

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

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20 Abstract

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

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

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44 discrimination, territorial defence

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47 In territorial behaviour, the so-called 'Dear Enemy phenomenon' (Fisher, 1954) is defined by the
48 pattern that territory owners tolerate familiar neighbours living on adjacent territories better than
49 unfamiliar intruders that represent a potential source of territorial threat. This way the residents
50 reduce inter-individual aggression and unnecessary conflicts. This phenomenon has already been
51 shown in a wide range of animals, but territorial bird species represent the most common and
52 best known examples for this phenomenon (Searcy, Akcay, Nowicki, & Becher, 2014; Temeles,
53 1994). Irrespective of the function and mechanism, including sensory modality, of the Dear
54 Enemy phenomenon, these processes include an ability to discriminate between familiar versus
55 unfamiliar individuals. For example, songbirds with a large repertoire may share some of their
56 song types with neighbours, which help in recognition of familiar neighbours (Briefer, Aubin,
57 Lehongre, & Rybak, 2008; Stoddard, 1996). Songbirds with a small repertoire may use unique
58 frequency characteristics for discrimination and recognition (Osiejuk, 2014). There is also an
59 increasing number of studies on non-oscine birds, which do not learn their songs, where the Dear
60 Enemy phenomenon was detected (e.g. Budka & Osiejuk, 2013; Hardouin, Tabel, & Bretagnolle,
61 2006; Mackin, 2005). However, neighbour-stranger discrimination (NSD) sometimes reveals
62 equal aggression toward neighbours and strangers when they show equal threat (Bard, Han,
63 Wikelski, & Wingfield, 2002; Battison, Wilson, Graham, Kovach, & Mennill, 2015), or even
64 works in reverse of the typical case (Brunton, Evans, Cope, & Ji, 2008).

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

69 Voslajerová, & Moskát, 2007), and consequently it monopolizes all food delivered by the foster-
70 parents (Anderson, Moskát, Bán, Grim, Cassey, & Hauber, 2009). Host nests serve as resources
71 for reproduction by female cuckoos, whereas males can be observed spatially aggregating near
72 females. A female cuckoo lays every second day, altogether ca. 20 eggs (up to 25) in a breeding
73 season (Wyllie, 1981), so their reproductive strategy could be characterized by an extended
74 laying cycle. Consequently, females primarily protect resources (host nests), while males protect
75 females directly or areas used by females. For this reason, we expect a developed territorial
76 signing and protection system in cuckoos. We hypothesise that individually distinctive calls
77 could enable neighbor-stranger discrimination, which could have a territorial function in
78 cuckoos. Territorial behaviour, including defence and inter annual use of the same sites, has also
79 been detected in several other brood parasitic species, including Horsfield's bronze cuckoos
80 (*Chalcites basalis*) in Australia (Langmore, Adcock, & Kilner, 2007) and brown-headed
81 cowbirds (*Molothrus ater*) in North America (Hauber, Strausberger, Feldheim, Lock, & Cassey,
82 2012). Furthermore, cuckoos seem to be polygamous (Marchetti, Nakamura, & Gibbs, 1998),
83 and this may explain why male cuckoos defend partly overlapping or shared territories. In
84 contrast, in the Horsfield's bronze cuckoo, genetic parentage analysis revealed that females were
85 monogamous and that males were also monogamous, or sequentially monogamous (Langmore,
86 Adcock, & Kilner, 2007). This may also have implications for the type of territoriality exhibited
87 by different brood parasitic taxa.

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

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

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

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

127

128 Methods

129

130 Study site

131 The experiments were carried out at around Apaj (47° 6' 53.9" N; 19° 5' 21.2" E) and Kunpeszér
132 (47° 3' 40.1" N; 19° 16' 31.3" E), ca. 40-60 km southeast of Budapest. We also recorded calls of
133 unfamiliar (stranger) cuckoos in the surrounding areas (~ 20 km). The study was conducted 7
134 May to 30 May 2016. The laying season of cuckoos follows the availability of host, great reed
135 warbler, nests and lasts about 60-70 days (Moskát, Barta, Hauber, & Honza, 2006) in our study
136 area. This laying season starts in the first half of May and ends in mid-July in Hungary (Moskát
137 & Honza, 2000), and it can be divided into three periods of similar length. In the first period

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

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

153

154 Sound recordings

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

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

170

171 Radio telemetry

172 We caught 14 male cuckoos by mist-netting and conducting playbacks and equipped with Pip3
173 transmitters (type 392 by Biotrack Ltd., Wareham, U.K.) for identifying neighbour cuckoos and
174 some of the strangers (i.e. more than 15-20 territories away) individually. To demonstrate the
175 organisation of cuckoo territories in our study area, we illustrated representative territory
176 positions along a channel section. These results were obtained by following radio-tracked
177 individuals, using the same equipment as mentioned above (Fig. 1a). We also show an example
178 of the territory-dynamics of cuckoos, using Pica GPS tags by Ecotone Ltd, Gdynia, Poland (Fig.
179 1b; Moskát et al. n.d., in preparation).

180

181 Playback experiments

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

184 from the same cuckoo individual, followed by a 15 s break. This set was repeated, and then the
185 30 s sound unit was added to finalize the playback sample (Fig. 2e). The sound files were edited
186 by the program Audacity version 2.1, and the sample file was produced by Raven 1.5 Pro (Fig.
187 2). No relevant manipulation was applied, but noise was filtered out below 300 Hz, and the
188 amplitude was standardised with respect to peak amplitude. The 2-min length of sound files was
189 chosen to attract nearby cuckoos effectively. To avoid potential pseudoreplication in data (c.f.
190 Kroodsma, 1989; Kroodsma, Byers, Goodale, Johnson, & Liu, 2001; McGregor, 2000), each
191 sound file (neighbour, stranger or control) was tested on one focal bird, and each cuckoo
192 individual was used in only one treatment (neighbour, stranger, or control). To standardize
193 playback sound files, we used only the most common and well-known cuckoo vocalisation, the
194 advertising call ("cu-coo"), and avoided the rarer sound types, such as the "gowk" and "guo"
195 calls (c.f. Lei et al., 2005) or the quicker "ka-ka-coo", etc. For "stranger" playbacks, we used
196 recordings of cuckoos obtained from geographically distant areas from the focal individuals
197 (mean distance to their recordings from focal birds: $22.18 \text{ km} \pm 55.14 \text{ S.D.}$; range: 9.49 - 30.6
198 km; $N = 15$), which is much larger than cuckoos' average territory size along the channels, ca. 1-
199 2 km (our unpublished data).

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

207 converted to dB scale using the following formula: $20 * \log (X / 23174)$. These analyses revealed
 208 no statistical difference between the two groups (absolute values of RMS measurements (median
 209 and range): $\text{RMS}_{\text{neighbours}}$: 16.22 (11-26.3), $\text{RMS}_{\text{strangers}}$: 13.69 (11.67-16.93); Mann-Whitney
 210 $U_{12,15} = 53.5$, $P = 0.75$).

211 We also assessed the number of syllables in our stranger and neighbour sets of playback
 212 sound files as a potential source of discrimination bias; again we found no significant differences
 213 between the playback categories: (median and range: 19 (9-24), $N_{\text{neighbours}}$: 21 (13-25), $N_{\text{strangers}}$:
 214 Mann-Whitney $U_{12,15} = 53.00$, $P = 0.75$). Finally, we compared both the number of syllables and
 215 RMS amplitudes with scores of the first two components, obtained through Principal Component
 216 Analysis, with linear regression. These analyses revealed no statistical pattern between
 217 behavioural components and number of syllables or RMS amplitudes. (RMS measurements for
 218 neighbours: $\text{PC1}_{\text{scores}} = 0.044 * X_{\text{RMS}} - 0.633$, $\text{Beta} = 0.107$, $t = 0.516$, $P = 0.611$; $\text{PC2}_{\text{scores}} = -$
 219 $0.107 * X_{\text{RMS}} + 1.545$, $\text{Beta} = -0.261$, $t = -1.297$, $P = 0.207$; number of syllables for neighbours:
 220 $\text{PC1}_{\text{scores}} = -0.003 * X_{\text{syllables}} + 0.049$, $\text{Beta} = -0.009$, $t = -0.045$, $P = 0.964$; $\text{PC2}_{\text{scores}} = 0.061 *$
 221 $X_{\text{syllables}} - 1.162$, $\text{Beta} = 0.223$, $t = 1.099$, $P = 0.283$). We, therefore, concluded that basic
 222 bioacoustics parameters of our sound samples used for experiments were statistically similar for
 223 strangers and neighbours, thus neither the sound pressure described by RMS amplitude
 224 measurements, nor the number of syllables affected the reported behavioural responses of
 225 cuckoos in this experiment.

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

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

236

237 Measuring cuckoo behaviour to playback

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

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

263

264 Statistical analysis

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

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

277

278 Ethical notes

279 We used radio telemetry for monitoring space use by individual cuckoos in the study area. We
280 caught cuckoos in mist nets. We kept handling to the minimum to reduce disturbance (typically <
281 5-10 min). We mounted Pip3 transmitters produced by Biotrac Ltd. (Wareham, U.K.) with 18 cm
282 length antennas on the central tail feathers of each cuckoo, secured by factory-provided strings
283 and glue (Loctate 4860). The transmitter's weight represented only ca. 1% of the cuckoo's body
284 weight (1.2 g versus 122.78 g mean \pm 5.65 SD; range: 116-136 g; $N = 14$). These tags were
285 designed to be lost during the next moulting cycle of the tail feathers. All work complied with
286 Hungarian law, and was approved by the Middle-Danube-Valley Inspectorate for Environmental
287 Protection, Nature Conservation and Water Management, Budapest (permit No. PE/KTF/17190-
288 3/2015).

289

290 Results

291

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

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

304 As the cuckoos' responses to controls were markedly different from both neighbour and
305 stranger playbacks across all of our metrics (see above), we analysed their behavioural responses
306 by PCA for the neighbour and stranger groups (Table 1), to reduce collinearity between the
307 different response metrics. Two eigenvalues were greater than 1, so we analysed the first two PC
308 scores. The two components explained 42% and 22% of the total variance, respectively.
309 Component 1 (PC1) was composed of distance variables (closest distance and standardised
310 distance difference), related to how cuckoos' approached the loudspeaker (termed a boldness
311 component, where positive values are related to stronger approaches). Component 2 (PC2) is
312 correlated with movement latency and vocalization latency; therefore, it expresses (the opposite
313 of) how quickly cuckoos respond to the intruders. A fifth variable, the number of birds attracted,
314 was not included in any principal component with a high loading value. This variable ("no. of
315 cuckoos attracted") showed similar values for neighbours and strangers (ANOVA: $F_{1,25} = 0.262$,
316 $P = 0.613$; Fig. 5).

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

321 than to neighbours' playbacks by coming closer to the playback when they are faced with the
322 new threat of an unknown intruder. A binary logistic regression analysis also selected only one
323 of the response variables (standardised distance difference), which affects the dependent variable
324 ($B = -11.387$, S.E. = 4.601, $Wald_1 = 6.125$, $P = 0.013$; where the Hosmer and Lemeshow test
325 indicated good fit of the model: $\chi^2_6 = 8.191$, $P = 0.224$).

326

327 Discussion

328

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

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

344 rate and cuckoos' density are unusually high (see above). Cuckoos lay eggs every second day
345 (Wyllie, 1981), and they lay in nests during the host egg-laying stage or in the pre-egg-laying
346 stage when the nest is still empty (Moskát & Honza, 2002) to ensure early hatching of their eggs
347 (Geltsch, Bán, Hauber, & Moskát, 2016). They cannot use all available host nests for parasitism
348 in a territory if there are many in the same state. For this reason sharing territories with other
349 cuckoos, either with males or females, is likely adaptive for individuals within a dense cuckoo
350 population. Our radio-telemetry study (unpublished data) on cuckoos revealed that a cuckoo
351 territory might overlap with a few other territories at a time (see also Nakamura & Miyazawa,
352 1997; Vogel et al., 2004). In our study area an average territory of male cuckoos was about 1-2
353 km long along a channel (Fig. 1), so a cuckoo could be the neighbour of different individuals at
354 different sections. Therefore a cuckoo may know and recognize up to a dozen cuckoos as
355 neighbours in the peak "hot spots" of our study area. We did not study if all cuckoos tolerate
356 different types of "neighbours" similarly (e.g. (i) two cuckoos share a territory, (ii) their
357 territories overlap partly, or (iii) two cuckoos have non-overlapping adjacent territories). This
358 feature of additional complexity in neighbour structure is a worthwhile direction for future
359 studies. Instead, here we chose neighbours from shared or overlapping territories to ensure daily
360 connection among cuckoos.

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

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

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

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

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

393

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401 References

402

403 Anderson, M. G., Moskát, C., Bán, M., Grim, T., Cassey, P., Hauber, M. E. (2009). Egg eviction
404 imposes a recoverable cost of virulence in chicks of a brood parasite. *PLoS One*, 4(11):
405 e7725.

406 Bard, S. C., Hau, M., Wikelski, M., & Wingfield, J. C. (2002). Vocal distinctiveness and
407 response to conspecific playback in the spotted antbird, a neotropical suboscine. *Condor*,
408 104, 387–394.

409 Battison, M. M., Wilson, D. R., Graham, B. A., Kovach, K. A., & Mennill, D. J. (2015). Rufous-
410 and-white wrens *Thryophilus rufalbus* do not exhibit a dear-enemy effects towards
411 conspecific or heterospecific competitors. *Current Zoology*, 61, 23–33.

- 412 Berezki, J., Tóth, J. P., Sramkó, G., & Varga, Z. (2014). Multilevel studies on the two
413 phenological forms of large blue (*Maculinea arion*) (Lepidoptera: Lycaenidae). *Journal*
414 *of Zoological Systematics and Evolutionary Research*, 52, 32-43.
- 415 Brenowitz, E. A. (1991). Evolution of the vocal control system in the avian brain. *Seminars in*
416 *the Neurosciences*, 3, 339–407.
- 417 Briefer, E., Aubin, T., Lehongre, K., & Rybak, F. (2008). How to identify dear enemies: the
418 group signature in the complex song of the skylark *Alauda arvensis*. *Journal of*
419 *Experimental Biology*, 211, 317–326.
- 420 Brunton, D. H., Evans, B., Cope, T., & Ji, W. (2008). A test of the dear enemy hypothesis in
421 female New Zealand bellbirds (*Anthornis melanura*): female neighbors as threats.
422 *Behavioral Ecology*, 19, 791–798.
- 423 Budka, M., & Osiejuk, T. S. (2013). Neighbour-stranger call discrimination in a nocturnal rail
424 species, the corncrake *Crex crex*. *Journal of Ornithology*, 154, 685–694.
- 425 Charif, R. A., Strickman, L. M., & Waack, A. M. (2010). *Raven Pro 1.4 User's Manual*. Ithaca,
426 N.Y., U.S.A.: The Cornell Lab of Ornithology.
- 427 Courvoisier, H., Camacho-Schlenker, S., & Aubin, T. (2014). When neighbours are not 'dear
428 enemies': a study in the winter wren, *Troglodytes troglodytes*. *Animal Behaviour*, 90,
429 229–235.
- 430 Daniel, C., Millar, C. D., Ismar, S. M. H., Stephenson, B., & Hauber, M. E. (2007). Evaluating
431 molecular and behavioural sexing methods for the Australasian gannet (*Morus serrator*).
432 *Australian Journal of Zoology*, 55, 377-382.
- 433 Davies, N. B. (2000). *Cuckoos, cowbirds and other cheats*. London, U.K.: T. & A. D. Poyser.

- 434 de la Colina, M. A., Hauber, M. E., Strausberger, B. M., Reboreda, J. C., & Mahler, B. (2016).
435 Molecular tracking of individual host use in the shiny cowbird - a generalist brood
436 parasite. *Ecology and Evolution* (published online) doi:10.1002/ece3.2234
- 437 Fisher, J. (1954). Evolution and bird sociality. In J. Huxley, A. C. Hardy, & E. B. Ford. (Eds.),
438 *Evolution as a process* (pp. 71–83). London, U.K.: Allen & Unwin.
- 439 Geltsch, N., Bán, M., Hauber, M. E., & Moskát, C. (2016). When should common cuckoos
440 *Cuculus canorus* lay their eggs in host nests? *Bird Study*, 63, 46–51.
- 441 Hardouin, L. A., Tabel, P., & Bretagnolle, V. (2006). Neighbour-stranger discrimination in the
442 little owl, *Athene noctua*. *Animal Behaviour*, 72, 105–112.
- 443 Hauber, M. E., Strausberger, B. M., Feldheim, K. A., Lock, J., & Cassey, P. (2012). Indirect
444 estimates of breeding and natal philopatry in an obligate avian brood parasite. *Journal of*
445 *Ornithology*, 153, 467–475.
- 446 Honza, M., Voslajerová, K., & Moskát, C. (2007). Eviction behaviour of the common cuckoo
447 *Cuculus canorus* chicks. *Journal of Avian Biology*, 38, 385–389.
- 448 Hinde, A. (1956). The biological significance of the territories of birds. *Ibis*, 98, 340–369.
- 449 Jung, W. J., Lee, J. W., & Yoo, J. C. (2014). “cu-coo”: Can you recognize my stepparents? - A
450 study of host-specific male call divergence in the Common Cuckoo. *PLoS one*, 9(3):
451 e90468.
- 452 Kiefer, S., Scharff, C., Hultsch, H., & Kipper, S. (2014). Learn it now, sing it later? Field and
453 laboratory studies on song repertoire acquisition and song use in nightingales.
454 *Naturwissenschaften*, 101, 955–963.
- 455 Kroodsma, D. E. (1989). Suggested experimental designs for song playbacks. *Animal Behaviour*,
456 37, 600–609.

- 457 Kroodsma, D. E., Byers, B. E., Goodale, E., Johnson, S., & Liu, W.-C. (2001). Pseudoreplication
458 in playback experiments, revisited a decade later. *Animal Behaviour*, *61*, 1029–1033.
- 459 Langmore, R. B., Adcock, G. J., & Kilner, R. M. (2007). The spatial organization and mating
460 system of Horsfield's bronze-cuckoos, *Chalcites basalis*. *Animal Behaviour*, *74*, 403–412.
- 461 Lei, F.-M., Zhao, H.-F., Wang, A.-Z., Yin, Z.-H., & Payne, R. B. (2005). Vocalizations of the
462 common cuckoo *Cuculus canorus* in China. *Acta Zoologica Sinica*, *51*, 31–37.
- 463 Mackin, W. A. (2005). Neighbor-stranger discrimination in Audubon's shearwater (*Puffinus l.*
464 *herminieri*) explained by a "real enemy" effect. *Behavioral Ecology and Sociobiology*,
465 *59*, 326–332.
- 466 Marchetti, K., Nakamura, H., & Gibbs, H. L. (1998). Host race formation in the common
467 cuckoo. *Science*, *282*, 471–472.
- 468 Marler, P. (1997). Three models of song learning: evidence from behaviour. *Journal of*
469 *Neurobiology*, *33*, 501–516.
- 470 Martinez, J. G., Soler, J. J., Soler, M., & Burke, T. (1998). Spatial patterns of egg laying and
471 multiple parasitism in a brood parasite: a non-territorial system in the great spotted
472 cuckoo (*Clamator glandarius*). *Oecologia*, *117*, 286–294.
- 473 McGregor, P. K. (2000). Playback experiments: design and analysis. *Acta Ethologica*, *3*, 3–8.
- 474 Moksnes, A., Røskaft, E., Rudolfson, G., Skjelseth, S., Stokke, B. G., Kleven, O., Gibbs, H. L.,
475 Honza, M., Taborsky, B., Teuschl, Y., Vogl, W., & Taborsky, M. (2008). Individual
476 female common cuckoos *Cuculus canorus* lay constant egg types but egg appearance
477 cannot be used to assign eggs to females. *Journal of Avian Biology*, *39*, 238–241.

- 478 Møller, A. P., Morelli, F., Mousseau, T. A., & Tryjanowski, P. (2016). The number of syllables
479 in Chernobyl cuckoo calls reliably indicate habitat, soil and radiation levels. *Ecological*
480 *Indicators*, *66*, 592–597.
- 481 Moskát, C., & Honza, M. (2000). Effect of nest and nest site characteristics on the risk of cuckoo
482 *Cuculus canorus* parasitism in the great reed warbler *Acrocephalus arundinaceus*.
483 *Ecography*, *23*, 335–341.
- 484 Moskát, C., & Honza, M. (2002). European cuckoo *Cuculus canorus* parasitism and host's
485 rejection behaviour in a heavily parasitized great reed warbler *Acrocephalus*
486 *arundinaceus* population. *Ibis*, *144*, 614–622.
- 487 Moskát, C., Bán, M., Bereczki, J., Fülöp, A., & Hauber, M. E. (n.d.) Bimodal habitat use of
488 common cuckoos (*Cuculus canorus*) as GPS telemetry revealed (in preparation)
- 489 Moskát, C., Barta, Z., Hauber, M. E., & Honza, M. (2006). High synchrony of egg laying in
490 common cuckoos (*Cuculus canorus*) and their great reed warbler (*Acrocephalus*
491 *arundinaceus*) hosts. *Ethology, Ecology & Evolution*, *18*, 159–167.
- 492 Moskát, C., Hauber, M. E., Avilés, J. M., Bán, M., Hargitai, R., & Honza, M. (2009). Increased
493 host tolerance of multiple cuckoo eggs leads to higher fledging success of the brood
494 parasite. *Animal Behaviour*, *77*, 1281–1290.
- 495 Nakamura, H., & Miyazawa, Y. (1997). Movements, space use and social organisation of
496 radiotracked common cuckoos during the breeding season in Japan. *Japanese Journal of*
497 *Ornithology*, *46*, 23–54.
- 498 Osiejuk, T. S. (2014). Differences in frequency of shared song types enables neighbour-stranger
499 discrimination in a songbird species with small song repertoire. *Ethology*, *120*, 893–903.

- 500 Scardamaglia, R., & Reboreda, J. C. (2014). Ranging behaviour of female and male shiny
501 cowbirds and screaming cowbirds while searching for host nests. *The Auk -*
502 *Ornithological Advances*, 131, 610–618.
- 503 Schulze-Hagen, K., Stokke, B. G., & Birkhead, T. R. (2009). Reproductive biology of the
504 European cuckoo *Cuculus canorus*: early insights, persistent errors and the acquisition of
505 knowledge. *Journal of Ornithology*, 150, 1–16.
- 506 Searcy, W. A., Akcay, C., Nowicki, S., & Beecher, M. D. (2014). Aggressive signalling in song
507 sparrow and other songbirds. In M. Naguib, L. Barrett, H. J. Brockmann, S. Healy, J. C.
508 Mitani, T. J. Roper, & L. W. Simmons (Eds.), *Advances in the Study of Behavior*, Vol. 46
509 (pp. 89–125). Burlington, M.A., U.S.A.: Academic Press.
- 510 Stoddard, P. K. (1996). Vocal recognition of neighbors by territorial passerines. In D. E.
511 Kroodsma, & E. H. Miller (Eds.), *Ecology and evolution of acoustic communication in*
512 *birds* (pp. 356–374). Ithaca, N.Y., U.S.A.: Cornell University Press.
- 513 Svensson, L., Mullaney, K., Zetterström, D., & Grant, P. J. (2010). *Collins Bird Guide*. 2nd
514 Edition. Harper Collins, London.
- 515 Temeles, E. J. (1994). The role of neighbours in territorial systems: when are they 'dear
516 enemies'? *Animal Behaviour*, 47, 339–350.
- 517 Tibbetts, E. A., & Dale, J. (2007). Individual recognition: it is good to be different. *Trends in*
518 *Ecology and Evolution*, 22, 529–537.
- 519 Vogl, W., Taborsky, B., Taborsky, M., Teuschl, Y., & Honza, M. (2004). Habitat and space use
520 of European cuckoo females during the egg laying period. *Behaviour*, 141, 881–898.
- 521 Wei, C., Jia, C., Dong, L., Wang, D., Xia, C., Zhang, Y., & Liang, W. (2015). Geographic
522 variation in the calls of the common cuckoo (*Cuculus canorus*): isolation by distance and

- 523 divergence among subspecies. *Journal of Ornithology*, 156, 533–542.
- 524 Wyllie, I. 1981. *The cuckoo*. London, U.K.: Batsford.
- 525 Zölei, A., Bán, M., & Moskát, C. (2015). No change in common cuckoo *Cuculus canorus*
- 526 parasitism and great reed warblers' *Acrocephalus arundinaceus* egg rejection after seven
- 527 decades. *Journal of Avian Biology*, 46, 570–576.
- 528 Zsebök, S., Moskát, C., & Bán, M. (2016). Individually distinctive vocalization in common
- 529 cuckoos (*Cuculus canorus*). *Journal of Ornithology* (in press)
- 530

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 <i>df</i> | Error <i>df</i> | <i>P</i> | Noncent. parameter | Observed 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

545 Legend to figures

546

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

559

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

567

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

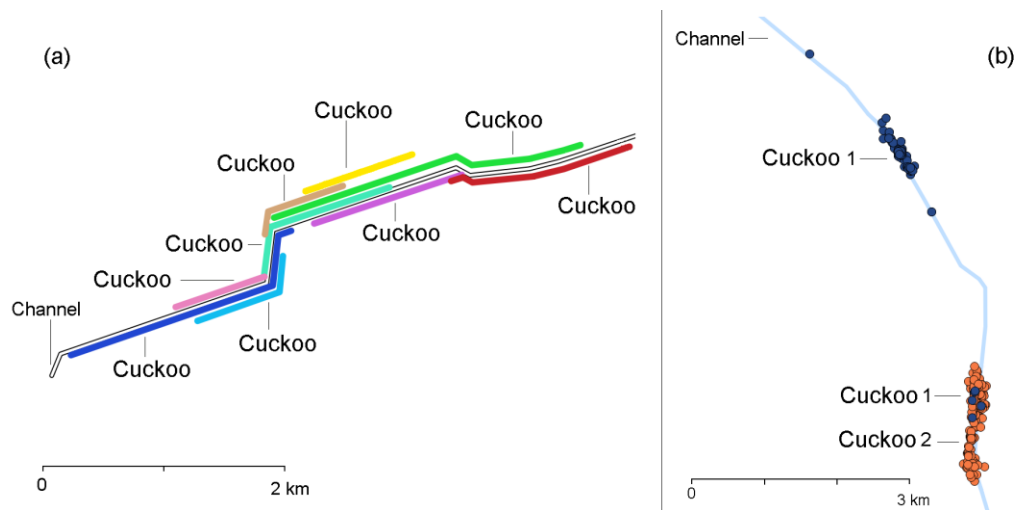
572 Figure 4 Cuckoos' quantitative responses to sound playback (means, standard errors and sample
573 sizes above bars are shown). (a) Distance of closest approach to the loudspeaker (closest
574 distance; m). (b) Movement toward loudspeaker from starting point (distance difference; m).

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

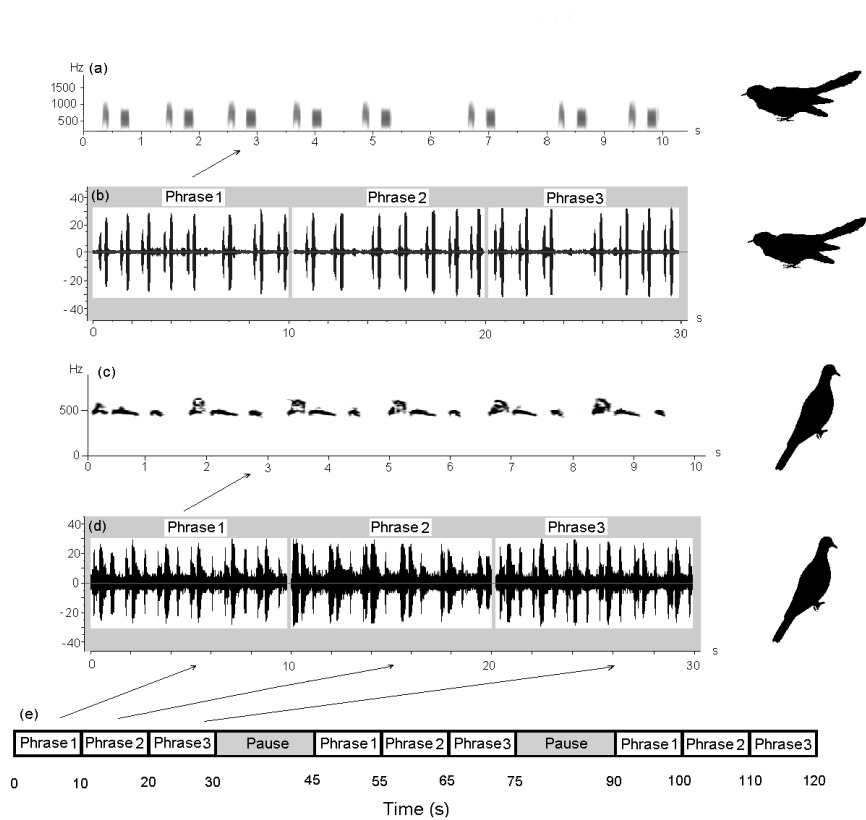
580 Fig.1



581

582

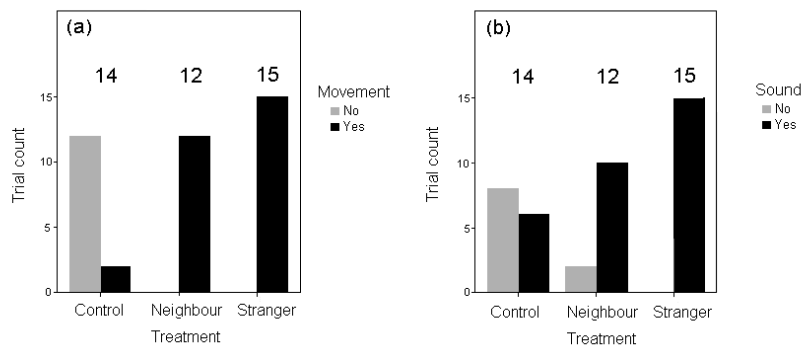
583 Fig. 2



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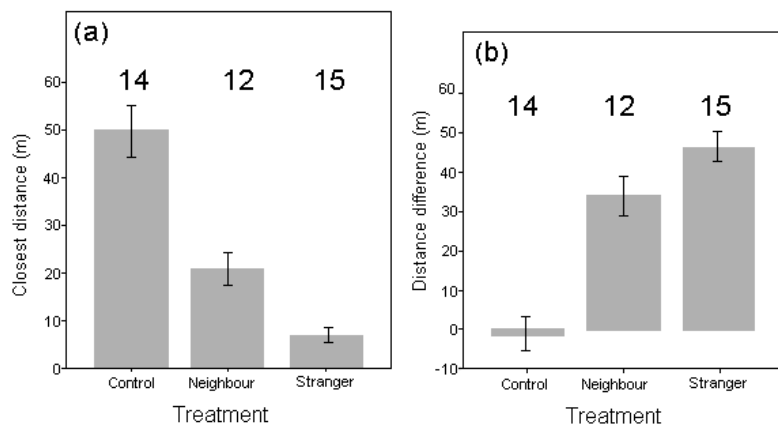
586 Fig. 3



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588

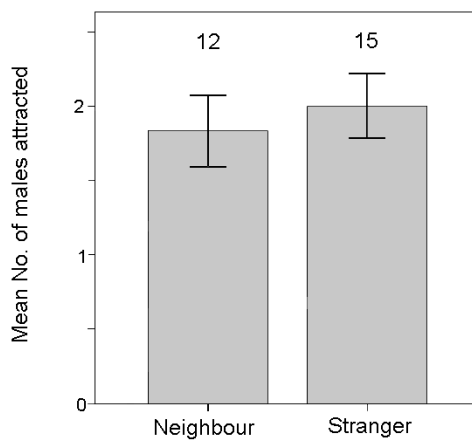
589 Fig. 4



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592 Fig. 5



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594