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- 2 Short running title: Female cuckoo calls
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- 22 YZ, WL designed the experiments; ZD participated in the field work; ZD, CX carried
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- 33 performed.
- 34

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Components of variation in female common cuckoo calls

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 canorous. 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 latitude, while differences in female call features were not associated with geographic 55 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 that the function of these female calls may be more variable and intricate than 58 previously thought. However, because research on female vocalizations is 59 60 underrepresented, future studies are still needed.

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

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63

65 **1. Introduction**

geographical variation; vocal activity.

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 territotries, 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 vocalisations vary in their complexity (Langmore, 1998; Cain and Langmore, 2016) 78 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 produced by individual males (Møller et al., 2016a, b). However, males of different 89 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 distraction of the attention cuckoo hosts. Consequently, we predicted that female calls 116 117 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

Fieldwork was conducted during June 8th to July 28th 2017 in the Liaohe Delta Nature 130 Reserve (41.034°N; 121.725°E), Liaoning Province, Northeast China. This region 131 132 represents one of the most important estuarine wetlands in China, with the largest area of reed-bed habitat along the coastal region of China, and consequently, extensive 133 nesting habitats for Oriental reed warbler Acrocephalus orientalis. Here, the common 134 135 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, to continuously record cuckoo calls from June 8th to July 28th. The minimal distance 144 145 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

recorded by two recorders. Recorders were attached to telegraph poles at a height of 3 m above ground, and set to record continuously at a sampling rate of 44.1 kHz, and a sampling accuracy of 16 bits. Recorders were checked every 10 days to replace the batteries and memory cards. Using mist nets, we also trapped and banded 20 individual adult common cuckoos (6 females and 14 males) around our recording sites, whilst daily observations also revealed many other unbanded individual cuckoos 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 bout (Fig. 1a). Female cuckoo calls consist of a series of rapidly repeated "kwik-kwik-176 177 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 male syllable or female bout, to Kaleidoscope Pro Software: frequency range from 189 190 400 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 by the Kaleidoscope Pro Software. Lastly, we manually checked all calls identified by 194 195 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

We used both our field recordings and recordings downloaded from Xeno-Canto to examine temporal patterns of vocal behavior by male and female cuckoos. Using our field recordings from June 8th to July 24th we first calculated the number of calls per 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 calls, and 36 recordings of female calls and calculated the number of recordings for 218 219 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

We used a TASCAM DR-100MKIII recorder and a Sennheiser MKH416 P48 external 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 individuals. To avoid pseudo-replication, we used a smaller data set only including six 245 recordings (corresponding to six individual females) recorded from different 246 247 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

We measured 35 female recordings from Xeno-Canto, and supplemented this datasetwith 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 day fluctuated widely, with a sharp decline after July 25th (Fig. 2). The number of 278 279 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 $11,867 \pm$ 899 syllables per day during bad weather (n = 22 days; independent samples t test, t_{45} 284 = 3.392, P = 0.001). Females uttered 61 ± 6 bouts summarized from data collected by 285 286 seven recorders per day in good weather (n = 25 days), significantly larger than 40 ± 7 bouts per day during bad weather (n = 22 days; independent samples t test, t_{45} = 2.406, P = 0.02). Data from both the Liaohe Delta Nature Reserve (Fig. 4a) and Xeno-Canto (Fig. 4b) revealed that peak call output occurred during the morning rather than in the afternoon, with noticeably little female call activity in the afternoon at the Liaohe Delta Nature Reserve. Number of calls per hour from Liaohe Delta Nature Reserve and Xeno-Canto were strongly positively correlated in both males (r = 0.769, P <

294

293

295 3.2 Individual consistency

(0.001) and females (r = 0.655, P = 0.001).

296 Males (n = 18) generally showed higher individual call consistency than females (n = 18)297 43) (Fig. 5). Pearson correlation coefficients of maximum frequency (male: r = 0.926; female: r = 0.618), duration (male: r = 0.488; female: r = 0.067), and number of 298 299 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 correlation coefficients of peak frequency were larger in females (r = 0.523) than in 301 males (r = 0.179), but the difference was not significant (Z test, Z = 1.32, P = 0.187). 302 303 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

510

311 3.3 Geographical variation

312 We found in female calls a maximum frequency of bouts of $2,228 \pm 40$ Hz; peak

frequency of bouts of 1,905 \pm 47 Hz; duration of bouts of 1.979 \pm 0.056 s, with 19.53

 ± 0.78 syllables. There was no significant linear trend of differences in female calls

- 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 sparverioides) 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*

Individual consistency as measured by repeatability is important in social behavior, 358 359 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* (Lynch and Lynch, 2017) and corncrake Crex crex (Budka and Osiejuk, 2017). One of 386 387 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 those populations (Irwin, 2000; Xing et al., 2013). This pattern can be caused by both 390 391 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 evidence of isolation by distance, and we found no significant linear trend in 396 397 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 Lougheed 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

In this study, we investigated three aspects (temporal patterns, call consistency,
geographical variation) concerning female common cuckoo calls. York and Davies
(2017) suggested that female common cuckoos mimic sparrowhawk vocalizations in
order to distract the host species after laying. In accordance with this prediction, we
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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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).

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504	
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, $r = -0.364$, $P = 0.016$, $n = 43$).











