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A weak individual signature might not allow chick call recognition by parent Stone Curlews *Burhinus oedicnemus*

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Offspring recognition has been confirmed in many bird species, and vocal signatures appear to be its major component. Up to now most studies dealt with colonial species, but recent findings indicate that similar recognition is also present in non-colonial birds. By integrating spectrographic analysis and playback experiments, we investigated for the first time parent–offspring vocal recognition in the Stone Curlew (*Burhinus oedicnemus*), a highly vocal, crepuscular-nocturnal species that usually rears a maximum of two precocial and nidifugous chicks. Even though the species is usually non-colonial and non-cooperative, in Taro River Regional Park, Parma, Italy, breeding territories are densely packed with distances between simultaneously active nests as close as 40 m, which creates the possibility that chicks might be exchanged. Our analysis identified two main vocalizations in the chick call repertoire: (1) the S-call, a brief strangled soft sound often uttered when parents and chicks were at close quarters; and (2) the C-call, a chirping sound with a complex structure, which seems to be a long-distance contact vocalization. Our acoustic analysis showed that, even though the structure was remarkably different between chicks, C-calls were an unreliable individual signature given their high intra-individual variability. This was confirmed by playback experiments. Indeed, when presented with simulated C-calling chicks, adults approached both their own and foreign chicks with equal responses. These results suggest that parent Stone Curlews are unable to recognize their offspring by voice. Furthermore, experiments are needed to test whether the chicks are able to recognize their parents' voice and, more generally, to understand the details of parent–offspring communication in this species.

Keywords: Stone Curlew; *Burhinus oedicnemus*; individual recognition; playback experiments; spectrographic analysis

Introduction

The theory of natural selection predicts that parents should not feed unrelated young, but rather should invest solely in their own offspring (Hepper 1986); this explains the evolution of parent–young recognition in many species whose young could intermingle while still dependent on parents (McArthur 1982). Many studies have demonstrated the existence of parent–offspring recognition in birds (Snow 1958; Peek et al. 1972; Miller and Emlen 1975; Burt 1977; Balda and Balda 1978; Rowley 1980; Beecher et al. 1981; McArthur 1982; Seddon and Van Heezik 1993; Searby et al. 2004; Mulard et al. 2008). In most cases, the recognition mechanism is based on individual vocal signatures, facilitating

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either mutual recognition (Lefevre et al. 1998; Insley 2003), recognition of parents by chicks (Charrier et al. 2001; Lengagne et al. 2001; Taylor and Perrin 2008) or recognition of chicks by parents (Stoddard and Beecher 1983; Lessells et al. 1991).

In colonially breeding birds, where young are mobile and the likelihood of intermingling is high, there should be strong selection for parent–offspring recognition. Indeed, the use of individual vocal signatures has been demonstrated for many colonial species (Lefevre et al. 1998; Jouventin and Aubin 2002). This phenomenon has been explored extensively in swallows (Beecher et al. 1981; Stoddard and Beecher 1983; Medvin et al. 1993). In two colonial species, Bank Swallow (*Riparia riparia*) and Cliff Swallow (*Hirundo pyrrhonota*), in which young form crèches that contain many dependent fledglings, offspring develop individually distinctive vocal signatures and parents learn the calls of their young. However, in two non-colonial species of swallows – Barn Swallow (*Hirundo rustica*) and Northern Rough-winged Swallow (*Stelgidopteryx serripennis*) – nestlings do not develop distinctive call features as fledglings of these species seldom intermingle with those of other broods. However, recent studies have found offspring vocal recognition in non-colonial species. Long-tailed tits (*Aegithalos caudatus*) breed independently in pairs, but most nests fail due to depredation. Failed breeders often re-nest, but may instead become helpers later in the season and preferentially care for close relatives. This kin-biased helping occurs in the absence of reliable spatial cues, and Sharp et al. (2005) showed that cooperatively breeding long-tailed tits can discriminate between kin and non-kin according to the individual characteristics of their contact calls. The Brown-headed Parrot (*Poicephalus cryptoxanthus*) nests in hollows in trees, but upon fledging the chicks are escorted to nursery areas in heavily foliated trees where they remain camouflaged. Therefore, although nest site recognition may be sufficient to identify chicks prior to fledging, a recognition problem arises when those chicks are mobile yet camouflaged. Taylor and Perrin (2008) showed that these chicks are able to recognize their parents by voice.

In colonial birds, the timing for development of recognition varies among species, but normally occurs just before chicks are able to leave the nest area (Taylor and Perrin 2008). It is interesting to note that the selective pressure acting in favour of (or against) offspring vocal recognition seems to be modulated by many factors which can change also within species (Falls 1982). For example, Rautenfeld (1978) demonstrated that for Herring Gulls (*Larus argentatus*) nesting on cliffs, where intermingling is impossible, recognition was lacking until the chicks were 14 days old, whereas Tinbergen (1953) showed that Herring Gulls nesting on the ground in proximity to other nests can recognize their chicks 5 days after hatching.

The aim of our work was to investigate parent–offspring vocal recognition in the Stone Curlew (*Burhinus oedicnemus*), a non-colonial and non-cooperative breeding species, characterized by a small clutch size (two eggs, ca. 19% of female body mass) and a relatively long fledgling period (usually > 40 days; Cramp and Simmons 1983; Nethersole-Thompson and Nethersole-Thompson 1986; D. Giunchi et al., unpublished data), which should favour the recognition between parents and chicks. We studied the population breeding in Taro River Regional Park, Parma, Italy. At this site, breeding territories of Stone Curlews are usually densely packed with distances between simultaneously active nests sometimes even < 40 m (nearest-neighbour distance between breeding pairs in the year 2010: median = 115 m, interquartile range = 95–160 m, $n = 91$; see also Giunchi et al. 2009). Such a high density might increase the likelihood of chicks swapping nests, given that even young chicks (< 10 days old) often move > 100 m from the nest sites, as observed by means of radiotracking (D. Giunchi et al., unpublished data). Moreover, selective pressure should

favour acoustic recognition if the probability of misdirected care is high. This might be the case for this species as the chicks are mobile (precocial and nidifugous: Cramp and Simmons 1983), active mainly during the night and rather cryptic during the day. Moreover, even though Stone Curlew parents do not directly feed their young, they actively care for chicks, e.g. by brooding them and by providing food items [1–3 times/h according to Barros (1994) in Vaughan and Vaughan-Jennings (2005)], which are placed on the ground near the chicks and often pointed out by the adult with its beak (Cramp and Simmons 1983). Furthermore, the Stone Curlew is a highly vocal species and which may be an additional condition favourable for parent–offspring recognition based on vocal individuality. A key behavioural component for recognition is an increase in vocal signature variation among individuals and an increased coefficient of variation of inter-individual/intra-individual signatures (Aubin et al. 2004), thus increasing information about individual identity (Beecher 1988). Therefore, we investigated both (1) the call repertoire of chicks and their vocal individuality and (2) the ability of adults to recognize their chick by voice. To our knowledge, this is the first in-depth analysis of chick call repertoire of the Stone Curlew, as previous studies (reviewed by Vaughan and Vaughan-Jennings 2005) reported only anecdotal descriptions without any quantitative results.

Materials and methods

Study area, recordings and spectrographic analysis

Recordings and playback experiments were carried out in the period 2008–2010 in the Taro River Regional Park, Parma, Italy (44.74°N, 10.17°E), where >80 pairs of Stone Curlews breed mainly in the dry gravel riverbed (Giunchi et al. 2009; Caccamo et al. 2011). Chicks were caught during the night using a lamp and a dip net. Vocalizations were recorded after ringing procedures. Birds were put singly in a wooden arena (35 × 35 × 37 cm) with a Sennheiser M67 shotgun microphone placed about 30 cm away, connected to a Sony Mini Disc MZ-R30 (2008–2009) or to a Fostex FR2-LE digital recorder (2010). Even though the distance between the microphone and the sound source was short, we used a shotgun microphone in order to reduce the environmental background noise and to increase the signal-to-noise ratio of the recordings. Each recording session lasted on average 15 min. Audio tracks were digitized at 44.1 kHz sampling rate, with 16-bit accuracy and stored on a PC in wav format (Pulse-Code Modulation (PCM)). Spectrograms were made using *SeeWave* 1.1 software (Pavan 1998) with parameters set as follows: FFT (Fast Fourier Transform) window size = 1024, overlap = 75%, window type = Gaussian, frequency resolution = 23 Hz and time resolution = 11 ms. Samples of call types mentioned in this paper may be downloaded from http://www.birdsongs.it/songs/burhinus_oedicnemus/burhinus_oedicnemus.html.

Chick call repertoire and vocal individuality

Call repertoire was studied by analysing the spectrograms of 66 recordings from chicks aged between 11 and 30 days old. Power spectra were obtained using the function *meanspec* from the *SeeWave* package v. 1.6.1 (Sueur et al. 2008) running under R environment (R Development Core Team 2011). This function returns the mean frequency spectrum (i.e. mean relative amplitude of frequency distribution) of a time wave; parameters of the *meanspec* function were sampling frequency = 44,100 Hz, FFT window length = 1024 and range of frequency axis = 1–10 kHz.

In 2010, 14 chicks (also used for playback experiments, see below) were recorded twice on average 9.4 days apart (range 9–12 days). Recordings from seven birds were

discarded because of the low number of calls (< 12) in one of these two recordings. The seven remaining chicks (age = 17–22 days) were used to assess within-individual variability and are identified hereafter as the W-GROUP. An additional 13 chicks (age = 14–29 days), recorded only once during the same year, identified hereafter as the A-GROUP, were used to assess between-individual variability.

Only one call type – the C-call used for long-distance contact with adults – was considered for this analysis (see “Results” section). Every chick was recorded for 15 min. Chicks uttered from a minimum of 25 to a maximum of 300 calls, consisting of only one syllable, with an irregular call rate, rather variable between and within chicks (see “Results” section). For each recording, we randomly selected 25 calls and then we cut twenty-five 250-ms audio samples, each containing one call. These samples were band-pass filtered between 1 and 8 kHz to clean background noise, because preliminary analyses of power spectra revealed that $> 95\%$ of the vocalization was below 8 kHz. Audio tracks were silenced before and after the call using Audacity v. 1.3.4 beta (Audacity Team, <http://audacity.sourceforge.net/>). Comparisons between two call series – belonging either to the same or different chick – were done by calculating a similarity index between each call pair by means of the function *covspectro* from the *SeeWave* package with the following settings: sampling frequency = 44,100 Hz, window length = 1024 points, window type = Hanning, number of covariance computed when sliding the two calls = 19. This function calculates the covariance between two spectrograms and returns the maximal covariance value (Hopp et al. 1998). For each chick from the W-GROUP, we calculated an index of intra-individual similarity (hereafter S-INTRA) by randomly pairing one call from the first recording and one call from the second recording, which was taken on average 9.4 days apart, as mentioned above. Each call was used only once. One advantage of this method is that the whole call is compared and no acoustic parameter is selected a priori, therefore avoiding subjective choices. Furthermore, the quantitative evaluation of the function *covspectro* is strongly influenced by the harmonic part of the vocalization, which is frequency as well as amplitude modulated over time and which may be relevant for individual recognition (Hopp et al. 1998; Sueur et al. 2008). The inter-individual similarity (hereafter S-INTER) indexes were calculated by comparing each bird of the W-GROUP with the remaining six chicks from the same group plus all the 13 chicks from the A-GROUP, yielding a total of 19 inter-individual comparisons. Similarity indexes were obtained by the same method described above, randomly pairing one of the two sets of 25 calls used in the intra-individual analysis, derived from either the first or the second recording (randomly chosen) of each chick from the W-GROUP, and 25 calls belonging to each of the other 19 chicks. In this way, for each W-GROUP bird, we got 19 sets of 25 S-INTER index values to compare with the series of 25 S-INTRA indexes previously calculated. Significantly lower values of S-INTER indexes with respect to the corresponding S-INTRA values would mean that the variability of vocal characteristics of calls within a chick is significantly lower than the variability between different chicks; clearly this is a pre-requisite for discrimination and individual recognition (Terry et al. 2005). It is important to notice that according to this analysis design, which involves two sources of random variation (i.e. random choice of one of the two call series used in the intra-individual analysis and random pairing of calls for each inter-individual comparison), the S-INTER indexes were always calculated with different data-sets, even if W-GROUP birds were considered twice in the analysis. For this reason, two pairwise comparisons involving the same birds (e.g. TH0375 vs. TH0384 and TH0384 vs. TH0375, see Table 1) did not necessarily yield the same results. This approach was

Table 1. Vocal similarity within and between chicks.

Group	Chick tags	TH0375	TH0384	TH0400	TJ2951	TJ2959	TJ2961	TJ2970
W-GROUP	TH0375	0.66 (0.10) ⁺⁺	0.57 (0.07) ⁺⁺	0.58 (0.10)	0.66 (0.07)	0.46 (0.1) ⁺⁺	0.53 (0.10) ⁺⁺	0.59 (0.09) ⁺⁺
	TH0384	0.51 (0.08) ⁺	0.67 (0.10) ⁺⁺	0.52 (0.12)	0.61 (0.14)	0.55 (0.16)	0.66 (0.11)	0.50 (0.17) ⁺⁺
	TH0400	0.57 (0.10) ⁺⁺	0.60 (0.10) ⁺	0.45 (0.13)	0.71 (0.08)	0.70 (0.11)	0.70 (0.10)	0.46 (0.11) ⁺⁺
	TJ2951	0.60 (0.10) ⁺	0.65 (0.15)	0.57 (0.13)	0.62 (0.11)	0.54 (0.20)	0.62 (0.13) ⁺⁺	0.66 (0.13)
	TJ2959	0.54 (0.07) ⁺⁺	0.42 (0.07) ⁺⁺	0.38 (0.08)	0.57 (0.11)	0.61 (0.11)	0.41 (0.10) ⁺⁺	0.67 (0.09)
	TJ2961	0.60 (0.08) ⁺	0.69 (0.09)	0.46 (0.14)	0.71 (0.08)	0.38 (0.14) ⁺⁺	0.71 (0.11)	0.61 (0.11) ⁺
	TJ2970	0.64 (0.08)	0.76 (0.05)	0.71 (0.07)	0.54 (0.14)	0.62 (0.07)	0.47 (0.11) ⁺⁺	0.69 (0.13)
	TJ2967	0.44 (0.14) ⁺⁺	0.50 (0.14) ⁺⁺	0.46 (0.14)	0.58 (0.14)	0.57 (0.12)	0.45 (0.14) ⁺⁺	0.63 (0.08) ⁺
	TJ2974	0.41 (0.07) ⁺⁺	0.32 (0.06) ⁺⁺	0.26 (0.05) ⁺⁺	0.34 (0.05) ⁺⁺	0.59 (0.06)	0.25 (0.05) ⁺⁺	0.45 (0.06) ⁺⁺
	P14_03A	0.53 (0.08) ⁺⁺	0.68 (0.12)	0.46 (0.21)	0.63 (0.08)	0.53 (0.18)	0.61 (0.14) ⁺⁺	0.60 (0.10) ⁺⁺
A-GROUP	TJ2955	0.62 (0.09)	0.64 (0.11)	0.60 (0.13)	0.67 (0.09)	0.50 (0.14) ⁺	0.59 (0.13) ⁺⁺	0.68 (0.09)
	TJ2956	0.60 (0.09) ⁺⁺	0.66 (0.12)	0.57 (0.16)	0.63 (0.10)	0.67 (0.07)	0.58 (0.14) ⁺⁺	0.65 (0.11)
	P17_01A	0.62 (0.08)	0.65 (0.08)	0.69 (0.10)	0.66 (0.09)	0.66 (0.11)	0.59 (0.11) ⁺⁺	0.63 (0.10)
	TH0395	0.43 (0.07) ⁺⁺	0.63 (0.04)	0.60 (0.08)	0.49 (0.15) ⁺⁺	0.22 (0.07) ⁺⁺	0.55 (0.06) ⁺⁺	0.45 (0.08) ⁺⁺
	TJ2962	0.59 (0.08) ⁺⁺	0.70 (0.06)	0.72 (0.06)	0.46 (0.09) ⁺⁺	0.73 (0.07)	0.40 (0.06) ⁺⁺	0.64 (0.07)
	TJ2965	0.51 (0.05) ⁺⁺	0.54 (0.07) ⁺⁺	0.76 (0.05)	0.62 (0.12)	0.31 (0.06) ⁺⁺	0.76 (0.04)	0.40 (0.06) ⁺⁺
	TJ2966	0.60 (0.05) ⁺⁺	0.63 (0.11)	0.58 (0.13)	0.69 (0.09)	0.66 (0.10)	0.59 (0.12) ⁺⁺	0.74 (0.10)
	TJ2969	0.61 (0.07) ⁺	0.76 (0.08)	0.63 (0.09)	0.64 (0.08)	0.67 (0.09)	0.61 (0.11) ⁺	0.65 (0.09)
	TJ2975	0.58 (0.08) ⁺⁺	0.64 (0.11)	0.62 (0.13)	0.66 (0.11)	0.47 (0.15) ⁺⁺	0.61 (0.14) ⁺⁺	0.66 (0.11)
	P14_04A	0.52 (0.09) ⁺⁺	0.56 (0.11) ⁺⁺	0.67 (0.06)	0.69 (0.06)	0.32 (0.11) ⁺⁺	0.71 (0.07)	0.42 (0.11) ⁺⁺

Notes: Average similarity index values (SD) of 25 C-calls randomly selected from 20 recordings belonging to different chicks. Bold data represent reference values calculated using two subsequent recordings of the same chick (S-INTRA indexes), and all other values are calculated on recordings belonging to different chicks (S-INTER indexes). Symbols refer to results of statistical comparison between S-INTRA and S-INTER indexes calculated by means of the GS test (see "Statistical analysis" section for further details). ⁺ $q < 0.05$; ⁺⁺ $q < 0.01$ (adjusted FDR values).

followed in order to reduce the degree of pseudoreplication (Hurlbert 1984) due to repeatedly considering the same bird in different comparisons.

Vocal individuality was also tested by carrying out a multivariate analysis on the same data-set (25 calls from 19 chicks) used for the calculation of the S-INTER indexes, i.e. 25 calls from each of the 13 A-GROUP chicks plus 25 calls derived from one of the two recordings (randomly chosen) of each chick from the W-GROUP. First and third frequency quartiles, peak frequency (frequency with the maximal energy) and duration were measured from each call using the *Seewave* functions *specprop* (for quartiles), *fpeaks* (for peak frequency) and *timer* (for call duration; amplitude threshold = 1).

Playback experiments

Playback experiments were carried out in 2010. Thirty-seven chicks belonging to 26 different pairs were captured (age = 12–26 days) and tagged with PIP3 single-celled leg mounting tags (Biotrack Ltd, Wareham, Dorset, UK; weight = 2.5 g \leq 3% of bird body mass). According to visual observations collected before and after the first capture, all chicks from each breeding pair were captured and tagged. After the ringing procedure, chicks were recorded using the techniques detailed in the previous paragraph. These recordings were then analysed and edited. We selected a 2-min audio sample with low background noise from each chick. The relative amplitude values (16-bits = track saturation) of these samples were digitally manipulated with slight increases or decreases to obtain samples with nearly equal maximal values, while variability of amplitude within samples was not changed. The call rate was left unchanged, as it could be an individually distinctive feature. Each 2-min sample was digitally filtered (high-pass filter at 1 kHz), and pauses between calls silenced by means of Audacity software to erase all background noises. From the 2-min samples, we prepared the following 15-min playback sequence, which was the same in all experiments:

Silence (4 min)	Calls (2 min)	Silence (2 min)	Calls (2 min)	Silence (1 min)	Calls (2 min)	Calls (2 min)
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Seventeen recordings from chicks captured in 2009, which were not offspring of tested adults, were similarly processed and used as foreign playback stimuli. For each experiment, a different foreign stimulus was randomly selected to avoid pseudoreplication (Kroodsma 1989; McGregor 2000). The order of presentation of offspring or foreign signals was systematically changed between tests. In case of sibling pairs, the call sequence of only one of the two chicks was randomly selected for playback.

Playback experiments were carried out on average 4.2 days (range = 2–8 days) after initial capture. Chicks were recaptured in the early morning (within 1 h after dawn) or in the late afternoon (within 2 h before dusk), put into cloth bags and then displaced > 200 m under the care of one researcher to avoid predation. The broadcast chain, consisting of (1) Sandisk Sansa m240 digital player, (2) Sonic Impact T-Amp integrated amplifier and (3) Ciare CT 263 loudspeakers (frequency range: 250 Hz to 25 kHz), was placed 20–40 m from the capture site and hidden under a relatively big patch of riverine bushes, as chicks often use this kind of vegetation as squatting place in response to any approaching danger (Cramp and Simmons 1983; D. Giunchi et al., personal observations). Signals were played back at 44.1 kHz sampling rate, with a 16-bit accuracy and at 80 dB Spl (sound pressure level, reference pressure 2×10^{-5} Pa), measured 1 m from the loudspeaker, with a AVM2050 analogue sound level meter (fast response, A-weighting). A videocamera (Sony DCR-SR55) and a recording apparatus (Sennheiser M67 shotgun microphone connected

to Fostex FR2-LE digital recorder) were put in front of the speaker at a distance of > 10 m. The speaker was placed in dense vegetation so as to prevent parents from approaching within 5 m from behind. Distance thresholds (5 and 1 m from the speaker) were indicated by means of stones or twigs used as markers. When possible, birds were also observed by means of a telescope (20–60 \times) from a hide located > 400 m from the speaker. In each experiment, the researcher started the videocamera and broadcast of playback sequence, walked away at least 150–200 m and hid in riparian vegetation. After 15 min, the same researcher returned to the speaker, started the second playback sequence and then returned to the same hiding place. Broadcasts of the two sequences were thus separated by about 10 min. At the end of the experiments, the apparatus was removed and chicks were returned to the parents' territory in the same location from where they were captured. On the whole, breeding pairs were disturbed for < 1 h.

Our initial sample size was 26 pairs, but results from one pair were discarded because the chick did not call at all during the first recording, and three more were discarded because chicks were predated before we could conduct the experiments. We carried out a total of 17 experiments, but two tests were discarded because of technical problems. Thus, 15 tests were included in the analyses, 8 in which parents were tested first with their own offspring signal and 7 with the signal of a foreign chick. As control, we carried out five blank tests using the same procedure, but without any broadcast during both the first and the second 15-min sequences.

Radio tags were removed on the day of the experiment, except for the 14 chicks recorded twice ("Chick call repertoire and vocal individuality"), which all survived the experiments and had tags removed the day of the second recording, on average 9.4 days after the first capture date (range 9–12 days).

Parental response was analysed from recorded videos by a researcher blind to the experimental protocol (i.e. order of playback sequences, experimental vs. control tests). Responses were recorded from the start of each sequence to its end 15 min later. Birds were considered as responding when they moved at least once towards the speaker to a distance of < 5 m. Given the secretive behaviour of the species (Vaughan and Vaughan-Jennings 2005) and its sensitivity to disturbance (Taylor et al. 2007), it was not possible to follow the birds from the start of playback, as parents hide within vegetation when disturbed. For this reason, we measured only the latency of first response (i.e. when birds moved for the first time towards the speaker to a distance of < 5 m) and the distance of nearest approach to the speaker (< 5 or < 1 m). The study was approved by the Animal Care Review Committee of the University of Pisa and complied with current Italian laws on animal welfare.

Statistical analysis

Pair-wise comparisons between S-INTRA and S-INTER indexes were carried out using the General Symmetry (GS) permutation test in the R-package *coin* 1.8-18 (Hothorn et al. 2006, 2008) which tests the symmetry of a response for repeated measurements in a complete block design. We considered the 25-call pairs used to calculate similarity indexes within and between chicks as blocks. All tests were one tailed under the hypothesis that S-INTRA indexes should be higher than S-INTER indexes. *p*-Values were calculated using 10,000 Monte-Carlo resample approximations. This approach produced one series of 19 tests (13 from the A-GROUP plus 6 from the W-GROUP – see Table 1) for each of the 7 W-GROUP chicks. To control for the family-wise error rate, *p*-values in each series were corrected by means of the False Discovery Rate (FDR; Benjamini and Hochberg 1995; Pike 2011), which controls the expected proportion of false discoveries among rejected hypotheses.

Multivariate analyses on the four parameters derived from chick calls (first and third frequency quartiles, peak frequency and duration) were carried out using the R-package *rrcov* 1.3-01 (Todorov and Filzmoser 2009). The comparison between inter- and intra-individual variability was done by means of the Wilks' Lambda Statistic based on the minimum covariance determinant (MCD) estimator, which is robust to the influence of outliers (Todorov and Filzmoser 2010). Robust linear discriminant analyses based on MCD were also carried out (Todorov and Filzmoser 2009), and the overall probability of misclassification was estimated by means of the leave-one-out cross-validation method.

Proportions were compared by means of χ^2 -tests with *p*-value calculated by Monte-Carlo simulations of 10,000 replicates. Significance of behavioural changes obtained from the two experimental signals (offspring and foreign) was examined using the exact version of McNemar's test (Sokal and Rohlf 1995; Fay 2010). Latencies to first approach to the stimulus were compared by means of the GS test using adult pairs as blocks.

Results

Chick call repertoire

Spectrographic analysis revealed that Stone Curlew chicks have two main call types: (1) the S-call, a brief strangled soft sound with a broadband spectrogram often uttered when parents and chicks were at close quarter (Figure 1); and (2) the C-call, a chirping sound with a complex structure, characterized by a modulated tonal-harmonic part and non-tonal components (Figure 2). Samples of both call types are given in the supplementary tab of the article's online page at <http://dx.doi.org/10.1080/09524622.2012.710394>. C-calls can be

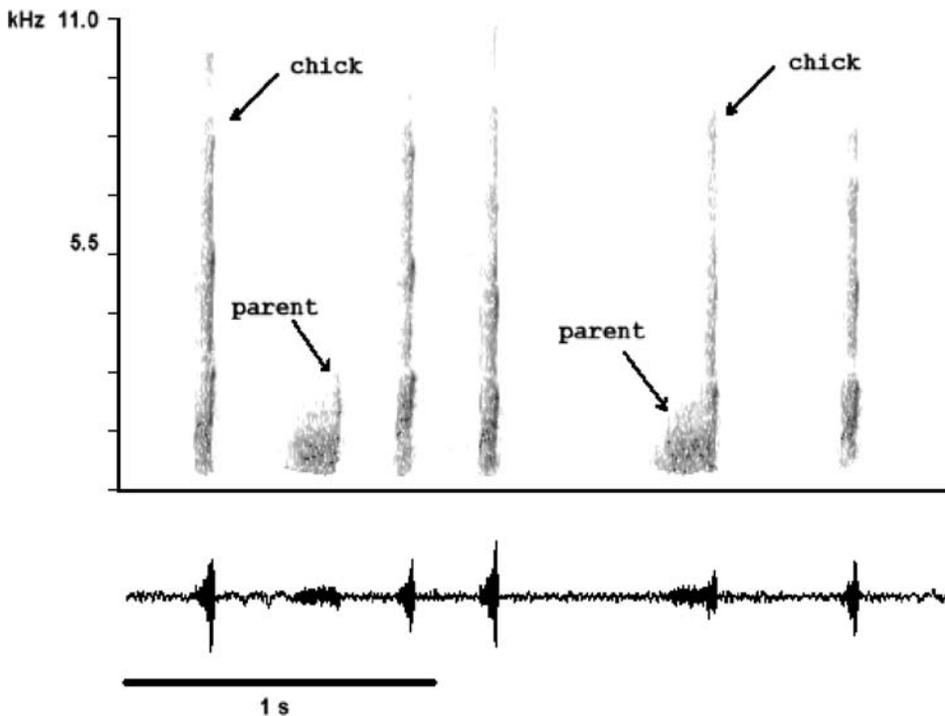


Figure 1. Chick call repertoire. Spectrogram (above) and oscillogram (below) of S-call, a brief strangled soft sound uttered by chicks near their parents and which was answered by parents.

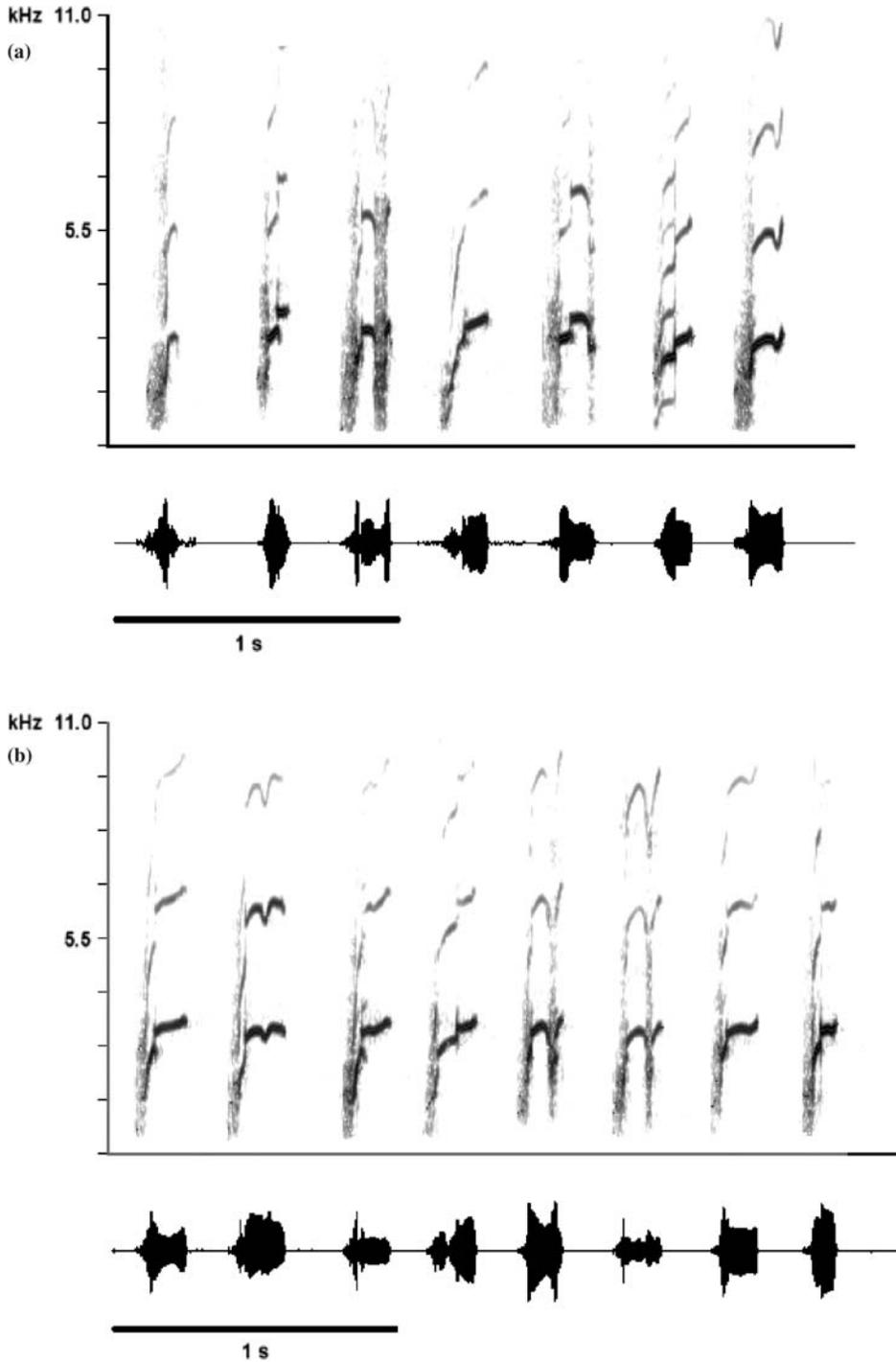


Figure 2. Chick call repertoire. Spectrogram (above) and oscillogram (below) of C-call, a chirping call characterized by a complex structure with modulated tonal-harmonic part and non-tonal components. (a) Seven samples from different chicks; note the very high inter-individual variability of these vocalizations. (b) Eight samples from the same chick; note the high intra-individual variability.

selectively stimulated by adult vocalizations, as indicated by the analysis of 24 audio tracks in which at least one call of distant adult(s) (< 100 m) was recorded in the background (Figure 3). Indeed, 1 min after a “kurlee” call (see Vaughan and Vaughan-Jennings 2005 for an account of adult call repertoire), the number of C-calls increased significantly with respect to the 1 min before ($Z = -3.04, p \ll 0.001, N = 14$, GS test). This is not true when considering all the other types of adult vocalization ($Z = 0.63, p = 1, N = 10$, GS test). Spectrograms in Figure 2(a) show a high variability of the C-call structure between different individuals, but we found variability within individuals too (Figure 2(b)). Mean power spectrum of all chicks, calculated over 25 randomly selected calls for each chick, shows that the third quartile is at 3.1 kHz, the peak frequency is at 2.7 kHz, >95% of the total power is below 8 kHz (Figure 4) and the average frequency range of the two first harmonics is from about 2 kHz to about 6.5 kHz (Figures 2 and 4).

Vocal individuality of chick calls

Table 1 gives the values of all S-INTRA and S-INTER indexes, showing the great variability in these data. In 68% of cases, the S-INTER index is lower than the S-INTRA index, as would be expected if vocal individuality exists, but in 32% of cases the situation is reversed.

On average 44% of the S-INTER indexes between chicks are significantly lower than the corresponding S-INTRA values (GS test, $q < 0.05$, adjusted FDR value, see Table 2). These

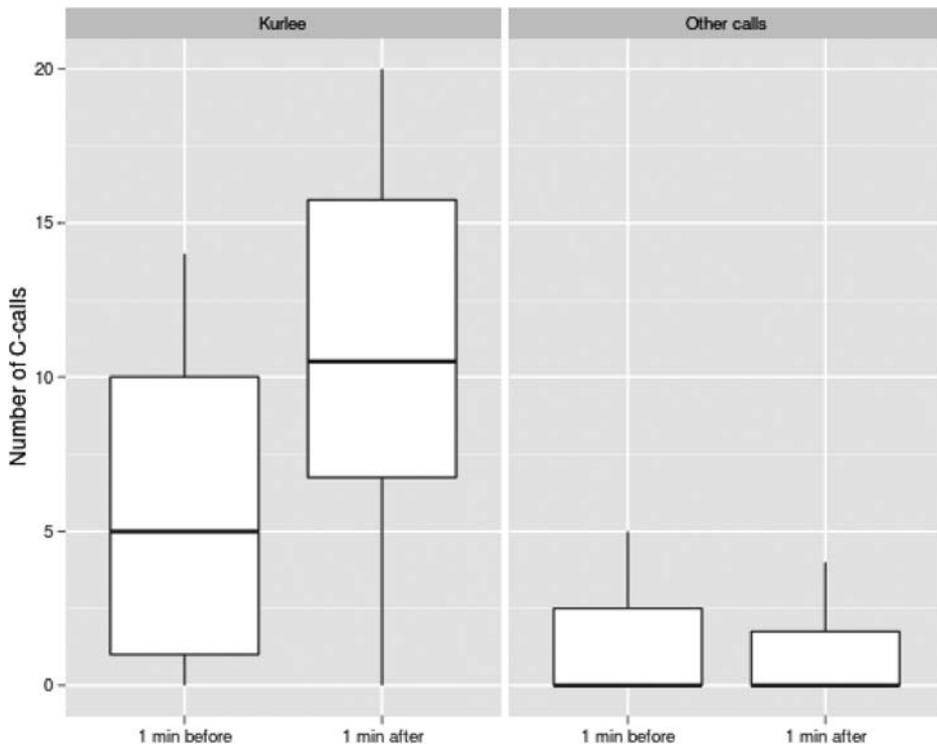


Figure 3. C-calls are stimulated by adult “kurlee” call. Boxplot of the number of C-calls 1 min before and 1 min after a distant (<100 m) adult uttered a “kurlee” ($N = 14$) or another call type ($N = 10$). “Hinges”, first and third quartiles; horizontal line, median; “whiskers”, ± 1.5 times the interquartile range.

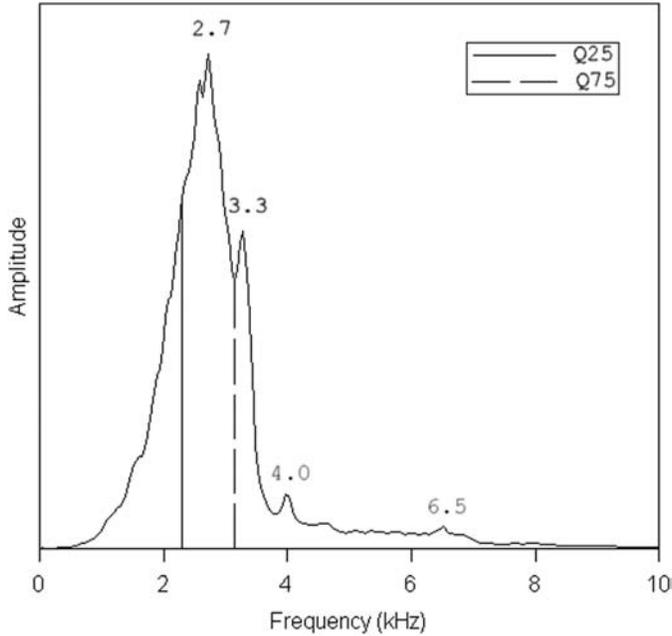


Figure 4. Power spectrum of C-call. Mean power spectrum of 25 C-calls randomly selected among all chicks ($N = 66$); y-axis represents relative amplitude of the frequency distribution; Q25, first quartile of the power distribution; Q75, third quartile; numbers represent peak values in kHz.

results indicate that according to this acoustic analysis, we can successfully recognize individual chicks in < 50% of cases (range in successful recognition from < 6% to > 80%, depending on the reference chick; see Table 2). It is interesting to note that even with chicks with a very high S-INTRA index (see for example chick TJ2961, Table 1), it is possible to find cases among the S-INTER index which are equal to or even greater than S-INTRA index.

The results of the robust one-way MANOVA calculated on the four parameters derived from chick calls indicate that the inter-individual variability was significantly higher than the intra-individual variability (Wilks' $\lambda = 0.045$, $\chi^2 = 1124.6$, $df = 68.4$, $p \ll 0.001$). However, the discriminating power of these parameters, estimated by means of the robust linear discriminant analysis, turned out to be very low (global misclassification

Table 2. Summary of number and percentage of statistically significant comparisons (GS test, $q < 0.05$, adjusted FDR values) showing vocal similarity to be lower between different chicks (as expected if vocal individuality exists) than within chick.

Chick tags	Number	Percentage
TH0375	16	84
TH0384	7	37
TH0400	1	5
TJ2951	3	16
TJ2959	7	37
TJ2961	15	79
TJ2970	10	53
Mean	8	44

probability = 0.63) and rather variable between chicks (range of misclassification probability at individual level = 0.20–0.96).

Playback experiments

In 10 out of 15 playback experiments, we recorded at least one positive response (i.e. one adult bird approached within 5 m of the speaker), whereas results from 5 control experiments without playback emission were all negative ($\chi^2 = 6.67$, $df = 1$, $p = 0.03$). Chick calls do stimulate the approach of parents.

Table 3 shows the results of the 15 playback experiments, each with one foreign and one own offspring stimulus in random sequence. In all but one successful trial, only one parent responded to the playback. Both type of stimuli and order of presentation did not have a significant effect on the number of positive responses (exact McNemar's test, $p = 0.68$ and $p = 0.69$, respectively). Latency to first approach recorded in the 10 tests with at least one positive response is shown in Figure 5. There is no statistically significant difference between own offspring and foreign chick playback ($Z = -1.07$, $p = 0.28$, GS test) nor between first and second playback calls ($Z = -0.22$, $p = 0.83$, GS test). We did not record any obvious behavioural differences by parents approaching the two types of

Table 3. Responses to playback stimulation.

Experiment	Tested birds	Responding birds
Answer to own chick	15	6
Answer to foreign chick	15	8
Answer to first test	15	8
Answer to second test	15	6

Note: Responding birds = adult(s) approaching within 5 m of speaker.

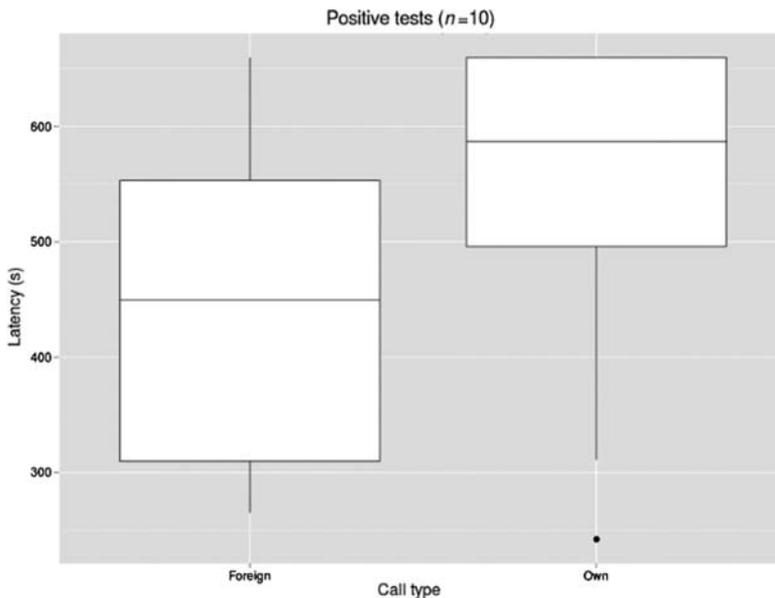


Figure 5. Latency of first response to playback. Boxplots of latency to first approach recorded in the 10 tests with at least one positive response. See Figure 3 for other details.

stimuli. The minimum approach distance to the speaker was always < 1 m, except in two cases (one towards the foreign chick stimulus and one towards their own offspring). Adults approached the speaker repeatedly and searched nearby, often uttering soft calls as is typical of vocal behaviour of parents getting close to their offspring (see Figure 1).

Discussion

We found at least two main vocalizations in the chick call repertoire of the Stone Curlew. In our experimental conditions, the S-call is often uttered in proximity to adults, and this finding is confirmed by some field observations of unrestrained chicks still receiving care from their parents (M. Dragonetti and D. Giunchi, unpublished data). Instead, we have used the C-call to investigate the vocal individuality of chicks and the parent–offspring recognition for the following reasons. This call is stimulated by distant adult calling and therefore seems to function as a long-distance contact vocalization. Spectrograms show a modulated harmonic structure which could be a good requisite for auditory discrimination, because birds are quite sensitive to changes in frequency of acoustic signals and they have an enhanced capacity in resolving the temporal fine structure of complex sounds, enabling discrimination of subtle differences in vocalizations (Marler and Slabbekoorn 2004). Furthermore, power spectrum analyses revealed that bandwidth of the first two harmonics of C-call is about 4 kHz; signal bandwidth exceeding 3 kHz has been shown to be critical in allowing discrimination and localization of sound sources in owls (Saber et al. 1999).

Vocal individuality was studied by comparing the acoustic structure of the C-call within and between chicks. Results showed a higher similarity within chicks than between chicks, allowing correct individual recognition in 44% of cases. However, C-calls show marked intra-individual variability too, which often prevented individual distinction between chicks by acoustic features alone (see Table 2, chicks TH0400 and TJ2951). Although acoustic analyses show that chick call structure is individually distinct, nevertheless it does not seem to be a reliable cue for identification, as both confirmed by cross-correlation and multivariate analysis of the four considered acoustic parameters.

Playback experiments suggest that C-calls of Stone Curlew chicks stimulate an approach by adults, but parent Stone Curlews respond to playback stimulus in the same way to calls of both their own offspring and stranger chicks: all parameters considered showed no statistical difference between the two groups. Since adult birds responded to both playback stimuli, a question arises about adult response to stranger calls. Might it be an aggressive response against an intrusion? There are several reasons to answer this negatively: we examined carefully all the videotapes of the experiments and no aggressive display (see Cramp and Simmons 1983) was ever observed; the behaviour of approaching adults was exactly the same in all experiments. Actually, in the Florida Scrub Jay, Barg and Mumme (1994) found an aggressive response towards unfamiliar young call with parameters clearly different from those recorded for their own offspring. Stone Curlew pairs that own a territory usually respond with an aggressive approach (e.g. “high-upright” posture: Cramp and Simmons 1983), to the vocalizations and/or the presence of an adult intruder (Cramp and Simmons 1983; M. Dragonetti and D. Giunchi, personal observations), but this has not been reported towards foreign chicks. In our playback experiments, chicks were caught and removed from their nesting territories for the whole duration of the test; therefore, any stimulus for adults to search for their own offspring would certainly be very strong.

To conclude, although it is always difficult to interpret non-significant results, as birds discrimination abilities might not always lead to a difference in response to playback (see McGregor 1992, 2000, for references), our results suggest that parent Stone Curlews

are unable to recognize their offspring by voice, confirming our findings about the unreliability of individual signatures of chick calls. This does not mean that a vocal recognition mechanism is absent in this species, because many researchers have found that in some bird species, chicks are able to recognize their parents vocally and this phenomenon may be mutual (Aubin and Jouventin 2002; Mulard et al. 2008) or not (Storey et al. 1992; Charrier et al. 2001; Lengagne et al. 2001; Taylor and Perrin 2008). Therefore, it is possible that Stone Curlew chicks are able to recognize their parent's voice and that parents use a recognition system based on different behaviour of their young. This kind of mechanism has been found in Herring Gulls by Knudsen and Evans (1986). Our finding that young Stone Curlews change their vocal behaviour on hearing adult calls is consistent with this hypothesis. However, further field experiments are needed to test whether chicks are able to recognize their parents' voice and by what mechanism.

Supplementary material

Supplementary material for this article is available via the supplementary tab of the article's online page at <http://dx.doi.org/10.1080/09524622.2012.710394>.

Samples of C- and S-calls of Stone Curlews *Burhinus oedicnemus*.

C_calls_TH0392.wav: C-calls.

S_calls_TH0387.wav: S-calls.

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