Can common cuckoos discriminate between neighbours and strangers by their calls?

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Abstract

Common cuckoos (*Cuculus canorus*) are brood parasites: they lay their eggs in the nests of other bird species, and let manipulate these hosts into incubation their eggs and feed and rear the

nestlings. Although cuckoos do not show parental care, they demonstrate complex social interactions, including territorial behaviours and male-to-male aggression. Cuckoos have a well-known and simple two-phase call ("cu" and "coo"), uttered by males during their breeding season. Previous studies suggested that the "cu-coo" call of males is individually unique, allowing discrimination between different classes of males. Using playback experiments in a dense population of radio-tagged cuckoos, we tested whether neighbouring males are tolerated more than unfamiliar intruders: the classic "Dear Enemy" phenomenon. Focal birds responded more aggressively to the calls of unfamiliar simulated intruders (strangers) than to the calls of conspecifics with whom they shared territorial boundaries (familiar neighbours). Cuckoos responded quickly, within an average of less than half a minute, they often approached the loudspeaker to a proximity of less than 5 - 10 m, even from further distances (up to 80 m), and used their "cu-coo" calls in response. Our results reveal that cuckoos were able to use their simple call for the discrimination of familiar versus unfamiliar individuals, and they did so specifically to aggressively protect their own territories. In turn, cuckoos showed tolerance to nearby conspecifics, e.g., neighbours with overlapping territories and did not respond to control playbacks. Finally, as typically more than one cuckoo was interested in the playbacks, this study confirmed the opportunity for brood parasitic birds to socialize during the breeding season.

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In territorial behaviour, the so-called 'Dear Enemy phenomenon' (Fisher, 1954) is defined by the pattern that territory owners tolerate familiar neighbours living on adjacent territories better than unfamiliar intruders that represent a potential source of territorial threat. This way the residents reduce inter-individual aggression and unnecessary conflicts. This phenomenon has already been shown in a wide range of animals, but territorial bird species represent the most common and best known examples for this phenomenon (Searcy, Akcay, Nowicki, & Becher, 2014; Temeles, 1994). Irrespective of the function and mechanism, including sensory modality, of the Dear Enemy phenomenon, these processes include an ability to discriminate between familiar versus unfamiliar individuals. For example, songbirds with a large repertoire may share some of their song types with neighbours, which help in recognition of familiar neighbours (Briefer, Aubin, Lehongre, & Rybak, 2008; Stoddard, 1996). Songbirds with a small repertoire may use unique frequency characteristics for discrimination and recognition (Osiejuk, 2014). There is also an increasing number of studies on non-oscine birds, which do not learn their songs, where the Dear Enemy phenomenon was detected (e.g. Budka & Osiejuk, 2013; Hardouin, Tabel, & Bretagnolle, 2006; Mackin, 2005). However, neighbour-stranger discrimination (NSD) sometimes reveals equal aggression toward neighbours and strangers when they show equal threat (Bard, Han, Wikelski, & Wingfield, 2002; Battison, Wilson, Graham, Kovach, & Mennill, 2015), or even works in reverse of the typical case (Brunton, Evans, Cope, & Ji, 2008).

Common cuckoos (*Cuculus canorus*; hereafter cuckoos) are well-known brood parasites (Davies, 2000; Schulze-Hagen, Stokke, & Birkhead, 2009) that lay eggs in the nests of other avian species (hosts), and leave these hosts to incubate the eggs and rear their offspring (Wyllie, 1981). The cuckoo hatchling evicts all eggs and other hatchlings from nest (Honza,

Voslajerová, & Moskát, 2007), and consequently it monopolizes all food delivered by the foster-parents (Anderson, Moskát, Bán, Grim, Cassey, & Hauber, 2009). Host nests serve as resources for reproduction by female cuckoos, whereas males can be observed spatially aggregating near females. A female cuckoo lays every second day, altogether ca. 20 eggs (up to 25) in a breeding season (Wyllie, 1981), so their reproductive strategy could be characterized by an extended laying cycle. Consequently, females primarily protect resources (host nests), while males protect females directly or areas used by females. For this reason, we expect a developed territorial signing and protection system in cuckoos. We hypothesise that individually distinctive calls could enable neighbor-stranger discrimination, which could have a territorial function in cuckoos. Territorial behaviour, including defence and inter annual use of the same sites, has also been detected in several other brood parasitic species, including Horsfield's bronze cuckoos (*Chalcites basalis*) in Australia (Langmore, Adcock, & Kilner, 2007) and brown-headed cowbirds (*Molothrus ater*) in North America (Hauber, Strausberger, Feldheim, Lock, & Cassey, 2012). Furthermore, cuckoos seem to be polygamous (Marchetti, Nakamura, & Gibbs, 1998), and this may explain why male cuckoos defend partly overlapping or shared territories. In contrast, in the Horsfield's bronze cuckoo, genetic parentage analysis revealed that females were monogamous and that males were also monogamous, or sequentially monogamous (Langmore, Adcock, & Kilner, 2007). This may also have implications for the type of territoriality exhibited by different brood parasitic taxa.

Cuckoos belong to an avian lineage which does not learn its songs (Brenowitz, 1991). This reduces the presence of individual differences due to cultural transmission. Neighbour-stranger discrimination is a case of familiarity recognition; it must be based on distinguishable characteristics of familiar versus unfamiliar individuals, for example in frequency, time, or

repertoire, and the ability to recognize these differences by the receivers (Tibbetts & Dale, 2007). The receiver also has to store the familiar, known song types in memory (Kiefer, Scharff, Hultsch, & Kipper, 2014; Marler, 1997). In songbird species with a small song repertoire, neighbour-stranger discrimination is typically based on differences in the frequency of shared song types (Osiejuk, 2014). In contrast, oscines with large song repertoires often learn syllable sequences from each other (Briefer et al., 2008). However, acoustic neighbour-stranger discrimination is less studied in non-songbirds, although there are some confirmatory results even in species using simple calls. For example, a playback study revealed that the nocturnal species, the corncrake (*Crex crex*), uses their simple calls for NSD (Budka & Osiejuk, 2013). In the little owl (*Athene noctua*) territory owners responded more aggressively to neighbour calls played at an unexpected part of their territory compared to the correctly positioned neighbour calls (Hardouin et al., 2006).

Common cuckoos have a very simple two-tone advertising call (Lei, Zhao, Wang, Yin, & Payne, 2005), the well-known "cu-coo" call. These calls are emitted by males during the breeding season (Jung, Lee, & Yoo, 2014). In a previous study we showed that this simple call type contains sufficient diversity for it to vary distinctively among different cuckoo individuals. Using sound analysis tools it is possible to discriminate different cuckoo individuals with high precision by their "cu-coo" calls (Zsebök, Moskát, & Bán, 2016). We hypothesize that these small differences in calls could be used for to discriminate among individuals in cuckoos, which may help the development of NSD in this species. We predict that cuckoos are more aggressive toward simulated stranger intruders than toward territorial neighbours. If the alternative hypothesis is true, i.e. cuckoos cannot use their simple calls for NSD, we predict similar aggression toward each male cuckoo. A second, alternative hypothesis is that the Dear Enemy

phenomenon works in reverse in parasitic cuckoos compared to the typical case, especially if male cuckoos' main competitors for females are in fact their immediate neighbours. If this is correct, we predict that cuckoos should be more aggressive toward neighbours than toward strangers. Here we have tested NSD in cuckoos, in a study site where the only known host species is the great reed warbler (*Acrocephalus arundinaceus*) and cuckoo parasitism rate is both unusually high (around 50% of host nests; Zölei, Bán, & Moskát, 2015) and where the different phenotypes of cuckoo eggs suggest that different female cuckoos lay eggs in the same nests (Moskát et al., 2009; see also Moksnes et al., 2008). Consequently, the laying areas of some of the female cuckoos may overlap. Multiple male cuckoos can also be located along short sections of channels. The dynamics of this unusually dense host-brood parasite interactions thus set the stage for us to study neighbour-stranger discrimination in cuckoos. As far as we know, there is no previous study on this type of cognitive skills in avian brood parasites.

Methods

Study site

The experiments were carried out at around Apaj (47° 6' 53.9" N; 19° 5' 21.2" E) and Kunpeszér (47° 3' 40.1" N; 19° 16' 31.3" E), ca. 40-60 km southeast of Budapest. We also recorded calls of unfamiliar (stranger) cuckoos in the surrounding areas (~ 20 km). The study was conducted 7 May to 30 May 2016. The laying season of cuckoos follows the availability of host, great reed warbler, nests and lasts about 60-70 days (Moskát, Barta, Hauber, & Honza, 2006) in our study area. This laying season starts in the first half of May and ends in mid-July in Hungary (Moskát & Honza, 2000), and it can be divided into three periods of similar length. In the first period

availability of host nests is high, in the second period it is much lower, and is highly reduced in the third period. As the phase of the breeding season may affect vocal responses of birds to playback (Courvoisier, Camacho-Schlenker, & Aubin, 2014), we conducted our fieldwork within the first three weeks of the breeding season (just after cuckoo territories have been established), when the availability of host nests for parasitism is typically high (Moskát et al., 2006).

In our study area cuckoos parasitize great reed warblers at a high rate (ca. 50%; Moskát & Honza, 2002; Zölei et al., 2015). Great reed warblers breed in reed beds grown on both sides of small channels. Typically, cuckoos can be observed in channel-side tree lines, in small woodland patches or sitting on poles and wires. This predominantly linear habitat is especially suitable for studying cuckoos' territorial and related behaviour as channels form a network in the area, and cuckoos distribution along the channels can be regarded as a quasi one-dimensional habitat (Fig. 1a). As a consequence of the dense host population and the high parasitism rate (see above), this dynamic host-brood parasite system is characterized by a high frequency of multiple parasitism (ca. 24-52% of parasitized nests, Zölei et al., 2015), and overlapping cuckoo territories (Fig. 1).

Sound recordings

Cuckoo calls were recorded using a Marantz PMD-620 MKII recorder (D&M Holdings Inc., Tokyo, Japan), connected to a Telinga Universal Parabola (Telinga Co., Tobo, Sweden) with a Sennheiser ME 62 microphone and a K6 powering module (Sennheiser Electronic GmbH & Co., Wedemark, Germany). As cuckoo calls are typically low frequency, in our study area they fall between 0.5 and 0.8 kHz (Zsebök et al., 2016), the parabola dishes developed for generic bird song recording amplify these sounds only moderately. For this reason we also used a FEL MX

mono preamp (FEL Communications Ltd., Sunbury-on-Thames, UK). By this equipment we were able to record cuckoo calls from 30-80 m in high quality (48 kHz sampling rate, 24 bit quality, .wav audio format). We also recorded the call of Eurasian collared doves (*Streptopelia decaocto*), a cuckoo-sized neutral species found in many parts of the study area and used for a control to our experiment. The vocalization of collared doves is somewhat similar to the cuckoo call ("coo"), and also similar in frequency (Fig. 2). We typically recorded calls when wind was negligible, often in the early mornings or evenings. Sample sound files were uploaded to the Xeno-Canto public library (common cuckoos: XC323683, XC323807, XC323954, XC323955; Eurasian collared doves: XC324006, XC324031).

Radio telemetry

We caught 14 male cuckoos by mist-netting and conducting playbacks and equipped with Pip3 transmitters (type 392 by Biotrack Ltd., Wareham, U.K.) for identifying neighbour cuckoos and some of the strangers (i.e. more than 15-20 territories away) individually. To demonstrate the organisation of cuckoo territories in our study area, we illustrated representative territory positions along a channel section. These results were obtained by following radio-tracked individuals, using the same equipment as mentioned above (Fig. 1a). We also show an example of the territory-dynamics of cuckoos, using Pica GPS tags by Ecotone Ltd, Gdynia, Poland (Fig. 1b; Moskát et al. n.d., in preparation).

Playback experiments

We played 2-min cuckoo calls to focal cuckoo individuals. The basic elements of a sound file contained 3 x 10 s long phrases (ca. 6-8 syllables in a phrase; altogether 30 s long sequence)

from the same cuckoo individual, followed by a 15 s break. This set was repeated, and then the 30 s sound unit was added to finalize the playback sample (Fig. 2e). The sound files were edited by the program Audacity version 2.1, and the sample file was produced by Raven 1.5 Pro (Fig. 2). No relevant manipulation was applied, but noise was filtered out below 300 Hz, and the amplitude was standardised with respect to peak amplitude. The 2-min length of sound files was chosen to attract nearby cuckoos effectively. To avoid potential pseudoreplication in data (c.f. Kroodsma, 1989; Kroodsma, Byers, Goodale, Johnson, & Liu, 2001; McGregor, 2000), each sound file (neighbour, stranger or control) was tested on one focal bird, and each cuckoo individual was used in only one treatment (neighbour, stranger, or control). To standardize playback sound files, we used only the most common and well-known cuckoo vocalisation, the advertising call ("cu-coo"), and avoided the rarer sound types, such as the "gowk" and "guo" calls (c.f. Lei et al., 2005) or the quicker "ka-ka-coo", etc. For "stranger" playbacks, we used recordings of cuckoos obtained from geographically distant areas from the focal individuals (mean distance to their recordings from focal birds: $22.18 \text{ km} \pm 55.14 \text{ S.D.}$; range: 9.49 - 30.6 km; $N = 15$), which is much larger than cuckoos' average territory size along the channels, ca. 1-2 km (our unpublished data).

In order to explore any potential amplitude-difference based discrimination bias caused by either the original sound recording or the standardisation method of the playback files, we compared the bioacoustics metric of the root-mean square (hereafter: RMS) amplitude between our playback categories. This approach enabled us to estimate the acoustic intensity (i.e. sound pressure on dB scale) of the playback stimuli. We completed these analyses in Raven Pro 1.5 (Bioacoustics Research Program, 2014) and calculated RMS amplitudes and the centre frequency for all sound samples (Charif et al., 2010). Every calculated RMS amplitude output was

converted to dB scale using the following formula: $20 * \log (X / 23174)$. These analyses revealed no statistical difference between the two groups (absolute values of RMS measurements (median and range): $\text{RMS}_{\text{neighbours}}$: 16.22 (11-26.3), $\text{RMS}_{\text{strangers}}$: 13.69 (11.67-16.93); Mann-Whitney $U_{12,15} = 53.5$, $P = 0.75$).

We also assessed the number of syllables in our stranger and neighbour sets of playback sound files as a potential source of discrimination bias; again we found no significant differences between the playback categories: (median and range: 19 (9-24), $N_{\text{neighbours}}$: 21 (13-25), $N_{\text{strangers}}$: Mann-Whitney $U_{12,15} = 53.00$, $P = 0.75$). Finally, we compared both the number of syllables and RMS amplitudes with scores of the first two components, obtained through Principal Component Analysis, with linear regression. These analyses revealed no statistical pattern between behavioural components and number of syllables or RMS amplitudes. (RMS measurements for neighbours: $\text{PC1}_{\text{scores}} = 0.044 * X_{\text{RMS}} - 0.633$, $\text{Beta} = 0.107$, $t = 0.516$, $P = 0.611$; $\text{PC2}_{\text{scores}} = -0.107 * X_{\text{RMS}} + 1.545$, $\text{Beta} = -0.261$, $t = -1.297$, $P = 0.207$; number of syllables for neighbours: $\text{PC1}_{\text{scores}} = -0.003 * X_{\text{syllables}} + 0.049$, $\text{Beta} = -0.009$, $t = -0.045$, $P = 0.964$; $\text{PC2}_{\text{scores}} = 0.061 * X_{\text{syllables}} - 1.162$, $\text{Beta} = 0.223$, $t = 1.099$, $P = 0.283$). We, therefore, concluded that basic bioacoustics parameters of our sound samples used for experiments were statistically similar for strangers and neighbours, thus neither the sound pressure described by RMS amplitude measurements, nor the number of syllables affected the reported behavioural responses of cuckoos in this experiment.

Sound files were played by a Lenovo NotePad (type TAB 2 A7; Lenovo Ltd., Morrisville, North Carolina, U.S.A.), and connected to a JBL Xtreme loudspeaker (40 W; Harman Incorp., Northridge, California, U.S.A.) with a 20 m audio cable. Peak sound volume was about 90 dB (A) SPL, measured at 1 m distance by a Voltcraft SL-100 sound level meter

(Conrad GmbH, Kalchreuth, Germany). Sound files were played to a focal bird by two people at a time, sheltered by a bush or reed stems. The loudspeaker was positioned ca. 20 m from the observers, about 30-60 m from the focal bird located by its radio signal; its location was also visually confirmed. One of the observers handled telemetry equipment and controlled the play of the sound file, and the other, who was blind to the type of call (neighbour or stranger), observed the cuckoos' behaviour.

Measuring cuckoo behaviour to playback

Since cuckoos have relatively large territories in our study area (Fig. 1), and male cuckoos typically move frequently within this area, it was difficult to start our playback treatments at a fixed distance from the loudspeaker. For this reason our starting distance between the focal bird and the loudspeaker varied typically between 30 and 60 m, and did not show any significant difference among the treatment groups (ANOVA $F_{2,38} = 0.541$, $P = 0.587$). Although we estimated this distance after training with a Bushnell rangefinder, we did not use these data in our analyses. Instead, we used distance of closest approach to the loudspeaker ("closest distance", m), and the difference between the starting and closest points ("distance difference", m). Both "closest distance" and "distance difference" are metrics for the level of aggression shown by the focal individual towards the playback. We standardized the latter variable by range ("standardized distance difference", calculated as distance difference/starting distance) to reduce the effect of starting point position. We also measured when the focal bird moved from its original position at the start of the treatment ("movement latency", s), and when the focal bird started calling ("sound latency", s). Our last response variable measured the number of male cuckoos that came to the experimental zone, ca. 50 m around the loudspeaker, during the

treatment ("no. of cuckoos present", *N*). No female cuckoos were attracted by our playbacks, except one case when four males and one female were observed during playback, although the female did not approach the loudspeaker. (The sex of cuckoos was typically identified by sound in the field, as the "bubbling call" is exclusively representative of females, and the "cuckoo" call of males. The sex of radio-tracked cuckoos was identified based on plumage characters when they were captured by mist-nets (Svensson, Mullarney, Zetterström, & Grant, 2010). We validated this method using molecular methods following the protocol of DNA analysis by Bereczki, Tóth, Sramkó, & Varga (2014), and identified the presence of avian W chromosomes (e.g. Daniel, Millar, Ismar, Stephenson, & Hauber, 2007) in blood samples taken from 24 males and 8 females in laboratory by J. Bereczki (Moskát et al. n.d., in preparation)).

Statistical analysis

We further analysed the relationships among the responses to playbacks based on the variables measured (see above) by principal component analysis (PCA). This technique reveals non-correlated (more precisely "independent") components of the variable structure. In the PCA the number of components was determined by the number of eigenvalues greater than 1.0, and no rotation was applied on component loadings. We performed a MANOVA on the PC scores for testing differences in experimental results between neighbours and strangers, and we also tested between subject effects by univariate ANOVAs, using the General Linear Model program in SPSS. We also used binary logistic regression with backward stepwise variable selection for the same playback response variables as used for PCA to reveal which variables are the most useful for separating the neighbour and stranger groups as the binary dependent variable. All statistical

analyses were performed by SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, Illinois, USA).

Ethical notes

We used radio telemetry for monitoring space use by individual cuckoos in the study area. We caught cuckoos in mist nets. We kept handling to the minimum to reduce disturbance (typically < 5-10 min). We mounted Pip3 transmitters produced by Biotrac Ltd. (Wareham, U.K.) with 18 cm length antennas on the central tail feathers of each cuckoo, secured by factory-provided strings and glue (Loctate 4860). The transmitter's weight represented only ca. 1% of the cuckoo's body weight (1.2 g versus 122.78 g mean $\pm$ 5.65 SD; range: 116-136 g; $N = 14$). These tags were designed to be lost during the next moulting cycle of the tail feathers. All work complied with Hungarian law, and was approved by the Middle-Danube-Valley Inspectorate for Environmental Protection, Nature Conservation and Water Management, Budapest (permit No. PE/KTF/17190-3/2015).

Results

We found only weak or no response by cuckoos to control playbacks. Overall, cuckoos were not interested in our control calls, and variation in their responses could probably be attributed to their normal behaviour, not guided specifically by conducting control playbacks. For example, cuckoos did not fly closer to the loudspeaker in 12 out of 14 cases, and even moved further in 7 cases, while all individuals came closer to the loudspeaker both in the neighbour and stranger groups (Fig. 3a). Cuckoos also showed no vocal response to control sounds (Fig. 3b). Cuckoos'

responses to playback showed a clear separation among the three treatment groups ("movement response" (Y/N): $\chi^2_2 = 30.26$, $P = 0.00001$; "vocal response" (Y/N): $\chi^2_2 = 13.37$, $P = 0.0013$). All of the other quantitative variables also showed much weaker response to control calls than to neighbour or stranger cuckoo calls (Fig. 4; Kruskal-Wallis tests of the treatments groups for the variable "closest distance": $\chi^2_2 = 24.23$, $P < 0.001$; "distance difference": $\chi^2_2 = 23.99$, $P < 0.001$; and "no. of cuckoos" attracted by playback: $\chi^2_2 = 12.74$, $P = 0.002$).

As the cuckoos' responses to controls were markedly different from both neighbour and stranger playbacks across all of our metrics (see above), we analysed their behavioural responses by PCA for the neighbour and stranger groups (Table 1), to reduce collinearity between the different response metrics. Two eigenvalues were greater than 1, so we analysed the first two PC scores. The two components explained 42% and 22% of the total variance, respectively. Component 1 (PC1) was composed of distance variables (closest distance and standardised distance difference), related to how cuckoos' approached the loudspeaker (termed a boldness component, where positive values are related to stronger approaches). Component 2 (PC2) is correlated with movement latency and vocalization latency; therefore, it expresses (the opposite of) how quickly cuckoos respond to the intruders. A fifth variable, the number of birds attracted, was not included in any principal component with a high loading value. This variable ("no. of cuckoos attracted") showed similar values for neighbours and strangers (ANOVA: $F_{1,25} = 0.262$, $P = 0.613$; Fig. 5).

A MANOVA test on component scores of neighbours and strangers revealed high statistical difference ($P = 0.001$) between treatment groups (Table 2). Subsequent ANOVAs showed that the component scores significantly separated along the first axis, only ($P = 0.001$; Table 3). This clearly suggests that cuckoos respond more aggressively to strangers' playbacks

than to neighbours' playbacks by coming closer to the playback when they are faced with the new threat of an unknown intruder. A binary logistic regression analysis also selected only one of the response variables (standardised distance difference), which affects the dependent variable ($B = -11.387$, S.E. = 4.601, $\text{Wald}_1 = 6.125$, $P = 0.013$; where the Hosmer and Lemeshow test indicated good fit of the model: $\chi^2_6 = 8.191$, $P = 0.224$).

Discussion

This study found a strong pattern of neighbour-stranger discrimination based on acoustic cues in a non-passerine brood parasitic bird species. It is a surprising result as it is based on very simple, two-tone calls ("cu-coo"), which is characteristic for this species over its entire breeding range in Eurasia (Lei et al., 2005; Wei et al., 2015).

Birds typically defend certain areas for resources, but territoriality may have several complex social functions. For example, it reduces aggressiveness among males (Hinde, 1956). In most cases defended resources represent the food and housing supply for adult birds, nestlings and fledglings. However, avian brood parasites do not exhibit parental care toward their offspring; they let the hosts to take care of this. Home ranges in avian brood parasites instead may serve as resources for host nests, although overlapping ranges might show a lack of active defence mechanisms in females (de la Colina, Hauber, Strausberger, Reboreda, & Mahler, 2016; Martinez, J. J. Soler, M. Soler, & Burke, 1998; Scardamaglia & Reboreda, 2014; Vogl, B. Taborsky, M. Taborsky, Teuschl, & Honza, 2004). As the cuckoos' ranging and mating systems may depend on the intensity of competition with conspecifics (Davies 2000), this might also explain why we found high aggression among cuckoos in our study area where the parasitism

rate and cuckoos' density are unusually high (see above). Cuckoos lay eggs every second day (Wyllie, 1981), and they lay in nests during the host egg-laying stage or in the pre-egg-laying stage when the nest is still empty (Moskát & Honza, 2002) to ensure early hatching of their eggs (Geltsch, Bán, Hauber, & Moskát, 2016). They cannot use all available host nests for parasitism in a territory if there are many in the same state. For this reason sharing territories with other cuckoos, either with males or females, is likely adaptive for individuals within a dense cuckoo population. Our radio-telemetry study (unpublished data) on cuckoos revealed that a cuckoo territory might overlap with a few other territories at a time (see also Nakamura & Miyazawa, 1997; Vogel et al., 2004). In our study area an average territory of male cuckoos was about 1-2 km long along a channel (Fig. 1), so a cuckoo could be the neighbour of different individuals at different sections. Therefore a cuckoo may know and recognize up to a dozen cuckoos as neighbours in the peak "hot spots" of our study area. We did not study if all cuckoos tolerate different types of "neighbours" similarly (e.g. (i) two cuckoos share a territory, (ii) their territories overlap partly, or (iii) two cuckoos have non-overlapping adjacent territories). This feature of additional complexity in neighbour structure is a worthwhile direction for future studies. Instead, here we chose neighbours from shared or overlapping territories to ensure daily connection among cuckoos.

Previously unusual cuckoo calls from many populations have been reported, and researchers were repeatedly able to recognize certain individuals by their aberrant calls within and between years (e.g. Wyllie, 1981). Møller, Morelli, Mousseau and Tryjanowski (2016) suggested that several ecological factors (e.g. habitat, soil, radioactive pollution) may slightly affect cuckoo calls or even increase the frequency of aberrant calls. In contrast, our study was

located along channels in central Hungary, where the habitat is more homogeneous and large-scale ecological effects are less expected to modulate individual cuckoo calls.

Our previous study suggested that calls of cuckoo individuals can be distinguished by sound analysis. It showed almost 100% accuracy based on seven sound variables, and still exceeded 90% when five variables were measured on spectrograms. We think that individual differences were coded in either the length or frequency of calls, probably in the first syllable (Zsebök et al., 2016). This previous study revealed the possibility that cuckoos use the individually distinguishable "cu-coo" calls for individual discrimination. The present study showed, by simulating neighbour and stranger intruder calls, that cuckoos actually use this information to discriminate neighbours from strangers in a manner similar to the classical Dear Enemy phenomenon. They tolerated the calls of neighbours better than those of strangers. They responded quickly to stranger playbacks, within an average of less than half a minute, and approached the speaker to within 5-10 m, or even flew over it.

Our ongoing study by GPS and radio telemetry on the territory use of cuckoos in our study area revealed that male cuckoos often have overlapping territories, at least in a population like ours, where the parasitism rate is high (see above; Moskát et al. n.d. in preparation). As typically more than one cuckoo was interested in a trial in the cuckoo playbacks (but not in the control playbacks), this study revealed the possibility of social life of brood parasitic birds during the breeding season. Cuckoos seem to tolerate other cuckoos with overlapping territories to some extent, although a reduced level of aggression was also observed in the territories. Our experiments on cuckoo behaviour also suggest the potential importance of cuckoos' individual call recognition (although not explicitly tested in this study). For example, we observed several times a radio-tagged, unusually shy individual that was silent in the vicinity of other cuckoos.

Future studies should clarify if multiply overlapping territories, quasi "cuckoo hotspots", are related to the presence of female cuckoos or driven by available host nests. Future studies should reveal exactly which parameter of the cuckoo calls is responsible for the presence of an individual sound signature.

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531 Table 1. Component loading matrix of cuckoos' behavioural responses to neighbour and stranger
 532 call playbacks revealed by principal component analysis (PCA).

Response variables	Component 1	Component 2
Movement latency (s)	0.195	0.673
Sound latency (s)	-0.390	0.684
Closest distance to loudspeaker (m)	0.932	0.195
Standardised distance difference (m)	0.919	0.123
Number of cuckoos attracted (n)	0.444	-0.360
Eigenvalues	2.100	1.104
Cumulative variance explained (%)	42	64

533

534

535 Table 2. MANOVA results of the separation of cuckoos' responses to neighbour and stranger call
 536 playbacks tested on PCA component scores.

537

	Value	<i>F</i>	Hypothesis	Error <i>df</i>	<i>P</i>	Noncent.	Observed
			<i>df</i>			parameter	power
Pillai's trace	0.453	9.101	2	22	0.001	27.697	0.989
Wilk's lambda	0.547	9.101	2	22	0.001	27.697	0.989
Hotelling's trace	0.827	9.101	2	22	0.001	27.697	0.989
Roy's largest root	0.827	9.101	2	22	0.001	27.697	0.989

538

539 Table 3. ANOVAs of the separation of cuckoos' responses to neighbour and stranger call
 540 playbacks tested on PCA component scores.

541

542

Variables	Type III sum of squares	<i>df</i>	Mean square	<i>F</i>	<i>P</i>	Noncent. parameter	Observed power
PC1 score	9.775	1	9.775	15.749	0.001	15.749	0.967
PC2 score	1.112	1	1.112	1.117	0.301	1.117	0.173

543

544

Legend to figures

Figure 1 Examples of space use of male common cuckoos in the breeding area. The local host species, the great reed warbler, breeds in reed-beds of small irrigation channels, whereas cuckoos occur along the same habitat and they are restricted to the reed-beds and channel-side stands of trees. (a) Cuckoo territories are packed in high-quality habitats. (Data were collected with classical radio telemetry on nine male cuckoos in 2014, where about 50% of the individuals were tagged.) (b) An example for how breeding territories are stable during the breeding season. The figure shows two males, both equipped with GPS telemetry. The GPS data logger of cuckoo-1 collected 110 geographic positions between 28 May 2015 and 17 June 2015, and the logger of cuckoo-2 stored 276 coordinates between 8 June 2015 and 25 June 2015. Cuckoo-1 also collected positions of 5 points in the breeding range of cuckoo-2 between 8 June 2015 and 17 June 2015, revealing that cuckoos may temporarily visit nearby territories (Moskát et al. n.d. in preparation).

Figure 2 The composition and duration of playback calls used for presentation to cuckoos in the field. (a) Spectrogram of a common cuckoo (*Cuculus canorus*) call. (b) Waveform of a common cuckoo (*Cuculus canorus*) call. (c) Spectrogram of an Eurasian collared dove (*Streptopelia decaocto*) call, used for control playback. (d) Waveform of a collared dove call. (e) The composition of playback files. An example for the three phrases, altogether 30 s in duration, is shown. The same composition of sound files was used for neighbour and stranger experimental trials, and also for the control trials.

568 Figure 3 Cuckoos' binary (yes/no per trial) responses to sound playback. (a) Movements (flight)
569 towards the playback speaker. (b) Vocal responses during playbacks. (Sample sizes are shown
570 above bars.)

571

572 Figure 4 Cuckoos' quantitative responses to sound playback (means, standard errors and sample
573 sizes above bars are shown). (a) Distance of closest approach to the loudspeaker (closest
574 distance; m). (b) Movement toward loudspeaker from starting point (distance difference; m).

575

576 Figure 5 Effect of playback treatment ("neighbour" or "stranger") on the number of cuckoos
577 attracted to the vicinity of the loudspeaker. (Means, standard errors, and sample sizes above bars
578 are shown.)

579

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