

SOCIALLY ACQUIRED HOST-SPECIFIC MIMICRY AND THE EVOLUTION OF HOST RACES IN HORSFIELD'S BRONZE-CUCKOO *CHALCITES BASALIS*

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Coevolution between parasites and their hosts typically leads to increasing specialization on host species by the parasite. Where multiple hosts are parasitized, specialization on each host can result in genetic divergence within the parasite population to create host races, and, ultimately, new species. We investigate how host-specific traits arise in Horsfield's bronze-cuckoo *Chalcites basalis* nestlings. Newly hatched cuckoos evict host young from the nest, yet in the absence of a model they accurately mimic the different begging calls of a primary host (superb fairy-wren, *Malurus cyaneus*) and a secondary host (buff-rumped thornbill, *Acanthiza reguloides*). Using cross-fostering experiments, we show that begging calls are modified after parasitism, through experience. Further, we demonstrate the mechanism by which mimetic calls are acquired. All cuckoo nestlings initially produced the call of their primary host. When cross-fostered as eggs to a secondary host, calls increased in variability and were rapidly modified to resemble those of the secondary host through shaping by host parents. We suggest that plasticity in the development of host-specific traits after parasitism is likely to reduce selection for host race formation.

KEY WORDS: Begging calls, brood parasitism, coevolution, learning, social shaping.

The reciprocal adaptations that take place in the process of coevolution between a social parasite and its various host species can lead to increasing specialization by the parasite (Davies 2000). As the host evolves defenses against parasitism, the parasite evolves ever more refined counter-measures (Payne 1977; Rothstein 1990; Davies 2000), thus becoming locally adapted to each of its different hosts (Thompson 2005). Local adaptation can facilitate reproductive isolation (Thompson 2005), and therefore contribute to the formation of host races (Brooke and Davies 1988; Gibbs et al. 2000) and even species (Davies 2000; Sorenson et al. 2003;

Thompson 2005). However, neither evolutionary outcome is an inevitable consequence of host specialization (e.g., Joseph et al. 2002; Als et al. 2004; Fanelli et al. 2005).

Here we consider how the development of host-specific traits in parasitic individuals might contribute to, or impede, the process of host race formation. The hypothesis we explore is that generalist parasites are unlikely to split into genetically distinct host races if they can acquire their host-specific adaptations soon after parasitism (Fanelli et al. 2005). Host races are much more likely to evolve if the specific adaptations for each host are innately

produced (Coyne and Orr 2004), and not subject to later modification following interaction with the host.

The hypothesis is based on evidence from insect social parasites. Their hosts bear distinctive cuticular hydrocarbon signatures, which the parasites must mimic to evade detection and rejection. Parasites may biosynthesize the appropriate hydrocarbon signature themselves or it may be acquired by mechanical transfer, or altered gene expression, soon after entering the host nest (Lenoir et al. 2001). Generalist parasitic species that manufacture an unmodifiable mimetic hydrocarbon signature appear to have radiated into several cryptic species as a result of their host specificity (e.g., Elmes et al. 1999; Schönrogge et al. 2002). By contrast, the generalist social parasites that acquire the mimetic traits for overcoming host defenses only after entering the host nest have not differentiated into genetically distinct host lineages (e.g., Akino et al. 1999; Als et al. 2004).

The same principle might also apply in the obligate avian brood parasites. Innately produced host-specific mimicry has been well characterized in the cuckoos: mimetic parasitic eggs are the phenotypic hallmarks of host races (e.g., common cuckoo *Cuculus canorus*, Brooke and Davies 1988; Diederik cuckoo *Chrysococcyx caprius* Rowan 1983; Pallid cuckoo *Cuculus pallidus* Starling et al. 2006) that show genetic differences (e.g., common cuckoo, Gibbs et al. 2000). Similarly, the innately produced host-specific mouth markings in nestling viduid finches differ among parasitic species (Payne 2005) and are associated with speciation (Sorenson et al. 2003). However, it is not known whether any brood parasites possess host-specific mimetic traits that can be modified after hatching to mimic different hosts.

Several generalist cuckoo species possess begging call polymorphisms (e.g., Diederik cuckoo *C. caprius*, Reed 1968; Long-tailed koel *Eudynamis taitensis*, McLean and Waas 1987; Great-spotted cuckoo *Clamator glandarius*, Redondo and Arias-de-Reyna 1988; Horsfield's bronze-cuckoo *Chalcites basalis*, Payne and Payne 1998), each morph resembling the nestling begging call of a different host species and apparently indicating the existence of host-specific races. Mimetic begging calls may have evolved as a result of selection on cuckoo nestlings to tune into existing channels of offspring–parent communication and thereby elicit optimal parental provisioning (e.g., Davies et al. 1998; Kilner et al. 1999) or to evade recognition and rejection by hosts (Langmore et al. 2003), but it is not known whether they are innately encoded or acquired after parasitism. Evidence to suggest the latter possibility is more likely comes from a recent study of the common cuckoo (Madden and Davies 2006). Common cuckoo chicks do not mimic the calls of host young, but their begging call structure does differ between host races. By cross-fostering cuckoo eggs between host species, Madden and Davies (2006) experimentally demonstrated that begging call structure is modified after parasitism to exploit the host most effectively.

Exactly how might cuckoo chicks acquire host-specific mimetic begging calls? In the Diederik cuckoo, the long-tailed koel and Horsfield's bronze-cuckoo, newly hatched cuckoo chicks evict any host eggs or young from the nest, so there is no opportunity for imitating host offspring. Instead, the cuckoo nestling may modify its begging call in response to provisioning rewards from the host parents (McLean and Waas 1987). We propose two processes by which mimetic nestling begging calls might develop, each of which generates a unique set of predictions.

SOCIAL SHAPING

This is a form of instrumental conditioning, in which an initially extensive repertoire is reduced to a set of calls that are most successful in eliciting social rewards (King and West 1983; West and King 1988a). Thus, regardless of its host, a newly hatched cuckoo nestling would produce a wide variety of different calls, but gradually eliminate those call types that are less successful in eliciting a high provisioning rate from their hosts, until only the mimetic calls that are reinforced by the host remain in older nestlings. Under this scenario we would predict that (1) cuckoo calls should become more similar to host calls with age, regardless of host species, and (2) whichever the host, cuckoo calls should be initially more variable than host calls, but similarly variable between older host and cuckoo chicks.

DEVELOPMENTAL SWITCH

Cuckoo nestlings possess two innate programs of vocal development and the one they follow is determined by an environmental switch. We imagine that newly hatched cuckoo nestlings would initially produce the nestling begging call of the primary host. In the nest of the primary host the begging call does not change and accurately mimics host young begging calls throughout the period of dependence. In the nest of a secondary host species, however, the cuckoo switches to a different developmental pathway. The switch could be state dependent if the nestling is not receiving adequate food. On the alternative developmental pathway, the cuckoo chick might (a) initially produce a range of begging calls but, through social shaping by the secondary host gradually eliminates those that are less effective at procuring food, until only those that mimic the calls of secondary host nestlings remain, or (b) switch to an innately produced alternative begging call that does not require social shaping. Under scenario (a), we would predict that (1) in primary hosts, cuckoo calls are similar to host calls at all ages; in secondary hosts cuckoo calls become more similar to host calls with increasing age, and (2) in primary hosts, cuckoo and host calls are similarly variable at all ages, whereas in secondary hosts, cuckoo calls are initially more variable than host calls. Under scenario (b) we would expect that following the developmental switch in the nest of a secondary host, the cuckoo would immediately produce a begging call that accurately mimics

that of the secondary host, with no increase in call variability and no gradual improvement in mimicry with age.

We investigated the development of begging call mimicry in the Horsfield's bronze-cuckoo by testing these predictions. This cuckoo primarily parasitizes fairy-wrens throughout its range (*Malurus* spp.), but also exploits a range of secondary host species, particularly thornbills (*Acanthiza* spp.) (see methods, Brooker and Brooker 1989a). Mimicry of the begging calls of both primary hosts and secondary hosts has been described for this species (Payne and Payne 1998), as has host specificity in individual females (Brooker and Brooker 1992), prompting researchers to suggest that host races might exist (Brooker and Brooker 1992; Joseph et al. 2002). Paradoxically, a recent study of the population genetics of Horsfield's bronze-cuckoos found low mtDNA diversity and a lack of phylogenetic structure, indicating a lack of evolutionarily long-term stable host races in this species (Joseph et al. 2002). We test the possibility that Horsfield's bronze-cuckoos have evolved an alternative pathway to host-specific adaptations, modification of calls after hatching to mimic different hosts, by cross-fostering cuckoo eggs from the nests of primary hosts, superb fairy-wrens *Malurus cyaneus*, to secondary hosts, buff-rumped thornbills *Acanthiza reguloides*.

Methods

STUDY SPECIES

Horsfield's bronze-cuckoos occur throughout Australia. They parasitize fairy-wrens primarily, but also a range of secondary hosts. For example, of 380 records of parasitism by Horsfield's bronze-cuckoos in southeastern Australia (where this study was conducted), the hosts were: fairy-wrens (41%), thornbills (*Acanthiza* spp. 34%), robins (*Petroica* spp., 11%) scrubwrens (*Sericornis* spp. 7%), and chats (*Epthianura*, 6%; Brooker and Brooker 1989a). Horsfield's bronze-cuckoos lay elongated pinkish-white eggs, speckled with red-brown spots, which mimic fairy-wren and thornbill eggs, but not those of some other secondary hosts (Brooker and Brooker 1989a, Langmore et al. 2003). They generally lay during the egg-laying period of their host, removing one host egg and replacing it with one of their own. The cuckoo chick hatches after about 12 days of incubation, one to two days before the host chicks, and ejects the host eggs or chicks from the nest within two days. It remains in the nest for 16–18 days. Hosts rarely reject cuckoo eggs (Brooker and Brooker 1989b, Langmore et al. 2003, 2005), but around 40% of superb fairy-wren females abandon cuckoo chicks in the first few days after hatching (Langmore et al. 2003).

Superb fairy-wrens (10 g) and buff-rumped thornbills (8 g) are small, cooperatively breeding, insectivorous passerines that build similar dome-shaped nests in similar nest sites (Langmore and Kilner 2007). Superb fairy-wren chicks fledge after 10–12

days in the nest, whereas buff-rumped thornbills fledge after 16–18 days.

STUDY SITE

Our study was conducted in eucalypt woodland in Campbell Park, Canberra, in southeastern Australia (149°9'E, 35°16'S) from 1999 to 2007. Horsfield's bronze-cuckoos primarily parasitize superb fairy-wrens at this site; only one case of natural parasitism of a buff-rumped thornbill occurred and no other potential hosts were parasitized (Langmore and Kilner 2007). Fairy-wren and thornbill nests were located by daily searching, and all parasitized and experimental nests were enclosed in a large, dome-shaped mesh cage that allowed access by host parents through the large mesh, but excluded larger predators.

CROSS-FOSTERED HORSFIELD'S BRONZE-CUCKOOS

Ten Horsfield's bronze-cuckoo eggs were transferred from superb fairy-wren nests to buff-rumped thornbill nests (four in 2002, five in 2006, and one in 2007). Eggs were transferred after 0 ($N = 2$), 1 ($N = 2$), 2 ($N = 1$), 4 ($N = 1$), 7 ($N = 2$), 8 ($N = 1$), and 9 ($N = 1$) days of incubation. All were accepted by their new foster parents. One egg was infertile, one was depredated, and eight hatched. Of the eight hatchlings, one died in the nest 10 days after hatching and seven fledged. Recordings started on day 2 or 3 (hatch day = day 0) when calls had sufficient amplitude for recording, and continued on alternate days until day 15 (weather permitting). Nestlings were recorded using a Sony tie-clip microphone ECM T6 and a Sony Professional Walkman WM-D6C (1999 to 2001) or a Sony DAT Walkman TCD-D100 (2002 to 2007) (Sony Corporation, Tokyo, Japan). The microphone was clipped to the wire of the cage, approximately 30 cm from the nest entrance.

NATURALLY REARED HORSFIELD'S BRONZE-CUCKOOS

Seventeen cuckoos were recorded in superb fairy-wren nests. Ten were reared in the nest in which they were laid, and seven were transferred as eggs from the nest in which they were laid to a different superb fairy-wren nest in the same manner as the eggs cross-fostered to thornbills. Eggs were transferred after 0 ($N = 2$), 2 ($N = 1$), 5 ($N = 1$), 6 ($N = 1$), and 10 ($N = 2$) days of incubation, and all were accepted by their new foster parents. We also collected recordings from the single Horsfield's bronze-cuckoo chick that was laid in a monitored buff-rumped thornbill nest. Recordings were made as described for the cross-fostered cuckoos, except that some chicks were recorded less frequently so as to minimize disruption to other data collection.

HOST CHICKS

Unmanipulated host broods ($N = 17$ superb fairy-wren and 12 buff-rumped thornbill broods) were also recorded on one to four occasions each in the same manner as above.

RELATEDNESS OF CUCKOO CHICKS

The analysis could be confounded if there was high relatedness between either the cross-fostered cuckoo chicks or the cuckoos reared by fairy-wrens. Microsatellite and mtDNA analysis (Langmore et al. 2007; 2006, 2007, unpubl. data) revealed the relatedness of 20 chicks. We had no genetic material from a further six chicks reared by fairy-wrens. Based on egg morphology (Langmore et al. 2007), we can infer that two of these from 1999 were the offspring of two different mothers and a third belonged to the same family as four chicks in 2006. Overall, the 17 chicks reared by fairy-wrens were the offspring of at least nine mothers, and the nine chicks reared by thornbills were the offspring of at least five mothers. Three offspring of two mothers shared the same father, and there was no shared paternity between the other families.

CALL ANALYSIS

We recognized the start of a nest visit by the sound of the host parent landing on the wire of the cage. Forty seconds of sound were then transferred to the Sound Analysis software package “Canary” version 1.2.4 (Cornell Bioacoustics Research Program, Ithaca, New York). From the point where the chick started begging in response to the parent’s arrival, 10 sec were converted to a spectrogram (Using default settings: Filter bandwidth = 1398.8, Frame length = 64 points, Grid resolution = 0.3628 msec, Overlap = 87.5%, Frequency = 172.3 Hz, fast Fourier transform = 128 points). Spectrograms were displayed on a full screen set to 11 kHz by 2.5 sec. Using “Canary,” measurements were taken of 10 calls, starting with the second call in the bout. Calls were defined as uninterrupted traces on the sonagram. We measured five variables for each call; call duration (sec), frequency bandwidth (kHz), peak frequency (the frequency at which the highest amplitude occurs, kHz), and maximum and minimum frequency (kHz). Where a call was obscured by other sounds or (in the case of host chicks) other chicks in the nest, it was skipped and measurements continued on the next unobscured call.

STATISTICAL ANALYSIS

Normality of call variables was confirmed using normal quantile plots and comparison of variances between species to confirm homogeneity. The five call variables were analyzed in a discriminant function model, using a stepwise procedure and incorporating significant variables only in the final model. We first aimed to determine whether the model could distinguish between Horsfield’s bronze-cuckoos reared by superb fairy-wrens (SFW-cuckoo) and those reared by buff-rumped thornbills (BRT-cuckoo) at different stages of the nestling period. We also used restricted maximum-likelihood models (REML) to test the same dataset, which allowed us to explore the effect of nestling age on call variables whilst controlling for year effects and repeated measures of individual chicks. We incorporated the fixed effects “host species”

and “nestling age,” with “year” and a nest identifier as random terms.

Second, we aimed to test whether the model could distinguish between the begging calls of the cuckoos and the hosts that reared them. Direct comparison of the begging calls of the cuckoos and their hosts was complicated by the fact that superb fairy-wrens are only in the nest for 10–12 days, whereas Horsfield’s bronze-cuckoos and buff-rumped thornbills are in the nest for 16–18 days. Recordings were made from day 2 to 9 in superb fairy-wrens and from day 2 to 15 in thornbills and cuckoos. Earlier recordings were not possible due to the low amplitude of the chicks’ calls, and later recordings were avoided due to the risk of provoking premature fledging. We made four comparisons; two in which the three species were matched for age (all species day 2–3, and all species day 5–7), one that compared the begging call structure of the three species in the first half of the nestling period (day 2–5 for fairy-wrens, day 2–8 for cuckoos and thornbills), and one that compared begging call structure in the second half of the nestling period (day 5–9 for superb fairy-wrens, day 9–15 for cuckoos and thornbills). A mean value for each of the five variables was calculated from all recording sessions made during the three periods at each nest ($N = 1–4$ recordings per nest).

To quantify changes in the variability of call parameters with nestling age, we calculated the coefficient of variation for each series of 10 calls from each nest visit. This was regressed against nestling age for each species separately and for cuckoos and their host species combined in a single analysis of variance.

If cuckoos change begging call structure in response to low provisioning rates, we would expect to see a growth cost during the early nestling period in cuckoos reared by the secondary host. We used a restricted maximum-likelihood model (REML) to test whether a significant amount of the variation in nestling cuckoo mass could be explained by the host species. “Host” and “nestling age” were fixed effects and a nest identifier was included as a random term to control for repeated weighing of individual chicks.

All statistical tests were carried out in JMP ver. 6.0, except REML analyses, which were performed using GENSTAT ver. 8.1 (VSNi Australia Pty Ltd., Helensburgh, Australia).

Results

DO HORSFIELD’S BRONZE-CUCKOOS CROSS-FOSTERED TO A DIFFERENT SPECIES PRODUCE A DIFFERENT BEGGING CALL?

Within a few days of hatching, Horsfield’s bronze-cuckoos that were cross-fostered as eggs from superb fairy-wren to buff-rumped thornbill nests produced a different begging call from cuckoos reared by superb fairy-wrens (Fig. 1). Visual inspection of sonagrams revealed that fairy-wren-reared cuckoos produced a short call with a relatively wide frequency bandwidth and a

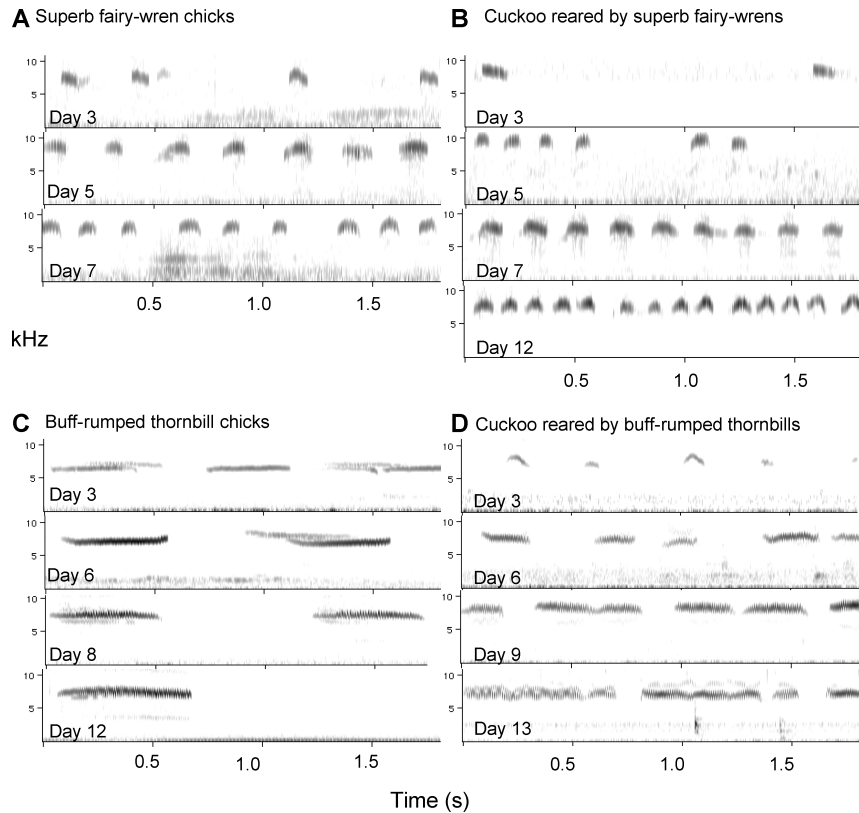


Figure 1. Sonograms of examples of nestling begging calls at different stages of the nestling period, for (A) superb fairy-wren chicks, (B) Horsfield's bronze-cuckoos reared by superb fairy-wrens, (C) buff-rumped thornbill chicks, (D) Horsfield's bronze-cuckoos laid in a superb fairy-wren nest, but cross-fostered to buff-rumped thornbills prior to hatching.

relatively high maximum frequency, due to a slightly “humped” shape (Fig. 1B). Young thornbill-reared cuckoos initially also produced a short call, but rapidly modified their call structure to show a narrower frequency bandwidth and increasing duration as the nestling period progressed (Fig. 1D). Three of the 17 cuckoos reared by superb fairy-wrens produced calls that resembled buff-rumped thornbill calls for at least part of the nestling period (one was recorded once on day 7, then depredated, one was recorded on days 10, 12, and 15, and the other was recorded on days 8 and 11). Although one possibility is that these cuckoos belonged to a thornbill host race, this seems unlikely because one nestling produced

fairy-wren-like calls until day 8, but switched to thornbill-like calls by day 11. It was also a full-sibling of four other cuckoos laid in fairy-wren nests, two of which were recorded and produced fairy-wren-like calls throughout the nestling period.

Using discriminant function analysis we were able to distinguish between BRT-cuckoos and SFW-cuckoos with 80–100% accuracy at each stage of the nestling period from day 3 onwards, with different features of the begging call contributing greatest significance to the model at different ages (Table 1). By the late nestling period the model could distinguish between SFW-cuckoos and the cross-fostered BRT-cuckoos with 100%

Table 1. Discriminant function analysis of the begging calls of Horsfield's bronze-cuckoo chicks cross-fostered to buff-rumped thornbills (BRT-cuckoo) and those reared by superb fairy-wrens (SFW-cuckoo) at each stage of the nestling period. Frequency is indicated by the abbreviation “F.” Significant results ($P < 0.05$) are shown in bold.

Nestling age	<i>N</i> BRT cuckoo	<i>N</i> SFW cuckoo	Significant variables	Assignment accuracy	Wilks' λ	Exact <i>F</i>	<i>P</i>
Day 3–5	7	6	F bandwidth	84.6%	0.50	11.02	0.007
Day 6–8	8	10	F bandwidth	83.3%	0.46	17.39	0.0008
Day 9–11	6	11	Max. F	82.4%	0.63	8.24	0.012
Day 12–15	6	8	Call duration F bandwidth	100%	0.22	17.43	0.0006

Table 2. REML model of the factors influencing variation in begging call structure in nestling cuckoos reared by superb fairy-wren and buff-rumped thornbill hosts. Significant results ($P < 0.05$) are shown in bold.

Call variable	Nestling age		Host		Nestling age \times Host	
	χ^2_1	P	χ^2_1	P	χ^2_1	P
Call duration	32.18	<0.001	17.0	0.01	7.11	0.01
Frequency bandwidth	40.54	<0.001	4.93	0.04	2.13	0.15
Maximum frequency	3.09	0.08	7.92	0.01	4.47	0.04
Minimum frequency	4.35	0.04	0.56	0.47	7.43	0.01
Peak frequency	0.31	0.58	14.34	0.01	4.21	0.04

accuracy. Similarly, using REML we found a significant effect of host species for all call variables except minimum frequency, and a significant effect of nestling age for all call variables except maximum and peak frequency (Table 2). There was also a significant interaction between host species and age for all call variables (except frequency bandwidth) indicating that the change in call variables with nestling age differed depending on which host reared the cuckoo (Table 2). By contrast, there were no significant differences in any call variables between SFW-cuckoos that were reared in the nest in which they were laid versus SFW-cuckoos that were moved from one SFW nest to another (REML; all $P > 0.18$).

DO HORSFIELD'S BRONZE-CUCKOOS MIMIC THEIR HOSTS?

We compared the begging calls of cuckoos and their hosts at four different stages of the nestling period; (1) day 2–3, chicks matched for age, (2) day 5–7, chicks matched for age, (3) during the first half of the nestling period, and (4) during the second half of the nestling period.

(1) Day 2–3, chicks matched for age.

The begging calls of nestling SFW-cuckoos and nestling superb fairy-wrens were structurally very similar, and a discriminant analysis failed to distinguish between them (all call variables, $P > 0.3$). By contrast, BRT-cuckoo begging calls were shorter than buff-rumped thornbill calls at an equivalent age (Figs. 1C,D) and a discriminant analysis could best distinguish between them (with 80% accuracy) by using call duration (Wilk's $\lambda = 0.47$, Exact $F_{1,8} = 8.9$, $P < 0.02$). Although the calls of very young BRT-cuckoos visually resembled those of superb fairy-wrens (Fig. 1), discriminant analysis distinguished between their calls on the basis of maximum frequency (with 100% accuracy, Wilk's $\lambda = 0.25$, Exact $F_{1,8} = 23.7$, $P = 0.001$), indicating that call modification had already occurred within the first few days in the nest.

(2) Day 5–7, chicks matched for age.

At five to seven days after hatching, the begging calls of SFW-cuckoos remained very similar to those of superb fairy-wrens,

and discriminant analysis did not distinguish between them ($P > 0.1$ for all call measures). BRT-cuckoo begging calls could still be distinguished from buff-rumped thornbill calls at an equivalent age (with 93% accuracy, Figs. 1C,D, Wilk's $\lambda = 0.19$, Exact $F_{1,12} = 26.7$, $P < 0.0001$) on the basis of call duration.

(3) Recordings from the first half of the nestling period.

In the first few days after hatching, the calls made by fairy-wrens and SFW-cuckoos were not significantly different (all $P > 0.2$). By contrast, BRT-cuckoo begging calls differed from those uttered by buff-rumped thornbills and their mean call parameters were intermediate between those of fairy-wrens and thornbills (Fig. 2A).

One call parameter, call duration, differed significantly between BRT-cuckoos and buff-rumped thornbills, and a discriminant analysis could distinguish between them with 98.9% accuracy (Wilk's $\lambda = 0.39$, Exact $F_{1,16} = 24.1$, $P = 0.0002$). Combining SFW-cuckoos, superb fairy-wrens, BRT-cuckoos, and buff-rumped thornbills into a single analysis, call duration and frequency bandwidth differed significantly among cohorts (Fig. 2A). Fifteen of 39 individuals were misclassified by the model. Of these, 10 (67%) were between fairy-wrens and SFW-cuckoos, indicating a high degree of similarity between these two groups, whereas only two (13%) misclassifications were made between thornbills and BRT-cuckoos.

(4) Recordings from the second half of the nestling period.

By the second half of the nestling period, the calls of BRT-cuckoos and SFW-cuckoos were similar to the calls of their rearing host's offspring, and differed significantly from one another (Fig. 2B). When all species were combined into a single analysis, two variables, call duration and frequency bandwidth, best distinguished between species (Fig. 2B). Of 15 misclassifications, 10 were between a cuckoo and the host that reared it (75%), suggesting a high level of mimicry at this stage, four (27%) were between BRT-cuckoos and SFW-cuckoos, and one SFW was misclassified as a BRT-cuckoo (7%).

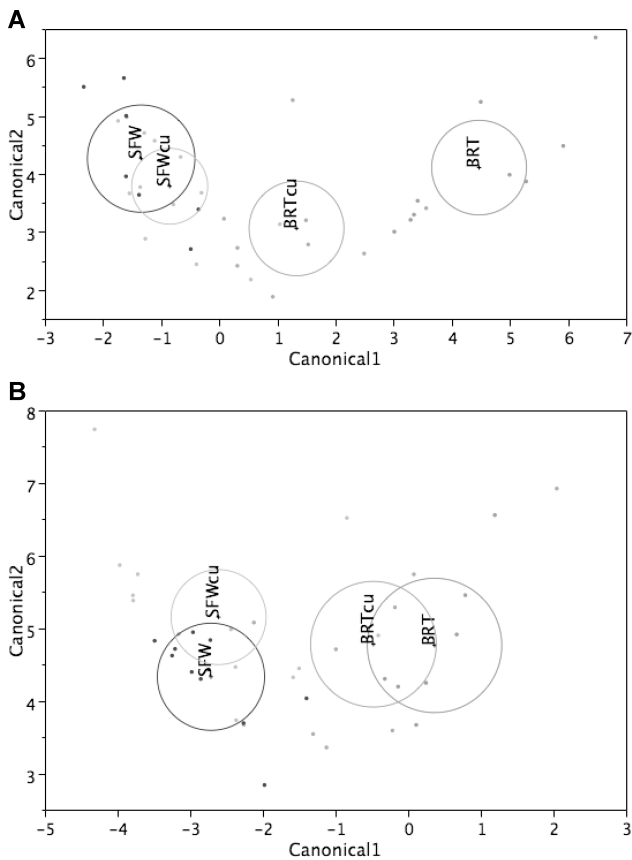


Figure 2. Canonical plots from discriminant function analysis separating the begging calls of buff-rumped thornbills, superb fairy-wrens, BRT-cuckoos, and SFW-cuckoos, (A) during the first half of the nestling period ($N = 8$ BRT-cuckoos, 12 SFW-cuckoos, 8 buff-rumped thornbill broods, and 7 superb fairy-wren broods), and (B) during the second half of the nestling period ($N = 7$ BRT-cuckoos, 12 SFW-cuckoos, 6 buff-rumped thornbill broods, and 9 superb fairy-wren broods). 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.

Discriminant function analysis could not distinguish between the begging calls of cross-fostered BRT-cuckoos and buff-rumped thornbills in the second half of the nestling period (all $P > 0.06$). Two call variables, minimum frequency and call duration, distinguished between SFW-cuckoos and superb fairy-wrens (Wilk's $\lambda = 0.6$, Exact $F_{2,22} = 6.8$, $P = 0.005$). When the two SFW-cuckoos that developed thornbill-type calls and survived into the second half of the nestling period were excluded from the dataset, only minimum frequency was significant (Wilk's $\lambda = 0.7$, Exact $F_{1,21} = 9.6$, $P = 0.005$).

HOW DO CUCKOO NESTLINGS LEARN THE BEGGING CALLS OF THEIR HOST?

Our results suggest a process of call learning whereby nestlings undergo a “developmental switch” in the nest of the secondary

host. By day 2–3 (when recordings were first audible), all nestling cuckoos produced a short begging call with a downward inflection (Fig. 1). However, the calls of thornbill-reared cuckoos differed from those of fairy-wrens in maximum frequency, suggesting that call modification had already commenced. In the primary host, SFW-cuckoo calls resembled those of fairy-wrens to a similar degree during the first and second half of the nestling period (Fig. 2). In addition, calls of both SFW-cuckoos and superb fairy-wrens were similarly variable throughout the nestling period. There was no significant change in the coefficient of variation for any call parameters with nestling age for either SFW-cuckoos (all $P > 0.1$) or superb fairy-wrens (all $P > 0.3$), and no significant differences between fairy-wrens and SFW-cuckoos in call variability with age for any call parameters, although call duration was borderline significant due to slightly increasing variability in call duration with age in SFW-cuckoos ($F_{3,51} = 2.53$, $P = 0.07$, all other $P > 0.4$).

By contrast, in nests of the secondary host, calls of cross-fostered BRT-cuckoos became increasingly similar to buff-rumped thornbill calls with nestling age (Fig. 2). BRT-cuckoos showed a significant decrease in call variation with nestling age for frequency bandwidth ($F_{1,40} = 5.2$, $P = 0.03$), maximum frequency ($F_{1,40} = 5.6$, $P = 0.02$) and peak frequency ($F_{1,40} = 7.4$, $P = 0.01$), and a significant increase in variation in call duration as call duration increased with nestling age ($F_{1,40} = 5.9$, $P = 0.02$), whereas buff-rumped thornbills showed no significant changes in call variability with nestling age for any call parameters (although minimum frequency was borderline significant $P = 0.08$, all other $P > 0.2$, Fig. 3). Consistent with these results, when coefficient of variation data from BRT-cuckoos and thornbills were combined in a single analysis of variance for each call parameter, there were significant or near-significant effects of “species” for all call parameters (Table 3). In general, call variability declined with age in BRT-cuckoos, but stayed relatively constant in thornbill young, although variability in call duration increased with age in BRT-cuckoos, and decreased slightly in thornbill young (Table 3).

Our analysis of nestling growth rates showed that cuckoos thrived equally well in the nests of their primary and secondary hosts (Wald statistic; nestling age effect $\chi^2_1 = 676.08$, $P < 0.001$, Host effect $\chi^2_1 = 0.56$, $P = 0.46$, Fig. 4).

Discussion

SOCIALLY ACQUIRED POLYMORPHIC MIMICRY

Our experiments demonstrate that host-specific, mimetic begging calls in the Horsfield's bronze-cuckoo can be socially acquired after parasitism, in the absence of a model, in nests of secondary hosts. All cuckoo chicks initially made short calls with a downward inflection, resembling those of fairy-wrens, and those reared by fairy-wrens produced calls that did not differ significantly from

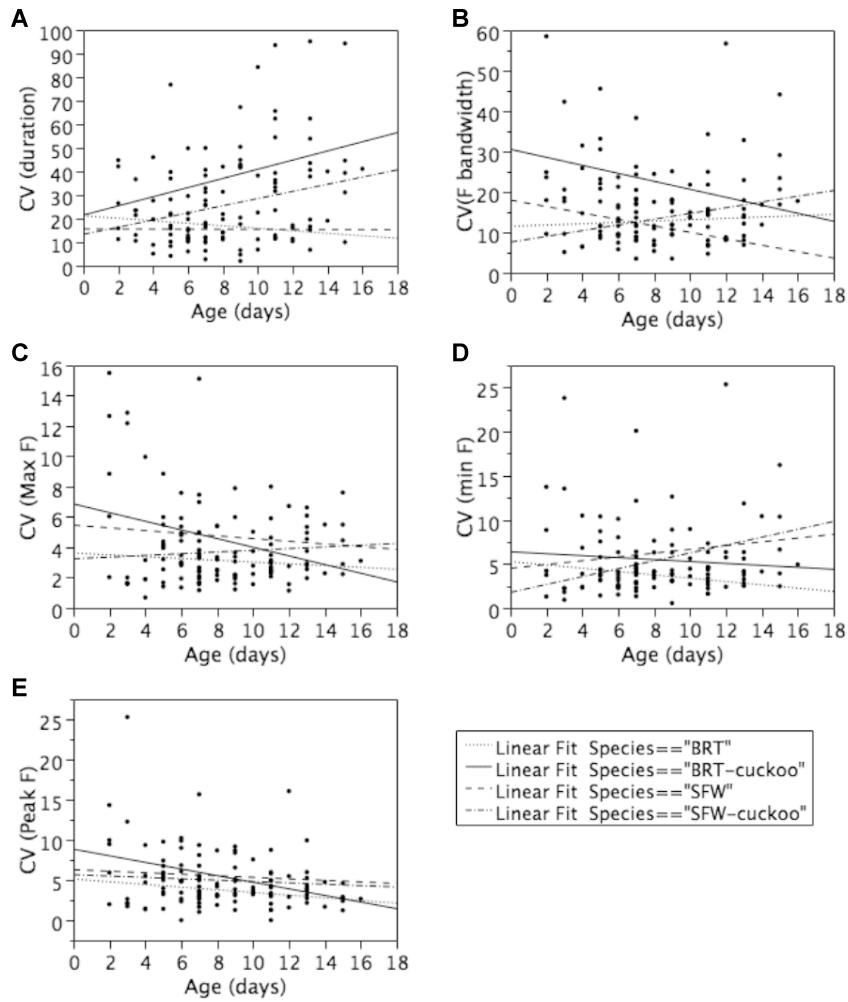


Figure 3. Regression plots for BRT-cuckoos, SFW-cuckoos, superb fairy-wrens, and buff-rumped thornbills, showing coefficients of variation by nestling age for (A) call duration, (B) frequency bandwidth, (C) maximum frequency, (D) minimum frequency, and (E) peak frequency.

those of their host at either the beginning or the end of the nestling period. By contrast, cuckoos reared by thornbills gradually modified their calls from a fairy-wren-like structure in the first few days after hatching toward a thornbill-like call structure, by decreasing the frequency bandwidth and increasing call duration.

The data are best explained by the “developmental switch” mechanism for acquired host-specific call mimicry. The cuckoo chick’s default strategy is to produce a begging call that closely mimics its primary hosts, the superb fairy-wren and its *Malurus* congeners (Brooker and Brooker 1989a). If it hatches in the nest of

Table 3. Comparison of changes in call variability with age for BRT-cuckoos and buff-rumped thornbills. Significant results are in bold type.

	Parameter estimates							
	Analysis of variance (ANOVA)		Age		Species		Age × Species	
	<i>F</i> _{3,59}	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
Call duration	9.6	<0.0001	1.13	0.26	-4.51	<0.0001	-1.98	0.05
Frequency bandwidth	6.5	0.0007	-1.20	0.23	-3.63	0.0006	1.68	0.1
Minimum frequency	1.4	0.26	-1.12	0.27	-1.81	0.08	-0.29	0.77
Maximum frequency	3.5	0.02	-1.81	0.08	-1.85	0.07	1.19	0.24
Peak frequency	4.3	0.008	-2.42	0.02	-1.84	0.07	1.03	0.31

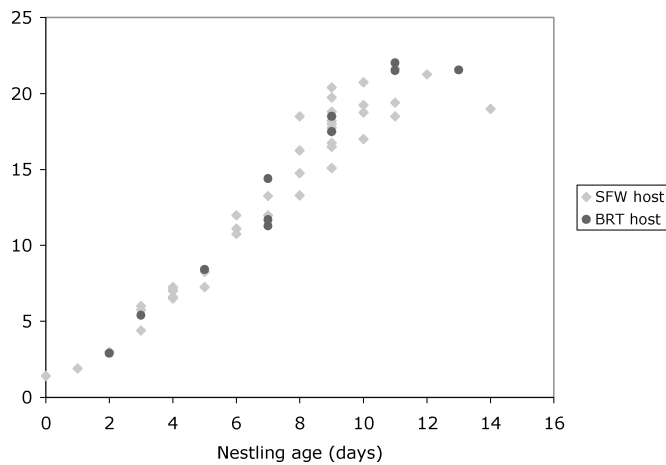


Figure 4. Mass of Horsfield's bronze-cuckoo nestlings (g) reared by superb fairy-wrens (diamonds) and buff-rumped thornbills (circles) in relation to nestling age.

a secondary host such as a thornbill (Brooker and Brooker 1989a), the cuckoo then modifies its begging call by producing variable notes initially, which are rapidly refined to resemble the calls of the secondary host. In the absence of a model, it seems likely that the calls are refined through reinforcement by host parents, specifically by selective provisioning in response to the more accurately mimetic calls. As a result of social shaping by its secondary host, the cuckoo chick ends up producing a call that closely mimics the begging notes produced by thornbill nestlings (Payne and Payne 1998; Fig. 1). Our results suggest that this process begins very soon after hatching, because differences could be detected between the calls of BRT-cuckoos and SFW-cuckoos as early as three days after hatching, and because BRT-cuckoos did not suffer a detectable growth cost as a consequence of undergoing the developmental switch (Fig. 4).

In buff-rumped thornbill nests, social shaping seems to have had the greatest influence on frequency bandwidth and call duration. A decrease in call frequency bandwidth can arise simply as a side effect of an increase in call rate, (Nowicki and Searcy 2005), which is common as chicks become hungrier (Madden and Davies 2006). In thornbill nests, however, cuckoo call rate decreased (Fig. 1), which rules out this alternative state-based explanation for call modification.

INDIVIDUAL VARIATION

Our experimental results add to a growing body of evidence that nestlings can modify their begging behavior after hatching to increase its profitability (e.g., Kedar et al. 2000; Kölliker and Richner 2004; Budden and Wright 2005; Madden and Davies 2006), although the learning mechanism we have identified is unique in this context (Grodzinski and Lotem 2008). The social shaping of the Horsfield's bronze-cuckoo's nestling begging call by secondary hosts is very similar to the social shaping of babbling

in human *Homo sapiens* babies by caregivers (Goldstein et al. 2003; King et al. 2005) and song in male brown-headed cowbirds *Molothrus ater* by female conspecifics (King and West 1983; West and King 1988a). Just as with these other species, we found considerable variation among individuals in their vocal development. Mimicry was achieved at different ages by different individuals and with variable accuracy. In brown-headed cowbirds, both the signaler (West and King 1988b) and the reinforcer (Smith et al. 2000) contribute to variation in vocal development and the same could be true for Horsfield's bronze-cuckoo nestlings. For example, host parents could vary in the degree to which they reward mimetic calls, with some hosts being more discriminating than others or some showing a greater responsiveness to begging in general than others. It is even possible that some superb fairy-wren hosts might strategically lower their provisioning rates in the days after hatching, to expose the cuckoo's true identity by forcing it onto a developmental pathway on which it will cease to mimic host nestlings.

In addition, there could be variation among individual cuckoo nestlings both in their propensity to produce variable calls and their ability to refine them after reinforcement. Individual nestlings could also vary in the threshold for switching from retaining a fairy-wren like begging call to making the more variable calls that will subsequently be shaped by social reinforcement. The three SFW-cuckoos that produced thornbill-like calls may have been particularly keen to switch developmental strategies, perhaps mistaking a relatively slow provisioning rate by their hosts for a reluctance to respond to fairy-wren-like begging calls. Setting a low threshold for this developmental switch could be beneficial if cuckoo mothers regularly exploit a mixture of primary and secondary hosts (Langmore and Kilner 2007), but it is costly because cuckoo chicks that fail to mimic host young may be more likely to be detected and abandoned by their superb fairy-wren hosts (Langmore et al. 2003). Temporal or spatial variation in the optimal balance between these costs and benefits could explain why individual variation in call development persists.

Similarly, cuckoo nestlings and host parents could each contribute to the variation in vocal development seen among Horsfield's bronze-cuckoo nestlings reared by different secondary host species. Payne and Payne (1998) observed that fledgling Horsfield's bronze-cuckoos reared by a different secondary host, the scarlet robin *Petroica multicolor*, did not mimic the whistling begging calls of fledgling scarlet robins, which exhibited a very wide frequency bandwidth (2.5 to 8 kHz), but instead produced a "whine" similar to that of thornbill-reared cuckoos. This might reflect a constraint on call variability in the cuckoo nestling. Such a constraint is plausible, because the acoustic differences between superb fairy-wren and buff-rumped thornbill begging calls are smaller (due to their similar and narrower frequency bandwidths and indistinguishable peak and minimum frequencies) than the

differences between these species and scarlet robins (Payne & Payne 1998). Alternatively, it is possible that scarlet robins did not discriminate against nonmimetic begging calls, and so did not reinforce any mimetic begging calls.

HOST RACE FORMATION

Mounting evidence suggests a lack of host races in Horsfield's bronze-cuckoos. First, they lack host-specific egg polymorphisms, a hallmark of host races in other cuckoo species (Davies 2000). Second, an Australia-wide population genetic study found no indication of evolutionarily stable host races throughout the Horsfield's bronze-cuckoo's extensive breeding range, although it did not rule out recently evolved ones (Joseph et al. 2002). Third, our preliminary field data support a lack of host races, because the single Horsfield's bronze-cuckoo laid in a thornbill nest at our site was a full-sibling of two cuckoo chicks laid in fairy-wren nests (Langmore et al. 2007) and because we could find no evidence from a mtDNA analysis for a genetically distinct superb fairy-wren race of Horsfield's bronze-cuckoos, when compared to Australia-wide patterns of variation ($N = 14$ females that parasitized superb fairy-wrens and 14 females that were captured outside the range of superb fairy-wrens during the breeding season, Langmore et al. unpubl. data). The apparent host races in the Horsfield's bronze-cuckoo indicated by host-specific begging calls (Payne and Payne 1998) therefore appear not to be the result of intraspecific genetic divergence. This could be confirmed if sufficient genetic samples from Horsfield's bronze-cuckoo nestlings in the nests of both a malurid and a nonmalurid host from the same study site could be obtained.

Our behavioral work shows how host-specific mimicry in the Horsfield's bronze-cuckoo is possible without the evolution of host races. The Horsfield's bronze-cuckoo is essentially a parasite that specializes in exploiting malurids (Brooker and Brooker 1989a, 1992; Langmore and Kilner 2007), but which possesses sufficient phenotypic flexibility to exploit a range of secondary hosts successfully. The cuckoo's pinkish-white egg, covered in fine red-brown speckling, is a universal approximate mimic of those laid by many of its potential hosts (Brooker and Brooker 1989a,b; Slater et al. 2000), although there is little indication that any of its current hosts are inclined to reject odd-looking foreign eggs anyway (Brooker and Brooker 1989b, 1996; Langmore et al. 2003, 2005). After hatching, there is greater selection for cuckoo nestlings to produce a call that mimics fairy-wren nestlings because those that do not may be at risk of abandonment by their hosts (Langmore et al. 2003). Any cuckoo chick that finds itself being reared by a secondary host, however, can rapidly modify its begging call, through social shaping, for effective communication with provisioning adults. It is possible that the Horsfield's bronze-cuckoo's ability to learn host-specific mimetic nestling begging calls, and its widely mimetic egg, together have relaxed selection

for host races and slowed the rate of evolutionary change (Paenke et al. 2007). The acquisition of host-specific adaptations after parasitism may even provide a more adaptive solution than host races to parasitism of multiple hosts, if a single species or genus is the primary host throughout the range of the parasite.

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