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Could prenatal sound discrimination predict vocal complexity later in life?

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Abstract

Background: Greater complexity of the learned vocal repertoire has been shown to increase mating and territory defence success in songbirds. Vocal learning in some songbird species begins in the egg and these songbird embryos can discriminate the sounds of different birds. Here, we test if prenatal sound discrimination positively correlates with song complexity in the Superb Fairy-wren (*Malurus cyaneus*). We use a habituation/dishabituation approach in natural and cross-fostered nests to measure prenatal sound discrimination of female vocalisations and later quantify observed song repertoire in fledgling sons and daughters.

Results: Superb Fairy-wren fledglings produced learned songs consisting of 6–11 different elements by 12 weeks of age. Using multiple regression analysis, both prenatal sound discrimination strength and parental song complexity (total number of vocal elements) positively correlated with a fledgling's song complexity. The number of parental vocal elements was unrelated to the embryos' sound discrimination score.

Conclusions: Prenatal sound discrimination strength was positively related to vocal complexity later in life. From previous research, we know that individuals with greater learned vocal complexity may have higher fitness. Therefore, characterizing the causes of prenatal sound discrimination can inform our understanding of fitness trajectories when phenotypes are shaped by learned cross-generational experience. Future research should explore causes of variance in prenatal sound discrimination.

Keywords: Oscines, Habituation, Dishabituation, Vocal learning, Repertoire, Complex song

Background

Songbirds are an excellent model system to study vocal learning because the structure and function of vocal learning can be tested across life stages. Using non-terminal sampling methods, neural response to song has been studied in songbirds [1], thereby allowing researchers to measure sound discrimination during the sensory and sensorimotor phases of vocal learning. Several key insights have emerged: (i) an auditory memory of the tutor song is acquired during the sensory phase well before song production, (ii) this auditory memory is stored within the forebrain auditory area (the caudal medial nidopallium, NCM), (iii) the NCM functions both in song discrimination and memory, and (iv) birds with better sound discrimination had higher vocal copy accuracy of the tutor song as adults [2]. In studies into songbird auditory discrimination, there was

high individual repeatability in learning speed across sound discrimination tasks [3]. Given recent evidence that some songbird embryos can discriminate sound [4, 5], we were interested in whether prenatal sound discrimination can predict vocal repertoire size later in life. This prediction is congruent with what is currently known about the role of sound discrimination for vocal learning, but extends the temporal window of the sensory phase and when auditory memory and sound discrimination could occur.

Fairy-wren (*Malurus spp.*) and Zebra Finch (*Taeniopygia guttata*) parents are known to call to their eggs during incubation [4, 6, 7]. In the fairy-wren system, embryos learned a vocal element from their mother's incubation call that they then produced as their begging call after hatching. Hatchlings with higher vocal copy accuracy of the learned begging call were fed more by the attending parents [7] and were less likely to be mistaken for a brood parasitic cuckoo hatchling and abandoned [4, 8]. In the Zebra Finch system, parents produced incubation calls when ambient temperature was higher, >

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26 °C [6]. Zebra Finch embryos that were experimentally exposed to incubation calls *in ovo* had altered developmental trajectory post-hatch, with smaller size under warmer ambient temperature and preference for warmer nest boxes as adults [6]. Maternal effects can significantly alter the developmental trajectory of offspring [9, 10]. Much remains to be learned about how female in-nest vocalization behavior during incubation could be a form of maternal effect that may influence offspring phenotype [11].

Prenatal exposure to incubation calls from attending females may have consequences for adult song learning [12]. For example, Red-backed Fairy-wrens (*M. melanocephalus*) produced song elements as fledglings that were similar to their mother's in-nest incubation call elements [12]. In the Zebra Finch, male embryos experimentally exposed to incubation calls from unrelated adults produced song with more non-paternal syllables and were more likely to approach females as adults [13], pointing to an effect of prenatal vocal experience on post-hatch social behavior. Whether an embryo can develop an auditory memory that would shape a learned vocalization during a subsequent sensorimotor phase is not known. What is known is that, similar to humans, some songbird embryos have prenatal sound learning. For example, Superb Fairy-wren (*M. cyaneus*) embryos habituated to repeated incubation calls of the same female and dishabituated to novel incubation calls of a new female [5]. Compared with northern hemisphere songbirds, Superb Fairy-wrens learn complex adult song relatively early in life. By 12 weeks of age, and hence 7–8 weeks after fledging, Superb Fairy-wren sons and

daughters produce song with ~8 vocal elements acquired from both parental tutors [14]. We have previously interpreted the shared song repertoire of the sibling fairy-wrens as a familect that could function to reduce inbreeding via kin recognition in this long-lived sedentary species [14]. But ontogenetic mechanisms underpinning such early vocal learning of a familect remain unknown.

Given that Superb Fairy-wren embryos habituate and dishabituate to female in-nest vocalisations [5], perceive adult chatter song *in ovo* [15], and sing a learned complex chatter song by 12 weeks of age [14], we aim to test if an embryo's prenatal sound discrimination score and its parental chatter song complexity correlate with fledgling vocal complexity.

Results

Habituation and dishabituation response in embryos

Average heart rate (\pm SD) was 284 ± 46 beats per minute at the start of the trial. The 58 embryos initially lowered their heart rate (baseline vs H1) to repeated exposure of 6 calls of the same female (paired t-test: $t = 9.75$, $df = 57$, $P < 0.001$) and then stopped changing heart rate to repeated exposure of 12–18 more calls of the same female (H2 vs H3) ($t = 1.02$, $df = 57$, $P = 0.311$), which indicates a habituation response (Fig. 1). We found the same pattern at genetic nests ($t = 0.64$, $df = 52$, $P = 0.526$) and cross-fostered nests ($t = 2.11$, $df = 6$, $P = 0.089$). During the subsequent dishabituation test phase, embryos were exposed to novel stimuli (6 incubation calls of a different female) (Fig. 1). There was a significant difference in the heart rate during the dishabituation test compared with

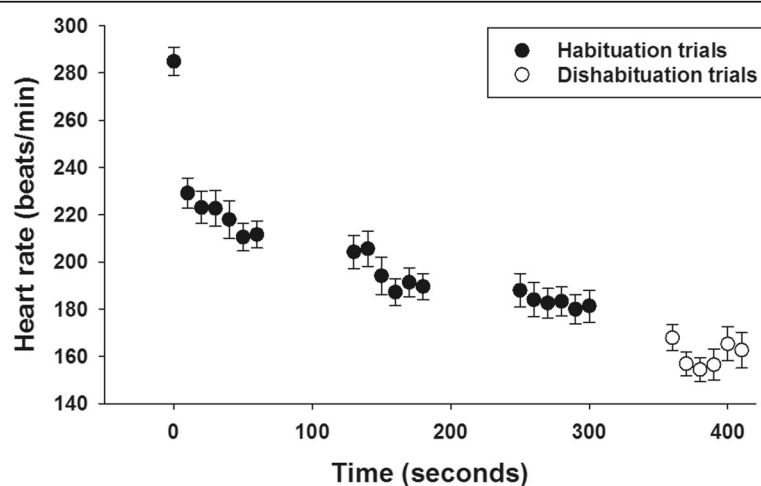


Fig. 1 The change in heart rate (HR) (mean \pm SE beats / minute) in Superb Fairy-wren embryos exposed to 18 different incubation calls of the same fairy-wren (filled circle; habituation trial) and 6 different incubation calls of a novel fairy-wren (hollow circle; dishabituation trial). Initially, embryos lowered HR during exposure to incubation calls every 10 s from 10 to 60 s (filled circles, H1 phase), lowered HR after renewed exposure to calls of the same female every 10 s from 120 to 180 s (filled circles, H2 phase), and then ceased to lower HR during renewed exposure to calls of the same female every 10 s from 240 to 300 s (filled circles, H3 phase). When embryos were exposed to calls of a novel female every 10 s from 360 to 420 s, they again lowered their HR (hollow circles, D phase)

the preceding H3 phase of the habituation trial ($t = 6.45$, $df = 58$, $P < 0.001$), whereby most embryos lowered their heart rate to a novel sound stimulus (Fig. 2).

We compared the dishabituation response in relation to egg age (day 11 to 13 of the incubation phase). Specifically, we tested for an effect of embryo age on the magnitude of the change in heart rate during exposure to a novel sound stimulus after the habituation phase. The change in heart rate during the dishabituation test was stronger in embryos at age d 13 (-22.2 ± 4.1 heart beats/min, $N = 33$) compared with d 12 (-13.2 ± 3.4 , $N = 15$) and d 11 (-11.2 ± 7.3 , $N = 10$) ($r_{\text{part}} = -0.343$, $P = 0.012$). We found the same pattern using data from natal nests and excluding the six cross-fostered nests ($r_{\text{part}} = -0.336$, $P = 0.019$). Therefore, we included egg age as a covariate in all subsequent analyses involving sound discrimination score.

Fledgling vocal repertoire

At natural nests with genetic parents ($N = 12$), parental song had 10.4 ± 0.5 vocal elements with a range of 7 to 14 vocal elements per pair. At cross-fostered nests with foster parents ($N = 6$), parental song had 10.2 ± 0.7 vocal elements with a range of 8 to 12 vocal elements per pair. This parental vocal complexity was comparable between natural and cross-fostered nests (t -test: $t = 0.34$, $P = 0.742$). At natural nests, fledglings produced song with 8.7 ± 0.4 vocal elements (range 6 to 11 elements) that contained $84 \pm 4\%$ (range 60 to 100%) of the elements in the songs of the attending adults. At cross-fostered nests, fledglings produced song with 8.0 ± 0.4 vocal

elements (range 7 to 10 elements) that had $79.7 \pm 5\%$ (range 66 to 91%) of the elements in the songs of the attending adults.

We compared fledgling song complexity (number of vocal elements) against the prenatal habituation and dishabituation (sound discrimination) response and parental song complexity (number of vocal elements). Better prenatal sound discrimination and increased parental song complexity predicted increased fledgling song complexity (multiple regression: $R^2 = 0.47$, $F = 4.19$, $P = 0.029$; sound discrimination: $r_{\text{part}} = -0.45$, $P = 0.046$; parental song complexity: $r_{\text{part}} = 0.61$, $P = 0.009$; habituation score: $r_{\text{part}} = -0.18$, $P = 0.398$) (Fig. 3). There was no statistically significant correlation between prenatal sound discrimination score and parental song complexity ($r = 0.21$, $P = 0.412$). Fledgling acquisition of parental song elements was also influenced by prenatal performance: embryos with better sound discrimination acquired a greater percentage of parental song elements in their song as fledglings (sound discrimination: $r_{\text{part}} = -0.55$, $P = 0.028$; habituation score: $r_{\text{part}} = -0.17$, $P = 0.466$) (Fig. 4).

Discussion

Nests with stronger prenatal sound discrimination produced fledglings with more complex song that also had a greater percentage of parental vocal elements in their song. Therefore, an embryo's sound discrimination *in ovo* may positively predict its vocal repertoire later in life. In addition to an embryo's sound discrimination performance, its parental song complexity appears to be

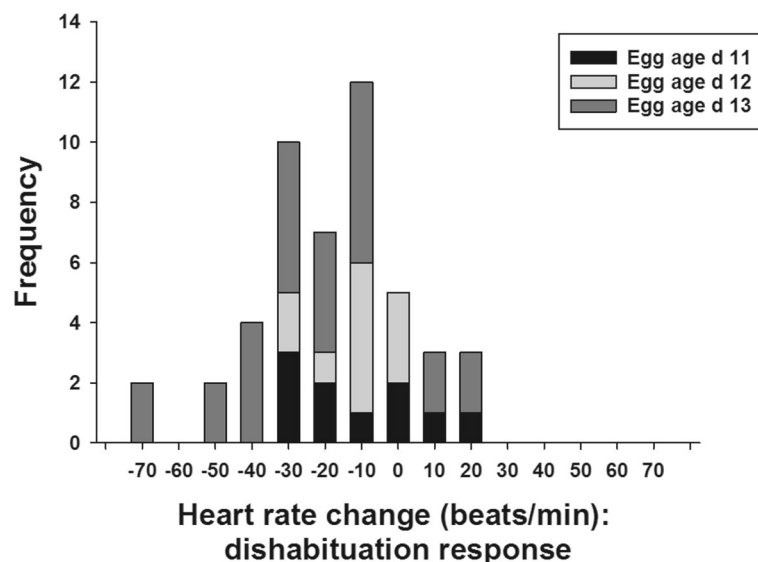


Fig. 2 The frequency of dishabituation response patterns in Superb Fairy-wren eggs exposed to incubation calls of a novel female following exposure to repeated calls of the same female. The data are calculated as the difference in average HR during D minus H3 and are shown per egg age (day 11, 12, 13 of incubation). We scored HR from the digital heart rate monitor during experimental trials. A larger negative HR value was interpreted as a stronger dishabituation response (sound discrimination score)

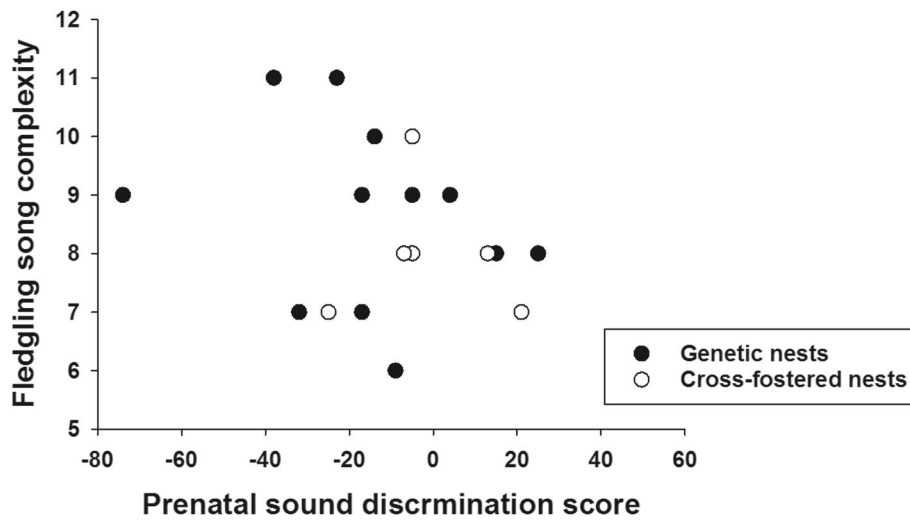


Fig. 3 The association between an embryo’s sound discrimination score (change in heart rate beats/min during dishabituation trial) and its observed song complexity (number of song elements) as a fledgling. Data are shown for 18 field nests (12 unmanipulated nests with genetic parents, 6 cross-fostered nests with foster parents) for which we have prenatal sound discrimination score and song recordings of both parents and fledglings recorded 7–8 weeks after leaving the nest. Fledglings acquired more song elements if they had stronger sound discrimination (negative value = strong response) as embryos

important for vocal development. Fledglings had greater song complexity if their genetic and foster parents had greater song complexity. Interestingly, most fledglings only acquired around 80% of their parental song repertoire, suggesting they may acquire additional elements from neighbors, helpers, or other sources [16, 17], which remains to be studied. We likely underestimated the vocal repertoire of fledglings given that our analysis of vocal complexity was based on 3–5 song recordings per

individual. We note that the maximum total parental vocal repertoire (14 different element types between the adult pair) may exceed what a single individual can retain, and hence also acknowledge that fledglings may prune their repertoire across the first year [18]. We fully acknowledge the limitations of the field study to draw firm conclusions. A controlled laboratory study could rear embryos with and without song exposure to elucidate the causal role (if any) of prenatal sound exposure for song learning.

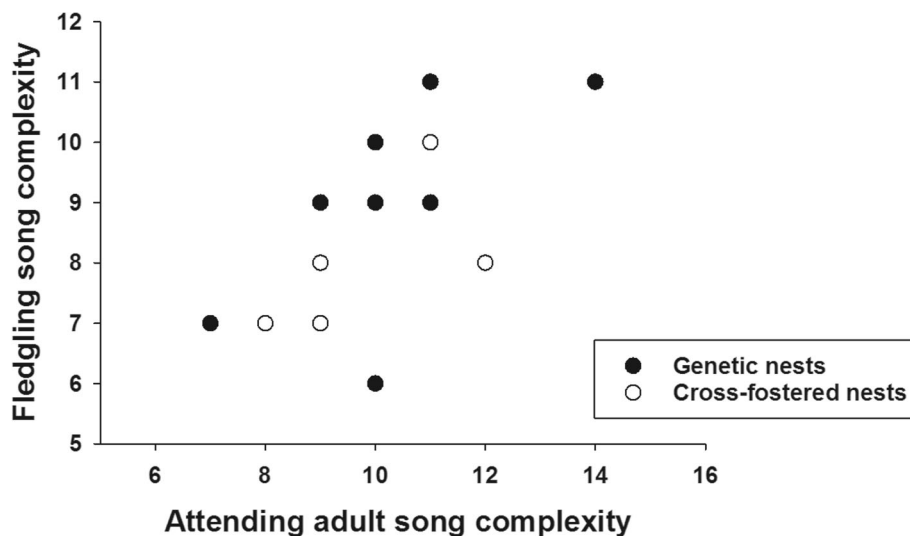


Fig. 4 The positive association between parental song complexity (total number of song elements in the attending adult pair) and fledgling song complexity (number of song elements per fledgling). Data are shown for 18 field nests (12 unmanipulated nests with genetic parents, 6 cross-fostered nests with foster parents)

Embryos varied in the magnitude of the dishabituation response. Perhaps unsurprisingly, older embryos had better sound discrimination, probably because neural structures underpinning sound perception are more developed in older embryos and/or because older embryos have acquired more auditory experience [19]. How sound exposure could affect development in songbirds is an emerging area of research. The precocial chicken is a long-standing model system for the study of avian hearing, including embryonic audition [20]. In contrast, at hatch, an altricial songbird is considered to be developmentally equivalent to a half-way developed precocial hatchling [21, 22], which raises evolutionary developmental questions about neural structures that underpin vocal learning and developmental rates across taxa. In the altricial Zebra Finch, the model system for song learning [23], no published study to date has examined *in ovo* response to sound directly, but embryos exposed to prenatal noise (their parents were also exposed to noise) had higher mortality, indicating some capacity to be impacted by prenatal sound [24]. Postnatal response to prenatal incubation calling was nevertheless found in Zebra Finch nestlings: embryos experimentally exposed to parental incubation calling developed into nestlings that vocalized while begging, while embryos reared without incubation calls were more likely to be silent [6]. In other lineages, Zebra Fish (*Danio rerio*) embryos showed a response to light at age 6–9 h, well before any neural structures for vision were apparent; these embryos had subsequent genetic upregulation of 6–4 photolyase mRNA as well as increased survival [25]. Much remains to be discovered about the role of early sensory exposure for the development of neural networks, anatomical structures and biochemical pathways for processing sensory input.

Given that pre-hearing and pre-vision exposure to sound and light upregulate subsequent investment into auditory and visual structures [25, 26], and the fact that many altricial songbird embryos must later produce complex learned vocalizations to attract a mate and defend a territory, it is reasonable to suggest that prenatal sound exposure may be particularly relevant for altricial songbird embryos with early onset of vocal learning and song production compared with delayed vocal production, as well as vocal ‘non-learners’. We predict there will be lineage-specific differences in the ontogeny of sound perception and vocal production that could influence rates of evolution in vocal learning lineages [27–31].

Our results provide observational evidence that, at least within altricial birds, an embryo’s capacity for sound discrimination may be positively correlated with the number of vocal elements learned later in life. It is possible that the prenatal sound discrimination forms part of the species-specific innate template for song

learning. Nature-nurture perspectives to explore possible causes and consequences of prenatal sound experience generate exciting evolutionary questions about the ontogeny of auditory memory and vocal learning [32, 33]. For example, isolation-reared Zebra Finches preferred conspecific song [34] and juveniles tutored by isolate-reared males with atypical song tended to revert to wild-type song after three to four generations [35], thereby demonstrating different lines of evidence for genetic predisposition for species-specific song. In addition to genetic predisposition for song type, other studies have revealed how experience can influence genetic variation and shape interindividual differences in learned song [36]. There is much to be discovered about factors that govern prenatal sound discrimination, including effects of instructive vocal experience before hatching, and how early sound experience can shape preference and behavior that directs vocal learning later in life.

Conclusions

Field studies are vital to inform the design of experimental research in behavioral ecology. The findings of this study add further support to the idea that an embryo’s acoustical environment may have profound impact on its subsequent developmental trajectory and behavior associated with song learning [13, 29]. Early sound discrimination capacity may be innate or it may be enhanced through exposure to prenatal vocal tutors. In precocial birds and humans, embryos have been shown to respond to sound, and to approach or respond to sound previously heard in the egg or uterus [reviewed in 11 & 13]. In a vocal learning songbird, prenatal sound discrimination and parental song complexity may predispose individuals to change behaviors relevant for song learning.

Methods

Study site

We monitored 234 wild and 26 cross-fostered Superb Fairy-wren nests during the fairy-wrens’ peak breeding period from September to January during the years 2012, 2013 and 2014. The study was conducted at two study sites in South Australia: (1) Cleland Wildlife Sanctuary (34°58’S, 138°41’E), 25kms SE of Adelaide; and (2) Newland Head Conservation Park (35°37’S, 138°29’E), a coastal area 15kms SW of Victor Harbor on the Fleurieu Peninsula [37]. Field studies are vital to maintain the biological relevance of questions (e.g. strength of selection, costs and benefits of behaviors for survivorship) and systems, but are extremely challenging due to high natural mortality [38, 39]. Of the 234 wild nests in this study, 169 (72%) were depredated; of the 26 cross-fostered nests, 20 (77%) were depredated. To cross-foster nest contents, we swapped entire clutches of eggs on day d 5 or d 6 after the

onset of incubation between two nests with same egg age. All tests involving prenatal sound exposure were done on d 11 to d 13 of the incubation phase. We describe each method and variable below, but provide a summary overview of sample size here, noting that sample size was impacted by predation at nests: (1) habituation/dishabituation responses of embryos to female incubation calls on d 11 to d 13 of incubation ($N = 52$ genetic nests, $N = 6$ cross-fostered nests); (2) nests with dishabituation response as an embryo and fledgling song repertoire ($N = 12$ genetic nests, $N = 6$ cross-fostered nests).

Study species

The Superb Fairy-wren is an insectivorous passerine that occurs in south-eastern Australia. Adult males and females are sexually dichromatic: males have bright blue crown, wing and tail plumage; females have grey crown and wing plumage, iridescent blue tails and red eye lore [40, 41]. The social mating system consists of breeding pairs or cooperative breeding, as some sons remain in the natal territory and become helper males [42]. The adult female builds a dome-shaped nest (usually 30–150 cm from the ground) and lays 2–3 eggs per nest [39]. The nesting period spans August to February with several breeding attempts per female per year [43]. The incubation phase lasts 12–15 days and the nestling phase 10–15 days. Females are uniparental incubators; all group members feed nestlings and dependent fledglings. Egg and nestling predation has been 24% to 90% across years, with subsequent predation risk for fledglings [39, 44]. While fledglings become independent of adult feeding after four weeks, they remain in the natal group for several months [41]. Males are philopatric and females disperse in the first year; a dispersing female moves an average 11.8 territories away from the natal territory and travels a distance of 1–10 km [45]. Recently fledged young of both sexes resemble adult females, but have paler legs and paler red lore [41]. Superb Fairy-wren fledglings produce subsong ~ 16 days after fledging and full song ~ 50 days post-fledging. This early song learning in fairy-wren fledglings is in stark contrast to northern hemisphere songbirds such as the Swamp Sparrow (*Melospiza georgiana*) that produce subsong ~ 272 days after fledging and full song ~ 334 days after fledging [17, 46].

Heart rate in embryos

We measured prenatal heart rate (HR) in the field using a digital egg monitor (Buddy™, Vetronic Services, UK) [5, 47, 48]. This device generates heart rate data by tracking light absorption changes due to embryonic blood flow, and can detect heart rate from ~ d 5 of the incubation period in Superb Fairy-wren. The egg was removed from its nest, placed in the heart rate monitor (that is, placed on a small portable heat pack to control

for temperature), and exposed at close proximity to broadcast of sound at ~ 60 dB. The experimental sound stimuli were broadcast to the egg as uncompressed files from an Apple iPod (Apple Inc., USA) connected to a Moshi Bass burger speaker (Moshi Corporation, USA; Frequency Response: 280 Hz - 16 kHz) placed 5–10 cm from the monitor. The embryo's HR is shown as beats per minute and appears on the external screen of the heart rate monitor; HR response was video recorded and/or hand-scored.

In an independent study in chickens (*Gallus domesticus*), researchers windowed eggs and measured HR in the digital monitor; lower HR occurred as a cardiac response but could also occur when the embryo moved [49]. Here, we did not window the eggs. Therefore, in our study, it is possible that the change in HR was cardiac change or occurred as the consequence of *in ovo* movement. In either case, the change in HR is indicative of a prenatal response. After each eight-minute playback experiment, the egg was returned to its natal nest. For tracking the individual identity of embryos, eggs were marked with black marker to be identified across sequential days and in relation to hatching.

Habituation/dishabituation tests in embryos

At 52 natural nests and 6 experimentally cross-fostered nests, we exposed d 11 to d 13 embryos to the incubation calls of unrelated females and measured their habituation/dishabituation response as change in heart rate. Our previous research used this approach and showed *in ovo* habituation after exposure to 18 calls from the same individual, followed by sound discrimination when exposed to different calls from a different individual [5]. In Zebra Finch, juveniles had a habituation response after exposure to ~ 15 songs from the same tutor bird [2].

Habituation test

We presented the embryo with 18 different incubation calls from the same female every 10 s; each trial lasted 1 min (habituation phase trials H1, H2, H3) and was followed by 1 minute of silence. Total duration was 6 min for the habituation test. We considered that an embryo habituated to the individual's call if it did not significantly change its HR during H3 (Fig. 1) and maintained comparable heart rate with the average HR of the preceding phase trial ($H2 = H3$) [5].

Dishabituation test

On the fourth trial (dishabituation phase trial D), we presented the embryo with 6 different incubation calls from a novel female every 10 s, also followed by one minute of silence. The dishabituation response was calculated as the difference in average HR during D minus

H3. A negative value indicates lower heart rate during D than H3, which is indicative of a renewed sound discrimination response to a novel stimulus [50].

We created the playback stimuli with Amadeus Pro 1.5 (Hairersoft Inc., Switzerland). We used female incubation calls that were previously recorded at our study sites since 2009 using a Zoom Handy Recorder H4n (Zoom Corporation, Australia) placed 10–30 cm under nest [4, 51]. We used calls that had no overlapping sound, normalized the stimuli (−15db), saved as uncompressed 16 bit broadcast wave files (.wav) and transferred them to the Apple iPod before the playback tests.

Observed song complexity in fledglings

Fairy-wrens are highly vocal and produce many different vocalization types [52] including complex type I (chatter) song [14, 53], type II song [54], alarm song [55], contact calls, alarm calls [56, 57], brood calls [41], and incubation calls with about 10 elements consisting of repeats of two different elements [4]. Here, we focus on chatter song, as this is produced by both females and males and is used for mate choice and territory defence [58, 59]. Chatter song is the most complex vocalization produced by this species; it typically lasts 3 s and contains ~ 50 elements that are repeats of ~ 10 different elements [14]. Females and males can have a comparable number of elements per song [14], though there may be geographical differences in complexity between males and females [60].

We recorded chatter song of 31 color-banded families with confirmed parentage from genetic relatedness analysis; we recorded song from the female, attending male and offspring 7–8 weeks after fledging. Partial recordings of adult family members exist for 136 nests, but 84 were depredated (62%); only 34 nests with recordings produced fledglings (25%) and we were unable to record the song of three family groups. In addition to complete family song recordings at 31 unmanipulated nests, we have song recordings for six cross-fostered families. We cross-fostered 26 clutches; six nests produced fledglings and 20 nests were depredated. For each nest, we used 3–5 song recordings per individual bird (dominant male, dominant female, fledged young). For our analysis of prenatal sound discrimination and postnatal song repertoire, the final sample size was 18 nests (12 unmanipulated nests, 6 cross-fostered) because we restricted the analysis to include only those nests at which embryos had shown a habituation response and for which we had song recordings of the entire family.

We recorded the chatter song of adult birds opportunistically throughout the breeding season. We recorded the songs of fledgling birds at a distance of 5–10 m from the bird every 14 days for eight weeks post-fledging. Recordings were made with a Telinga Twin Science parabolic microphone (Telinga Microphones, Sweden) connected to

a portable Sound Device 722 digital audio recorder (Sound Devices, USA). Sound files were recorded as broadcast wave files (24 bit, 48 kHz). We transcribed all sound files to an Apple MacBookPro for editing with Amadeus Pro 2.1.2 and analysis with Raven Pro 1.5. Spectrograms were created using the Hann algorithm (filter bandwidth 270 Hz, size 256 samples, time grid overlap 50%, grid resolution 2.67 ms, 188 Hz, DTF 256 samples). For each spectrogram, we scored the number of total elements and the number of different element types. We defined the vocal complexity of a bird as the total number of different element types sung by the individual from 3 to 5 songs recorded and analyzed. In this study, we did not quantify the absolute element repertoire size. We used observed song elements with the average number of different element types per song, and refer to this as vocal complexity.

We categorized the different element types per spectrogram according to the element library of Evans and Kleindorfer [14]. We printed spectrograms and assigned element types visually according to the library of element categories. This method was chosen because humans outperform machines for tasks like the visual recognition of sonogram element types [61, 62]. Three people (two naïve assessors and the person who scored the spectrograms for this study) independently reviewed 20 randomly chosen spectrograms (identity of bird unknown) and classified the different element types. The average similarity rate was 95.7% for the scoring of the number of different element types. The song complexity per fledgling was calculated as the total number of different song elements. We also calculated the percentage of song elements present in fledgling song that were also present in the combined parental song repertoire (attending male and female).

Statistical analyses

We used IBM SPSS 22 for Windows (SPSS Inc., Chicago, U.S.A) for statistical analyses. We examined the data for normality and homogeneity of variance. Heart rate data were normally distributed. For the habituation/dishabituation experiment, we averaged heart rate data scored every 10 s per 1 min trial. We used a paired t-test to analyze the habituation phase responses for each embryo. We statistically compared an embryo's average heart rate during the H2 phase against its average heart rate during the H3 phase. For the dishabituation response, we statistically compared an embryo's average heart rate during the D phase with its average heart rate during the H3 phase.

We used multiple regression analysis weighted by Nest ID to test the association between habituation response and dishabituation response against egg age. To examine vocal complexity in adult vocal tutors at genetic and cross-fostered nests, we used an independent samples t-test to compare the number of vocal elements. We

used multiple regression analysis to test for effects of prenatal sound discrimination score (D minus H3), habituation response (H3 minus H2), and parental vocal complexity (number of different vocal elements) on fledgling vocal complexity (number of vocal elements).

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Authors' contributions

SK designed the study; SK, DCN and CE performed field work; CE collected the field song recordings and did the song analysis; MEH and DCN designed the habituation/dishabituation test; SK led the writing of the manuscript; all authors worked on the manuscript draft; all authors gave final approval for publication.

Ethics approval

The present study complies with institutional, national, and international ethical guidelines. This study on wild Superb Fairy-wrens was approved by the Animal Welfare Committee of Flinders University (E325–404) and adhered to a Department of Environment, Wildlife and Natural Resources (DEWNR) scientific permit to conduct the research (Z24699).

Competing interests

The authors declare that they have no competing interests.

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References

- Prather JF, Peters S, Nowicki S, Mooney R. Precise auditory–vocal mirroring in neurons for learned vocal communication. *Nature*. 2008;451:305–10.
- Phan ML, Pytte CL, Vicario DS. Early auditory experience generates long-lasting memories that may subserve vocal learning in songbirds. *PNAS*. 2006;103:1088–93.
- Kriengwatana B, Spierings MJ, ten Cate C. Auditory discrimination learning in zebra finches: effects of sex, early life conditions and stimulus characteristics. *Anim Behav*. 2016;116:99–112.
- Colombelli-Négrel D, Hauber ME, Robertson J, Sulloway FJ, Hoi H, Griggio M, Kleindorfer S. Embryonic learning of vocal passwords in superb fairy-wrens reveals intruder cuckoo nestlings. *Curr Biol*. 2012;22:2155–60.
- Colombelli-Négrel D, Hauber ME, Kleindorfer S. Prenatal learning in an Australian songbird: habituation and individual discrimination in superb fairy-wren embryos. *Proc R Soc Lond B*. 2014;281:20141154.
- Mariette MM, Buchanan KL. Prenatal acoustic communication programs offspring for high posthatching temperatures in a songbird. *Science*. 2016;353:812–4.
- Colombelli-Négrel D, Webster MS, Dowling JL, Hauber ME, Kleindorfer S. Vocal imitation of mother's calls by begging red-backed Fairywren nestlings increases parental provisioning. *Auk*. 2016;133:273–85.
- Kleindorfer S, Evans C, Colombelli-Négrel D. Females that experience threat are better teachers. *Biol Lett*. 2014;10:20140046.
- Mousseau TA, Uller T, Wapstra E, Badyaev AV. Evolution of maternal effects: past and present. *Proc R Soc Lond B*. 2009;364:1035–8.
- Duckworth RA, Belloni V, Anderson SR. Cycles of species replacement emerge from locally induced maternal effects on offspring behavior in a passerine bird. *Science*. 2015;347:875–7.
- Rivera M, Louder MI, Kleindorfer S, Liu W-C, Hauber ME. Avian prenatal auditory stimulation: progress and perspectives. *Behav Ecol Sociobiol*. 2018;72:112.
- Dowling JL, Colombelli-Négrel D, Webster MS. Kin signatures learned in the egg? Red-backed fairy-wren songs are similar to their Mother's in-Nest calls and songs. *Frontiers Ecol Evol*. 2016;4:48.
- Katsis A, Davies M, Buchanan K, Kleindorfer S, Hauber ME, Mariette M. Prenatal exposure to incubation calls affects song learning in the zebra finch. *Sci Rep*. 2018;8:15232.
- Evans C, Kleindorfer S. Superb fairy-wren (*Malurus cyaneus*) sons and daughters acquire song elements of mothers and social fathers. *Frontiers Ecol Evol*. 2016;4:9.
- Colombelli-Négrel D, Kleindorfer S. Prenatal environment affects embryonic response to song. *Biol Lett*. 2017;13:20170302.
- Baptista LF, Morton ML. Song learning in montane white-crowned sparrows: from whom and when. *Anim Behav*. 1988;36:1753–64.
- Lapierre JM, Mennill DJ, MacDougall-Shackleton EA. Spatial and age-related variation in use of locally common song elements in dawn singing of song sparrows *Melospiza melodia*: old males sing the hits. *Behav Ecol Sociobiol*. 2011;65:2149–60.
- Ho WW, Connolly SG, Reynolds PL, Cornelius JM, MacDougall-Shackleton EA, Morton ML, Pereyra ME, Hahn TP. Song exposure during juvenile dispersal in mountain white-crowned sparrows. *Auk*. 2014;131:208–14.
- Gottlieb G. Roles of early experience in species-specific perceptual development. *Dev Percept*. 1981;1:5–44.
- Jones TA, Jones SM, Paggett KC. Emergence of hearing in the chicken embryo. *J Neurophysiol*. 2006;96:128–41.
- Oppenheim RW. Prehatching and hatching behaviour in birds: a comparative study of altricial and precocial species. *Anim Behav*. 1972;20:644–55.
- Brittan-Powell EF, Dooling RJ. Development of auditory sensitivity in budgerigars (*Melopsittacus undulatus*). *J Acoustic Soc Am*. 2004;115:3092–102.
- Zann RA. The zebra finch: a synthesis of field and laboratory studies. New York: Oxford University Press; 1996:352.
- Potvin DA, MacDougall-Shackleton SA. Traffic noise affects embryo mortality and nestling growth rates in captive zebra finches. *J Exp Zool A: Ecol Genetics Physiol*. 2015;323:722–30.
- Tamai TK, Vardhanabhuti V, Foulkes NS, Whitmore D. Early embryonic light detection improves survival. *Curr Biol*. 2004;14:R104–5.
- Chaudhury S, Nag TC, Jain S, Wadhwa S. Role of sound stimulation in reprogramming brain connectivity. *J Biosci*. 2013;38:605–14.
- Kisilevsky BS. Fetal auditory processing: implications for language development? In: Reissland N, Kisilevsky B, editors. *Fetal Development*. Cham: Springer; 2016. p. 133–52.
- Greenhill SJ, Atkinson QD, Meade A, Gray RD. The shape and tempo of language evolution. *Proc R Soc Lond B*. 2010;277:2443–50.
- Spencer KA, Minderman. Developmental programming via activation of the hypothalamic–pituitary–adrenal Axis: a new role for acoustic stimuli in shaping behavior? *Adv Study Behav*. 2018;50:87–126.
- Lee MS, Cau A, Naish D, Dyke GJ. Sustained miniaturization and anatomical innovation in the dinosaurian ancestors of birds. *Science*. 2014;345:562–6.
- Mason NA, Burns KJ, Tobias JA, Claramunt S, Seddon N, Derryberry EP. Song evolution, speciation, and vocal learning in passerine birds. *Evolution*. 2017;71:786–96.
- Gottlieb G. *Synthesizing nature-nurture: prenatal roots of instinctive behavior*. New York: Psychology Press; 2014:200.
- West MJ, King AP. Settling nature and nurture into an ontogenetic niche. *Dev Psychobiol*. 1987;20:549–62.
- Braaten RF, Reynolds K. Auditory preference for conspecific song in isolation-reared zebra finches. *Anim Behav*. 1999;58:105–11.

35. Fehér O, Wang H, Saar S, Mitra PP, Tchernichovski O. De novo establishment of wild-type song culture in the zebra finch. *Nature*. 2009;459:564.
36. Mets DG, Brainard MS. Genetic variation interacts with experience to determine interindividual differences in learned song. *PNAS*. 2017;115:421–6.
37. Schlotfeldt BE, Kleindorfer S. Adaptive divergence in the superb fairy-wren (*Malurus cyaneus*): a mainland versus island comparison of morphology and foraging behaviour. *Emu-Austral Ornithol*. 2006;106:309–19.
38. Kleindorfer S, Hoi H, Evans C, Mahr K, Robertson J, Hauber ME, Colombelli-Négrel D. The cost of teaching embryos in superb fairy-wrens. *Behav Ecol*. 2014;25:1131–5.
39. Colombelli-Négrel D, Kleindorfer S. Nest height, nest concealment, and predator type predict nest predation in superb fairy-wrens (*Malurus cyaneus*). *Ecol Res*. 2009;24:921–8.
40. Mahr K, Evans C, Thonhauser KE, Griggio M, Hoi H. Multiple ornaments-multiple signaling functions? The importance of song and UV plumage coloration in female superb fairy-wrens (*Malurus cyaneus*). *Frontiers Ecol Evol*. 2016;4:43.
41. Rowley I, Russell EM. Fairy-wrens and grasswrens: Maluridae. New York: Oxford University Press; 1997:274.
42. Brouwer L, van de Pol M, Aranzamendi NH, Bain G, Baldassarre DT, Brooker LC, Brooker MG, Colombelli-Négrel D, Enbody E, Gielow K, Hall ML. Multiple hypotheses explain variation in extra-pair paternity at different levels in a single bird family. *Mol Ecol*. 2017;26:6717–29.
43. Cockburn A, Brouwer L, Margraf N, Osmond HL, Van de Pol Martijn. Superb fairy-wrens: Making the worst of a good job. In: Koenig WD, Dickinson JL. *Cooperative Breeding: Studies of Ecology, Evolution and Behaviour*. Cambridge University Press; 2016. p. 133–49.
44. Colombelli-Négrel D, Robertson J, Sulloway FJ, Kleindorfer S. Extended parental care of fledglings: parent birds adjust anti-predator response according to predator type and distance. *Behaviour*. 2010;147:853–70.
45. Double M, Peakall R, Beck N, Cockburn A. Dispersal, philopatry, and infidelity: dissecting local genetic structure in superb fairy-wrens (*Malurus cyaneus*). *Evolution*. 2005;59:625–35.
46. Reid JM, Arcese P, Cassidy AL, Hiebert SM, Smith JN, Stoddard PK, Marr AB, Keller LF. Song repertoire size predicts initial mating success in male song sparrows, *Melospiza melodia*. *Anim Behav*. 2004;68:1055–63.
47. Lierz M, Gooss O, Hafez HM. Noninvasive heart rate measurement using a digital egg monitor in chicken and Turkey embryos. *J Avian Med Surg*. 2006;20:141–6.
48. Sheldon EL, McCowan LS, McDiarmid CS, Griffith SC. Measuring the embryonic heart rate of wild birds: an opportunity to take the pulse on early development. *Auk*. 2017;135:71–82.
49. Pollard A, Pitsillides A, Portugal S. Validating a noninvasive technique for monitoring embryo movement in Ovo. *Physiol Biochem Zool*. 2016;89:331–9.
50. Hauber ME, Pearson HE, Reh A, Merges A. Discrimination between host songs by brood parasitic brown-headed cowbirds (*Molothrus ater*). *Anim Cogn*. 2002;5:129–37.
51. MacLeod KJ, Brouwer L. Social context-dependent provisioning rules in red-winged fairy-wrens do not vary with signals of increased chick need. *Anim Behav*. 2018;143:105–11.
52. Greig E, Pruett-Jones S. Splendid songs: the vocal behaviour of splendid fairy-wrens (*Malurus splendens melanotus*). *Emu-Austral Ornithol*. 2008;108:103–14.
53. Kleindorfer S, Evans C, Mahr K. Female in-nest chatter song increases predation. *Biol Lett*. 2016;12:20150513.
54. Langmore NE, Mulder RA. A novel context for bird song: predator calls prompt male singing in the Kleptogamous superb fairy-wren, *Malurus cyaneus*. *Ethology*. 1992;90:143–53.
55. Colombelli-Négrel D, Robertson J, Kleindorfer S. Risky revelations: superb fairy-wrens *Malurus cyaneus* respond more strongly to their mate's alarm song. *J Ornithol*. 2011;152:127–35.
56. Magrath RD, Bennett TH. A micro-geography of fear: learning to eavesdrop on alarm calls of neighbouring heterospecifics. *Proc R Soc Lond B*. 2011;279:902–9.
57. Colombelli-Négrel D, Evans C. Superb fairy-wrens respond more to alarm calls from mate and kin compared to unrelated individuals. *Behav Ecol*. 2017;28:1101–12.
58. Cooney R, Cockburn A. Territorial defence is the major function of female song in the superb fairy-wren, *Malurus cyaneus*. *Anim Behav*. 1995;49:1635–47.
59. Cain KE, Cockburn A, Langmore NE. Female song rates in response to simulated intruder are positively related to reproductive success. *Frontiers Ecol Evol*. 2015;3:119.
60. Kleindorfer S, Evans C, Mihailova M, Colombelli-Négrel D, Hoi H, Griggio M, Mahr K, Robertson J. When subspecies matter: resident superb fairy-wrens (*Malurus cyaneus*) distinguish the sex and subspecies of intruding birds. *Emu-Austral Ornithol*. 2013;113:259–69.
61. Keen S, Ross JC, Griffiths ET, Lanzone M, Farnsworth A. A comparison of similarity-based approaches in the classification of flight calls of four species of north American wood-warblers (*Parulidae*). *Ecol Informatics*. 2014;21:25–33.
62. Von Ahn L. Human computation. In: *Proceedings of the 2008 IEEE 24th international conference on data engineering, IEEE Computer Society*; 2008. p. 1–2.

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