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24 **Within-season decline in the call consistency of individual male Common Cuckoo**

25 *(Cuculus canorus)*

26 **Abstract**

27 Numerous studies have identified individually distinctive vocal characteristics and call consistency in  
28 different bird species. If these are to be utilised as non-invasive markers for monitoring purposes, then  
29 these vocal characteristics must remain stable over time. Three recent studies have shown that it is  
30 possible to identify individual male Common Cuckoo (*Cuculus canorus*) based on vocal characteristics  
31 but whether these are stable over the duration of a breeding season, remains unknown. We recorded  
32 1032 syllables from 30 male Common Cuckoos in a Northeast Asian population. We colour-banded six  
33 of these males and made repeated recordings of their *cu-coo* advertisement call across a 19-day period  
34 of the breeding season in China. We used three methods to identify individuals: discriminant function  
35 analyses (DFA), correlation analysis (CA) and spectrographic cross-correlation (SPCC). We also used  
36 repeatability analysis to test whether call consistency (the number of syllables in each calling bout) was  
37 repeatable within individuals. Based on the same day recordings, calls from the same male were more  
38 similar in their characteristics than those of different males, and yielded correct rates of classifying  
39 individuals of 93.6% (SPCC), 90.8 % (DFA), and 71.5% (CA). However, these rates declined to 40.5%  
40 (SPCC), 40.7% (DFA) and 27% (CA) when using recordings over the 19-day period. Call consistency  
41 was repeatable within individuals across two successive calling bouts, but this individual repeatability  
42 disappeared when several (more than two) calling bouts from the same day or bouts from the different  
43 days were included in the analyses. Declines in the correct rate of identifying individual male cuckoos  
44 and call consistency in this study raises concerns that individual male cuckoo calls may be more  
45 variable than previously thought.

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46 **Key words:** call consistency; Common Cuckoo; temporal stability; vocal individuality; vocal signals

47

48 **Introduction**

49 Recognition based on individually distinctive vocalizations is a functionally important aspect of  
50 signaling amongst many animal species (Falls 1982; Stoddard et al. 1996; Tibbets and Dale 2007).  
51 Numerous studies have shown the presence of individually distinctive vocal characteristics in different  
52 bird species (Terry et al. 2005) i.e. the presence of vocal characteristics that are less variable within  
53 individuals than between individuals (e.g. Galeotti and Pavan 1991; Rebbeck et al. 2001; Puglisi and  
54 Adamo 2004; Policht et al. 2009). Since bird vocalizations function for the long-distance broadcast of  
55 fitness related information (Catchpole and Slater 2008; Cramer 2013a), individual identification may  
56 benefit other birds (i.e. the signal receivers) in assessing the quality or behavioural state of individuals,  
57 territory occupation, or simply to maintain relationships with neighbouring individuals (e.g. Delgado et  
58 al. 2013; Sandoval et al. 2014). Many studies have examined the utility of individual vocal  
59 characteristics as non-invasive markers for monitoring individuals or populations (e.g. Laiolo et al.  
60 2007; Kirschel et al. 2011; Budka et al. 2015), or as a complimentary method to more traditional forms  
61 of monitoring (Blumstein et al. 2011), particularly for secretive or rare bird species (e.g. Kemp and  
62 Kemp 1989; Gilbert et al. 1994; Grava et al. 2008) for which monitoring will be essential for effective  
63 conservation management (Terry et al. 2005; Klenova et al. 2008). Furthermore, the number of  
64 syllables produced by calling males of some passerine and non-passerine bird species has also been  
65 found to be remarkably consistent over short periods of time (Catchpole and Slater 2008) suggesting  
66 that call length may also serve as a form of signaling for individual fitness during the breeding season  
67 (Møller et al. 2016a, b).

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68        If unique vocal characteristics are to be used as markers for monitoring purposes, and if call  
69 consistency is a reliable indicator of male quality, then it is essential that calls remain unchanged i.e.  
70 remain ‘stable’ over significant periods of time (Terry et al. 2005; Dawson and Efford 2009; Linhart  
71 and Šálek 2017) such as the duration of a single breeding season, or even between different seasons.  
72 However, demonstrating such vocal stability over time has proven difficult because ideally, the acoustic  
73 analyses should be conducted on known individuals that have been individually marked e.g. using  
74 colour rings (Terry et al. 2005), but this is not always feasible. Some studies dealing with individual  
75 acoustic signals are based on recordings made from only a few days sampling within a single season  
76 (e.g. Li et al. 2017). Studies of the European Eagle Owl (*Bubo bubo*) found that within-year rates of  
77 correct classification of individuals varied from 60-100%, but between years, only 41.8% of ‘hoots’  
78 were correctly attributed to the territory owner in the previous year, with the between-year correct  
79 classification ranging from 0 to 100% (Grava et al. 2008). Even studies of the long-term stability of  
80 individual vocal characteristics of mammals have recorded reclassification rates of <50% (e.g.  
81 Jorgensen and French 1998). This has led some authors to conclude that the correct rate of acoustically  
82 identify individual birds over significant time periods will be lower (Linhart and Šálek 2017; Průchová  
83 et al. 2017) because of temporal changes in vocal characteristics caused by physiological changes,  
84 changes to the physical environment, social status, repertoire size and breeding stage (Delgado et al.  
85 2013).

86        The Common Cuckoo (*Cuculus canorus*) is a highly charismatic species widely known for its  
87 parasitic life history (Thorogood and Davies 2012; Yang et al. 2015). Male cuckoo advertisement calls  
88 show a highly stereotypical acoustic structure, consisting of two elements (‘*cu-coo*’) across their entire  
89 geographic range (Wei et al. 2015; Zsebók et al. 2017). Both male and female cuckoos utter loud, far-

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90 carrying vocal signals during the breeding season to communicate with conspecifics (Moskát et al.  
91 2017) or misdirect hosts (York and Davies 2017; but see Liang et al. 2017). Surveying and monitoring  
92 populations of cuckoos during the breeding season using more traditional methods (e.g. point counts,  
93 transects) remains problematic due to the secretive life-history traits (Williams et al. 2015), and mist-  
94 netting surveys to date, tend to catch so few individuals. Surveys for this species carry added  
95 significance because monitoring cuckoo abundance and distribution may serve as an indicator of  
96 overall bird community composition (Morelli et al. 2015, 2017; Tryjanowski and Morelli 2015).  
97 Identifying individual male cuckoos based on vocal signals may represent a promising method to  
98 generate new information on the abundance and life history of this species, and three recent studies  
99 have kindled this hope (Jung et al. 2014; Li et al. 2017; Zsebök et al. 2017). These authors found the  
100 between-individual variation in male cuckoos' calls was much greater than within-individual variation,  
101 and that it was possible to identify individual male cuckoos based on specific call characteristics (Jung  
102 et al. 2014; Li et al. 2017; Zsebök et al. 2017). Furthermore, there appears to be a high degree of  
103 consistency in the number of syllables produced within individual males (e.g. Møller et al. 2016a, b),  
104 and these measures could be utilized to assess environmental conditions (e.g. Møller et al. 2016a, b).  
105 Despite these encouraging findings, all surveys to date have been conducted during just a short period  
106 of the breeding season, with the longest period of acoustic recordings taken over a 5-day period (Li et  
107 al. 2017), whilst the two other studies used recordings of calling males from just one occasion (Jung et  
108 al. 2014; Zsebök et al. 2017). One of these studies revealed a rate of correct classification calls to  
109 individual male of 91.9% from recordings made on one day, but this declined to 50% for recordings  
110 made more than two days apart (Li et al. 2017), suggesting that male vocal characteristics may not be  
111 temporally stable within a single breeding season.

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112 In this study, we assess the feasibility of using vocal characteristics to identify individual male  
113 Common Cuckoos over a 19-day period during the breeding season based on the repeated recordings  
114 from male cuckoos in a northeast Asian population. We used three methods to identify individual  
115 males: discriminant function analyses, correlation analysis, and spectrographic cross-correlation. We  
116 also examined the consistency in the number of syllables produced by calling males during the  
117 breeding season by using acoustic data recorded from successive calling bouts, several (more than two)  
118 bouts recorded during the same day, and calling bouts recorded from different days during the breeding  
119 season.

120

## 121 **Methods**

### 122 *Study area and sound recording*

123 Field work was conducted from June 10th to July 29th 2017 in the Liaohe Delta Nature Reserve  
124 (41.034°N; 121.725°E), Liaoning Province, northeast China. This region represents one of the most  
125 important estuarine wetland in the country, which contains the largest area of reed-bed habitat along the  
126 coastal region of China, and consequently, extensive nesting habitat for Oriental Reed Warbler  
127 (*Acrocephalus orientalis*). Here, the Common Cuckoo is a summer breeding species, and  
128 predominantly parasitizes Oriental Reed Warbler nests during late May to early August (Li et al. 2016).  
129 Using mist nets, we trapped 20 individual cuckoos from June 9th to July 6th 2017. All individuals were  
130 banded with a numbered metal band, and fitted with a backpack radio transmitter (Biotrack Co., UK)  
131 weighing 2.12g (approximately 2.3% of the cuckoo's weight), using the method described by Rappole  
132 and Tipton (1991). This enabled us to track and observe cuckoos during the breeding season to obtain  
133 repeated recordings from known individuals.



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134 All cuckoo vocalizations were recorded using a TASCAM DR-100MKIII recorder (Tascam Co.,  
135 Japan) and a Sennheiser MKH416 P48 external directional microphone (Sennheiser Co., Germany),  
136 with a sampling rate of 44.1 kHz and a sampling accuracy of 16 bits. In the study area, male cuckoos  
137 regularly call when perching on electrical wires (Li et al. 2016), which enabled us to approach within  
138 10-30 m of calling males and obtain the best possible recording with minimal background noise. In  
139 total, we recorded vocalizations of 30 different males, six of which were individually marked (banded)  
140 before recording. The fate of the other 14 banded cuckoos was unknown. We avoided repeated  
141 sampling of the remaining 24 unbanded males by observing the movements of each recorded male, and  
142 then travelling by motorcycle along one of the main roads until we encountered another male. We  
143 traveled each road only once, so we were sure that we recorded different males. This method for  
144 avoiding repeated sampling the same individual was also adopted in previous research (e.g. Li et al.  
145 2017; Zsebök et al. 2017). Due to bad weather or a lack of vocalizations on some of the survey days,  
146 both the number of days from which vocalizations were recorded, and the duration from the first day of  
147 recording to the last day, varied for the six banded males. In summary, we obtained recordings for each  
148 of the six banded males from 5 days across a nine day sampling period, 4 days across a ten day  
149 sampling period, 5 days across an eleven day sampling period, 7 days across a thirteen day sampling  
150 period, 9 days across a thirteen day sampling period, and from 11 days across a twenty day sampling  
151 period, respectively.

152

### 153 *Sound measurements*

154 We used Avisoft-SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany) to resample the  
155 recordings with 6 kHz and created spectrograms with the following settings: sample size, 16 bits; Fast

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156 Fourier transform length 256 points; Hamming window with a frame size of 100% and an overlap of  
157 50%; frequency resolution 23 Hz; and time resolution of 21.3 ms. Male cuckoo advertisement calls  
158 consist of a repeated series of ‘*cu- coo*’ syllables, with each syllable composed of two distinctive  
159 elements (Møller et al. 2016a, b; Møller et al. 2017). We manually separated each element of each  
160 syllable (see Fig. 1) represented by a continuous trace in the spectrogram, and used Avisoft-SASLab  
161 Pro software to measure call features automatically following Li et al. (2017). We first automatically  
162 search the maximum amplitude in each element, and then determine the start and end points of each  
163 element at 19 dB level lower than the maximum amplitude. We selected 19 dB (rather than 16 dB in Li  
164 et al. 2017) because the characteristics of all syllables were explicit and clearly audible above the  
165 background noise on all recordings, allowing us to obtain comparable syllable parameters  
166 independently of the absolute intensity of the calls and the background noise level (Zollinger et al.  
167 2012). The following variables were then measured: duration of the element ( $T_{dur1}$ ,  $T_{dur2}$ ); duration  
168 from the start of element to the point of maximum amplitude within that element ( $T_{dis1}$ ,  $T_{dis2}$ );  
169 frequency at the start point of the element ( $F_{sta1}$ ,  $F_{sta2}$ ); frequency at the end point of the element  
170 ( $F_{end1}$ ,  $F_{end2}$ ); minimum frequency of the element ( $F_{min1}$ ,  $F_{min2}$ ); maximum frequency of the  
171 element ( $F_{max1}$ ,  $F_{max2}$ ); frequency of the maximum amplitude within the element ( $F_{peak1}$ ,  $F_{peak2}$ );  
172 time interval between the first and second element ( $T_{int}$ ) (Supplementary Material Fig. 1). In total, we  
173 measured 1032 syllables from 30 males: 750 syllables for 6 banded males and 282 syllables for 24 un-  
174 banded males (all original measurements of call features can be seen in Supplementary Material file 1).

175 We count the number of syllables within each calling bout based on the number visible from the  
176 spectrograms. The pause between successive bouts was always larger than 2 s, which is obvious greater  
177 than pause between successive syllables within one calling bout (see Fig. 1). We were unable to obtain

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178 a complete calling bout for 8 unbanded males. Consequently, we measured a total of 317 calling bouts  
179 from 22 males (6 banded and 16 unbanded). The data on syllable numbers for each calling bout are  
180 presented in Supplementary Material file 2.

181

182 *Data analyses – identification of vocal individuality*

183 We separated our acoustic data into two data sets. The first of these contained 368 syllables from 6  
184 banded males and 24 un-banded males, and every syllable from each individual in this data set was  
185 recorded on the same day. This first data set was used to construct discriminant functions, and to  
186 calculate the correct rate of acoustically identify individuals within one day (see below). The second  
187 data set contained 664 syllables recorded from the 6 banded males from all other days of field work.  
188 This second set was used to calculate the within-season correct rate of acoustically identify individuals  
189 (i.e. more than one day). All analyses were performed using R v. 3.4.1 (R Core Development Team,  
190 2017) with significance assumed at  $P < 0.05$ .

191 Discriminant function analyses (DFA) is a multivariate technique widely used to identify vocal  
192 individuality in birds (e.g. Delgado et al. 2013; Linhart and Šálek 2017) by combining variables with  
193 weighting coefficients to create a set of functions that can discriminate groups and classify new data  
194 into one of any number of pre-existing groups (Williams and Titus 1988; Mundry and 2007). We used  
195 calls from the first cuckoo data set to construct discriminant functions and examined the power of  
196 functions to correctly classify each syllable to an individual using a jack-knife analysis (Manly 1986;  
197 Galeotti and Sacchi 2001). The prior probability for each individual was set equal in DFA. For the  
198 second data set, we used the 30 discriminant functions constructed (corresponding to 30 males) based  
199 on the first data set to classify syllables of 6 banded males recorded across different days. The number

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200 of syllables was not equal for each male in the first set, and varied from 6 to 20, with a mean of 12  
201 syllables per male. Due to the possibility that the variables used for identifying individuality in males  
202 from the first data set were not similar to those necessary for identifying males over the duration of the  
203 breeding season, we calculated the rate of correct classification in the second data set using the 30  
204 discriminant functions constructed from the first data set based on all combinations of variables (each  
205 time, only a subset of variables were used in discriminant functions). The highest correct rate and their  
206 corresponding combinations of variables were reported.

207 Correlation analysis (CA) was used to calculate the similarity of all pairs of syllables using  
208 Pearson's  $R$  based on call variables, and then identified individuals based on this similarity value  
209 (Budka et al. 2015). Using the first data set, we calculated coefficients of variation (CV) for each  
210 variable to compare differences within (CV<sub>w</sub>) and between (CV<sub>b</sub>) individuals (Robisson et al. 1993).  
211 We computed CV for each male based on syllables belonging to that male, and then calculated the  
212 mean CV for each male as CV<sub>w</sub>. We used the average value for each male to compute CV<sub>b</sub>. The ratio  
213 of CV<sub>b</sub> / CV<sub>w</sub> is the measurement of potential individual coding (PIC) which shows the importance of  
214 each variable used in identifying individuals (Charrier et al. 2001; Charrier et al. 2003). PIC value of  
215 Tdis2, Tdur1 and Tdis2 were less than or nearly equal to one (Table 1), meaning that these variables  
216 showed greater or similar variation within an individual than between individuals. Consequently, these  
217 three variables were not included in the subsequent analysis. Since call variables have different orders  
218 of magnitude e.g. the frequency of cuckoo syllables range in the hundreds Hz, while duration of  
219 syllables last nearly a tenth of a second, we standardized the variables using the formula: (value –  
220 mean) / standard deviation, and used these standardized variables to calculate the similarity of all pairs  
221 of syllables using Pearson correlation for both within male and between males. Based on the first data

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222 set, independent samples t-test were used to compare the similarity of pairs of syllables from the same  
223 male to similarity of pairs of syllables from the different males. Each syllable was assigned to an  
224 individual, whose syllable (except the one being classified) has the maximum similarity value with the  
225 syllable to be assigned. The correct classification of individuals within a single day was expressed as  
226 the percentage of syllables correctly assigned. To calculate the correct rate of acoustically identify  
227 individuals within the breeding season, we first calculated the similarity of all pairs of syllables, one  
228 from the first data set and one from the second data set. We then assigned the syllable in the second set  
229 to one male in the first set, whose syllable has the maximum similarity value with the syllable to be  
230 assigned. The correct rate was expressed as the percentage of syllables correctly assigned.

231 Spectrographic cross-correlation (SPCC) is another widely used technique for identifying vocal  
232 individuality in bird species (e.g. McDonald and Wright 2011; Cramer 2013a). SPCC involves cross-  
233 correlating two spectrograms frame by frame as matrices of amplitude values that are incrementally  
234 overlapped over time (Clark et al. 1987; Radford 2005), then using the resultant peak correlation scores  
235 as measures of similarity (Khanna et al. 1997; Terry et al. 2001). We conducted the SPCC using the  
236 *'template cross correlation on short files'* function in Avisoft-SASLab Pro software. Firstly, we  
237 intercepted every syllable in the spectrograms and saved as *.son* files, and calculated peak similarity  
238 values for all pairs of syllables within the first data set, and between both sets. The sound used in SPCC  
239 was removed the background noise bellow 400 Hz, and the frequency deviation was set as 0 Hz in  
240 SPCC. Based on the first data set, we used independent samples t-test to compare the SPCC similarity  
241 of pairs of syllables from the same male to similarity of pairs of syllables from different males. Each  
242 syllable was assigned to an individual, whose syllable (except the one being classified) has the  
243 maximum SPCC similarity value with the syllable to be assigned. The correct classification of

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244 individuals within a single day was expressed as the percentage of syllables correctly assigned. To  
245 calculate the correct rate of individual identification within the breeding season, we assigned the  
246 syllable from the second data set to one male in the first set, whose syllable has the maximum SPCC  
247 similarity value with the syllable to be assigned. The correct rate was expressed as the percentage of  
248 syllables correctly assigned.

249

#### 250 *Data analysis - call consistency ('repeatability') of syllable numbers*

251 We estimated repeatability in the number of syllables within bout using the *rpt* function in the R  
252 package *rptR* (Stoffel et al. 2017), which estimates repeatability as the proportion of among-individual  
253 variance out of the total variance (the sum of among-individual variance and within-individual  
254 variance), using a generalized linear mixed model framework (Nakagawa and Schielzeth 2010; Stoffel  
255 et al. 2017). The number of syllable recorded from all calling bouts followed a Poisson distribution  
256 (Supplementary Material Fig. 2), so we used a logit link function with individual males as the random  
257 effect. We used parametric bootstrapping (1000 iterations) to evaluate standard errors (SE), and  
258 likelihood-ratio test to evaluate the statistical significance of repeatability  $> 0$  against the null  
259 hypothesis repeatability = 0 as suggested by Stoffel et al. (2017). We calculated the repeatability ( $R$ ) of  
260 the number of syllables from: (1) two successive calling bouts (using 44 bouts from the 22 males); (2)  
261 several (more than two) calling bouts from the same day (using 159 bouts from 22 males); (3) calling  
262 bouts from different days across the breeding season surveys (using 256 bouts from 6 males)  
263 respectively.

264

## 265 **Results**

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266 *Acoustic identification of individuals from one day of sampling*

267 Based on the first data set, both CA and SPCC revealed a higher similarity value of pairs of syllables

268 from the same individual than different individuals (Figs. 2 and 3). Pearson correlation similarity of

269 paired syllables from the same male was  $0.66 \pm 0.30$  (mean  $\pm$  SD) which was significantly higher

270 (independent samples t-test,  $t_{2634} = 102.95$ ,  $P < 0.001$ ) than the similarity of paired syllables from

271 different males ( $0.00 \pm 0.45$ ). SPCC similarity of paired syllables from the same male was  $0.87 \pm 0.08$ ,

272 which was significantly higher (independent samples t-test,  $t_{2864} = 194.29$ ,  $P < 0.001$ ) than the

273 similarity of paired syllables from different males ( $0.50 \pm 0.18$ ). The rate of correct classification of all

274 30 individual males based on DFA, CA and SPCC was 90.8%, 71.5 % and 93.6 %, respectively

275 (Supplementary Material Fig. 3). The rate of correct classification of the six banded males based on

276 DFA, CA and SPCC were quite similar at 87.2 %, 75.6 % and 92.8 %, respectively.

277

278 *Acoustic identification of individuals within the breeding season*

279 The correct rate of individual identification decreased with increasing number of days from which

280 recordings were made within the breeding season, for DFA (Fig. 4a), CA (Fig. 4b), and SPCC (Fig. 4c).

281 The correct rate of individual identification based on recordings across multiple days within the

282 breeding season from all three measures declined significantly to 40.7 % (DFA), 27.0 % (CA) and

283 40.5 % (SPCC). The highest correct rate of individual identification (43.0 %) was achieved when using

284 the following variables to construct the discriminant functions: Tdur1, Fsta1, Fend1, Fmin1, Fmax1,

285 Tint, Tdur2, Tdis2, Fend2, Fpeak2, Fmin2.

286

287 *Call consistency ('repeatability') of syllable numbers*

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288 We found significant repeatability in the number of syllables within individual male cuckoos when  
289 analyzing data from recordings of two successive calling bouts (repeatability =  $0.55 \pm 0.18$  mean  $\pm$  SE;  
290 95% confidence interval range from 0.22 to 0.73;  $P = 0.001$ ). However, analyses of several (more than  
291 two) calling bouts from the same day (repeatability =  $0 \pm 0.02$ ; 95% confidence interval range from 0  
292 to 0.06;  $P = 0.436$ ) and calling bouts from different days (repeatability =  $0 \pm 0.01$ ; 95% confidence  
293 interval range from 0 to 0.02;  $P = 0.500$ ) revealed that the number of syllables were not significantly  
294 repeatable. In other words, we found that syllable number was not stable within individual males within  
295 the breeding season.

296

## 297 **Discussion**

298 Based on the same day recordings, we found that syllables from the same calling male were more  
299 similar in their characteristics than syllables from different males, and our analyses yielded high correct  
300 rates of classification of individuals from 93.6% (SPCC), 90.8 % (DFA), and 71.5% (CA). These  
301 results support the findings of the three previous studies (Jung et al. 2014; Li et al. 2017; and Zsebök et  
302 al. 2017) which found inter-individual variation of male cuckoo calls was much greater than intra-  
303 individual variation, and it was possible to identify individual male cuckoos based on call  
304 characteristics within one day. However, our results failed to provide evidence that male call structure  
305 is sufficiently stable to allow re-identification of individuals even within the same breeding season.

306 Based on repeated recordings from the 6 banded males, we found the correct rate of classifying  
307 syllables to individual males declined dramatically to 40.7 % from the DFA, 27.0 % from the CA, and  
308 40.5 % from the SPCC. Thus, male cuckoos recorded singing across the longer duration of the breeding  
309 season were more likely to have their calls incorrectly assigned than were males recorded from the



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310 same day. Furthermore, based on all combinations of variables used to construct discriminant  
311 functions, the highest correct rate of individual identification during the breeding season was only  
312 43.0 % from the DFA, suggesting that there is no single call variable or combination of variables that  
313 can be used to consistently identify individuals in this study. Among the 6 banded males, two males  
314 (represented by circle and reversed triangle symbols in Fig. 4) had consistently ~~reported~~ higher correct  
315 rate of classification than other males. ~~So~~Thus, in our study population, average call individuality  
316 ~~does~~did not ~~seem to~~ decline in all males, ~~but that~~. Instead, there ~~are~~were males with more consistent  
317 and males with less consistent calls.

318 We used three methods to identify individual male cuckoos based on vocal characteristics. Among  
319 these methods, DFA is the most popular analytical method applied to classify bird individuals based on  
320 call characteristics (Terry et al. 2005). The use of DFA is dependent on collecting an adequate number  
321 of calls per male to get a robust discriminant function (Williams and Titus 1988), so individuals with an  
322 insufficient number of calls were removed from the DFA, e.g. three males with less than ten calls were  
323 not include in the analysis in Zsebők et al. (2017). CA can SPCC can be conducted with much smaller  
324 sample sizes (two calls for each individual in theory) (Budka et al. 2015), thus reducing the need to  
325 omit males with fewer recordings from the analyses. The advantage of using SPCC is that whole  
326 spectrograms are used for the analyses (Terry et al. 2001), while CA use only the partial information  
327 (the measurement variables) (Budka et al. 2015), so the discriminative power of SPCC is always better  
328 than other methods (Xia et al 2011; Cramer 2013b; but not in Rogers and Paton 2005). However, SPCC  
329 is easily affected by background noise (Khanna et al 1997), and consequently only high signal-to-noise  
330 recordings can be used, which may limit the usage of SPCC.

331 Despite the use of different analytical techniques, we found that the correct rate of acoustic

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332 identification is highly consistent across all three techniques based on the recordings from a single day,  
333 but quickly declines when using recordings from multiple days within the same breeding season.  
334 Studies have identified vocal individuality and stability within a single breeding season for a number of  
335 different bird species (e.g. Kennedy et al. 2009; Wilson and Mennill 2010) or over subsequent breeding  
336 seasons for the same species (e.g. Kirschel et al. 2011). However, other studies have failed to find these  
337 stability (e.g. Feher et al. 2009; Kipper and Kiefer 2010; Zdenek et al. 2017). E.g. DFA correctly  
338 assigned 59% of female White-throated Magpie-Jay (*Calocitta formosa*) begging calls to individual  
339 females, but this correct rate declined sharply to less than 20% for some individuals when using  
340 recordings made over seven days within the same season (Ellis 2008). Calls of individual European  
341 Bitterns (*Botaurus stellaris*) recorded on a single day can be used to distinguish individual males but  
342 this discrimination by DFA declines when vocalisations from multiple days within the same season  
343 were used (Puglisi and Adamo 2004). DFA correctly assigned 65% of calls of American Crows  
344 (*Corvus brachyrhynchos*) to the correct individuals but these calls varied even over a period of just a  
345 few days (Yorzinski et al. 2006).

346       Temporary or permanently changes to vocal features does not necessarily prevent the  
347 identification and monitoring of individuals (Fox 2008; Kirschel et al. 2011). For example, Brownish-  
348 flanked Bush Warbler (*Horornis fortipes*) songs show variation in song characteristics, but the correct  
349 rate of acoustically identify individuals across the whole breeding season using DFA was 98% (Xia et  
350 al. 2010). Further, the coefficients of variation between individual bush warblers was always larger  
351 than 0.1 for 46 of the 52 measured variables (Xia et al. 2012), while the coefficients of variation  
352 between individuals in cuckoos was less than 0.07 in ten of 12 variables demonstrating the potential  
353 individual coding based on the first data set. Examination of the calls from the six banded males from

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354 all recording days suggests that only 7 variables show potential individual coding (with PIC value > 1),  
355 and 6 of these have a coefficients of variation between individuals of less than 0.04 (Supplementary  
356 Material Table 1), confirming that calls from different male cuckoos are very similar (see also the  
357 spectrograms in Supplementary Material Fig. 4). It is possible that slight changes of acoustic features  
358 within individual males may result in declines in correct rate of acoustically identify individuals in a  
359 long term (e.g. Průchová et al. 2017). Poor weather conditions throughout a breeding season can also  
360 modify the reliability of acoustic information contained within an individual's vocalizations,  
361 influencing their calling behaviour (e.g. Lengagne and Slater 2002) and this merits further investigation  
362 within our study population.

363 We found that the number of syllables produced by male cuckoos from two successive bouts was  
364 strongly repeatable within individual males, matching the findings of Møller et al. (2016a, b), but the  
365 consistency declined when using data from several (more than two) bouts within the same day, or when  
366 bouts from the different days were considered. These declines could be attributed to differences in the  
367 motivation for calling from one recording period to another, depending on the male's status in the  
368 breeding cycle. Call consistency in male cuckoos can vary in response to the number of males and  
369 females present in the immediate neighbourhood, with males able to increase the number of syllables in  
370 the presence of females or conspecific males (Møller et al. 2016a, b) and discriminate between  
371 neighbour and stranger males based on their calls (Moskát et al. 2017). Density of cuckoos is high in  
372 our study population (Li et al. 2016), where several individuals often occur in close proximity (less  
373 than 10 m) to each other (Supplementary Material Fig. 5). Thus, female choice, male-male competition  
374 and density may play a significant role in syllable repeatability in our male cuckoo population (e.g.  
375 Moskát et al. 2017). Male call consistency is also known to vary with environmental conditions (Møller

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376 et al. 2016a, b), as individuals compete for better quality sites with greater primary productivity i.e.  
377 better soil quality, food resources and an abundance of potential hosts. Little is known about the  
378 distribution of individual males across different gradients of habitat and soil conditions at our study  
379 site, but it remains plausible that male cuckoos may need more variable vocal signals in our population  
380 in order to adjust to changing social relationships and across a gradient of different environmental  
381 conditions.

382

### 383 **Authors' contributions**

384 Zhuqing Deng, Huw Lloyd, Canwei Xia, Donglai Li, Yanyun Zhang  
385 CX, YZ designed the experiments; ZD, DL participated in the field work; ZD, CX carried out the analyses;  
386 ZD, CX drafted the earlier version of the manuscript and HL, DL, YZ revised it. All authors have read  
387 and approved the final manuscript.

388

### 389 **Availability of data and materials**

390 Data generated or analysed during this study can be seen in Supplementary Material file 1 and  
391 Supplementary Material file 2. All recordings in this study are available from the corresponding author  
392 (xiacanwei@bnu.edu.cn) on reasonable request.

393

### 394 **Competing interests**

395 The authors declare that they have no competing interests.

396

### 397 **Ethical standards**

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398 Our research protocol was approved by the Animal Management Committee at the College of Life  
399 Sciences, Beijing Normal University under license number CLS-EAW-2016-017. Bird capture and  
400 banding were permitted by the National Bird-banding Center of China under license number  
401 H20110042. The experiments comply with the current laws of the country in which they were  
402 performed.

403

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564 **Table 1** Coefficients of variation (CV) and potential individual coding (PIC) for 15 acoustic variables  
565 based on 368 syllables from 30 males. CV<sub>w</sub> = coefficient of variation within males; CV<sub>b</sub> = coefficient  
566 of variation between males

567

568 **Fig. 1** Spectrogram of male common cuckoo call showing two successive calling bouts and six  
569 syllables

570

571 **Fig. 2** Distribution of similarity values for syllable pairs using Pearson's R from the same male (a) and  
572 different males (b). Similarity of paired syllables from the same male was higher than the similarity of  
573 paired syllables from different males

574

575 **Fig. 3** Distribution of similarity values for syllable pairs calculated by spectrographic cross-correlation  
576 from the same male (a) and different males (b). Similarity of paired syllables from the same male was  
577 higher than the similarity of paired syllables from different males

578

579 **Fig. 4** Correct ~~rate~~identification of ~~acoustic identify~~ individuals based on (a) discriminant function  
580 analyses (DFA), (b) correlation analysis (CA), and (c) spectrographic cross-correlation. Different  
581 symbol indicates different males

582

583 **Supplementary material figure 1** The measured variables in common cuckoo call (following Li et al.  
584 2017 and Møller et al. 2016a, b): duration of the element (t<sub>1</sub>, t<sub>3</sub>); time interval between the first and  
585 second element (t<sub>2</sub>); duration from the start of element to the point of maximum amplitude within that

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586 element ( $t_4$ ,  $t_5$ ); frequency at the start point of the element ( $f_1$ ,  $f_6$ ); frequency at the end point of the  
587 element ( $f_2$ ,  $f_7$ ); minimum frequencies of the element ( $f_3$ ,  $f_8$ ); maximum frequency of the element ( $f_4$ ,  
588  $f_9$ ); frequency of the maximum amplitude within the element ( $f_5$ ,  $f_{10}$ ).

589

590 **Supplementary material figure 2** The Poisson distribution of syllable numbers within calling bouts of  
591 male common cuckoos

592

593 **Supplementary material figure 3** Confusion matrix of classification based on (a) discriminant  
594 function analyses (DFA), (b) correlation analysis (CA), and (c) spectrographic cross-correlation.

595

596 **Supplementary material figure 4** Spectrograms of the advertisement call from six banded male  
597 cuckoos, showing the variation within and between individuals. Spectrograms represent in one square  
598 frame was from the same male, and was recorded on different days.

599

600 **Supplementary material figure 5** Four common cuckoos gathered together in close proximity.

601

602 **Supplementary material Table 1** Coefficients of variation (CV) and potential individual coding (PIC)  
603 for 15 acoustic variables based on 750 syllables from 6 banded male cuckoos across all recording days.

604  $CV_w$  = coefficient of variation within males;  $CV_b$  = coefficient of variation between males

605

606 **Supplementary Material file 1** Original measurement data of 1032 syllables from 30 males. See main  
607 text for explanations of variables

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608

609 **Supplementary Material file 2** Syllable numbers for 317 bouts from 22 males







24 **Within-season decline in the call consistency of individual male Common Cuckoo**

25 *(Cuculus canorus)*

26 **Abstract**

27 Numerous studies have identified individually distinctive vocal characteristics and call consistency in  
28 different bird species. If these are to be utilised as non-invasive markers for monitoring purposes, then  
29 these vocal characteristics must remain stable over time. Three recent studies have shown that it is  
30 possible to identify individual male Common Cuckoo (*Cuculus canorus*) based on vocal characteristics  
31 but whether these are stable over the duration of a breeding season, remains unknown. We recorded  
32 1032 syllables from 30 male Common Cuckoos in a Northeast Asian population. We colour-banded six  
33 of these males and made repeated recordings of their *cu-coo* advertisement call across a 19-day period  
34 of the breeding season in China. We used three methods to identify individuals: discriminant function  
35 analyses (DFA), correlation analysis (CA) and spectrographic cross-correlation (SPCC). We also used  
36 repeatability analysis to test whether call consistency (the number of syllables in each calling bout) was  
37 repeatable within individuals. Based on the same day recordings, calls from the same male were more  
38 similar in their characteristics than those of different males, and yielded correct rates of classifying  
39 individuals of 93.6% (SPCC), 90.8 % (DFA), and 71.5% (CA). However, these rates declined to 40.5%  
40 (SPCC), 40.7% (DFA) and 27% (CA) when using recordings over the 19-day period. Call consistency  
41 was repeatable within individuals across two successive calling bouts, but this individual repeatability  
42 disappeared when several (more than two) calling bouts from the same day or bouts from the different  
43 days were included in the analyses. Declines in the correct rate of identifying individual male cuckoos  
44 and call consistency in this study raises concerns that individual male cuckoo calls may be more  
45 variable than previously thought.

46 **Key words:** call consistency; Common Cuckoo; temporal stability; vocal individuality; vocal signals

47

## 48 **Introduction**

49 Recognition based on individually distinctive vocalizations is a functionally important aspect of  
50 signaling amongst many animal species (Falls 1982; Stoddard et al. 1996; Tibbets and Dale 2007).  
51 Numerous studies have shown the presence of individually distinctive vocal characteristics in different  
52 bird species (Terry et al. 2005) i.e. the presence of vocal characteristics that are less variable within  
53 individuals than between individuals (e.g. Galeotti and Pavan 1991; Rebbeck et al. 2001; Puglisi and  
54 Adamo 2004; Policht et al. 2009). Since bird vocalizations function for the long-distance broadcast of  
55 fitness related information (Catchpole and Slater 2008; Cramer 2013a), individual identification may  
56 benefit other birds (i.e. the signal receivers) in assessing the quality or behavioural state of individuals,  
57 territory occupation, or simply to maintain relationships with neighbouring individuals (e.g. Delgado et  
58 al. 2013; Sandoval et al. 2014). Many studies have examined the utility of individual vocal  
59 characteristics as non-invasive markers for monitoring individuals or populations (e.g. Laiolo et al.  
60 2007; Kirschel et al. 2011; Budka et al. 2015), or as a complimentary method to more traditional forms  
61 of monitoring (Blumstein et al. 2011), particularly for secretive or rare bird species (e.g. Kemp and  
62 Kemp 1989; Gilbert et al. 1994; Grava et al. 2008) for which monitoring will be essential for effective  
63 conservation management (Terry et al. 2005; Klenova et al. 2008). Furthermore, the number of  
64 syllables produced by calling males of some passerine and non-passerine bird species has also been  
65 found to be remarkably consistent over short periods of time (Catchpole and Slater 2008) suggesting  
66 that call length may also serve as a form of signaling for individual fitness during the breeding season  
67 (Møller et al. 2016a, b).

68           If unique vocal characteristics are to be used as markers for monitoring purposes, and if call  
69 consistency is a reliable indicator of male quality, then it is essential that calls remain unchanged i.e.  
70 remain ‘stable’ over significant periods of time (Terry et al. 2005; Dawson and Efford 2009; Linhart  
71 and Šálek 2017) such as the duration of a single breeding season, or even between different seasons.  
72 However, demonstrating such vocal stability over time has proven difficult because ideally, the acoustic  
73 analyses should be conducted on known individuals that have been individually marked e.g. using  
74 colour rings (Terry et al. 2005), but this is not always feasible. Some studies dealing with individual  
75 acoustic signals are based on recordings made from only a few days sampling within a single season  
76 (e.g. Li et al. 2017). Studies of the European Eagle Owl (*Bubo bubo*) found that within-year rates of  
77 correct classification of individuals varied from 60-100%, but between years, only 41.8% of ‘hoots’  
78 were correctly attributed to the territory owner in the previous year, with the between-year correct  
79 classification ranging from 0 to 100% (Grava et al. 2008). Even studies of the long-term stability of  
80 individual vocal characteristics of mammals have recorded reclassification rates of <50% (e.g.  
81 Jorgensen and French 1998). This has led some authors to conclude that the correct rate of acoustically  
82 identify individual birds over significant time periods will be lower (Linhart and Šálek 2017; Průchová  
83 et al. 2017) because of temporal changes in vocal characteristics caused by physiological changes,  
84 changes to the physical environment, social status, repertoire size and breeding stage (Delgado et al.  
85 2013).

86           The Common Cuckoo (*Cuculus canorus*) is a highly charismatic species widely known for its  
87 parasitic life history (Thorogood and Davies 2012; Yang et al. 2015). Male cuckoo advertisement calls  
88 show a highly stereotypical acoustic structure, consisting of two elements (‘*cu-coo*’) across their entire  
89 geographic range (Wei et al. 2015; Zsebók et al. 2017). Both male and female cuckoos utter loud, far-

90 carrying vocal signals during the breeding season to communicate with conspecifics (Moskát et al.  
91 2017) or misdirect hosts (York and Davies 2017; but see Liang et al. 2017). Surveying and monitoring  
92 populations of cuckoos during the breeding season using more traditional methods (e.g. point counts,  
93 transects) remains problematic due to the secretive life-history traits (Williams et al. 2015), and mist-  
94 netting surveys to date, tend to catch so few individuals. Surveys for this species carry added  
95 significance because monitoring cuckoo abundance and distribution may serve as an indicator of  
96 overall bird community composition (Morelli et al. 2015, 2017; Tryjanowski and Morelli 2015).  
97 Identifying individual male cuckoos based on vocal signals may represent a promising method to  
98 generate new information on the abundance and life history of this species, and three recent studies  
99 have kindled this hope (Jung et al. 2014; Li et al. 2017; Zsebök et al. 2017). These authors found the  
100 between-individual variation in male cuckoos' calls was much greater than within-individual variation,  
101 and that it was possible to identify individual male cuckoos based on specific call characteristics (Jung  
102 et al. 2014; Li et al. 2017; Zsebök et al. 2017). Furthermore, there appears to be a high degree of  
103 consistency in the number of syllables produced within individual males (e.g. Møller et al. 2016a, b),  
104 and these measures could be utilized to assess environmental conditions (e.g. Møller et al. 2016a, b).  
105 Despite these encouraging findings, all surveys to date have been conducted during just a short period  
106 of the breeding season, with the longest period of acoustic recordings taken over a 5-day period (Li et  
107 al. 2017), whilst the two other studies used recordings of calling males from just one occasion (Jung et  
108 al. 2014; Zsebök et al. 2017). One of these studies revealed a rate of correct classification calls to  
109 individual male of 91.9% from recordings made on one day, but this declined to 50% for recordings  
110 made more than two days apart (Li et al. 2017), suggesting that male vocal characteristics may not be  
111 temporally stable within a single breeding season.

112 In this study, we assess the feasibility of using vocal characteristics to identify individual male  
113 Common Cuckoos over a 19-day period during the breeding season based on the repeated recordings  
114 from male cuckoos in a northeast Asian population. We used three methods to identify individual  
115 males: discriminant function analyses, correlation analysis, and spectrographic cross-correlation. We  
116 also examined the consistency in the number of syllables produced by calling males during the  
117 breeding season by using acoustic data recorded from successive calling bouts, several (more than two)  
118 bouts recorded during the same day, and calling bouts recorded from different days during the breeding  
119 season.

120

## 121 **Methods**

### 122 *Study area and sound recording*

123 Field work was conducted from June 10th to July 29th 2017 in the Liaohe Delta Nature Reserve  
124 (41.034°N; 121.725°E), Liaoning Province, northeast China. This region represents one of the most  
125 important estuarine wetland in the country, which contains the largest area of reed-bed habitat along the  
126 coastal region of China, and consequently, extensive nesting habitat for Oriental Reed Warbler  
127 (*Acrocephalus orientalis*). Here, the Common Cuckoo is a summer breeding species, and  
128 predominantly parasitizes Oriental Reed Warbler nests during late May to early August (Li et al. 2016).  
129 Using mist nets, we trapped 20 individual cuckoos from June 9th to July 6th 2017. All individuals were  
130 banded with a numbered metal band, and fitted with a backpack radio transmitter (Biotrack Co., UK)  
131 weighing 2.12g (approximately 2.3% of the cuckoo's weight), using the method described by Rappole  
132 and Tipton (1991). This enabled us to track and observe cuckoos during the breeding season to obtain  
133 repeated recordings from known individuals.

134 All cuckoo vocalizations were recorded using a TASCAM DR-100MKIII recorder (Tascam Co.,  
135 Japan) and a Sennheiser MKH416 P48 external directional microphone (Sennheiser Co., Germany),  
136 with a sampling rate of 44.1 kHz and a sampling accuracy of 16 bits. In the study area, male cuckoos  
137 regularly call when perching on electrical wires (Li et al. 2016), which enabled us to approach within  
138 10-30 m of calling males and obtain the best possible recording with minimal background noise. In  
139 total, we recorded vocalizations of 30 different males, six of which were individually marked (banded)  
140 before recording. The fate of the other 14 banded cuckoos was unknown. We avoided repeated  
141 sampling of the remaining 24 unbanded males by observing the movements of each recorded male, and  
142 then travelling by motorcycle along one of the main roads until we encountered another male. We  
143 traveled each road only once, so we were sure that we recorded different males. This method for  
144 avoiding repeated sampling the same individual was also adopted in previous research (e.g. Li et al.  
145 2017; Zsebök et al. 2017). Due to bad weather or a lack of vocalizations on some of the survey days,  
146 both the number of days from which vocalizations were recorded, and the duration from the first day of  
147 recording to the last day, varied for the six banded males. In summary, we obtained recordings for each  
148 of the six banded males from 5 days across a nine day sampling period, 4 days across a ten day  
149 sampling period, 5 days across an eleven day sampling period, 7 days across a thirteen day sampling  
150 period, 9 days across a thirteen day sampling period, and from 11 days across a twenty day sampling  
151 period, respectively.

152

### 153 *Sound measurements*

154 We used Avisoft-SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany) to resample the  
155 recordings with 6 kHz and created spectrograms with the following settings: sample size, 16 bits; Fast

156 Fourier transform length 256 points; Hamming window with a frame size of 100% and an overlap of  
157 50%; frequency resolution 23 Hz; and time resolution of 21.3 ms. Male cuckoo advertisement calls  
158 consist of a repeated series of ‘*cu- coo*’ syllables, with each syllable composed of two distinctive  
159 elements (Møller et al. 2016a, b; Møller et al. 2017). We manually separated each element of each  
160 syllable (see Fig. 1) represented by a continuous trace in the spectrogram, and used Avisoft-SASLab  
161 Pro software to measure call features automatically following Li et al. (2017). We first automatically  
162 search the maximum amplitude in each element, and then determine the start and end points of each  
163 element at 19 dB level lower than the maximum amplitude. We selected 19 dB (rather than 16 dB in Li  
164 et al. 2017) because the characteristics of all syllables were explicit and clearly audible above the  
165 background noise on all recordings, allowing us to obtain comparable syllable parameters  
166 independently of the absolute intensity of the calls and the background noise level (Zollinger et al.  
167 2012). The following variables were then measured: duration of the element ( $T_{dur1}$ ,  $T_{dur2}$ ); duration  
168 from the start of element to the point of maximum amplitude within that element ( $T_{dis1}$ ,  $T_{dis2}$ );  
169 frequency at the start point of the element ( $F_{sta1}$ ,  $F_{sta2}$ ); frequency at the end point of the element  
170 ( $F_{end1}$ ,  $F_{end2}$ ); minimum frequency of the element ( $F_{min1}$ ,  $F_{min2}$ ); maximum frequency of the  
171 element ( $F_{max1}$ ,  $F_{max2}$ ); frequency of the maximum amplitude within the element ( $F_{peak1}$ ,  $F_{peak2}$ );  
172 time interval between the first and second element ( $T_{int}$ ) (Supplementary Material Fig. 1). In total, we  
173 measured 1032 syllables from 30 males: 750 syllables for 6 banded males and 282 syllables for 24 un-  
174 banded males (all original measurements of call features can be seen in Supplementary Material file 1).

175 We count the number of syllables within each calling bout based on the number visible from the  
176 spectrograms. The pause between successive bouts was always larger than 2 s, which is obvious greater  
177 than pause between successive syllables within one calling bout (see Fig. 1). We were unable to obtain



178 a complete calling bout for 8 unbanded males. Consequently, we measured a total of 317 calling bouts  
179 from 22 males (6 banded and 16 unbanded). The data on syllable numbers for each calling bout are  
180 presented in Supplementary Material file 2.

181

#### 182 *Data analyses – identification of vocal individuality*

183 We separated our acoustic data into two data sets. The first of these contained 368 syllables from 6  
184 banded males and 24 un-banded males, and every syllable from each individual in this data set was  
185 recorded on the same day. This first data set was used to construct discriminant functions, and to  
186 calculate the correct rate of acoustically identify individuals within one day (see below). The second  
187 data set contained 664 syllables recorded from the 6 banded males from all other days of field work.  
188 This second set was used to calculate the within-season correct rate of acoustically identify individuals  
189 (i.e. more than one day). All analyses were performed using R v. 3.4.1 (R Core Development Team,  
190 2017) with significance assumed at  $P < 0.05$ .

191 Discriminant function analyses (DFA) is a multivariate technique widely used to identify vocal  
192 individuality in birds (e.g. Delgado et al. 2013; Linhart and Šálek 2017) by combining variables with  
193 weighting coefficients to create a set of functions that can discriminate groups and classify new data  
194 into one of any number of pre-existing groups (Williams and Titus 1988; Mundry and 2007). We used  
195 calls from the first cuckoo data set to construct discriminant functions and examined the power of  
196 functions to correctly classify each syllable to an individual using a jack-knife analysis (Manly 1986;  
197 Galeotti and Sacchi 2001). The prior probability for each individual was set equal in DFA. For the  
198 second data set, we used the 30 discriminant functions constructed (corresponding to 30 males) based  
199 on the first data set to classify syllables of 6 banded males recorded across different days. The number

200 of syllables was not equal for each male in the first set, and varied from 6 to 20, with a mean of 12  
201 syllables per male. Due to the possibility that the variables used for identifying individuality in males  
202 from the first data set were not similar to those necessary for identifying males over the duration of the  
203 breeding season, we calculated the rate of correct classification in the second data set using the 30  
204 discriminant functions constructed from the first data set based on all combinations of variables (each  
205 time, only a subset of variables were used in discriminant functions). The highest correct rate and their  
206 corresponding combinations of variables were reported.

207 Correlation analysis (CA) was used to calculate the similarity of all pairs of syllables using  
208 Pearson's  $R$  based on call variables, and then identified individuals based on this similarity value  
209 (Budka et al. 2015). Using the first data set, we calculated coefficients of variation (CV) for each  
210 variable to compare differences within (CV<sub>w</sub>) and between (CV<sub>b</sub>) individuals (Robisson et al. 1993).  
211 We computed CV for each male based on syllables belonging to that male, and then calculated the  
212 mean CV for each male as CV<sub>w</sub>. We used the average value for each male to compute CV<sub>b</sub>. The ratio  
213 of CV<sub>b</sub> / CV<sub>w</sub> is the measurement of potential individual coding (PIC) which shows the importance of  
214 each variable used in identifying individuals (Charrier et al. 2001; Charrier et al. 2003). PIC value of  
215 Tdis2, Tdur1 and Tdis2 were less than or nearly equal to one (Table 1), meaning that these variables  
216 showed greater or similar variation within an individual than between individuals. Consequently, these  
217 three variables were not included in the subsequent analysis. Since call variables have different orders  
218 of magnitude e.g. the frequency of cuckoo syllables range in the hundreds Hz, while duration of  
219 syllables last nearly a tenth of a second, we standardized the variables using the formula: (value –  
220 mean) / standard deviation, and used these standardized variables to calculate the similarity of all pairs  
221 of syllables using Pearson correlation for both within male and between males. Based on the first data

222 set, independent samples t-test were used to compare the similarity of pairs of syllables from the same  
223 male to similarity of pairs of syllables from the different males. Each syllable was assigned to an  
224 individual, whose syllable (except the one being classified) has the maximum similarity value with the  
225 syllable to be assigned. The correct classification of individuals within a single day was expressed as  
226 the percentage of syllables correctly assigned. To calculate the correct rate of acoustically identify  
227 individuals within the breeding season, we first calculated the similarity of all pairs of syllables, one  
228 from the first data set and one from the second data set. We then assigned the syllable in the second set  
229 to one male in the first set, whose syllable has the maximum similarity value with the syllable to be  
230 assigned. The correct rate was expressed as the percentage of syllables correctly assigned.

231 Spectrographic cross-correlation (SPCC) is another widely used technique for identifying vocal  
232 individuality in bird species (e.g. McDonald and Wright 2011; Cramer 2013a). SPCC involves cross-  
233 correlating two spectrograms frame by frame as matrices of amplitude values that are incrementally  
234 overlapped over time (Clark et al. 1987; Radford 2005), then using the resultant peak correlation scores  
235 as measures of similarity (Khanna et al. 1997; Terry et al. 2001). We conducted the SPCC using the  
236 *'template cross correlation on short files'* function in Avisoft-SASLab Pro software. Firstly, we  
237 intercepted every syllable in the spectrograms and saved as *.son* files, and calculated peak similarity  
238 values for all pairs of syllables within the first data set, and between both sets. The sound used in SPCC  
239 was removed the background noise bellow 400 Hz, and the frequency deviation was set as 0 Hz in  
240 SPCC. Based on the first data set, we used independent samples t-test to compare the SPCC similarity  
241 of pairs of syllables from the same male to similarity of pairs of syllables from different males. Each  
242 syllable was assigned to an individual, whose syllable (except the one being classified) has the  
243 maximum SPCC similarity value with the syllable to be assigned. The correct classification of

244 individuals within a single day was expressed as the percentage of syllables correctly assigned. To  
245 calculate the correct rate of individual identification within the breeding season, we assigned the  
246 syllable from the second data set to one male in the first set, whose syllable has the maximum SPCC  
247 similarity value with the syllable to be assigned. The correct rate was expressed as the percentage of  
248 syllables correctly assigned.

249

250 *Data analysis - call consistency ('repeatability') of syllable numbers*

251 We estimated repeatability in the number of syllables within bout using the *rpt* function in the R  
252 package *rptR* (Stoffel et al. 2017), which estimates repeatability as the proportion of among-individual  
253 variance out of the total variance (the sum of among-individual variance and within-individual  
254 variance), using a generalized linear mixed model framework (Nakagawa and Schielzeth 2010; Stoffel  
255 et al. 2017). The number of syllable recorded from all calling bouts followed a Poisson distribution  
256 (Supplementary Material Fig. 2), so we used a logit link function with individual males as the random  
257 effect. We used parametric bootstrapping (1000 iterations) to evaluate standard errors (SE), and  
258 likelihood-ratio test to evaluate the statistical significance of repeatability  $> 0$  against the null  
259 hypothesis repeatability = 0 as suggested by Stoffel et al. (2017). We calculated the repeatability (*R*) of  
260 the number of syllables from: (1) two successive calling bouts (using 44 bouts from the 22 males); (2)  
261 several (more than two) calling bouts from the same day (using 159 bouts from 22 males); (3) calling  
262 bouts from different days across the breeding season surveys (using 256 bouts from 6 males)  
263 respectively.

264

265 **Results**

266 *Acoustic identification of individuals from one day of sampling*

267 Based on the first data set, both CA and SPCC revealed a higher similarity value of pairs of syllables  
268 from the same individual than different individuals (Figs. 2 and 3). Pearson correlation similarity of  
269 paired syllables from the same male was  $0.66 \pm 0.30$  (mean  $\pm$  SD) which was significantly higher  
270 (independent samples t-test,  $t_{2634} = 102.95$ ,  $P < 0.001$ ) than the similarity of paired syllables from  
271 different males ( $0.00 \pm 0.45$ ). SPCC similarity of paired syllables from the same male was  $0.87 \pm 0.08$ ,  
272 which was significantly higher (independent samples t-test,  $t_{2864} = 194.29$ ,  $P < 0.001$ ) than the  
273 similarity of paired syllables from different males ( $0.50 \pm 0.18$ ). The rate of correct classification of all  
274 30 individual males based on DFA, CA and SPCC was 90.8%, 71.5 % and 93.6 %, respectively  
275 (Supplementary Material Fig. 3). The rate of correct classification of the six banded males based on  
276 DFA, CA and SPCC were quite similar at 87.2 %, 75.6 % and 92.8 %, respectively.

277

278 *Acoustic identification of individuals within the breeding season*

279 The correct rate of individual identification decreased with increasing number of days from which  
280 recordings were made within the breeding season, for DFA (Fig. 4a), CA (Fig. 4b), and SPCC (Fig. 4c).  
281 The correct rate of individual identification based on recordings across multiple days within the  
282 breeding season from all three measures declined significantly to 40.7 % (DFA), 27.0 % (CA) and  
283 40.5 % (SPCC). The highest correct rate of individual identification (43.0 %) was achieved when using  
284 the following variables to construct the discriminant functions: Tdur1, Fsta1, Fend1, Fmin1, Fmax1,  
285 Tint, Tdur2, Tdis2, Fend2, Fpeak2, Fmin2.

286

287 *Call consistency ('repeatability') of syllable numbers*

288 We found significant repeatability in the number of syllables within individual male cuckoos when  
289 analyzing data from recordings of two successive calling bouts (repeatability =  $0.55 \pm 0.18$  mean  $\pm$  SE;  
290 95% confidence interval range from 0.22 to 0.73;  $P = 0.001$ ). However, analyses of several (more than  
291 two) calling bouts from the same day (repeatability =  $0 \pm 0.02$ ; 95% confidence interval range from 0  
292 to 0.06;  $P = 0.436$ ) and calling bouts from different days (repeatability =  $0 \pm 0.01$ ; 95% confidence  
293 interval range from 0 to 0.02;  $P = 0.500$ ) revealed that the number of syllables were not significantly  
294 repeatable. In other words, we found that syllable number was not stable within individual males within  
295 the breeding season.

296

## 297 **Discussion**

298 Based on the same day recordings, we found that syllables from the same calling male were more  
299 similar in their characteristics than syllables from different males, and our analyses yielded high correct  
300 rates of classification of individuals from 93.6% (SPCC), 90.8 % (DFA), and 71.5% (CA). These  
301 results support the findings of the three previous studies (Jung et al. 2014; Li et al. 2017; and Zsebök et  
302 al. 2017) which found inter-individual variation of male cuckoo calls was much greater than intra-  
303 individual variation, and it was possible to identify individual male cuckoos based on call  
304 characteristics within one day. However, our results failed to provide evidence that male call structure  
305 is sufficiently stable to allow re-identification of individuals even within the same breeding season.

306 Based on repeated recordings from the 6 banded males, we found the correct rate of classifying  
307 syllables to individual males declined dramatically to 40.7 % from the DFA, 27.0 % from the CA, and  
308 40.5 % from the SPCC. Thus, male cuckoos recorded singing across the longer duration of the breeding  
309 season were more likely to have their calls incorrectly assigned than were males recorded from the

310 same day. Furthermore, based on all combinations of variables used to construct discriminant  
311 functions, the highest correct rate of individual identification during the breeding season was only  
312 43.0 % from the DFA, suggesting that there is no single call variable or combination of variables that  
313 can be used to consistently identify individuals in this study. Among the 6 banded males, two males  
314 (represented by circle and reversed triangle symbols in Fig. 4) had consistently higher correct rate of  
315 classification than other males. Thus, in our study population, average call individuality did not decline  
316 in all males. Instead, there were males with more consistent and males with less consistent calls.

317 We used three methods to identify individual male cuckoos based on vocal characteristics. Among  
318 these methods, DFA is the most popular analytical method applied to classify bird individuals based on  
319 call characteristics (Terry et al. 2005). The use of DFA is dependent on collecting an adequate number  
320 of calls per male to get a robust discriminant function (Williams and Titus 1988), so individuals with an  
321 insufficient number of calls were removed from the DFA, e.g. three males with less than ten calls were  
322 not include in the analysis in Zsebök et al. (2017). CA can SPCC can be conducted with much smaller  
323 sample sizes (two calls for each individual in theory) (Budka et al. 2015), thus reducing the need to  
324 omit males with fewer recordings from the analyses. The advantage of using SPCC is that whole  
325 spectrograms are used for the analyses (Terry et al. 2001), while CA use only the partial information  
326 (the measurement variables) (Budka et al. 2015), so the discriminative power of SPCC is always better  
327 than other methods (Xia et al 2011; Cramer 2013b; but not in Rogers and Paton 2005). However, SPCC  
328 is easily affected by background noise (Khanna et al 1997), and consequently only high signal-to-noise  
329 recordings can be used, which may limit the usage of SPCC.

330 Despite the use of different analytical techniques, we found that the correct rate of acoustic  
331 identification is highly consistent across all three techniques based on the recordings from a single day,

332 but quickly declines when using recordings from multiple days within the same breeding season.  
333 Studies have identified vocal individuality and stability within a single breeding season for a number of  
334 different bird species (e.g. Kennedy et al. 2009; Wilson and Mennill 2010) or over subsequent breeding  
335 seasons for the same species (e.g. Kirschel et al. 2011). However, other studies have failed to find these  
336 stability (e.g. Feher et al. 2009; Kipper and Kiefer 2010; Zdenek et al. 2017). E.g. DFA correctly  
337 assigned 59% of female White-throated Magpie-Jay (*Calocitta formosa*) begging calls to individual  
338 females, but this correct rate declined sharply to less than 20% for some individuals when using  
339 recordings made over seven days within the same season (Ellis 2008). Calls of individual European  
340 Bitterns (*Botaurus stellaris*) recorded on a single day can be used to distinguish individual males but  
341 this discrimination by DFA declines when vocalisations from multiple days within the same season  
342 were used (Puglisi and Adamo 2004). DFA correctly assigned 65% of calls of American Crows  
343 (*Corvus brachyrhynchos*) to the correct individuals but these calls varied even over a period of just a  
344 few days (Yorzinski et al. 2006).

345       Temporary or permanently changes to vocal features does not necessarily prevent the  
346 identification and monitoring of individuals (Fox 2008; Kirschel et al. 2011). For example, Brownish-  
347 flanked Bush Warbler (*Horornis fortipes*) songs show variation in song characteristics, but the correct  
348 rate of acoustically identify individuals across the whole breeding season using DFA was 98% (Xia et  
349 al. 2010). Further, the coefficients of variation between individual bush warblers was always larger  
350 than 0.1 for 46 of the 52 measured variables (Xia et al. 2012), while the coefficients of variation  
351 between individuals in cuckoos was less than 0.07 in ten of 12 variables demonstrating the potential  
352 individual coding based on the first data set. Examination of the calls from the six banded males from  
353 all recording days suggests that only 7 variables show potential individual coding (with PIC value > 1),



354 and 6 of these have a coefficients of variation between individuals of less than 0.04 (Supplementary  
355 Material Table 1), confirming that calls from different male cuckoos are very similar (see also the  
356 spectrograms in Supplementary Material Fig. 4). It is possible that slight changes of acoustic features  
357 within individual males may result in declines in correct rate of acoustically identify individuals in a  
358 long term (e.g. Průchová et al. 2017). Poor weather conditions throughout a breeding season can also  
359 modify the reliability of acoustic information contained within an individual's vocalizations,  
360 influencing their calling behaviour (e.g. Lengagne and Slater 2002) and this merits further investigation  
361 within our study population.

362 We found that the number of syllables produced by male cuckoos from two successive bouts was  
363 strongly repeatable within individual males, matching the findings of Møller et al. (2016a, b), but the  
364 consistency declined when using data from several (more than two) bouts within the same day, or when  
365 bouts from the different days were considered. These declines could be attributed to differences in the  
366 motivation for calling from one recording period to another, depending on the male's status in the  
367 breeding cycle. Call consistency in male cuckoos can vary in response to the number of males and  
368 females present in the immediate neighbourhood, with males able to increase the number of syllables in  
369 the presence of females or conspecific males (Møller et al. 2016a, b) and discriminate between  
370 neighbour and stranger males based on their calls (Moskát et al. 2017). Density of cuckoos is high in  
371 our study population (Li et al. 2016), where several individuals often occur in close proximity (less  
372 than 10 m) to each other (Supplementary Material Fig. 5). Thus, female choice, male-male competition  
373 and density may play a significant role in syllable repeatability in our male cuckoo population (e.g.  
374 Moskát et al. 2017). Male call consistency is also known to vary with environmental conditions (Møller  
375 et al. 2016a, b), as individuals compete for better quality sites with greater primary productivity i.e.

376 better soil quality, food resources and an abundance of potential hosts. Little is known about the  
377 distribution of individual males across different gradients of habitat and soil conditions at our study  
378 site, but it remains plausible that male cuckoos may need more variable vocal signals in our population  
379 in order to adjust to changing social relationships and across a gradient of different environmental  
380 conditions.

381

### 382 **Authors' contributions**

383 Zhuqing Deng, Huw Lloyd, Canwei Xia, Donglai Li, Yanyun Zhang

384 CX, YZ designed the experiments; ZD, DL participated in the field work; ZD, CX carried out the analyses;

385 ZD, CX drafted the earlier version of the manuscript and HL, DL, YZ revised it. All authors have read

386 and approved the final manuscript.

387

### 388 **Availability of data and materials**

389 Data generated or analysed during this study can be seen in Supplementary Material file 1 and

390 Supplementary Material file 2. All recordings in this study are available from the corresponding author

391 (xiacanwei@bnu.edu.cn) on reasonable request.

392

### 393 **Competing interests**

394 The authors declare that they have no competing interests.

395

### 396 **Ethical standards**

397 Our research protocol was approved by the Animal Management Committee at the College of Life

398 Sciences, Beijing Normal University under license number CLS-EAW-2016-017. Bird capture and  
399 banding were permitted by the National Bird-banding Center of China under license number  
400 H20110042. The experiments comply with the current laws of the country in which they were  
401 performed.

402

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- 562

563 **Table 1** Coefficients of variation (CV) and potential individual coding (PIC) for 15 acoustic variables  
564 based on 368 syllables from 30 males. CV<sub>w</sub> = coefficient of variation within males; CV<sub>b</sub> = coefficient  
565 of variation between males

566

567 **Fig. 1** Spectrogram of male common cuckoo call showing two successive calling bouts and six  
568 syllables

569

570 **Fig. 2** Distribution of similarity values for syllable pairs using Pearson's R from the same male (a) and  
571 different males (b). Similarity of paired syllables from the same male was higher than the similarity of  
572 paired syllables from different males

573

574 **Fig. 3** Distribution of similarity values for syllable pairs calculated by spectrographic cross-correlation  
575 from the same male (a) and different males (b). Similarity of paired syllables from the same male was  
576 higher than the similarity of paired syllables from different males

577

578 **Fig. 4** Correct identification of individuals based on (a) discriminant function analyses (DFA), (b)  
579 correlation analysis (CA), and (c) spectrographic cross-correlation. Different symbol indicates different  
580 males

581

582 **Supplementary material figure 1** The measured variables in common cuckoo call (following Li et al.  
583 2017 and Møller et al. 2016a, b): duration of the element (t<sub>1</sub>, t<sub>3</sub>); time interval between the first and  
584 second element (t<sub>2</sub>); duration from the start of element to the point of maximum amplitude within that

585 element (t4, t5); frequency at the start point of the element (f1, f6); frequency at the end point of the  
586 element (f2, f7); minimum frequencies of the element (f3, f8); maximum frequency of the element (f4,  
587 f9); frequency of the maximum amplitude within the element (f5, f10).

588

589 **Supplementary material figure 2** The Poisson distribution of syllable numbers within calling bouts of  
590 male common cuckoos

591

592 **Supplementary material figure 3** Confusion matrix of classification based on (a) discriminant  
593 function analyses (DFA), (b) correlation analysis (CA), and (c) spectrographic cross-correlation.

594

595 **Supplementary material figure 4** Spectrograms of the advertisement call from six banded male  
596 cuckoos, showing the variation within and between individuals. Spectrograms represent in one square  
597 frame was from the same male, and was recorded on different days.

598

599 **Supplementary material figure 5** Four common cuckoos gathered together in close proximity.

600

601 **Supplementary material Table 1** Coefficients of variation (CV) and potential individual coding (PIC)  
602 for 15 acoustic variables based on 750 syllables from 6 banded male cuckoos across all recording days.

603 CV<sub>w</sub> = coefficient of variation within males; CV<sub>b</sub> = coefficient of variation between males

604

605 **Supplementary Material file 1** Original measurement data of 1032 syllables from 30 males. See main  
606 text for explanations of variables

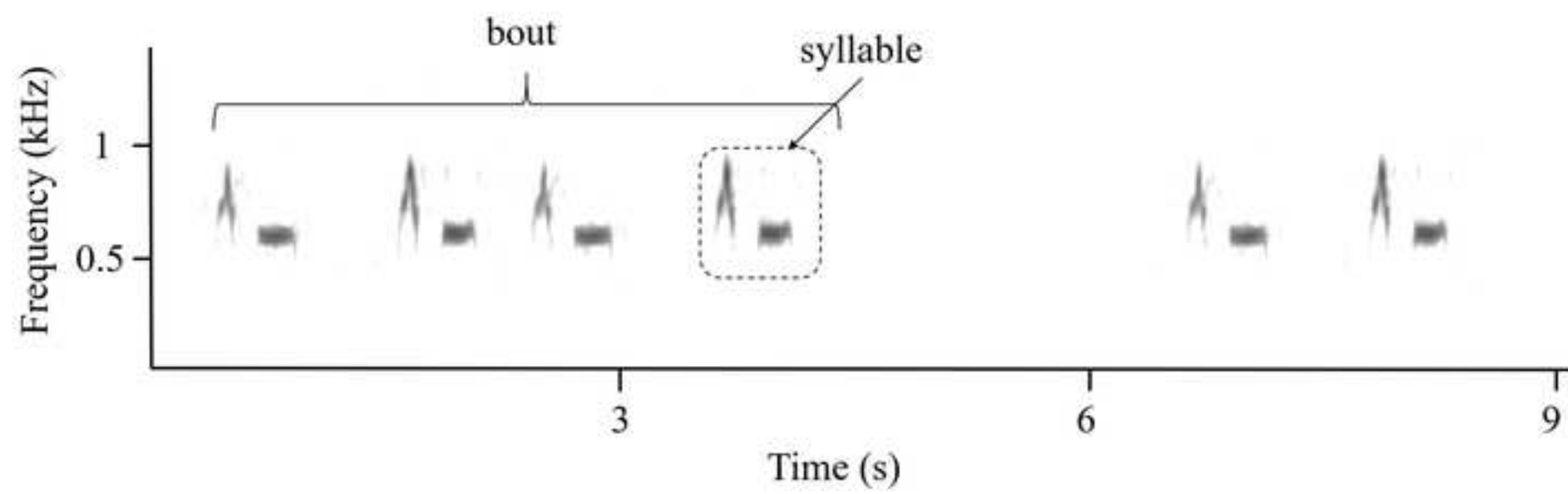
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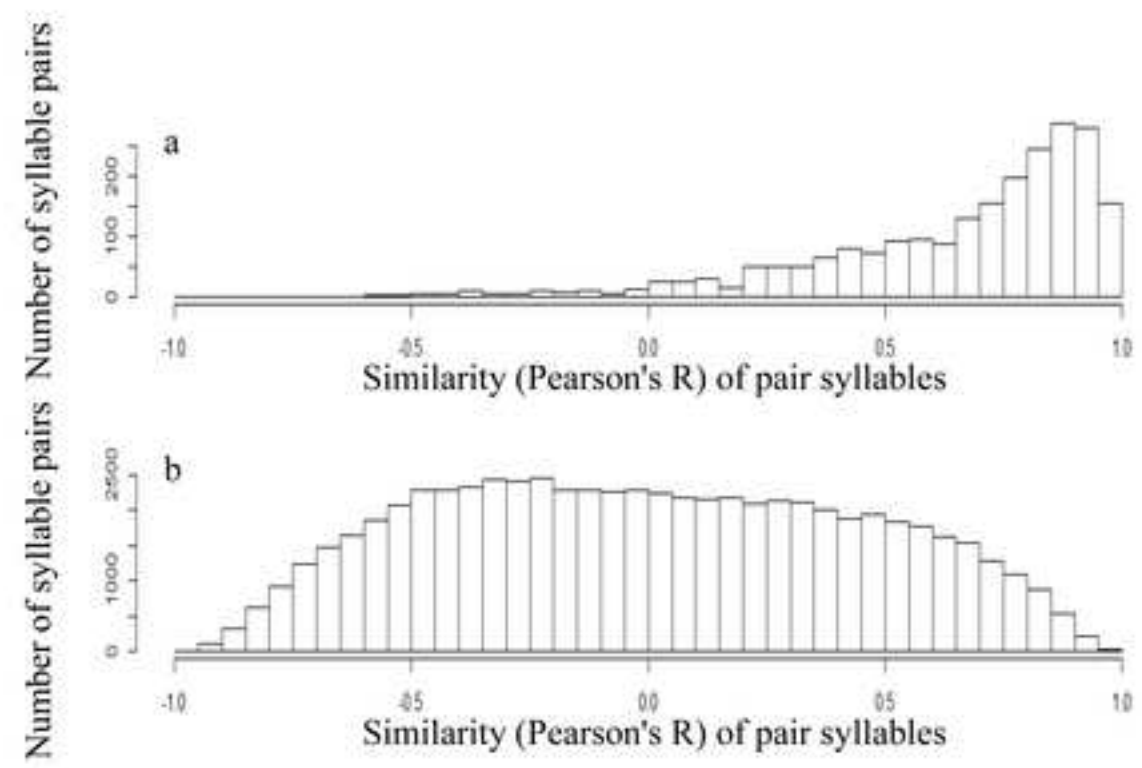
608 **Supplementary Material file 2** Syllable numbers for 317 bouts from 22 males

Table1

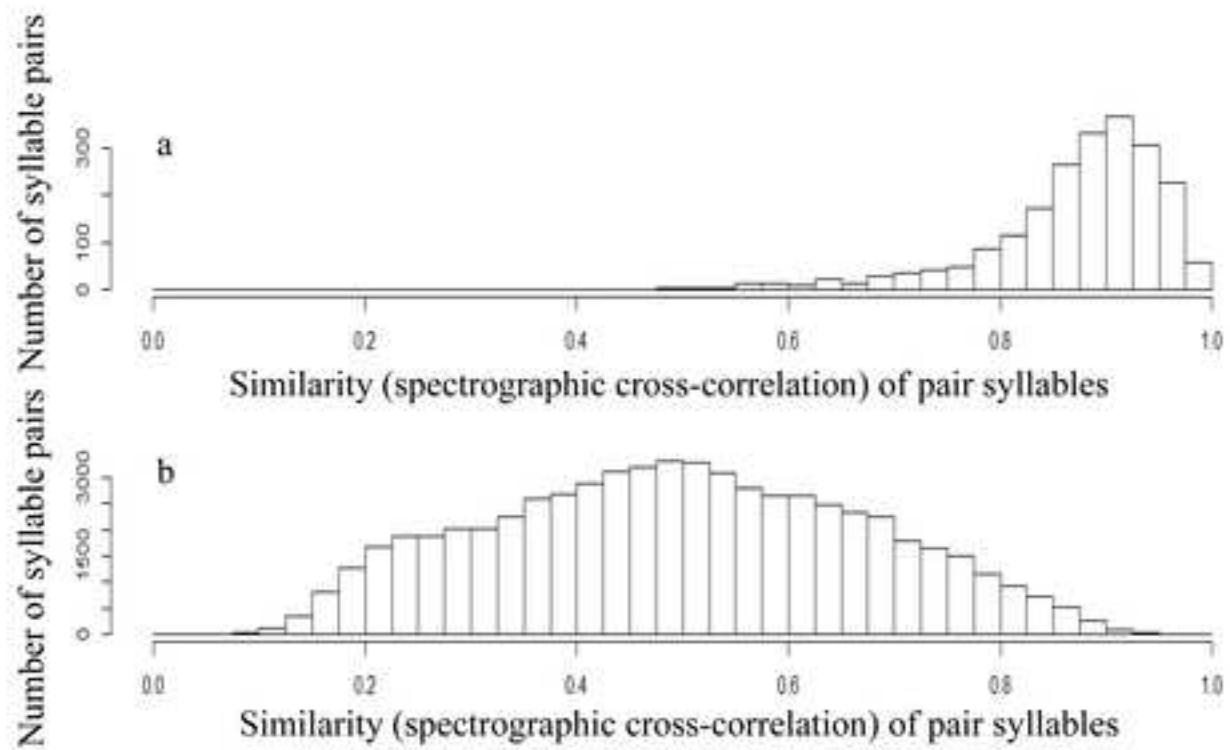
Variables*	CV <sub>w</sub>	CV <sub>b</sub>	PIC
Tdur1	0.152	0.167	1.097
Tdis1	0.315	0.322	1.021
Fsta1	0.036	0.062	1.734
Fend1	0.046	0.056	1.22
Fpeak1	0.019	0.057	2.942
Fmin1	0.037	0.049	1.343
Fmax1	0.016	0.054	3.308
Tint	0.068	0.137	2.01
Tdur2	0.089	0.143	1.606
Tdis2	0.25	0.213	0.85
Fsta2	0.015	0.043	2.921
Fend2	0.016	0.045	2.823
Fpeak2	0.008	0.045	5.604
Fmin2	0.013	0.044	3.315
Fmax2	0.011	0.045	4.232

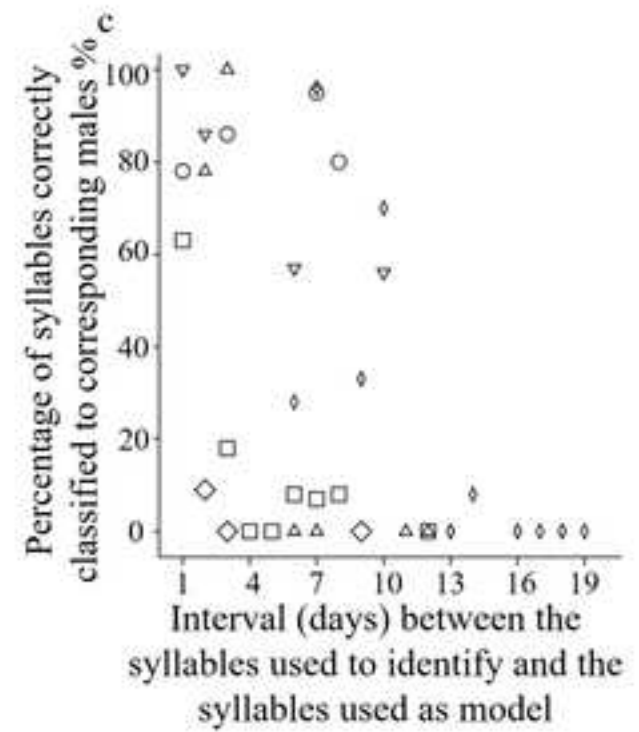
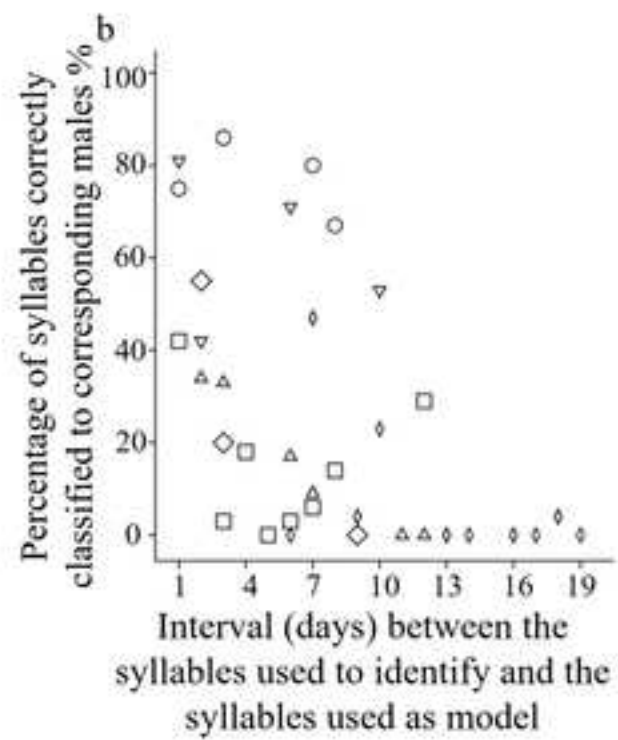
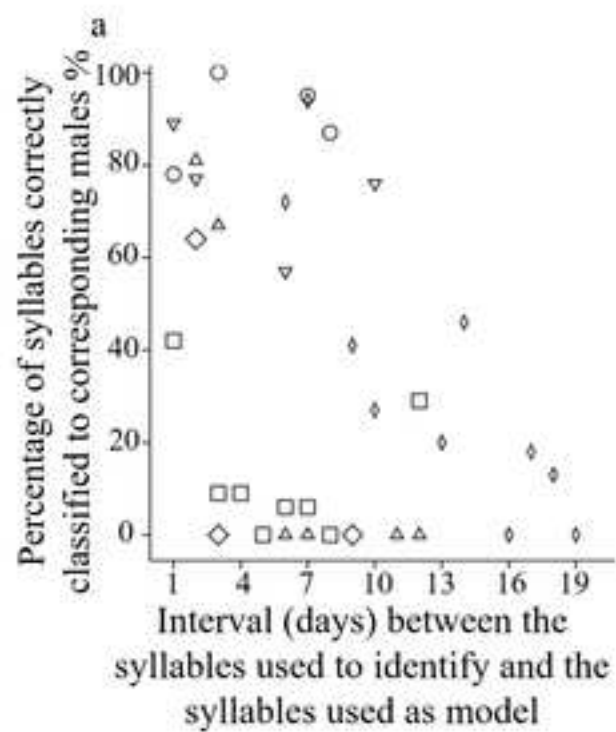
\* variable names are given in the main text.













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