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Within-season decline in the call consistency of individual male Common Cuckoo (*Cuculus canorus*)

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Abstract

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

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

Introduction

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

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

The Common Cuckoo (*Cuculus canorus*) is a highly charismatic species widely known for its parasitic life history (Thorogood and Davies 2012; Yang et al. 2015). Male cuckoo advertisement calls show a highly stereotypical acoustic structure, consisting of two elements (‘*cu-coo*’) across their entire geographic range (Wei et al. 2015; Zsebők et al. 2017). Both male and female cuckoos utter loud, farcarrying vocal signals during the breeding season to communicate with conspecifics (Moskát et al. 2017) or misdirect hosts (York and Davies 2017; but see Liang et al. 2017). Surveying and monitoring populations of cuckoos during the breeding season using more traditional methods (e.g. point counts, transects) remains problematic due to the secretive life-history traits (Williams et al. 2015), and mist-netting surveys to date, tend to catch so few individuals. Surveys for this species carry added significance because monitoring cuckoo abundance and distribution may serve as an indicator of overall bird community composition (Morelli et al. 2015, 2017; Tryjanowski and Morelli 2015). Identifying individual male cuckoos based on vocal signals may represent a promising method to generate new information on the abundance and life history of this species, and three recent studies have kindled this hope (Jung et al. 2014; Li et al. 2017; Zsebők et al. 2017). These authors found the between-individual variation in male cuckoos' calls was much greater than within-individual variation, and that it was possible to identify individual male cuckoos based on specific call characteristics (Jung et al. 2014; Li et al. 2017; Zsebők et al. 2017). Furthermore, there appears to be a high degree of consistency in the number of syllables produced within individual males (e.g. Møller et al. 2016a, b), and these measures could be utilized to assess environmental conditions (e.g. Møller et al. 2016a, b). Despite these encouraging findings, all surveys to date have been conducted during just a short period of the breeding season, with the longest period of acoustic recordings taken over a 5-day period (Li et al. 2017), whilst the two other studies used recordings of calling males from just one occasion (Jung et al. 2014; Zsebők et al. 2017). One of these studies revealed a rate of correct classification calls to individual male of 91.9% from recordings made on one day, but this declined to 50% for recordings made more than two days apart (Li et al. 2017), suggesting that male vocal characteristics may not be temporally stable within a single breeding season.

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

Methods

Study area and sound recording

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

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

Sound measurements

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

Fourier transform length 256 points; Hamming window with a frame size of 100% and an overlap of 50%; frequency resolution 23 Hz; and time resolution of 21.3 ms. Male cuckoo advertisement calls consist of a repeated series of '*cu- coo*' syllables, with each syllable composed of two distinctive elements (Møller et al. 2016a, b; Møller et al. 2017). We manually separated each element of each syllable (see Fig. 1) represented by a continuous trace in the spectrogram, and used Avisoft-SASLab Pro software to measure call features automatically following Li et al. (2017). We first automatically search the maximum amplitude in each element, and then determine the start and end points of each element at 19 dB lever lower than the maximum amplitude. We selected 19 dB (rather than 16 dB in Li et al. 2017) because the characteristics of all syllables were explicit and clearly audible above the background noise on all recordings, allowing us to obtain comparable syllable parameters independently of the absolute intensity of the calls and the background noise level (Zollinger et al. 2012). The following variables were then measured: duration of the element (Tdur1, Tdur2); duration from the start of element to the point of maximum amplitude within that element (Tdis1, Tdis2); frequency at the start point of the element (Fsta1, Fsta2); frequency at the end point of the element (Fend1, Fend2); minimum frequency of the element (Fmin1, Fmim2); maximum frequency of the element (Fmax1, Fmax2); frequency of the maximum amplitude within the element (Fpeak1, Fpeak2); time interval between the first and second element (Tint) (Supplementary Material Fig. 1). In total, we measured 1032 syllables from 30 males: 750 syllables for 6 banded males and 282 syllables for 24 un-banded males (all original measurements of call features can be seen in Supplementary Material file 1).

We count the number of syllables within each calling bout based on the number visible from the spectrograms. The pause between successive bouts was always larger than 2 s, which is obvious greater than pause between successive syllables within one calling bout (see Fig. 1). We were unable to obtain a complete calling bout for 8 unbanded males. Consequently, we measured a total of 317 calling bouts from 22 males (6 banded and 16 unbanded). The data on syllable numbers for each calling bout are presented in Supplementary Material file 2.

Data analyses – identification of vocal individuality

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

Discriminant function analyses (DFA) is a multivariate technique widely used to identify vocal individuality in birds (e.g. Delgado et al. 2013; Linhart and Šálek 2017) by combining variables with weighting coefficients to create a set of functions that can discriminate groups and classify new data into one of any number of pre-existing groups (Williams and Titus 1988; Mundry and 2007). We used calls from the first cuckoo data set to construct discriminant functions and examined the power of functions to correctly classify each syllable to an individual using a jack-knife analysis (Manly 1986; Galeotti and Sacchi 2001). The prior probability for each individual was set equal in DFA. For the second data set, we used the 30 discriminant functions constructed (corresponding to 30 males) based on the first data set to classify syllables of 6 banded males recorded across different days. The number of syllables was not equal for each male in the first set, and varied from 6 to 20, with a mean of 12 syllables per male. Due to the possibility that the variables used for identifying individuality in males from the first data set were not similar to those necessary for identifying males over the duration of the breeding season, we calculated the rate of correct classification in the second data set using the 30 discriminant functions constructed from the first data set based on all combinations of variables (each time, only a subset of variables were used in discriminant functions). The highest correct rate and their corresponding combinations of variables were reported.

Correlation analysis (CA) was used to calculate the similarity of all pairs of syllables using Pearson's *R* based on call variables, and then identified individuals based on this similarity value (Budka et al. 2015). Using the first data set, we calculated coefficients of variation (CV) for each variable to compare differences within (CVw) and between (CVb) individuals (Robisson et al. 1993). We computed CV for each male based on syllables belonging to that male, and then calculated the mean CV for each male as CVw. We used the average value for each male to compute CVb. The ratio of CVb / CVw is the measurement of potential individual coding (PIC) which shows the importance of each variable used in identifying individuals (Charrier et al. 2001; Charrier et al. 2003). PIC value of Tdis2, Tdur1 and Tdis2 were less than or nearly equal to one (Table 1), meaning that these variables showed greater or similar variation within an individual than between individuals. Consequently, these three variables were not included in the subsequent analysis. Since call variables have different orders of magnitude e.g. the frequency of cuckoo syllables range in the hundreds Hz, while duration of syllables last nearly a tenth of a second, we standardized the variables using the formula: (value − mean) / standard deviation, and used these standardized variables to calculate the similarity of all pairs of syllables using Pearson correlation for both within male and between males. Based on the first data set, independent samples t-test were used to compare the similarity of pairs of syllables from the same male to similarity of pairs of syllables from the different males. Each syllable was assigned to an individual, whose syllable (except the one being classified) has the maximum similarity value with the syllable to be assigned. The correct classification of individuals within a single day was expressed as the percentage of syllables correctly assigned. To calculate the correct rate of acoustically identify individuals within the breeding season, we first calculated the similarity of all pairs of syllables, one from the first data set and one from the second data set. We then assigned the syllable in the second set to one male in the first set, whose syllable has the maximum similarity value with the syllable to be assigned. The correct rate was expressed as the percentage of syllables correctly assigned.

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

Data analysis - call consistency (‘repeatability’) of syllable numbers

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

Results

Acoustic identification of individuals from one day of sampling

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

Acoustic identification of individuals within the breeding season

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

Call consistency ('repeatability') of syllable numbers

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

Discussion

Based on the same day recordings, we found that syllables from the same calling male were more similar in their characteristics than syllables from different males, and our analyses yielded high correct rates of classification of individuals from 93.6% (SPCC), 90.8 % (DFA), and 71.5% (CA). These results support the findings of the three previous studies (Jung et al. 2014; Li et al. 2017; and Zsebők et al. 2017) which found inter-individual variation of male cuckoo calls was much greater than intra-individual variation, and it was possible to identify individual male cuckoos based on call characteristics within one day. However, our results failed to provide evidence that male call structure is sufficiently stable to allow re-identification of individuals even within the same breeding season. Based on repeated recordings from the 6 banded males, we found the correct rate of classifying syllables to individual males declined dramatically to 40.7 % from the DFA, 27.0 % from the CA, and 40.5 % from the SPCC. Thus, male cuckoos recorded singing across the longer duration of the breeding season were more likely to have their calls incorrectly assigned than were males recorded from the same day. Furthermore, based on all combinations of variables used to construct discriminant functions, the highest correct rate of individual identification during the breeding season was only 43.0 % from the DFA, suggesting that there is no single call variable or combination of variables that can be used to consistently identify individuals in this study. Among the 6 banded males, two males (represented by circle and reversed triangle symbols in Fig. 4) had consistently ~~reported~~ higher correct rate of classification than other males. ~~So~~Thus, in our study population, average call individuality ~~does~~did not ~~seem to~~ decline in all males~~, but that~~. Instead, there ~~are~~were males with more consistent and males with less consistent calls.

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

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

Temporary or permanently changes to vocal features does not necessarily prevent the identification and monitoring of individuals (Fox 2008; Kirschel et al. 2011). For example, Brownish-flanked Bush Warbler (*Horornis fortipes*) songs show variation in song characteristics, but the correct rate of acoustically identify individuals across the whole breeding season using DFA was 98% (Xia et al. 2010). Further, the coefficients of variation between individual bush warblers was always larger than 0.1 for 46 of the 52 measured variables (Xia et al. 2012), while the coefficients of variation between individuals in cuckoos was less than 0.07 in ten of 12 variables demonstrating the potential individual coding based on the first data set. Examination of the calls from the six banded males from all recording days suggests that only 7 variables show potential individual coding (with PIC value > 1), and 6 of these have a coefficients of variation between individuals of less than 0.04 (Supplementary Material Table 1), confirming that calls from different male cuckoos are very similar (see also the spectrograms in Supplementary Material Fig. 4). It is possible that slight changes of acoustic features within individual males may result in declines in correct rate of acoustically identify individuals in a long term (e.g. Průchová et al. 2017). Poor weather conditions throughout a breeding season can also modify the reliability of acoustic information contained within an individual's vocalizations, influencing their calling behaviour (e.g. Lengagne and Slater 2002) and this merits further investigation within our study population.

We found that the number of syllables produced by male cuckoos from two successive bouts was strongly repeatable within individual males, matching the findings of Møller et al. (2016a, b), but the consistency declined when using data from several (more than two) bouts within the same day, or when bouts from the different days were considered. These declines could be attributed to differences in the motivation for calling from one recording period to another, depending on the male's status in the breeding cycle. Call consistency in male cuckoos can vary in response to the number of males and females present in the immediate neighbourhood, with males able to increase the number of syllables in the presence of females or conspecific males (Møller et al. 2016a, b) and discriminate between neighbour and stranger males based on their calls (Moskát et al. 2017). Density of cuckoos is high in our study population (Li et al. 2016), where several individuals often occur in close proximity (less than 10 m) to each other (Supplementary Material Fig. 5). Thus, female choice, male-male competition and density may play a significant role in syllable repeatability in our male cuckoo population (e.g. Moskát et al. 2017). Male call consistency is also known to vary with environmental conditions (Møller et al. 2016a, b), as individuals compete for better quality sites with greater primary productivity i.e. better soil quality, food resources and an abundance of potential hosts. Little is known about the distribution of individual males across different gradients of habitat and soil conditions at our study site, but it remains plausible that male cuckoos may need more variable vocal signals in our population in order to adjust to changing social relationships and across a gradient of different environmental conditions.

Authors' contributions

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

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

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Ethical standards

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

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Table 1 Coefficients of variation (CV) and potential individual coding (PIC) for 15 acoustic variables based on 368 syllables from 30 males. CVw = coefficient of variation within males; CVb = coefficient of variation between males

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

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

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

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

Supplementary material figure 1 The measured variables in common cuckoo call (following Li et al. 2017 and Møller et al. 2016a, b): duration of the element (t1, t3); time interval between the first and second element (t2); duration from the start of element to the point of maximum amplitude within that element (t4, t5); frequency at the start point of the element (f1, f6); frequency at the end point of the element (f2, f7); minimum frequencies of the element (f3, f8); maximum frequency of the element (f4, f9); frequency of the maximum amplitude within the element (f5, f10).

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

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

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

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

Supplementary material Table 1 Coefficients of variation (CV) and potential individual coding (PIC) for 15 acoustic variables based on 750 syllables from 6 banded male cuckoos across all recording days. CVw = coefficient of variation within males; CVb = coefficient of variation between males

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

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

Within-season decline in the call consistency of individual male Common Cuckoo (*Cuculus canorus*)

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Within-season decline in the call consistency of individual male Common Cuckoo (*Cuculus canorus*)

Abstract

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

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

Introduction

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

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

The Common Cuckoo (*Cuculus canorus*) is a highly charismatic species widely known for its parasitic life history (Thorogood and Davies 2012; Yang et al. 2015). Male cuckoo advertisement calls show a highly stereotypical acoustic structure, consisting of two elements ('*cu-coo*') across their entire geographic range (Wei et al. 2015; Zsebők et al. 2017). Both male and female cuckoos utter loud, farcarrying vocal signals during the breeding season to communicate with conspecifics (Moskát et al. 2017) or misdirect hosts (York and Davies 2017; but see Liang et al. 2017). Surveying and monitoring populations of cuckoos during the breeding season using more traditional methods (e.g. point counts, transects) remains problematic due to the secretive life-history traits (Williams et al. 2015), and mist-netting surveys to date, tend to catch so few individuals. Surveys for this species carry added significance because monitoring cuckoo abundance and distribution may serve as an indicator of overall bird community composition (Morelli et al. 2015, 2017; Tryjanowski and Morelli 2015). Identifying individual male cuckoos based on vocal signals may represent a promising method to generate new information on the abundance and life history of this species, and three recent studies have kindled this hope (Jung et al. 2014; Li et al. 2017; Zsebők et al. 2017). These authors found the between-individual variation in male cuckoos' calls was much greater than within-individual variation, and that it was possible to identify individual male cuckoos based on specific call characteristics (Jung et al. 2014; Li et al. 2017; Zsebők et al. 2017). Furthermore, there appears to be a high degree of consistency in the number of syllables produced within individual males (e.g. Møller et al. 2016a, b), and these measures could be utilized to assess environmental conditions (e.g. Møller et al. 2016a, b). Despite these encouraging findings, all surveys to date have been conducted during just a short period of the breeding season, with the longest period of acoustic recordings taken over a 5-day period (Li et al. 2017), whilst the two other studies used recordings of calling males from just one occasion (Jung et al. 2014; Zsebők et al. 2017). One of these studies revealed a rate of correct classification calls to individual male of 91.9% from recordings made on one day, but this declined to 50% for recordings made more than two days apart (Li et al. 2017), suggesting that male vocal characteristics may not be temporally stable within a single breeding season.

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

Methods

Study area and sound recording

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

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

Sound measurements

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

Fourier transform length 256 points; Hamming window with a frame size of 100% and an overlap of 50%; frequency resolution 23 Hz; and time resolution of 21.3 ms. Male cuckoo advertisement calls consist of a repeated series of '*cu- coo*' syllables, with each syllable composed of two distinctive elements (Møller et al. 2016a, b; Møller et al. 2017). We manually separated each element of each syllable (see Fig. 1) represented by a continuous trace in the spectrogram, and used Avisoft-SASLab Pro software to measure call features automatically following Li et al. (2017). We first automatically search the maximum amplitude in each element, and then determine the start and end points of each element at 19 dB lever lower than the maximum amplitude. We selected 19 dB (rather than 16 dB in Li et al. 2017) because the characteristics of all syllables were explicit and clearly audible above the background noise on all recordings, allowing us to obtain comparable syllable parameters independently of the absolute intensity of the calls and the background noise level (Zollinger et al. 2012). The following variables were then measured: duration of the element (Tdur1, Tdur2); duration from the start of element to the point of maximum amplitude within that element (Tdis1, Tdis2); frequency at the start point of the element (Fsta1, Fsta2); frequency at the end point of the element (Fend1, Fend2); minimum frequency of the element (Fmin1, Fmim2); maximum frequency of the element (Fmax1, Fmax2); frequency of the maximum amplitude within the element (Fpeak1, Fpeak2); time interval between the first and second element (Tint) (Supplementary Material Fig. 1). In total, we measured 1032 syllables from 30 males: 750 syllables for 6 banded males and 282 syllables for 24 un-banded males (all original measurements of call features can be seen in Supplementary Material file 1).

We count the number of syllables within each calling bout based on the number visible from the spectrograms. The pause between successive bouts was always larger than 2 s, which is obvious greater than pause between successive syllables within one calling bout (see Fig. 1). We were unable to obtain a complete calling bout for 8 unbanded males. Consequently, we measured a total of 317 calling bouts from 22 males (6 banded and 16 unbanded). The data on syllable numbers for each calling bout are presented in Supplementary Material file 2.

Data analyses – identification of vocal individuality

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

Discriminant function analyses (DFA) is a multivariate technique widely used to identify vocal individuality in birds (e.g. Delgado et al. 2013; Linhart and Šálek 2017) by combining variables with weighting coefficients to create a set of functions that can discriminate groups and classify new data into one of any number of pre-existing groups (Williams and Titus 1988; Mundry and 2007). We used calls from the first cuckoo data set to construct discriminant functions and examined the power of functions to correctly classify each syllable to an individual using a jack-knife analysis (Manly 1986; Galeotti and Sacchi 2001). The prior probability for each individual was set equal in DFA. For the second data set, we used the 30 discriminant functions constructed (corresponding to 30 males) based on the first data set to classify syllables of 6 banded males recorded across different days. The number of syllables was not equal for each male in the first set, and varied from 6 to 20, with a mean of 12 syllables per male. Due to the possibility that the variables used for identifying individuality in males from the first data set were not similar to those necessary for identifying males over the duration of the breeding season, we calculated the rate of correct classification in the second data set using the 30 discriminant functions constructed from the first data set based on all combinations of variables (each time, only a subset of variables were used in discriminant functions). The highest correct rate and their corresponding combinations of variables were reported.

Correlation analysis (CA) was used to calculate the similarity of all pairs of syllables using Pearson's *R* based on call variables, and then identified individuals based on this similarity value (Budka et al. 2015). Using the first data set, we calculated coefficients of variation (CV) for each variable to compare differences within (CVw) and between (CVb) individuals (Robisson et al. 1993). We computed CV for each male based on syllables belonging to that male, and then calculated the mean CV for each male as CVw. We used the average value for each male to compute CVb. The ratio of CVb / CVw is the measurement of potential individual coding (PIC) which shows the importance of each variable used in identifying individuals (Charrier et al. 2001; Charrier et al. 2003). PIC value of Tdis2, Tdur1 and Tdis2 were less than or nearly equal to one (Table 1), meaning that these variables showed greater or similar variation within an individual than between individuals. Consequently, these three variables were not included in the subsequent analysis. Since call variables have different orders of magnitude e.g. the frequency of cuckoo syllables range in the hundreds Hz, while duration of syllables last nearly a tenth of a second, we standardized the variables using the formula: (value − mean) / standard deviation, and used these standardized variables to calculate the similarity of all pairs of syllables using Pearson correlation for both within male and between males. Based on the first data set, independent samples t-test were used to compare the similarity of pairs of syllables from the same male to similarity of pairs of syllables from the different males. Each syllable was assigned to an individual, whose syllable (except the one being classified) has the maximum similarity value with the syllable to be assigned. The correct classification of individuals within a single day was expressed as the percentage of syllables correctly assigned. To calculate the correct rate of acoustically identify individuals within the breeding season, we first calculated the similarity of all pairs of syllables, one from the first data set and one from the second data set. We then assigned the syllable in the second set to one male in the first set, whose syllable has the maximum similarity value with the syllable to be assigned. The correct rate was expressed as the percentage of syllables correctly assigned.

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

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

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

Results

Acoustic identification of individuals from one day of sampling

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

Acoustic identification of individuals within the breeding season

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

Call consistency ('repeatability') of syllable numbers

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

Discussion

Based on the same day recordings, we found that syllables from the same calling male were more similar in their characteristics than syllables from different males, and our analyses yielded high correct rates of classification of individuals from 93.6% (SPCC), 90.8 % (DFA), and 71.5% (CA). These results support the findings of the three previous studies (Jung et al. 2014; Li et al. 2017; and Zsebők et al. 2017) which found inter-individual variation of male cuckoo calls was much greater than intra-individual variation, and it was possible to identify individual male cuckoos based on call characteristics within one day. However, our results failed to provide evidence that male call structure is sufficiently stable to allow re-identification of individuals even within the same breeding season. Based on repeated recordings from the 6 banded males, we found the correct rate of classifying syllables to individual males declined dramatically to 40.7 % from the DFA, 27.0 % from the CA, and 40.5 % from the SPCC. Thus, male cuckoos recorded singing across the longer duration of the breeding season were more likely to have their calls incorrectly assigned than were males recorded from the same day. Furthermore, based on all combinations of variables used to construct discriminant functions, the highest correct rate of individual identification during the breeding season was only 43.0 % from the DFA, suggesting that there is no single call variable or combination of variables that can be used to consistently identify individuals in this study. Among the 6 banded males, two males (represented by circle and reversed triangle symbols in Fig. 4) had consistently higher correct rate of classification than other males. Thus, in our study population, average call individuality did not decline in all males. Instead, there were males with more consistent and males with less consistent calls.

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

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

Temporary or permanently changes to vocal features does not necessarily prevent the identification and monitoring of individuals (Fox 2008; Kirschel et al. 2011). For example, Brownish-flanked Bush Warbler (*Horornis fortipes*) songs show variation in song characteristics, but the correct rate of acoustically identify individuals across the whole breeding season using DFA was 98% (Xia et al. 2010). Further, the coefficients of variation between individual bush warblers was always larger than 0.1 for 46 of the 52 measured variables (Xia et al. 2012), while the coefficients of variation between individuals in cuckoos was less than 0.07 in ten of 12 variables demonstrating the potential individual coding based on the first data set. Examination of the calls from the six banded males from all recording days suggests that only 7 variables show potential individual coding (with PIC value > 1), and 6 of these have a coefficients of variation between individuals of less than 0.04 (Supplementary Material Table 1), confirming that calls from different male cuckoos are very similar (see also the spectrograms in Supplementary Material Fig. 4). It is possible that slight changes of acoustic features within individual males may result in declines in correct rate of acoustically identify individuals in a long term (e.g. Průchová et al. 2017). Poor weather conditions throughout a breeding season can also modify the reliability of acoustic information contained within an individual's vocalizations, influencing their calling behaviour (e.g. Lengagne and Slater 2002) and this merits further investigation within our study population.

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

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

Authors' contributions

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

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

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Ethical standards

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

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

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Table 1 Coefficients of variation (CV) and potential individual coding (PIC) for 15 acoustic variables based on 368 syllables from 30 males. CVw = coefficient of variation within males; CVb = coefficient of variation between males

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

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

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

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

Supplementary material figure 1 The measured variables in common cuckoo call (following Li et al. 2017 and Møller et al. 2016a, b): duration of the element (t1, t3); time interval between the first and second element (t2); duration from the start of element to the point of maximum amplitude within that element (t4, t5); frequency at the start point of the element (f1, f6); frequency at the end point of the element (f2, f7); minimum frequencies of the element (f3, f8); maximum frequency of the element (f4, f9); frequency of the maximum amplitude within the element (f5, f10).

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

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

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

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

Supplementary material Table 1 Coefficients of variation (CV) and potential individual coding (PIC) for 15 acoustic variables based on 750 syllables from 6 banded male cuckoos across all recording days. CVw = coefficient of variation within males; CVb = coefficient of variation between males

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

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

Variables*	CVw	CVb	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.

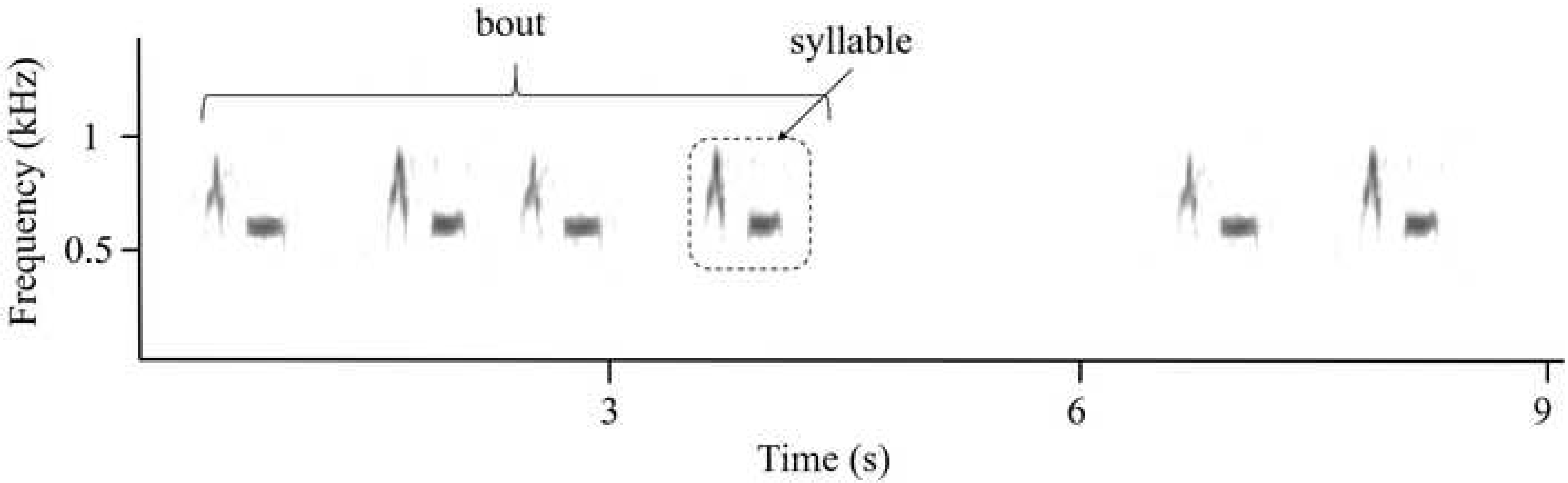
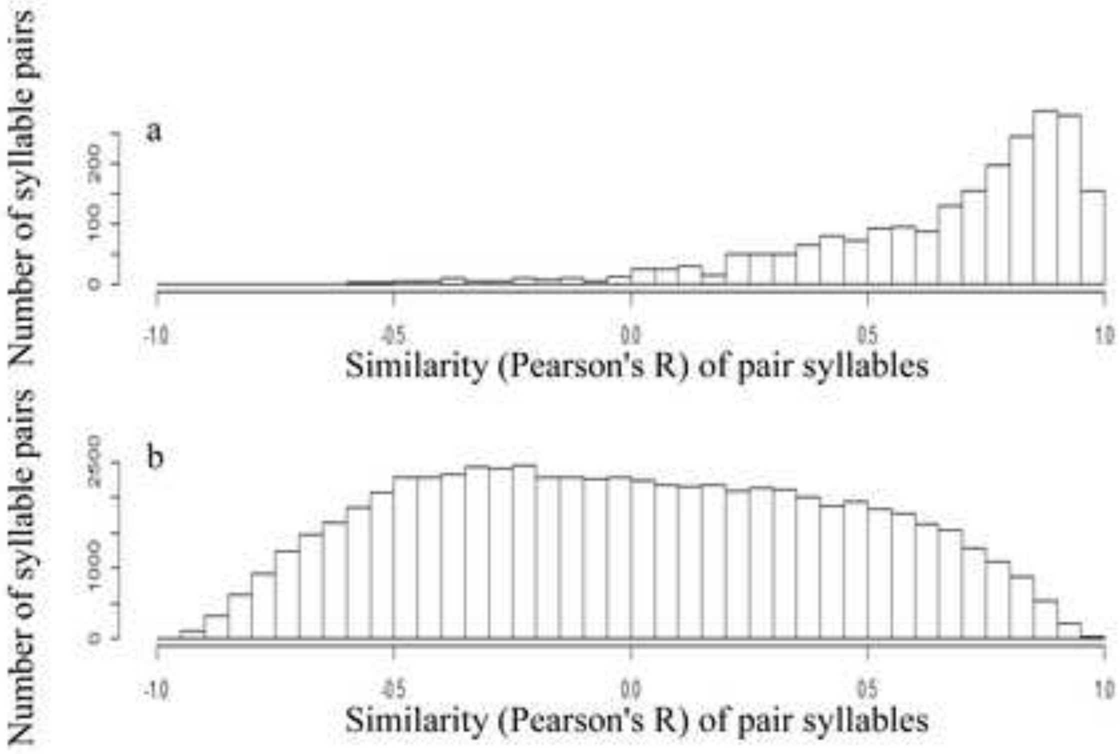

bout
syllable
Frequency (kHz)
1
0.5
3
6
9
Time (s)

Fig2


a
Number of syllable pairs
0
100
200
-1.0
-0.5
0.0
0.5
1.0
Similarity (Pearson's R) of pair syllables
b
Number of syllable pairs
0
1000
2500
-1.0
-0.5
0.0
0.5
1.0
Similarity (Pearson's R) of pair syllables

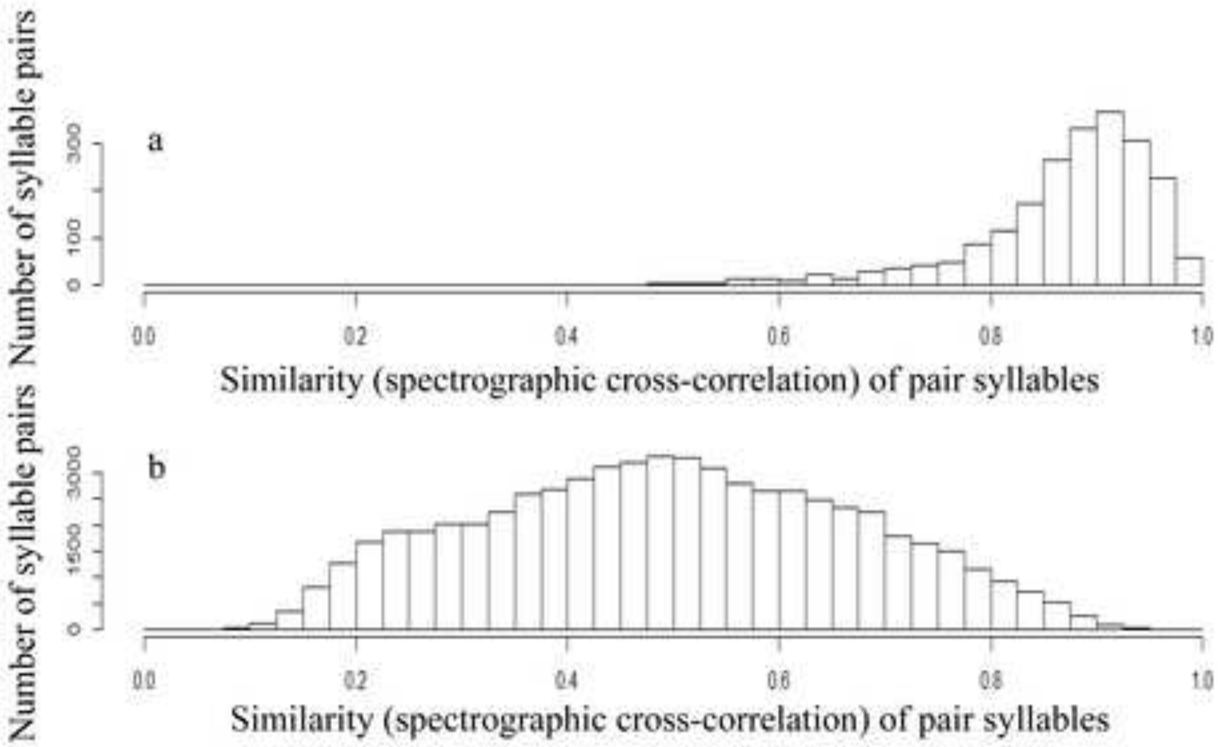
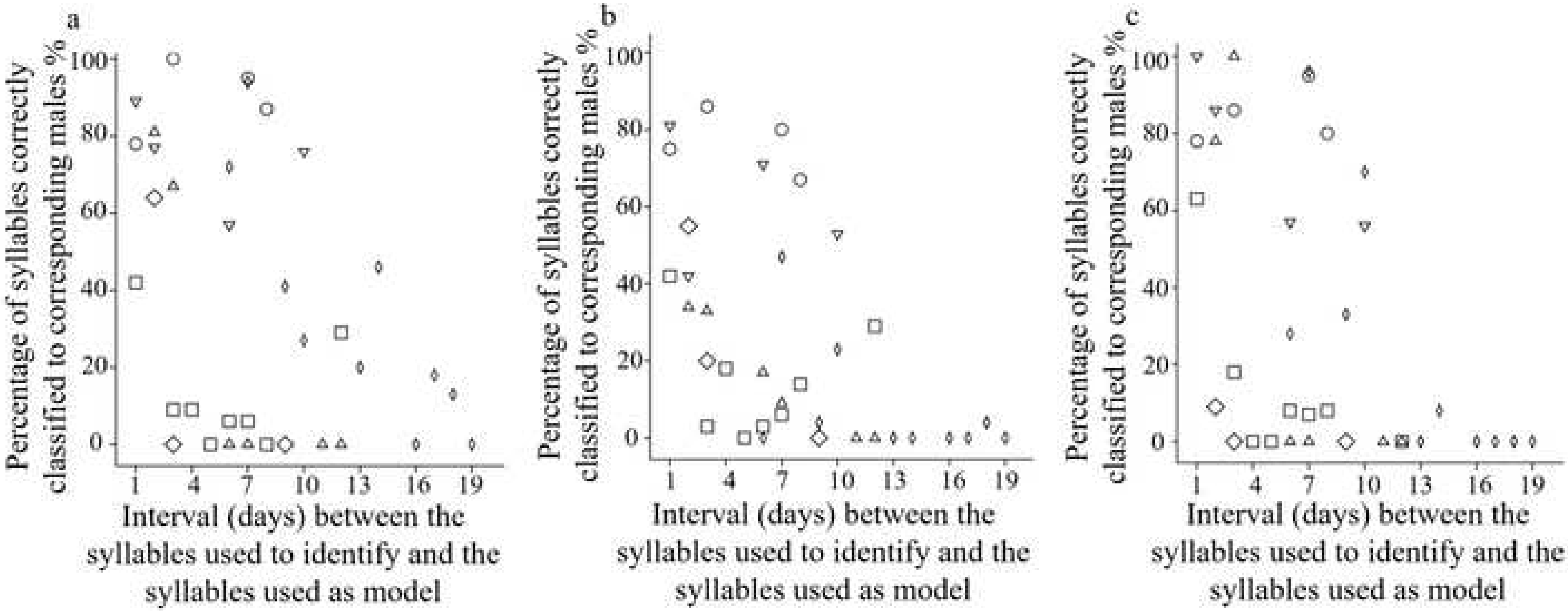

a
Number of syllable pairs
0
100
300
0.0
0.2
0.4
0.6
0.8
1.0
Similarity (spectrographic cross-correlation) of pair syllables
b
Number of syllable pairs
0
1500
3000
0.0
0.2
0.4
0.6
0.8
1.0
Similarity (spectrographic cross-correlation) of pair syllables

Fig4


a
Percentage of syllables correctly classified to corresponding males %
100
80
60
40
20
0
1
4
7
10
13
16
19
Interval (days) between the syllables used to identify and the syllables used as model
b
Percentage of syllables correctly classified to corresponding males %
100
80
60
40
20
0
1
4
7
10
13
16
19
Interval (days) between the syllables used to identify and the syllables used as model
c
Percentage of syllables correctly classified to corresponding males %
100
80
60
40
20
0
1
4
7
10
13
16
19
Interval (days) between the syllables used to identify and the syllables used as model

Supplementary material fig1

Supplementary material fig2

Supplementary material fig3

Supplementary material fig4

Supplementary material fig5

Supplementary Material file 1

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Electronic Supplementary Material

Supplementary Material file 1.csv

Supplementary Material file 2

Supplementary material Table 1