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1 **Title:** The function of three main call types in common cuckoo

2

3 **Short running title:** Function of cuckoo calls

4

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37 Xia, and Xiaomeng Zhao analyzed the data. Canwei Xia, Huw Lloyd and Anders Pape
38 Møller wrote the manuscript.

39

40 **Conflict of Interest Statement:** The authors declared that they have no conflict of
41 interest for this work.

42

43 **Ethical standards:** Our research protocol was approved by the Animal Management
44 Committee at the College of Life Sciences, Beijing Normal University under license
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46 National Bird-banding Center of China under license number H20110042. The
47 experiments comply with the current laws of the country in which they were
48 performed.

49

50 **Abstract:** Acoustic signals play a key role in shaping the relationships in birds.
51 Common cuckoos *Cuculus canorus* are known to produce various call types, but the
52 function of these calls has only been studied recently. Here, we used a combination of
53 field recordings (conducted in 2017) and playback experiments (conducted in 2018)
54 to investigate the functional significance of common cuckoo calls. We found
55 significant differences in the characteristics between male two-element ‘*cu-coo*’ and
56 three-element ‘*cu-cu-coo*’ calls, with these two call types being used in different
57 contexts. The three-element male ‘*cu-cu-coo*’ calls were associated with females
58 emitting their ‘bubbling’ call. Playback experiments revealed that both males and

59 females exhibit stronger responses to playing female “bubbling” calls than with the
60 calls of Eurasian sparrowhawk (*Accipter nisus*) serving as a control suggesting a
61 significant intraspecific communication function for this call type. However, we did
62 not find any evidence to support mate attraction in male calls, as females were not
63 stimulated by playback of male calls compared to sparrowhawk calls in the control
64 group.

65

66 **Key words:** acoustic signals; China; common cuckoo; female vocalization; playback;
67 sparrowhawk.

68

69 **Introduction**

70 Relationships among individuals are adjusted by the transmission of signals
71 (Boughman, 2002; Hurd & Enquist, 2005; Partan, 2013). There are several channels
72 for signal transmission, such as acoustic, visual, chemical and tactile (Partan, 2013;
73 Rubi & Stephens, 2016). Among these channels, acoustic signals can take effect in
74 darkness, penetrate through objects, and can be transmitted over long distances
75 (Lemon, 1977; Slabbekoorn & Smith, 2002). Therefore, not surprisingly, acoustic
76 signals play a key role in adjusting relationships in birds (Todt & Naguib, 2000;
77 Slater, 2003). Passeriformes adopt versatile vocations to attract a partner and/or deter
78 rivals (Catchpole & Slater, 2008). In non-Passeriformes, vocalizations are often
79 simple and stereotyped, but messages can also be encoded in different call types. For
80 examples, adult African penguin (*Spheniscus demersus*) employ four vocal categories
81 for use in different circumstances (Favaro et al., 2014); male corncrakes (*Crex crex*)
82 are known to express low or high levels of aggressive motivation in broadcast calls
83 and soft calls, respectively (Rek & Osiejuk, 2011); and male ural owls (*Strix*
84 *uralensis*) use different call types for territorial advertisement and for duetting with
85 female (Lundberg, 1980).

86

87 For cuckoos, a well-studied brood parasitic avian species, the temporal and frequency
88 variables of male ‘cu-coo’ calls (Fig. 1a) are well-known (Lei et al., 2005). This call
89 type is sufficient to provide individual information (Jung et al., 2014; Li et al., 2017;
90 Zsebok et al., 2017), which can be used to distinguish between neighbors and
91 strangers (Moskát et al., 2017, 2018): male cuckoos show less aggressive response to
92 familiar ‘*cu-coo*’ calls from neighbors than unfamiliar ‘*cu-coo*’ calls from strangers
93 during playback experiments. Besides the characteristic and conspicuous two-element
94 ‘*cu-coo*’ calls, males can also utter a three-element ‘*cu-cu-coo*’ call (Fig. 1b) which
95 contains a repeat of the first element of the regular ‘*cu-coo*’ call (Lei et al., 2005;
96 Payne, 2005). Based on our experience and previous research (e.g. Lei et al., 2005),
97 ‘*cu-coo*’ calls can be heard very often at our study site, but ‘*cu-cu-coo*’ calls are much
98 rarer. The exact function of this three-element call is unknown but is thought to be
99 associated with female activity (Payne, 2005; Erritzøe et al., 2012; Tryjanowski et al.,
100 2018), however empirical evidence for this is scant. Different cuckoo calls have also
101 been a common component of folklore (Møller et al., 2017), suggesting that not only
102 cuckoos, but also humans respond to differences in cuckoo calls.

103

104 Female cuckoos give a conspicuous ‘bubbling’ (or ‘chuckle’) call (Fig. 1c) (Lei et
105 al., 2005; Payne, 2005). One recent study suggested that these ‘bubbling’ calls mimic
106 those of the Eurasian sparrowhawk (*Accipiter nisus*), serving primarily as a distraction
107 of host species after laying (York & Davies, 2017). However, a more recent study has
108 suggested that female cuckoo calls may have other functions besides distraction of
109 nest hosts (Deng et al., 2019), as female cuckoos predominantly lay their eggs in the
110 afternoon (Payne, 2005; Erritzøe et al., 2012), but vocal activity of female ‘bubbling’
111 calls peak in the morning, with nearly no call output in the afternoon (Deng et al.,
112 2019). Besides, Benedetti et al. (2018) found the number of syllables in male cuckoos
113 call was negatively correlated with the presence of female ‘bubbling’ calls, which
114 implies intraspecific communication function of the female ‘bubbling’ calls.

115

116 In this study, we investigate the function of three cuckoo call types using a series
117 of playback experiments and new field recordings of both male and female calls. Our
118 first aim was to quantify the features of the three-element male ‘*cu-cu-coo*’ call, and
119 to determine whether this call was associated with female activity. The second aim
120 was to investigate the intersexual function of the male two-element ‘*cu-coo*’ calls
121 using playback of male calls to female cuckoos. The function of the male ‘*cu-coo*’ call
122 is assumed to deter territorial rivals and attract mates (Payne, 2005). Deterring
123 territorial rivals has been repeatedly demonstrated in previous cuckoo studies (Moskát
124 et al., 2017, 2018; Tryjanowski et al., 2018). However, empirical field tests
125 concerning the function of attracting females is still in its infancy. We predicted that
126 females were attracted by male calls. Our third and final aim was to determine the
127 intraspecific communication function of the female ‘bubbling’ calls using playback
128 trials of this call type to both male and female cuckoos. We predicted that if there is
129 an intraspecific effect in this call type, then we would detect a significant behavioral
130 changes after playback of this call type.

131

132 **Methods**

133 Study area

134 Fieldwork was conducted during the breeding season (May to July) in 2017 and 2018
135 at the Liaohe Delta Nature Reserve (41.034°N; 121.725°E), Liaoning Province,
136 Northeast China. This region has a semiarid continental monsoon climate with rainfall
137 usually occurring from July to September, and it represents one of the most important
138 estuarine wetlands, with the largest area of reed-bed habitat (about 800 km²) along the
139 coastal region of China. Due to oil fields in the wetlands, energy projects are being
140 constructed. The oil field infrastructures have resulted in installation of electrical
141 wires, which provide perch sites for the cuckoos. Here, the common cuckoo is a
142 summer breeding species, and it predominantly parasitizes Oriental reed warbler

143 (*Acrocephalus orientalis*) nests (Li et al., 2016). The breeding season for the common
144 cuckoo lasts from May to July in this area, and typically the first individual cuckoos
145 are seen around the middle May, and the last Oriental reed warbler nests found around
146 late July each year (Li et al., 2016).

147

148 Sound recording

149 We used a recorder (DR-100MKIII, Tascam Co., Japan) and an external directional
150 microphone (MKH416 P48, Sennheiser Co., Germany), with a sampling rate of 44.1
151 kHz and a sampling accuracy of 16 bits, to record cuckoo vocalizations. Further
152 recordings were made using seven passive acoustic recorders (SM4, Wildlife
153 Acoustics Inc., USA) placed at seven different locations, separated by a minimal
154 distance of 200 m, to continuously record cuckoo calls from June 8th to July 28th
155 2017. Recorders were attached to telegraph poles at a height of 3 m above ground,
156 and set to record continuously at a sampling rate of 44.1 kHz, and a sampling
157 accuracy of 16 bits. Recorders were checked every 10 days to replace the batteries
158 and memory cards. In addition, we used mist nets to trap and band 20 individual adult
159 common cuckoos (6 females and 14 males) around our recording sites, whilst daily
160 observations also revealed the presence of many other unbanded individual cuckoos at
161 these same locations during data collection. These 20 individuals were banded with
162 metal rings and backpack radio transmitters (weight 2.12g, Biotrack, UK).

163

164 Sound measurements

165 All recordings were re-sampled with 6 kHz, and saved as .wav files. We used Avisoft-
166 SASLab Pro 5.2 audio analysis software (Avisoft Bioacoustics, Germany) to generate
167 spectrograms with the following settings: fast Fourier transform length 256 points;
168 Hamming window with a frame size of 100% and an overlap of 50%; frequency
169 resolution 23 Hz; and time resolution of 21 ms. By checking the spectrograms, we
170 found a total of 24 three-element 'cu-cu-coo' calls recorded from 11 banded males: 3

171 'cu-cu-coo' calls were recorded in 3 males, 2 'cu-cu-coo' calls were recorded in 7
172 males, and one 'cu-cu-coo' call was recorded in one male. We measured four
173 variables for each 'cu-cu-coo' call: duration, minimum frequency, maximum
174 frequency, and peak frequency (frequency of the maximum amplitude). We also
175 measured 24 'cu-coo' calls from the same 11 banded males, all of which were
176 recorded immediately before or after the 'cu-cu-coo' calls. For these calls, we
177 measured the same four variables: duration, minimum frequency, maximum
178 frequency, and peak frequency.

179

180 In order to determine whether male 'cu-cu-coo' calls are associated with female
181 activity (emitting 'bubbling' call), we compare the number of two-element 'cu-coo'
182 and three-element 'cu-cu-coo' calls 30 s before or after female 'bubbling' calls.
183 Firstly, we used Kaleidoscope Pro software (Wildlife Acoustics Inc., USA) to create
184 recognizers for identifying all female 'bubbling' calls from recordings collected with
185 seven passive acoustic recorders. We entered the following acoustic features to
186 Kaleidoscope Pro Software: frequency range from 600 to 2900 Hz; duration ranges
187 from 1.6 to 4 s. These acoustic features are slightly larger than actual parameters of
188 female 'bubbling' calls, but this was done simply to increase the detectability of these
189 calls by the Kaleidoscope Pro Software. Then, we manually checked all calls
190 identified by the recognizer based on visual inspection of the spectrograms, resulting
191 in 2,407 female 'bubbling' calls. Next, we selected all female 'bubbling' call bouts
192 which were separated by a minimum interval of 30 mins with other female call bouts,
193 to ensure male calls are affected by the target 'bubbling' call, rather than adjacent
194 'bubbling' call, resulting in 144 'bubbling' calls for subsequent analyses. Lastly, we
195 manually counted the number of 'cu-coo' and 'cu-cu-coo' calls 30 s before or after
196 female 'bubbling' calls from spectrograms. We were unable to automatically detect
197 male calls using Kaleidoscope Pro Software due to the difficulty in distinguishing
198 between the three-element and two-element calls which may be attributed to the

199 shared ‘*cu*’ and ‘*coo*’ elements in both types of calls.
200
201 Playback experiments
202 Playback experiments were conducted in two periods defined here as the early
203 breeding season (28th May to 8th June) and late breeding season (5th to 28th July) in
204 2018. We used 12 sound files belonging to 4 call types (i.e.3 sound files in each call
205 type): two-element male ‘*cu-coo*’ calls (Fig. 1a), three-element male ‘*cu-cu-coo*’ calls
206 (Fig. 1b), female ‘*bubbling*’ calls (Fig. 1c), and Eurasian sparrowhawk calls (Fig. 1d)
207 to act as a control. The Eurasian sparrowhawk is an uncommon predator of small
208 songbirds in the study area, but there are no reports of it being a threat to adult
209 common cuckoos. Each sound file lasted 2 min. The basic elements of a sound file
210 contained some repeated bouts from the same individual, composing a 30 s unit,
211 followed by a 15 s break. This set was repeated, and then the whole 30 s sound unit
212 was added to complete the playback sound file. The duration of different bouts varied
213 so we also varied the number of bouts (from 3 to 9 bouts) in each 30 s unit of the
214 different sound files, whilst ensuring that the proportion of total calls duration in 30 s
215 units were uniform in all sound files. Subsequently each sound file was edited using
216 Goldwave 5.25 (GoldWave Inc., Canada). To avoid pseudoreplication, we used calls
217 from different individuals to create different sound files. Cuckoos calls were recorded
218 during the 2017 breeding season in the study area, and sparrowhawk calls were
219 downloaded from Xeno-Canto (<http://www.xeno-canto.org>, ID XC107015, ID
220 XC120729, ID XC143459, recorded in Kyrgyzstan and Poland), a non-profit website
221 set up to share recordings of sounds of wild birds.

222

223 For playbacks, we used a smartphone player (Honor 8; Huawei Technologies Co.,
224 China) connected to a loudspeaker (SME-AFS; Saul Mineroff Electronics Inc., USA),
225 with the playback amplitude set to normal call amplitude of cuckoos, approximately
226 95 dB SPL measured at 1 m with a sound level meter (NL-20; Rion Co., Japan). All

227 trials were conducted between 0700 and 1900 h, after the end of the dawn chorus and
228 before the peak of the dusk chorus. Playback experiments were started in the close
229 vicinity of cuckoos observed. The loudspeaker was positioned about 20 m from the
230 observers, about 68 ± 10 m (mean \pm SD, range 39 to 95 m) from the target cuckoo,
231 which always perched on electrical wires. As most target cuckoos for our trials were
232 not banded, we conducted playback experiments targeting individuals separated by at
233 least 1 km to try our best to avoid repeated sampling from the same individual in each
234 period (either early breeding season or late breeding season). Each target cuckoo was
235 only used once in each period, played with one randomly selected sound file.

236

237 Following the method used by Moskát et al. (2017, 2018), we measured the
238 following four variables during the playback experiments: starting distance (distance
239 between the focal cuckoo and the loudspeaker at the beginning of playback); closest
240 distance (the distance of closest approach to the loudspeaker during 2 min playback);
241 movement latency (when the focal cuckoo moved from its original position at the start
242 of the playback); and sound latency (when the focal cuckoo started calling). Starting
243 distance and closest distance were measured with a range finder (ELITE 1500;
244 Bushnell Corp. USA). Movement latency and sound latency were measured with a
245 stopwatch (Tianfu PC396; Shenzhen Huibo Industry & Trade Co., China). If a target
246 individual cuckoo did not move or call during 2 minutes of playback, movement
247 latency or sound latency was recorded as 120 s. If an individual cuckoo did not call
248 during playback, we continued observations until it called, and recorded the sex based
249 on the call type (males utter ‘*cu-coo*’ or ‘*cu-cu-coo*’ calls, while females utter
250 ‘bubbling’ calls). We discarded 6 playback trials from subsequent analyses due to
251 fighting between cuckoos (3 occasions), or because the target individual was chased
252 by Oriental reed warblers (2 occasions), or they flew away in response to passing
253 vehicles (1 occasion) during the playback trail. Overall, we successfully conducted
254 playback experiments to 74 individuals during early breeding season, and 58

255 individuals in late breeding season. Cuckoos calls used in the playback experiments
256 were collected in the same area occupied by the focal individual cuckoos, so there is a
257 possibility that these calls were from neighboring individuals to the focal cuckoos. In
258 addition, focal cuckoos may show specific response to these familiar calls, however,
259 the large sample sizes obtained in this study can minimize the chance of such cases.

260

—

261 Data analyses

262 We measured four variables: duration, minimum frequency, maximum frequency, and
263 peak frequency for both the two-element '*cu-coo*' call and three-element '*cu-cu-coo*'
264 calls. When comparing these call variables, four linear mixed models were used. In
265 each model, one of the call variables was the response variable, with male call type
266 ('*cu-coo*' or '*cu-cu-coo*') as the fixed effect and bird ID as the random effect. For
267 comparing the number of two-element and three-element male calls 30 s before or
268 after a female call, we used the number of '*cu-coo*' and '*cu-cu-coo*' calls as the
269 response variable in each model, with period (30 s before or after female 'bubbling'
270 calls) as the fixed effect, and recorder ID as the random effect.

271

272 Following the method used by Moskát et al. (2017, 2018), we used distance
273 difference (starting distance minus closest distance), movement latency, and sound
274 latency to reflect the degree of excitement during playback. Since these three
275 variables are highly correlated (KMO and Bartlett's test, KMO value = 0.71, $P <$
276 0.001), we applied principal component analysis and used the first principal
277 component with eigenvalue = 2.25 to reflect the original variables. Playback
278 experiment data were then divided into four data sets based on the sex of target
279 cuckoos and period: males in early breeding season, males in late breeding season,
280 female in early breeding season, female in late breeding season. We used four linear
281 mixed models for these four data sets, with the first principal component entered as
282 the response variable, sound type used in playback, date of the playback experiment

283 and time as fixed effects (due to the fact that bird activity may be influenced by
284 breeding condition and daily activity), and the ID of the sound file as the random
285 effect.

286

287 Considering that censored data were used for measuring behavior during
288 playback experiments, e.g. a target individual cuckoo did not move or call during 2
289 min playback, movement latency or sound latency was recorded as 120 s. We also
290 employed Mann-Whitney test to analyze playback experiment data: comparing the
291 responses among different groups. Mann-Whitney test based on ranks rather than
292 original values is generally less sensitive to censored data. The results are quite
293 similar to the results in the linear mixed models described above, and presented in
294 Appendix 1.

295

296 All analyses were performed using R software (R Core Development Team,
297 2018), with the linear mixed model conducted in package *lme4* (Douglas et al., 2015).
298 Data are presented as mean \pm SD and *P* values less than 0.05 were considered
299 statistically significant.

300

301 **Results**

302 Our recordings revealed that the structure of the two-element ‘*cu-coo*’ and three-
303 element ‘*cu-cu-coo*’ calls were similar (Fig. 1a and b). However, three-element calls
304 were of significantly longer duration and had significantly higher minimum,
305 maximum and peak frequency (Table 1). Output from the linear mixed models
306 revealed that the two call types were used in different contexts. There were
307 significantly less ($t_{280} = 2.03$, $P = 0.044$) two-element male ‘*cu-coo*’ calls emitted after
308 (3.68 ± 4.93) than before (5.10 ± 6.81) female calls, while there was a significantly
309 greater ($t_{280} = 3.96$, $P < 0.001$) number of three-element male ‘*cu-cu-coo*’ calls
310 emitted following female calls (1.12 ± 1.59) than before (0.49 ± 1.09 ; Fig. 2).

311

312 The three observed variables in playback experiments are highly correlated (KMO
313 and Bartlett's test, KMO value = 0.71, $P < 0.001$). Therefore, we applied principal
314 component analysis and used the first principal component, with eigenvalue = 2.25
315 explaining 74.9 % of the variance in the original variables, as a measure of the degree
316 of activity. This principal component was positively related to the difference in
317 distance (correlation coefficient = 0.84), and negatively related to both movement
318 latency (correlation coefficient = -0.90) and sound latency (correlation coefficient = -
319 0.86). Thus, we named the principal component 'excitement', with large values
320 indicating a high degree of activity (i.e. approach the loudspeaker, fly early, call early)
321 during playback.

322

323 Playback experiments revealed that males show higher degree of excitement in
324 response to the playback of female 'bubbling' calls than those of the control (playing
325 sparrowhawk calls) during both the early breeding season and late breeding season
326 (Fig. 3, Table 2). Males exhibited a higher degree of excitement to male two-element
327 'cu-coo' calls during playback, than toward the sparrowhawk calls but only during the
328 early breeding season (Fig. 3, Table 2). In response to playback of the three-element
329 male 'cu-cu-coo' calls, males did not show a significant difference compared to
330 playback of the sparrowhawk calls either during the early or late breeding season (Fig.
331 3, Table 2). In contrast, females exhibited a higher degree of excitement in response to
332 playback of female 'bubbling' calls than the controls during both early and late
333 breeding season (Fig. 4, Table 3). Females did not show a significant difference in
334 response to both types of male calls when compared to sparrowhawk calls in either
335 the early breeding season or late breeding season (Fig. 4, Table 3).

336

337 **Discussion**

338 Is the male 'cu-cu-coo' call associated with female activity?

339 Despite the three-element calls having previously been quantitatively described by Lei
340 et al. (2005), this call type has been omitted as abnormal calls in other studies (e.g.
341 Wei et al., 2015). Historically this three-element call type was considered to be
342 associated with female ‘bubbling’ calls (Payne, 2005; Lei et al., 2005), but there has
343 been no empirical study to support this suggestion. In this study we compared the
344 number of calls emitted 30 s before or after a female ‘bubbling’ call which we found
345 was the only call type that stimulated males during both early and late breeding
346 season amongst the call types tested. We found that the number of male three-element
347 ‘*cu-cu-coo*’ calls increased significantly following the female ‘bubbling’ call while the
348 number of two-element male ‘*cu-coo*’ calls decreased. Our findings support the idea
349 that the three-element male ‘*cu-cu-coo*’ call is associated with females emitting
350 ‘bubbling’ calls. In addition, we found that the three-element ‘*cu-cu-coo*’ call is
351 significantly longer in duration and with higher frequency, compared with the two-
352 element ‘*cu-coo*’ call. In some Passeriformes species, long and higher frequency song
353 types are associated with sexual selection (Gil et al., 2007; Cardoso, 2012; Nelson &
354 Poesel, 2012) and are more efficient in attracting a mate. It could be that male
355 common cuckoos also adopt this tactic.

356

357 Is the function of the male call to attract females?

358 In non-Passeriformes, vocalizations are often simple and stereotyped, with no song
359 behavior (Catchpole & Slater, 2008). However, some non-Passeriformes, such as
360 cuckoos (*Cuculus* spp.), utilize a song that, although stereotyped, is important during
361 the breeding season (Kroodsma & Miller, 1996). Call features always served as an
362 important basis for interspecies classification in different cuckoo species (Xia et al.,
363 2016; Kim et al., 2017). Although it is tacitly assumed that cuckoo vocalizations
364 during the breeding season serve as a means of mate attraction (King, 2005; Xia, et al.
365 2016), this crucial assumption remains untested empirically. In this study we did not
366 find any evidence that females were stimulated by playback of male calls compared to

367 the control playback of sparrowhawk calls. Interestingly, we did find an association
368 between the three-element male ‘*cu-cu-coo*’ call and female ‘bubbling’ call. However,
369 the female did not show a higher degree of excitement in response to playback of the
370 three-element ‘*cu-cu-coo*’ call, and, consequently, did not find any evidence to
371 support the suggestion that the function of the male call is to attract females.
372 However, we could not rule out a female response in some subtle ways, e.g. change in
373 posture and heart rate during playback. Even in Passeriformes, direct evidence for a
374 mate attraction function in male song is far less abundant (Kroodsma & Byers, 1991).
375 The majority of studies supporting the mate attraction function has been collated from
376 laboratory-based studies, with contradictory observations about mate attraction by
377 male song from field-based studies (Byers & Kroodsma, 2009; Soma & Garamszegi,
378 2011). For common cuckoos, whether male calls function to attract females remains
379 an open question.

380

381 The function of the female ‘bubbling’ call

382 Historically, most attention to variation in avian vocal signals has been directed
383 towards males (Beecher & Brenowitz, 2005). However, female vocalizations are also
384 widespread in birds (Garamszegi et al., 2007; Yang et al., 2011; Odom et al., 2014)
385 e.g. female song sparrows (*Melospiza melodia*) sing during territorial conflicts with
386 other females (Arcese et al., 1988), and female blue tits (*Cyanistes caeruleus*) are
387 known to sing when their nests are approached by sparrowhawks (Mahr et al., 2016).
388 One recent experimental playback study has suggested that the female cuckoo
389 bubbling call primarily serves as a distraction of the host parent species (York and
390 Davies, 2017). Such a function would enable female cuckoos to benefit from reducing
391 their egg rejection rate through distraction of the attention of hosts (York & Davies,
392 2017). Based on playback experiments in this study, clearly common cuckoos are able
393 to distinguish between female ‘bubbling’ calls and that of the sparrowhawk since both
394 male and female common cuckoos show higher degree of excitement in response to

395 playback of the female call. Despite this, the female ‘bubbling’ call may mean
396 different things to the two sexes. During daily observations, we found that males often
397 flew with females after a female called. Thus, female calls may function as a signal to
398 attract males. For females, the ‘bubbling’ call may function as a signal to defend
399 resources e.g. host nests. Similar phenomena have also been discovered in a recent
400 research (Moskát & Hauber, 2019). Based on these findings, and those of our
401 previous research, which found that vocal activity of female common cuckoos in the
402 same study population peaked in the morning (Deng et al., 2019), we suggest that the
403 primary function of the female ‘bubbling’ call in this population is intraspecific
404 communication, rather than distraction of nest hosts.

405

406 **Conclusion**

407 In this study, we used call recordings and playback experiments to determine the
408 function of different common cuckoo call types. Firstly, we demonstrated that the
409 three-element male ‘*cu-cu-coo*’ call is associated with the female ‘bubbling’ call.
410 Secondly, we did not find clear evidence supporting the proposed function of mate
411 attraction in male calls. Finally, we suggest that intraspecific communication is the
412 primary function of the female ‘bubbling’ call in our study population, as both male
413 and female common cuckoos show higher degree of excitement in response to
414 playback of female calls.

415

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541

542 Table 1. Call features (mean \pm SD) of 24 '*cu-coo*' calls and 24 '*cu-cu-coo*' calls from
543 11 individual cuckoos. Call features were compared using linear mixed models, with
544 individual ID as the random effect.

Call features	' <i>cu-coo</i> ' call	' <i>cu-cu-coo</i> ' call	t value	<i>P</i>
Duration (s)	0.43 \pm 0.04	0.62 \pm 0.05	17.12	< 0.001
Minimum frequency (kHz)	0.62 \pm 0.03	0.66 \pm 0.03	5.98	< 0.001
Maximum frequency (kHz)	1.01 \pm 0.06	1.18 \pm 0.08	8.45	< 0.001
Peak frequency (kHz)	0.83 \pm 0.12	0.94 \pm 0.13	12.81	< 0.001

545

546

547 Table 2. Male cuckoos responded differently to playback of different call types.

548 Playback of sparrowhawk calls was used as a control.

Variables	Early breeding season			Late breeding season		
	Coefficient ± SE	t	P	Coefficient ± SE	t	P
Playback male ' <i>cu-coo</i> ' calls	1.59 ± 0.36	4.37	0.002	0.68 ± 0.4	1.71	0.126
Playback male ' <i>cu-cu-coo</i> ' calls	0.7 ± 0.37	1.91	0.092	-0.09 ± 0.42	-0.21	0.842
Playback female 'bubbling' calls	1.81 ± 0.36	5.09	0.001	1.42 ± 0.38	3.78	0.005
Time	-0.3 ± 0.58	-0.51	0.612	-2.51 ± 1.34	-1.88	0.073
Date	-0.06 ± 0.03	-2.14	0.040	-0.01 ± 0.02	-0.50	0.625

549

550

551 Table 3. Female cuckoos responded differently to playback of different call types.
 552 Playback of sparrowhawk calls was used as a control.

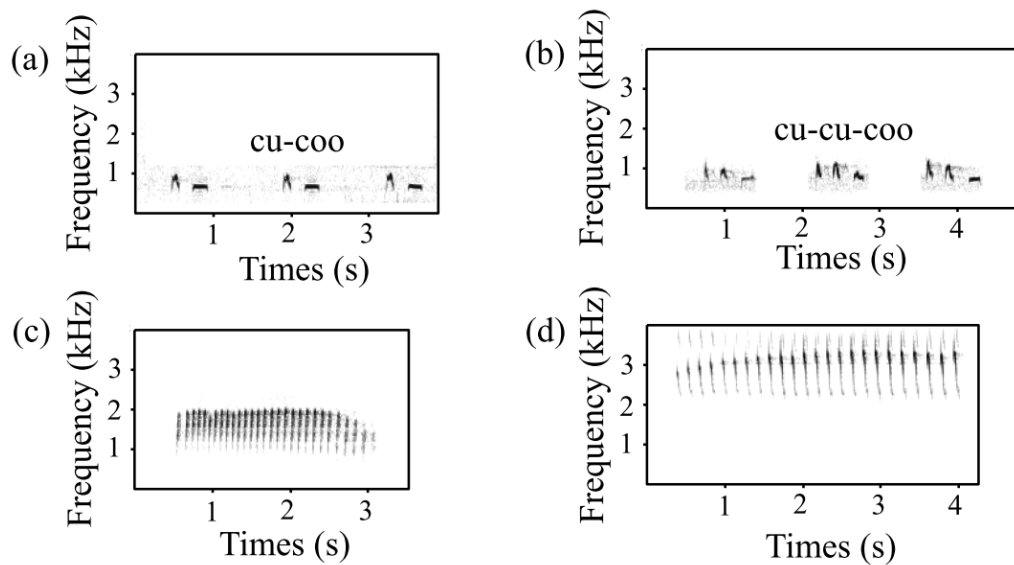
Variables	Early breeding season			Late breeding season		
	Coefficient ± SE	t	<i>P</i>	Coefficient ± SE	t	<i>P</i>
Playback male ' <i>cu-coo</i> ' calls	-0.32 ± 0.5	-0.63	0.555	0.03 ± 0.54	0.06	0.951
Playback male ' <i>cu-cu-coo</i> ' calls	-0.18 ± 0.48	-0.38	0.723	0.39 ± 0.55	0.72	0.490
Playback female 'bubbling' calls	1.44 ± 0.47	3.06	0.028	1.56 ± 0.52	3.00	0.017
Time	-0.17 ± 1.16	-0.14	0.888	0.27 ± 1.9	0.14	0.893
Date	-0.06 ± 0.05	-1.21	0.244	0 ± 0.03	-0.15	0.884

553

554

555 **Figure legends:**

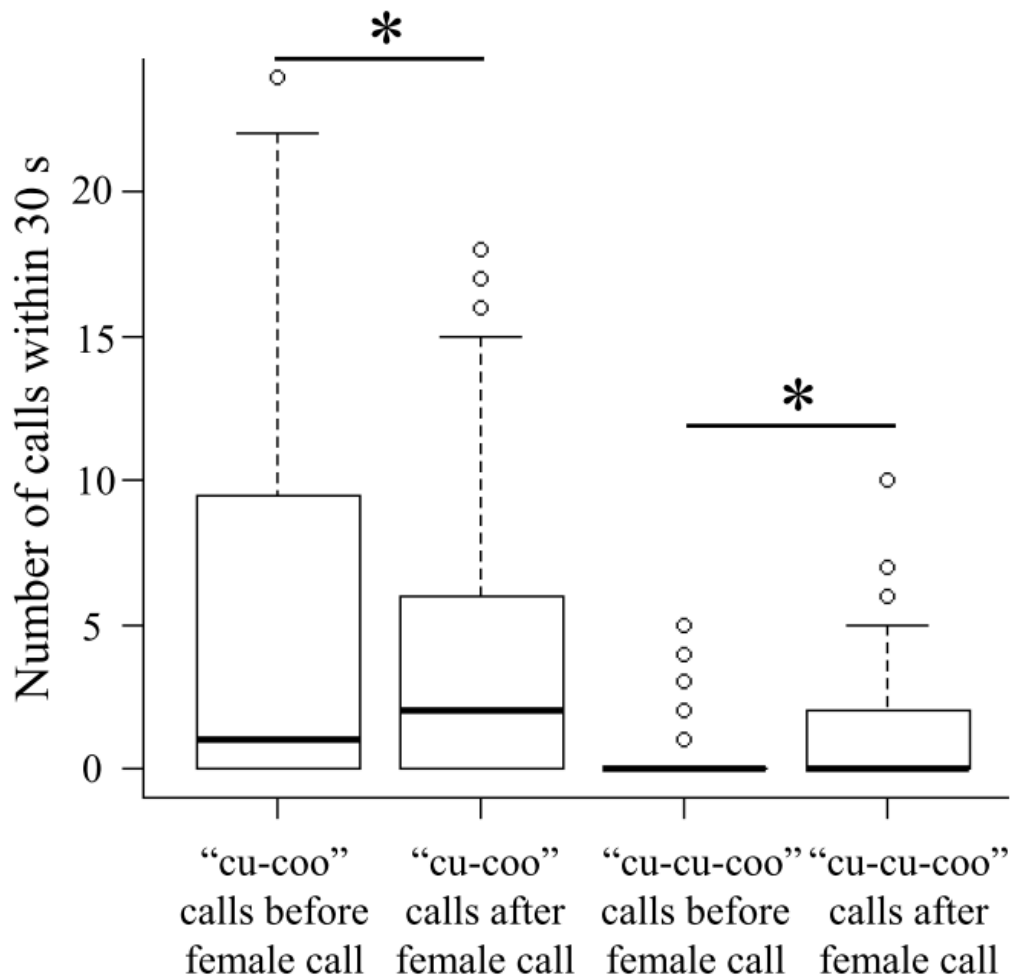
556 Figure 1. Spectrogram of male common cuckoo '*cu-coo*' call (a); male common
557 cuckoo '*cu-cu-coo*' call (b); female common cuckoo 'bubbling' call (c); and
558 sparrowhawk call (d).



559

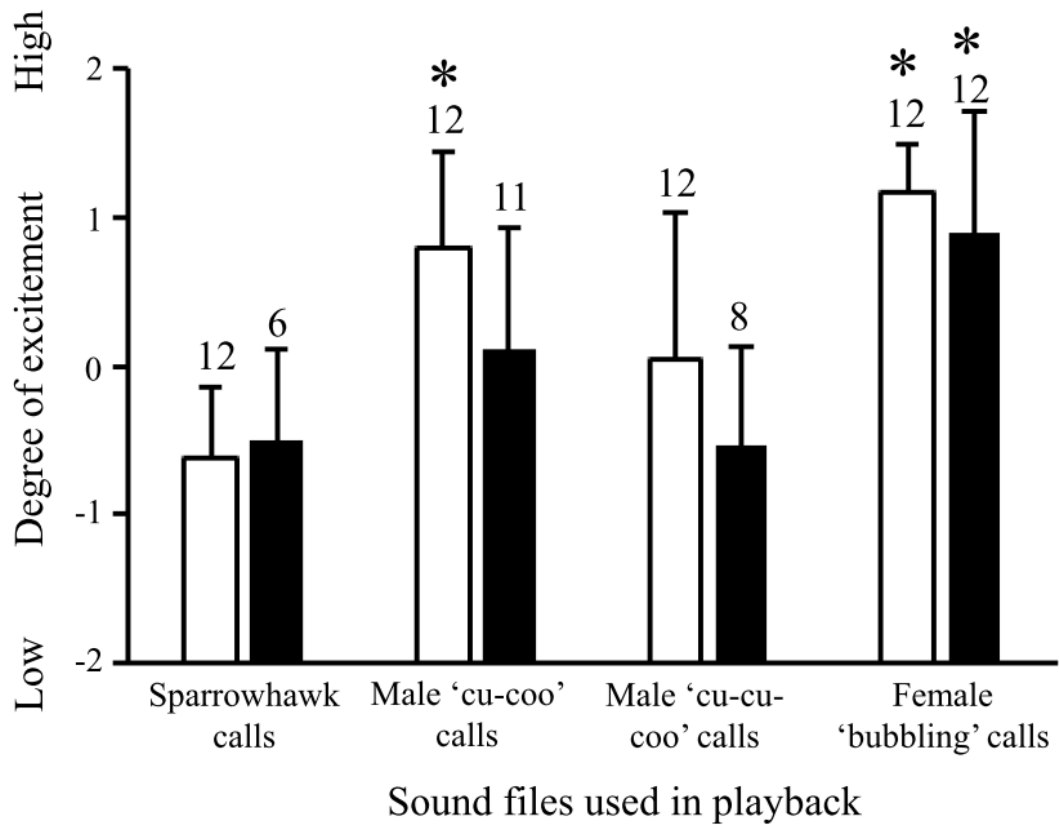
560

561 Figure 2. Comparison of the number of male '*cu-coo*' and '*cu-cu-coo*' calls recorded
562 30 s before or after playback of female 'bubbling' calls. Asterisk indicates significant
563 difference based on linear mixed models, with recorder ID as the random effect.



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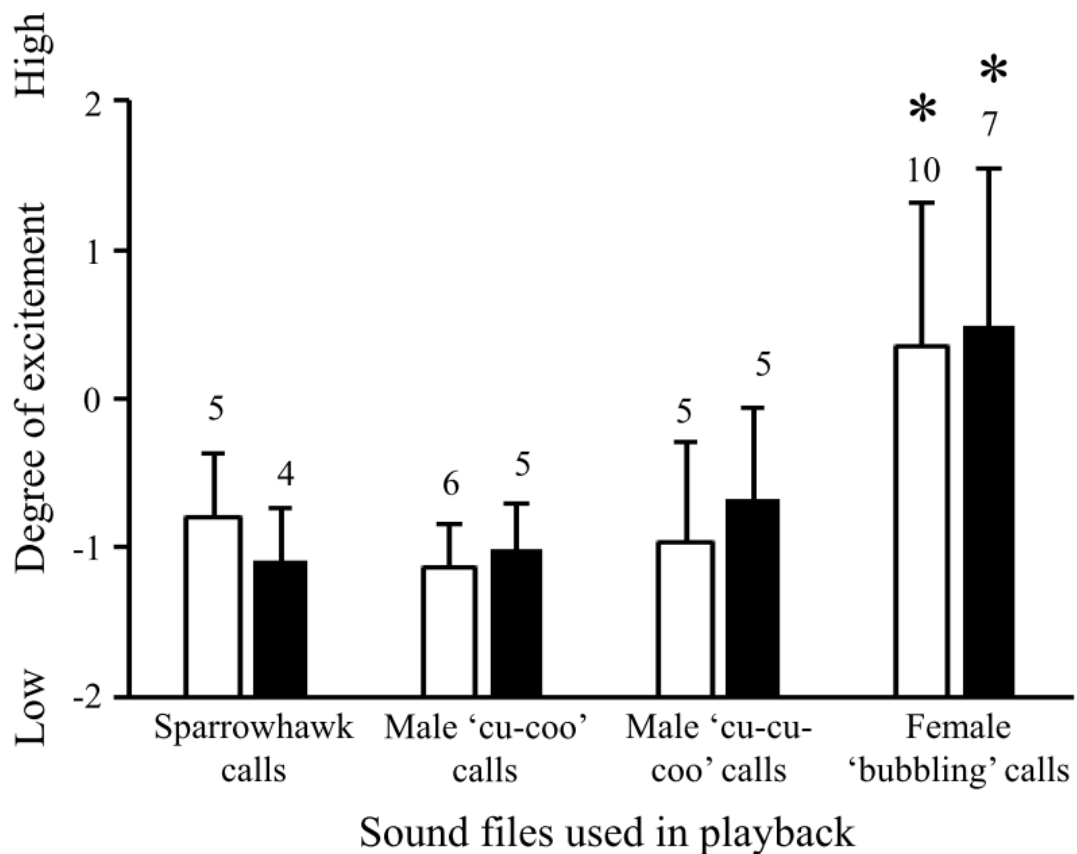
566 Figure 3. Response of male common cuckoos to playback (measured as degree of
567 excitement). White bar indicates playback experiments in early breeding season (28th
568 May to 8th June in 2018), while black bars indicate playback experiments in late
569 breeding season (5th to 28th July in 2018). Sample sizes are shown above each bar.
570 Asterisk indicates significant difference compared to control (playing sparrowhawk
571 calls).



572

573

574 Figure 4. Response of female common cuckoos to playback (measured as degree of
 575 excitement). White bars indicate playback experiments in early breeding season (28th
 576 May to 8th June in 2018), while black bars indicate playback experiments in late
 577 breeding season (5th to 28th July in 2018). Sample sizes were shown above each bar.
 578 Asterisk indicates significant difference comparing to control (playing sparrowhawk
 579 calls).



580

581

582 **SUPPORTING INFORMATION**

583 Appendix 1. Cuckoos responded differently to playback of different call types.

584 Playback of sparrowhawk calls was used as a control. Analysis is based on Mann-

585 Whitney test.

Groups	Early breeding season				Late breeding season			
	Male		Female		Male		Female	
	Z	P	Z	P	Z	P	Z	P
Playback male 'cu-coo' calls	3.64	< 0.001	1.38	0.247	1.50	0.151	0.81	0.556
Playback male 'cu-cu-coo' calls	1.16	0.260	0.82	0.548	0.24	0.864	1.07	0.413
Playback female 'bubbling' calls	4.16	< 0.001	2.33	0.019	2.53	0.010	2.22	0.024

