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1 **Title:** Components of variation in female common cuckoo calls

2 **Short running title:** Female cuckoo calls

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21 **Authors' contributions:**

22 YZ, WL designed the experiments; ZD participated in the field work; ZD, CX carried
23 out the analyses; CX drafted the earlier version of the manuscript and HL, AM, ZD
24 revised it. All authors have read and approved the final manuscript.

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26 available from the corresponding author (xiacanwei@126.com) on reasonable request.

27 **Declarations of interest:** none

28 **Ethical standards:** Our research protocol was approved by the Animal Management

29 Committee at the College of Life Sciences, Beijing Normal University under license
30 number CLS-EAW-2016-017. Bird capture and banding were permitted by the
31 National Bird-banding Center of China under license number H20110042. The
32 experiments comply with the current laws of the country in which they were
33 performed.

34

35 **Components of variation in female common cuckoo calls**

36

37 **Highlights**

38 Larger intra-individual variation in female than in male cuckoo calls

39 Peak frequency of female cuckoo call was significantly negative related to latitude

40 Cuckoos were more vocally active in sunny weather than rainy weather

41 The peak in vocal activity (both male and female cuckoos) was in the morning

42

43 **Abstract:** Investigations on bird vocalizations have largely focused on males. Female
44 vocalizations are widespread in birds but few studies have investigated female vocal
45 characteristics, particularly in non-Passeriformes. In this study, we use new field
46 recordings from China, and calls available from an online sound library to examine
47 temporal patterns, call consistency and geographical variation in vocalizations of
48 female common cuckoos *Cuculus canorows*. The peak in vocal activity (both male and
49 female) was in the morning, which contrasts to what would be predicted if the sole
50 function of the female call was to distract the attention of hosts after parasitizing a host's
51 nest in the afternoon. Both male and female common cuckoos were more vocally active
52 in sunny weather, than rainy weather. We also found larger intra-individual variation in
53 female rather than in male calls, which may benefit female cuckoos by increasing
54 stimulation to host species. Peak frequency of female calls decreased with increased
55 latitude, while differences in female call features were not associated with geographic
56 distance. In summary, our findings that female calls are used in the morning, rather than
57 at peak egg laying, yet are highly variable and show little geographic patterns suggest
58 that the function of these female calls may be more variable and intricate than
59 previously thought. However, because research on female vocalizations is
60 underrepresented, future studies are still needed.

61

62 **Key words:** acoustic signals; call consistency; common cuckoo; female vocalization;

63 geographical variation; vocal activity.

64

65 **1. Introduction**

66 Studies of bird vocalizations have been critical in shaping our understanding of the
67 relationship between avian signal evolution and species differentiation (Andersson,
68 1994). Historically, most attention on variation in avian acoustic signals has been
69 directed towards males (Beecher and Brenowitz, 2005), with many bird populations
70 showing regional differences in male vocal characteristics over local and/or wider
71 geographical scales (Irwin, 2000; Boughman, 2002; Kaluthota et al., 2016). However,
72 female vocalizations are also widespread in both temperate and tropical species
73 (Garamszegi et al., 2007; Yang et al., 2011; Odom et al., 2014; Mahr et al., 2016).
74 Whilst females of some species use their acoustic signals to defend territories,
75 coordinate breeding activities and attract mates (Langmore, 1998). Mate attraction
76 across bird species does not appear to be the primary function of female vocalizations
77 (Dabelsteen et al., 1998; Cain and Langmore, 2016; Krieg and Getty, 2016). Female
78 vocalisations vary in their complexity (Langmore, 1998; Cain and Langmore, 2016)
79 but few studies have examined whether female vocal characteristics vary
80 geographically, particularly for non-Passeriformes (Odom and Benedict, 2018). Given
81 the prevalence of female vocalizations, more research should be conducted to build a
82 comprehensive understanding of the function and evolution of bird vocalizations.

83 Male common cuckoos *Cuculus canorous* utter loud simple and stereotypic
84 “cuck-ooo” calls during the breeding season. Temporal and frequency variables
85 among different male cuckoo calls are sufficient to provide individual information
86 (Jung et al., 2014; Li et al., 2017; Zsebok et al., 2017), which can be used to
87 distinguish between neighbors and strangers (Moskat et al., 2017). Furthermore, there
88 appears to be a high degree of consistency in the number of syllables in each bout
89 produced by individual males (Møller et al., 2016a, b). However, males of different
90 subspecies differ significantly in their calls (Wei et al., 2015), even within the same

91 subspecies, male calls from populations in the same habitat are more similar to each
92 other than those from populations in different habitats (Fuisz and de Kort, 2007). In
93 comparison, our knowledge of the characteristics and function of female cuckoo calls
94 have, until recently, been largely overlooked. Female cuckoos often give a
95 conspicuous 'chuckle' call (Payne, 2005). One recent experimental playback study
96 suggests that the chuckle call primarily serves as a distraction of the host parent
97 species (e.g. reed warbler *Acrocephalus scirpaceus*) since female chuckle calls had
98 the same effect on the attention of host and non-host species, as playback of the calls
99 of sparrowhawk *Accipiter nisus* (York and Davies, 2017). In contrast, male common
100 cuckoo calls had no such effect. Such a function would enable female cuckoos to
101 benefit from reducing their egg rejection rate through distraction of the attention of
102 hosts (York and Davies, 2017). Other aspects of female cuckoo calls remain
103 unquantified in the peer-review literature (Liang, 2017; Kim et al., 2017).

104 In this study, we use new recordings and those deposited in the online avian
105 acoustic sound library Xeno-Canto, to examine temporal patterns, individual
106 consistency, and geographical variation in the chuckle calls of female common
107 cuckoos. Unlike most birds, which lay egg in the morning, female cuckoos
108 predominantly lay their eggs in the afternoon (Payne, 2005), and the function of
109 female cuckoo calls is to distract the host species after laying (York and Davies,
110 2017). Therefore, we predicted that peak female cuckoo calls should occur during the
111 afternoon. Since sound signals are more easily distorted in bad weather (e.g. rain or
112 strong winds) than good weather (e.g. sunny) (Lengagne and Slater, 2002), we
113 predicted that cuckoo calls would be more frequent on days with good weather.
114 Theoretically, elaborate signals could increase stimulation of sensory perception
115 (Akre and Johnsen, 2014; Cui et al., 2016), which may reduce habituation in the
116 distraction of the attention cuckoo hosts. Consequently, we predicted that female calls
117 should be more variable than male calls. Besides the potential difference in call
118 characteristics among different subspecies and populations in different habitats, there

119 may also be differences in female call characteristics among different host races
120 within the same population. As female cuckoo calls functionally mimic hawk calls
121 (York and Davies, 2017), different populations may exhibit different call
122 characteristics due to different geographic variation in hawk calls and/or hawk species
123 with different calls occurring within the resident bird community. Thus, our final
124 objective was to determine whether female chuckle calls exhibit geographic variation,
125 but because this could be more complex than those for males, we made no specific
126 prediction about geographic variation in female cuckoo calls.

127

128 **2. Methods**

129 *2.1 Study area and sound recording*

130 Fieldwork was conducted during June 8th to July 28th 2017 in the Liaohe Delta Nature
131 Reserve (41.034°N; 121.725°E), Liaoning Province, Northeast China. This region
132 represents one of the most important estuarine wetlands in China, with the largest area
133 of reed-bed habitat along the coastal region of China, and consequently, extensive
134 nesting habitats for Oriental reed warbler *Acrocephalus orientalis*. Here, the common
135 cuckoo is a summer breeding species, and it predominantly parasitizes Oriental reed
136 warbler nests (Li et al., 2016). Density of cuckoos is high in our study population (Li
137 et al. 2016), where several individuals often occur in close proximity (less than 10 m)
138 to each other but data on territory size is lacking. We used a TASCAM DR-100MKIII
139 recorder (Tascam Co., Japan) and a Sennheiser MKH416 P48 external directional
140 microphone (Sennheiser Co., Germany), with a sampling rate of 44.1 kHz and a
141 sampling accuracy of 16 bits, to record cuckoo vocalizations. Further recordings were
142 made using seven passive acoustic recorder SM4 Songmeters (Wildlife Acoustics Inc.
143 USA) placed at seven different locations, separated by a minimal distance of 200 m,
144 to continuously record cuckoo calls from June 8th to July 28th. The minimal distance
145 between these recorders is larger than the effective recording range (100 m for
146 cuckoos call, seen in Huang et al., 2017), in order to avoid the same call being

147 recorded by two recorders. Recorders were attached to telegraph poles at a height of 3
148 m above ground, and set to record continuously at a sampling rate of 44.1 kHz, and a
149 sampling accuracy of 16 bits. Recorders were checked every 10 days to replace the
150 batteries and memory cards. Using mist nets, we also trapped and banded 20
151 individual adult common cuckoos (6 females and 14 males) around our recording
152 sites, whilst daily observations also revealed many other unbanded individual cuckoos
153 at our recording sites during data collection.

154 To examine geographic variation in female cuckoo song, we downloaded all
155 recordings of female common cuckoo from the online sound library Xeno-Canto
156 (<http://www.xeno-canto.org>). For multiple recordings collected on the same day at the
157 same location such that individuals could not be identified, we randomly selected one
158 recording for analysis to avoid pseudo-replication. We also used four recordings that
159 we collected from Liaohe Delta Nature Reserve, and four recordings collected from
160 Beijing Wild Duck Lake National Wetland Park (40.410°N; 115.829°E), which is
161 situated approximately 500 km from the Liaohe Delta Nature Reserve. These eight
162 recordings were made in June 2017 with the same equipment mentioned before.

163

164 *2.2 Acoustic measurements*

165 All recordings were re-sampling with 8 kHz, and saved as .wav files. We used
166 Avisoft-SASLab Pro 5.2 audio analysis software (Avisoft Bioacoustics, Germany) to
167 generate spectrograms with the following settings: Fast Fourier transform length 256
168 points; Hamming window with a frame size of 100% and an overlap of 50%;
169 frequency resolution 31 Hz; and time resolution of 16 ms. Here the phrase ‘number of
170 calls’ refers to number of syllables in males, and the number of calling bouts in
171 females. Male cuckoo calls consist of a repeated series of ‘*cu-coo*’ syllables (Møller et
172 al., 2016a; Li et al., 2017) i.e. both ‘cu’ and ‘coo’ elements constitute a single syllable
173 with several successive syllables constituting a bout. The pause between successive
174 male calling bouts was always longer than 2 s in our recordings, which is obviously

175 greater than the time interval between successive ‘*cu-coo*’ syllables within one calling
176 bout (Fig. 1a). Female cuckoo calls consist of a series of rapidly repeated “*kwik-kwik-*
177 *kwik*” notes (York and Davies, 2017), which we named a bout (Fig. 1b). Each “*kwik*”
178 represented a syllable, corresponding to the terminology used for male calls. For each
179 bout (both male and female), we measured maximum frequency, minimum frequency,
180 peak frequency, duration, and the number of syllables. Peak frequency refers to the
181 frequency associated with the maximum energy. Setting a standard to measure
182 minimum frequency was problematic because energy decreases gradually towards low
183 frequency in female cuckoo calls. Consequently, we did not include minimum
184 frequency in the subsequent analyses.

185 To describe the temporal patterns of male and female cuckoo vocal activity, we
186 used Kaleidoscope Pro Software (Wildlife Acoustics Inc. USA) to create recognizers
187 for finding all male and female calls from recordings collected with the SM4 Song
188 meters. Firstly, we entered the following acoustic features of our target sound, either a
189 male syllable or female bout, to Kaleidoscope Pro Software: frequency range from
190 400 to 1200 Hz for a male syllable, and 600 to 2900 Hz for a female calling bout;
191 duration ranges from 0.3 to 0.5 s for a male syllable, and 1.6 to 4 s for a female
192 calling bout. These acoustic features are slightly larger than actual parameters of male
193 and female cuckoo calls, but this was done to increase the detectability of these calls
194 by the Kaleidoscope Pro Software. Lastly, we manually checked all calls identified by
195 the recognizer based on visual inspection of the spectrograms. In total, we obtained
196 701,661 male syllables, and 2407 female bouts after manual check.

197

198 *2.3 Temporal patterns of vocal activity*

199 We used both our field recordings and recordings downloaded from Xeno-Canto to
200 examine temporal patterns of vocal behavior by male and female cuckoos. Using our
201 field recordings from June 8th to July 24th we first calculated the number of calls per
202 hour using absolute time since we lacked data on twilight time. Our prediction was

203 that female cuckoos had a peak call output in the afternoon, based on the primary
204 function of female calls being the distraction of hosts during egg laying by cuckoos.
205 Thus, we assumed that the use of absolute time, rather than twilight time, would have
206 little or no effect on tests of our prediction. We used a generalized linear mixed model
207 (GLMM) to estimate the repeatability of number of calls per hour, based on the
208 function *rpt* in the R package *rptR* (Stoffel et al., 2017). After confirming that most
209 variation occurred among hours, rather than during the same hour on different days
210 (repeatability of number of calls per hour in male 0.763 ± 0.056 mean \pm SE; 95%
211 confidence interval ranged from 0.623 to 0.828; $P < 0.001$; in female = 0.872 ± 0.087
212 mean \pm SE; 95% confidence interval ranged from 0.609 to 0.910; $P < 0.001$), we
213 pooled the data by calculating the mean number of calls per hour, and we used these
214 data to illustrate temporal patterns of cuckoo vocal activity.

215 For a second data source, we used the time of cuckoo calls from the recordings
216 downloaded from Xeno-Canto, again using absolute rather than twilight time for the
217 analysis. From this second source, we obtained the time of 359 recordings of male
218 calls, and 36 recordings of female calls and calculated the number of recordings for
219 every two-hour period. Pearson correlation coefficient based on the number of calls
220 (or recordings) in each hour from the Liaohe Delta Nature Reserve recordings and
221 those from Xeno-Canto were used to determine patterns of similarity in vocal activity
222 from the two separate data sources. Finally, we related patterns of vocal activity from
223 the first data source to localized weather conditions. We defined weather condition
224 based on the information from Weather China (www.weather.com.cn), and classified a
225 day as a bad i.e. days with rain or strong winds (with wind speed greater than 8m/s) or
226 good i.e. days without rain and strong winds.

227

228 *2.4 Individual call consistency*

229 We used a TASCAM DR-100MKIII recorder and a Sennheiser MKH416 P48 external
230 directional microphone to obtain recordings from 18 males in the Liaohe Delta Nature

231 Reserve during June and July 2017; six of these males were color-banded from our
232 mist-netting efforts, whilst further recordings were made from unbanded males from
233 locations separated by at least 2 km from each other, in an effort to reduce the
234 likelihood of repeatedly sampling the same individual twice. Since female calls were
235 much rarer than male calls in our study area (Fig. 2), we supplemented the dataset
236 with recordings made using the passive acoustic recorder SM4 Songmeters. Since we
237 could not be certain which individual calls belonged to the same individual in passive
238 acoustic recordings, we defined two bouts recorded by the same recorders within 1
239 min and with similar amplitude as being from the same individual because we found
240 that it is rare to hear different female calls at the same time in adjacent sites. Using
241 this definition, we obtained 43 recordings, each containing two bouts, from 6
242 songmeters. These recordings were recorded in different locations, or at the same
243 location on different days. Since common cuckoos are abundant in our study area (Li
244 et al., 2017) we treated these 43 recordings as being derived from 43 different female
245 individuals. To avoid pseudo-replication, we used a smaller data set only including six
246 recordings (corresponding to six individual females) recorded from different
247 locations, randomly selected from the original 43 recordings. We calculated the
248 Pearson correlation coefficient from measurements on two successive bouts, and used
249 this to reflect individual call consistency. We compared the correlation coefficients
250 between males and females in the larger data set ($n = 43$), using the Fisher z -
251 transformation to assess the significance of the difference between two correlation
252 coefficients. We did not calculate the significance of the difference between
253 correlation coefficients between males and females in the smaller data set because of
254 the small sample size in the smaller data set ($n = 6$).

255

256 *2.5 Geographical variation*

257 We measured 35 female recordings from Xeno-Canto, and supplemented this dataset
258 with four recordings from Liaohe Delta Nature Reserve, and four recordings from

259 Wild Duck Lake National Wetland Park. Although different equipment was used to
260 collect recordings by Xeno-Canto recorders, we assume that any effects of equipment
261 on recordings should only cause noise in the data set, and there is no reason to expect
262 bias in such effects. We restricted our analyses to one bout in each recording, as most
263 recordings downloaded from Xeno-Canto only contained one bout. Since acoustic
264 measures vary by different orders of magnitude e.g. the frequency of cuckoo syllables
265 ranges in the hundreds of Hz, while the duration of syllables lasts nearly a tenth of a
266 second, acoustic measures were transformed into z-scores, and then used to calculate
267 the Euclidean distance of all pairs of bouts. We used Mantel tests to assess the
268 correlation between Euclidean distance and geographic distance and Pearson
269 correlation to compared the acoustic measures with latitude, as acoustic
270 characteristics are known to change with latitude in many bird species (Kaluthota et
271 al., 2016; Wei et al., 2017). All statistical analyses were performed using R software,
272 v. 3.4.1 (R Core Development Team, 2017). Data are presented as mean \pm standard
273 deviation. Differences with P values less than 0.05 were considered significant.

274

275 **3. Results**

276 *3.1 Temporal variation in common cuckoo calls*

277 At the Liaohe Delta Nature Reserve, we found that the number of cuckoo calls per
278 day fluctuated widely, with a sharp decline after July 25th (Fig. 2). The number of
279 male and female cuckoo calls per day was significantly positively correlated (Pearson
280 correlation, $r = 0.075$, $P < 0.001$, $n=47$) and both male and female common cuckoos
281 showed higher vocal activity during good weather (Fig. 3). Males on average uttered
282 $17,192 \pm 1,241$ (mean \pm SD) syllables summarized from data collected by seven
283 recorders per day in good weather ($n = 25$ days), significantly more than the $11,867 \pm$
284 899 syllables per day during bad weather ($n = 22$ days; independent samples t test, t_{45}
285 $= 3.392$, $P = 0.001$). Females uttered 61 ± 6 bouts summarized from data collected by
286 seven recorders per day in good weather ($n = 25$ days), significantly larger than 40 ± 7

287 bouts per day during bad weather ($n = 22$ days; independent samples t test, $t_{45} = 2.406$,
288 $P = 0.02$). Data from both the Liaohe Delta Nature Reserve (Fig. 4a) and Xeno-Canto
289 (Fig. 4b) revealed that peak call output occurred during the morning rather than in the
290 afternoon, with noticeably little female call activity in the afternoon at the Liaohe
291 Delta Nature Reserve. Number of calls per hour from Liaohe Delta Nature Reserve
292 and Xeno-Canto were strongly positively correlated in both males ($r = 0.769$, $P <$
293 0.001) and females ($r = 0.655$, $P = 0.001$).

294

295 *3.2 Individual consistency*

296 Males ($n = 18$) generally showed higher individual call consistency than females ($n =$
297 43) (Fig. 5). Pearson correlation coefficients of maximum frequency (male: $r = 0.926$;
298 female: $r = 0.618$), duration (male: $r = 0.488$; female: $r = 0.067$), and number of
299 syllables (male: $r = 0.609$; female: $r = 0.087$) were significantly larger for males than
300 for females (Z test, $Z = 3, 1.98, 2.05$; $P = 0.003, 0.048, 0.040$, respectively). Pearson
301 correlation coefficients of peak frequency were larger in females ($r = 0.523$) than in
302 males ($r = 0.179$), but the difference was not significant (Z test, $Z = 1.32$, $P = 0.187$).
303 Pearson's correlation coefficients for females in the smaller data set (6 recordings
304 made from different locations) showed a similar trend as Pearson's correlation
305 coefficients for females in the larger data set (43 recordings made from different
306 locations, or at the same location on different days): If Pearson correlation coefficients
307 for females in the larger data set was larger (or lower) than Pearson correlation
308 coefficients for males, Pearson's correlation coefficients for females in the smaller
309 data set was also larger (or lower) than in males for each variable (Fig. 5).

310

311 *3.3 Geographical variation*

312 We found in female calls a maximum frequency of bouts of $2,228 \pm 40$ Hz; peak
313 frequency of bouts of $1,905 \pm 47$ Hz; duration of bouts of 1.979 ± 0.056 s, with 19.53
314 ± 0.78 syllables. There was no significant linear trend of differences in female calls

315 and geographic distance (Mantel test, $P = 0.256$) (Fig. 6a). Peak frequency decreased
316 with increased latitude (Pearson's correlation coefficients, $r = -0.364$, $P = 0.016$, $n =$
317 43) (Fig. 6b), while maximum frequency ($r = -0.166$, $P = 0.289$), duration ($r = -0.031$,
318 $P = 0.842$), and number of syllables ($r = -0.094$, $P = 0.547$) showed no significantly
319 relationship with latitude.

320

321 **4. Discussion**

322 *4.1 Temporal patterns of female vocal activity*

323 Based on the field recordings from Liaohe Delta Nature Reserve and others deposited
324 on the online sound library Xeno-Canto, we found that vocal activity of female
325 cuckoos peaked in morning rather than in the afternoon. We admit that the call
326 recordings in Xeno-Canto are influenced by human activities (Blackburn et al., 2014)
327 e.g. observers are more likely to spend more time recording birds in good weather and
328 in the morning, which may lead to bias in the interpretation of daily vocal behavior
329 patterns. In addition, to avoid potential bias due to differences in time across the
330 different time zones represented in the Xeno-Canto recordings, we used the regional
331 local time rather than twilight time as this would have little or no effect on tests of our
332 prediction of peak call output in the afternoon. We used automatic recognizers
333 generated from our field recordings to automatically identify potential cuckoo calls,
334 all of which were then subsequently checked manually by visual inspection of the
335 spectrograms. Previous research on Large Hawk Cuckoo (*Hierococcyx sparveroides*)
336 found that only about 50 % of all cuckoos calls were correctly identified by automatic
337 recognizers from 96 hours of recordings (Huang et al., 2017). Most of the common
338 cuckoo calls that were not detected by the automatic recognizers in this study
339 occurred during the dawn chorus, and were largely masked by the dawn songs of
340 oscine passerines. Thus, female cuckoos may have higher peak of call output in the
341 morning than we observed.

342 The peak of female call output in the morning is in contrast to what would be

343 predicted if the only function of the female cuckoo call was to distract the attention of
344 hosts after parasitizing a host's nest i.e. peak in call activity should occur during the
345 afternoon (see York and Davies, 2017). Perhaps, female calls may be used to find
346 nests of hosts in the morning. As most birds in the morning lay eggs or sit on eggs,
347 female cuckoos produce calls which cause that host leave the nest, and then locate
348 nests of hosts. It is also likely that female common cuckoo calls at our study site have
349 other functions besides distraction hosts (Liang, 2017). For example, females calling
350 in the morning may attract the attention of males (e.g. Langmore, 1998).
351 Alternatively, it may be the case that potential host species at Liaohe Delta Nature
352 Reserve are able to distinguish between female common cuckoo calls from hawk
353 calls, or even use female calls as a predictor of risk of parasitism or increased
354 probability of egg rejection, although further experimental playback research is
355 needed.

356

357 *4.2 Individual call consistency*

358 Individual consistency as measured by repeatability is important in social behavior,
359 but also as an upper limit for heritability and hence micro-evolution (Bell et al. 2009;
360 Nakagawa and Schielzeth 2010). Male common cuckoo call features are consistent
361 within individuals (Jung et al., 2014; Li et al., 2017; Zsebok et al., 2017), which can
362 facilitate neighbor - stranger discrimination in birds (Moskat et al., 2017). For
363 females, York and Davies (2017) reported that the primary function of calls is to
364 distract the attention of host species. Elaborate signals could increase stimulation of
365 sensory perception (Akre and Johnsen, 2014; Cui et al., 2016), which may reduce
366 habituation of distraction of the attention of hosts. In agreement with this prediction,
367 females generally show lower individual consistency in call characteristics than
368 males: specifically, Pearson's correlation coefficients of maximum frequency,
369 duration, and number of syllables in females are significantly lower than those of
370 males. These results were consistent across both the larger (43 recordings recorded in

371 different locations, or at the same location on different days) and the smaller (6
372 recordings recorded in different locations) data sets thus we believe that our sample
373 sizes were sufficient to evaluate both within- and between-individual differences.
374 Pearson's correlation coefficients of peak frequency in female calls was larger than in
375 males, although the difference was not significant. For many bird species, peak
376 frequency is determined by body size (Fletcher, 2004; Rodriguez et al., 2015) and is
377 consistently higher in male cuckoo calls (Jung et al., 2014; Li et al., 2017; Zsebok et
378 al., 2017). This acoustic feature could potentially be used for monitoring female
379 cuckoos.

380

381 *4.3 Geographical variation in female cuckoo calls*

382 Geographic variation in bird vocalizations is common, and may affect mate choice,
383 pair bonding, and territory defense in both passerine species such as stonechat
384 *Saxicola torquata* (Mortega et al., 2014) and coal tit *Periparus ater* (Pentzold et al.
385 2016), and also non-passerine species such as gentoo penguin *Pygoscelis papua*
386 (Lynch and Lynch, 2017) and corncrake *Crex crex* (Budka and Osiejuk, 2017). One of
387 the more common patterns in bird acoustic geographic variation can be described in
388 terms of the 'isolation by distance' model (Podos and Warren, 2007), in which
389 vocalization differences in paired populations increase with the distance between
390 those populations (Irwin, 2000; Xing et al., 2013). This pattern can be caused by both
391 cultural drift and genetic differences, as there are few chances for culture and genetic
392 exchange among remote populations (Irwin et al., 2008; Stewart and MacDougall-
393 Shackleton, 2008; Ramsay and Otter, 2015). In accordance with this model,
394 differences in calls and geographic distance are known to be closely correlated in
395 male common cuckoos (Wei et al., 2015). However, in our study we found no
396 evidence of isolation by distance, and we found no significant linear trend in
397 differences in female cuckoo calls with geographic distance. For female cuckoos,
398 there may be differences in call characteristics among different races within the same

399 population, besides potential differences in call characteristics among different
400 subspecies and populations in different habitats. If the purpose of female cuckoo calls
401 is to functionally mimic hawk calls (York and Davies, 2017), different populations
402 may have different call features due to different hawk species with different calls
403 occurring in the local bird community. These factors could lead to complexity in
404 geographic variation of female cuckoo calls, and mask any linear trend between
405 differences in calls and geographic distance as predicted in the isolation by distance
406 model.

407 Peak frequency is thought to correlate negatively with body size (Fletcher, 2004;
408 Rodriguez et al., 2015) but it is not unusual to find evidence to the contrary to this
409 rule in some oscines (e.g. rufous-collared sparrow *Zonotrichia capensis* Handford and
410 Loughheed 1991; dark-eyed junco *Junco hyemalis* and serin *Serinus serinus* Cardoso et
411 al. 2008), due to song learning and sexual selection (Patel et al., 2010; Cardoso,
412 2012). However, this rule is generally supported in suboscines and non-passerines,
413 particularly for species with large body size and low acoustic frequency in
414 vocalizations such as doves (Tubaro and Mahler, 1998), tinamous (Bertelli and
415 Tubaro, 2002), and antbirds (Seddon, 2005). In this study, we found that peak
416 frequency of female cuckoo calls decreased at higher latitude. In cuckoos, both male
417 and female body size is known to increase at higher latitudes (Payne, 2005; Erritzøe et
418 al., 2012), and this may lead to the negative relationship between peak frequency and
419 latitude.

420

421 **5. Conclusions**

422 In this study, we investigated three aspects (temporal patterns, call consistency,
423 geographical variation) concerning female common cuckoo calls. York and Davies
424 (2017) suggested that female common cuckoos mimic sparrowhawk vocalizations in
425 order to distract the host species after laying. In accordance with this prediction, we
426 found larger intra-individual variation in female calls, which may increase stimulation

427 of the host species. Besides, we found peak frequency of female calls decreased with
428 increased latitude, while differences in female call features were not associated with
429 geographic distance. However, the daily vocal pattern contrasts with what would be
430 predicted according to York and Davies (2017). If the only function of the female
431 common cuckoo call was to distract the attention of hosts after parasitizing a host's
432 nest, peak call output should occur during the afternoon because female common
433 cuckoos predominantly lay their eggs in the afternoon (Payne, 2005). Data from both
434 the Liaohe Delta Nature Reserve and Xeno-Canto revealed that peak call output
435 occurred during the morning rather than in the afternoon. Based on this result, we
436 infer that female common cuckoo calls have other functions besides distraction hosts.

437

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447

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- 579
- 580 **Figure captions:**
- 581
- 582 **Figure 1.** Spectrogram of male (A), and female (B) common cuckoo calls, recorded in
583 Liaohe Delta Nature Reserve (41.034°N; 121.725°E).

584

585 **Figure 2.** The number of calls by male and female common cuckoos per day during
586 the 2017 breeding season, summarized from data collected by seven recorders.

587

588 **Figure 3.** Call output (number of syllables summarized from data collected by seven
589 recorders per day \pm SE) by (A) male and (B) female common cuckoos in
590 relation to different weather conditions. Asterisk indicates significant
591 difference at $P < 0.05$ (independent samples t test).

592

593 **Figure 4.** Daily temporal pattern of common cuckoo call (A) based on recordings
594 from seven recorders in Liaohe Delta Nature Reserve; (B) 359 male
595 recordings and 36 female recordings from the online sound library Xeno-
596 Canto. Error bars are SE.

597

598 **Figure 5.** Pearson correlation coefficients from measurements of vocalization features
599 in common cuckoos in two successive bouts. Asterisk indicates significant
600 difference at $P < 0.05$ (Z test). n is the number of individuals and error bars are
601 SE.

602

603 **Figure 6.** (A) Lack of significant linear trend in female common cuckoo calls with
604 geographic distance (Mantel test, $P = 0.256$); and (B) significant negative
605 correlation between peak frequency and latitude (Pearson's correlation
606 coefficients, $r = -0.364$, $P = 0.016$, $n = 43$).











