

# 1 Female cuckoo calls misdirect host defences towards the wrong enemy

2 Jenny E. York\* and Nicholas B. Davies

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4 Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK.

5 \*Correspondence to: [jy364@cam.ac.uk](mailto: jy364@cam.ac.uk)

6  
7 **Prey are sensitive to even subtle cues of predation risk which provides the evolutionary**  
8 **potential for parasites to exploit host risk perception. Brood parasitic common cuckoos**  
9 **(*Cuculus canorus*) lay their eggs in the nests of host species and their secretive laying**  
10 **behaviour enables them to evade host defences. Therefore, it seems paradoxical that**  
11 **female cuckoos often give a conspicuous “chuckle” call after parasitizing a host’s clutch.**  
12 **Here we show that this hawk-like chuckle call increases the success of parasitism by**  
13 **diverting host parents’ attention away from the clutch and towards their own safety. Our**  
14 **field experiments reveal that reed warbler (*Acrocephalus scirpaceus*) hosts paid no more**  
15 **attention to the “cuck-oo” call of the male common cuckoo than to the call of a harmless**  
16 **dove. However, the chuckle call of the female cuckoo had the same effect as the call of a**  
17 **predatory hawk in distracting the warblers’ attention and reducing rejection of a foreign**  
18 **egg. Our results show that the cuckoo enhances her success by manipulating a**  
19 **fundamental trade-off in host defences between clutch- and self-protection.**

20 Parasites evolve not only to evade host defences but also to manipulate host behaviour<sup>1</sup>.  
21 Endo-parasites do this inside the bodies of their hosts by physiological manipulation of host  
22 risk-taking to enhance parasite transmission<sup>2</sup>. Here we test whether a brood parasitic cuckoo  
23 manipulates host perception of predation risk using an acoustic signal, a hawk-like call, that  
24 might misdirect host defences and thereby reduce the chance that hosts detect parasitism. It is  
25 well known that adult birds distinguish threats to themselves from those to their offspring<sup>3</sup>; for

26 example, parents flee from hawks but readily attack nest predators of no direct threat to the  
27 adults themselves<sup>4</sup>. In theory, cuckoos could exploit this fundamental trade-off in host defences  
28 by deceptive signals.

29 Obligate brood parasites lay their eggs in the nests of other species, the hosts, which are  
30 then tricked into raising parasite young at the expense of some, or all, of their own offspring<sup>5</sup>.  
31 Previous studies have shown that hosts of the common cuckoo, *Cuculus canorus*, defend against  
32 parasitism by mobbing adult cuckoos<sup>6</sup> (a first line of defence)<sup>7</sup> and by rejecting eggs that differ  
33 from their own<sup>8-10</sup>. Hosts also monitor cuckoo activity in the vicinity of their nest and vary these  
34 defences in relation to local parasitism risk.<sup>11-14</sup> In response, cuckoos have evolved host egg  
35 mimicry<sup>15</sup> and remarkable secrecy and speed when they parasitize a host nest<sup>16</sup>. Therefore, it  
36 seems paradoxical that female cuckoos often call while they monitor host nests, and especially  
37 just after parasitizing a clutch<sup>16</sup>. Their chuckle (or ‘bubble’)<sup>16</sup> call, a rapidly repeated “*k-wik-k-wik-*  
38 *k-wik...*” is similar in fundamental frequency and rate to the “*k-iii-k-iii-k-iii...*” call of *Accipiter*  
39 hawks and strikingly different from the familiar two-note call of the male cuckoo (Fig. 1a).

40 We propose that the female cuckoo chuckle call tricks the hosts into responding  
41 vigilantly as if they were exposed to danger from a hawk, instead of from a cuckoo. This would  
42 divert host attention from clutch-protection to self-protection<sup>3</sup>, and so reduce the chance that  
43 the hosts detect that they have been parasitized. As noted by Alfred Russel Wallace (1889)<sup>17</sup>,  
44 many parasitic cuckoos also resemble hawks in appearance. Indeed, experiments have shown  
45 that this visual resemblance makes hosts more reluctant to mob<sup>18</sup>. An alternative hypothesis  
46 therefore is that the female cuckoo’s chuckle call might provide an additive benefit to enhance  
47 her hawk-mimicry in order to bypass the hosts’ first line of defence. In this study, we test both  
48 of these potential benefits of the female cuckoo chuckle call in overcoming the host’s mobbing  
49 and egg-rejection defences.

50

51

## 52 Results

53 First, we tested whether female cuckoo calls provoke vigilance in reed warblers, a  
54 favourite cuckoo host in marshland<sup>8</sup>. Our playback experiment had four treatments (Fig. 1a): call  
55 of female cuckoo (a threat to the clutch but not to adults), call of Eurasian sparrowhawk,  
56 *Accipiter nisus* (a threat to the adults but not to the clutch), call of male cuckoo (no direct threat to  
57 the clutch nor to adults, but a potential cue to parasitism risk), and call of collared dove,  
58 *Streptopelia decaocto* (a harmless control). All four calls are frequently encountered on the study site.  
59 At 24 nests where reed warblers were incubating a recently completed clutch, we placed a  
60 speaker 5 m from the nest and recorded host responses on video to each of the four calls in  
61 sequence (see Methods: Fig. 1d). There were marked differences in response across the four  
62 treatments (Fig. 1b; Table 1, Experiment 1). As predicted, reed warblers were more likely to  
63 become vigilant (scanning surrounds for danger; see Methods) during hawk calls than during  
64 dove calls (Generalized Linear Mixed-effects Model (GLMM):  $\chi^2 = 12.02$ ;  $p < 0.001$ ). There was  
65 little response to male cuckoo calls and this did not differ from that to dove calls ( $\chi^2 = 0.37$ ;  $p =$   
66  $0.54$ ). By contrast, hosts responded strongly to female cuckoo calls (Figure 1b) and this did not  
67 differ from that to hawk calls ( $\chi^2 = 0.62$ ;  $p = 0.43$ ). When vigilance responses occurred they  
68 were rapid, occurring within the first few syllables of playback (see Methods).

69 The increase in vigilance to both the female cuckoo and hawk calls may arise from their  
70 acoustic similarity or because both are independently recognized as a threat to reed warblers. We  
71 therefore repeated the playbacks to tits (Paridae), frequent victims of sparrowhawks<sup>19</sup> but  
72 typically unsuitable hosts for cuckoos in Europe. Hence, they should not respond to female  
73 cuckoo calls as a threat unless they mistake them for hawk calls. We presented the playbacks to  
74 60 individually recognizable tits at experimental feeders (28 blue tits, *Cyanistes caeruleus*, and 32  
75 great tits, *Parus major*). Each individual experienced just one of the four treatments broadcast  
76 from a speaker 5 m from the feeder. Playback order was randomized and we recorded responses

77 on video to each of the four calls in separate trials (see Methods; Fig. 1e). There were no  
78 differences in responses between blue and great tits (Generalised Linear Model (GLM):  $\chi^2 =$   
79 1.62,  $p = 0.20$ ). Once again, responses occurred rapidly and there were the same marked  
80 differences in vigilance as for reed warblers (Fig. 1c; Table 1, Experiment 2). Tits were more  
81 likely to become vigilant during hawk calls than during dove calls ( $\chi^2 = 9.36$ ;  $p = 0.002$ ), the  
82 response to male cuckoo calls was no different from that to dove calls ( $\chi^2 = 0.83$ ;  $p = 0.36$ ),  
83 whereas female cuckoo calls increased vigilance as much as hawk calls ( $\chi^2 = 2.00$ ;  $p = 0.16$ ). As  
84 cuckoos are no threat to tits, their similar response to the calls of female cuckoos and hawks is  
85 likely to result from perceived acoustic similarity.

86         Next, we tested whether exposure to the four calls influenced reed warbler nest defences  
87 (egg rejection and mobbing). We removed one egg at random from 72 reed warbler clutches on  
88 the day they laid their fourth egg, when they would still be vulnerable to parasitism, painted it  
89 brown and then returned it to the nest to simulate parasitism (Fig. 2a; female cuckoos typically  
90 remove a host egg and then lay their own egg in its place; see Methods). We then placed a balsa  
91 wood model of an adult cuckoo on the nest with a speaker concealed next to it. Each reed  
92 warbler pair then received just one of the four playbacks. We measured host mobbing responses  
93 (mandible snaps and rasp calls) for one minute after the first member of the pair returned to  
94 within 1 m of the nest, then playback was triggered remotely and we recorded host mobbing  
95 responses for another minute (see Methods). This experiment allowed us to test whether the  
96 female chuckle influences the first line of defence (mobbing) and/or egg rejection defences.

97         Playback treatment had a marked effect on egg rejection (Figure 2b; Table 1, Experiment 3).  
98 When we checked the nests one day after the trial, two clutches had been depredated and of the  
99 remaining 70 clutches, the foreign egg had been rejected in 32 cases (one by nest desertion and  
100 all others by targeted ejection from the nest). As predicted from our hypothesis that increased  
101 vigilance diverts host attention away from the clutch, reed warblers that had been exposed to

102 hawk or female cuckoo calls were more likely to accept the foreign egg (Figure 2b). The effect of  
103 playback treatment was still apparent when we checked clutches again three days after the trial,  
104 after opportunity for delayed rejection ( $n = 68$  nests, two clutches were depredated since day 1).  
105 Reed warblers were still more than twice as likely to retain a foreign egg in their clutch after  
106 female cuckoo calls compared to male cuckoo calls ( $\chi^2 = 5.99$ ;  $p = 0.014$ ).

107 By contrast, call type did not affect mobbing responses (Table 1, Experiment 3;  
108 Supplementary Information Figure 1). Neither propensity to mob after playback (GLM:  $\chi^2 =$   
109 4.84,  $n = 72$  nests,  $p = 0.18$ ) nor mobbing intensity ( $F = 0.76$ ,  $n = 44$  nests,  $p = 0.52$ ) differed  
110 significantly across the treatments. As in previous studies, individual mobbing responses also did  
111 not predict egg rejection<sup>12</sup>; current study:  $\chi^2 = 0.69$ ,  $p = 0.40$ ). These results are perhaps not  
112 surprising given that mobbing is a generalized defence against all intruders at the nest, where  
113 individuals show consistent differences in mobbing intensity, not specific to cuckoos<sup>20</sup>.

114

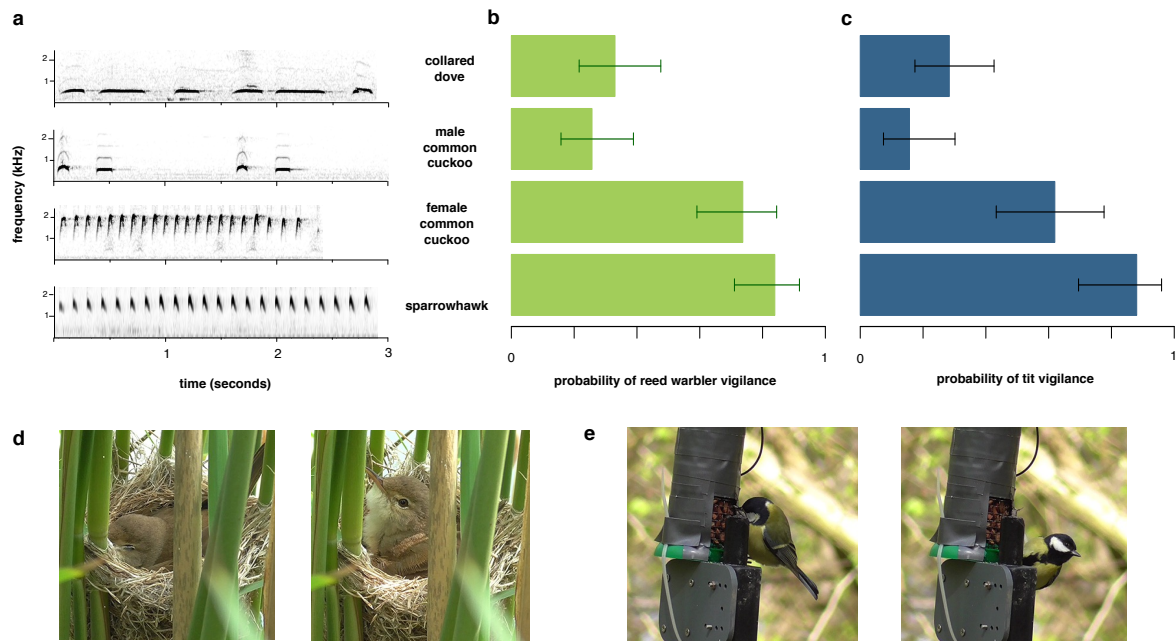
## 115 **Discussion**

116 Why did male cuckoo calls have no more effect on host responses than a harmless dove  
117 control? Male cuckoos call conspicuously from exposed perches to repel rival males<sup>21</sup> and attract  
118 females<sup>22</sup>, but their calls are likely to be a poor predictor of local parasitism risk because males  
119 roam widely and call frequently even when females are scarce<sup>22</sup>. Conversely, the presence of a  
120 female cuckoo is a strong predictor of parasitism risk,<sup>6</sup> which explains why they are more  
121 secretive than males and call less frequently.<sup>16</sup> This would reduce the potential for hosts to learn  
122 to discriminate female cuckoo chuckles from hawk calls. Our results also explain why female  
123 cuckoos typically call just after laying,<sup>16,22</sup> which is precisely when it would pay them to distract  
124 host attention from the clutch<sup>24</sup>. A female cuckoo can choose an opportune time to glide down  
125 to the nest when the hosts are away, but there is an increasing probability that the hosts will  
126 return or at least see her leaving, when it might be most beneficial to distract them with a call.

127 Similar vocal trickery has been demonstrated in kleptoparasitic drongos (*Dicrurus adsimilis*), whose  
128 false alarm calls enable them to steal food by distracting the attention of foragers.<sup>23</sup>

129 Hawk-like calls are typical for female cuckoos of the *Cuculus* genus and are quite unlike  
130 the male calls, which are simple coos and whistles. A comparison across the cuckoo subfamily  
131 Cuculinae suggests that sexually dimorphic calls have evolved with parasitism: 19 of 58 parasitic  
132 species exhibit sex-differences, whereas none of the 32 non-parasitic species do so<sup>25</sup>. In many  
133 species sex-specific calls have socially selected functions, for example to attract mates and repel  
134 rivals<sup>26</sup>. Female cuckoos rarely call<sup>22</sup> which suggests the calls are not important for territory  
135 defence, though they may function in attracting males. However, their timing (after laying),  
136 acoustic similarity with hawk calls, and our experimental results reported here all suggest that  
137 their calls have been shaped by host defences. Our results suggest that female chuckles play an  
138 important role in a suite of specialised female traits associated with a brood-parasitic lifestyle,  
139 including: secretive behaviour to avoid alerting hosts,<sup>8,16</sup> polymorphic plumage to confuse host  
140 recognition<sup>27,28</sup> and brain specialization to facilitate spatial memory of the locations of host  
141 nests<sup>29</sup>.

142 To the human ear, there are clear differences between female chuckle calls and hawk  
143 calls. Nevertheless, manipulation by imperfect mimicry is frequent in the natural world, and  
144 resemblance to hawk calls in some key features might be sufficient to trick hosts<sup>30,31</sup>. If hosts  
145 respond to a female cuckoo call as though it were a hawk, they will be less likely to reject a  
146 cuckoo egg, but if they fail to respond to a hawk call they may lose their life. Predators are  
147 secretive so it is not surprising that even brief encounters, including auditory cues, can have  
148 long-lasting effects on prey behaviour<sup>19,32,33</sup>. The benefits of a more rapid response to hawk-like  
149 signals inevitably leads to increased discrimination errors<sup>34</sup> and so leaves hosts vulnerable to  
150 exploitation by cuckoo chuckles. As a result, the female cuckoo might have ‘the last laugh’ in this  
151 particular battle between host defence and parasite trickery.



152

153 **Figure 1 | Reed warblers and tits were more likely to become vigilant in response to**

154 **female cuckoo and hawk calls than to calls of a male cuckoo or dove. a,** Examples of call

155 types used in playback experiments (collared dove “*coo-cooo-coo*”, male common cuckoo “*cuck-oo*”,

156 female common cuckoo “*kwik-kwik-kwik...*”, and sparrowhawk “*kiii-kiii-kiii...*”) displayed as

157 spectrograms. **b,** Probability of reed warblers’ becoming vigilant during the playback trial was

158 greater during exposure to female cuckoo or sparrowhawk calls compared to dove or male

159 cuckoo calls (Table 1, Experiment 1; n = 96 observations at 24 nests; predicted means ±

160 standard errors shown). **c,** Probability of blue and great tits’ becoming vigilant during the

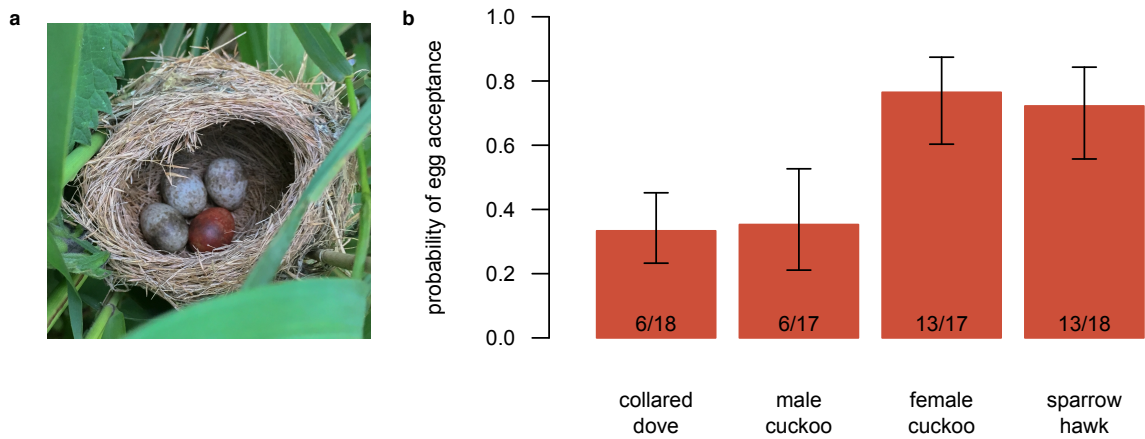
161 playback trial was greater across individuals during exposure to female cuckoo (n = 17) or

162 sparrowhawk (n = 13) calls compared to dove (n = 16) or male cuckoo (n = 14) calls (Table 1,

163 Experiment 2, n = 60 observations of 60 individuals; predicted means ± standard errors shown).

164 **d,** An incubating reed warbler at rest (left) and vigilant, scanning the surroundings (right). **e,** A

165 great tit on an experimental feeder foraging (left) and vigilant (right).



166

167 **Figure 2 | Reed warblers were more likely to accept a foreign egg after playback of**  
 168 **female cuckoo or hawk calls than after the calls of a male cuckoo or dove. a, A reed**  
 169 **warbler clutch with one egg painted brown to simulate parasitism. b, The probability of reed**  
 170 **warblers' accepting a foreign egg one day after the experiment was greater after exposure to**  
 171 **female cuckoo or hawk calls compared to dove or male cuckoo calls (Table 1, Experiment 3, n =**  
 172 **70 nests, predicted means  $\pm$  standard errors shown; raw proportions of nests in which foreign**  
 173 **eggs were accepted also shown at the base of each bar). Male cuckoo calls had no more effect**  
 174 **than control dove calls ( $\chi^2 = 0.015$ ;  $p = 0.90$ ), whereas female cuckoo calls reduced egg rejection**  
 175 **as much as hawk calls ( $\chi^2 = 0.083$ ;  $p = 0.77$ ).**



176 **Table 1** | Outcomes of GLMM and GLM to investigate the effect of playback treatment on  
 177 vigilance and egg acceptance in experiments 1, 2 and 3.

Analysis	Response	Predictor (reference)	LRT		Parameter Estimates	
			$\chi^2$	p	Factor levels	Mean $\pm$ SE
<b>Experiment 1</b> Reed warbler vigilance (GLMM)	Scan during playback? (yes/no)	Intercept				-0.69 $\pm$ 0.60
		Playback order (first)	2.52	0.47	second	0.45 $\pm$ 0.68
					third	0.93 $\pm$ 0.69
					fourth	<-0.01 $\pm$ 0.67
		Call type (dove)	<b>*23.66</b>	<b>*&lt;0.001</b>	male cuckoo	-0.37 $\pm$ 0.61
					female cuckoo	1.72 $\pm$ 0.66
			sparrowhawk	2.34 $\pm$ 0.76		
<b>Experiment 2</b> Tit vigilance (GLM)	Scan during playback? (yes/no)	Intercept				-0.93 $\pm$ 0.63
		Tit species (blue)	1.62	0.20	great	0.79 $\pm$ 0.63
		Call type (dove)	<b>*17.68</b>	<b>*&lt;0.001</b>	male cuckoo	-0.76 $\pm$ 0.84
					female cuckoo	1.41 $\pm$ 0.76
					sparrowhawk	2.92 $\pm$ 1.17
<b>Experiment 3</b> Egg acceptance at 1 day (GLM)	Accept foreign egg? (yes/no)	Intercept				-0.69 $\pm$ 0.50
		Call type (dove)	<b>*11.72</b>	<b>*0.008</b>	male cuckoo	0.087 $\pm$ 0.71
					female cuckoo	1.87 $\pm$ 0.76
					sparrowhawk	1.65 $\pm$ 0.73
Egg acceptance at 3 days (GLM)	Accept foreign egg? (yes/no)	Intercept				-0.96 $\pm$ 0.53
		Call type (dove)	6.81	0.078	male cuckoo	-1.06 $\pm$ 0.92
					female cuckoo	0.96 $\pm$ 0.73
					sparrowhawk	0.60 $\pm$ 0.72
Propensity to mob (GLM)	Mob (yes/no)	Intercept				-0.52 $\pm$ 0.65
		Mob before (no)	<b>*33.98</b>	<b>*&lt;0.001</b>	yes	3.68 $\pm$ 0.84
		Call type (dove)	4.84	0.18	male cuckoo	-1.68 $\pm$ 1.08
					female cuckoo	-0.14 $\pm$ 0.89
					sparrowhawk	-1.66 $\pm$ 1.02
Mobbing intensity (GLM)	Mobbing rate (calls/min)	Intercept				5.98 $\pm$ 10.30
		Mob rate before	<b>*130.87</b>	<b>*&lt;0.001</b>	rate before	0.95 $\pm$ 0.08
		Call type (dove)	0.76	0.52	male cuckoo	13.16 $\pm$ 14.2
					female cuckoo	-6.74 $\pm$ 14.1
			sparrowhawk	8.75 $\pm$ 15.07		

178  
 179 The *P* value for each term is based on the chi-squared test (likelihood ratio test (LRT)) for  
 180 change in deviance when comparing models with or without that term. The mean estimates  $\pm$   
 181 s.e.m. are reported for all terms in the full model, and those terms that resulted in a significant  
 182 change in deviance when removed are indicated by an asterisk. GLM, generalized linear model;  
 183 GLMM, generalized linear mixed-effects model.

184

185 **Methods**

186

187 **Study species and field sites.** Our experiments were conducted from March to July in 2016 at  
188 three field sites in Cambridgeshire, UK. Playback experiments with great tits and blue tits were  
189 conducted in the Cambridge University Botanic Garden (52°19'35"N, 0°12'58"E) and Madingley  
190 Wood (52°21'71"N, 0°04'89"E). Experiments with reed warblers were conducted on Wicken  
191 Fen (52°18'29"N, 0°16'50"E), where we have studied reed warblers and cuckoos since 1985<sup>12</sup>.  
192 Each year, circa 300 pairs of reed warblers nest along the reed fringes of waterways and defend  
193 11-35 m linear territories. On average, ~5% of these nests are parasitized by cuckoos who  
194 monitor host nests from perches in trees and large shrubs near the reeds. Our experiments  
195 closely follow procedures detailed elsewhere<sup>12</sup> and are described briefly here.

196

197 **Playback stimuli.** Each exemplar was extracted from original uncompressed WAV files  
198 obtained from XenoCanto recordists (<http://www.xeno.canto.org>; Supplementary information).  
199 For all three playback experiments we used the same exemplars of each call type: four different  
200 exemplars for each call type (16 in total). Each playback track of female cuckoo or sparrowhawk  
201 call comprised one natural phrase of repeated syllables extracted from the recordings, while for  
202 the male cuckoo each exemplar comprised of three natural “*cuck-oo*” phrases and for the dove  
203 call two natural “*coo-cooo-coo*” phrases. Average duration across tracks was  $3.06 \pm 0.17$  seconds  
204 (mean  $\pm$  standard error; dove:  $3.11 \pm 0.18$ ; male cuckoo:  $3.73 \pm 0.21$ , female cuckoo:  $2.18 \pm 0.13$ ,  
205 sparrowhawk:  $3.21 \pm 0.31$  seconds). Visual inspection of the data revealed no consistent  
206 difference between playback exemplars of a given call type on responses in any of our  
207 experiments. Vigilance responses by both reed warblers and tits occurred rapidly, within the first  
208 few syllables of the playback (see below), so small differences in playback duration did not affect  
209 the results. Each playback track was composed using Cool Edit Pro (v. 2.0). First, we filtered low  
210 frequency background noise (below 100Hz) from each track, and then added 10 seconds of

211 silence before and after each call clip (to allow the observers time to prepare to record  
212 responses). All responses were measured from onset of the playback call and not the onset of the  
213 track. The tracks were then standardised to a peak amplitude of -15db and saved in stereo format  
214 as uncompressed WAV files.

215       Given that we used the calls of three different species, and there is no information available  
216 on natural production amplitudes for female cuckoo calls, it was not possible to exactly match  
217 playback amplitudes to natural levels in our experiment. Instead, we standardized all playback  
218 amplitudes to the same level, and based our amplitude levels on those used in previous studies  
219 using hawk call playbacks<sup>35</sup>. Playback amplitude was standardized within and across treatments  
220 by calibration of peak amplitude (HandyMAN TEK1345 sound level meter, Metrel UK Ltd) at  
221 the distance of the fixed location for all subjects (either the nest or feeder, see experiments 1-3  
222 below for further details). The example spectrograms in Figure 1a were generated in Raven Pro<sup>36</sup>  
223 1.4 with Hamming window, 1024 points 56 Hz, 96% overlap, 0.99 ms.

224

225 **Video recording and analysis.** Behavioural responses were recorded on video files (HC-  
226 V270EB-K HD Camcorder, Panasonic, UK; 50 FPS, resolution 1920 x 1080). The videos were  
227 scored blind to treatment by first marking the time-point of the playback call onset, and then  
228 subsequently watching muted videos slowed to x 0.25 recorded speed VLC (VideoLan  
229 Organisation). The 'Jump to time' (v 2.1) extension was used to identify the exact frame in which  
230 the response began (see Supplementary Information for video examples).

231

232 **Experiment 1: vigilance in reed warblers (cuckoo hosts).** At Wicken Fen and adjacent  
233 waterways we conducted a repeated-measures playback experiment at 24 reed warbler nests (May  
234 to June 2016), with each nest exposed to all four treatments in an order defined by latin square  
235 to remove the potential effects of order exposure. In addition, a 10-minute rest period was given  
236 between each trial and no effects of playback order were found suggesting that this period was

237 adequate (Table 1; see below). Calls were broadcast using a Pignose 7100 field speaker (PigNose,  
238 NV, U.S.A.) placed at 1 m above the ground and calibrated to 80 dBA peak amplitude at 1 m  
239 from the speaker. Nests were sufficiently separated in space or time to avoid effects of the  
240 playbacks on neighboring nests<sup>12</sup>.

241 While incubating, reed warblers typically sit deep in the nest cup with their head below the  
242 nest rim. Occasionally, they stretch their necks to peer out over the nest rim and such vigilance  
243 scans are associated with approaching threats, for example, when a human or dog approaches  
244 the nest. The bird will subsequently leave the nest if the threat persists. On average these  
245 vigilance scans last 2.5 seconds and range between 489 milliseconds and 11.5 seconds (n = 25  
246 scans measured from baseline incubation behaviour (using methods described above). We  
247 categorized subjects as exhibiting vigilance behaviour if they were observed to scan peering over  
248 the nest rim with neck extended for more than 1 second continuously during the trial. Vigilance  
249 responses (58 of 96 trials) began rapidly with 52 (90%) beginning within 500 ms of the playback  
250 call onset (which equates to during the first 1-5 syllables: see Figure 1a), and all but two began  
251 within the first second of the call playback.

252

253 **Experiment 2: vigilance in great tits and blue tits (not cuckoo hosts).** We conducted a  
254 second experiment at Cambridge University Botanic Garden and Madingley Wood, using 60  
255 individually identifiable (colour-banded or pit-tagged) free-living parids: 32 great tits, (*Parus*  
256 *major*), and 28 blue tits, (*Cyanistes caeruleus*). These species nest in tree holes, inaccessible to female  
257 cuckoos, and in Europe they are not parasitized (though there are records of cuckoo parasitism  
258 in Asia<sup>37</sup>). We used experimental peanut feeders as a standardized location from which to  
259 conduct the playback trials during March and April 2016 (before cuckoos had arrived in the  
260 region). Trials began when a bird had been on the feeder for at least 10 seconds, and when no  
261 other tits were present on the feeder or in close proximity. The speaker was located 5 m away  
262 from the feeder and playbacks were broadcast in a randomized order across individuals and at a

263 standardized amplitude (as in experiment 1). Given unpredictable visits by individuals to feeders,  
264 it was not possible to ensure that each individual received all treatments, so each individual  
265 received just one playback treatment.

266 Behavioural responses were recorded on video files (as above). When feeding on peanut  
267 feeders, tits regularly survey the surroundings with short, regular ‘look-ups’ that last for on  
268 average 539 milliseconds (range 172-3303 milliseconds; n = 50 look-ups measured from baseline  
269 feeding activity). Vigilance behaviour was defined as the subject scanning the surroundings for  
270 more than 1 second continuously during the trial, or scanning the surroundings before  
271 immediately leaving the feeder during the trial. Vigilance responses (33 of 60 trials) began rapidly  
272 with 30 (91%) beginning within 500 ms of the onset of the playback call onset (which equates to  
273 during syllables 1-5: see Figure 1a), and all began within the first second of the call playback.

274

275 **Experiment 3: nest defences in reed warbler hosts.** At 72 reed warbler nests at Wicken Fen  
276 and adjacent waterways, we conducted an experiment on the day the fourth egg was laid (most  
277 pairs lay a clutch of four eggs)<sup>8</sup>. We simulated parasitism with a foreign egg using previously  
278 validated methods<sup>8,12</sup>. We selected one egg from the nest at random, painted it uniform brown  
279 with Rowney acrylic ‘burnt sienna’ paint, and then replaced the egg in the nest. This simulates  
280 the behaviour of female cuckoos, who first remove a host egg before they lay their own egg in  
281 the nest<sup>8</sup>. We used “non-mimetic” brown eggs for two reasons. First, these are similar to the  
282 eggs laid by some female cuckoos on our study site<sup>8</sup>. Second, reed warblers have reduced their  
283 propensity to reject eggs over the last three decades, in concert with the decline in cuckoos and  
284 hence a decline in parasitism risk<sup>11</sup>. Highly mimetic eggs are now rarely rejected and so responses  
285 to non-mimetic eggs give a better measure of host rejection<sup>11</sup>.

286 Having “parasitized” the nest with a foreign egg, we then placed a model adult cuckoo on top  
287 of the nest. As in previous experiments<sup>12</sup>, we alternated between two virtually identical balsa  
288 wood cuckoo models, which did not differ in their effects on responses. The models were

289 painted with grey upper-parts and pale under-parts with barring. Grey females are the most  
290 common morph on our study site, and are similar to males in appearance. Response to these  
291 models correlated strongly with those to taxidermic cuckoo mounts and were similar to those to  
292 a live cuckoo<sup>38,39</sup>. We concealed a small speaker (Altec Lansing<sup>12</sup>) next to the nest and broadcast  
293 the playbacks calibrated to a peak amplitude of 75 dBA at 1 meter, the distance from the nest at  
294 which subjects were when playbacks began. Each nest received just one playback treatment  
295 chosen at random. Female cuckoos typically produce one chuckle phrase after laying so our  
296 playbacks mimic natural call production<sup>16,22</sup>. Once again, nests were sufficiently separated in  
297 space or time to avoid effects of the playbacks on neighboring nests<sup>12</sup> and were different pairs  
298 from those tested in experiment one.

299 We retreated from the nest to observe the pairs' behavioural response to the model cuckoo,  
300 following previous protocols<sup>6</sup>, after which we remotely triggered one of the four playback  
301 treatments chosen at random and recorded behavioural responses to the playback for another  
302 minute afterwards. At the end of the minute, we removed the model and playback speaker. We  
303 then checked nest contents one day after the trial and again three days after the trial to assess  
304 whether the painted egg was 'accepted' (painted egg still present in the nest and clutch being  
305 incubated) or 'rejected' (painted egg no longer present and clutch being incubated or clutch  
306 deserted and pair beginning a replacement nest nearby). Our previous work has shown that most  
307 rejections of real cuckoo eggs<sup>8</sup>, and all rejections of experimental painted eggs<sup>12</sup>, occur within  
308 three days.

309

310 **Statistics.** All statistical analyses were conducted using the statistical software R v 3.3.2<sup>40</sup> and  
311 were two-sided. Models were checked for normality of residuals, homogeneity of variance, and  
312 over-dispersion by both manual visual inspection and using R package DHARMA<sup>41</sup>. Statistical  
313 modelling utilized a full model approach for the mixed-effects model (package lme4<sup>42</sup>) and the  
314 GLM analyses: all terms of interest were fitted, and then significance testing was performed via

315 likelihood ratio tests to determine which factors resulted in a significant reduction in explanatory  
316 power when removed (Table 1). The significance of the factor levels for ‘call type’ was  
317 determined using likelihood ratio tests to assess whether collapsing the two levels of interest (for  
318 e.g. ‘hawk’ and ‘female cuckoo’) resulted in a significant reduction in the explanatory power of  
319 the model compared to a model with all four levels<sup>43</sup>.

320 For experiment 1 (vigilance in reed warbler hosts), a GLMM<sup>42</sup> with a binomial error structure  
321 and logit-link function was used to test the prediction that female cuckoo calls provoke vigilance  
322 behaviour in reed warblers. Responses were coded as ‘vigilant’ (Yes/No) according to the  
323 definition above. “Nest identity” was fitted as a random term to control for repeated measures at  
324 each nest for each of the four playback call types. “Call type” (dove, male cuckoo, female  
325 cuckoo, hawk) and “order of playback” (first, second, third, fourth) were each fitted as a four-  
326 level fixed effect.

327 For experiment 2 (vigilance in tits), a GLM with a binomial error structure and logit-link  
328 function was used to test the prediction that female cuckoo calls provoke vigilance behaviour in  
329 tits. Again, responses were coded as ‘vigilant’ (Yes/No) according to the definition above, and  
330 “call type” (dove, male cuckoo, female cuckoo, hawk) was fitted as a four-level fixed effect and  
331 “species” (blue tit or great tit) was fitted as a two-level fixed effect.

332 For experiment 3 (nest defences in reed warbler hosts), two GLMs with binomial error  
333 structure and logit-link function were used to test the prediction that female cuckoo calls reduce  
334 egg rejection by reed warbler hosts by one day and by three days after the trial. Egg rejection  
335 responses were coded as ‘reject’ = 0 or ‘accept’ = 1. “Call type” (dove, male cuckoo, female  
336 cuckoo, hawk) was fitted as a single four-level fixed effect. A GLM with a binomial error  
337 structure and logit-link function was used to test the prediction that female cuckoo calls reduce  
338 mobbing behaviour by reed warbler hosts. Mobbing propensity responses were coded as ‘mob  
339 after playback’ (Yes/No) based on whether or not parents mobbed the model or not after the  
340 playback. “Call type” (dove, male cuckoo, female cuckoo, hawk) was fitted as a four-level fixed

341 effect and “mob before playback” (Yes/No) was fitted as a two-level fixed effect. For those  
342 individuals that did mob after playback, an additional GLM with normal distribution was used to  
343 investigate effects of playback treatment on mobbing intensity (call rate). The response term for  
344 ‘mobbing intensity’ was the mobbing rate (number mobbing calls per minute) after the playback.  
345 Again, “call type” (dove, male cuckoo, female cuckoo, hawk) was fitted as a four-level fixed  
346 effect and “mobbing call rate before the playback” was fitted as a covariate, to control for the  
347 marked variation between individuals in mobbing responses often observed in this species<sup>12</sup>.

348

349 **Data availability:** Data are available in the Supplementary Information.

350 **Ethics statement:** All protocols were reviewed and licenced by Natural England.

351

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435

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442

## 443 **Author contributions**

444 J.E.Y and N.B.D contributed equally to the field experiments and writing of the manuscript.  
445 J.E.Y analysed the data.

446

## 447 **Additional information**

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## 453 **Competing interests**

454 The authors declare no competing financial interests.