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Report

Embryonic Learning of Vocal Passwords in Superb Fairy-Wrens Reveals Intruder Cuckoo Nestlings

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Summary

How do parents recognize their offspring when the cost of making a recognition error is high [1–3]? Avian brood parasite-host systems have been used to address this question because of the high cost of parasitism to host fitness. We discovered that superb fairy-wren (*Malurus cyaneus*) females call to their eggs, and upon hatching, nestlings produce begging calls with key elements from their mother's "incubation call." Cross-fostering experiments showed highest similarity between foster mother and nestling calls, intermediate similarity with genetic mothers, and least similarity with parasitic Horsfield's bronze-cuckoo (*Chalcites basalis*) nestlings. Playback experiments showed that adults respond to the begging calls of offspring hatched in their own nest and respond less to calls of other wren or cuckoo nestlings. We conclude that wrens use a parent-specific password [4] learned embryonically to shape call similarity with their own young and thereby detect foreign cuckoo nestlings.

Results and Discussion

Recognition is a fundamental mechanism to facilitate behavioral discrimination. Yet, recognition can be inadequate, as seen in the failure of parent-offspring discrimination when avian hosts accept brood parasites such as cuckoos [1]. Despite extensive evidence for coevolved patterns of parasitic mimicry of eggs and nestlings of hosts [1, 5–7], there has been strong theoretical opposition and—until recently (see for example [8, 9, 10])—scant empirical evidence that parents can directly recognize offspring [2, 3]. The theoretical objection stems from the extremely high cost of mistakenly rejecting one's own young, especially young that are larger, beg more intensively, and hence appear the healthiest [11, 12]. In the model cuckoo species (*Cuculus canorus*), most cuckoos are recognized by the host at the egg stage [1, 2, 13]. Yet, some hosts apparently do not detect cuckoo eggs but detect and reject parasitic young. For example, some Australasian warblers (*Gerygone* spp.) eject visually mimetic bronze-cuckoo (*Chalcites* spp.) nestlings from their nest while continuing to care for the remaining brood [8, 9]. Similarly, superb fairy-wrens (*Malurus cyaneus*) can recognize visually mimetic bronze-cuckoo hatchlings and then abandon the nest to start a new breeding attempt [5, 14, 15]. Critically, those parasitic bronze-cuckoo young that are not rejected by their host parents go on to develop a host-mimetic begging vocalization, even though they evict all of the host's young [5, 14, 16]. This recognition arms race indicates an escalation of the sensory and cognitive bases of coevolved host-parasite recognition systems [14].

To identify possible developmental sources of parent-offspring recognition cues, we focused on the coevolutionary dynamics of an acoustical arms race in the superb fairy-wren and its most common brood parasite, the Horsfield's bronze-cuckoo (*Chalcites basalis*). It has been suggested that fairy-wrens discriminate cuckoo nestlings by using an acoustic recognition template and that the Horsfield's bronze-cuckoo selectively mimics the begging calls of its host [14]. Conversely, the shining bronze-cuckoo (*C. lucidus*) is a less frequent brood parasite of superb fairy-wrens, and—because its calls do not mimic the host begging calls—this cuckoo is consistently detected by its wren foster parents, leading to abandonment of the parasitized brood [14]. Critically, wrens are more likely to abandon a single chick in years when adult Horsfield's bronze-cuckoos have been detected in the breeding area [11], suggesting a flexible and experience-dependent rejection threshold by adult hosts [11, 17, 18]. However, the developmental and perceptual mechanisms underlying patterns of brood rejection remain unknown [9, 11]. Ideas about cuckoo discrimination have largely rested on functional tests of visual or acoustical discrimination of intruder eggs or nestlings [13, 19]. Here, we show that call learning starts inside the avian egg and functions in nestling discrimination. Embryonic call learning may be the mechanism underpinning the arms race for acoustical discrimination between parents and offspring after hatching. We also show that females communicate the vocal password to their male mate and that males discriminate between foreign and cuckoo nestling calls, too.

We recorded 15 nests 24 hours per day during the whole nesting period with a new audiovisual nest-monitoring network [20] and discovered a previously undescribed vocalization in superb fairy-wrens, which we termed the "incubation call" (see Audio S1, Audio S2, and Audio S3 available online). This call was a 1.8 ± 0.1 s (mean ± SE) trill with 19.1 ± 2.6 distinct elements, with a minimum frequency of 5,782.8 ± 149.3 Hz and a maximum frequency of 11,011.2 ± 140.5 Hz. Females produced on average 16 ± 2 calls per hour when they were alone and incubating their eggs during the late incubation period, 4–5 days prior to hatching. Specifically, females produced the incubation call at days 9–10 of incubation and stopped at the end of the incubation period or as soon as the eggs hatched (Figure 1). Four of 15 females produced incubation calls on the morning that their eggs hatched, but the incubation call was never recorded on days thereafter.

We obtained recordings from 12 nests of both the female and her respective nestlings (Audio S1, Audio S2, and Audio S3). A visual inspection of the spectrogram of each brood's begging calls showed that nestlings produced a single element

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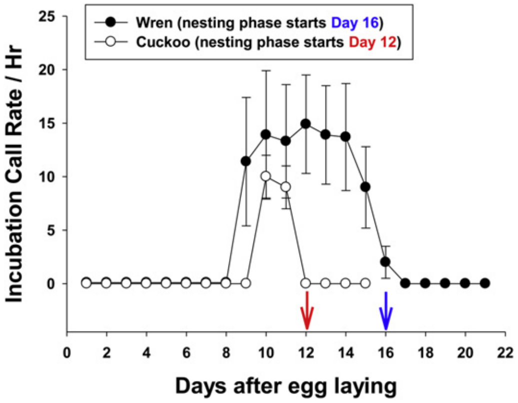

Figure 1. Onset and Intensity of Female Incubation Calls across the Nesting Phase

Data are given as mean ± SE number of female wren incubation calls per hour in relation to the day of the nesting phase (shown as number of days after egg laying due to differences in the onset of the nesting phase for parasitized and nonparasitized nests). Sample size for this figure is six nonparasitized nests and two parasitized nests for which we have daily recordings from the onset of incubation to the onset of nestling begging calls (both cuckoo nestlings were abandoned on day 3 of the nesting phase). The wren incubation duration is ~15 days; the cuckoo incubation duration is ~11 days. Females start incubation calling circa day 10 after egg laying and generally stop on the day of hatching, which was day 12 for the parasitized nests (red arrow) and day 16 for the nonparasitized nests (blue arrow).

repeated several times when begging. Spectrogram cross-correlation analysis [21] showed that begging call structure differed significantly across broods (Table 1). The nestling call's begging element was identified in the mother's incubation call as the female's "signature element." When nestlings begged, they repeated the single begging call element (mean 6.2; range 1–12). The signature element of each female was significantly different from the signature element of any other female (Table 1). We used cross-correlation analysis to examine the similarity between the females' signature elements and their nestlings' begging calls. Each female's signature element was significantly more similar to the begging call element of their own nestlings than to the begging call element recorded from any other brood (Table 1).

If chicks learn the mother's signature call element while in the egg, we could expect that those chicks that hear the mother's call more often will be able to better match those elements (using the coordinate values [CV] as similarity values—see Experimental Procedures for details). We found that females with higher call rates subsequently had higher call similarity to their nestlings' begging calls for two of the five coordinate values (linear regression CV1: $r = 0.30$, $t_{48} = 2.15$, $p = 0.04$; CV2: $r = 0.26$, $t_{48} = -1.89$, $p = 0.06$; CV3: $r = 0.48$, $t_{48} = 3.81$, $p < 0.0001$; CV4: $r = 0.15$, $t_{48} = 1.06$, $p = 0.29$; CV5: $r = 0.10$, $t_{48} = 0.70$, $p = 0.49$). Averaging the five coordinate values into a single measure, we found that the call rate per hour was a significant predictor of the overall relationship between the call structure of the mothers' signature elements and the nestlings' begging calls ($r = 0.35$, $t_{48} = 2.57$, $p = 0.01$; see Table S1 available online).

Table 1. Statistical Results for Call Similarity

	Coordinate Values	df	F	p	eta^2
Nestling begging calls (n = 12)	1	11	11.50	<0.0001	0.72
	2	11	15.77	<0.0001	0.78
	3	11	10.99	<0.0001	0.72
	4	11	8.58	<0.0001	0.66
	5	11	2.75	0.008	0.40
Female signature elements (n = 15)	1	14	76.66	<0.0001	0.95
	2	14	38.85	<0.0001	0.90
	3	14	12.01	<0.0001	0.74
	4	14	8.45	<0.0001	0.66
	5	14	8.79	<0.0001	0.67
Similarity between females and nestling calls (n = 12)	1	11	39.14	<0.0001	0.90
	2	11	30.39	<0.0001	0.87
	3	11	4.02	<0.0001	0.48
	4	11	24.26	<0.0001	0.85
	5	11	7.03	<0.0001	0.62
Similarity between foster females and nestlings (n = 8)	1	7	1104.17	<0.0001	1.00
	2	7	5.80	<0.0001	0.56
	3	7	0.05	1.00	0.01
	4	7	0.84	0.56	0.15
	5	7	0.25	0.97	0.05
Similarity between genetic females and nestlings (n = 7)	1	6	11.18	<0.0001	1.00
	2	6	0.004	1.00	0.001
	3	6	0.05	0.99	0.10
	4	6	0.01	1.00	0.003
	5	6	0.002	1.00	<0.0001

Spectrographic cross-correlation and principal coordinates analysis (PCoA) (Raven Pro 1.3) of begging calls 3–4 days after hatching and signature elements from incubation calls at day 10–12 of incubation. The data presented are for the 15 nonparasitized fairy-wren nests recorded between 2007 and 2011 (top three data groups) and the additional 8 nests used in our cross-fostering experiments (bottom two data groups). PCoA ordinates the distance values in multidimensional space and coordinates the sound position in reduced space. The table presents the results (and eta^2, a measure of effect size) for the multivariate ANOVA on the first five coordinate values provided by the PCoA. df, degrees of freedom.

To explicitly test the hypothesis that begging calls are learned from the mother's incubation calls, we experimentally cross-fostered complete clutches (three eggs) of superb fairy-wren eggs of similar age between nests at the beginning of the incubation period (no later than 4 days after clutch completion) when the females were not yet producing any incubation call. If begging call structure is learned, we predict a positive correlation between foster mother call rate and similarity to nestling begging calls, less similarity between host and parasite calls, no similarity between genetic mother and begging calls, and similarity between foster mother call and nestling begging call. If begging call structure is more strongly influenced by genetic mechanisms, then we predict similarity between genetic mother call and nestling begging call, and no correlation between foster mother call and nestling begging call.

We obtained recordings of foster females and their cross-fostered nestlings from eight nests, and recordings of original females and their biological nestlings at seven nests. Cross-correlation analysis of female signature elements and their nestlings' begging calls revealed higher call similarity between females and their fostered young (Table 1) than between females and their biological young (Table 1; Figure 2). Call similarity was positively correlated with incubation call rate of the foster mother for two (CV1 and CV2) of the five coordinate values (linear regression CV1: $r = 0.38$, $t_{39} = 2.52$, $p = 0.02$; CV2: $r = 0.32$, $t_{39} = 2.03$, $p = 0.049$; CV3: $r = 0.05$, $t_{39} = 0.33$, $p = 0.74$; CV4: $r = 0.17$, $t_{39} = 1.05$, $p = 0.30$; CV5: $r = 0.09$, $t_{39} = 0.55$, $p = 0.58$). Averaging the five separate coordinate values into a single measure, we found that call rate per hour was a significant positive predictor of call similarity between the foster mother's signature element and her nestlings' begging call ($r = 0.85$, $t_7 = 3.87$, $p = 0.008$; Figure S1).

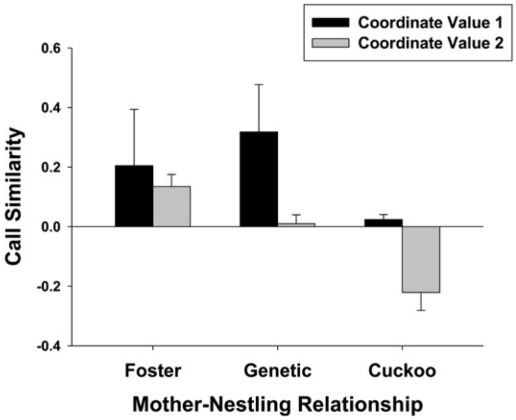

Figure 2. Call Similarity

Data are given as mean ± SE for the first two coordinate values CV1 and CV2 between female incubation call and nestling begging call for the following categories of nestling: foster-reared (n = 8), genetic (n = 7), and cuckoo (n = 2). Results are shown for CV1 and CV2 because these variables were significantly different between foster and genetic nests for the cross-fostered treatment.

For two cases of parasitism by Horsfield's bronze-cuckoo, we found low call similarity between the female signature element and cuckoo begging call (Figure 2). We used linear regression (focused contrast) [22–24] to compare call similarity for mother-nestling relationships, which were predicted to be highest for foster nests (1), intermediate at nests with genetic young (0), and lowest at nests with cuckoo nestlings (−1). Call similarity was significantly higher between foster females and the unrelated nestlings in their own nests than their genetic offspring in different nests or cuckoo nestlings for CV2 (CV1: $r = 0.29$, $t_{17} = 1.24$, $p = 0.24$; CV2: $r = 0.81$, $t_{17} = 5.70$, $p < 0.001$; Figure 2).

We experimentally tested via playback experiments whether adult wrens behaviorally discriminate nestling begging calls. First we showed that males have the opportunity to learn the signature element of the females, and then we used playback experiments to show that adults respond differently to the calls of own nestling versus foreign nestling (foreign wren or cuckoo nestling). Female fairy-wrens were often heard using "solicitation calls" to beg for food from males away from the nest during the incubation and nestling periods. At eight nests in 2011, we observed these solicitation calls, and in all cases the females included the signature elements within their calls to males. We used playback experiments and presented all attending adults (males, females, and helpers, if any) with (1) the begging calls of their own nestlings and those of an unfamiliar (stranger) wren nestling from another nest (n = 13; paired experiments) and (2) the begging calls of a wren nestling from another nest (stranger) or those of a cuckoo nestling (n = 18; paired experiments). We matched nestlings in experimental nests for age. Adult wrens use the presence of adult cuckoos in the area as a cue to alter rates of abandonment of a single nestling [11, 17]. Perhaps adult wrens use the occurrence of a foreign begging call as a cue to search the area for cuckoos, which would increase their vigilance and lower feeding rates. These predictions were supported in our experiments: adult male and female wrens increased their time scanning outside the nest (df = 12; t = 3.00; p = 0.01) as well as their time between visits to the nest (df = 12; t = −2.10; p = 0.05), fed the nestlings less (df = 12; t = 3.42; p = 0.005), and produced more alarm calls (df = 12; t = −4.52; p = 0.001) in response to the stranger nestling calls compared to trials with their own nestlings. There were no differences in the responses of adult wrens to the playback of stranger or cuckoo begging calls (df = 17; all p > 0.06).

We propose that we have found a learned parent-and-offspring-specific password [4] that functions in offspring discrimination; the password is produced by the female and learned by the embryo. The occurrence of the learned password, which appears to be subject to temporal sensitivity for accurate production, can be used to detect intruder nestlings or nestlings that have not learned the password. Parents can use the absence of the password as a mechanism to detect the presence of a costly, intruding, brood-parasitic cuckoo nestling. This is also the first study to show that both females and males can detect acoustic differences in nestling begging calls, which we argue arises from females sharing the password within a feeding solicitation call to males away from the nest.

Why don't cuckoo chicks also learn the signature elements from the host female's incubation calls? We show that the begging calls of the parasitic cuckoo nestlings had low call similarity to the female's incubation calls (Figure 2). As a cuckoo egg is laid among the host eggs, the cuckoo embryo is exposed to the host incubation calls—but the cuckoo egg is exposed for less time than the host eggs are. Female wrens begin producing the incubation call circa day 10 of incubation and stop producing the incubation call after the first nestling hatches (Figures 1 and 3). The wren nestlings hatch after ∼15 days of incubation; wren embryos have therefore been exposed to the incubation call for ∼5 days in a nonparasitized nest. The cuckoo nestling hatches after ∼12 days of incubation and expels the remaining wren eggs ([11, 14]; this study). The cuckoo embryo would therefore only have been exposed to the incubation call for ∼2 days. We show that nestling call similarity correlates positively with female call rate. Perhaps call similarity is also influenced by cumulative long-term exposure to the female's incubation call. The early hatching by cuckoo chicks reduces the amount of potential time they would have to learn these calls, but this remains to be tested, given that cuckoo embryos could learn the password in fewer days. Perhaps the next step in the acoustical arms race between the superb fairy-wren and Horsfield's bronze-cuckoo will be fine tuning of the sensory and cognitive developmental stages for the learning of the password used by parents to discriminate between offspring.

Horsfield's bronze-cuckoo nestlings in host nests adjust their begging call structure during the first 2 days posthatching to match the call structure of the host using trial-and-error learning [15]. This call matching is adaptive for the generalist Horsfield's bronze-cuckoo because the cuckoo embryo "does not know" what host species' nest it will hatch in, and therefore it opportunistically trials several begging call structures that maximize the host parental provisions [15]. We argue that the wren host species has changed the arms race by calling to the embryos circa day 10 and not earlier; the temporal onset of incubation calls late in the incubation phase could have been favored by selection given the shorter incubation duration of the cuckoo embryo. We show lower call similarity posthatching when embryos have been exposed for less time or lower rates to the female incubation call. We also argue that call similarity is the "rule of thumb" proxy, or discrimination cue, by which parent wrens assess the payoff of parental investment.

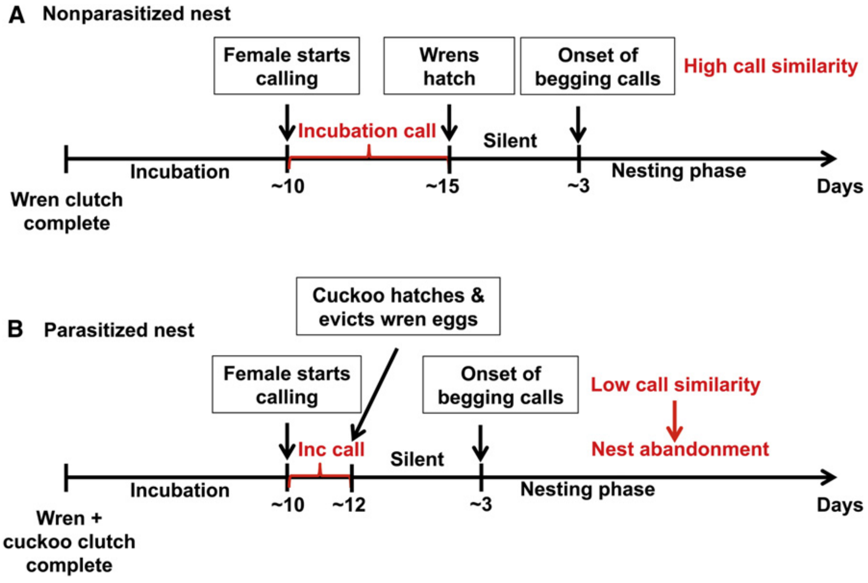

Figure 3. Hypothetical Timeline for Incubation and Begging Calls

A hypothesized schematic timeline for onset of the incubation call by the female wren, onset of the nestling begging call, and outcome of call similarity in nests not parasitized by cuckoos (A) and in nests parasitized by cuckoos (B). The onset of female incubation calls occurs circa day 10 of incubation; cuckoos hatch circa day 12 after egg laying; wrens hatch circa day 15 after egg laying. Therefore, cuckoos are exposed to the "password" in the female incubation call for ~2 days, whereas fairy-wren embryos in nonparasitized nests are exposed to the incubation call for ~5 days before hatching.

There is now no doubt that some seemingly innate traits are the result of experience during the embryonic stages [25]. Precocial avian embryos in eggs are known to respond to stimuli, including signals from their parents [26, 27]. Our cross-fostering experiments showed a role for both genetic and learned components in the call matching that we found. The first coordinate value had high call similarity between genetic and foster mothers and their offspring, whereas the second coordinate value had significantly higher call similarity in the cross-fostered nestlings, and the similarity was stronger when mothers called frequently to the embryos (for another example of learned recognition, see [10]). Furthermore, females at cross-fostered nests presented with begging calls from their genetic young raised by a different mother did not recognize the nestlings as their own offspring and behaved as if hearing unfamiliar nestlings (all $p > 0.09$; data not shown).

Birds were the founding model system for our current understanding of neural plasticity in adults, including humans (e.g., [28]; see also [29]). This initial work on neural plasticity in birds was followed by many studies on neural pathways that underpin muscle and thought control in rats [30], evidence for neural/robotic interface in monkeys [31], and most recently neural control of thought to initiate robotic arms to assist humans [32]. Our results here on prenatal learning in wrens highlight the point that birds are also an excellent model system in which to study prenatal sensory and cognitive development and their underlying neural organization, because embryonic developmental stages can be easily observed and stimulated in the egg (see also [33]). Model systems that use noninvasive approaches (such as MRI) to study embryonic neural and cognitive development are likely to stimulate new fields of inquiry over the coming decades.

Experimental Procedures

Study Species and Sites

The endemic superb fairy-wren is a common iconic southeastern Australian insectivorous passerine that occurs in woodlands and open scrub [34, 35]. The wrens are opportunistic cooperative breeders with male helpers at the nest from previous broods; in this study, we had one or two male helpers at 16 of 24 nests (66%) [36]. The birds breed between August and January, with one to three broods per year, each with a clutch of two or three eggs [34, 35]. In this study, clutch size was always three. Horsfield's bronze-cuckoos in other study areas have been shown to parasitize 13%–37% of superb fairy-wren nests [11, 14]. In our study sites in South Australia, only 6 of 138 nests (4%) had evidence of brood parasitism. All cuckoo nestlings were abandoned as soon as they were alone in the nest and started vocalizing. Cuckoo nestlings evict host young within 2 days after hatching [11, 14, 16]. This study was conducted over four breeding seasons (September–January 2007, 2009, 2010 and 2011) at Newland Head Conservation Park, a coastal area 15 km southwest of Victor Harbor on the Fleurieu Peninsula of South Australia (35°37′S, 138°29′E); Scott Creek Conservation Park (35°05′S, 138°41′E) and Cleland Wildlife Sanctuary (34°58′S, 138°41′E), respectively 35 km and 25 km southeast of Adelaide; and Flinders Chase National Park (35°56′S, 136°44′E) at the west of Kangaroo Island.

Audio Recording and Analysis

We monitored a total of 138 superb fairy-wren nests over the four years. We obtained audio and video recordings of the vocalizations produced by all individuals present at the nest during the whole nesting period for a total of 15 nonparasitized fairy-wren nests, and continuous recording 24 hr per day at 6 of those 15 nests. The recording system is described in [20], and the microphones were placed approximately 30 cm under the nest. The recorded vocalizations were broadcast wave files (16 bit/48 kHz) that were transcribed to an Apple Mac Pro for editing with BIAS Peak (Berkley Integrated Audio Software) and analysis with Raven Pro 1.3 [21]. Spectrograms of audio recordings were created using the Hann algorithm (filter bandwidth 124 Hz, size 512 samples, time grid overlap 50%, grid resolution 5.8 ms, 86.1 Hz, discrete Fourier transform 512 samples).

For each nest, we used five signature elements from the female's incubation call recorded at day 10–12 of incubation and five begging calls from the nestlings on day 3–4 after hatching. Begging calls were only analyzed when one chick was calling, to avoid noise from overlapping calls.

We compared spectrograms with spectrographic cross-correlation (SPCC) using batch correlation in Raven Pro 1.3, which cross-correlates pairs of vocalizations frame by frame in the time-frequency domains, thus comparing two matrices of frequency × time. Using correlation coefficients at each increment of overlap, the SPCC highlights peak values where the two matrices are most similar. The matrix of similarity thus produces a matrix of distance (D) according to the transformation [$D = (1 - S)\ 0.5$] [37]. This matrix of similarity (S) was evaluated by principal coordinates analysis (PCoA) using the R package for multivariate and spatial analysis, version 4.0 [38] following the general approach described in [39] and generally used for acoustic population differentiation, such as in dialects [39, 40]. PCoA, like principal component analysis (PCA), uses a linear (Euclidean) mapping of the distance or dissimilarities between objects in the ordination space [37]. Shepard diagrams [39] indicated that the Euclidean representation of the principal coordinates preserves the distance relationships of the original multidimensional space. However, unlike PCA, PCoA works with any dissimilarity measure, and its components, instead of being linear combinations of the original variables as in PCA, are complex functions of the original variables depending on the selected dissimilarity measure. Thus, PCoA gives several coordinate values per call, not per individual, and the first five coordinate values explain most of the data set [40]. We used the PCoA coordinate values in a multivariate ANOVA (MANOVA), with individuals or nests as the between-subjects factor. Spectrogram cross-correlation analysis of whole incubation calls and of the signature elements showed the same statistical results when testing for individual differences between females. Therefore, only the signature elements' analysis is presented here.

Using a linear regression, we then analyzed (1) each coordinate value separately and (2) all five values averaged into one per call as the dependent variable in relation to the average number of calls per hour per female (call rate) as the independent variable, to test for a possible role of learning. SPSS 18.0 for Windows (SPSS Inc.) was used for statistical analysis.

Cross-Fostering Experiments

In 2011, we found 57 nests during incubation and cross-fostered 22 complete clutches of eggs of similar age (early incubation; 1–3 days old). Clutches were removed, temporarily replaced with model eggs, and then quickly swapped so that the nests were apparently unchanged but contained eggs that were unrelated to the female that incubated them (see also [41]). Of those 22 nests, 8 survived to the nestling stage, giving a complete data set for analysis. Vocalizations at the nest were recorded with a Behringer C2 condenser microphone (Behringer International GmbH) connected to a portable Sound Devices 722 digital audio recorder (Sound Devices, LLC), a Fostex FR-2LE digital recorder (Fostex Co.), or a Korg M1000 digital recorder (Korg Inc.). We recorded all sound files as broadcast wave files (24 bit/48 kHz). Incubation and begging call data from the cross-fostered nests were analyzed as described above. In addition, we used focused contrast linear regression [22–24] to test call similarity for the predicted relationship between female incubation calls and nestling begging calls in foster nestlings, genetic nestlings, and cuckoo nestlings.

Playback Experiments

To test whether adults (males, females, and helpers if any) discriminate unfamiliar begging calls that are broadcast when their own nestlings are begging, we used playback of nestling begging calls (own versus stranger) from loudspeakers under 13 nonparasitized nests. We used playback of stranger and cuckoo nestling calls at 7 of those 13 nests, as well as at 11 additional nests.

From recordings, we chose begging calls with the best signal-to-noise ratio without overlapping sound and pasted four at natural intervals ($\sim$0.3 s) to create a playback stimulus. Sounds below 1.5 kHz were filtered out, and playbacks were normalized at −15 dB and saved as uncompressed 16-bit broadcast wave files (.wav) using Amadeus Pro 1.5 (HairerSoft). We used three different types of cuckoo begging calls: (1) three cuckoo begging calls recorded in the Kimberley region of Western Australia from Horsfield's bronze-cuckoos raised by purple-crowned fairy-wrens (*Malurus coronatus*; cuckoos aged 4–6 days; number of nests tested = 7), (2) one cuckoo begging call recorded in Western Australia from a Horsfield's bronze-cuckoo raised by splendid fairy-wrens (*Malurus splendens*; cuckoo aged 7 days; n = 4), and (3) two cuckoo begging calls recorded at our study sites from Horsfield's bronze-cuckoos raised by superb fairy-wrens (cuckoos aged 3–6 days; n = 7). The stranger begging calls were recorded at other wren nests at our study sites.

The playback experiments were carried out on 24 nests with two or three nestlings that were 4–7 days old over two consecutive days during morning feeding between 0600 and 1000. Each time an adult was at the nest, a stimulus was broadcast for 15 s using a remotely controlled FOXPRO Scorpion X1B system (FOXPRO Inc.) placed under the nest. Each experiment lasted a total of 30 min, and the order of the stimuli was randomized. The experiments were video monitored with a Jaycar monochrome CCD security camera attached at the top entrance of the nest and powered by a 12V, 12 Ah sealed lead-acid battery. The video signal was digitized by a Canopus ADVC110A/D converter (Canopus Co., Ltd.) and recorded with Apple QuickTime Pro on an Apple 13" MacBook Pro. The nests were also audio monitored using a Behringer C2 condenser microphone connected to a portable Sound Devices 722 digital audio recorder. During the recording, observers could monitor the wren reactions from a distance of 15 m or more. We recorded all sound files as broadcast wave files (24 bit/48 kHz).

The adult responses were measured as (1) total number of visits (with and without feeding); (2) time spent at nest during feeding; (3) head movements while outside the nest (head movements per second); (4) time between visits; (5) percentage of successful feeds (parent at nest rim inserted beak into nestling's gape); (6) time spent $\leq$50 cm from the nest, scanning outside the nest; and (7) number of mobbing alarm calls in response to the playback.

Data were corrected for the number of males at the nest if necessary and then separated between male and female. We analyzed these data with a logistic regression for each playback type (own, stranger, cuckoo) to test for sex difference in behavior. None of the behaviors were significantly different between sexes (all $p > 0.09$), but in response to the stranger playback, females tended to have more successful feeds than males ($\beta = 4.03$, n = 26 nests, SE = 2.27, $p = 0.09$). Because these results matched our previous study that found no difference between sexes, the data were pooled (see [42]).

To ensure that the responses of the individuals were not due to possible mismatched calls (with stranger nestlings and own nestlings both being heard at the same time by parents), we quantified the level of mismatched calls per nest and per playback type. We used a linear regression analysis and found no statistical difference in the behavioral responses between nests in relation to the level of mismatch calls (linear regression: all $p > 0.10$).

Finally, we tested for an effect of cuckoo playback type for difference in response using a MANOVA. We found no significant differences between the three types of cuckoo playback (all $p \geq 0.07$), and therefore all the cuckoo data were analyzed together. A paired t test was used for statistical analysis between the two playback types (own versus stranger or stranger versus cuckoo).

Supplemental Information

Supplemental Information includes one table, one figure, and three audio files and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.09.025.

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