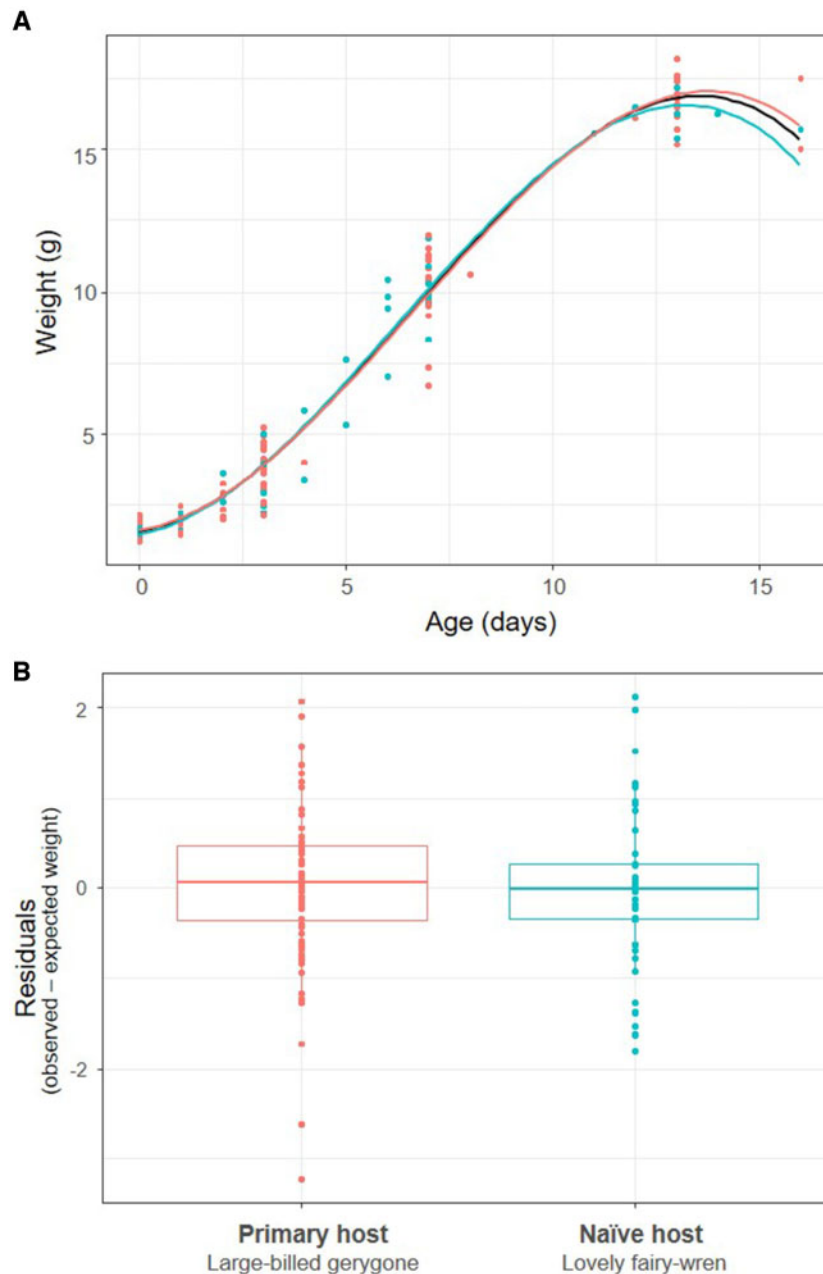


Figure 6. (A) Growth of LBC chicks in LBG nests (primary host, $n=28$, red points and line) and LFW nests (naïve host, $n=14$, blue points and line). The average growth curve from all data points is shown in black. (B) Boxplot of residuals of each data point from the average growth curve.

cuckoo is a generalist that exploits several different genera (Brooker and Brooker 1989). The hosts of Horsfield's bronze-cuckoos, therefore, show greater diversity in begging call structure than those of LBCs (Langmore et al. 2008), and Horsfield's bronze-cuckoos may be under greater selection from hosts to produce a plastic begging call that can be modified to match those of its various hosts. In the specialist LBC, there may be developmental costs to maintaining such vocal plasticity, which presumably requires repeated sampling of environmental cues to achieve accuracy (Jamie and Kilner 2017).

An alternative explanation for the lack of modification of LBC begging calls in the nest of a new host is that this particular new host failed to provide a trigger for call modification. The proposed mechanism underlying call modification in Horsfield's bronze-

cuckoos was that a nonmimetic begging call failed to stimulate provisioning by the host, triggering a change in call structure in the cuckoo chick (Langmore et al. 2008). Our results showed that the nestling growth patterns of cuckoos reared by LBGs and LFWs did not differ, suggesting that LFWs did not reduce provisioning in response to a nonmimetic call (Figure 6). LFWs may have maintained an adequate provisioning rate when faced with a cuckoo chick because either (1) they respond to the same cues as LBGs (e.g. they might provide more food with increasing call rate, regardless of call structure) or (2) they are not a primary host of any cuckoo species, so they have not evolved discrimination based on call structure. This may suggest that the cuckoo begging call itself effectively stimulated provisioning in the nests of a nonhost species, the LFW. However,

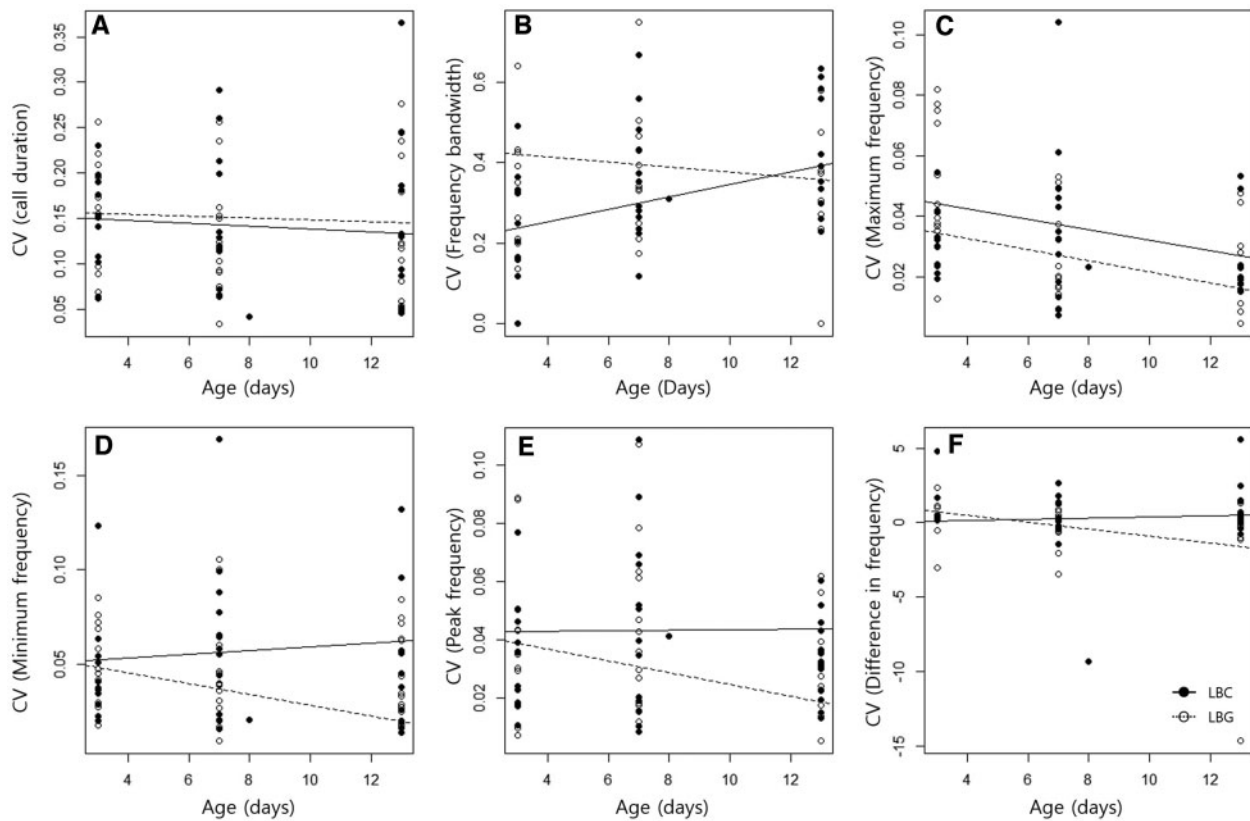


Figure 7. Regression plots for LBC and LBG showing coefficients of variation by nestling age for (A) call duration, (B) frequency bandwidth, (C) maximum frequency, (D) minimum frequency, (E) peak frequency, and (F) difference in frequency.

this does not preclude the possibility that their begging calls are plastic, because it is possible that LBC chicks are able to modify their begging calls if triggered by reduced provisioning by the host (Langmore et al. 2008). This possibility requires further investigation, by cross-fostering LBCs to a secondary host species that has both a different begging call structure from LBGs and shows reduced provisioning in response to a nonmimetic begging call.

In conclusion, LBCs show imperfect mimicry of host begging calls; nestling calls are most similar to those of gerygone chicks in the first days after hatching, but they become less similar as chicks age. During the period when cuckoo chick rejection occurs (within 2–3 days of hatching), cuckoo chicks which produced less similar begging calls were more likely to be rejected. This suggests that cuckoo nestlings are under strong selection for mimicry during the period when rejection of cuckoo chicks by hosts occurs, but that this selection pressure is relaxed later in the nestling period. LBCs do not modify their begging calls when they are reared by a naïve host (the LFW). We propose that this is either because the vocal development of begging calls of LBCs is fixed, or that the naïve host in this case failed to provide an appropriate trigger for call modification.

Ethical Approval Statement

All experiments were conducted under approval of the Australian National University Experimentation Ethics Committee Protocol number A201539 and A2016/16.

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conflict of interest

The authors declare no competing financial interests.

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