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**INTER- AND INTRASPECIFIC ACOUSTIC ADAPTATIONS IN AN
AVIAN BROOD PARASITE-HOST COEVOLUTIONARY SYSTEM**

Thesis for the Degree of Doctor of Philosophy (PhD)

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I hereby declare that I prepared this dissertation within the Doctoral Council of Natural Sciences and Information Technology, Juhász-Nagy Pál Doctoral School, University of Debrecen to obtain a PhD Degree in Natural Sciences/Informatics at the University of Debrecen. The results published in the dissertation are not reported in any other PhD dissertation.

16th February 2023, Debrecen

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PhD candidate

I hereby confirm that PhD candidate Attila Marton conducted his studies with my supervision within the Biodiversity Programme of the Juhász-Nagy Pál Doctoral School between 2017 and 2020. The independent studies and research work of the candidate significantly contributed to the results published in the dissertation. I also declare that the results published in the dissertation are not reported in other theses. I support the acceptance of the dissertation.

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Dr. Csaba Moskát

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doctoral (PhD) degree in Biology (Natural Sciences)

Written by Attila Marton, certified Biologist, within the framework of
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ESSAY SECTION

"Now, here, you see, it takes all the running you can do, to
keep in the same place."

Lewis Carroll: Through the Looking-Glass (1871)

1. Introduction and objectives

1.1. Avian brood parasitic systems

Brood parasites form a functionally distinct group of natural enemies on the predator-parasite continuum, which hijack host reproductive resources and divert them towards their own reproduction by alloparental care (Pollock *et al.* 2021). This reproductive strategy evolved several times throughout the history of Life as a means of increasing individual fitness benefits by imposing the costs related to reproduction on conspecific or heterospecific individuals. Obligate brood parasitism, as a form of extreme interspecific alloparental care evolved on multiple occasions in social insects (Osváth-Ferencz *et al.* 2016; Tartally *et al.* 2019; Rabeling 2020), at least once in fishes (cuckoo catfish *Synodontis multipunctatus*; Sato 1986; Blažek *et al.* 2018; Cohen *et al.* 2019; Polačik *et al.* 2019), and at least six times in birds (Davies 2000, 2015; Payne 2005).

The 109 obligate brood parasitic bird species belong to five different families: Anatidae (black-headed duck *Heteronetta atricapilla*), Indicatoridae (17 species of honeyguides and honeybirds), Viduidae (20 species including indigobirds, whydahs and the parasitic weaver *Anomalospiza imberbis*), Cuculidae (65 species in the Cuculinae subfamily, and 3 species in the Neomorphinae subfamily) and Icteridae (6 species belonging to the *Molothrus* genus). These bird species lay their eggs in the nests of other species, termed hosts, and trick them into incubating their eggs and rearing their offspring. Host species attempt to evade the costs of brood parasitism by evolving multi-level defences and maintaining an evolutionary arms race with their brood parasites (Dawkins & Krebs 1979). These defences can be roughly split in two categories: preventive measures (also called frontline defences; Feeney *et al.* 2012; Medina & Langmore 2016; Noh *et al.* 2021) and mitigatory measures (Davies & Brooke

1988; Grim *et al.* 2003; Noh *et al.* 2018). The former evolved to lower the chances of brood parasitism, while the latter evolved to reduce the negative effects of brood parasitism once the brood parasitic egg is laid in the host's nest.

Frontline defences include nesting in areas with low brood parasitic pressure (Øien *et al.* 1996; Stokke *et al.* 2007; Fiorini *et al.* 2009; Stumpf *et al.* 2012; Méré *et al.* 2015, 2023; Medina & Langmore 2019), building well-concealed or inaccessible nests (Banks & Martin 2001; Rutila *et al.* 2002; Fiorini *et al.* 2012; Samaš *et al.* 2016), behaving secretively during nest building and egg laying (Banks & Martin 2001; McLaren & Sealy 2003; York & Davies 2017), assessing the presence of brood parasites through social learning (Soler & Soler 1999; Feeney & Langmore 2013; Campobello *et al.* 2017), or recognizing and subsequently mobbing adult brood parasites (Požgayová *et al.* 2009; Ma *et al.* 2018; Šulc *et al.* 2020; Jelínek *et al.* 2021, Zhao *et al.* 2022). Brood parasites evolved a plethora of traits to counter these adaptations: cuculids resemble both visually and acoustically to common predators of their hosts (Welbergen & Davies 2011; Trnka & Prokop 2012; York & Davies 2017; Marton *et al.* 2021), combat hosts' visual discrimination by evolving polymorphic plumage (Thorogood & Davies 2012; Trnka & Grim 2013; Trnka *et al.* 2015), and lay their egg with an uncanny speed to minimize their contact with hosts (Guigueno & Sealy 2011; Jelínek *et al.* 2021). After locating a suitable host nest for laying, brood parasites monitor the nest in order to seize the proper moment for laying their egg without being caught by the hosts (Clotfelter 1998; Honza *et al.* 2002). After a successful parasitic attempt, host can attenuate the costs of rearing non-kin nestling by recognizing and removing parasitic eggs or nestlings from their nest or by abandoning the nest altogether (Colombelli-Négrel *et al.* 2012; Šulc *et al.* 2016a; Noh *et al.* 2018; Tosi-Germán *et al.* 2020).

Brood parasites and their hosts provided important insight into the operation of evolution in general, and of coevolution, in particular. The selective pressure imposed by the costs of rearing non-kin individuals gave rise to evolutionary adaptations that aim to minimize these costs, but also locked these dyads of hosts and parasites into (almost) endless arms races (Dawkins & Krebs 1979). A recent call for integration of coevolutionary biology of brood parasitism by Thorogood *et al.* (2019) highlighted that the majority of the work performed on these systems in the past 35 years revolved around the fitness consequences of brood parasitism (i.e., Tinbergen's (1963) third question: "what's it for?"). The studies presented in the doctoral dissertation at hand focus on the functions of different behavioural and ecological traits of a brood

parasite and its host, but I will also strive to discuss the mechanisms, ontogeny, and phylogenetical aspects of our findings.

Science is a collaborative endeavour and the studies presented here are products of our research group led by my supervisors, Dr. Csaba Moskát and Dr. Miklós Bán, therefore I will consistently use the plural first-personal pronoun “we” whenever I refer to our work.

I divided my dissertation into two main parts: an essay section and annexes, the latter containing the four research articles that are discussed in the former part. In the subchapters of the essay section, I will briefly describe the ecology of the two central species of my dissertation, the common cuckoo *Cuculus canorus* and the great reed warbler *Acrocephalus arundinaceus*. These will be followed by two subchapters regarding interspecific and intraspecific acoustic communication, in which I will succinctly present the state of art, hypothesis and the predictions of our work, as presented in Figure 1. I present two studies in each subchapter, succeeded by two chapters describing the methodological aspects and the results of our studies, placing each result into context in subsequent paragraphs. I will conclude the dissertation by discussing our most important findings and identifying possible future avenues of research regarding the topics discussed. The links pertaining to the recordings marked with superscripts are accessible through the endnotes listed at the end of the dissertation.

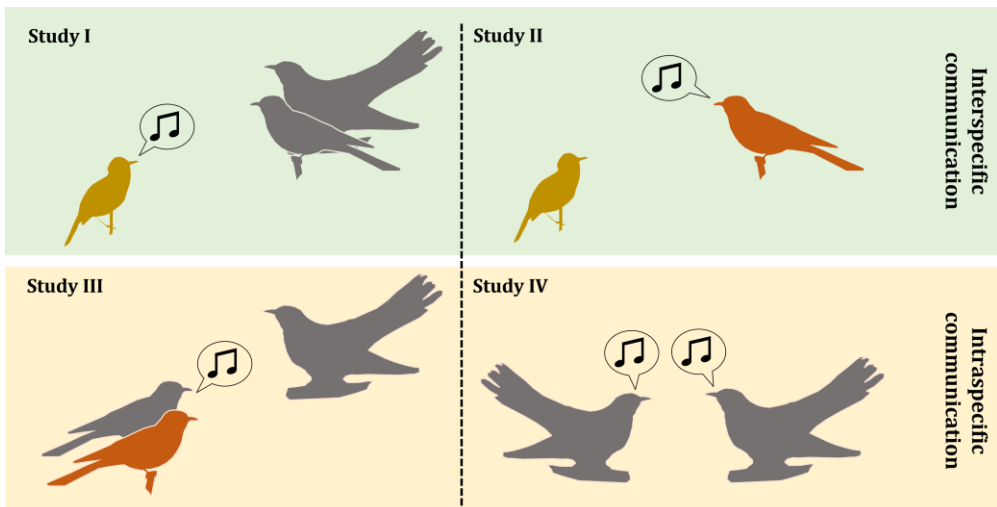


Fig. 1: Schematic representation of the studies discussed in the dissertation. Brown birds depict great reed warblers, rufous and grey birds represent the two morphs of female common cuckoo, while the grey birds with erected tails represent male cuckoos.

1.2. The common cuckoo

The common cuckoo (or simply cuckoo, from hereafter) is the best-known brood parasitic species, described by Aristotle (384—322 B.C.) as a bird that “do[es] not sit, nor hatch, nor bring up [its] young, but when the young bird is born it casts out of the nest those with whom it has so far lived” (Hett 1936). Most interestingly, its breeding habits were forgotten and rediscovered by Edward Jenner (1788), sparking outrage in the scientific community of the day regarding the lack of maternal care characteristic to the species (Davies, 2015).

The common cuckoo is one of the 65 brood parasitic species from the monophyletic Cuculinae subfamily, Cuculidae family (Payne 2005). It is widely distributed throughout the Western Palearctic, breeding from Portugal and the United Kingdom throughout Europe and Asia, including Japan and the Kamchatkan Peninsula, but mostly absent on the Tibetan Plateau and on the Indian Peninsula. Cuckoos have grey beaks with a yellow base, yellow irises surrounded by a yellow periorbital ring, and have short, zygodactylous legs. Males weigh around 100-115 g, and have a grey head and upperparts, grey bib, coarsely barred whitish breast and a long black tail with white spots and a white, narrow terminal band. Females are smaller than males, weighing 90-100 g, and are polymorphic: grey females have a plumage identical to that of males, but have no well-defined grey bib, while rufous (or hepatic) females are chestnut brown, with coarsely marked upperparts and tail. Fledged juveniles are somewhat similar to adults but are more variable regarding plumage colour than adults and sport a white nape patch (Payne 2005).

Cuckoos are specialists regarding their diet, consuming hairy caterpillars and other invertebrates, snails or rarely fruits also, but often depredate the eggs of their hosts too. Cuckoos are migratory, wintering mainly in Sub-Saharan Africa regardless of the location of their breeding grounds (Willemoes *et al.* 2014; Jacobsen *et al.* 2017; Bán *et al.* 2018; Thorup *et al.* 2020). Second year birds are already sexually mature and return to the breeding grounds in the middle of April, where both males and females hold partly overlapping territories (Nakamura *et al.* 2005; Moskát *et al.* 2019; Koleček *et al.* 2021).

Common cuckoo males have a relatively small, innate repertoire of calls, the typical disyllabic “cu-coo” being uttered most often¹ (Fig. 2a). These 1-3 s long calls are repeated up to 300 times, with a call rate of 40 calls/min on average (Elek *et al.* 2021). The frequency of the first syllable spans between 0.60-0.75 kHz, while the second has a narrower range, spanning between 0.5-0.6 kHz (Zsebők *et al.* 2017). The other two main calls are the trisyllabic “cu-cu-coo”

usually uttered in excitement as reaction to female calls (Xia *et al.* 2019; Moskát & Hauber 2021), and the so-called “gowk” call, sometimes uttered in short burst (Moskát & Hauber 2022a). Female cuckoos produce a repetitive bubbling call (Fig. 2b), similar to the calls of hawk species *Accipiter spp.* (Fig. 2c), although the bubbling call² is constructed of a short burst (2-5 s) of repetitive elements ranging from 0.07 to 0.2 kHz, while the calls of the sparrowhawk³ *Accipiter nisus* are much longer (8-12 s) and are higher pitched (0.2-0.35 kHz).

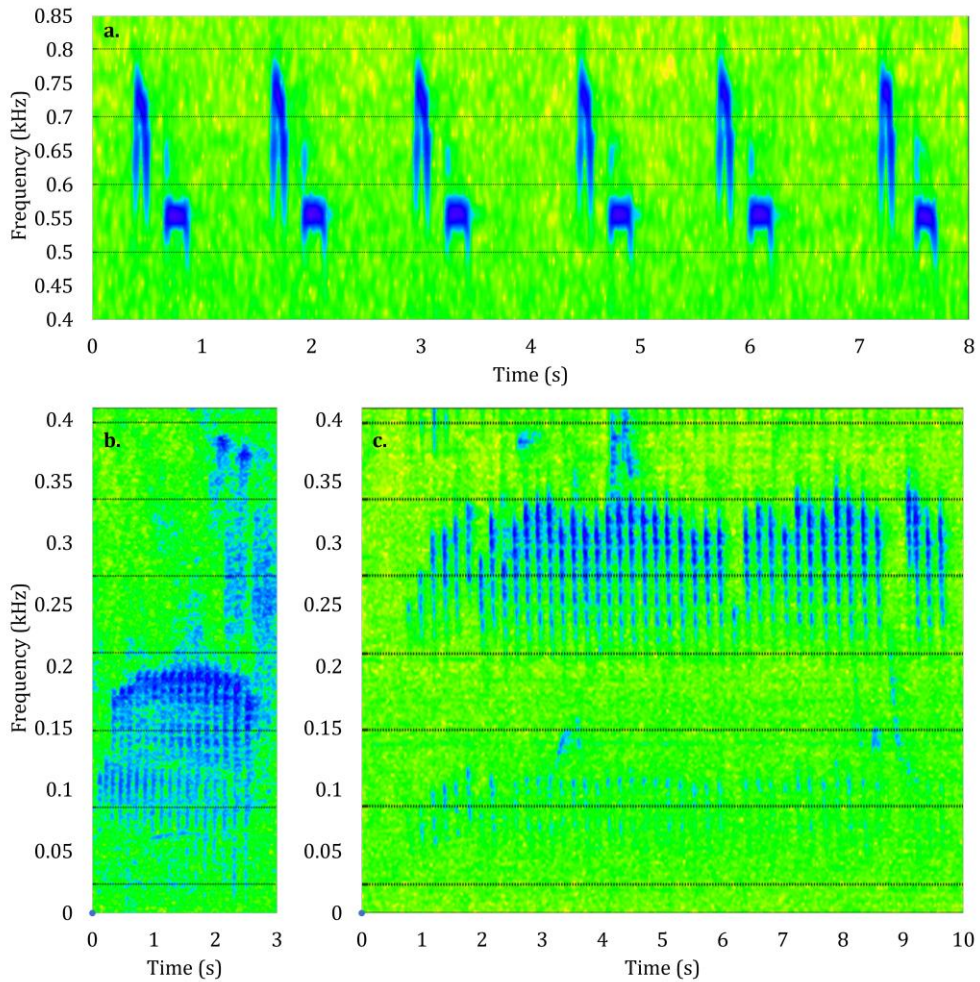


Fig. 2: Spectrograms of a typical (a.) male common cuckoo call sequence, (b.) a female cuckoo's bubbling call and (c.) the call of a sparrowhawk.

As obligate brood parasites, cuckoos lay their eggs in the nests of a large variety of Passerines, ranging in size from the Eurasian wren *Troglodytes troglodytes* weighing only 5 g to the azure-winged magpie *Cyanopica cyana*, weighing up to 96 g. The most commonly used host species in Europe are the great reed warbler, marsh warbler *Acrocephalus palustris*, Eurasian reed warbler *Acrocephalus scirpaceus*, and Eastern olivaceous warbler *Iduna pallida* in riparian habitats, the robin *Erithacus rubecula* and the common redstart *Phoenicurus phoenicurus* in forest habitats, and the tree pipit *Anthus trivialis*, meadow pipit *Anthus pratensis*, water pipit *Anthus spinoletta*, pied wagtail *Motacilla alba* and dunnocks *Prunella modularis* in open-field habitats (Glue & Morgan 1972; Mikulica *et al.* 2017; Stokke *et al.* 2018). Rarely used hosts include the red-backed shrike *Lanius collurio*, great grey shrike *Lanius excubitor*, corn bunting *Emberiza calandra*, skylark *Alauda arvensis*, or the barn swallow *Hirundo rustica* (Mikulica *et al.* 2017).

In contrast with the host species that lay their eggs in the morning, cuckoos parasitise nests mostly in the afternoon, laying one egg every second day in up to 25 different host nests (Davies 2015). Female cuckoos lurk around the vicinity of the nest chosen to be parasitised on any given day of laying, and glide to the nest when it is not attended by any of the host pair (Honza *et al.* 2002). Upon landing on the rim of the nest, cuckoos take one egg randomly out of the nest and substitute it with one of their own (Šulc *et al.* 2016b). Egg laying usually takes place incredibly fast; 8-10 s long successful parasitism events having been recorded on video multiple times (Wang *et al.* 2020, 2021; Jelínek *et al.* 2021). As the wide range of hosts produce a large variety of egg phenotypes, each egg phenotype is matched by one of different cuckoo maternal lineages, termed gentes (*gens* in singular; Marchetti *et al.* 1998; Krüger 2007; Antonov *et al.* 2010). Therefore, it is essential for female cuckoos to recognise their own host species, in order to increase their chances for a successful parasitism.

Evolving distinctly marked eggs by the hosts is a prerequisite for recognising and removing parasitic eggs. Hosts inspect their clutches regularly, and upon discovering an egg deemed as not one of their own, they abandon the nest or puncture and eject the foreign egg. Therefore, only the parasitic eggs best resembling the host's own eggs are accepted (Honza *et al.* 2004; Požgayová *et al.* 2011). This results in fast convergence of egg phenotypes between hosts and their parasites, as hosts exert a strong selective pressure on parasitic egg phenotypes, unwittingly selecting for eggs that best resemble their own (Hauber *et al.* 2006; Antonov *et al.* 2010; Vikan *et al.* 2011; Spottiswoode &

Busch 2019; Stoddard *et al.* 2019). Intriguingly, the dunnock is the only host species that we know to share a long evolutionary past with the cuckoo, without evolving egg phenotype recognition, therefore accepting brood parasitic eggs irrespective of their dissimilarity to its own eggs (Davies 2000, 2015).

If the host fails to recognise and remove the brood parasitic egg from its clutch, the cuckoo chick will hatch after 11 days of incubation, one day earlier than the host's own offspring (Birkhead *et al.* 2011). Within hours after hatching, the blind and featherless cuckoo nestling proceeds to evict the host's own eggs from the nest, by balancing them on its back and pushing them over the nest's rim (Honza *et al.* 2007). Cuckoo nestlings are reared in the nest for around three weeks and are continued to be fed by the hosts for a couple of days after leaving the nests. Therefore, rearing a cuckoo chick imposes fitness costs for the host, as it precludes the possibility of rearing the host's own progeny during that breeding season.

The common cuckoo is listed as Least Concern according to the International Union for Conservation of Nature Red List of Threatened Species (IUCN 2019), due to its wide range of distribution and large global population, although its populations is decreasing throughout its breeding range. According to data provided by the Pan-European Common Bird Monitoring Scheme, the European population of common cuckoos decreased by 43% since 1980 (Keller *et al.* 2020). The Hungarian population is estimated to consist of 62-63000 singing males and has a stable national long-term population trend ($-0.7\% \pm 0.8\%$) but decreasing in our study area (Czirák 2021).

1.3. The great reed warbler

The great reed warbler is one of the largest regularly used host species by the cuckoo, and the largest *Acrocephalus* warbler breeding in Europe. Its breeding range spans from Portugal and Spain from west, until South Scandinavia in the north and east to Kazakhstan. The species does not breed in Great Britain (Keller *et al.* 2020).

The great reed warbler is an unstreaked, monomorphic species, that has a brown head with whitish supercilium and a white throat, brown or greyish brown upperparts, wings and tail, and a paler breast and belly. Its legs are reddish browns or bluish in juveniles. Fledged juveniles resemble adults, but they can be identified in their first year based on their fresh plumage (i.e.,

compared to the worn plumage of adults in the same period) and by their two tongue-spots visible until October (Svensson *et al.* 2011).

The great reed warbler is a typical edge species, breeding in the outer edge of compact, homogenous reed beds or along irrigation canals (Báldi 1999; Moskát & Honza 2000). Males perch on the top of reed stems and sing a complex, highly repetitive, crackling “kara-kara-kara-khri-khri” song⁴ ranging between 0.2 and 0.4 kHz, with whistling elements intertwined reaching a peak frequency up to 0.5 kHz (Fig 3a). This song is a territoriality signal, and it does not indicate the location of the nest to brood parasites, as males often sing near the boundaries of their territories (Capek *et al.* 2017). Males start building a nest out of grass and reed leaves above water, woven on reed stems, and show it to females looking for a mate. After securing a mate, females continue building the nest by themselves. (Leisler & Schulze-Hagen 2011).

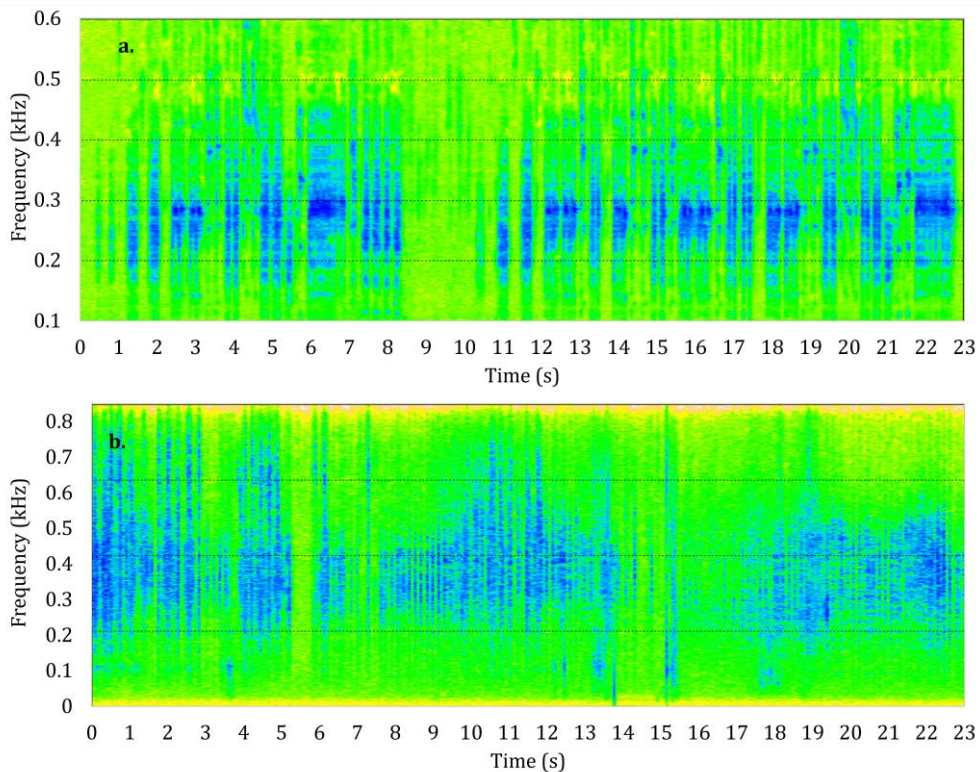


Fig. 3: Spectrograms of a typical (a.) male great reed warbler song, and (b.) of an intensive alarm sequence uttered by a pair of great reed warblers at the nest, when presented with a common cuckoo decoy.

Great reed warblers are facultatively polygynous, with high-quality males having two or more females (Catchpole *et al.* 1985). Females start laying an egg daily after mating, until a clutch of 4-6 eggs is completed. Females are responsible of clutch inspection and the removal of brood parasitic eggs, while males invest more in nest defence and the mobbing of brood parasites (Požgayová *et al.* 2009, 2015). Incubation starts upon clutch completion and lasts 12 days. Nestlings are fed with damselflies, crane flies, spiders, and other invertebrates found in the breeding habitat. While polygyny has some genetic advantages, the clutches of secondary females face lower provisioning rates from males and are more prone to be parasitised by brood parasites (Hasselquist *et al.* 1996; Trnka & Prokop 2011; Požgayová *et al.* 2015). Nestlings fledge before properly developing their flight feathers and spend their first days after fledging on the reed stems near the nest.

The great reed warbler is a long distant migrant species, wintering in South-Saharan Africa (Liechti *et al.* 2018; Brlík *et al.* 2020; Požgayová *et al.* 2022) and is listed by IUCN as Least Concerned (IUCN 2019). Interestingly, the European population of the species shows a mild increase of 12% on the long-term (1982-2019), although the population size decreased in the past by 6% in the past 10 years after reaching a peak (Keller *et al.* 2020). The Hungarian breeding population consists of 208-221000 pairs, moderately decreasing on the long term ($-2.6\% \pm 1.0\%$; Csörgő & Gyurácz 2021).

1.4. Acoustic components of brood parasite–host interactions

In his seminal work, Claude Shannon (1948) described communication systems as systems in which a transmitter issues a coded signal (i.e., message) through a medium (i.e., channel) in order to be perceived by a receiver. The message might be distorted by noise (i.e., any kind of interference that can impede the decoding of the signal) or intercepted by unintended receivers (i.e., eavesdroppers). Meaningful communication ensues when the message is decoded and acted upon by the receiver, or more precisely, if the signaller (i.e., transmitter) benefits from affecting the behaviour of an intended receiver through a broadcasted signal (Magrath *et al.* 2015). Therefore, the benefits of signalling should surpass the costs of producing the signal (e.g., physiological costs) and the costs entailed by the interception of the signal by eavesdroppers (Dabelsteen *et al.* 1998; Maynard-Smith & Harper 2003).

Within this framework, alarm calls are signals produced with the intent of deterring enemies or alerting nearby conspecific and heterospecific individuals

about the presence of a common threat. Alarming can be costly if it reveals the location of the signaller to its predators, or if it's not reciprocated by receivers (i.e., signaller-receiver roles are not interchangeable). The structure and intensity of the alarm call might convey information about the identity of the enemy (e.g., aerial, or terrestrial predator; Strnad *et al.* 2012), its location or distance (Krytofkov *et al.* 2011), or the nature of the threat (e.g., predator of adult birds, nest predator or brood parasite; Trnka & Prokop 2010; Campobello & Sealy 2018; Lawson *et al.* 2020; Krausová *et al.* 2022). For example, great tits *Parus major* produce longer "dee" calls with more elements when presented with their main predator, the sparrowhawk *Accipiter nisus*, in comparison to calls produced against the less-threatening tawny owl *Strix aluco* (Kalb & Randler 2019). Similarly, Kleindorfer *et al.* (2005) found that moustached warblers *Acrocephalus melanopogon*, Eurasian reed warblers and great reed warblers defended their nests more vigorously when the perceived threats (snake, stoat *Mustela erminea*, or marsh harrier *Circus aeruginosus*) were closer to their nests. Nest defence varied also by nest height and predator type: birds breeding closer to the water surface had a higher reaction score towards snakes, while birds nesting higher up above the water surface alarmed stronger against aerial threats (i.e., marsh harrier).

Nest defence behaviours received an increased level of interest from evolutionary ecologists studying alarm calls, as it entailed behavioural responses that impact reproductive outputs: by responding aggressively to threats near the nest (e.g., mobbing predators or brood parasites) focal individuals invest more in short-term benefits (i.e., current reproduction) at the detriment of potential future reproductive gains (i.e., fleeing the nest). This decision might prove to be especially difficult for the hosts of the common cuckoo, as recognition errors might bear extra costs: failure to recognise and mob a cuckoo might result in a successful parasitism and the loss of the current brood, while mistaking a sparrowhawk for a cuckoo might lead to predation (Ellis-Felege *et al.* 2013).

Nest defence inherently takes place near the nest (Da Cunha *et al.* 2017), and as shown above, the intensity of mobbing increases if the threat is closer to the nest (Kleindorfer *et al.* 2005; Krytofkov *et al.* 2011). Placed into the context of Claude Shannon's (1948) Theory of Communication, the host (i.e., signaller) benefits from banishing enemies from the nest, as uttering alarm calls (i.e., signal) and mobbing enemies elicits a change in the receivers' behaviour. However, this behaviour of the host near its nest might bear a high informational value and might be exploited by eavesdropping cuckoos, as it

reveals the location of the host's nest. Although two studies performed on brown-headed cowbird hosts (Uyehara & Narins 1995; Clotfelter 1998) and a written account of an anecdotal observation on cuckoos (Seppä 1969) indicate that brood parasites might rely on host alarm calls when searching for nests, this assumption was never before experimentally tested. Therefore, we tested if common cuckoos recognise and respond to the mobbing behaviour of their hosts, by performing two experimental studies (**Study I**). In these experiments, we predicted that female cuckoos would approach mobbing hosts both when the great reed warbler hosts are seen and heard mobbing an enemy, and also when the hosts' alarm calls⁵ (Fig. 3b) are broadcasted without any visual signals. We also analysed data regarding parasitism risk and mobbing behaviour, gathered during the monitoring of great reed warbler nests. We expected to find higher parasitism rates at nests where the great reed warblers uttered alarm calls more readily upon facing a potential nest predator (i.e., observers during nest visits).

Being mobbed near the hosts' nest also poses risks for cuckoos: alarm calls might attract additional mobbers, and increase clutch inspection and brood parasitic egg removal both by the mobbing host and by its neighbours (Feeney & Langmore 2013; Thorogood & Davies 2016). Therefore, it has been suggested that the hawk-like plumage pattern evolved to reduce mobbing by the hosts to some extent (Welbergen & Davies 2011). Hosts vary in their ability to discriminate cuckoos from sparrowhawks based on visual cues, and usually mob cuckoos (or cuckoo decoys) vigorously at their nests but are reluctant to approach sparrowhawk mounts (Duckworth 1991; Davies & Welbergen 2008; Li *et al.* 2015; Yu *et al.* 2017; Lawson *et al.* 2021; Krausová *et al.* 2022; Attwood *et al.* 2023). York & Davies (2017) presented experimental evidence that the hawk-mimicry has an acoustic component too. The bubbling call of the female cuckoo is similar in structure and pitch to the mating calls of the sparrowhawk and increases the attentiveness of the hosts when broadcasted to incubating Eurasian reed warblers (York & Davies 2017). Furthermore, great tits and blue tits *Cyanistes caeruleus*, two non-host species regularly depredated by sparrowhawk (Panter & Amar 2021), respond similarly to cuckoo bubbling calls and sparrowhawk calls at bird feeders during winter. This suggests that passerines are unable to discriminate between the calls of cuckoo females and sparrowhawks, irrespective of the nature of the threat they pose (i.e., brood parasite or predator). Interestingly, these results were further confirmed in a non-host and non-prey species also: Jiang *et al.* (2020) found that free-ranging chicken *Gallus gallus domesticus* became more vigilant when treated with the

bubbling call of the female cuckoo or the calls of a sparrowhawk, while this was not the case when male cuckoo calls and dove calls were broadcasted.

Larger hosts are known to be able to seriously harm or even kill brood parasites, therefore, cuckoos evolved fast egg laying to avoid costly confrontations with the hosts (Ma *et al.* 2018; Šulc *et al.* 2020; Jelínek *et al.* 2021). As the experiment carried out by York & Davies was performed on Eurasian reed warblers, we carried out our own experiment to test if the female cuckoo's bubbling call reduces host aggression (**Study II**). We predicted that great reed warblers would mob cuckoo decoys less when the bubbling call is broadcasted, compared to the time period when the cuckoo decoy is presented without the call, and compared to responses exhibited to dove decoys and calls. We used cuckoo decoys painted to resemble the rufous cuckoo morph, in order to exclude the potential confounding effect of the hawk-like plumage mimicry.

1.5. Intraspecific acoustic signalling in common cuckoos

The Malthusian principle of economics and population growth integrated in evolutionary theory states that finite resources impede populations in their indefinite growth, creating competition among individuals and therefore creating the premises for natural selection to take place (Malthus 1798; Darwin 1873). The competition for resources might entail costly antagonistic interactions; therefore, animals evolved morphological and behavioural traits to advertise their quality and avoid costly confrontations (Maynard-Smith & Harper 2003).

The Zahavi-Grafen condition-dependent signalling hypothesis (Zahavi 1977; Grafen 1990; reviewed in Penn & Számadó 2020), which was at the renewed refoundation of sexual selection theory in the past half a century, states that these signals are honest signals (i.e., they truly reflect the quality of its bearer) precisely because they are costly to produce and maintain. These traits can be visual (e.g., plumage), acoustic (i.e., song and calls), behavioural (e.g., courtship feeding) or external (e.g., the nests constructed by bowerbirds belonging to the Ptilonorhynchidae family). For instance, since birds cannot synthesize carotenoids *de novo*, they must acquire it through diet (Olson & Owens 2005; Costantini & Møller 2008), therefore, plumage carotenoid content can be informative regarding the ability of individuals to find and protect carotenoid-rich feeding grounds. These constraints need not be extrinsic: some signals can be phenotype-specific and might be under genetic control. The melanocortin hypothesis reviewed in Ducrest *et al.* (2008) and San-Jose &

Roulin (2018) states that the genes that regulate the synthesis of melanin-based plumage traits also modulate behavioural and physiological traits through pleiotropic effects.

Plumage polymorphism is common in some bird families and rarer in others (e.g., 33% in Strigiformes, 12% in Cuculiformes, but < 1% in Piciformes and Passeriformes; Galeotti *et al.* 2003). Plumage polymorphism is most often related to melanin-based pigmentation: some morphs have a higher amount of eumelanin in their plumage responsible for producing grey and black colours (i.e., dark morphs), some are preponderantly brown or chestnut due to pheomelanin pigments (i.e., rufous morphs), while others are characterised by the lack of both melanin-based pigments (i.e., light morphs). According to the melanocortin hypothesis, phenotypes with more eumelanic plumages tend to be more dominant and sexually active, cope better with physiological stress, and tend to have larger territories and dispersal distances than pheomelanic phenotypes (Ducrest *et al.* 2008; Roulin & Ducrest 2011).

Plumage polymorphism is restricted to females in common cuckoos, which supposedly evolved as a trickery against hosts (Thorogood & Davies 2012, 2013). Females belonging to the rufous morph receive less aggression from the hosts in sites where this morph is rare (Honza *et al.* 2006; Trnka & Grim 2013), but are attacked with similar versatility at sites where they are as frequent as the grey morph (Honza *et al.* 2006). Lee *et al.* (2019) recently proposed that female colour polymorphism in cuckoos evolved as a defence against male sexual harassment. This hypothesis was experimentally tested in an Asian population where the rufous morph is nearly absent, males attempting to copulate more often with decoys resembling the more common grey morph (Noh *et al.* 2016; Lee *et al.* 2019).

The number of available host nests is a finite resource that entails competition between brood parasites, especially in the case of Cuculids, as the first hatched cuckoo chick evicts all eggs from the nest, including those laid by concurrent female cuckoos (Marton 2021). Knowing that negative density dependent selection favours the rarer rufous morph, as this morph is seldom recognised by hosts (Honza *et al.* 2006; Trnka & Grim 2013) or by sexually harassing males (Lee *et al.* 2019), we would assume that it poses similar advantages regarding female-female interaction, as females of the grey morph fail to recognise rufous females as competition. We devised an experimental study in order to test if female cuckoos react differently to simulated territorial intrusions with bubbling call broadcasts and decoys of the rufous and grey cuckoo morphs (**Study III**). As the two morphs are equally common at our

study site, we expected female cuckoos to respond with a similar interest towards both types of decoys, but not so towards the collared dove *Streptopelia decaocto* decoys used as a control.

Calls are innate acoustic signals simpler in their construction than the songs of Passerines, that could also function as age-, sex- and condition-dependent traits (Catchpole 1986; Galeotti *et al.* 1997; Murphy *et al.* 2008). For example, the hoots of male tawny owls are supposed to reflect the endoparasitic load of the individuals, which in turn predict response speed against simulated territorial intrusions (Appleby & Redpath 1997; Galeotti 1998; Redpath *et al.* 2000).

The speed with which calls are uttered, expressed as the number of calls produced per unit of time (i.e., call rate), the peak frequency of the call, its frequency bandwidth, or the length of a call sequence might be constrained by vocal tract limitations, lung capacity (i.e., allometric body size) or call structure and complexity. These constraints, coupled with extrinsic factors (e.g., calling in mid-flight vs. perched) might ensure the honest condition-dependency of these signals (Gil & Gahr 2002; Maynard-Smith & Harper 2003). Of these call characteristics, call rates are easiest to quantify, are known to correlate with individual quality and have fitness consequences in many bird species (e.g., grey partridge *Perdix perdix*, see Beani & Dessì-Fulgheri 1995; hoopoe *Upupa epops*, see Martín-Vivaldi *et al.* 1998; Martín-Vivaldi *et al.* 1999; brown skuas *Catharacta antarctica lonnbergi* see Janicke *et al.* 2008).

Male cuckoos utter a simple two-note “cu-coo” call throughout the breeding season, mostly during early mornings and in the evening (Yoo *et al.* 2020). Although call characteristics were proposed as a signal of male quality in polluted habitats (Møller *et al.* 2016), calls are prone to syntax errors (Moskát *et al.* 2021; Moskát & Hauber 2022b) and decline in consistency throughout the breeding season (Deng *et al.* 2019). We devised a correlative and an experimental study to test if male cuckoo call rates reflect individual male body size and mass, as proxies of male quality (**Study IV**). In line with previous studies, we predicted that larger males would be able to produce more calls per unit of time (i.e., higher call rates) and that playbacks of experimentally increased call rates would elicit a more aggressive response from free-ranging male cuckoos than calls with a decreased call rate.

2. Materials and methods

The occurrence of obligate interspecific brood parasitism might vary greatly (0-70%), depending on the brood parasitic species in question, the number and density of hosts species available to parasitise, and on the accessibility of host nests (Stokke *et al.* 2007; Jelínek *et al.* 2014; Medina & Langmore 2019). Due to the constraints of having a statistically relevant sample size, most research groups that study brood parasitism work in locations with high parasitism rates (10-35%), therefore our knowledge regarding the natural rate of brood parasitism in the wild might be biased (Marton 2021). For example, parasitism rates by common cuckoos varied between 6 and 21% among 16 pan-European populations of Eurasian reed warblers depending on habitat and local host density (Stokke *et al.* 2007). Common cuckoos parasitised ca. 26-28% of marsh warbler and Eastern olive-backed warbler, but only 9% of corn bunting nests in north-western Bulgaria (Antonov *et al.* 2006a; b, 2007a), 20% of common redstart pairs breeding in nest-boxes in Finland (Ruttila *et al.* 2002), 22-34% of oriental reed warbler *Acrocephalus orientalis* nests in Eastern China (Yang *et al.* 2014).

The extensive network of irrigation canals at our study site, located near Apaj village in central Hungary (N 47.113°, E 19.087°), harbours a large, but decreasing population of great reed warblers. The 2-7 m narrow habitat stripes of common reed *Phragmites australis* are lined with poplars *Populus alba*, willows *Salix alba*, black locusts *Robinia pseudoacacia* and silverberry bushes *Eleagnus angustifolia*, suitable to be used by cuckoos as perching sites during nest searching (Øien 2000; Antonov *et al.* 2007b; Marton *et al.* 2019). Great reed warblers breeding here experience a parasitism rate of 50-60% annually, the highest parasitism rate described in the scientific literature (Moskát & Honza 2000; Zölei *et al.* 2015). The study site functions as a sink for the local great reed warbler population due to the extreme risk of brood parasitism, and might heavily rely on the influx of newly settled individuals recruited from the unparasitised reed beds found in the gravel pits adjacent to the irrigation canals (Barabás *et al.* 2004).

All studies presented here were performed at this study site in May, during the egg-laying season of the great reed warbler and the common cuckoo, between 2016 and 2020. The data analysed for the correlational study presented in **Study I** were collected in 2013-2014 and 2018, while the calls of male and female cuckoos used in **Study II**, **Study III** and **Study IV** were recorded between 2015 and 2018 in the same area. The calls were recorded with the equipment described in Moskát and Hauber (2021): a Telinga

Universal parabola dish with Rycote Hi Wind Cover, a Marantz PMD-620 MKII sound recorder (48 kHz sampling rate, 24-bit quality, .wav format), a Sennheiser ME62 microphone, with a K6 powering module and a FEL MX mono preamp and were replayed on a JBL Xtreme loudspeaker (40 W; 90 dB).

The four life-size common cuckoo (one resembling the grey morph and three similar to the rufous morph) and three collared dove decoys used in **Study I**, **Study II**, and **Study III** were printed with an Ultimaker 2+ 3D printer using transparent Ultimaker PLA filament based on a model file supplied by 3D QuickPrinting (UK) and painted with acrylics to resemble their real-life counterparts. All replicates (i.e., the three rufous cuckoo and the three collared dove decoys) were painted to look identical to each other as best as possible.

In **Study I** we tested our hypothesis that host alarm calls function as cues for the cuckoos during nest searching, by performing two experiments and a correlative study. In the first experiment we placed a 3D printed cuckoo resembling the grey morph next to known great reed warbler nests ($n = 51$) to elicit a strong mobbing behaviour from the hosts⁶. We scored the reactions of male and female cuckoos towards the visual and acoustic stimuli provided by the mobbing hosts for 2 minutes before and 2 minutes during the mobbing on a four-level scale:

- 0: no reaction,
- 1: typical male or female cuckoo call produced by perching at a distance closer than 100 m,
- 2: flying closer to the mobbing host without calling and
- 3: flying closer to the mobbing hosts while producing the typical sex-specific cuckoo calls.

To avoid testing the same cuckoos on multiple occasions, we performed consecutive trials at minimum 500 m from each other. We expected the reactions exhibited by cuckoos during the mobbing period to be stronger than prior to mobbing, therefore we analysed the data collected using one-tailed Fisher's exact test in the R statistical environment (R Core Team 2017).

We carried out a second experiment to test the reaction of cuckoos solely to the alarm calls of their hosts, without the visual signalling produced by hosts during mobbing. We broadcasted host alarm calls ($n = 16$) and collared dove calls used as control ($n = 16$) at 32 sites where female cuckoos were observed. The playback of either treatment call started 2 minutes after the presence of a female cuckoo was confirmed based on its typical bubbling call (Wyllie 1981)

and lasted for 2 minutes. The movements of all cuckoos, irrespective of sex, were recorded during the 2 minutes of playback and for the subsequent 2-minute period. We considered a response positive if a focal bird approached the playback device compared to the location at which it was observed initially, and neutral if the individual in focus had no reaction or departed from the study area during the experiment. Both male and female cuckoos hold ca. 1 km long, partially overlapping territories along the irrigation canals throughout the breeding season (Moskát *et al.* 2017, 2018, 2019), therefore, to ensure that all cuckoos are tested only once, playback trials were conducted at sites at least 2 km apart from each other. We expected the cuckoos to approach the experimental setup during the playback of host alarm calls, but not during the playback of collared dove calls, therefore we analysed the data collected using one-tailed Fisher's exact test in the R statistical environment (R Core Team 2017).

Study I also contained a correlational study which aimed to establish a link between the risk of brood parasitism and the behaviour of the hosts (i.e., the tendency of hosts to defend their nest by mobbing). We tested this assumption by collecting data on great reed warbler nests ($n = 170$) monitored during the egg-laying period ($n = 330$ visits in total, mean number of visits per nest: 1.94, $SD = 1.10$). During these visits we determined the day the first egg was laid, clutch composition (i.e., number of great reed warbler and common cuckoo eggs at each visit) and the presence or absence of alarm calls towards the observer during nest checking. Great reed warblers are fiercely territorial during the breeding period and defend their nests against human intruders with an aggressivity similar to that directed against cuckoos and nest predators (Požgayová *et al.* 2009; Trnka & Prokop 2010). All nest visits were performed during the peak activity period of the great reed warblers (6:00-11:00 AM) by four, previously trained observers. We constructed a generalised linear mixed-effect model (GLMM; Chambers 1992) with a binomial error distribution to test if great reed warbler pairs that were more readily alarmed during nest visits faced a higher risk of brood parasitism. The presence or absence of brood parasitism at given visits was included in the model as a binary response variable, while alarm call (two-level factor), parasitism risk (two-level factor), time of visit (expressed as minutes elapsed after midnight, standardised with Z-transformation to mean = 0 and $SD = 1$) and the second-order interactions of alarm call and the other variables listed above were entered as fixed terms. We included observer ID and nest ID nested under site ID (i.e., the different irrigation canals where the nests were found) as random factors in the model. As cuckoos are known to parasitise host nests more often right after the first

eggs are laid in a nest (Moskát & Honza 2002; Moskát *et al.* 2006) compared to the time period when the clutch is completed, parasitism risk was determined as ‘high’ if the visit was performed during the first three days after clutch initiation, or ‘low’ after 4-6 days from the clutch initiation date, when the clutch nears its completion. We simplified the model by stepwise backwards elimination, dropping at each step the fixed effect with highest p value, until reaching the minimal adequate model containing only significant and marginally significant ($p < 0.1$) variables. We tested the multicollinearity of both full and minimal adequate models using the ‘*vif.mer*’ function (Chambers 1992; Frank 2014) and found that the variance inflation factor (VIF) was less than 2.09 for all variables in the full model, and less than 1.12 for all variables in the minimal adequate models. The GLMMs were performed using the ‘*glmer*’ function from the ‘lme4’ package (Fox & Weisberg 2011; Bates *et al.* 2014) in the R statistical environment (R Core Team 2017).

In **Study II** we tested if the sex-specific call produced by female cuckoos alters the mobbing intensity of the hosts near the nest, facilitating the deposition of the parasitic egg. To test the effect of the acoustic mimicry (i.e., the bubbling call) and to discard the potentially confounding effect of visual mimicry (i.e., the resemblance of grey female cuckoos to sparrowhawks), we performed experimental trials with 3D printed cuckoos painted to resemble female cuckoos belonging to the rufous morph, and with 3D printed collared dove decoys. Great reed warblers and allies are highly aggressive towards intruders near their nest, especially against cuckoos (Moskát 2005; Li *et al.* 2015; Ma *et al.* 2018; but see also Trnka & Prokop 2012). We randomly placed a cuckoo or a dove decoy and a JBL Xtreme loudspeaker next to great reed warbler nests and registered the response of the hosts with Nikon Coolpix AW130 digital camera placed at 3-5 m from the nest. We recorded the behaviour of great reed warbler hosts⁷ for 2 minutes after arriving to the nest and discovering the decoy, after which we broadcasted 2-minute long female cuckoo bubbling calls for the trials with cuckoo decoys, and collared dove calls for the control trials (for the structure of the call files, see Moskát & Hauber 2019; Moskát *et al.* 2020). The experimental trials ($n = 22$) were performed with three rufous cuckoo decoys and six locally recorded bubbling call files, while the control trials ($n = 20$) were performed with three dove decoys and six locally recorded dove calls, in order to reduce pseudoreplication as a source of bias in the dataset (Kroodsma 1989; Kroodsma *et al.* 2001). Great reed warblers reacted to the decoys with a wide range of aggressive behaviours: by producing “churr” calls, snapping their bills, pecking, and physically attacking the decoys. We expressed total mobbing intensity as the sum of all these

different aggressive behaviours during the 2-minute presentation of the cuckoo or dove decoys both without sounds, and accompanied with their specific calls, respectively. In some control trials, great reed warblers continued to incubate their clutch without reacting in any manner to the decoy. This lack of reactions to control decoys zero-inflated the data collected, therefore, were analysed the data by constructing a general linear mixed-effect model with zero-inflated Poisson error distribution (Bolker *et al.* 2009; Brooks *et al.* 2017). We introduced total mobbing intensity as the response variable in this model, and we included the number of hosts present (i.e., one or both members of the pair), the playback period (i.e., before or during the call broadcasting), the treatment (i.e., cuckoo or dove decoys and calls) and its two-way interactions with the other two variables as fixed effects, and irrigation canal ID ($n = 4$) and nest ID as random factors. We eliminated nonsignificant variables from the full model by stepwise backwards elimination process until reaching a minimal adequate model including only significant interactions, significant main effects or non-significant main effects involved in significant interactions ($p > 0.05$). We performed all statistical analysis in the R statistical environment (R Core Team 2017).

The methods deployed for the experimental study described in **Study III** were similar as those used in **Study I** and **Study II**, with the main difference that this experiment was conducted to test the intraspecific use and impact of acoustic and visual signals. Common cuckoos spend their time either in their breeding habitats, such as reed beds alongside irrigation canals, or at feeding and roosting sites, which are usually forest patches near breeding sites (Moskát *et al.* 2019). Both male (Moskát *et al.* 2017, 2018) and female (Moskát & Hauber 2019) cuckoos restrict their breeding movements to ca. 2 km-long territories along the irrigation canals, therefore we predicted that both sexes are able to recognise grey morphs and the rufous female morph of their species.

We performed field trials in known female cuckoo territories to test if the aggressive responses of female cuckoos differ towards the grey morph, compared to the rufous morph. Therefore, we placed rufous ($n = 18$), or grey ($n = 17$) cuckoo decoys coupled with previously locally recorded bubbling calls, or dove decoys ($n = 17$) accompanied with collared dove calls as a control treatment, and recorded the following variables during the 2-minute-long call playback period, and a 2-minute long post-playback period:

- acoustic response from cuckoos or movement or lack thereof within a 50 m radius (binary variable),
- distance from the experimental setup at the time of first detection (m),
- closest distance to the experimental setup during the playback period (m),
- elapsed time between the trials' start and the moment of closest distance to the loudspeaker (s),
- the time elapsed until the first movement of the focal bird (i.e., movement latency, s),
- the time elapsed until the first call by the focal bird (i.e., calling latency, s),
- the duration of continuous calling (s),
- the number of calls uttered by female cuckoos or number of call types uttered by males (as described in Moskát & Hauber 2019),
- the number of flights toward the experimental setup, and the total number of cuckoos observed during the 4-minute-long trial, divided by sexes identified based plumage characteristics and sex-specific calls.

We performed Fisher's exact tests on the binary variable coding cuckoo responses or the lack of thereof, to test if female and male cuckoos responded differently to the experimental treatments (i.e., cuckoo decoys) than to the control treatments (i.e., dove decoys). Subsequently, we constructed generalised linear models with binomial distribution (GLM; Bolker *et al.* 2009) separately for the two sexes, to test if common cuckoos reacted differently to decoys representing grey or rufous female cuckoos. We entered treatment type (i.e., grey, or rufous cuckoo decoy) as a response variable in these models, and the time-based variables (i.e., time at first detection, time at closest detection, latency of calling, length of continuous calling), distance-based variables (i.e., distance at first detection, closest distance), and numeric variables (i.e., number of calls, number of flights, number of birds) as explanatory variables. We selected the most parsimonious models that explained our data based on the AICc information criterion (Bolker *et al.* 2009) by fitting all logistic models with all explanatory variables, and sequentially removing the variables with the least explanatory power, until we reached the optimal number of model parameters based on the AICc (Bolker *et al.* 2009; Bates *et al.* 2014). Similarly, we extracted the composite variables which best describe the behavioural and acoustic traits by performing principal component analyses (PCA) on the two sex-specific

subsets of data (i.e., responses of female and male cuckoos, respectively), to test if the responses towards the two decoy morphs were different on this dimension-reduced composite variables. Only components with a corresponding eigenvalue >1.0 were retained. Fisher's exact tests and generalised linear models were fitted in the R statistical environment (v. 3.6.1, R Core Team 2017), while principal component analyses (PCA) were performed in SPSS Statistics (v. 17.0, SPSS Inc. Chicago, IL, USA).

The well-known "cu-coo" call of male common cuckoos also plays an important role during intraspecific communication, and it was at the focus point of many research groups working with this specific brood parasitic species for the last years (Moskát *et al.* 2017; Benedetti *et al.* 2018; Moskát & Hauber 2019; Xia *et al.* 2019; Moskát *et al.* 2021). Male cuckoos advertise themselves and lay territorial claims by uttering their typical "cu-coo" call (Moskát *et al.* 2017; Tryjanowski *et al.* 2018; Yoo *et al.* 2020) or by tail waiving, but interestingly not by both (Esposito *et al.* 2021). As male common cuckoos repeat the same simple two-note call throughout the breeding season, calling continuously for prolonged periods of time, mostly during dusk or dawn (Yoo *et al.* 2020), the rate the calls are produced might reflect the condition of the emitting individuals. We performed a correlative and an experimental study (**Study IV**) to test this hypothesis. We captured male common cuckoos using Ecotone mist-nets and territorial call playbacks as a lure, during the breeding seasons between 2017 and 2019, and fitted them with Pip3 radio transmitters (1.2 g, type 392, produced by Biotrack Ltd; as described in Moskát *et al.* 2017) attached to their central tail feather. Upon capture, we ringed and sexed all individuals based on morphological characters (Svensson *et al.* 2011) and measured the following biometrics: wing length (to the nearest 1 mm, with ruler), tail length (to the nearest 1 mm, with ruler), tarsus length (to the nearest 0.1 mm, with a digital calliper), and body mass (to the nearest 1 g, with a Pesola spring scale), and released them on site. We calculated a residual physiological condition index by regressing body mass on body size and extracting residuals as a proxy for a size-independent condition (Jakob *et al.* 1996).

The male cuckoos tagged with radio transmitters were identified in the field based on the unique radio frequencies emitted by the Pip3 tags, using a Sika receiver attached to a Yagi antenna, both produced by Biotrack Ltd. We recorded the calls produced by tagged males using a Telinga Universal parabola dish mounted with Rycote Hi Wind Cover, on a Marantz PMD-620 MKII sound recorder (48 kHz sampling rate, 24-bit quality, wav format), and a Sennheiser ME62 microphone after 2-5 days after ringed. We discarded all recordings

during which we identified in the field confounding effects that might impact the call rate (e.g., arrival of a female Common Cuckoo or of concurrent males). We identified complete sequences without pause and containing only the “cu-coo” calls and calculated natural call rates by dividing the number of calls per unit of time on a sample of randomly selected recordings ($n = 18$) recorded from 23 radio-tagged cuckoos. Several studies used the maximum number of calls as proxies of individual or habitat quality, erroneously naming single “cu-coo” calls as syllables (Møller *et al.* 2016, 2017; Tryjanowski *et al.* 2018). We opted not to calculate maximum number of calls as the density of common cuckoos is particularly high at our study sites, and elevated call rates produced due to the presence of females or male competitors were common (Moskát & Honza 2000). We constructed general linearised models with quasi-Poisson error distribution (Bolker *et al.* 2009) in the R statistical environment (v. 3.6.1; R Core Team 2017), in which natural call rate was treated as a dependent variable, and the recorded biometric parameters (i.e., wing-, body- and tail length, and body mass), the year of capture, and their second order interactions were included as fixed effects.’

Besides the correlational study described above, we also devised a playback experiment in which we broadcasted natural call rates, altered call rates (i.e., experimentally increased, and decreased call rates), and dove calls used as a control to free-ranging male cuckoos. We used the same general structure of the audio files as in previous experiments: 2-minute-long audio files, containing a 30 s long call sequence repeated three times, with 15 s pauses between the repeated sequences. We manipulated recordings with natural call rates with the Audacity recording and audio editing software (v. 2.1.0; Audacity Team 2017), by increasing (i.e., “quicker” treatment) or decreasing (i.e., “slower” group) the interval of time between two consecutive “cu-coo” calls. The call rates of the 30 s long sequences that were repeated three times were 20.4 ± 0.76 (mean \pm SD; range: 9-24) for the unaltered (i.e., “normal” rate) calls, 28.0 ± 1.3 (mean \pm SD; range: 18-36) for the “quicker”, and 15.2 ± 0.69 (mean \pm SD; range: 9-18) for the “slower” group. Thus, the 2-minute-long recording contained 61.2 ± 2.28 (mean \pm SD; range: 27-72) calls on the audio files of the “normal” treatment, 84.0 ± 3.9 (mean \pm SD; range: 54-108) calls in the “quicker” treatment, and 45.6 ± 2.07 (mean \pm SD; range: 27-54) in the “slower” treatment. All experimental treatments differed significantly among each other regarding call rates: “normal” vs. “quicker” (Mann-Whitney U test: $z_{15,17} = -3.888$, $p < 0.001$), “normal” vs. “slower” (Mann-Whitney U test: $z_{15,16} = -3.959$, $p < 0.001$), and “quicker” vs. “slower” (Mann-Whitney U test: $z_{17,16} = -4.843$, $p < 0.001$).

The experimental trials for the “quicker” and “slower” call rate treatments were performed in May 2018, when cuckoo males reach their peak activity. For the “normal” call rate treatment and the control trials, we reused data collected in May 2016 for a previous experiment, collected under identical circumstances (Moskát *et al.* 2017). All data were collected in days with good weather conditions, between 6 and 11 AM, when cuckoo males tend to be most active (Yoo *et al.* 2020). In all trials, treatment or control calls were broadcasted from a JBL Xtreme loudspeaker in sites where male cuckoos were seen or heard, just after observing them. We placed the loudspeaker on a tree at a height of ca. 1.5 m and recorded the following movements and behavioural responses of common cuckoos during the 2-minute-long playback period:

- movement or lack thereof within a 50 m radius (binary variable),
- closest distance (i.e., by flight or perching between the focal bird and the experimental setup, m),
- distance to first detection (i.e., distance from the experimental setup at the moment when the focal individual was first observed, m),
- latency of first detection (i.e., elapsed time between the trial’s start and the moment the bird was first observed, s),
- latency of closest detection (i.e., elapsed time between the trial’s start and the moment the bird was closest to the experimental setup, s),
- latency of calling (i.e., the time elapsed until the first call by the focal bird, s),
- length of continuous calling (i.e., the duration of the longest continuous calling sequence, s),
- the number of flights toward the experimental setup, and
- the total number of male cuckoos observed in the vicinity of the loudspeaker during the 2-minute-long playback.

We performed Fisher’s tests to assess if male cuckoos reacted differently towards experimental (i.e., normal, “quicker” or “slower” call rate) and control treatments. We constructed a generalised linear model with binary logistic regression (Chambers 1992) in SPSS Statistics (v. 17.0, SPSS Inc. Chicago, IL, USA) to test if male cuckoos responded differently to the altered calls of conspecific males. The model contained the two treatments with altered call rates (i.e., “quicker” or “slower”) as dependent variables, while the variables listed above were retained as fixed effects. Similarly, we performed a principal component analysis in the same statistical environment (SPSS Statistics, v. 17.0, SPSS Inc. Chicago, IL, USA) to analyse the behavioural responses exhibited by

male cuckoos on a lower dimensionality scale. We retained the first three principal components (all components having an eigenvalue > 1), which explained cumulatively 65.8% of the variance within the data. We created ordination plots based on the first two principal components without rotating the component loadings on the plots.

3. Results and discussion

The acoustic components of the coevolutionary interactions between avian obligate brood parasites and their hosts are varied and subtle, and might impact the breeding success of both host and brood parasite in a similar manner as the more well-studied adaptations based on visual cues. Two such surprising acoustic adaptations are the ability of brood parasitic females to recognise and seek out hosts based on the host's alarm calls (as hypothesised in **Study I**), and to deter hosts from their nests with their own sex-specific bubbling call (as hypothesised in **Study II**).

During the 51 experimental trials performed for the first experiment described in **Study I**, both female ($n = 14$) and male cuckoos ($n = 30$) reacted strongly to the visual and acoustic mobbing signals of great reed warbler pairs, compared to the period prior the trials (females: Fisher's exact test: $p = 0.004$; males: Fisher's exact test: $p < 0.001$). This strong reaction remained unchanged when we excluded the visual component of the host's mobbing behaviour, by replaying host alarm calls on a loudspeaker. We recorded 5 positive responses from female cuckoos during the 16 experimental trials (i.e., playback of host alarm calls), and no positive responses during the control trials (i.e., playback of collared dove calls; Fisher's exact test: $p = 0.021$). In a similar fashion, male cuckoos reacted positively during 7 experimental trials but only once during the control trials (Fisher's exact test: $p = 0.018$).

The intensity of male reactions in both experiments performed for **Study I** is still open for debate, especially since great reed warblers are one of the largest cuckoo hosts and can injure or even kill adult brood parasites (Molnár 1944; Šulc *et al.* 2020; Jelínek *et al.* 2021). To the best of our knowledge there are no studies regarding the role of cuckoo males in the post-copulatory stage of the breeding process. Based on the interest exhibited by cuckoo males towards the mobbing behaviour of the hosts, it is sensible to suggest that males may gain fitness advantages by pointing out host territories for females, even by distracting aggressive hosts from their nests (Davies 2000; Mikulica *et al.* 2017). This seems to be somewhat supported by a study performed by

Marchetti *et al.* (1998) in Japan, in which maternity and paternity assignment of common cuckoo chicks revealed that most of the local females mated with only one male, and most males sired offspring with only one or two females. More than half of the adult male and female cuckoos sampled did not sire any of 136 nestlings in the 7 km² study area.

Although it is difficult to test whether cuckoo males benefit from recognising and reacting to host mobbing, our correlative study described in **Study I** suggests that hosts pay the costs of mobbing. Alarming behaviour of the hosts towards human observers was positively associated with the probability of brood parasitism in the monitored host nests ($\beta = 2.748$, $SE = 1.222$, $z = 2.248$, $p = 0.024$). We put forward several mutually non-exclusive explanations for this association without implying causality between the propensity of host alarming and risk of brood parasitism. A plausible explanation for these results, in concordance with our main hypothesis, would be that more aggressive hosts inadvertently reveal the location of their nests to brood parasites, by alarming usually near their nests (Uyehara & Narins 1995; Clotfelter 1998; Da Cunha *et al.* 2017). Given that the presence or absence of alarms call was recorded when the nest was visited and the parasitism took place before the nest visit, an opposite relationship might be equally plausible: great reed warbler pairs became more aggressive against any kind of nest disturbance after a successful parasitism event (and a possible encounter with a brood parasite at the nest). Encountering a brood parasite at the nest increases the probability for the hosts to recognise and reject parasitic eggs (Bártol *et al.* 2002; Davies & Welbergen 2009; Welbergen & Davies 2009; Thorogood & Davies 2016; York & Davies 2017), although unsuccessful parasitism due to egg recognition is fairly low (~30%) in our study population (Moskát & Honza 2000; Zölei *et al.* 2015). There is a case to be made for conjectures when an adaptive trait, mobbing, functions in a maladaptive manner: under a scenario with low brood parasite and predator density, host can successfully deter enemies by mobbing, thus increasing their breeding success (Welbergen & Davies 2009; Šulc *et al.* 2020). If the local density of predators or brood parasites is high, deterring a predator or brood parasite from the nest might be observed by other predators or brood parasites lurking nearby. This seems to be the case at our study site also, where great reed warblers face high parasitism and predation rates, which may render selection favouring nest defence and mobbing behaviours ineffective or counterproductive (Mérő *et al.* 2013; Mérő & Žuljević 2015, 2019).

It is a long-held supposition that common cuckoos search for suitable host nests visually, perching on trees used as vantage points (Øien *et al.* 1996;

Moskát & Honza 2000; Dyrce & Haupka 2006; Antonov *et al.* 2007b). From a functional point of view, eavesdropping on host alarm calls might be similarly, or even more effective than searching for nests visually. Combining information from multiple sources might be especially important when host species breed in large, homogenous habitats with scarce vantage points (e.g., *Acrocephalus* species breeding in compact reed habitats).

Host nests, once found, are monitored by female cuckoos, to be parasitised at the proper moment (Honza *et al.* 2002; Moskát *et al.* 2006, 2019; Geltsch *et al.* 2016). The incubation period of common cuckoo chicks is 11 days, one day shorter than the incubation of most of their non-conspecific nest mates (Davies 2000; Birkhead *et al.* 2011). Hosts adjust their egg rejection behaviour based on the perceived risk of parasitism, therefore, the timing and swiftness of the egg laying by female cuckoos is essential. Therefore, cuckoos evolved fast egg-laying to minimise the contact with the hosts and to ensure a successful parasitic attempt: the substitution of a host egg with a parasitic egg usually occurs in 8-20 seconds, even if the female cuckoos are vigorously mobbed by the hosts (Jelínek *et al.* 2021). Our results described in **Study II** support the hypothesis put forward by York and Davies (2017), according to which the bubbling call produced by female cuckoos near the host nest scares the hosts, due to its similarity to the calls of common avian predators (*Accipiter* species). Upon placing a 3D printed collared dove decoy near the great reed warblers' nest, we found no significant difference in the hosts' total mobbing intensity towards the dove decoy before and during the playback of dove calls ($\beta = 0.336$, $SE = 0.420$, $t = 0.799$, $p = 0.427$). However, in trials where we placed 3D printed cuckoos belonging to the rufous morph next to the hosts' nest, hosts mobbed the decoy significantly less when the bubbling call was broadcasted compared to the silent period before the broadcast ($\beta = -0.607$, $SE = 0.052$, $t = -11.692$, $p < 0.001$). Interestingly, total mobbing intensity did not increase with the number of mobbing hosts (i.e., only one host or both individuals of the pair the nest belonged to), suggesting that the mobbing activity is unevenly distributed within the host pair. Studying the same host species, Požgayová *et al.* (2009) found that males were responsible for defending the nest and mobbing intruders, while females performed regular nest checking and ejected brood parasitic eggs.

Our results suggest that female cuckoos producing their bubbling call mimic common avian raptor species acoustically, to decrease their hosts' vigilance and to increase their own breeding success. This acoustic mimicry might function in junction with the visual mimicry of sparrowhawks deployed

by cuckoo males and females belonging to the grey morph (Trnka & Prokop 2012; Trnka *et al.* 2012), placing the grey morph in advantage over the rufous females, which resemble avian predators less (Trnka & Grim 2013; Trnka *et al.* 2015).

Besides playing an important role as an acoustic component of the interspecific evolutionary arms race between brood parasites and their hosts, the bubbling call uttered by female common cuckoos might also function as an important intraspecific acoustic signal. The bubbling call might bear a high informational value as a mating call uttered to attract the attention of conspecific males, or as a territorial signal for other brood parasitic females. However, this acoustic signal might be somewhat distorted by the visual discrepancy created by the female dichromatism specific to common cuckoos. In an experimental study performed in Eastern Asia, Lee *et al.* (2019) attracted cuckoo males by broadcasting bubbling calls, and presented these males with the opportunity to choose a female belonging either to the grey or the rufous morph as their mating partner. Cuckoo males exhibited a clear preference towards the locally common grey morph, even attempting to copulate with the decoys. Therefore, the combination of visual and acoustic signals has the potential to impact mating success, hindering the chances of females belonging to the less common rufous morph to easily find a mate.

Density dependent selection should favour the recognition of the more common morph both by conspecific males attempting to breed with these females, and the hosts species attempting to evade brood parasitism. This would translate in a stronger selection for the more common morph by male cuckoos (Lee *et al.* 2019), and in a stronger selection for the less common morph from the hosts, as hosts fail to recognise the rarer morph (Thorogood & Davies 2012). The local abundance of females belonging to the rufous morph in our study area is unusually high, estimated to make up 60% of the female cuckoos breeding in the area (Honza *et al.* 2006). As expected, in places where the local ratio of the two morphs is not skewed in any direction, as it is at our study site, great reed warbler hosts defend their nests similarly against both morphs and attack both with the same intensity (Honza *et al.* 2006), while common cuckoos also respond with an equal level of interest towards both morphs (**Study III**).

We performed 52 experimental trials with grey ($n = 17$) and rufous ($n = 18$) common cuckoo decoys accompanied by bubbling call playbacks, and dove decoys ($n = 17$) accompanied by collared dove playbacks used as a control. Both male and female common cuckoos responded more aggressively to both cuckoo

morph decoys as compared to the dove decoys (Fisher's exact test: $p < 0.001$), although neither sex responded differently to the two colour morphs (females: Fisher's exact test: $p = 0.658$; males: Fisher's exact test: $p = 0.677$). These results remained unchanged when only vocal responses were considered: cuckoo vs. dove decoys (both sex: Fisher's exact test: $p < 0.001$), rufous morph vs. grey morph decoys (both sex: Fisher's exact test: $p = 1.000$). Furthermore, we found no statistically relevant differences in female and male common cuckoo responses against decoys belonging to either the grey or the rufous morph neither in logistic regression models, nor during principal component analysis (PCA). Interestingly, both approaches revealed sex-specific behavioural characteristics against the two colour morphs. We performed AIC based stepwise logistic regression for both models containing the responses of common cuckoo males and females. We found that the most parsimonious model for female responses retained time of closest detection, latency of calling, maximal continuous calling, and the number of flights as best explanatory variables (all $p > 0.050$), while it retained distance in first detection, closest distance, and time of closest detection in the model containing male reactions (all $p > 0.050$). Principal component analysis revealed that time at first detection and latency of calling showed the highest positive loadings for PC1 in female responses, whereas males' distance at first detection and closest distance were positioned on the same axis as the number of flights performed, indicating that males either flew closer to the experimental setup, or performed more flights around the cuckoo decoys.

As the density of cuckoos throughout their breeding range is generally low, studies of female-female interactions are scarce. Only a handful of studies reported direct antagonist interactions between female cuckoos, usually as empirical observations (Riddiford 1986; Dröscher 1988; Lee *et al.* 2019; Moskát & Hauber 2019). Our results show that female common cuckoos are territorial and respond to the presence of intruding females by approaching them and producing their sex-specific calls. These results complement the findings of Koleček *et al.* (2021), which show that female cuckoos have locally distinct laying territories. As available host nests represent a limited resource for brood parasites, protecting these territories from conspecific competitors is essential, especially in places where the rate of multiple parasitism is high (Takasu *et al.* 2009; Zölei *et al.* 2015; Marton 2021) and if the brood parasites have not evolved to recognise and remove competitor parasitic eggs during parasitism (Šulc *et al.* 2016b).

Territoriality and acoustic territorial signalling have been at the centre of focus for many research groups working with brood parasites. Our research group contributed with valuable pieces of information to this study subject, showing that common cuckoo males can discriminate between local males and intruders based on their calls (Moskát *et al.* 2017, 2018), are prone to aberrant calls and syntax errors but these do not disturb effective communication (Moskát *et al.* 2021; Moskát & Hauber 2022b) and use specific calls to duet with conspecific females (Moskát & Hauber 2021). However, the informational value of these simple calls regarding the individual condition of the signallers is still unknown in common cuckoos. As calling is costly in terms of energy expenditure and self-exposure to predators (Oberweger & Goller 2001; Grunst *et al.* 2014), we tested if the call rate (i.e., number of calls per unit of time) might function as an honest signal regarding the quality of the males uttering the calls. Our results described in **Study IV** show that the call rate of free-ranging radio-tagged cuckoo males ($n = 23$) do not differ from those of untagged individuals ($n = 18$; Mann-Whitney U test: $z_{23,18} = -0.775$, $p = 0.438$). Body mass, wing-, tarsus- and body length were not associated with the call rates produced by radio-tagged common cuckoo males in any of the generalised linear models with quasi-Poisson error distribution. Although body mass correlated positively with call rate in the full model, its effect was not significant in the minimal adequate model.

We also performed a field experiment during which we broadcasted common cuckoo calls with normal call rate ($n = 15$), with an enhanced call rate (i.e., “quicker”; $n = 17$), or with a reduced call rate (i.e., “slower”; $n = 16$) and collared dove call broadcasted as control trials ($n = 14$). Cuckoo males responded to the playback calls of conspecific males in almost all trials (94-100%) but responded to dove calls only during two trials (all cuckoos vs. dove controls: Fisher’s exact test: $p < 0.001$). Cuckoo males approached the experimental setup with the same level of high interest irrespective of the broadcasted calls’ rate (Kruskal-Wallis test: $\chi^2_{17,15,16} = 2.415$, $p = 0.299$), further suggesting that call rate is not a signal of male quality. This result remained unchanged when we compared the reactions of cuckoos towards the two most dissimilar calls (i.e., “quicker” vs. “slower” call rate) with two additional methods: none of the time-based and distance-based variables were associated with either “quicker” or “slower” call rate in the binary logistic regression models constructed (all $p > 0.050$), and “quicker” and “slower” groups did not segregate in the PCA space in the dimensionality reduction analysis (independent sample t test, PC1: $t_{31} = 0.495$, $p = 0.624$; PC2: $t_{31} = -0.462$, $p = 0.647$).

Our results presented in **Study IV** suggest that the uttering rate of the “cu-coo” call is not an indicator of male quality by itself, although it plays an important role in territorial disputes between males (Moskát *et al.* 2017, 2018, 2021; Xia *et al.* 2019). Males could advertise their quality during mate searching by other means also, using visual displays and flight speed when chasing females mid-air (Lee *et al.* 2019) or tail wagging (Esposito *et al.* 2021). Other vocal characteristics than call rate might also play an important role in self-advertisement, as previously shown in other non-passerine species: For example, the mating success of brown skuas was higher in individuals that were able to produce a higher call rate while also being able to produce consistently more notes over a broad range of sound frequency (i.e., had a fast and consistently variable call; Janicke *et al.* 2008). Similarly, magnificent frigatebird *Fregata magnificens* males had a higher mating success if they were able to produce a quicker call rate by generating a drumming sound that resonated in their inflated, bright red neck pouch, the colour of which is also a signal of male quality (Madsen *et al.* 2007).

4. Conclusions and potential future avenues of research

In **Study I** we showed that common cuckoos recognise and react to the alarm calls of their local host, the great reed warbler, and that great reed warblers that alarm more often expose the location of their nests to brood parasites. Our results suggest that alarm calls might constitute good candidate cues for brood parasitic nestlings to imprint on, in order to recognise the host of their gens later on in life. The study was published in Scientific Reports (Marton *et al.* 2019).

Future studies should test if cuckoos react to the alarm calls of their hosts in places where different potential hosts breed alongside each other and should test by experimental means if cuckoos imprint on different sounds during early life stages and react to these sounds later on. Although it would be difficult to conduct, cross-fostering experiments between different hosts species would elucidate if brood parasites imprint on some characteristics of their hosts or the nest, or on the particularities of the breeding habitat.

In **Study II** we showed that the bubbling call of female cuckoos decreased mobbing propensity of great reed warbler hosts, disarming hosts during mobbing. The number of hosts present did not influence mobbing intensity, suggesting an uneven division of mobbing activity between male and female great reed warblers. The study was published in Ethology (Marton *et al.* 2021).

Future studies should aim to replicate these results on other host species, with emphasis on utilising decoys of both morphs in study sites where the ratio of the two morphs is skewed. The proximal mechanisms of the relationship between brood parasitic calls and host behavioural responses would also be of interest, with focus on hormonal stress responses (e.g., changes in corticosterone or testosterone levels). A case is to be made also for the necessity of a phylogenetic comparative study on the sound characteristics of raptor species, in order to identify common call elements that can be used as general threat signals for the host species.

In **Study III** we showed that neither male nor female cuckoo discriminated between the two morphs of the female cuckoo (i.e., grey, and rufous) in a study area, where both phenotypes are equally common. The study was published in *The Science of Nature* (Moskát *et al.* 2020).

Future studies should aim to decouple the effects of visual signals (i.e., cuckoo decoys) from those of acoustic signals (i.e., broadcasted bubbling calls) in order to truly test female reactions towards the two distinct cuckoo morphs. The differences in success of two co-occurring morphs were largely ignored until recently, and the advances made on other polymorphic species (e.g., black sparrowhawks *Accipiter melanoleucus*, barn owls *Tyto alba* or tawny owls) could provide a roadmap for exploring the adaptive differences between the two rufous cuckoo morphs. Since female-restricted polymorphism is rare in the animal kingdom, the identification of the genes responsible for such colour polymorphism could become the basis of future ontogenetic studies.

In **Study IV** we showed that the rate with which male cuckoos produced their typical “cu-coo” calls were independent of their allometric size, mass, or body condition. We also showed that male cuckoos do not respond differently to calls with experimentally increased or decreased call rates. The study was published in the *Journal of Ornithology* (Elek *et al.* 2021).

Future studies should focus on measuring other characteristics of male calls as call consistency or peak frequency of calls, alongside with conspicuous plumage characteristics (e.g., tail spots, breast pattern, or colour of bare parts) and behavioural traits that might signal individual quality. Studies regarding the mating system of the species might also prove as a fruitful avenue of research in this question, as species with high promiscuity (i.e., with many voluntary or forced copulatory events) might invest more in sperm competition rather than external visual signals.

5. Personal contribution to the studies presented

Although we worked as a team under the supervision and guidance of Dr. Csaba Moskát, the contribution of each team member differed between the studies. All 3D-printed decoys were printed and painted by myself, and I contributed to the ringing and radio-tagging of all cuckoos caught between 2017 and 2019. I devised the experimental setup and performed the experiments for the first experiment of **Study I** together with Dr. Miklós Bán, while Dr. Csaba Moskát and Dr. Zoltán Elek devised and performed the second experiment presented in **Study I**. I devised the experimental setup of **Study II** with the help of both my supervisor and performed the experiment by myself. I wrote the manuscript for both of these studies with considerable input from the rest of the team members. The experiments presented in **Studies III** and **IV** were devised and performed by Dr. Csaba Moskát and Dr. Zoltán Elek, using the 3D-printed decoys created by myself. Some of the cuckoos tested in the trials were ringed and radio-tagged by Dr. Miklós Bán, Dr. Attila Fülöp and me. The manuscripts afferent to these studies was written by Dr. Csaba Moskát, with valuable inputs from the rest of the co-authors, myself included.

6. Összefoglaló (Summary in Hungarian)

A költésparaziták egy funkcionálisan elkülönülő természetes ellenség-csoportot alkotnak a ragadozó-parazita kontinuum mentén, amelyek nemrokon egyedek szülői gondozását kihasználva növelik saját szaporulatukat (Pollock és mtsai. 2021). Az obligát interspecifikus költésparazitizmus – a költésparazita életmód szélsőséges formája – több alkalommal is kialakult az élet evolúciója során: megtaláljuk szociális rovaroknál (Osváth-Ferencz és mtsai. 2016; Tartally és mtsai. 2019; Rabeling 2020), halakban (kakukkharcsa, *Synodontis multipunctatus*; Sato 1986; Blažek és mtsai. 2018; Cohen és mtsai. 2019; Polačik et al. 2019), és madarakban is (Davies 2000, 2015; Payne 2005).

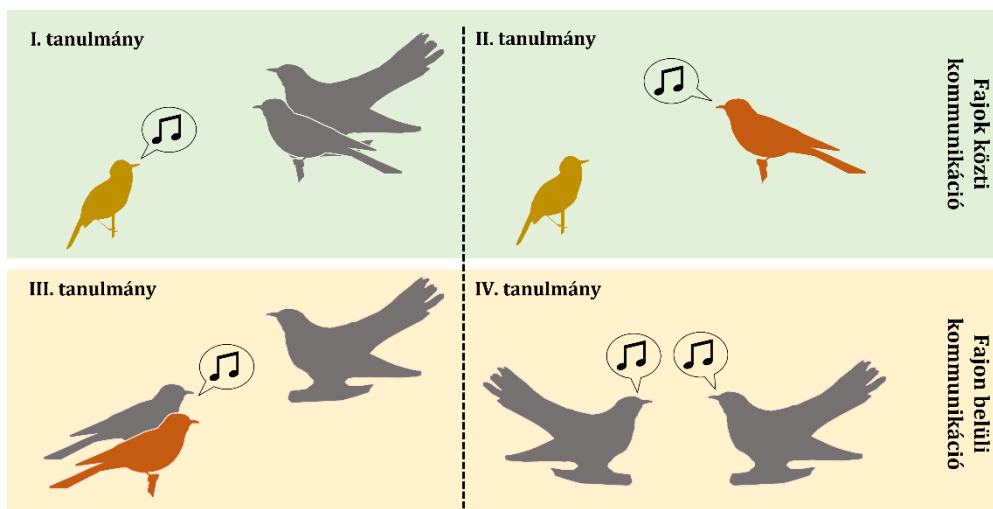
A 109 obligát költésparazita madárfaj öt különböző családba tartozik: a feketefejú réce *Heteronetta atricapilla* (Anatidae család), 17 mézkalauz faj (Indicatoridae család), 20 vidapinty-féle és a kakukk-szövőmadár *Anomalospiza imberbis* (Viduidae család), 65 óvilági kakukk (Cuculidae család, Cuculinae alcsalád), 3 újvilági kakukk (Cuculidae család, Neomorphinae alcsalád) és 6 gulyajáró faj (*Molothrus* nemzetség, Icteridae család). Ezeknek a madárfajoknak a közös jellemzője az, hogy tojásaikat más fajok (úgynevezett gazdák) fészkebe rakják, a parazita tojásaik költésére és utódaik felnevelésére kényszerítve azokat. A gazdafajok többrétű védekezést fejlesztettek ki a költésparazita fajok ellen, amely óhatatlanul egy evolúciós fegyverkezési versenyhez vezetett (Dawkins és Krebs 1979). Ezek a védekezések lehetnek megelőző intézkedések (úgynevezett frontvonal védelem; Feeney és mtsai. 2012; Medina és Langmore 2016; Noh és mtsai. 2021) vagy parazitizmust enyhítő intézkedések (Davies és Brooke 1988; Grim és mtsai. 2003; Noh és mtsai. 2018). Az előbbieket a költésparazita parazitálási esélyét csökkentik, míg az utóbbi a már bekövetkezett költésparazitizmus negatív hatásait hivatott csökkenteni.

A potenciális gazdafajok azáltal előzhetik meg a költésparazitizmust, hogy olyan helyen fészkelnek, ahol annak nyomása alacsony (Øien és mtsai. 1996; Stokke és mtsai. 2007; Fiorini és mtsai. 2009), jól elrejtett vagy megközelíthetetlen fészkekben költenek (Banks és Martin 2001; Rutila és mtsai. 2002), valamint felismerik és megtámadják az adult költésparazitákat (Ma és mtsai. 2018; Šulc és mtsai. 2020). A költésparaziták válaszként több olyan adaptív jelleget fejlesztettek ki, amelyek ellensúlyozzák a gazdák védekezését: a kakukkfélék vizuálisan és akusztikusan is hasonlítanak a gazdafajok gyakori ragadozóira (Welbergen és Davies 2011; Trnka és Prokop 2012; York és Davies 2017), polimorf tollazatot fejlesztettek ki a gazdafajok megtévesztésére (Thorogood és Davies 2012; Trnka és Grim 2013; Trnka és mtsai. 2015), és elképesztő sebességgel tojják le tojásaikat, a gazdákkal való találkozás idejét

csökkentve (Guigueno és Sealy 2011). Amennyiben a költésparazita sikeresen becsempészi tojását a gazda fészkébe, a gazdaegyed azzal csökkentheti a nemrokon fiókák felnevelésével járó költségeit, hogy felismeri és eltávolítja a parazita tojásokat vagy fiókákat a fészkből (Colombelli-Négrel és mtsai. 2012; Šulc és mtsai. 2016a; Noh és mtsai. 2018).

Jelen doktori disszertációban olyan tanulmányokat mutatok be, amelyek a költésparazita kakukk *Cuculus canorus* és egyik gazdája, a nádirigó *Acrocephalus arundinaceus*, különböző viselkedési és ökológiai tulajdonságait vizsgálják. A modern tudomány együttműködésen alapszik, az itt bemutatott tanulmányok a témavezetőim, Dr. Moskát Csaba és Dr. Bán Miklós által vezetett kutatócsoport tudományos termékei, ezért következetesen a többes szám első személyt fogom használni, valahányszor közös munkánkra utalok.

Disszertációm két fő részre osztottam: az angol nyelvű áttekintő részre, és egy függelékeket tartalmazó részre, amely az első részben tárgyalt négy tanulmányt tartalmazza. Az áttekintő rész alfejezeteiben bemutatom a költésparazita rendszereket, és vizsgálataink két célfajának, a kakukknek és a nádirigónak, az ökológiáját. Ezeket egy a fajok közötti-, és egy fajon belüli akusztikus kommunikációról szóló alfejezet követ, melyekben ismertetem hipotéziseinket és predikcióinkat. Mindkét alfejezethez két-két tanulmány leírása társul, amelyek módszertani vonatkozásait és eredményeit két további fejezetben mutatom be (1. ábra).



1. ábra: A doktori disszertáció alapját képező négy vizsgálat sematikus ábrázolása.

6.1. I. tanulmány (Marton és *mtsai*. 2019)

A gazdafajok fészekvédelmezési tevékenysége az egyik leghatékonyabb védekezés a költsésparazitizmus ellen, és intenzitása a fenyegető veszélyforrás típusától és fészektől való távolságától függ (Kleindorfer és *mtsai*. 2005; Krytofkov és *mtsai*. 2011). Mivel a fészekvédelmezés inherens módon a fészek közelében történik (Da Cunha és *mtsai*. 2017), fontos információforrás lehet az épp parazitálандó fészkeket kereső hallgatódzó kakukkok számára.

Két kísérlettel és egy korrelatív vizsgálattal teszteltük azt a hipotézisünket, amely szerint a kakukk felismeri a gazda riasztóhangját. Az első kísérlet során hím és tojó kakukkok fészük közelében levő nádírigók erős riasztása iránt tanúsított érdeklődését vizsgáltuk. A végrehajtott 51 kísérleti próba során a tojó ($n = 14$) és hím kakukkok ($n = 30$) erősen reagált a nádírigók vizuális és akusztikus riasztására, a kísérleteket megelőző időszakhoz képest (tojók: Fisher egzakt teszt: $p = 0,004$; hímek: Fisher egzakt teszt: $p < 0,001$). Ez az erős reakció változatlanul megmaradt akkor is, amikor kizártuk a gazda riasztási tevékenységének viselkedésének vizuális komponensét, és riasztását csak hangszórón játszottuk le. A 16 kísérleti próba során 5 pozitív reakciót regisztráltunk a tojó kakukkoknál, míg a kontrollként bejátszott balkáni gerle hangok esetében nem volt reakció (Fisher egzakt teszt: $p = 0,021$). Hasonló módon, a hím kakukkok 7 próba során reagáltak pozitívan, de csak egyszer a kontroll-próbák alatt (Fisher egzakt teszt: $p = 0,018$).

A tanulmányban leírt korrelatív vizsgálatunk eredménye arra utal, hogy a riasztás költséggel jár a gazdák számára. A gazdák emberi megfigyelőkkel szembeni riasztó viselkedése pozitív kapcsolatban állt a gazdafészkekben megfigyelt költsésparazitizmus valószínűségével ($\beta = 2,748$, $SE = 1,222$, $z = 2,248$, $p = 0,024$). Több, egymást nem kizáró magyarázatot is felvetettünk erre az összefüggésre, anélkül, hogy ok-okozati összefüggést feltételeznénk a gazda riasztási hajlandósága és a költsésparazitizmus kockázata között. Az eredmények egyik plauzibilis magyarázata, amely összhangban van fő hipotézisünkkel az, hogy az agresszívebb gazdák véletlenül felfedik fészük helyét a költsésparaziták számára, mivel általában a fészük közelében riasztanak (Uyehara és Narins 1995; Clotfelter 1998; Da Cunha és *mtsai*. 2017). Tekintettel arra, hogy a riasztás jelenlétét vagy hiányát a fészek meglátogatásakor rögzítettük, és a parazitálás megelőzte a fészeklátogatást, ugyanilyen valószínű az is, hogy a nádírigó-párok agresszívebbek lettek a potenciális zavarótényezőkkel szemben egy sikeres parazitálási esemény után. A fészkenél egy költsésparazitával való találkozás növeli annak valószínűségét, hogy a gazdák felismerik és elutasítják a parazita tojásokat (Bártol és *mtsai*. 2002; Davies és Welbergen 2009; Welbergen és Davies

2009; Thorogood és Davies 2016; York és Davies 2017), bár a tojásfelismerés miatti sikertelen parazitizmus meglehetősen alacsony (~30%) a vizsgált populációkban (Moskát és Honza 2000; Zölei és *mtsai*. 2015).

Régóta fennálló feltételezés, hogy a kakukkok magaslatokról nézelődve, vizuálisan keresik a megfelelő gazdafészkeket (Øien és *mtsai*. 1996; Moskát és Honza 2000; Dyrce és Haupka 2006; Antonov és *mtsai*. 2007b). Funkcionális szempontból a gazdafaj riasztóhangjainak felismerése azonos mértékben vagy akár még hatékonyabb jelezheti a gazdafészkek hollétét, mint a vizuális jelzések. A több forrásból származó információk kombinálása különösen akkor lehet fontos, ha a gazdafajok nagy, homogén élőhelyeken költenek (pl. mint a sűrű nádasokban költő *Acrocephalus* fajok).

Eredményeink azt mutatják, hogy a gazda fészekvédelmező tevékenysége jelzésértékű lehet a költésparazita faj számára, és az akusztikus jeleken alapuló fészekkeresés fontos szerepet játszhat a kakukkok költési sikerének biztosításában.

6.2. II. tanulmány (Marton és *mtsai*. 2021)

A gazdák fészekvédelmező tevékenysége informatív lehet a környéken fészkelő egyéb gazdaegyedek számára, a riasztóhang további hatására a szomszédosan költő gazdák fokozottan figyelnek a saját fészkeik tartalmára és nagyobb valószínűséggel eltávolítják a kakukktojásokat a fészkekből (Feeney és Langmore 2013; Thorogood és Davies 2016). Korábbi vizsgálatok eredményei azt sugallják, hogy a kakukk karvalyszerű tollazata azért fejlődött ki, hogy csökkentse a védekező gazdák általi agresszív támadások gyakoriságát (Welbergen és Davies 2011). Az egyes gazdafajok különböző mértékben képesek megkülönböztetni a kakukkot a karvalytól, és általában erőteljesen támadják a kakukkokat (vagy kakukkot imitáló maketteket kísérletek során), viszont tartózkodóan közelítik meg a karvaly-maketteket (Duckworth 1991; Davies és Welbergen 2008; Lawson és *mtsai*. 2021; Krausová és *mtsai*. 2022; Attwood és *mtsai*. 2023). York és Davies (2017) kísérletes módon bizonyította, hogy egyes gazdafajok nem képesek elkülöníteni a tojó kakukk karvalyszerű hangját a karvaly hangjától. Sőt, a téli madáretetés során végzett kísérletekben is azt találták, hogy a karvaly gyakori zsákmányállatai, a széncinege *Parus major* és kékcinege *Cyanistes caeruleus* sem képes a két hangot megkülönböztetni egymástól, holott a kakukk nem jelent veszélyt számukra.

A nagyobb testméretű gazdák súlyosan megsebesíthetik vagy akár vízbe is taszíthatják a kakukkokat, ezért a kakukkok gyors tojásrakással csökkentik a gazdák fészkenél töltött időt (Ma és *mtsai*. 2018; Šulc és *mtsai*. 2020; Jelínek és

mtsai. 2021). Mivel a York és Davies (2017) által végzett kísérletekben a gazdafaj a kisebb és jámborabb cserregő nádiposzáta *Acrocephalus scirpaceus* volt, elvégeztünk egy kísérletet annak érdekében, hogy kiderítsük, hogy a nagyobb és sokkal agresszívabb nádirigó is hasonlóan reagál-e a tojó kakukk hangjára. Azt prediktáltuk, hogy a nádirigók kevesebb alkalommal fogják megtámadni a vörös színváltozatú tojó kakukkot imitáló maketteket akkor, amikor a tojó kakukokra jellemző hangot is lejátszunk a fészek közelében, míg a kontrollként használt balkáni gerle hang nem vált ki csökkentett agressziót a balkáni gerle *Streptopelia decaocto* makett ellen.

Eredményeink alátámasztják York és Davies (2017) hipotézisét, miszerint a nőstény kakukkok által hallatott karvalyszerű hang megijeszti a gazdákat a fészkük közelében, ezzel csökkentve az agresszív interakciók gyakoriságát. A nádirigó fészkekhez helyezett balkánigerle-makettek nem váltottak ki agresszív reakciót a nádirigókban sem a balkáni gerle hang bejátszása előtt, sem utána ($\beta = 0,336$; $SE = 0,420$; $t = 0,799$; $p = 0,427$). Ezzel ellentétben, a nádirigók jelentősen kevesebb alkalommal támadták meg a kakukk-makettet akkor, amikor kakukk tojó hangját is lejátszottuk ($\beta = -0,607$; $SE = 0,052$; $t = -11,692$; $p < 0,001$). Érdekes módon a riasztás gyakorisága nem nőtt a jelenlevő riasztó nádirigók számával, ami arra utal, hogy a fészekvédelmező tevékenység egyenetlenül oszlik el a nádirigó páron belül. Ugyanazon gazdafajt tanulmányozva Požgayová és mtsai. (2009) is azt találták, hogy a hímek felelősek a fészek védelméért, míg a tojók rendszeres fészekellenőrzést végeznek, és felelősek a kakukktojások eltávolításáért.

Eredményeink azt mutatják, hogy a tojó kakukk hangja fontos szerepet játszik a költésparazitizmus során, csökkentve a gazdafajok agresszivitását. Ennek értelmében az akusztikus mimikri megerősítheti a szürke színváltozatú kakukk tojók vizuális mimikriét, míg a vörös színváltozathoz tartozó kakukkok csak az akusztikus mimikri lehetőségével élhetnek (Trnka és Prokop 2012; Trnka és mtsai. 2012).

6.3. III. tanulmány (Moskát és mtsai. 2020)

A tollazati polimorfizmus némely madárcsaládban gyakori, míg másokban ritkább (pl. bagolyfélék 33%-ára és kakukkfélék 12%-ára jellemző, míg a harkályfélék és énekesmadarak családjában nem haladja meg az 1%-os gyakoriságot; Galeotti és mtsai. 2003). Kakukkoknál a tollazati polimorfizmus a tojókra korlátozódik, és feltehetően azért fejlődött ki, hogy ezzel a tojók nehezebben felismerhetőbbek legyenek a gazdafajok számára (Thorogood és Davies 2012, 2013). A vörös színváltozathoz tartozó tojókat ritkábban

támadják meg a gazdamadarak azokon a költőhelyeken, ahol ez a színváltozat ritka (Honza és mtsai. 2006; Trnka és Grim 2013), de ez az előny eltűnik azokon a költőterületeken, ahol a vörös és szürke színváltozat egyaránt gyakori (Honza és mtsai. 2006).

A kakukk tojók rendelkezésére álló nádirigó fészkek száma egy olyan véges erőforrást jelképez, amelyet a kakukk tojóknak védelmezniük kell a saját költési sikerük biztosítása érdekében. Ez főként amiatt is fontos, mert a kakukkok által többszörösen parazitált fészkekben az első kikelő kakukk más kakukkok tojásait is kitaszítja a fészekből (Marton 2021).

Ismerve azt, hogy a negatív sűrűségfüggő szelekció a ritkább, vörös színváltozatnak kedvez azokon a területeken, ahol a gazdafajok ritkábban találkoznak vele (Honza és mtsai. 2006; Trnka és Grim 2013), illetve ez a színváltozat a hímek szexuális zaklatásának is kevésbé kitett (Lee és mtsai. 2019), azt feltételezhetjük, hogy a vörös színváltozatú kakukkok előnyt élvezhetnek a szürke tojókkal szemben is, mivel a szürke tojók sem ismerik fel a vörös tojókat vetélytársként. Kísérleti vizsgálatot terveztünk annak érdekében, hogy megvizsgáljuk a tojó kakukkok reakciói közti különbséget a vörös és szürke színváltozatú tojók általi szimulált territoriális behatolásokra. Mivel a két színváltozat egyformán elterjedt vizsgálati területünkön, arra számítottunk, hogy tojó kakukkok hasonló érdeklődést mutatnak mindkét színváltozatú maketre, ellentétben a kontrollként használt balkáni gerle makettekkel.

Az elvégzett kísérletek alátámasztották predikcióinkat, a tojó és hím kakukkok egyaránt nagyobb érdeklődést mutattak a kakukk-makettek iránt, mint a balkánigerle-makettek iránt (Fisher-féle egzakt teszt, mindkét ivar esetében $p < 0,001$), viszont egyik ivar sem tett különbséget a két különböző színváltozatú kakukk-makett között (tojók: Fisher-féle egzakt teszt: $p = 0,658$; hímek: Fisher-féle egzakt teszt: $p = 0,677$). A két színváltozat iránti reakcióbeli különbséget nem sikerült kimutatni az egyik ivarnál sem logisztikus regressziós modellel és főkomponens analízis módszerével sem (minden változó esetben $p > 0,05$).

Mivel a kakukkok sűrűsége más vizsgálati területeket rendszerint alacsony, a tojók közti interakciók kevésbé ismertek. Csak néhány tanulmány számolt be a kakukkok tojók közti közvetlen antagonista kölcsönhatásokról, és ezek a beszámolók rendszerint csupán empirikus megfigyelések (Riddiford 1986; Dröscher 1988; Lee és mtsai. 2019; Moskát és Hauber 2019). Eredményeink azt mutatják, hogy a kakukk tojók a hímekhez hasonlóan territóriumokat tartanak fenn, és hasonlóan reagálnak a területükre behatoló tojók jelenlétére akár a

hímek: megközelíti az idegen tojókat és ivarspecifikus hangjukkal jelzik jelenlétüket. Eredményeink kiegészítik Koleček és mtsai. (2021) eredményeit, melyek szerint a kakukk tojók lokálisan elkülönülő tojásrakási területeket tartanak fenn. Mivel a rendelkezésre álló gazdafészek száma egy korlátozott erőforrást jelent a költésparaziták számára, elengedhetetlen ezeknek a területeknek a védelme a fajtársaikkal szemben, különösen olyan helyeken, ahol magas a többszörös parazitizmus aránya (Takasu és mtsai. 2009; Zölei és mtsai. 2015; Marton 2021).

A tojók aktív territórium-védő viselkedése nélkülözhetetlen a megfelelő mennyiségű parazitálandó gazdafészek biztosítása érdekében, főleg egy olyan költésparazita fajnál, amely nem tesz különbséget a vetélytársak és a gazdák tojásai között (Šulc és mtsai. 2016b).

6.4. IV. tanulmány (Elek és mtsai. 2021)

A hívóhangok olyan veleszületett akusztikus jelek, amelyek kor-, ivar- és állapotfüggő tulajdonságjelző szereppel járhatnak (Catchpole 1986; Galeotti és mtsai. 1997; Murphy és mtsai. 2008). Például a hím macskabaglyok *Strix aluco* huhogása az egyedek endoparazitákkal való terheltségét tükrözi, ami viszont prediktálja a szimulált territoriális behatolások esetében mutatkozó reakciókészséget (Appleby és mtsai. 1998; Redpath és mtsai. 2000).

A hívóhangok képzésének sebessége (vagyis az egységnyi idő alatt kiadott hangok számát), a hívóhang csúcsfrekvenciáját, a frekvencia sáv szélességét vagy a hívássorozat hosszát korlátozhatják a hangcsatorna sajátossága, a tüdőkapacitás (azaz allometrikus testméret) vagy a hívóhang szerkezete és összetettsége. Ezek a megszorítások külső tényezőkkel párosulva (pl. repülés közbeni hangadás) biztosíthatják azt, hogy ezek a jelek őszintén tükrözzék a hangadó egyed minőségét (Gil és Gahr 2002; Maynard-Smith és Harper 2003). Korábbi vizsgálatok kimutatták, hogy a hívóhang hangképzési sebessége kapcsolatban áll az egyedi minőségével, és befolyásolhatja az egyedek fitnessét több faj esetében (pl. fogoly *Perdix perdix*, Beani és Dessì-Fulgheri 1995; búbosbanka *Upupa epops*, Martín-Vivaldi és mtsai. 1998, 1999; barna halfarkas *Catharacta antarctica lonnbergi*, Janicke és mtsai. 2008).

A hím kakukkok a költési periódus teljes hossza alatt, főként kora reggel és késő este hallatják egyszerű, két szótagú „ka-kukk” hívóhangjukat (Yoo és mtsai. 2020). A hívóhangok ismétlésének hossza jelzi a hímek minőségét szennyezett élőhelyeken (Møller és mtsai. 2016), bár a hangadási konzisztenciája csökken a költési időszak vége felé haladva (Deng és mtsai. 2019).

Kidolgoztunk egy korrelatív és kísérleti vizsgálatot azért, hogy megvizsgáljuk, hogy az általunk megjelölt hím kakukkok hangképzési sebessége tükrözi-e a hím minőség mérőszámaként használt testméretet és testtömeg adatokat. A korábbi tanulmányokkal összhangban azt prediktáltuk, hogy a nagyobb hímek hívóhang képzési sebessége nagyobb (azaz többször kakukkolnak egységnyi idő alatt), illetve, a kísérlet során a szabadon mozgó hím kakukkok erősebb érdeklődést mutatnak az egységnyi idő alatt többet kakukkoló hangfelvétel hallatán, mint az egységnyi idő alatt kevesebbet kakukkoló hangfelvétel lejátszásakor.

Korrelatív vizsgálatunk eredményei azt mutatják, hogy a rádiójeladóval jelölt kakukk hímek ($n = 23$) hangadási rátája nem különbözik a jelöletlen egyedek hangadási rátájától ($n = 18$; Mann-Whitney U teszt: $z_{23,18} = -0,775$, $p = 0,438$). A kvázi-Poisson hibaeloszlású általánosított lineáris modellek egyikében sem találtunk kapcsolatot a kakukk hímek hangadási rátája és testtömege vagy szárny-, csüd- és testhossza között. Bár a testtömeg pozitívan társult a hangadási rátához a teljes modellben, hatása nem volt szignifikáns a minimál-modellben.

Az elvégzett terepi kísérletekben szabadon mozgó kakukk hímeknek játszottunk be olyan hím kakukk hangfelvételeket, amelyek természetes hangadási rátával szóltak ($n = 15$), illetve amelyeknek hangadási rátáját mesterségesen növeltük (azaz „gyorsabban kakukkoltak”; $n = 17$), vagy csökkentett (azaz „lassabban kakukkoltak”; $n = 16$). Kontrollként balkáni gerle nászhangot sugároztunk ($n = 14$). A hím kakukk minden kísérletben erős pozitív reakciót mutattak a fajtársaik bejátszott hangjára, ellentétben a balkáni gerle hangfelvételekkel (mindhárom kakukk hangadási ráta vs. balkáni gerle kontroll: Fisher-féle egzakt teszt: $p < 0,001$). A kakukk hímek, a sugárzott hívások hangadási rátájától függetlenül, egyformán nagy érdeklődéssel közelítették meg a kísérleti berendezést (Kruskal-Wallis teszt: $\chi^2_{17,15,16} = 2,415$, $p = 0,299$), ami arra utal, hogy a hangadási ráta nem tükrözi az egyes hímek minőségét. Ez az eredmény nem változott akkor sem, ha az egymástól legkülönbözőbb hangadási rátára (azaz a „gyorsabb” vs. „lassabb” kakukkolásra) adott válaszokat hasonlítottuk össze két további módszerrel: az időalapú és a távolság alapú változók közül egyik sem társult a „gyorsabb” vagy „lassabb” hangadási rátával a bináris logisztikus regressziós modellekben (minden $p > 0,050$). A „gyorsabb” és „lassabb” csoportok nem különültek el a főkomponens-analízis során sem a főkomponens térben sem (független mintás t teszt, PC1: $t_{31} = 0,495$, $p = 0,624$; PC2: $t_{31} = -0,462$, $p = 0,647$).

A tanulmányban bemutatott eredményeink arra utalnak, hogy a „ka-kukk” hang hangadási rátája önmagában nem egy hím minőségjelző szignál, bár fontos szerepet játszik a hímek közötti területharcokban (Moskát és *mtsai.* 2017, 2018, 2021; Xia és *mtsai.* 2019). A hímek párkeresés során más módon is a tojók tudtára adhatják azt, hogy jó minőségű egyedek, például vizuális bélyegek vagy maximális repülési sebesség segítségével akkor, amikor a tojókat a levegőben üldözik (Lee és *mtsai.* 2019), vagy olyankor amikor kiülő-ágakon farokcsóválást végeznek (Esposito és *mtsai.* 2021). A hangadási rátán kívül más hangjellemzők is fontos szerepet játszhatnak az minőségjelzésben, amint azt korábban más fajoknál is kimutatták: így például azoknak a barna halfarkasoknak volt magasabb a párzási sikere, amelyek magasabb hangadási rátával rendelkeztek miközben konzisztensen több hangot is képesek voltak előállítani a hangfrekvencia széles tartományában (azaz gyors és moduláló hangjuk volt; Janicke és *mtsai.* 2008). Hasonlóképpen, a pompás fregattmadár *Fregata magnificens* hímek párzási sikere nagyobb volt, ha gyorsabb hangadási sebességet mellett élénkebb piros nyaki lebennnyel is rendelkeztek (Madsen és *mtsai.* 2007).

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8. Bibliography

Antonov, A., Stokke, B. G., Moksnes, A. & Røskift, E. (2006a) Egg rejection in marsh warblers *Acrocephalus palustris* heavily parasitized by common cuckoos *Cuculus canorus*. *The Auk*, **123**, 419–430.

Antonov, A., Stokke, B. G., Moksnes, A. & Røskift, E. (2006b) Coevolutionary interactions between common cuckoos and corn buntings. *The Condor*, **108**, 414–422.

Antonov, A., Stokke, B. G., Moksnes, A. & Røskift, E. (2007a) First evidence of regular common cuckoo, *Cuculus canorus*, parasitism on Eastern olivaceous warblers, *Hippolais pallida elaeica*. *Naturwissenschaften*, **94**, 307–312.

Antonov, A., Stokke, B. G., Moksnes, A. & Røskift, E. (2007b) Factors influencing the risk of common cuckoo *Cuculus canorus* parasitism on marsh warblers *Acrocephalus palustris*. *Journal of Avian Biology*, **38**, 390–393.

Antonov, A., Stokke, B. G., Vikan, J. R., Fossøy, F., Ranke, P. S., Røskift, E., Moksnes, A., Møller, A. P. & Shykoff, J. A. (2010) Egg phenotype differentiation in sympatric cuckoo *Cuculus canorus* gentes. *Journal of Evolutionary Biology*, **23**, 1170–1182.

Appleby, B. M. & Redpath, S. M. (1997) Indicators of male quality in the hoots of tawny owls *Strix aluco*. *Journal of Raptor Research*, **31**, 65–70.

Attwood, M. C., Lund, J., Nwaogu, C. J., Moya, C., Spottiswoode, C. N. (2023) Aggressive hosts are undeterred by a cuckoo's hawk mimicry, but probably make good foster parents. *Proceedings of the Royal Society B: Biological Sciences*, **290**, 20221506.

Audacity Team. (2017) Audacity(R): Free Audio Editor and Recorder. Version 3.0.0 retrieved March 17th 2021 from <https://audacityteam.org/>

Báldi, A. (1999) Microclimate and vegetation edge effects in a reedbed in Hungary. *Biodiversity and Conservation*.

Bán, M., Moskát, C., Fülöp, A. & Hauber, M. E. (2018) Return migration of common cuckoos *Cuculus canorus* between breeding grounds in Hungary and wintering grounds in Africa as documented by non-PTT GPS technology. *Journal of Ornithology*, **159**, 337–344.

Banks, J. & Martin, T. E. (2001) Host activity and the risk of nest parasitism by brown-headed cowbirds. *Behavioral Ecology*, **12**, 31–40.

Barabás, L., Gilicze, B., Takasu, F. & Moskát, C. (2004) Survival and anti-parasite defense in a host metapopulation under heavy brood parasitism: A source-sink dynamic model. *Journal of Ethology*, **22**, 143–151.

Bártol, I., Karcza, Z., Moskát, C., Røskift, E. & Kisbenedek, T. (2002) Responses of great reed warblers *Acrocephalus arundinaceus* to experimental brood parasitism: The

effects of a cuckoo *Cuculus canorus* dummy and egg mimicry. *Journal of Avian Biology*, **33**, 420–425.

Bates, D., Mächler, M., Bolker, B. M. & Walker, S. (2014) Fitting Linear Mixed-Effects Models using lme4. *Journal of Statistical Software*, **67**, 1–48.

Beani, L. & Dessì-Fulgheri, F. (1995) Mate choice in the grey partridge, *Perdix perdix*: role of physical and behavioural male traits. *Animal Behaviour*, **49**, 347–356.

Benedetti, Y., Slezak, K., Møller, A. P., Morelli, F. & Tryjanowski, P. (2018) Number of syllables in cuckoo *Cuculus canorus* calls: A test using a citizen science project. *Scientific Reports*, **8**, 12872.

Birkhead, T. R., Hemmings, N., Spottiswoode, C.N., Mikulica, O., Moskát, C., Bán, M. & Schulze-Hagen, K. (2011) Internal incubation and early hatching in brood parasitic birds. *Proceedings of the Royal Society B: Biological Sciences*, **278**, 1019–1024.

Blažek, R., Polačik, M., Smith, C., Honza, M., Meyer, A. & Reichard, M. (2018) Success of cuckoo catfish brood parasitism reflects coevolutionary history and individual experience of their cichlid hosts. *Science Advances*, **4**, eaar4380.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H. & White, J. S. S. (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution*, **24**, 127–35.

Brlík, V., Malmiga, G., Dimitrov, D., Emmenegger, T., Gavrilov, A., Hasselquist, D., Peev, S., Willemoes, M., Yohannes, E., Hahn, S., Hansson, B. & Procházka, P. (2020) Population-specific assessment of carry-over effects across the range of a migratory songbird. *Behavioral Ecology and Sociobiology*, **74**, 143.

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M. & Bolker, B. M. (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R Journal*, **9**, 378–400.

Campobello, D. & Sealy, S. G. (2018) Evolutionary significance of antiparasite, antipredator and learning phenotypes of avian nest defence. *Scientific Reports*, **8**, 1–10.

Campobello, D., Sealy, S. G. & Welbergen, J. A. (2017) Anti-brood parasite defences: the role of individual and social learning. *Avian Brood Parasitism: Behaviour, Ecology, Evolution and Coevolution*, pp. 421–436.

Capek, M., Petrusková, T., Šebelíková, Z., Campos Serrano, J., Procházka, P., Honza, M. & Požgayová, M. (2017) Great reed warbler singing behavior and conspicuous song structures are not nest-location cues for the common cuckoo. *Journal of Ornithology*, **158**, 925–933.

Catchpole, C. K. (1986) Song repertoires and reproductive success in the great reed warbler *Acrocephalus arundinaceus*. *Behavioral Ecology and Sociobiology*, **19**, 439–445.

Catchpole, C., Leisler, B. & Winkler, H. (1985) Polygyny in the great reed warbler *Acrocephalus arundinaceus*: a possible case of deception. *Behavioral Ecology and Sociobiology*, **16**, 285–291.

Chambers, J. M. (1992) Linear models. *Statistical Models in S* (ed. Chambers, J. M. & Hastie, T. J.), Wedsworth & Brooks. London.

Csörgő, T. & Gyurácz, J. (2021) Nádirigó *Acrocephalus arundinaceus* (Linnaeus, 1758). *Magyarország Madáratlasza* (ed. Szép, T., Csörgő T., Halmos, G., Lovászi, P., Nagy, K., & Schmidt, A.), Agrárminisztérium & Magyar Madártani és Természetvédelmi Egyesület, Budapest.

Czirák, Z. (2021) Kakukk *Cuculus canorus* (Linnaeus, 1758). *Magyarország Madáratlasza* (ed. Szép, T., Csörgő T., Halmos, G., Lovászi, P., Nagy, K., & Schmidt, A.), Agrárminisztérium & Magyar Madártani és Természetvédelmi Egyesület, Budapest.

Clotfelter, E. D. (1998) What cues do brown-headed cowbirds use to locate red-winged blackbird host nests? *Animal Behaviour*, **55**, 1181–1189.

Cohen, M. S., Hawkins, M. B., Stock, D. W. & Cruz, A. (2019) Early life-history features associated with brood parasitism in the cuckoo catfish, *Synodontis multipunctatus* (Siluriformes: Mochokidae). *Philosophical Transactions of the Royal Society B: Biological Sciences*, **374**, 20180205.

Colombelli-Négrel, D., Hauber, M. E., Robertson, J., Sulloway, F. J., Hoi, H., Griggio, M. & Kleindorfer, S. (2012) Embryonic learning of vocal passwords in superb fairy-wrens reveals intruder cuckoo nestlings. *Current Biology*, **22**, 2155–2160.

Costantini, D. & Møller, A. P. (2008) Carotenoids are minor antioxidants for birds. *Functional Ecology*, **22**, 367–370.

Da Cunha, F. C. R., Fontenelle, J. C. R. & Griessera, M. (2017) Predation risk drives the expression of mobbing across bird species (ed. Griffin, A.). *Behavioral Ecology*, **28**, 1517–1523.

Dabelsteen, T., McGregor, P. K., Lampe, H. M., Langmore, N. E. & Jo, H. (1998) Quiet song in song birds: an overlooked phenomenon. *Journal of Bioacoustics*, **9**, 89–105.

Darwin, C. R. (1873) *A Fajok Eredete a Természeti Kiválás Útján Vagyis Az Előnyös Válfajok Fenmaradása a Létérti Küzdelemben*. Természettudományi Társulat, Budapest.

Davies, N. B. (2000) *Cuckoos, Cowbirds and Other Cheats*. T. & A. D. Poyster Ltd., London.

Davies, N. B. (2015) *Cuckoo: Cheating by Nature*. Bloomsbury, New York.

Davies, N. B. & Brooke, M. de L. (1988) Cuckoos versus reed warblers: adaptations and counteradaptations. *Animal Behaviour*, **36**, 262–284.

Davies, N. B. & Welbergen, J. A. (2008) Cuckoo-hawk mimicry? An experimental test. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 1817–1822.

Davies, N. B. & Welbergen, J. A. (2009) Social transmission of a host defense against cuckoo parasitism. *Science*, **324**, 1318–1320.

Dawkins, R. & Krebs, J. R. (1979) Arms races between and within species. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **205**, 489–511.

Deng, Z., Lloyd, H., Xia, C., Li, D. & Zhang, Y. (2019) Within-season decline in call consistency of individual male common cuckoos *Cuculus canorus*. *Journal of Ornithology*, **160**, 317–327.

Dröscher, L. (1988) A study on radio-tracking of the European cuckoo *Cuculus canorus canorus*. Proceedings of the International 100th Deutsche Ornithologische-Gesellschaft Meeting, Deutsche Ornithologen-Gesellschaft, (ed. van den Elzen, R., Schuchmann, K.L. & Schmidt-Koenig, K.), pp. 187–193.

Duckworth, J. W. (1991) Responses of breeding reed warblers *Acrocephalus scirpaceus* to mounts of sparrowhawk *Accipiter nisus*, cuckoo *Cuculus canorus* and jay *Garrulus glandarius*. *Ibis*, **133**, 68–74.

Ducrest, A. L., Keller, L. & Roulin, A. (2008) Pleiotropy in the melanocortin system, coloration and behavioural syndromes. *Trends in Ecology and Evolution*, **23**, 502–510.

Dyrce, A. & Haupka, L. (2006) Great reed warbler *Acrocephalus arundinaceus* and reed warbler *Acrocephalus scirpaceus* respond differently to cuckoo dummy at the nest. *Journal of Ornithology*, **147**, 649–652.

Elek, Z., Bán, M., Fülöp, A., Marton, A., Hauber, M. E. & Moskát, C. (2021) Call rate in common cuckoos does not predict body size and responses to conspecific playbacks. *Journal of Ornithology*, **162**, 1183–1192.

Ellis-Felege, S. N., Burnam, J. S., Palmer, W. E., Sisson, D. C. & Carroll, J. P. (2013) Fight or flight: parental decisions about predators at nests of northern bobwhites *Colinus virginianus*. *Auk*, **130**, 637–644.

Esposito, M., Ceraulo, M., Tuliozi, B., Buscaino, G., Mazzola, S., Sala, L., Dal Zotto, M. & Campobello, D. (2021) Decoupled acoustic and visual components in the multimodal signals of the common cuckoo *Cuculus canorus*. *Frontiers in Ecology and Evolution*, **9**, 725858.

Feeney, W. E. & Langmore, N. E. (2013) Social learning of a brood parasite by its host. *Biology Letters*, **9**, 20130443.

Feeney, W. E., Welbergen, J. A. & Langmore, N. E. (2012) The frontline of avian brood parasite-host coevolution. *Animal Behaviour*, **84**, 3–12.

Feldon, D. F., Shukla, K. D. & Maher, M. A. (2016) Faculty–student coauthorship as a means to enhance STEM graduate students’ research skills. *International Journal for Researcher Development*, **7**, 178–191.

Fiorini, V. D., Tuero, D. T. & Reboreda, J. C. (2009) Host behaviour and nest-site characteristics affect the likelihood of brood parasitism by shiny cowbirds on chalk-browed mockingbirds. *Behaviour*, **146**, 1387–1403.

Fiorini, V. D., Tuero, D. T. & Reboreda, J. C. (2012) Dense canopy cover over House Wren *Troglodytes aedon* nests increases latency of brood parasitism by Shiny Cowbirds *Molothrus bonariensis*. *Emu*, **112**, 55–59.

Fox, J. & Weisberg, S. (2011) Mixed-Effects Models in R. *An R Companion to Applied Regression*, 1–54.

Frank, A. F. (2014) R-hacks/mer-utils.R. URL <https://github.com/aufrank/R-hacks/blob/master/mer-utils.R> [accessed 22 January 2018]

Galeotti, P. (1998) Correlates of hoot rate and structure in male tawny owls *Strix aluco*: implications for male rivalry and female mate choice. *Journal of Avian Biology*, **29**, 25.

Galeotti, P., Rubolini, D., Dunn, P. O. & Fasola, M. (2003) Colour polymorphism in birds: causes and functions. *Journal of Evolutionary Biology*, **16**, 635–646.

Galeotti, P., Saino, N., Sacchi, R. & Møller, A. P. (1997) Song correlates with social context, testosterone and body condition in male barn swallows. *Animal Behaviour*, **53**, 687–700.

Geltsch, N., Bán, M., Hauber, M. E. & Moskát, C. (2016) When should common cuckoos *Cuculus canorus* lay their eggs in host nests? *Bird Study*, **63**, 46–51.

Gil, D. & Gahr, M. (2002) The honesty of bird song: multiple constraints for multiple traits. *Trends in Ecology and Evolution*, **17**, 133–141.

Glue, D. & Morgan, R. (1972) Cuckoo hosts in British habitats. *Bird Study*, **19**, 187–192.

Grafen, A. (1990) Biological signals as handicaps. *Journal of Theoretical Biology*, **144**, 517–546.

Grim, T., Kleven, O. & Mikulica, O. (2003) Nestling discrimination without recognition: a possible defence mechanism for hosts towards cuckoo parasitism? *Proceedings of the Royal Society B: Biological Sciences*, **270**, S73–S75.

Grunst, M. L., Grunst, A. S. & Rotenberry, J. T. (2014) Phenotypic plasticity in nest departure calls: Weighing costs and benefits. *Animal Behaviour*, **90**, 31–39.

Guigueno, M. F. & Sealy, S. G. (2011) Aggression towards egg-removing cowbird elicits clutch abandonment in parasitized yellow warblers, *Dendroica petechia*. *Animal Behaviour*, **81**, 211–218.

Hasselquist, D., Bensch, S. & Von Schantz, T. (1996) Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature*, **381**, 229–232.

Hauber, M. E., Moskát, C. & Bán, M. (2006) Experimental shift in hosts' acceptance threshold of inaccurate-mimic brood parasite eggs. *Biology Letters*, **2**, 177–180.

Hett, W. S. (1936) *Aristotle: Minor Works. On Marvellous Things Heard*. Heinemann, London.

Honza, M., Grim, T., Capek, M., Moksnes, A. & Røskft, E. (2004) Nest defence, enemy recognition and nest inspection behaviour of experimentally parasitized reed warblers *Acrocephalus scirpaceus*. *Bird Study*, **51**, 256–263.

Honza, M., Šicha, V., Procházka, P. & Ležalová, R. (2006) Host nest defense against a color-dimorphic brood parasite: great reed warblers *Acrocephalus arundinaceus* versus common cuckoos *Cuculus canorus*. *Journal of Ornithology*, **147**, 629–637.

Honza, M., Taborsky, B., Taborsky, M., Teuschl, Y., Vogl, W., Moksnes, A. & Røskft, E. (2002) Behaviour of female common cuckoos *Cuculus canorus*, in the vicinity of host nests before and during egg laying: A radiotelemetry study. *Animal Behaviour*, **64**, 861–868.

Honza, M., Vošlajerová, K. and Moskát, C. (2007), Eviction behaviour of the common cuckoo *Cuculus canorus* chicks. *Journal of Avian Biology*, **38**, 385–389.

IUCN. (2019) The IUCN Red List of Threatened Species. Version 2019-3.

Jacobsen, L. B., Jensen, N. O., Willemoes, M., Hansen, L., Desholm, M., Fox, A.D., Tøttrup, A. P. & Thorup, K. (2017) Annual spatiotemporal migration schedules in three larger insectivorous birds: European nightjar, common swift and common cuckoo. *Animal Biotelemetry*, **5**, 1–11.

Jakob, E. M., Marshall, S. D. & Uetz, G. W. (1996) Estimating fitness: a comparison of body condition indices. *Oikos*, **77**, 61.

Janicke, T., Hahn, S., Ritz, M. S. & Peter, H. U. (2008) Vocal performance reflects individual quality in a nonpasserine. *Animal Behaviour*, **75**, 91–98.

Jelínek, V., Procházka, P., Požgayová, M. & Honza, M. (2014) Common cuckoos *Cuculus canorus* change their nest-searching strategy according to the number of available host nests. *Ibis*, **156**, 189–197.

Jelínek, V., Šulc, M., Štětková, G. & Honza, M. (2021) Fast and furious: host aggression modulates behaviour of brood parasites. *Ibis*, 1–10.

Jenner, E. (1788) Observation on the natural history of the cuckoo. *Philosophical Transactions of the Royal Society of London*, **78**, 219–237.

Jiang, X., Zhang, C., Liu, J. & Liang, W. (2020) Female cuckoo calls elicit vigilance and escape responses from wild free-range chickens. *Ethology Ecology and Evolution*, **33**, 37–48.

Kalb, N. & Randler, C. (2019) Behavioral responses to conspecific mobbing calls are predator-specific in great tits *Parus major*. *Ecology and Evolution*, **9**, 9207–9213.

Keller, V., Herrando, S., Vorisek, P. & Franch, M. (2020) European Breeding Bird Atlas 2: Distribution, Abundance and Change (ed. Keller, V., Herrando, S., Voříšek, P., Franch, M., Kipson, M., Milanese, P., Martí, D., Anton, M., Klvaňová, A., Kalyakin, M. V., Bauer, H. G., & Foppen, R. P. B.). *European Breeding Bird Atlas 2. Distribution, Abundance and Change*, 86–87.

Kleindorfer, S., Fessler, B. & Hoi, H. (2005) Avian nest defence behaviour: Assessment in relation to predator distance and type, and nest height. *Animal Behaviour*, **69**, 307–313.

Koleček, J., Piálková, R., Piálek, L., Šulc, M., Hughes, A. E., Brlík, V., Procházka, P., Požgayová, M., Capek, M., Sosnovcová, K., Štětková, G., Valterová, R. & Honza, M. (2021) Spatiotemporal patterns of egg laying in the common cuckoo. *Animal Behaviour*, **177**, 107–116.

Krausová, L., Veselý, P., Syrová, M., Antonová, K., Fišer, O., Chlumská, V., Pátková, M., Pužej, Š., & Fuchs, R. (2022). Red-backed shrike *Lanius collurio* versus common cuckoo *Cuculus canorus*: an example of ineffective cuckoo-hawk mimicry. *Ecology and Evolution*, **12**, e9664.

Kroodsma, D. E. (1989) Suggested experimental designs for song playbacks. *Animal Behaviour*, **37**, 600–609.

Kroodsma, D. E., Byers, B. E., Goodale, E., Johnson, S. & Liu, W. C. (2001) Pseudoreplication in playback experiments, revisited a decade later. *Animal Behaviour*, **61**, 1029–1033.

Krüger, O. (2007) Cuckoos, cowbirds and hosts: adaptations, trade-offs and constraints. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **362**, 1873–1886.

Krytofkov, M., Haas, M. & Exnerov, A. (2011) Nest Defense in blackbirds *Turdus merula*: effect of predator distance and parental sex. *Acta Ornithologica*, **46**, 55–63.

Lawson, S. L., Enos, J. K., Antonson, N. D., Gill, S. A. & Hauber, M. E. (2021) Do hosts of avian brood parasites discriminate parasitic vs. predatory threats? A meta-analysis. *Advances in the Study of Behavior*, **53**, 63–95.

Lawson, S. L., Leuschner, N., Gill, B. J., Enos, J. K. & Hauber, M. E. (2020) Loss of graded enemy recognition in a whitehead population allopatric with brood-parasitic long-tailed cuckoos. *Avocetta*, **44**, 3–10.

Lee, J. W., Kim, H. N., Yoo, S. & Yoo, J. C. (2019) Common cuckoo females may escape male sexual harassment by color polymorphism. *Scientific Reports*, **9**, 1–9.

Leisler, B. & Schulze-Hagen, K. (2011) *The Reed Warblers. Diversity in a Uniform Bird Family*. KNNV Uitgeverij.

Li, D., Wei, H., Zhang, Z., Liang, W. & Stokke, B. G. (2015) Oriental reed warbler *Acrocephalus orientalis* nest defence behaviour towards brood parasites and nest predators. *Behaviour*, **152**, 1601–1621.

Liechti, F., Bauer, S., Dhanjal-Adams, K. L., Emmenegger, T., Zehtindjiev, P. & Hahn, S. (2018) Miniaturized multi-sensor loggers provide new insight into year-round flight behaviour of small trans-Saharan avian migrants. *Movement Ecology*, **6**, 19.

Ma, L., Yang, C. & Liang, W. (2018) Hawk mimicry does not reduce attacks of cuckoos by highly aggressive hosts. *Avian Research*, **9**, 35.

Madsen, V., Valkiunas, G., Iezhova, T. A., Mercade, C., Sanchez, M. & Osorno, J. L. (2007) Testosterone levels and gular pouch coloration in courting magnificent frigatebird *Fregata magnificens*: variation with age-class, visited status and blood parasite infection. *Hormones and Behavior*, **51**, 156–163.

Magrath, R. D., Haff, T. M., Fallow, P. M. & Radford, A. N. (2015) Eavesdropping on heterospecific alarm calls: from mechanisms to consequences. *Biological Reviews*, **90**, 560–586.

Malthus, T. R. (1798) *An Essay on the Principle of Population*. J. Johnson, London.

Marchetti, K., Nakamura, H. & Gibbs, H. L. (1998) Host-race formation in the common cuckoo. *Science*, **282**, 471–472.

Martín-Vivaldi, M., Palomino, J. J. & Soler, M. (1998) Song structure in the hoopoe *Upupa epops* - strophe length reflects male condition. *Journal fur Ornithologie*, **139**, 287–296.

Marín-Vivaldi, M., Palomino, J. J., Soler, M. & Martínez, J. G. (1999) Song strophe-length and reproductive success in a non-passerine bird, the hoopoe *Upupa epops*. *Ibis*, **141**, 670–679.

Marton, A. (2021) Quintuple parasitism of a great reed warbler nest by common cuckoos. *Ecology and Evolution*, **11**, 8420–8423.

Marton, A., Fülöp, A., Bán, M., Hauber, M. E., Moskát, C., Fülöp, A., Bán, M., Hauber, M.E. & Moskát, C. (2021) Female common cuckoo calls dampen the mobbing intensity of great reed warbler hosts. *Ethology*, **127**, 286–293.

Marton, A., Fülöp, A., Ozogány, K., Moskát, C. & Bán, M. (2019) Host alarm calls attract the unwanted attention of the brood parasitic common cuckoo. *Scientific Reports*, **9**, 18563.

Maynard-Smith, J. & Harper, D. (2003) *Animal Signals*. Oxford University Press.

McLaren, C. M. & Sealy, S. G. (2003) Factors influencing susceptibility of host nests to brood parasitism. *Ethology Ecology and Evolution*, **15**, 343–353.

Medina, I. & Langmore, N. E. (2016) Batten down the thatches: front-line defences in an apparently defenceless cuckoo host. *Animal Behaviour*, **112**, 195–201.

Medina, I. & Langmore, N. E. (2019) Host density predicts the probability of parasitism by avian brood parasites. *Philosophical Transactions of the Royal Society B*, **374**, 20180204.

Mérő, T. O. & Žuljević, A. (2015) Nest position and reed density influence nest defence behaviour of great reed warbler. *Ethology, Ecology and Evolution*, **29**, 94–101.

Mérő, T. O. & Žuljević, A. (2019) The effects of reed density, surface and management on the probability of cuckoo *Cuculus canorus* parasitism on great reed warbler *Acrocephalus arundinaceus* nests. *Ethology, Ecology and Evolution*, **31**, 98–104.

Mérő, T. O., Žuljević, A., Varga, K. & Lengyel, S. (2013) Breeding of the brood parasitic common cuckoo *Cuculus canorus* in reed habitats in nw Vojvodina, Serbia. *Natura Croatica*, **22**, 265–273.

Mérő, T. O., Žuljević, A., Varga, K. & Lengyel, S. (2015) Habitat use and nesting success of the great weed warbler *Acrocephalus arundinaceus* in different reed habitats in Serbia. *The Wilson Journal of Ornithology*, **127**, 477–485.

Mérő, T. O., Žuljević, A. & Lengyel, S. (2023) The role of reed management and habitat quality on brood parasitism and chick survival of the brood parasitic common cuckoo. *Ecology and Evolution*, **13**, e9705.

Mikulica, O., Grim, T., Schulze-Hagen, K. & Stokke, B. G. (2017) *The Cuckoo: The Uninvited Guest*. Wild Nature Press.

Møller, A. P., Morelli, F., Mousseau, T. A. & Tryjanowski, P. (2016) The number of syllables in Chernobyl cuckoo calls reliably indicate habitat, soil and radiation levels. *Ecological Indicators*, **66**, 592–597.

Møller, A. P., Morelli, F. & Tryjanowski, P. (2017) Cuckoo folklore and human well-being: cuckoo calls predict how long farmers live. *Ecological Indicators*, **72**, 766–768.

Molnár, B. (1944) The cuckoo in the Hungarian Plain. *Aquila*, **51**, 100–112.

Moskát, C. (2005) Nest defence and egg rejection in great reed warblers over the breeding cycle: are they synchronised with the risk of brood parasitism? *Annales Zoologici Fennici*, **42**, 579–586.

Moskát, C., Bán, M., Fülöp, A., Bereczki, J. & Hauber, M. E. (2019) Bimodal habitat use in brood parasitic common cuckoos *Cuculus canorus* revealed by GPS telemetry. *Auk*, **136**, 1–12.

Moskát, C., Barta, Z., Hauber, M. E. & Honza, M. (2006) High synchrony of egg laying in common cuckoos *Cuculus canorus* and their great reed warbler *Acrocephalus arundinaceus* hosts. *Ethology Ecology and Evolution*, **18**, 159–167.

Moskát, C., Elek, Z., Bán, M., Geltsch, N. & Hauber, M. E. (2017) Can common cuckoos discriminate between neighbours and strangers by their calls? *Animal Behaviour*, **126**, 253–260.

Moskát, C. & Hauber, M. E. (2019) Sex-specific responses to simulated territorial intrusions in the common cuckoo: a dual function of female acoustic signaling. *Behavioral Ecology and Sociobiology*, **73**.

Moskát, C. & Hauber, M. E. (2021) Male common cuckoos use a three-note variant of their “cu-coo” call for duetting with conspecific females. *Behavioural Processes*, **191**, 104472.

Moskát, C. & Hauber, M. E. (2022a) Quantitative analysis of vocalisation types in male common cuckoos’ “gowk” call complex. *Zoology*, **154**, 126043.

Moskát, C. & Hauber, M. E. (2022b) Syntax errors do not disrupt acoustic communication in the common cuckoo. *Scientific Reports*, **12**, 1568.

Moskát, C., Hauber, M. E., Bán, M., Fülöp, A., Geltsch, N., Marton, A. & Elek, Z. (2018) Are both notes of the common cuckoo’s call necessary for familiarity recognition? *Behavioural Processes*, **157**, 685–690.

Moskát, C., Hauber, M. E., Růžicková, J., Marton, A., Bán, M. & Elek, Z. (2020) Female-female aggression and male responses to the two colour morphs of female common cuckoos. *Science of Nature*, **107**, 28.

Moskát, C. & Honza, M. (2000) Effect of nest and nest site characteristics on the risk of cuckoo *Cuculus canorus* parasitism in the great reed warbler *Acrocephalus arundinaceus*. *Ecography*, **23**, 335–341.

Moskát, C. & Honza, M. (2002) European cuckoo *Cuculus canorus* parasitism and host's rejection behaviour in a heavily parasitized Great reed warbler *Acrocephalus arundinaceus* population. *Ibis*, **144**, 614–622.

Moskát, C., Taylor, D. M. & Hauber, M. E. (2021) Effective conspecific communication with aberrant calls in the common cuckoo *Cuculus canorus*. *Behavioral Ecology and Sociobiology*, **75**, 7.

Murphy, M. T., Sexton, K., Dolan, A. C. & Redmond, L. J. (2008) Dawn song of the eastern kingbird: an honest signal of male quality? *Animal Behaviour*, **75**, 1075–1084.

Nakamura, H., Miyazawa, Y. & Kashiwagi, K. (2005) Behavior of radio-tracked common cuckoo females during the breeding season in Japan. *Ornithological Science*, **4**, 31–41.

Noh, H. J., Gloag, R. & Langmore, N. E. (2018) True recognition of nestlings by hosts selects for mimetic cuckoo chicks. *Proceedings of the Royal Society B: Biological Sciences*, **285**, 20180726.

Noh, H. J., Jacomb, F., Gloag, R. & Langmore, N. E. (2021) Frontline defences against cuckoo parasitism in the large-billed gerygones. *Animal Behaviour*, **174**, 51–61.

Noh, H. J., Lee, J. W. & Yoo, J. C. (2016) Color morph variation in two brood parasites: common cuckoo and lesser cuckoo. *Ornithological Science*, **15**, 109–117.

Oberweger, K. & Goller, F. (2001) The metabolic cost of birdsong production. *Journal of Experimental Biology*, **204**, 3379–3388.

Øien, I. J. (2000) How do cuckoos *Cuculus canorus* find host nests? *Ornis Norvegica*, **23**, 3–7.

Øien, I. J., Honza, M., Moksnes, A. & Røskaft, E. (1996) The risk of parasitism in relation to the distance from reed warbler nests to cuckoo perches. *The Journal of Animal Ecology*, **65**, 147.

Olson, V. A. & Owens, I. P. F. (2005) Interspecific variation in the use of carotenoid-based coloration in birds: diet, life history and phylogeny. *Journal of Evolutionary Biology*, **18**, 1534–1546.

Osváth-Ferencz, M., Czekes, Z., Molnár, G., Markó, B., Vizauer, T. C., Rákossy, L. & Nowicki, P. (2016) Adult population ecology and egg laying strategy in the 'cruciata' ecotype of the endangered butterfly *Maculinea alcon* (Lepidoptera: Lycaenidae). *Journal of Insect Conservation*, **20**, 255–264.

Panther, C. T. & Amar, A. (2021) Sex and age differences in the diet of the Eurasian sparrowhawk *Accipiter nisus* using web-sourced photographs: exploring the feasibility of a new citizen science approach. *Ibis*, **163**, 928–947.

Payne, R. B. (2005) *The Cuckoos*. Oxford University Press, New York.

Penn, D. J. & Számadó, S. (2020) The Handicap Principle: how an erroneous hypothesis became a scientific principle. *Biological Reviews*, **95**, 267–290.

Polačik, M., Reichard, M., Smith, C. & Blažek, R. (2019) Parasitic cuckoo catfish exploit parental responses to stray offspring. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **374**, 20180412.

Pollock, H. S., Hoover, J. P., Uy, F. M. K. & Hauber, M. E. (2021) Brood parasites are a heterogeneous and functionally distinct class of natural enemies. *Trends in Parasitology*, **37**, 588–596.

Požgayová, M., Beňo, R., Procházka, P., Jelínek, V., Abraham, M. M. & Honza, M. (2015) Lazy males and hardworking females? Sexual conflict over parental care in a brood parasite host and its consequences for chick growth. *Behavioral Ecology and Sociobiology*, **69**, 1053–1061.

Požgayová, M., Koleček, J., Honza, M. & Procházka, P. (2022) Is there an effect of fostering a brood parasite on the timing of host autumn migration? *Journal of Ornithology*, **1**, 1–7.

Požgayová, M., Procházka, P. & Honza, M. (2009) Sex-specific defence behaviour against brood parasitism in a host with female-only incubation. *Behavioural Processes*, **81**, 34–38.

Požgayová, M., Procházka, P., Polačiková, L. & Honza, M. (2011) Closer clutch inspection—quicker egg ejection: timing of host responses toward parasitic eggs. *Behavioral Ecology*, **22**, 46–51.

R Core Team. (2017) R: A language and environment for statistical computing.

Rabeling, C. (2020) Social Parasitism. *Encyclopedia of Social Insects*, 1–23.

Redpath, S. M., Appleby, B. M. & Petty, S. J. (2000) Do male hoots betray parasite loads in tawny owls? *Journal of Avian Biology*, **31**, 457–462.

Riddiford, N. (1986) Why do cuckoos *Cuculus canorus* use so many species of hosts? *Bird Study*, **33**, 1–5.

Roulin, A. & Ducrest, A. L. (2011) Association between melanism, physiology and behaviour: a role for the melanocortin system. *European Journal of Pharmacology*, **660**, 226–233.

Rutla, J., Latja, R. & Koskela, K. (2002) The common cuckoo *Cuculus canorus* and its cavity nesting host, the redstart *Phoenicurus phoenicurus*: a peculiar cuckoo-host system? *Journal of Avian Biology*, **33**, 414–419.

Samaš, P., Rutla, J. & Grim, T. (2016) The common redstart as a suitable model to study cuckoo-host coevolution in a unique ecological context. *BMC Evolutionary Biology*, **16**, 1–13.

San-Jose, L. M. & Roulin, A. (2018) Toward understanding the repeated occurrence of associations between melanin-based coloration and multiple phenotypes. *American Naturalist*, **192**, 111–130.

Sato, T. (1986) A brood parasitic catfish of mouthbrooding cichlid fishes in Lake Tanganyika. *Nature*, **323**, 58–59.

Seppä, J. (1986) The cuckoo's ability to find a nest where it can lay an egg. *Ornis Fennica*, **46**, 78–80.

Shannon, C. E. (1948) A Mathematical Theory of Communication. *Bell System Technical Journal*, **27**, 623–656.

Soler, M. & Soler, J. J. (1999) Innate versus learned recognition of conspecifics in great spotted cuckoos *Clamator glandarius*. *Animal Cognition*, 97–102.

Spottiswoode, C. N. & Busch, R. (2019) Vive la difference! Self/non-self recognition and the evolution of signatures of identity in arms races with parasites. *Philosophical Transactions of the Royal Society B*, **374**, 20180206.

Stoddard, M. C., Hogan, B. G., Stevens, M. & Spottiswoode, C. N. (2019) Higher-level pattern features provide additional information to birds when recognizing and rejecting parasitic eggs. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **374**, 20180197.

Stokke, B. G., Hafstad, I., Rudolfson, G., Bargain, B., Beier, J., Bigas Campàs, D., Dyrce, A., Honza, M., Leisler, B., Pap, P. L., Patapavičius, R., Procházka, P., Schulze-Hagen, K., Thomas, R., Moksnes, A., Møller, A. P., Røskft, E. & Soler, M. (2007) Host density predicts presence of cuckoo parasitism in reed warblers. *Oikos*, **116**, 913–922.

Stokke, B. G., Ratikainen, I. I., Moksnes, A., Røskft, E., Schulze-Hagen, K., Leech, D. I., Møller, A. P. & Fossøy, F. (2018) Characteristics determining host suitability for a generalist parasite. *Scientific Reports*, **8**, 6285.

Strnad, M., Němec, M., Veselý, P. & Fuchs, R. (2012) Red-backed shrikes *Lanius collurio* adjust the mobbing intensity, but not mobbing frequency, by assessing the potential threat to themselves from different predators. *Ornis Fennica*, **89**, 206–215.

Stumpf, K. J., Theimer, T. C., McLeod, M. A. & Koronkiewicz, T. J. (2012) Distance from riparian edge reduces brood parasitism of southwestern willow flycatchers,

whereas parasitism increases nest predation risk. *The Journal of Wildlife Management*, **76**, 269–277.

Šulc, M., Procházka, P., Capek, M. & Honza, M. (2016a) Birds use eggshell UV reflectance when recognizing non-mimetic parasitic eggs. *Behavioral Ecology*, **27**, 677–684.

Šulc, M., Procházka, P., Capek, M. & Honza, M. (2016b) Common cuckoo females are not choosy when removing an egg during parasitism. *Behavioral Ecology*, **27**, 1642–1649.

Šulc, M., Štětková, G., Procházka, P., Požgayová, M., Sosnovcová, K., Studecký, J. & Honza, M. (2020) Caught on camera: circumstantial evidence for fatal mobbing of an avian brood parasite by a host. *Journal of Vertebrate Biology*, **69**, 1–6.

Svensson, L., Mullarney, K., Zetterström, D. & Grant, P.J. (2011) *Collins Bird Guide*, 2nd ed. Collins, London.

Takasu, F., Moskát, C., Muñoz, A. R., Imanishi, S. & Nakamura, H. (2009) Adaptations in the common cuckoo *Cuculus canorus* to host eggs in a multiple-hosts system of brood parasitism. *Biological Journal of the Linnean Society*, **98**, 291–300.

Tartally, A., Thomas, J. A., Anton, C., Balletto, E., Barbero, F., Bonelli, S., Bräu, M., Casacci, L. Pietro, Csósz, S., Czeke, Z., Dolek, M., Dziekańska, I., Elmes, G., Fürst, M. A., Glinka, U., Hochberg, M. E., Höttinger, H., Hula, V., Maes, D., Munguira, M. L., Musche, M., Nielsen, P. S., Nowicki, P., Oliveira, P. S., Peregovits, L., Ritter, S., Schlick-Steiner, B. C., Settele, J., Sielezniew, M., Simcox, D. J., Stankiewicz, A. M., Steiner, F. M., Švitra, G., Ugelvig, L. V., Van Dyck, H., Varga, Z., Witek, M., Woyciechowski, M., Wynhoff, I. & Nash, D. R. (2019) Patterns of host use by brood parasitic *Maculinea* butterflies across Europe. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **374**, 20180202.

Thorogood, R. & Davies, N. B. (2012) Cuckoos combat socially transmitted defenses of reed warbler hosts with a plumage polymorphism. *Science*, **337**, 578–580.

Thorogood, R. & Davies, N. B. (2013) Hawk mimicry and the evolution of polymorphic cuckoos. *Chinese Birds*, **4**, 39–50.

Thorogood, R. & Davies, N. B. (2016) Combining personal with social information facilitates host defences and explains why cuckoos should be secretive. *Scientific Reports*, **6**, 10–15.

Thorogood, R., Spottiswoode, C. N., Portugal, S. J. & Gloag, R. (2019) The coevolutionary biology of brood parasitism: a call for integration. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **374**, 20180190.

Thorup, K., Vega, M. L., Snell, K. R. S., Lubkovskaia, R., Willemoes, M., Sjöberg, S., Sokolov, L. V. & Bulyuk, V. (2020) Flying on their own wings: young and adult cuckoos

respond similarly to long-distance displacement during migration. *Scientific Reports*, **10**, 1–8.

Tinbergen, N. (1963) On aims and methods of Ethology. *Zeitschrift für Tierpsychologie*, **20**, 410–433.

Tosi-Germán, R. A., Tassino, B. & Reboreda, J. C. (2020) Female and male rufous horneros eject shiny cowbird eggs using a mental template of the size of their own eggs. *Behavioural Processes*, **178**, 104152.

Trnka, A. & Grim, T. (2013) Color plumage polymorphism and predator mimicry in brood parasites. *Frontiers in Zoology*, **10**, 1–10.

Trnka, A. & Prokop, P. (2010) Does social mating system influence nest defence behaviour in great reed warbler *Acrocephalus arundinaceus* males? *Ethology*, **116**, 1075–1083.

Trnka, A. & Prokop, P. (2011) Polygynous great reed warblers *Acrocephalus arundinaceus* suffer more cuckoo *Cuculus canorus* parasitism than monogamous pairs. *Journal of Avian Biology*, **42**, 192–195.

Trnka, A. & Prokop, P. (2012) The effectiveness of hawk mimicry in protecting cuckoos from aggressive hosts. *Animal Behaviour*, **83**, 263–268.

Trnka, A., Prokop, P. & Grim, T. (2012) Uncovering dangerous cheats: how do avian hosts recognize adult brood parasites? *PLoS ONE*, **7**, e37445.

Trnka, A., Trnka, M. & Grim, T. (2015) Do rufous common cuckoo females indeed mimic a predator? *Biological Journal of the Linnean Society*, **116**, 134–143.

Tryjanowski, P., Morelli, F., Osiejuk, T. S. & Møller, A. P. (2018) Functional significance of cuckoo *Cuculus canorus* calls: responses of conspecifics, hosts and non-hosts. *PeerJ*, **2018**, e5302.

Uyehara, J. C. & Narins, P. M. (1995) Nest defense by willow flycatchers to brood-parasitic intruders. *The Condor*, **97**, 361–368.

Vikan, J. R., Fossøy, F., Huhta, E., Moksnes, A., Røskft, E. & Stokke, B. G. (2011) Outcomes of brood parasite-host interactions mediated by egg matching: common cuckoos *Cuculus canorus* versus *Fringilla* finches. *PLoS ONE*, **6**, e19288.

Wang, L., Yang, C., He, G., Liang, W. & Møller, A. P. (2020) Cuckoos use host egg number to choose host nests for parasitism: cuckoos are sensitive to egg number. *Proceedings of the Royal Society B: Biological Sciences*, **287**, 20200343.

Wang, L., Zhang, Y., Liang, W. & Møller, A. P. (2021) Common cuckoo females remove more conspicuous eggs during parasitism. *Royal Society Open Science*, **8**, 201264.

Welbergen, J. A. & Davies, N. B. (2009) Strategic variation in mobbing as a front line of defense against brood parasitism. *Current Biology*, **19**, 235–240.

Welbergen, J. A. & Davies, N. B. (2011) A parasite in wolf's clothing: hawk mimicry reduces mobbing of cuckoos by hosts. *Behavioral Ecology*, **22**, 574–579.

Willemoes, M., Strandberg, R., Klaassen, R. H. G., Tøttrup, A. P., Vardanis, Y., Howey, P. W., Thorup, K., Wikelski, M. & Alerstam, T. (2014) Narrow-front loop migration in a population of the common cuckoo *Cuculus canorus*, as revealed by satellite telemetry. *PLoS ONE*, **9**, e83515.

Yyllie, I. (1981) *The Cuckoo*. Universe Pub, London.

Xia, C., Deng, Z., Lloyd, H., Møller, A. P., Zhao, X. & Zhang, Y. (2019) The function of three main call types in common cuckoo. *Ethology*, **125**, 652–659.

Yang, C. C., Li, D. L., Wang, L. W., Liang, G. X., Zhang, Z. W. & Liang, W. (2014) Geographic variation in parasitism rates of two sympatric cuckoo hosts in China. *Zoological Research*, **35**, 67–71.

Yoo, S., Kim, H. N., Lee, J. W. & Yoo, J. C. (2020) Seasonal and diurnal patterns of population vocal activity in avian brood parasites. *Ibis*, **162**, 1001–1011.

York, J. E. & Davies, N. B. (2017) Female cuckoo calls misdirect host defences towards the wrong enemy. *Nature Ecology and Evolution*, **1**, 1520–1525.

Yu, J., Xing, X., Jiang, Y., Liang, W., Wang, H. & Møller, A. P. (2017) Alarm call-based discrimination between common cuckoo and Eurasian sparrowhawk in a Chinese population of great tits. *Ethology*, **123**, 542–550.

Zahavi, A. (1977) The cost of honesty (further remarks on the handicap principle). *Journal of Theoretical Biology*, **67**, 603–605.

Zhao, H., Luo, H., Yan, H., He, G., Wang, L. & Liang, W. (2022) Fatal mobbing and attack of the common cuckoo by its warbler hosts. *Ecology and Evolution*, **12**, e9649.

Zölei, A., Bán, M. & Moskát, C. (2015) No change in common cuckoo *Cuculus canorus* parasitism and great reed warblers' *Acrocephalus arundinaceus* egg rejection after seven decades. *Journal of Avian Biology*, **46**, 570–576.

Zsebők, S., Moskát, C. & Bán, M. (2017) Individually distinctive vocalization in common cuckoos *Cuculus canorus*. *Journal of Ornithology*, **158**, 213–222.

ANNEXES

9. Study I:

Host alarm calls attract the unwanted attention of the brood parasitic common cuckoo

Marton, A., Fülöp, A., Ozogány, K., Moskát, C. & Bán, M.





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Abstract

It is well known that avian brood parasites lay their eggs in the nests of other bird species, called hosts. It remains less clear, however, just how parasites are able to recognize their hosts and identify the exact location of the appropriate nests to lay their eggs in. While previous studies attributed high importance to visual signals in finding the hosts' nests (e.g., nest building activity or the distance and direct sight of the nest from vantage points used by the brood parasites), the role of host acoustic signals during the nest searching stage has been largely neglected. We present experimental evidence that both female and male common cuckoos *Cuculus canorus* pay attention to their host's, the great reed warbler's *Acrocephalus arundinaceus* alarm calls, relative to the calls of an unparasitized species used as controls. Parallel to this, we found no difference between the visibility of parasitized and unparasitized nests during drone flights, but great reed warblers that alarmed more frequently experienced higher rates of parasitism. We conclude that alarm calls might be advantageous for the hosts when used against enemies or for alerting conspecifics, but can act in a detrimental manner by providing important nest location cues for eavesdropping brood parasites. Our results suggest that host alarm calls may constitute a suitable trait on which cuckoo nestlings can imprint on to recognize their primary host species later in life. Our study contributes to the growing body of knowledge regarding the context-dependency of animal signals, by providing a novel example of a beneficial acoustic trait intercepted by a heterospecific and used against the emitter.

OPEN

Host alarm calls attract the unwanted attention of the brood parasitic common cuckoo

Attila Marton ^{1,2*}, Attila Fülöp ^{2,3}, Katalin Ozogány¹, Csaba Moskát ^{4,5} & Miklós Bán ^{1,3,5}

It is well known that avian brood parasites lay their eggs in the nests of other bird species, called hosts. It remains less clear, however, just how parasites are able to recognize their hosts and identify the exact location of the appropriate nests to lay their eggs in. While previous studies attributed high importance to visual signals in finding the hosts' nests (e.g. nest building activity or the distance and direct sight of the nest from vantage points used by the brood parasites), the role of host acoustic signals during the nest searching stage has been largely neglected. We present experimental evidence that both female and male common cuckoos *Cuculus canorus* pay attention to their host's, the great reed warbler's *Acrocephalus arundinaceus* alarm calls, relative to the calls of an unparasitized species used as controls. Parallel to this, we found no difference between the visibility of parasitized and unparasitized nests during drone flights, but great reed warblers that alarmed more frequently experienced higher rates of parasitism. We conclude that alarm calls might be advantageous for the hosts when used against enemies or for alerting conspecifics, but can act in a detrimental manner by providing important nest location cues for eavesdropping brood parasites. Our results suggest that host alarm calls may constitute a suitable trait on which cuckoo nestlings can imprint on to recognize their primary host species later in life. Our study contributes to the growing body of knowledge regarding the context-dependency of animal signals, by providing a novel example of a beneficial acoustic trait intercepted by a heterospecific and used against the emitter.

Interspecific avian brood parasitism affects the reproductive success of hosts, as brood parasites hijack parental investment causing this to be misdirected towards an unrelated nestling, whilst the hosts' investment into their own brood is either greatly diminished or lost altogether^{1–3}. In order to counter the negative effects of this phenomenon, host species have evolved a range of adaptive traits which can reduce parasitism risk prior to egg-laying (i.e. frontline defenses), as well as during egg-, nestling-, and fledgling stages¹. Host frontline defenses include secretive female behavior during the breeding season, inaccessible or well-concealed nests, and active nest defense behavior against brood parasites^{4,5}. Furthermore, nest defense behavior can also act as a social cue to other potential hosts within the population, greatly enhancing any response against the brood parasite^{6,7} and increasing the hosts' egg-rejection rates⁸. In turn, brood parasites have evolved traits to bypass these host defenses, leading to a significant variation of responses among brood parasitic study systems^{9–14}.

Several studies performed on Nearctic brood parasitic systems address the question regarding the informational value of the host's activity and behavior for the brood parasites during the nest searching process^{9,11,15–17}. While host activity near the nest is essential for the brood parasite to locate the nest^{9,11,15}, only two of these studies found a positive correlation between host vocalization (i.e. males singing, and calls uttered near the nest) and the probability of parasitism by cowbirds^{16,17}. Brown-headed cowbirds *Molothrus ater* preferred to parasitize willow flycatcher *Empidonax traillii* females with a higher vocalization rate over females that called less often during egg-laying and early incubation¹⁶. Similarly, higher cowbird parasitism rates were observed at the nests of red-winged blackbird *Agelaius phoeniceus* females that produced more often a call uttered typically when leaving or arriving to the nest, and which was used to coordinate male vigilance and nest defense¹⁷. In contrast, common

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cuckoos *Cuculus canorus* exhibited a clear preference to parasitize great reed warblers *Acrocephalus arundinaceus* based on their nest size and nest visibility^{12,18} rather than based on behavioral traits, such as male singing activity and song repertoire^{12,13}. To the best of our knowledge, an empirical observation by a Finnish ornithologist originating from 1930, discussed below in detail, constitutes the only description that common cuckoos might rely on a host behavioral trait during host selection or nest searching¹⁹.

The common cuckoo is one of the most well-studied avian brood parasites^{2,20}. Female cuckoos are divided into races (i.e. gentes) that differ with respect to the host species they parasitize, having less than 20 known gentes in Europe^{21,22}. Female cuckoos within different gentes are able to recognize their own hosts (e.g. sexually monomorphic, reed-dwelling *Acrocephalus* species with cryptic plumage), and utilize non-primary hosts only if the number of suitable primary host nests is insufficient, despite the striking similarity in appearance between these closely related species^{23–26}. Highly accurate species-level host identification was observed also in Edgar Chance's famous 'Cuckoo A', which laid its eggs in tree pipit *Anthus trivialis* nests only if meadow pipit *Anthus pratensis* nests were unavailable, despite the striking degree of similarity between meadow pipits and tree pipits²⁷. Therefore, host recognition must be an important fitness component for the cuckoo, given that a mismatch in egg phenotype between hosts and parasites leads to reproductive failure for the cuckoo due to egg rejection^{28–30}. Although the recognition of the gent-specific host species and the localization of potential nests is of paramount importance for the fitness of brood parasites, the mechanisms utilized to recognize primary host species and locate their nests remains incompletely understood.

Female cuckoos monitor their potential hosts from vantage points, from where they locate suitable nests to lay their egg in³⁴. As cuckoos do not parasitize nests without host activity³¹, some of the host's behavioral traits are of crucial importance for the brood parasite during the nest searching stage. Also, the nest-site characteristics of hosts are expected to influence the probability of parasitism, and indeed, previous studies have shown that both distance from a vantage point and nest visibility affect the probability of brood parasitism, while vegetation type, cover, nest size, and nest position within the reed has no effect on parasitism rates^{18,32,33}. The nest searching strategy utilized by a female might change in accordance with host nest availability: in periods when the abundance of suitable host nests is high, well-concealed nests tend to be less parasitized, while if host nest density is low, cuckoos seemingly invest more effort in nest searching and also parasitize well-hidden nests³⁴. Previous studies that have attempted to identify nest localization strategies have focused mainly on visual signals, such as the degree of visibility (e.g. direct nest view, no nest view) of a nest from the nearest cuckoo perch^{18,33,34}, while the link between the hosts' acoustic signals and the ability of brood parasitic cuculids to find suitable host nests has been largely neglected. To the best of our knowledge, only one study was conducted regarding host vocalization and the nest searching strategy of common cuckoos, which found that the conspicuous and easily recognizable song of the male great reed warbler was not a good cue for a female cuckoo when searching for a preferred host's nest¹⁵.

The host's alarm calls, however, are uttered usually close to the nest and could offer important information to a cuckoo regarding the nest's location. In a study conducted on blackbirds *Turdus merula*, the focal pairs mounted intensive antipredator responses towards magpies *Pica pica* in the close vicinity of their nest (i.e. 1.5 m), but not in cases when the predator was far from the nest (i.e. 6–7 m)³⁵. Likewise, willow flycatchers were more likely to defend their nests by chasing away female cowbirds and non-cowbird species when the intruders were close to the nest (i.e. < 2 m), compared to trials when the intruder was not in the close vicinity of the nest (i.e. 2–10 m)¹⁶. A similar response was also found in three *Acrocephalus* species, which exhibited intensive mobbing and produced alarm calls when their potential predators (i.e. snake, stoat *Mustela erminea*, and marsh harrier *Circus aeruginosus*) were closer to their nests (i.e. 1 m) than in cases when the mounts were placed at a distance of 5 m³⁶. Thus, we hypothesize that cuckoos may eavesdrop on host alarm calls to identify the location of their nests, or even elicit them while actively searching for nests if host density is low^{34,37}. Recognizing the hosts' alarming acoustic signals might also enhance the success rate of finding well-hidden nests in inaccessible habitats with tall or dense vegetation (e.g. reed beds). One anecdotal observation from 1930 by Jussi Seppä, a Finnish ornithologist, suggests that common cuckoos use alarm calls to locate suitable host nests much as in the popular children's game 'hot and cold': the closer a cuckoo gets to the correct location of the host's nest, the more intensive an alarm call will be¹⁹. Alarm calls might therefore serve as reliable cues for cuckoos in the identification of preferred host nest's exact location.

To test this hypothesis, we carried out a correlative study and performed two experiments to assess the role of alarm calls uttered by the host and intercepted by the common cuckoo in the nest searching process. Previous studies performed on common cuckoos emphasized on the importance of visual cues such as nest size or the visibility of the nest from the nearest perching site that can be used by the brood parasite to locate the hosts' nest^{18,32,33}. To further corroborate the importance of host alarm calls in the nest searching process of the common cuckoo, we tested how visible the host nests were from the air and from cuckoo perching sites, by performing unmanned aerial vehicle (i.e. drone) flights above parasitized and unparasitized nests.

Based on this hypothesis, we predicted that female cuckoos would respond positively to alarming great reed warblers, seemingly the only host species in our study area^{38,39}, by approaching alarming hosts (Experiment 1) or the loudspeaker used for playing back host alarm calls (Experiment 2). Although it is expected that only female common cuckoos search for host nests⁴⁰, some cooperation could exist between the sexes during nest searching. For example, some authors suggested that male cuckoos might play a role in nest searching similarly to other brood parasitic species (e.g. greater spotted cuckoos *Clamator glandarius* and Asian koels *Eudynamis scolopacea*), where males may provoke hosts, distracting them from the nest while the female lays her egg(s)⁴¹.

Beside the two experiments, we devised a correlative study to test if the potential interest of the cuckoos towards the host's alarm calls would lead to an increase in parasitic attempts which would translate into higher parasitism rates in the hosts. Here, we predicted that great reed warblers which mob potential nest predators (i.e. observers perceived as nest predators^{42,43}) or brood parasites, unwittingly advertise the proximity of their nests and thus increase their odds of being parasitized.

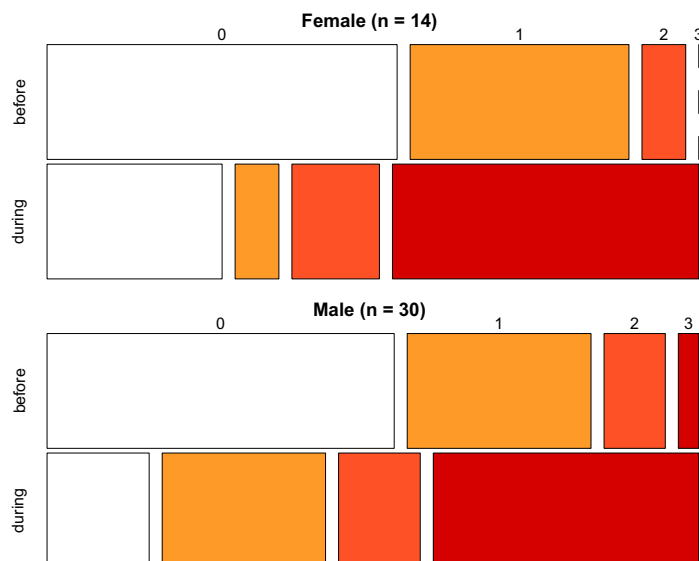


Figure 1. Mosaic plot showing that both female ($n = 14$) and male ($n = 30$) common cuckoos exhibited intensive responses during the 2 minutes when their host, the great reed warbler, engaged in nest defense activities, compared to the 2 minutes prior to the reed warblers alarming. Both female and male cuckoo responses were scored on the following scale: 0 – no response; 1 – typical female or male call within 100 m; 2 – flying towards the alarming great reed warblers; 3 – flying towards the alarming great reed warblers and vocalizing.

Also, to estimate the chances of locating host nests based on direct visual cues as suggested by previous studies^{18,32,33}, we performed drone flights from the nearest perching site to selected nests, above the nests in mid-air and parallel to the nests on the inner side of the reed bed (i.e. above the water) and measured nest site characteristics (i.e. nest size, distance from reed edges, and vegetation cover)¹⁸. Here, we predicted that parasitized great reed warbler nests were more visible to human observers on the aerial footage than the unparasitized nests, that are expected to be well concealed in the dense reed. Furthermore, in accordance with a previous study conducted in our study area¹⁸, we predicted that nests built high above the water surface and with a larger volume are more often parasitized, while nests built further from the inner or outer edge of the reed bed, which have a high vegetation above the cup and are further from cuckoo perches are less likely to be parasitized. We also predicted that host nests that are directly visible from the nearest cuckoo perch are more often parasitized than those that are not visible from the perch.

Results

Experiment 1: Cuckoos respond to alarming hosts. To test if common cuckoos were attracted by the alarm calls and mobbing displays (i.e. perching on the top of the reed when alarming and jumping between reed stems) of their hosts, we elicited mobbing reactions from the great reed warblers at their nests, using playback recordings of conspecific alarm calls and a 3D printed cuckoo decoy. During the 51 experimental trials, we recorded 14 cases when a female cuckoo was present and 30 cases when at least one male cuckoo was present. Both male and female cuckoo responses were stronger during the 2 minutes of playback when the great reed warblers were alarming than in the 2-minute pre-alarming period (Fig. 1; females: Fisher's exact test: $p = 0.004$; males: Fisher's exact test: $p < 0.001$). We note here that most cuckoos typically flew closer to the mobbing hosts and vocalized, while one male even hovered above the experimental setup for a prolonged period. The number of birds in each response category for both sexes is presented in Table S2 of the Supplementary Material.

Experiment 2: Cuckoos respond to alarm call playbacks. To remove the effect of the host's visual displays during mobbing, we tested the response of common cuckoos to playbacks of the host's alarm call and the calls of a neutral control species, the Eurasian collared dove *Streptopelia decaocto*. Female cuckoos approached the speaker more often during the experimental playback trials than during control trials (Fig. 2; 5/16 positive responses in experimental trials and 0/16 in control trials, i.e. no response; Fisher's exact test: $p = 0.021$). One of the females exhibited a weaker response to the experimental playback compared to the other females, but the statistical results were similar if we treated this response as a neutral reaction (i.e. 4 positive and 12 neutral responses to the experimental playback; Fisher's exact test: $p = 0.043$). The same result was found for male cuckoos (7/16 positive responses in experimental trials and 1/16 response in controls trials; Fisher's exact test: $p = 0.018$).

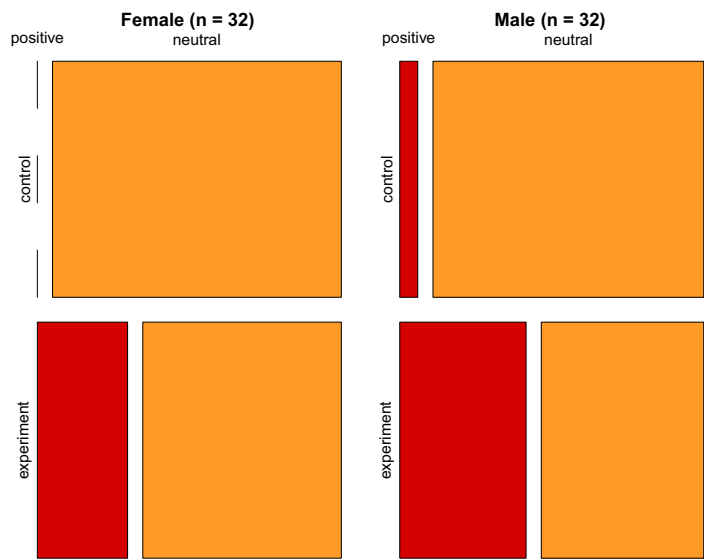


Figure 2. Mosaic plot showing that both female ($n = 32$) and male ($n = 32$) common cuckoos responded positively (i.e. flew closer to the playback device compared to the position where they were initially observed) to the alarm call of great reed warblers by approaching the loudspeaker, compared to the trials when the calls of collared doves were played as a control treatment.

Fixed effects	Estimate	SE	z value	Wald χ^2	df	p	Variance
(Intercept)	−10.504	1.342	−7.823	61.197	1	<0.001	
Host alarm call	2.748	1.222	2.248	5.054	1	0.024	
Time of nest visit	−0.862	0.457	−1.885	3.551	1	0.059	
Random effects							
Site: Nest identity							428.600
Observer identity							0.000

Table 1. Results of the minimal adequate model showing that alarming great reed warblers were parasitized more often by common cuckoos than conspecifics which were less keen to alarm human observers during regular nest visits. Values for the fixed factor ‘host alarm call’ indicate the difference in the probability of brood parasitism of alarming hosts compared to non-alarming great reed warblers, while ‘time of nest visit’ is a continuous variable, standardized with Z-transformation to mean = 0 and SD = 1, showing the effect of the time of the nest visit on the probability of parasitism. The full model from which the minimal adequate model was derived is presented in Table S1 of the Supplementary Material.

Correlative study: Alarming hosts experience higher parasitism rate. To assess the risk great reed warblers expose themselves to by alarming, we quantified parasitism rates and the presence of alarm calls during regular nest visits. The probability of brood parasitism correlated with the presence of host alarm calls directed against human observers. Great reed warblers that uttered alarm calls during regular nest visits encountered a higher probability of parasitism than conspecifics less prone to alarm (Table 1, Fig. 3) and time of the nest visit had a near significant negative effect (Table 1). Laying stage (i.e. first 3 days of laying or second 3 days of laying), and the interactions of alarm call presence with time of nest visit, and alarm call presence with laying stage had no effect on the probability of parasitism (full model presented in Table S1 of the Supplementary Material).

Drone flights. We performed drone flights to test if visual cues alone are sufficient for the common cuckoo to locate the hosts’ nest ($n = 16$). Contrary to previous results^{26,27}, parasitized nests were not more visible than unparasitized nests (0/8 parasitized nests and 1/8 unparasitized nests were visible during the drone flights; Fisher’s test: $p = 1.000$), but nests closer to the channel’s bank were more likely to be parasitized than nests that were built further from the bank (Wilcoxon rank sum test: $W = 50.5$, $p = 0.025$). Other nest site characteristics (i.e. nest volume, nest distance from the water, nest height above the water, height of the vegetation above the nest and distance of the nearest cuckoo perch) did not differ significantly between parasitized and unparasitized nests (Table S3 in the Supplementary Material). In general, host nests were well-concealed in the dense reed beds that are characteristic to our study area: only one great reed warbler nest out of 16 was visible on the drone footage.

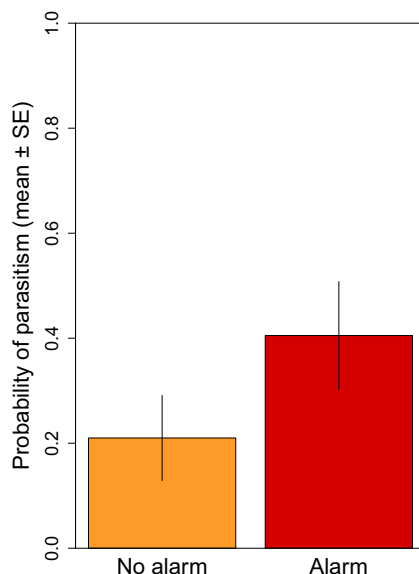


Figure 3. Barplot showing that alarming great reed warblers experience higher probability of brood parasitism by common cuckoos than non-alarming conspecifics. Values indicating the probability of brood parasitism are predicted based on the minimal adequate model (see Table 1) from the correlative study (see main text). Mean predicted values \pm standard error (SE) are shown.

This later nest was unparasitized by cuckoos, despite being built in a narrow reed bed, and being classified in the field as directly visible from the nearest perch.

Discussion

In this study we tested if brood parasites rely on the alarm calls produced by their hosts, rather than on visual nest searching^{18,33,34} when seeking for nests to lay their eggs in¹⁹. We found that the nests of the great reed warbler, the host of the brood parasitic common cuckoo in our study area, are hardly visible in the dense reed bed from the nearest cuckoo perches and from mid-air. In contrary, the alarm calls uttered by the hosts are likely to be good nest location cues for the brood parasites, since both female and male common cuckoos exhibited interest towards alarming hosts and alarm call playbacks. Moreover, hosts that were more likely to alarm when approached by human observers during nest visits experienced a higher probability of parasitism.

To the best of our knowledge, our study is the first to provide evidence that brood parasites exploit the alarm calls of their hosts when searching for nests to parasitize, and vigilant hosts expose themselves to the costs of revealing the location of their nests during its defense. Although, if the brood parasites find a host nest it would not necessarily mean that they will parasitize it, the results of our correlative study highlight that the nests of great reed warblers that were prone to alarm at intruders were much more likely to be parasitized by the common cuckoos. An alternative explanation of the results of our correlative study is that the common cuckoos attempted or succeeded to parasitize the great reed warblers in the early stages of the breeding period, which increased the vigilance of the hosts, and thus, increased the probability of alarming at potential threats (i.e. observers during nest visits).

Both female and male common cuckoos exhibited strong interest in host alarm calls by flying closer to the experimental setup and vocalizing, when hosts were present and performed visual displays of aggression (Experiment 1). This interest from the cuckoos was similar also during the playback trials when there was no great reed warbler in sight (Experiment 2), underlining the importance of audio signals compared to visual stimuli of the host. The hosts' nest defense behavior may also be triggered by male brood parasites, which pose no direct risk for the host, but can provide valuable information for female cuckoos lurking nearby^{41,44}.

There are no studies regarding the post-copulatory role played by common cuckoo males in the breeding process. A possible explanation for the unexpected interest of cuckoo males towards the alarm calls of the host parasitized by the cuckoo females might be related to the mating success of males. In a pivotal study regarding the mating system of the common cuckoo performed in Japan, researchers have found that in a study site of 7 km², the vast majority of females mated with only one male, while most males mated only with one or two females⁴⁵. Even more importantly, 65% of the cuckoo males and 50% of the females were not assigned as parents to any of the 136 nestlings sampled. This suggests that some kind of cooperation during the nest searching process between the top female and male cuckoos would result in direct fitness advantages for the individuals with high mating success, as it is in the case other cooperatively breeding brood parasites^{3,41}.

Frontline defenses are the most widespread host counter-adaptations against brood parasites⁵: hosts that invest in anti-parasitic defenses (e.g. secretive breeding behavior, well concealed nest) lower their chance of being

parasitized, or at least save time and energy to restart the breeding attempt if they are parasitized⁴³. These nest defense behaviors usually occur in the close vicinity of the nests^{16,35,36}, and are employed against nest predators or brood parasites, sometimes resulting in adverse effects. For example, one recently published study performed on reed warblers and yellow warblers *Setophaga petechia* found that intensive nest defense behavior lowered the risk of brood parasitism only in the case of the reed warblers, but also attracted the attention of predators¹⁴. Nest defense might also act as a social cue for conspecifics, stimulating them to mount a collective response (e.g. collective mobbing) against the predator or brood parasite, further enhancing its adaptive value^{7,42,46}.

Only a few studies dealt with the potential effect of a conspicuous behavior of the host regarding the risk of brood parasitism. These studies found that some conspicuous behaviors, as the calls uttered near the nest, intra-pair signaling or nest-building activity are cues used by brown-headed cowbirds to identify host nests^{9,15–17}. Contrary to these, studies performed on common cuckoos and its hosts found that the conspicuous singing of male great reed warblers is neither a reliable nest location cue for the common cuckoo¹³, nor a reliable proxy of the future parental investment of the host¹². However, neither of these studies examined the effect of alarm calls on the probability of brood parasitism.

If host alarm calls are exploited by brood parasites during nest searching, nest defense as a behavior is likely to survive only if it is adaptive in a different context, rendering the overall benefits higher than its costs. Nest defense can be benefic if it is directed towards a range of enemies: snakes, small carnivores, avian predators posing a risk to the nest (e.g. jays *Garrulus glandarius*, magpies, marsh harriers *Circus aeruginosus*) or to the adult birds (e.g. sparrowhawks *Accipiter nisus*), and brood parasites^{14,36,42}. Hosts' alarm calls act as a social cue and attract the attention of conspecifics from the adjacent territories⁴⁷, which results in a more effective mobbing of the brood parasite⁷ and might increase the probability that a host ejects a parasitic egg^{8,46}. Albeit, alarming might be useful for keeping conspecifics or predators at bay¹⁴, if the alarm calls are uttered near the nest^{5,16,35}, alarming might impose the cost of revealing the whereabouts of the host's nest to eavesdroppers. It is also worth noting, that at sites where the rate of brood parasitism is high³⁹, the aggressive behavior towards the cuckoos can be effective only in some cases, the focal nest being successfully parasitized by the brood parasite (or even by multiple brood parasitic individuals) after several parasitism attempts.

An alternative explanation for the persistence of a defensive trait (i.e. alarm calls) acting in a seemingly maladaptive manner (i.e. increasing the likelihood of brood-parasitism) might arise from the breeding ecology of the great reed warbler. The great reed warbler is a typical 'edge-species' which prefers to breed on the edge of the reed beds and in the narrow reed-stripes found alongside irrigation channels^{47,48}. These high-quality habitats, despite being quickly occupied by early-arriving, large-winged and presumably higher-quality males, are characterized by higher rates of brood parasitism^{49–51}. Therefore, irrigation channels function as ecological traps^{50,51} and habitats for sink populations, dependent on the influx of naïve individuals from the habitats with low parasitism rates⁵². These two factors, coupled with the fact that the irrigation channels are artificial habitats created in the past 100 years, prevent natural selection to root out alarm calls, despite the negative fitness consequences suggested by our results.

One implication of our results resides in the potential role of alarm calls in the recognition of the host species by the brood parasites. Passerine nestlings recognize the species-specific alarm calls of their parents⁵³, but for brood parasitic nestlings the imprinting on the alarm calls of their foster parents might be important later in life, when they return to the breeding area as adults. This is supported by a recent study showing that common cuckoos imprinted on their hosts as nestlings, but not on the habitat or nest site where they were raised^{31,54}. Some commonly used hosts of the cuckoo live in dense habitats, have cryptic behavior or live in sympatry with other similar species. Nevertheless, female cuckoos tend to recognize and parasitize the host species of their own genes^{23,24}, and parasitize the nest of alternative hosts only if the nests of their main host are in limited supply^{2,27}. Based on these findings, it is likely that cuckoos imprint on the alarm call of their foster parents during their nestling or fledgling stages, and they use the alarm calls later during their life to differentiate among hosts similar in appearance, like those belonging to the *Acrocephalus* or *Anthus* genus.

We conclude that common cuckoos can utilize their hosts' alarm calls when searching for host nests, which represents a novel aspect of the evolutionary arms race between avian brood parasites and their hosts. Common cuckoos eavesdrop on the alarm calls of their host, the great reed warbler, during nest searching, and great reed warblers that alarm more often face higher rates of parasitism. Thus, while alarming plays an important role in keeping nest predators at bay, it can be intercepted by brood parasites, leading to major fitness consequences for both hosts and parasites.

Methods

Study area. We performed the different parts of the present study near Apaj village, central Hungary (47.113° N, 19.087° E), between early May and mid-June in 2013, 2014 and 2018. This period coincides with the peak availability (i.e. number) of host nests and the highest rate of parasitism in our study area^{18,55}. Here, great reed warblers breed in 1–3 m wide reed beds located along irrigation channels, and experience an unusually high rate of cuckoo parasitism: nests are parasitized in a proportion of 50–70%³⁹, compared to the parasitism rates of several hosts ranging between 10–35% at other study sites across the distribution range of the common cuckoo^{2,33,56,57}.

All of the work reported here complied with the Hungarian laws and was conducted under the auspices of research permit No. PE/KTF/17190-3/2015 issued by the Middle-Danube-Valley Inspectorate for Environmental Protection, Nature Conservation and Water Management, Budapest.

Experiment 1: Cuckoos respond to alarming hosts. We performed an experiment at great reed warbler nests to test if common cuckoos are attracted to the audio (i.e. alarm call) and visual components (i.e. mobbing) of their host's nest defense behavior. To test our hypothesis, we scored the activity of female and male cuckoos at active great reed warbler nests ($n = 51$) during 2 minutes of silence (i.e. pre-alarming period) and 2 minutes of elicited mobbing by the hosts. We scored the activity of the cuckoos on a 0–3 scale: female and male cuckoo



Figure 4. The 3D printed common cuckoo decoy used in our study. The decoy was painted with acrylic paint and mounted on a pole supplied with a noiseless electric motor to stimulate horizontal movement.

exhibited no reaction (0), produced their characteristic female bubbling call or male call on a perch closer than 100 m (1), exhibited stronger responses by flying silently towards the focal great reed warblers (2), or by flying towards the great reed warblers while producing intense sex-specific calls (3). We performed the trials at great reed warbler nests in the order of which these nests were found, but kept a 500 m distance between nests involved in consecutive trials to minimize the probability of testing the same cuckoos. To elicit mobbing and intense alarming from the focal great reed warbler pair (i.e. intense movement and alarm calls), ensuring both visual and audio stimuli for the cuckoos, we played previously recorded great reed warbler alarm calls using a loudspeaker and a 3D cuckoo decoy bird. We placed the 3D cuckoo decoy at a distance of ca. 0.5 m from the host nest, well concealed by the reed and out of site for any cuckoos, to avoid having a reaction to the 3D decoy instead of the host's alarm calls and visual displays. This setup ensured the lack of pseudo-replication^{58,59}, since the pre-recorded alarm calls ($n = 10$) coupled with the alarm calls of the focal great reed warbler pairs were unique for all trials. The alarm calls were played on a JBL Xtreme loudspeaker (40 W; at about 90 dB, measured by Volcraft SL-100 sound level meter from 1 m distance), while the 3D life-size cuckoo model ($313 \times 92 \times 200$ mm) supplied by 3D QuickPrinting UK was printed with an Ultimaker 2+ 3D printer using transparent Ultimaker PLA filament. We painted the transparent 3D cuckoo with acrylics to resemble a cuckoo, and fitted it on a standing support supplied with a silent robotic motor to stimulate the display movements of cuckoos (Fig. 4., additional video in the Supplementary Material). Experiments were carried out by two observers (AM and MB).

Experiment 2: Cuckoos respond to alarm call playbacks. During our first experiment, perching common cuckoos were exposed to both the sound and sight of their hosts in the vicinity of the nest, but the informational value of the two types of stimuli was unknown. To test if cuckoos truly responded to the alarm calls uttered by their hosts and not to their aggressive displays, we carried out a second experiment. In this second experiment, we played host alarm calls ($n = 16$) and collared dove calls as a neutral control ($n = 16$) in a random order, at 32 different sites where female cuckoos were heard (i.e. the presence of individuals was confirmed). Cuckoos were observed usually at distance of 40–50 m, and females were identified based on their typical bubbling call⁴⁰. The playback began within 2 minutes after the female cuckoo was heard, lasted for 2 minutes and was followed by a 2 minute post-playback period, while the movements of both female and male cuckoos were quantified. A response was considered positive if a focal bird approached the playback device compared to the distance it was initially observed, and neutral if it did not react or increased their distance (i.e. flew away) from the playback device compared to their initial position. Both female and male cuckoos are typically territorial in the breeding season, and previous studies with VHS and GPS telemetry typically revealed less than 1 km long, partly overlapping cuckoo territories along the channels in our study area^{44,60,61}. Thus, in order to reduce potential pseudo-replication^{58,59} in our data, we used every sound file only once (both experimental and control files) at sites at least 2 km apart. The great reed warbler alarm calls ($n = 16$) and collared dove calls ($n = 16$) were recorded during the breeding season in 2018 and each of them was played only once, using a JBL Xtreme loudspeaker (40 W), at about 90 dB. All trials were performed by CM.

Correlative study: Alarming hosts experience higher parasitism rates. We collected data on the breeding performance and alarm calls of great reed warblers, a facultatively polygynous^{42,62} host widely used by cuckoos across several parts of Europe^{21,22,39,63,64}.

We visited great reed warbler nests ($n = 170$) in the egg-laying stage on multiple occasions ($n = 330$ visits in total, mean number of visits \pm SD per nest: 1.94 ± 1.10) and quantified clutch initiation date (i.e. day 0 = laying date of the first egg), clutch size (i.e. sum of great reed warbler and cuckoo eggs at the time of each visit), and the reaction (i.e. alarm calls) of hosts to the human observer, as an indicator of nest defense intensity. Great reed warblers respond aggressively to human intrusion similarly to the intrusion of cuckoos or nest predators^{42,56} and produce a typical alarming call. Alarming behavior of hosts during nest visits was assessed by four observers on a

binary scale (0: no alarm call, 1: at least one great reed warbler alarmed at the nest). All four observers (AM, MB and two field assistants) were trained to recognize the great reed warbler alarm calls prior to the observations and all nest visits were performed in the morning hours, between 6:00 AM and 11:00 AM, covering the peak activity period of great reed warblers¹⁶.

Drone flights. Previous studies^{18,32,33} show that cuckoos perch on trees serving as vantage points, when searching for suitable host nests. To check whether nests are visible from such vantage points or from above the reed, we conducted drone flights above 8 parasitized and 8 unparasitized randomly chosen focal nests from one of the channels in our study site. Flights were carried out with a DJI Phantom 4 drone set to record aerial videos at 4k resolution and 25 fps, based on the following protocol, simulating cuckoo flights: first, the drone ascended to 6–8 m (i.e. depending on the height of the closest cuckoo vantage point), then performed a slow descent approaching the focal nest, until reaching the area above the nest (i.e. 1 m above the reed bed). After this operation, the drone descended to the inner side of the reed bed and hovered above the water in order to get a clear view of the reed bed from the inner side. While performing the drone flights, the exact location of the nest was pointed out with a 1 m long ruler by one observer (AM or MB) standing in the reed near the focal nest.

Nest volume (expressed in cm³ as the volume of a cylinder with the height equal to the nest's height and its base diameter equal to width of the nest), and the following nest site characteristics were measured similarly as described in a previous study¹⁸: nest height above the water surface (cm), nest distance from the bank and from open water (cm), vegetation height above nest (cm) and nest visibility from the nearest cuckoo perch (scored on a three-level factor: direct nest view, indirect nest view or no nest view). The recorded videos were screened for nests using VLC media player (v. 3.0.8. Vetinari) at normal speed by an assistant person with no previous knowledge about the aims of the study to ensure the blind evaluation of the recordings, and subsequently by the first author of the study, rendering the same results.

Statistical analyses. We hypothesized that cuckoos would exhibit a stronger response during the alarming period compared to the pre-alarming period (see above). Thus, the data from Experiment 1 were analyzed using one-tailed Fisher's exact test⁶³. Prior to the analysis, we excluded all trials in which no cuckoo responses were recorded neither before, nor during the alarming period.

Data from Experiment 2 were analyzed with one-tailed Fisher's exact test⁶⁵, due to our hypotheses that cuckoos would mount a stronger response to the alarm calls compared to the control calls.

Data gathered during our correlative study were analyzed using a generalized linear mixed-effect model (GLMM) with binomial error distribution⁶⁶. Host alarm calls might have higher informational value for the brood parasite in the first days of laying of the host, which could ensure brood parasites that their progeny would hatch before those of the hosts⁶⁷ or of the concurrent cuckoos, as it is the case at our study site, where multiple parasitism is common³⁹. For the Eurasian reed warblers, a close relative of the great reed warbler, the first three days of laying clearly represents a high-risk period, but later on in the laying period the risk is reduced². Therefore, we divided the egg-laying period into two subperiods forming two groups: "high risk" (i.e. first 3 days of laying) and "low risk" (i.e. the second 3 days of laying) groups.

We entered the occurrence of brood parasitism of individual nests at every visit as a binary response variable (0: no cuckoo eggs in the nest, 1: at least one cuckoo egg in the nest) in the model. The presence of absence of host alarm calls (factor with two levels), risk of parasitism (factor with two levels), time of visit within the day (expressed in number of minutes elapsed from midnight, standardized with Z-transformation to mean = 0 and SD = 1 to improve model convergence), and the second-order interactions between the presence of alarm call and the variables listed above were included as fixed terms in the model. Observer ID and nest ID nested under site ID (i.e. different irrigation channels hosting the great reed warblers) were all included as random terms. After constructing the full model containing all the predictors listed above and their interactions, we simplified the model utilizing a stepwise backwards elimination procedure based on the significance level ($p < 0.050$) of the predictors, in each step dropping the predictor with the highest p value, until reaching the minimal adequate model containing only significant or marginally significant ($p < 0.100$) effects. We tested the full and the minimum adequate model for multicollinearity between predictors using the 'vif.mer' function^{66,68}, which calculates the variance inflation factor (VIF) for each predictor separately. Since VIF was less than 2.09 for all variables for the full model and 1.12 for the variables in the minimal model, we concluded that there was no multicollinearity between predictors. The GLMM were performed using the function 'glmer', from the R package 'lme4'⁶⁹, using Laplace approximation for parameter estimation⁷⁰ and 'bobyqa' algorithm for model optimization⁷¹. Data handling, date and time conversions were performed using the packages 'chron'⁷² and 'doBy'⁷³ in the R statistical environment⁶⁵.

The numerical data collected during the drone study, namely the nest site characteristics (i.e. nest volume, nest distance from the bank and from the water, nest height of above the water surface, height of the reed above the nest cup and the distance to the nearest cuckoo perch) of parasitized and unparasitized nests were analyzed using one-tailed Wilcoxon rank sum tests⁷⁴ in accordance with the predicted direction for each variable presented in the Introduction and summarised in Table S4 of the Supplementary Material, while the nest view expressed in a three-level factor (i.e. direct nest view, indirect nest view and no nest view) was analyzed using one-tailed Fisher's exact test⁶⁵.

All statistical analyses were conducted in RStudio version 1.0.153⁷¹, running R version 3.3.3⁷⁵.

Data availability

All data from this study are included in this publication and its Supplementary Material.

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References

- Soler, M. (ed.) *Avian Brood Parasitism: Behaviour, Ecology, Evolution and Coevolution* (Springer International Publishing, 2017).
- Davies, N. B. *Cuckoo: Cheating by Nature* (Bloomsbury Publishing Plc., 2015).
- Davies, N. B. *Cuckoos Cowbirds and Other Cheats* (Poyser, 2000).
- Soler, M. Brood Parasitism in Birds: A Coevolutionary Point of View in *Avian Brood Parasitism: Behaviour, Ecology, Evolution and Coevolution* (ed. Soler, M.) 1–19 (Springer International Publishing, 2017).
- Feeney, W. E., Welbergen, J. A. & Langmore, N. E. The frontline of avian brood parasite—host coevolution. *Anim. Behav.* **84**, 3–12 (2012).
- Davies, N. B. & Welbergen, J. A. Social transmission of a host defense against cuckoo parasitism. *Science* **324**, 1318–1320 (2009).
- Campobello, D. & Sealy, S. G. Use of social over personal information enhances nest defense against avian brood parasitism. *Behav. Ecol.* **22**, 422–428 (2011).
- Davies, N. B. & Brooke, M. L. Cuckoos versus reed warblers: Adaptations and counteradaptations. *Anim. Behav.* **36**, 262–284 (1988).
- Banks, A. J. & Martin, T. E. Host activity and the risk of nest parasitism by brown-headed cowbirds. *Behav. Ecol.* **12**, 31–40 (2001).
- Parejo, D. & Avilés, J. M. Do avian brood parasites eavesdrop on heterospecific sexual signals revealing host quality? A review of the evidence. *Anim. Cogn.* **10**, 81–88 (2007).
- Fiorini, V. D., Tuelo, D. T. & Reboreda, J. C. Host behaviour and nest-site characteristics affect the likelihood of brood parasitism by shiny cowbirds on chalkbrowed mockingbirds. *Behaviour* **146**, 1387–1403 (2009).
- Avilés, J. M., Moskát, C., Bán, M., Hargitai, R. & Parejo, D. Common cuckoos *Cuculus canorus* do not rely on indicators of parental abilities when searching for host nests: The importance of host defenses. *Auk* **126**, 431–438 (2009).
- Capek, M. et al. Great reed warbler singing behavior and conspicuous song structures are not nest-location cues for the common cuckoo. *J. Ornithol.* **158**, 925–933 (2017).
- Campobello, D. & Sealy, S. G. Evolutionary significance of antiparasite, antipredator and learning phenotypes of avian nest defence. *Sci. Rep.* **8**, 10569 (2018).
- Robinson, W. D. & Robinson, T. R. Is host activity necessary to elicit brood parasitism by cowbirds. *Ethol. Ecol. Evol.* **13**, 161–171 (2001).
- Uyehara, J. C. & Narins, P. M. Nest defense by willow flycatchers to brood-parasitic intruders. *Condor* **97**, 361–368 (1995).
- Clotfelter, E. D. What cues do brown-headed cowbirds use to locate red-winged blackbird host nests? *Anim. Behav.* **55**, 1181–1189 (1998).
- Moskát, C. & Honza, M. Effect of nest and nest site characteristics on the risk of cuckoo *Cuculus canorus* parasitism in the great reed warbler *Acrocephalus arundinaceus*. *Ecography* **23**, 335–341 (2000).
- Seppä, J. The cuckoo's ability to find a nest where it can lay an egg. *Ornis Fennica* **46**, 78–80 (1969).
- Erritzoe, J., Mann, C. E., Brammer, F., Fuller, R. A. *Cuckoos of the World* (Helm, 2012).
- Moksnes, A. & Roskaft, E. Egg-morphs and host preference in the common cuckoo *Cuculus canorus*: an analysis of cuckoo and host eggs from European museum collections. *J. Zool.* **236**, 625–648 (1995).
- Stokke, B. G. et al. Characteristics determining host suitability for a generalist parasite. *Sci. Rep.* **8**, 6285 (2018).
- Skjelseth, S. et al. Parentage and host preference in the common cuckoo *Cuculus canorus*. *J. Avian Biol.* **35**, 21–24 (2004).
- Honza, M. et al. Behaviour of female common cuckoos *Cuculus canorus*, in the vicinity of host nests before and during egg laying: A radiotelemetry study. *Anim. Behav.* **64**, 861–868 (2002).
- Kleven, O. et al. Breeding success of common cuckoos *Cuculus canorus* parasitising four sympatric species of *Acrocephalus* warblers. *J. Avian Biol.* **35**, 394–398 (2004).
- Sklepowitz, B. & Halupka, L. The use of sympatric reed warblers *Acrocephalus scirpaceus* and marsh warblers *Acrocephalus palustris* as breeding hosts: parasitism rates and breeding success of common cuckoos *Cuculus canorus*. *Acta Ornithol.* **44**, 177–184 (2009).
- Chance, E. P. *The Truth about the Cuckoo* (Country Life, 1940).
- Davies, N. B. & Brooke, M. L. An experimental study of coevolution between the cuckoo *Cuculus canorus*, and its hosts. I. Host egg discrimination. *J. Anim. Ecol.* **58**, 207–224 (1989).
- Davies, N. B. & Brooke, M. L. An experimental study of coevolution between the cuckoo *Cuculus canorus*, and its hosts. II. Host egg markings, chick discrimination, and general discussion. *J. Anim. Ecol.* **58**, 225–236 (1989).
- Manna, T., Moskát, C. & Hauber, M. E. Cognitive decision rules for egg rejection in *Avian Brood Parasitism: Behaviour, Ecology, Evolution and Coevolution* (ed. Soler, M.) 437–448 (Springer International Publishing, 2017).
- Yang, C., Wang, L., Liang, W. & Möller, A. P. How cuckoos find and choose host nests for parasitism. *Behav. Ecol.* **28**, 859–865 (2017).
- Öien, I. J., Honza, M., Moksnes, A. & Roskaft, E. The risk of parasitism in relation to the distance from reed warbler nests to cuckoo perches. *J. Anim. Ecol.* **65**, 147–153 (1996).
- Antonov, A., Stokke, B. G., Moksnes, A. & Roskaft, E. Factors influencing the risk of common cuckoo *Cuculus canorus* parasitism on marsh warblers *Acrocephalus palustris*. *J. Avian Biol.* **38**, 390–393 (2007).
- Jelínek, V., Procházka, P., Požgayová, M. & Honza, M. Common cuckoos *Cuculus canorus* change their nest-searching strategy according to the number of available host nests. *Ibis* **156**, 189–197 (2014).
- Kryštofková, M., Haas, M. & Exnerová, A. Nest defense in blackbirds *Turdus merula*: effect of predator distance and parental sex. *Acta Ornithol.* **46**, 55–63 (2011).
- Kleindorfer, S., Fessl, B. & Hoi, H. Avian nest defence behaviour: Assessment in relation to predator distance and type and nest height. *Anim. Behav.* **69**, 307–313 (2005).
- Ma, L. et al. Costs of breeding far away from neighbors: Isolated host nests are more vulnerable to cuckoo parasitism. *Behav. Process.* **157**, 327–332 (2018).
- Moskát, C. & Honza, M. European cuckoo *Cuculus canorus* parasitism and host's rejection behaviour in a heavily parasitized great reed warbler *Acrocephalus arundinaceus* population. *Ibis* **144**, 614–622 (2002).
- Zölei, A., Bán, M. & Moskát, C. No change in common cuckoo *Cuculus canorus* parasitism and great reed warblers' *Acrocephalus arundinaceus* egg rejection after seven decades. *J. Avian Biol.* **46**, 570–576 (2015).
- Wyllie, I. *The Cuckoo* (Universe Books, 1981).
- Mikulica, O., Grim, T., Schulze-Hagen, K., Stokke, B. G. *The Cuckoo: The Uninvited Guest* (Wild Nature Press, 2017).
- Trnka, A. & Prokop, P. Does social mating system influence nest defence behaviour in great reed warbler *Acrocephalus arundinaceus* males? *Ethology* **116**, 1075–1083 (2010).
- Davies, N. B. Cuckoo adaptations: Trickery and tuning. *J. Zool.* **284**, 1–14 (2011).
- Moskát, C., Bán, M., Fülöp, A., Bereczki, J. & Hauber, M. E. Bimodal habitat use in brood parasitic common cuckoos *Cuculus canorus* revealed by GPS telemetry. *Auk* **136**, 1–12 (2019).
- Marchetti, K., Nakamura, H. & Gibbs, H. L. Host-race formation in the common cuckoo. *Science* **282**, 471–472 (1998).
- Bártol, I., Karcza, Z., Moskát, C., Roskaft, E. & Kisbenedek, T. Responses of great reed warblers *Acrocephalus arundinaceus* to experimental brood parasitism: the effects of a cuckoo *Cuculus canorus* dummy and egg mimicry. *J. Avian Biol.* **33**, 420–425 (2002).
- Báldi, A. Microclimate and vegetation edge effect in a reedbed in Hungary. *Biodivers. Conserv.* **8**, 1697–1706 (1999).
- Báldi, A. & Kisbenedek, T. Species-specific distribution of reed-nesting passerine birds across reed-bed edges: effect of spatial scale and edge type. *Acta Zool. Hung.* **45**, 97–114 (1999).

49. Mérő, T. O., Žuljević, A., Varga, K. & Lengyel, S. Wing size-related habitat selection by great reed warbler *Acrocephalus arundinaceus* males. *Auk* **133**, 205–2012 (2016).
50. Mérő, T. O., Žuljević, A., Varga, K. & Lengyel, S. Reed management influences philopatry to reed habitats in the great reed warbler *Acrocephalus arundinaceus*. *Condor* **120**, 94–105 (2018).
51. Mérő, T. O., Žuljević, A., Varga, K. & Lengyel, S. Habitat use and nesting success of the great reed warbler *Acrocephalus arundinaceus* in different reed habitats in Serbia. *Wilson J. Ornithol.* **127**, 477–485 (2015).
52. Barabás, L., Gilicze, B., Takasu, F. & Moskát, C. Survival and anti-parasite defense in a host metapopulation under heavy brood parasitism: a source-sink dynamic model. *J. Ethol.* **22**, 143–151 (2004).
53. Davies, N. B., Madden, J. R. & Butchart, S. H. M. Learning fine-tunes a specific response of nestlings to the parental alarm calls of their own species. *P. Roy. Soc. B. Biol. Sci.* **271**, 2297–2304 (2004).
54. Yang, C., Liang, W. & Möller, A. P. Do cuckoos imprint on hosts, micro-habitats, or nest sites? Parasitism preferences in the common cuckoo *Cuculus canorus*. *Behav. Ecol. Sociobiol.* **72**, 126–130 (2018).
55. Moskát, C., Barta, Z., Hauber, M. E. & Honza, M. High synchrony of egg laying in common cuckoos *Cuculus canorus* and their great reed warbler *Acrocephalus arundinaceus* hosts. *Ethol. Ecol. Evol.* **18**, 159–167 (2006).
56. Požgayová, M., Procházka, P. & Honza, M. Sex-specific defence behaviour against brood parasitism in a host with female-only incubation. *Behav. Process.* **81**, 34–38 (2009).
57. Yang, C. *et al.* Geographic variation in parasitism rates of two sympatric cuckoo hosts in China. *Zool. Res.* **35**, 67–71 (2014).
58. Kroodsmas, D. E. Inappropriate experimental designs impede progress in bioacoustic research: A reply. *Anim. Behav.* **38**, 717–719 (1989).
59. Kroodsmas, D. E., Byers, B. E., Goodale, E., Johnson, S. & Liu, W. C. Pseudoreplication in playback experiments revisited a decade later. *Anim. Behav.* **61**, 1029–1033 (2001).
60. Moskát, C., Elek, Z., Bán, M., Geltsch, N. & Hauber, M. E. Can common cuckoos discriminate between neighbours and strangers by their calls? *Anim. Behav.* **126**, 253–260 (2017).
61. Moskát, C. *et al.* Are both notes of the common cuckoo's call necessary for familiarity recognition? *Behav. Process.* **157**, 685–690 (2018).
62. Požgayová, M., Procházka, P. & Honza, M. Is shared male assistance with antiparasitic nest defence costly in the polygynous great reed warbler? *Anim. Behav.* **85**, 615–621 (2013).
63. Honza, M., Procházka, P., Šícha, V. & Požgayová, M. Nest defence in a cuckoo host: Great reed warblers risk themselves equally for their own and parasitic chicks. *Behaviour* **147**, 741–756 (2010).
64. Mérő, T. O. & Žuljević, A. The effects of reed density, surface and management on the probability of cuckoo *Cuculus canorus* parasitism on great reed warbler *Acrocephalus arundinaceus* nests. *Ethol. Ecol. Evol.* **31**, 98–104 (2019).
65. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, <https://www.R-project.org> (2017).
66. Chambers, J. M. Linear models in *Statistical Models in S* (eds Chambers, J. M., Hastie, T. J.) 95–138 (Chapman & Hall, 1992).
67. Geltsch, N., Bán, M., Hauber, M. E. & Moskát, C. When should common cuckoos *Cuculus canorus* lay their eggs in host nests? *Bird Study* **63**, 46–51 (2016).
68. Frank, A. F. R-hacks/mer-utils.R. GitHub, <https://github.com/aufrank/R-hacks/blob/master/mer-utils.R> (2014).
69. Bates, D., Mächler, M., Bolker, B. M. & Walker, S. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48 (2014).
70. Bolker, B. M. *et al.* Generalized linear mixed models: A practical guide for ecology and evolution. *Trends Ecol. Evol.* **24**, 127–35 (2009).
71. Fox, J., Weisberg, S. Mixed-effects models in R in *An R Companion to Applied Regression* 2nd Edition (eds Fox, J., Weisberg, S.) 1–54 (Sage Publications, 2011).
72. James, D., Hornik, K. Chron: chronological objects which can handle dates and times. R Core Team, <https://cran.r-project.org/web/packages/chron/index.html> (2017).
73. Hojsgaard, S., Halekoh, U. DoBy: groupwise statistics, LSmeans, linear contrasts, utilities, <http://people.math.aau.dk/~sorenh/software/doBy> (2016).
74. Hothorn, T., Hornik, K. ExactRankTests: exact distributions for rank and permutation tests, <https://cran.r-project.org/web/packages/exactRankTests/index.html> (2017).
75. RStudio Team. RStudio: Integrated development for R. *RStudio Inc*, <http://www.rstudio.com> (2016).

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Author contributions

A.M. and M.B. collected data for the correlative study, A.M., C.M. and M.B. conceived and performed the experiments with K.O. A.M. and C.M., analyzed the data with considerable input from A.F. A.M. wrote the manuscript with considerable input from all authors. All authors approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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10. Study II:

Female common cuckoo calls dampen the mobbing intensity of great reed warbler hosts

Marton, A., Fülöp, A., Bán, M., Hauber, M. E. & Moskát, C.

Ethology, 127, 286–293 (2021)

Abstract

To avoid mobbing attacks by their hosts during egg laying, some avian brood parasites have evolved traits to visually and/or acoustically resemble predator(s) of their hosts. Prior work established that reed warblers *Acrocephalus scirpaceus*, a small host species of the brood parasitic common cuckoo *Cuculus canorus*, delayed returning to the nest when confronted by either the calls of the female cuckoo or that of the predatory sparrowhawk *Accipiter nisus*. It remains less clear, however, whether female cuckoo calls also suppress the nest defences of larger and more aggressive hosts. Such hosts typically attack vigorously, and can even hurt the brood parasitic intruders, instead of fleeing in the face of danger. Here, we tested whether the female cuckoo calls dampen mobbing intensity in a much larger *Acrocephalus* host of the common cuckoo, the great reed warbler *A. arundinaceus*. We presented great reed warbler pairs with female common cuckoo models at their nests without and then with playing back the female-specific bubbling calls of the cuckoo. As controls, we tested the hosts' responses to harmless collared dove *Streptopelia decaocto* models, also without and then with the playbacks of dove calls. We found that the playback of female brood parasite calls reduced the aggression of hosts towards the cuckoo models as compared to model presentations without female calls, but we detected no such effect of the control calls with dove models. Our results revealed that female cuckoo calls effectively suppress the antiparasitic responses of great reed warbler hosts, which could aid parasites to approach the nest undiscovered and to evade the costly attacks of this large host. Therefore, the female call can be regarded as a general part of the cuckoo's trickery repertoire for successful parasitism.

RESEARCH ARTICLE

Female common cuckoo calls dampen the mobbing intensity of great reed warbler hosts

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Abstract

To avoid mobbing attacks by their hosts during egg laying, some avian brood parasites have evolved traits to visually and/or acoustically resemble predator(s) of their hosts. Prior work established that reed warblers (*Acrocephalus scirpaceus*), a small host species of the brood parasitic common cuckoo (*Cuculus canorus*), delayed returning to the nest when confronted by either the calls of the female cuckoo or that of the predatory sparrowhawk (*Accipiter nisus*). It remains less clear, however, whether female cuckoo calls also suppress the nest defences of larger and more aggressive hosts. Such hosts typically attack vigorously, and can even hurt the brood parasitic intruders, instead of fleeing in the face of danger. Here, we tested whether the female cuckoo calls dampen mobbing intensity in a much larger *Acrocephalus* host of the common cuckoo, the great reed warbler (*A. arundinaceus*). We presented great reed warbler pairs with female common cuckoo models at their nests without and then with playing back the female-specific bubbling calls of the cuckoo. As controls, we tested the hosts' responses to harmless collared dove (*Streptopelia decaocto*) models, also without and then with the playbacks of dove calls. We found that the playback of female brood parasite calls reduced the aggression of hosts towards the cuckoo models as compared to model presentations without female calls, but we detected no such effect of the control calls with dove models. Our results revealed that female cuckoo calls effectively suppress the antiparasitic responses of great reed warbler hosts, which could aid parasites to approach the nest undiscovered and to evade the costly attacks of this large host. Therefore, the female call can be regarded as a general part of the cuckoo's trickery repertoire for successful parasitism.

KEYWORDS

acoustic mimicry, brood parasitism, front-line defence, host aggression, nest defence

1 | INTRODUCTION

Enemy recognition plays a pivotal role in shaping the coevolutionary relationship between brood parasites and their hosts, eliciting either fighting or fleeing behavioural responses from the hosts. This behavioural dichotomy gains a focal function in the breeding

season (Ellis-Fellege et al., 2013; Pavel, 2006; Zhang et al., 2020), when the benefits of protecting the current brood (i.e., fighting) are put in balance against the benefits of potential future broods (i.e., fleeing). This trade-off implies that any investment by parents in their current offspring decreases their ability to invest in future offspring (Trivers, 1974). Therefore, parental recognition and

engagement of a potential brood parasite require a decision by the parent.

Decision errors (e.g., failing to recognise a parasite, or conversely, identifying an avian predator as a nest parasite) might be especially costly for the hosts of the brood parasitic Cuculidae cuckoos, since many of these brood parasitic species evolved vocal and plumage traits to resemble their hosts' avian predators in an effort to successfully parasitise host nests (Gluckman & Mundy, 2013). In theory, if a host identifies a threat as a brood parasite, the best response is to fight and drive the brood parasite away from the nest, therefore increasing current fitness without threatening parental survival (i.e., front-loaded antiparasitic defences; Feeney & Langmore, 2013). In contrast, if the parent identifies the threat as a predator, the best action is to flee in order to secure parental survival and, hence, future fitness. Misidentifying a brood parasite as a predator and fleeing might bring about negative fitness consequences through a higher chance of being parasitised, while misidentifying a predator as a brood parasite and attacking it might lead to an early demise of the adult host due to predation.

When the nest is approached by a heterospecific enemy, parents rely on a wide array of cues to assess the predatory vs. parasitic threat posed by the intruder. Some of the cues and contexts influencing the decision to flee or to stay and protect the brood include the stage of the brood (Campobello & Sealy, 2010; Moskát, 2005; Lawson et al., 2020), the identity of the enemy (e.g., reptile, bird or mammal; Kleindorfer et al., 2005; Strnad et al., 2012), its distance from the focal individual or its nest (Kryštofková et al., 2011), the type of threat (e.g., predator of adult birds, nest predator or brood parasite; Campobello & Sealy, 2018; Kleindorfer et al., 2005; Trnka & Prokop, 2010; Lawson et al., 2020), prior information regarding the occurrence of the threat (both on an individual and social level; Fontain & Martin, 2006; Davies & Welbergen, 2009; Campobello et al., 2017) and/or the reproductive senescence of parents (Clutton-Brock, 1984; Graham & Shuttler, 2019).

The common cuckoo (*Cuculus canorus*, "cuckoo" from hereafter) is a well-known obligate brood parasite that lays its eggs in the nests of other bird species, coercing these hosts to incubate parasitic eggs, and feed and rear the lone cuckoo nestling at the cost of their own brood's failure (Wyllie, 1981). Hosts evade brood parasitism by evolving traits to prevent parasitism (e.g., secretive nesting, mobbing adult parasites; Feeney et al., 2012; Moksnes & Røskaft, 1988; Welbergen & Davies, 2011) or to lessen costs once their nests have been parasitised (e.g., the recognition and ejection of brood parasitic eggs; Bártol et al., 2002). Intense aggression towards the brood parasite (reviewed in Campobello et al., 2017) might ensure host reproductive success even in species where no egg or chick rejection has evolved (Medina & Langmore, 2015). In turn, cuckoos have evolved several adaptations to overcome host defences, such as rapid egg deposition and laying in the afternoons (instead of mornings, like their hosts do), egg mimicry and resemblance to avian predators, giving rise to a coevolutionary arms race between brood parasites and their hosts (Davies, 2015). Whether visual resemblance to predators has adaptive value is still debated (Davies & Welbergen, 2008; Li

et al., 2015; Ma et al., 2018; Trnka & Prokop, 2012; Trnka et al., 2012, 2015; Welbergen & Davies, 2011).

While the adaptive similarity in appearance between the adult cuckoo and the sparrowhawk (*Accipiter nisus*) was one of the foci of the past two decades of research on brood parasites and their hosts (Davies, 2015; Trnka et al., 2012; Welbergen & Davies, 2011), the acoustic components of this similarity were only recently examined. These studies have now revealed that sounds emitted by brood parasites play an important role in conspecific communication (Moskát et al., 2017, 2020; Moskát & Hauber, 2019; Yu et al., 2019), and also as a means to increase the breeding success of the brood parasite. For example, a recent study demonstrated that cuckoos likely eavesdrop on the alarm calls of their hosts to locate host nests (Marton et al., 2019), while another study revealed that female cuckoos manipulate the behaviour of their hosts through their calls which are similar to those of the sparrowhawk (York & Davies, 2017). This latter study provided experimental evidence that a small host species, the reed warbler (*Acrocephalus scirpaceus*, ca. 12 g), increased its vigilance, returned to their nest later and was more likely to accept foreign eggs when threatened by the calls of female cuckoos, a sound similar to that of their aerial predator, the sparrowhawk, than when presented with the calls of the collared dove (*Streptopelia decaocto*; "dove" from hereafter) used as a sympatric, harmless control. Moreover, the female cuckoo calls had the same effect as sparrowhawk calls had on the attentiveness of wintering great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) outside the breeding season, two species that are only seldom parasitised by the cuckoo (Grim et al., 2014). Tits constitutes an important part of the sparrowhawk's diet during winter (Krams et al., 2020; Newton, 1986), when the cuckoos are on their sub-Saharan wintering grounds (Bán et al., 2018).

The reed warbler avoided possible predatory threats (e.g., sparrowhawk) through increased vigilance (i.e., peeking out of the nest) and an increased latency to return to the nest once flushed (York & Davies, 2017). However, the great reed warbler (*Acrocephalus arundinaceus*, ca. 32 g), a relatively larger host of the common cuckoo (ca. 100 g), is typically more aggressive towards both predators and brood parasites than the smaller warbler species, and attacks cuckoos near its nest more often (Moksnes et al., 1991; Røskaft et al., 2002; Trnka & Prokop, 2010, 2012), sometimes with fatal consequences for the brood parasites (Molnár, 1944; Šulc et al., 2020). Therefore, the coping strategy of a larger and more aggressive host might differ markedly and predictably from the behavioural response of a smaller, more threat-avoidant host.

To test whether or not the bubbling call of the female cuckoo has a greater effect on the aggressive mobbing behaviours of the larger great reed warblers than it had on a smaller host (e.g., reed warblers), we performed experimental cuckoo intrusion trials at great reed warbler nests. Specifically, we measured the aggressiveness (i.e., mobbing intensity) of great reed warblers towards cuckoo and dove decoys, before and during the playback of female cuckoo bubbling calls, and the calls of the dove used as control, respectively. Based on prior work on several host species (Li et al., 2015; Ma et al., 2018;

Trnka & Prokop, 2012), we predicted greater response intensity against the 3D-printed model of the cuckoo relative to the control decoy. In turn, given that the bubbling call of the female cuckoo mimics the sparrowhawk's calls (York & Davies, 2017), we predicted reduced response intensity by the hosts to the cuckoo model presentations coupled with the female call's playback vs. without the playback, and a generally low level of mobbing intensity towards the dove decoy, irrespective of the presence or absence of playbacks of dove calls.

2 | MATERIAL AND METHODS

2.1 | Study area

We carried out our study in central Hungary, around Apaj village (47°6'53.9"N; 19°5'21.2"E), ca. 40 km south of Budapest. In this region, great reed warblers face high rates of parasitism by cuckoos since decades (ca. 50%; Zölei et al., 2015). Here, hosts nest in narrow reed beds running along both sides of small irrigation channels, lined with tall trees serving as vantage points for eavesdropping cuckoos (Moskát & Honza, 2000; Marton et al., 2019).

2.2 | Experimental set-up

To test whether the female cuckoo's bubbling call (Moskát & Hauber, 2019) has a negative effect on host mobbing intensity, we presented 3D-printed common cuckoo or dove models as a control to great reed warblers at their nests (following Marton et al., 2019). We performed experimental trials at great reed warbler nests ($n = 61$), during the laying and early incubation stages. Great reed warblers and, other closely related *Acrocephalus* species maintain a high level of aggression towards adult cuckoos during their breeding cycle (Moskát, 2005; Li et al., 2015; Ma et al., 2018; but see Trnka & Prokop, 2012), likely because cuckoos may predate active nests to force hosts to restart breeding and to secure suitable laying opportunities for themselves (Schulze-Hagen, 1992; Soler et al., 2017). We had to exclude 19 trials (~30%) from the final data set; these were conducted at nests where great reed warblers altogether failed to turn up after 20 min, as these nests were subsequently deemed as deserted. We collected information regarding the number of great reed warbler and cuckoo eggs per clutch, clutch initiation date, and date and time of the experiments, with all experiments being performed in May 2019, between 7:00 and 12:00 or 17:00 and 20:00 hrs local time.

Cuckoo males are always grey, whereas females are polymorphic, being either grey or rufous, or of some intermediary form between (Moskát et al., 2020). The rufous female morph is common at our study area (up to 60%) and is mobbed by great reed warblers with an intensity similar to the grey morph (Honza et al., 2006). We used 3D-model cuckoo decoys painted with acrylics to resemble rufous cuckoo females, similarly to Lee et al. (2019). These decoys

were produced by printing life-size common cuckoo models (3D Quick Printing, UK) and collared dove models downloaded from Thingiverse.com (modified to fit the support pole of the cuckoo models), using an Ultimaker 2+ 3D printer and plain white Ultimaker PLA filament (see Marton et al., 2019 for more details). Researchers extensively use model birds in experiments with brood parasites and hosts (see a review in Campobello et al., 2017). Although the surface texture of the decoy may affect the intensity of host reactions (e.g., Némec et al., 2015), several studies have established that taxidermic mounts of cuckoos, decoys made of polystyrene or real live cuckoos were similarly attacked by different songbird species in field experiments (Honza et al., 2006; Tryjanowski et al., 2018).

We placed a randomly selected cuckoo or dove decoy next to a great reed warbler nest (cuckoo trials: $n = 22$; dove trials: $n = 20$), at a distance of ca. 20 cm, and a Nikon Coolpix AW130 Digital Camera Recorder, at 3–5 m from the nest. We recorded the mobbing behaviours of great reed warblers for 2 min upon discovering the cuckoo or dove decoy, after which we played back the female cuckoo's bubbling calls for the cuckoo decoy, or the call of the dove for the dove decoy for 2 min, on a JBL Xtreme 40 W Loudspeaker. The average time lag (\pm SD) between the two 2-min periods was 31 ± 33 s. We used 2-min-long acoustic files for playback, each containing ca. 2-s-long bubbling calls (three calls within 30 s), and this section was repeated two times with 15-s inter-section silent periods. A similar structure was also used for the 2-min dove control files (see more details of the playback in Moskát & Hauber, 2019; Moskát et al., 2020). To reduce pseudoreplication, we used three different rufous cuckoo and three dove decoys, and six different bubbling call and six dove call recordings chosen randomly. All calls were recorded by us locally between 2014 and 2018. We subsequently analysed the videos by counting the numbers of adult great reed warblers present during the trial and the numbers of different mobbing behaviours separately: "churr" alarm calls, bill snaps, pecks and physical contact attacks against the decoys per the 2-min trials.

2.3 | Statistical analyses

To test the effect of the female cuckoo bubbling call on the mobbing intensity of the hosts, we first calculated the total mobbing intensity of the hosts towards the 3D decoys both before and during the 2-min-long period when great reed warblers were exposed to playback calls. We expressed total mobbing intensity by summing the number of "churr" calls, bill snaps, pecks and attacks during 2 min. We built a general linear mixed-effects model with a zero-inflated Poisson error distribution, with total mobbing intensity as a dependent variable, and three two-level fixed factors as explanatory variables: the number of great reed warblers mobbing the 3D decoy (one member or both members of the pair), treatment (cuckoo or dove) and playback call (before or during playback). Additionally, we tested all the two-way interactions with the fixed factor treatment. Nest ID and irrigation channel (site) ID ($n = 4$) were entered as random factors in the model to control both for repeated measurements at

the same nests during trial periods and for potential spatial autocorrelation in the parasitism rate among irrigation channels. The initial model that contained all the above-listed variables (i.e., full model) was then simplified by sequentially removing variables with non-significant effects ($p > .05$) until reaching the minimal adequate model that included only significant interactions, significant main effects or non-significant main effects involved in significant interactions.

We performed all statistical analyses using the R statistical environment (ver. 4.0.2, R Core Team, 2020). Mixed-effects modelling was carried out using the R package "glmmTMB" (Brooks et al., 2017). Predictor significance levels were calculated using the Wald tests with an ANOVA (type II) as implemented in the "car" R package (Fox & Weisberg, 2019). Model fit assumptions for both full and minimal models were assessed using diagnostic plots and functions included in the "DHARMA" R package (Hartig, 2020). *Post-hoc* tests for significant interactions were carried out using the R package "emmeans" (Lenth, 2020).

3 | RESULTS

Mobbing intensity showed extensive variation between treatments and playback periods, and great reed warblers used different forms of mobbing with varying intensities in response to the different treatments (Table 1).

We found a significant interaction between treatment and playback period (Table 2). *Post-hoc* comparison of contrasts between

mobbing levels for the different periods (i.e., before and during playback) of the two treatment types (i.e., cuckoo vs. dove) revealed that there was no significant difference in total mobbing intensity before and during the dove call playback ($\beta = 0.336$, $SE = 0.420$, $t = 0.799$, $p = .427$), whereas great reed warblers mobbed significantly less during the cuckoo bubbling call playback than before it was broadcast ($\beta = -0.607$, $SE = 0.052$, $t = -11.692$, $p < .001$; Figure 1). Although total mobbing intensity seemed to be greater when both members of the great reed warbler pair were present, compared to when only one of the hosts was mobbing the decoys, after removing the non-significant interaction between treatment \times number of great reed warblers present from the full model, the main effect of the number of great reed warblers present also became non-significant in the minimal model (Table 2).

4 | DISCUSSION

Our results revealed that the female cuckoo bubbling call decreased the levels of aggression displayed by great reed warbler hosts towards cuckoos at their nests. This is in line with the results of a study performed on a smaller and less aggressive host species, the reed warbler. This smaller host, when experimentally exposed to the bubbling calls of female cuckoos or to the calls of the sparrowhawk, reduced its nest attendance and avoided a potential predator by returning later to the nest, as well as accepted foreign eggs more often compared to when the calls of the collared dove or common cuckoo

TABLE 1 Mean, median, standard deviation (SD) and range of mobbing variables produced by great reed warblers at their nests, before and during the playback of the female common cuckoo bubbling call at a rufous morph cuckoo decoy and of the calls of the collared dove at a collared dove decoy, as counted from video recordings.

Treatment	Variable	Before playback				During playback				V	p	p_{adj}
		Mean	Median	SD	Range	Mean	Median	SD	Range			
Cuckoo	No. of "churr" calls	0.41	0.00	1.71	0–8	0.00	0.00	0.00	0–0	3	.500	.580
	No. of bill snaps	29.09	19.00	31.04	0–123	14.46	2.00	26.73	0–120	169.5	<.001	<.001
	No. of pecks	7.32	0.00	15.99	0–70	5.41	0.00	15.74	0–72	45.5	.290	.580
	No. of attacks	14.05	6.50	19.98	0–80	6.41	0.00	16.25	0–67	120	<.001	<.001
	<i>Total mobbing intensity</i>	<i>50.86</i>	<i>43.00</i>	<i>50.54</i>	<i>0–203</i>	<i>26.27</i>	<i>7.00</i>	<i>43.96</i>	<i>0–191</i>			
Dove	No. of "churr" calls	0.75	0.00	1.45	0–5	1.10	0.00	4.08	0–18	11	.938	1.000
	No. of bill snaps	0.45	0.00	1.40	0–5	0.10	0.00	0.45	0–2	3	.500	1.000
	No. of pecks	0.10	0.00	0.45	0–2	0.00	0.00	0.00	0–0	1	1.000	1.000
	No. of attacks	0.55	0.00	1.82	0–8	0.00	0.00	0.00	0–0	6	.250	1.000
	<i>Total mobbing intensity</i>	<i>1.85</i>	<i>0.00</i>	<i>3.18</i>	<i>0–12</i>	<i>1.20</i>	<i>0.00</i>	<i>4.07</i>	<i>0–18</i>			

Note: Comparisons of mobbing behaviours were carried out using paired Wilcoxon signed rank tests, and significance levels (p) were corrected for multiple comparisons using Holm correction (p_{adj}). Significant differences ($p \leq .05$) are marked with bold, while variable names and values marked with italics are composite variables and values showing the total mobbing intensity of great reed warblers expressed through the sum of the different mobbing responses recorded.

TABLE 2 Results of the full and minimal adequate models showing that great reed warblers (GRW) exhibited a higher mobbing intensity towards the cuckoo decoys than towards the dove decoys and that mobbing intensity towards the cuckoo decoys decreased if the cuckoo female bubbling call was played back

Predictors	Full model						Minimal adequate model					
	β	SE	z value	χ^2	df	p	β	SE	z value	χ^2	df	p
Conditional model												
Intercept	1.543	0.845	1.826				3.363	0.352	9.562			
GRW present (both members)	2.023	0.920	2.199	4.089	1	.043						
Treatment (dove)	-2.255	1.006	-2.243	17.764	1	<.001	-3.867	0.616	-6.277	35.276	1	<.001
Playback (with playback)	-0.608	0.052	-11.706	133.143	1	<.001	-0.607	0.052	-11.692	132.392	1	<.001
Treatment (dove):GRW present (both members)	-1.412	1.305	-1.082	1.170	1	.279						
Treatment (dove):playback (with playback)	0.911	0.434	2.101	4.413	1	.036	0.943	0.423	2.228	4.963	1	.026
Zero-inflated model												
Intercept	-1.257	0.405	-3.105				-1.107	0.355	-3.120			
<i>Random effects</i>												
Residual variance	1.78						1.85					
Nest ID	2.3						2.36					
Channel ID	0						0					
N (Nest ID)	42						42					
N (Channel ID)	4						4					
Observations	84						84					

Significant effects ($p \leq .05$) are marked with bold.

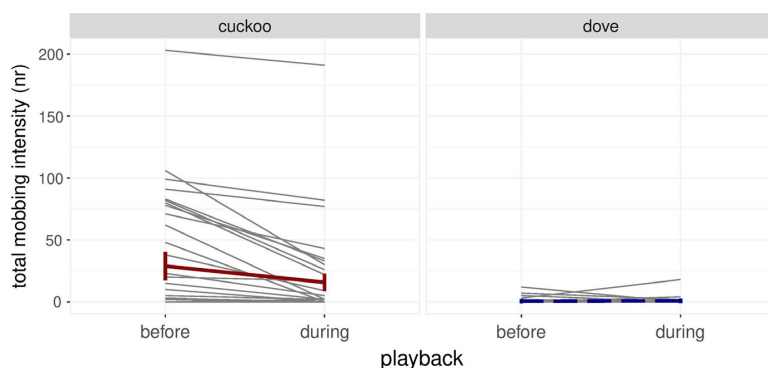


FIGURE 1 Change in the total mobbing intensity (i.e., sum of all mobbing responses) of great reed warblers against adult rufous common cuckoo and collared dove decoys, for the two periods of 2 min before and during the playback of female cuckoo bubbling calls or dove calls, respectively. Grey lines indicate the changes at the hosts' responses at individual nests before and during species-specific playback calls, whereas the red continuous and the dashed blue lines indicate changes in average mobbing intensity against the cuckoo or dove decoys (model predicted means \pm SE) [Colour figure can be viewed at wileyonlinelibrary.com]

male calls (non-bubbling) were played back to them as a control (York & Davies, 2017). Although in the aforementioned study the mobbing intensity of reed warblers towards the cuckoo decoy, measured as mobbing call rate, did not differ before and during playbacks, in our study the focal species is more aggressive and 2–3 times heavier than the reed warbler (Cramp, 1992; Leisler & Schulze-Hagen, 2011).

Also, apart from producing a very loud mobbing call, great reed warblers readily pecked and attacked the cuckoo decoy before the onset of the bubbling playbacks, whereas its total mobbing intensity still dropped by more than half when the bubbling calls were initiated.

Although our results show a consistent drop in total mobbing intensity against the cuckoo decoy during the period when the bubbling

call was broadcast compared to the period without the bubbling call, we acknowledge that our study design has some shortcomings also. Specifically, our experimental set-up lacked a fully factorial design due to the unusually low number of active great reed warbler nests in our study area in 2019, and was not symmetrical (i.e., silent periods always preceded playbacks). This latter issue, however, might have a negligible effect on our results, because although the bubbling call playback decreased the mobbing intensity of great reed warblers against the cuckoo decoy, we found no such effect during the trials with dove decoys and the dove playbacks, albeit they were presented in same order as in the case of the cuckoo treatment. Also, it is reasonable to assume that a symmetrical design (i.e., having a treatment group with playback preceding the silent period) would have resulted in a biased sampling, since it is likely that the less aggressive great reed warblers would not have approached the cuckoo decoy coupled with bubbling calls. As a possible improvement for future studies with similar design and facing a parallel design conundrum, we recommend inserting a second silent (non-playback) period after the playback periods.

The great reed warbler is a socially monogamous species, in which high-quality males mate with multiple females (Sejberg et al., 2000). Monogamous great reed warbler males invest more in nest defence than polygamous males do, while females defend their nests more vigorously than males, and do so regardless of their mating status (Trnka & Prokop, 2010). The mating status of great reed warblers in our study was unknown, but we recorded the number of adults mobbing the decoys. Since this number of mobbing great reed warblers at the nest was not a statistical predictor of total mobbing intensity, we assume that most of the mobbing was performed by females. Additionally, the treatment (i.e., cuckoo vs. dove) was assigned randomly to each nest; therefore, it is likely that the social mating status of the hosts did not confound our findings.

It is worth noting that, despite the few known cases when the great reed warblers managed to kill a cuckoo by drowning it in water (Molnár, 1944; Šulc et al., 2020), the mobbing of live female cuckoos by great reed warblers seemingly has little effect on the success rate of parasitic attempts, as cuckoos still manage to lay eggs in the host nests, even when both parents of this larger host are attacking them fiercely (Jelínek et al., 2020).

These results suggest that the acoustic mimicry of predatory sparrowhawk calls by female cuckoos has an adaptive function, which is additional to the raptorial plumage mimicry of the grey morph of adult cuckoos from both sexes (Trnka & Grim, 2013; Trnka et al., 2012, 2015). Critically, the acoustic mimicry functions for both the grey (York & Davies, 2017) and rufous (this study) morph of adult female cuckoos. Grey cuckoos appear similar to sparrowhawks (e.g., Trnka & Prokop, 2012; Trnka et al., 2012), and this mimicry may already defend cuckoos from host aggression to some extent (Welbergen & Davies, 2011), although plumage mimicry is less effective against the more aggressive and larger *Acrocephalus* species (Li et al., 2015; Ma et al., 2018; Trnka & Prokop, 2012). Rufous female cuckoos, in turn, despite having barred underparts and yellow eyes, do not resemble sparrowhawks or small falcons in colouration (Trnka & Grim, 2013; Trnka et al., 2015). Therefore, the lack of

sparrowhawk-plumage mimicry could create an uneven playing field against rufous females relative to females of the grey morph, might hinder successful parasitism and might explain why this morph is usually rarer than the grey morph. Future studies should aim to address these differences, by experimentally testing the reactions of various host species towards the two female cuckoo colour morphs (e.g., Thorogood & Davies, 2012), presented to the hosts alongside the bubbling call or the control calls of a harmless species.

The female cuckoo's bubbling call seems to be a multifunctional signal, being important in interspecific cuckoo–host relationships, as well as in conspecific communication (Deng et al., 2019; Moskát & Hauber, 2019). The bubbling call might play a role in the context of mate attraction (Moskát & Hauber, 2019; Xia et al., 2019) and could also function as a territorial signal for other conspecific females (Davies, 2015; Moskát et al., 2020). A previous study (York & Davies, 2017) tested an interspecific function of female cuckoo calls against a small host, the reed warbler, while our results revealed that this call also effectively dampened the mobbing responses of a large host, the great reed warbler. Our results point to the adaptive value of acoustic mimicry of female cuckoo calls even against more aggressive hosts, especially against those host species where the raptorial plumage mimicry of the grey cuckoo morph is less effective (Li et al., 2015; Ma et al., 2018; Trnka & Prokop, 2012).

ETHICS STATEMENT

All applicable international, national and/or institutional guidelines for the care and use of animals were followed. Local ethical regulations and agreements were followed for fieldwork. All work complied with Hungarian law and the Middle-Danube-Valley Inspectorate for Environmental Protection, Nature Conservation and Water Management, Budapest, provided permission for research (permit no. PE/KTFO/3097-10/2020).

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CONFLICT OF INTEREST

We declare no competing interests.

AUTHOR CONTRIBUTIONS

AM, CM and MEH devised the study, AM and MB collected the data, AF performed the statistical analyses, and AM wrote the first draft and revised manuscript with considerable input and the approval of the final version by all authors.

DATA AVAILABILITY STATEMENT

The data analysed in this study is available online at Figshare.com [https://doi.org/10.6084/m9.figshare.13489209].

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REFERENCES

- Bán, M., Moskát, C., Fülöp, A., & Hauber, M. E. (2018). Return migration of common cuckoos (*Cuculus canorus*) between breeding grounds in Hungary and wintering grounds in Africa as documented by non-PTT GPS technology. *Journal of Ornithology*, 159, 337–344. <https://doi.org/10.1007/s10336-017-1508-x>
- Bártol, I., Karcza, Z., Moskát, C., Røskft, E., & Kisbenedek, T. (2002). Responses of great reed warblers *Acrocephalus arundinaceus* to experimental brood parasitism: The effects of a cuckoo *Cuculus canorus* dummy and egg mimicry. *Journal of Avian Biology*, 33, 420–425. <https://doi.org/10.1034/j.1600-048X.2002.02945.x>
- Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modelling. *The R Journal*, 9, 378–400. <https://doi.org/10.3929/ethz-b-000240890>
- Campobello, D., & Sealy, S. G. (2010). Enemy recognition of reed warblers (*Acrocephalus scirpaceus*): Threats and reproductive value act independently in nest defence modulation. *Ethology*, 116, 498–508. <https://doi.org/10.1111/j.1439-0310.2010.01764.x>
- Campobello, D., & Sealy, S. G. (2018). Evolutionary significance of antiparasite, antipredator and learning phenotypes of avian nest defence. *Scientific Reports*, 8, 10569. <https://doi.org/10.1038/s41598-018-28275-3>
- Campobello, D., Sealy, S. G., & Welbergen, J. A. (2017). Anti-brood parasite defences: The role of individual and social learning. In Soler, M. (Ed.), *Avian brood parasitism: Behaviour, ecology, evolution and coevolution* (pp. 421–436). Springer International Publishing. https://doi.org/10.1007/978-3-319-73138-4_23
- Clutton-Brock, T. H. (1984). Reproductive effort and terminal investment in iteroparous animals. *American Naturalist*, 123, 212–229. <https://doi.org/10.1086/284198>
- Cramp, S. (Ed.) (1992). *Handbook of the birds of Europe, the Middle East and North Africa*, Vol. 6. Oxford University Press.
- Davies, N. B. (2015). *Cuckoo. Cheating by nature*. Bloomsbury Publishing.
- Davies, N. B., & Welbergen, J. A. (2008). Cuckoo-hawk mimicry? An experimental test. *Proceedings of the Royal Society B: Biological Sciences*, 275, 1817–1822. <https://doi.org/10.1098/rspb.2008.0331>
- Davies, N. B., & Welbergen, J. A. (2009). Social transmission of a host defense against cuckoo parasitism. *Science*, 324, 1318–1320. <https://doi.org/10.1126/science.1172227>
- Deng, Z., Lloyd, H., Xia, C., Møller, A. P., Liang, W., & Zhang, Y. (2019). Components of variation in female common cuckoo calls. *Behavioural Processes*, 158, 106–112. <https://doi.org/10.1016/j.beproc.2018.10.007>
- Ellis-Fellege, S. N., Burnam, J. S., Palmer, W. E., Clay Sisson, D., & Carroll, J. P. (2013). Fight or flight: Parental decisions about predators at nests of northern bobwhites (*Colinus virginianus*). *The Auk*, 130, 637–644. <https://doi.org/10.1525/auk.2013.12227>
- Feeney, W. E., & Langmore, N. E. (2013). Social learning of a brood parasite by its host. *Biology Letters*, 9, 20130443. <https://doi.org/10.1098/rsbl.2013.0443>
- Feeney, W. E., Welbergen, J. A., & Langmore, N. E. (2012). The frontline of avian brood parasite-host coevolution. *Animal Behavior*, 84, 3–12. <https://doi.org/10.1016/j.anbehav.2012.04.011>
- Fontain, J. J., & Martin, T. E. (2006). Parent birds assess nest predation risk and adjust their reproductive strategies. *Ecology Letters*, 9, 428–434. <https://doi.org/10.1111/j.1461-0248.2006.00892.x>
- Fox, J., & Weisberg, S. (2019). *An R companion to applied regression*, 3rd ed. Sage. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- Gluckman, T.-L., & Mundy, N. I. (2013). Cuckoos in raptors' clothing: Barred plumage illuminates a fundamental principle of Batesian mimicry. *Animal Behavior*, 86, 1165–1181. <https://doi.org/10.1016/j.anbehav.2013.09.020>
- Graham, K. F., & Shutler, D. (2019). How robust are risk-taking associations in incubating birds? A test and a review. *Behavioral Ecology and Sociobiology*, 73, 79. <https://doi.org/10.1007/s00265-019-2692-x>
- Grim, T., Samas, P., Procházka, P., & Rutila, J. (2014). Are tits really unsuitable hosts for the common cuckoo? *Ornis Fennica*, 91, 166–177.
- Hartig, F. (2020). DHARMa: Residual diagnostics for hierarchical (multi-level / mixed) regression models. R package version 0.3.2.0. <https://CRAN.R-project.org/package=DHARMa>
- Honza, M., Šícha, V., Procházka, P., & Ležalová, R. (2006). Host nest defense against a color-dimorphic brood parasite: Great reed warblers (*Acrocephalus arundinaceus*) versus common cuckoos (*Cuculus canorus*). *Journal of Ornithology*, 147, 629–637. <https://doi.org/10.1007/s10336-006-0088-y>
- Jelínek, V., Šulc, M., Štětková, G., & Honza, M. (2020). Fast and furious: Host aggression modulates behaviour of brood parasites. *bioRxiv*, 2020.04.27.063933, 1–15. <https://doi.org/10.1101/2020.04.27.063933>
- Kleindorfer, S., Fessl, B., & Hoi, H. (2005). Avian nest defence behaviour: Assessment in relation to predator distance and type and nest height. *Animal Behavior*, 69, 307–313. <https://doi.org/10.1016/j.anbehav.2004.06.003>
- Krams, I. A., Krama, T., Freeberg, T. M., Krams, R., & Sieving, K. E. (2020). Attacks of songbirds in mixed-species flocks by Eurasian sparrowhawks: Strategies of predators and potential prey. *Journal of Field Ornithology*, 91, 1–8. <https://doi.org/10.1111/jof.12350>
- Kryštofková, M., Haas, M., & Exnerová, A. (2011). Nest defense in blackbirds *Turdus merula*: Effect of predator distance and parental sex. *Acta Ornithologica*, 46, 55–63. <https://doi.org/10.3161/000164511X589938>
- Lawson, S. L., Enos, J. K., Mendes, N. C., Gill, S. A., & Hauber, M. E. (2020). Heterospecific eavesdropping on an anti-parasitic referential alarm call. *Communications Biology*, 3, 143. <https://doi.org/10.1038/s42003-020-0875-7>
- Lawson, S. L., Leuschner, N., Gill, B. J., Enos, J. K., & Hauber, M. E. (2020). Loss of graded enemy recognition in a whitehead population allopatric with brood-parasitic Long-tailed Cuckoos. *Avocetta*, 44, 3–10. <https://doi.org/10.30456/AVO.2020104>
- Lee, J. W., Kim, H. N., Yoo, S., & Yoo, J. C. (2019). Common cuckoo females may escape male sexual harassment by color polymorphism. *Scientific Reports*, 9, 7515. <https://doi.org/10.1038/s41598-019-44024-6>
- Leisler, B., & Schulze-Hagen, K. (2011). *Reed warblers: Diversity in a uniform bird family*. Leiden, The Netherlands. KNNV Publishing. <https://doi.org/10.1163/9789004278028>
- Lenth, R. (2020). emmeans: Estimated marginal means, aka least-squares means. R Package Version 1.4.6. <https://CRAN.R-project.org/package=emmeans>
- Li, D., Wei, H., Zhang, Z., Liang, W., & Stokke, B. G. (2015). Oriental reed warbler (*Acrocephalus orientalis*) nest defence behaviour towards brood parasites and nest predators. *Behaviour*, 152, 1601–1621. <https://doi.org/10.1163/1568539x-00003295>
- Ma, L., Yang, C., & Liang, W. (2018). Hawk mimicry does not reduce attacks of cuckoos by highly aggressive hosts. *Avian Research*, 9, 35. <https://doi.org/10.1186/s40657-018-0127-4>

- Marton, A., Fülöp, A., Ozogány, K., Moskát, C., & Bán, M. (2019). Host alarm calls attract the unwanted attention of the brood parasitic common cuckoo. *Scientific Reports*, 9, 18563. <https://doi.org/10.1038/s41598-019-54909-1>
- Medina, I., & Langmore, N. E. (2015). Batten down the thatches: Front-line defences in an apparently defenceless cuckoo host. *Animal Behavior*, 112, 195–201. <https://doi.org/10.1016/j.anbehav.2015.12.006>
- Moksnes, A., & Røskft, E. (1988). Responses of fieldfares *Turdus pilaris* and bramblings *Fringilla montifringilla* to experimental parasitism by the cuckoo *Cuculus canorus*. *Ibis*, 130, 535–539. <https://doi.org/10.1111/j.1474-919X.1988.tb02719.x>
- Moksnes, A., Røskft, E., Braa, A. T., Korsnes, L., Lampe, H. M., & Pedersen, H. C. (1991). Behavioural response of potential hosts towards artificial cuckoo eggs and dummies. *Behaviour*, 116, 64–89. <https://doi.org/10.1163/156853990X00365>
- Molnár, B. (1944). The cuckoo in the Hungarian plain. *Aquila*, 51, 100–112.
- Moskát, C. (2005). Nest defence and egg rejection in great reed warblers over the breeding cycle: Are they synchronised with the risk of brood parasitism? *Annales Zoologici Fennici*, 42, 579–586.
- Moskát, C., Elek, Z., Bán, M., Geltsch, N., & Hauber, M. E. (2017). Can common cuckoos discriminate between neighbours and strangers by their calls? *Animal Behavior*, 126, 253–260. <https://doi.org/10.1016/j.anbehav.2017.02.013>
- Moskát, C., & Hauber, M. E. (2019). Sex-specific responses to simulated territorial intrusions in the common cuckoo: A dual function of female acoustic signalling. *Behavioral Ecology and Sociobiology*, 73, 60. <https://doi.org/10.1007/s00265-019-2665-0>
- Moskát, C., Hauber, M. E., Růžicková, J., Marton, A., Bán, M., & Elek, Z. (2020). Female-female aggression and male responses to the two colour morphs of female common cuckoos. *The Science of Nature*, 107, 28. <https://doi.org/10.1007/s00114-020-01680-3>
- Moskát, C., & Honza, M. (2000). Effect of nest and nest site characteristics on the risk of cuckoo *Cuculus canorus* parasitism in the great reed warbler *Acrocephalus arundinaceus*. *Ecography*, 23, 335–341. <https://doi.org/10.1111/j.1600-0587.2000.tb00289.x>
- Němec, M., Syrová, M., Dokoupilová, L., Veselý, P., Šmilauer, P., Landová, E., Lišková, S., & Fuchs, R. (2015). Surface texture and priming play important roles in predator recognition by the red-backed shrike in field experiments. *Animal Cognition*, 18, 259–268. <https://doi.org/10.1007/s10071-014-0796-2>
- Newton, I. (1986). *The sparrowhawk*. T. and A. D. Poyser.
- Pavel, V. (2006). When do altricial birds reach maximum of their brood defence intensity? *Journal of Ethology*, 24, 175–179. <https://doi.org/10.1007/s10164-005-0178-y>
- R Core Team (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Røskft, E., Moksnes, A., Stokke, B. G., Bičič, V., & Moskát, C. (2002). Aggression to dummy cuckoos by potential European cuckoo hosts. *Behaviour*, 139, 613–628. <https://doi.org/10.1163/15685390260136735>
- Schulze-Hagen, K. (1992). Parasitierung und Brutverluste durch Kuckuck (*Cuculus canorus*) bei Teich- und Sumpfrohrsänger (*Acrocephalus scirpaceus*, *A. palustris*) in Mittel- und Westeuropa. *Journal of Ornithology*, 133, 237–249. <https://doi.org/10.1007/BF01645635>
- Sejberg, D., Bensch, S., & Hasselquist, D. (2000). Nestling provisioning in polygynous great reed warblers (*Acrocephalus arundinaceus*): Do males bring larger prey to compensate for fewer nest visits? *Behavioral Ecology and Sociobiology*, 47, 213–219. <https://doi.org/10.1007/s002650050658>
- Soler, M., Pérez-Contreras, T., & Soler, J. J. (2017). Brood parasites as predators: Farming and mafia strategies. In Soler, M. (Ed.), *Avian brood parasitism: Behaviour, ecology, evolution and coevolution* (pp. 271–286). Springer International Publishing. https://doi.org/10.1007/978-3-319-73138-4_15
- Strnad, M., Nimec, M., Veselý, P., & Fuchs, R. (2012). Red-backed shrikes (*Lanius collurio*) adjust the mobbing intensity, but not mobbing frequency, by assessing the potential threat to themselves from different predators. *Ornis Fennica*, 89, 206–215.
- Šulc, M., Štětková, G., Procházka, P., Požgayová, M., Sosnovcová, K., Studecký, J., & Honza, H. (2020). Caught on camera: Circumstantial evidence for fatal mobbing of an avian brood parasite by a host. *Journal of Vertebrate Biology*, 69, 1–6. <https://doi.org/10.25225/jvb.20027>
- Thorogood, R., & Davies, N. B. (2012). Cuckoos combat socially transmitted defenses of reed warbler hosts with a plumage polymorphism. *Science*, 337, 578. <https://doi.org/10.1126/science.1220759>
- Trivers, L. R. (1974). Parent-offspring conflict. *American Zoologist*, 14, 249–264. <https://doi.org/10.1093/icb/14.1.249>
- Trnka, A., & Grim, T. (2013). Color plumage polymorphism and predator mimicry in brood parasites. *Frontiers in Zoology*, 10, 25. <https://doi.org/10.1186/1742-9994-10-25>
- Trnka, A., & Prokop, P. (2010). Does social mating system influence nest defence behaviour in great reed warbler *Acrocephalus arundinaceus* males? *Ethology*, 116, 1075–1083. <https://doi.org/10.1111/j.1439-0310.2010.01821.x>
- Trnka, A., & Prokop, P. (2012). The effectiveness of hawk mimicry in protecting cuckoos from aggressive hosts. *Animal Behavior*, 83, 263–268. <https://doi.org/10.1016/j.anbehav.2011.10.036>
- Trnka, A., Prokop, P., & Grim, T. (2012). Uncovering dangerous cheats: How do avian hosts recognize adult brood parasites? *PLoS ONE*, 7, e37445. <https://doi.org/10.1371/journal.pone.0037445>
- Trnka, A., Trnka, M., & Grim, T. (2015). Do rufous common cuckoo females indeed mimic a predator? An experimental test. *Biological Journal of the Linnean Society*, 116(1), 134–143. <https://doi.org/10.1111/bij.12570>
- Tryjanowski, P., Morelli, F., Kwieciński, Z., Indykiewicz, P., & Møller, A. P. (2018). Birds respond similarly to taxidermic models and live cuckoos *Cuculus canorus*. *Journal of Ethology*, 36, 243–249. <https://doi.org/10.1007/s10164-018-0554-z>
- Welbergen, J. A., & Davies, N. B. (2011). A parasite in wolf's clothing: Hawk mimicry reduces mobbing of cuckoos by hosts. *Behavioral Ecology*, 22, 574–579. <https://doi.org/10.1093/beheco/arr008>
- Wyllie, I. (1981). *The cuckoo*. Batsford.
- Xia, C., Deng, Z., Lloyd, H., Møller, A. P., Zhao, X., & Zhang, Y. (2019). The function of three main call types in common cuckoo calls. *Ethology*, 125, 652–659. <https://doi.org/10.1111/eth.12918>
- York, J. E., & Davies, N. B. (2017). Female cuckoo calls misdirect host defences towards the wrong enemy. *Nature Ecology and Evolution*, 1, 1520–1525. <https://doi.org/10.1038/s41559-017-0279-3>
- Yu, J., Lu, H., Sun, W., Liang, W., Wang, H., & Møller, A. P. (2019). Heterospecific alarm-call recognition in two warbler hosts of common cuckoos. *Animal Cognition*, 22, 1149–1157. <https://doi.org/10.1007/s10071-019-01307-9>
- Zhang, L., Liu, J., Zhang, H., Wan, D., Liang, W., & Møller, A. P. (2020). Fight or flight: Geographic variation in antipredator defenses by cinereous tits. *Global Ecology and Conservation*, 24, e01207. <https://doi.org/10.1016/j.gecco.2020.e01207>
- Zölei, A., Bán, M., & Moskát, C. (2015). No change in common cuckoo *Cuculus canorus* parasitism and great reed warblers' *Acrocephalus arundinaceus* egg rejection after seven decades. *Journal of Avian Biology*, 46, 570–576. <https://doi.org/10.1111/jav.00673>

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11. Study III:

Female-female aggression and male responses to the two colour morphs of female common cuckoos

Moskát, C., Hauber, M. E., Růžicková, J., Marton, A., Bán, M. & Elek, Z.

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Abstract

Female-only colour polymorphism is rare in birds, but occurs in brood parasitic cuckoos (Cuculidae). Obligate brood parasites leave incubation and parental care to other species (hosts), so female-female interactions can play a role in how parasites guard critical resources (host nests) within their laying areas. The plumage of adult female common cuckoos *Cuculus canorus* is either rufous (typically rare) or grey (common), whereas adult male conspecifics are monochromatic (grey). In previous studies, hosts and conspecific males responded with less intensity toward the rare female morph in support of a negative frequency-dependent benefit of female plumage polychromatism. Here, we assessed responses of both conspecific females and males to vocal playbacks of female calls, coupled with one of two 3D models of the different morphs of female cuckoos. At our study population, the rufous female morph was as common as the grey morph; therefore, we predicted similarly high rates of conspecific responses in both treatments. Both female and male cuckoos responded to playbacks acoustically, which demonstrated the primary role of acoustic communication in social interactions amongst cuckoos. Following this, some cuckoos flew closer to the models to inspect them visually. As predicted, no significant differences were detected between the live cuckoos' responses toward the two colour morphs in this population. We conclude that dichromatism in female cuckoos evolved to serve one or more functions other than conspecific signalling.



Female-female aggression and male responses to the two colour morphs of female common cuckoos

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Abstract

Female-only colour polymorphism is rare in birds, but occurs in brood parasitic cuckoos (Cuculidae). Obligate brood parasites leave incubation and parental care to other species (hosts), so female-female interactions can play a role in how parasites guard critical resources (host nests) within their laying areas. The plumage of adult female common cuckoos (*Cuculus canorus*) is either rufous (typically rare) or grey (common), whereas adult male conspecifics are monochromatic (grey). In previous studies, hosts and conspecific males responded with less intensity toward the rare female morph in support of a negative frequency-dependent benefit of female plumage polychromatism. Here, we assessed responses of both conspecific females and males to vocal playbacks of female calls, coupled with one of two 3D models of the different morphs of female cuckoos. At our study population, the rufous female morph was as common as the grey morph; therefore, we predicted similarly high rates of conspecific responses in both treatments. Both female and male cuckoos responded to playbacks acoustically, which demonstrated the primary role of acoustic communication in social interactions amongst cuckoos. Following this, some cuckoos flew closer to the models to inspect them visually. As predicted, no significant differences were detected between the live cuckoos' responses toward the two colour morphs in this population. We conclude that dichromatism in female cuckoos evolved to serve one or more functions other than conspecific signalling.

Keywords Acoustic playback · Colour polymorphism · 3D model · Female-female aggression · Territory

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Introduction

Colour polymorphism (or polychromatism) refers to the existence of two or more discrete spectral phenotypes of individuals in a population (Caro 2005; Roulin 2004; White and Kemp 2016). Such polymorphism may be controlled genetically and/or developmentally and may vary between populations, habitats, sexes, life-history stages, and/or age classes. Animal colour polymorphism occurs in diverse invertebrate (Ajuria-Ibarra and Reader 2013) and vertebrate (Hubbard et al. 2010) lineages. Colour morphs may be adaptive for concealment, interspecific and intraspecific communication (which includes sexual selection), and/or for several physiological aspects (e.g. reflecting or adsorbing heat or other types of radiation; Caro 2005). When polychromatic individuals occur in different proportions in a population, negative frequency dependence may favour the maintenance of the rarer morphs, which provides stabilising selection for polymorphism (Galeotti et al. 2003; Roulin 2004).

Colour polymorphism is well-known in birds, which includes 33.3% of the species in the order Strigiformes and about

10% of Cuculiformes, Upupiformes, Galliformes, and Ciconiiformes (Galeotti et al. 2003). It is more frequent in lineages that live in both open and closed habitats and in species that show extended daily activity patterns, under variable light conditions (Galeotti et al. 2003). For example, the lighter, white morph of the barn owl (*Tyto alba*) is able to capture prey more efficiently under brighter moonlight conditions than the darker, reddish morph (San-Jose et al. 2019). Colour polymorphism may also be related to personality, as seen in the Gouldian finch (*Erythrura gouldiae*), where red-headed individuals were more aggressive than the black-headed morph, but black-headed individuals appeared to be bolder (i.e. approaching and touching novel objects more often) and less risk-averse (i.e. more likely to return to a feeder after the experimental presentation of a predator) (Williams et al. 2012).

Colour polymorphism can be restricted to only one of the sexes in a species (Cuthill et al. 2017). However, this type of colour polymorphism is rare in female birds and occurs in only about 0.2% of avian species (Galeotti et al. 2003). Parasitic cuckoo species (*Cuculus* spp.) are one example of female-restricted, colour polymorphism in adults (Erritzøe et al. 2012; Payne 1967, 2005; Sato et al. 2015; Tanaka 2016). In these species, the ratio of eumelanin and pheomelanin plays a key role in generating grey and rusty colour morphs (Toral et al. 2008; see also in general McGraw et al. 2005; Ducrest et al. 2008). The common cuckoo (*Cuculus canorus*), a widely studied obligate brood parasite, is a typical example of adult female-specific, plumage polymorphism (e.g. Payne 2005; Mikulica et al. 2017; Wyllie 1981). This species meets the criteria defined by Galeotti et al. (2003) that predict the appearance of plumage polymorphism, i.e. it lives in a variety of habitats, which include semi-open habitats (Røskaft et al. 2002b), but shows a strong diurnal activity pattern (Moskát et al. 2019; Wyllie 1981; Yoo et al. 2019). Adult males are grey (monomorphic) and adult females are polymorphic independent of age, either individually grey (typically common in most cuckoo populations) or rufous (typically rare) (Fig. 1).

Most previous research focused on the potential role of cuckoo colour polymorphism in cuckoo-host relationships. Specifically, the grey morph of common cuckoos is thought to mimic the Eurasian sparrowhawk (*Accipiter nisus*), in what is known as the sparrowhawk mimicry hypothesis (e.g. Thorogood and Davies 2012; Gluckman and Mundy 2013). This sparrowhawk is a predator of small passerines, which includes cuckoo hosts, and this aggressive mimicry deters hosts from attacking the female cuckoo as a front-loaded antiparasitic defence strategy (Welbergen and Davies 2011). Interestingly, sparrowhawk mimicry cannot prevent cuckoos from being attacked by hosts altogether, neither in reed warblers (*Acrocephalus scirpaceus*) (Campobello and Sealy 2010; Welbergen and Davies 2011) nor in other passerine cuckoo hosts (Liang and Møller 2015; Moksnes et al. 1991;

Røskaft et al. 2002a; Tryjanowski et al. 2018a, b), which include larger and more aggressive *Acrocephalus* species (Bártol et al. 2002; Dyrce and Halupka 2006; Li et al. 2015; Ma et al. 2018; Marton et al. 2019). Although the rufous morph of female common cuckoos is somewhat similar to common kestrels (*Falco tinnunculus*) (the kestrel mimicry hypothesis, Voipio 1953), an experimental study rejected the idea that the rufous plumage of female common cuckoos was an adaptation to mimic this raptor species that also preys on small passerines (Trnka et al. 2015). At sites where the grey morph was common, the rufous morph received less aggression by hosts (Honza et al. 2006; Trnka and Grim 2013). However, this advantage disappeared when the rufous morph was more widespread in a population and had a similar frequency to that of the grey morph (Honza et al. 2006).

Apart from the well-studied role of colour polymorphism in cuckoo-host relationships, this polymorphism could also have intraspecific functions. First, adult female polychromatism may reduce sexual harassment of females by males in a reproductive and/or territorial context by preventing males from recognizing the rarer colour morph as a conspecific female individual. A recent study tested this hypothesis in an Asian population of common cuckoos where the rusty morph was nearly absent amongst adult females (Lee et al. 2019). In that study, which was conducted as a field experiment, males tried to copulate with female models of the grey colour morph more than with the rufous morph (Lee et al. 2019).

Female common cuckoos typically parasitise the nests of small songbirds within a specific area – an individual laying area – (Chance 1940; Wyllie 1981). However, individual laying areas of multiple females sometimes partly overlap (Moskát et al. 2019; Nakamura and Miyazawa 1997; Wyllie 1975). Female common cuckoos defend their laying area from rival conspecific females, which show at least some degree of territorial-like behaviour when guarding their potential host nests (e.g. Moskát and Hauber 2019). Researchers disagree on the degree of territoriality of female common cuckoos: this species has been defined by some researchers as strictly territorial (Dröscher 1988; Honza et al. 2002; Nakamura et al. 2005), but others termed it as mostly territorial (Gärtner 1981; Riddiford 1986) or non-territorial (Vogl et al. 2004). Nonetheless, a second hypothesis for the intraspecific role of female adult colour polymorphism suggested that this may help to reduce intrasexual aggression caused by the territorial-like behaviour of breeding females (Horton et al. 2012) that compete for critical resources: namely, available host nests within their laying areas. Although this second hypothesis has not yet been studied in common cuckoos, a study by Svensson et al. (2009) on lizards and damselflies concluded that female colour polymorphism functioned intraspecifically to avoid both



Fig. 1 Colour variants of live common cuckoos (*Cuculus canorus*) and decoys used for model presentation experiments, including Eurasian collared dove (*Streptopelia decaocto*) used for a neutral control. Decoys: **a**: grey cuckoo, **b**: rufous cuckoo, **c**: collared dove; live common cuckoos: **d**: adult male, always grey; **e**: grey adult female, and

a series of rufous adult females: **f**: brownish female; **g**: red-brown female; and **h**: bright orange-red female. Birds were caught by mist-netting at our study site in Hungary. Photo credits: **(a)** and **(c)**: Zoltán Elek; **(b)**, **(d)**, **(f)** and **(h)**: Csaba Moskát; **(e)** and **(g)**: Miklós Bán

intersexual harassment and intrasexual competition for critical resources.

Both sets of these hypotheses assume that the rarer morph, which is typically the rufous morph in common cuckoos, has an evolutionary advantage of not being mobbed/harassed as heavily as the more common grey female cuckoo morph (Lee et al. 2019; Li et al. 2015; Trnka and Grim 2013). For instance, the appearance of a novel predatory species may have caused an initial recognition failure in prey birds (Carlson et al. 2017; Vesely et al. 2016) and may have favoured the rarer morphs. Similarly, a recognition problem is expected to occur toward a new or rare colour variant of brood parasites by their hosts. The role of social learning by hosts to recognise parasites may further increase the adaptive benefit of the rarer colour morph in female cuckoos (Thorogood and Davies 2013).

Morph ratios of female cuckoos appear to vary geographically, and this variation has been thought to be related to ecological factors that keep them at equilibrium at these different ratios (Thorogood and Davies 2012).

Overall, however, the typically rarer rufous colour variant does not appear to be growing in frequency in most cuckoo populations where it is tracked over time, even though it is thought to garner a greater fitness advantage than the more common grey morph (Mappes and Lindström 2012). Yet, in some populations, the rufous morph has become as common as the grey morph, including at our study site in Hungary (Honza et al. 2006). In such a stable but unbiased sex-ratio situation, it is assumed that each of the colour morphs has a specific fitness advantage over the other in regarding a specific physiological function (e.g. different costs of rusty vs. grey pigment production) and/or regarding an ecological context (e.g. camouflage from hosts in a specific microhabitat), and these effects are summed to yield similar fitness payoffs (Galeotti et al. 2003; Roulin 2004). Alternatively, when selection pressure is weak, different and even unsuitable alternative morphs may co-exist for extended periods due to stochastic effects, such as population perturbation, colonisation,

or range expansion (Excoffier et al. 2009; Johansson and Butlin 2017).

Although many aspects of brood parasitism are well understood in birds (Soler 2017), how brood parasitic birds recognise their conspecifics remains poorly understood (Göth and Hauber 2004). Only a handful of experiments have addressed the ontogeny and the mechanisms (e.g. the phenotypic and vocal cues) used by brood parasites to recognise their conspecifics (e.g. Soler and Soler 1999; Hauber et al. 2000; Payne et al. 2000; Louder et al. 2019). In this study, we specifically address a territorial conflict scenario that assumes that the rarer morph would have an advantage amongst adult female cuckoos when competing for critical breeding resources, such as host territories or nests. This advantage is expected to disappear when both colour morphs are widespread in a population, which is similar to the predator mimicry and sexual harassment hypotheses.

Here we tested this territorial conflict hypothesis (i.e. how female common cuckoos respond to the two female colour morphs by using playbacks and model (decoy) cuckoo presentations). We predicted strong responses to the cuckoo models relative to control (Eurasian collared dove *Streptopelia decaocto*) treatments, but we also predicted no preferential social responses toward either the grey or the rufous morphs because their frequencies were similar in our study population at Apaj, Hungary (Honza et al. 2006), and females would defend their resources (host nests) from as many females as possible, irrespective of morph colour. We also predicted that plumage colour discrimination is not biased by sex because males in our population should court and mate with as many females as possible, irrespective of colour. Regarding the dominant sensory modality of intraspecific communication, we hypothesised that acoustic cues play a primary role in both male and female cuckoos' recognition of and responses to potential mates or intruders over visual cues and physical proximity. Thus, we predicted that male cuckoos would respond quickly to female cuckoo calls, first acoustically, and only then by approaching to inspect the newcomer visually. We also predicted that female cuckoos would try to avoid direct, physical aggressive contacts with intruding females and would respond primarily to unfamiliar female cuckoos' bubbling calls acoustically. Given that in our study area the frequencies of the two colour morphs of female cuckoos were similar, our results could serve as reference for future similar studies with uneven frequencies of adult female cuckoo colour morphs.

Study area and methods

The study was conducted in a 20 × 40 km area around the village of Apaj (47° 6' 53.9" N; 19° 5' 21.2" E), in central Hungary ca. 50 km south of Budapest. This area contained a

dense network of narrow irrigation and flood-relief channels. The channels were typically surrounded by banks that were 2 to 5 m high and covered with trees and bushes on one or both sides of the channels. In this semi-open habitat cuckoos and their movements were easily visible to researchers. In this area, common cuckoos parasitised great reed warblers (*Acrocephalus arundinaceus*), which bred in 2- to 5-m-wide reed beds along both sides of the channels (Moskát and Honza 2000). The frequency of parasitism was high in the area (ca. 50% of nests had one or more cuckoo eggs; Zölei et al. 2015). Cuckoos parasitised this host species where trees hybrid poplars, white poplars (*Populus alba*), willows (*Salix alba*), black locusts (*Robinia pseudoacacia*), and Russian olives (*Eleagnus angustifolia*) were present along the channels, which were used by cuckoos as perches to locate and to monitor breeding activities of potential hosts (Moskát and Honza 2000). Both sexes of adult cuckoos seem to have high intra- and interannual breeding site fidelity (Bán et al. 2018; Moskát et al. 2019). Rufous adult female plumage morphs were common in this cuckoo population, with a frequency of ca. 60% (Honza et al. 2006).

For playback experiments, we recorded female common cuckoo calls ("bubbling calls") between 2015 and 2018. Bubbling calls are short (ca. 2 s long), sex-specific calls that are quite different from the "cu-coo" calls of males (Deng et al. 2019; Moskát and Hauber 2019; Xia et al. 2019). We also recorded calls of the Eurasian collared dove within our study area as control vocalisations during that same period. The collared dove is a harmless, sympatric species of cuckoos and their great reed warbler hosts, and they have often been used as controls for field experiments with common cuckoos as taxidermic mounts (e.g. Bártl et al. 2002; Davies and Welbergen 2008; Lovász and Moskát 2004; Trnka et al. 2015) or as playback calls (Moskát et al. 2017; York and Davies 2017).

We coupled our playback experiment with the presentation of 3D plastic models that were printed on an Ultimaker 2+ 3D printer using standard white ColorFabb PLA filament. The source file of the life-size cuckoo model was supplied by 3D Quick Printing Service (Golden Green Barn, Sandpitts Lane, Coventry, UK). The source file of the dove was downloaded from Thingiverse (<https://www.thingiverse.com/>), which is a free repository for 3D models. Three common cuckoo models (two of the more variable rufous morph and one of the less variable grey morph) and two collared doves, acrylic-painted models were used for the experiments (Fig. 1) (see also Marton et al. 2019 for a description of the decoys). Although we only had one model specimen of the grey morph, which may have resulted in visual but not acoustic pseudoreplication, this same decoy had already been used in a previous experiment where great reed warbler hosts aggressively attacked it more than controls (Marton et al. 2019).

The plumage colour of live rufous female cuckoos showed some variation, which ranged from brown to orange-reddish (Fig. 1). For our experiments, we chose the orange-reddish form because it was easily discernible by a researcher in the field. Many birds perceive a wider spectrum of light than humans (Stoddard and Hauber 2017) because they have a fourth, UV-sensitive cone in their retinas. However, the visually perceivable spectral range of cuckoos is likely more similar to humans, which was suggested by a genetic study of the short wavelength-sensitive type 1 (SWS1) opsin gene in shining cuckoos (*Chalcites lucidus*) and long-tailed cuckoos (*Urodynamis taitensis*) that indicated the presence of violet-sensitive (VS) and not ultraviolet-sensitive (UVS) cones (Aidala et al. 2012). The lack of UVS sensitivity was also supported indirectly through feather light-reflectance analyses that showed no strong reflectance in the UV range of the cuckoo's plumage (Mullen and Pohland 2008; see also Koleček et al. 2019). Here, we measured avian-visible reflectances of adult cuckoos' feathers and the 3D models (Fig. 2) with a USB 2000 spectrophotometer (Ocean Optics, Europe)

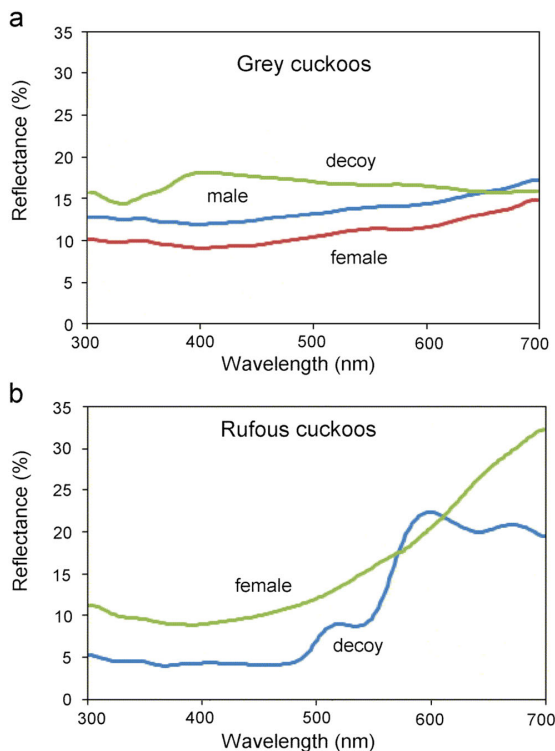


Fig. 2 Spectral reflectance of common cuckoo (*Cuculus canorus*) feathers and 3D model cuckoos. (a): feathers of grey male and grey female, and grey decoy; (b): feather of rufous female and rufous decoy. All curves show mean values of six measurements. Reflectances are shown in the 300–700-nm interval as typical, although cuckoos are expected not to be sensitive for the ultraviolet range (i.e. <380 nm; Aidala et al. 2012; for more details see “Study area and methods”)

with a DH-2000 deuterium light source and R400-7 bifurcated fibre-optic probe. The probe was oriented at a 90° to the surface (see for more details in Laczi et al. 2011).

The structure of our playback sound files was the same as the one used by our team in a recent similar study (Moskát and Hauber 2019). The short, 2-s female cuckoo bubbling call was repeated thrice in a 30-s period, followed by a 15-s break, and then repeated twice for a total duration of 2 min (without a final 15-s break). The last bubbling call unit was instead followed by a 2-min silent observational period (only the model bird was presented for visual cues). The same experimental design and playback file structure were used for the control stimuli of the dove models and calls.

We initiated a playback experiment within 2 min at a site after we heard the bubbling call of a female cuckoo ≤ 50 m away. We set up a loudspeaker (model: JBL Xtreme 40 W; volume was ca. 90 dB at 1 m distance measured by Voltcraft SL-100 sound meter by Conrad GmbH, Kalchreuth, Germany) on a tree at ca. 1–1.5-m height on the bank of the channel (typically above of the top of the reeds in the channel), which was connected by a 20-m audio cable to a Lenovo TAB 2 A7 tablet that contained the playback files in 16-bit .wav format.

We recorded our observations on cuckoos on a Tascam dr-05 ver2 sound recorder by verbally narrating the details of our observations. A second observer recorded cuckoo calling behaviours using a Marantz PMD-620 MKII audio recorder, a Sennheiser ME66 shotgun microphone, a FEL-MX mono pre-amp, an AKG K141 MKII headphone, a Rode PG2 pistol grip, and a Rode WS6 Deluxe windshield. After we adapted to the quick movements of the birds, distances of perching and flying cuckoos were estimated visually after observers had trained with a Bushnell Yardage Pro 800 rangefinder to estimate distances in the field.

Previous studies on common cuckoos that used VHF radio telemetry (Moskát et al. 2017) or GPS tags (Moskát et al. 2019) revealed that male common cuckoos maintained and defended territories from conspecific males during their breeding season in our study area. Similarly, female cuckoos also appeared to maintain territories during the breeding season, as evidenced by GPS data (Moskát et al. 2019) and playback experiments (Moskát and Hauber 2019). Here, we attempted to reduce the chance of collecting data on the same focal bird twice for the same type of trial. This is because “experienced birds” (Budka et al. 2019) may reduce their response or increase their response to repeated simulated territorial intrusions (Sprau et al. 2014). Therefore, we moved slowly by car from the first playback site along the irrigation channels to the next territory if we heard two females simultaneously calling from two such sites. In other cases, we moved by car > 1 km away along the channel, where we conducted the next experimental trial with a calling female if she was present. This pattern of site selection was implemented to reduce

pseudoreplication (Hurlbert 1984; Kroodsma 1989). For the same reason, we used each playback file only once, and did not use multiple recordings from the same individuals.

We applied three different treatments: (i) rufous cuckoo model with female cuckoo bubbling calls, (ii) grey cuckoo model with bubbling calls, and (iii) collared dove model with dove calls. We observed the activity of cuckoos for 4 min (2-min playback and the next 2-min post-playback period) while hid behind bushes (see for more details in Moskát and Hauber 2019). For direct comparisons of responses to playbacks and model presentations, we used binary (yes/no) variables that expressed response/lack of response by wild cuckoos to the experimental trials whether they responded acoustically or by moving within the 50-m radius around the model bird. We also measured the following variables in the same way for both the experimental and the control trials: distance from loudspeaker at first detection (m), closest distance (m) during playback, time elapsed between the start of the trial and the time of the closest distance (s), the time of the first movement of the focal bird (“movement latency”, s), the time of the first calling by the focal bird (“calling latency”, s), the duration of continuous calling (s), the number of calls uttered, the number of flights toward the model, and the total number of cuckoos observed. We identified sexes by plumage (rufous: all females) and/or calls (bubbling calls: all females, cu-coo calls: all males). All variables were recorded for both sexes, except that the number of calls for males was replaced by the number of call types (see details in Moskát and Hauber 2019): these included the “cu-coo” advertising call (sensu Lei et al. 2005) and the mate attraction vocalizations of the quick “cu-cu-coo” (Lei et al. 2005; Xia et al. 2019) and “gowk” (Lei et al. 2005) calls, respectively (latter category also included the difficult-to-distinguish “guo” calls (sensu Wyllie 1981)).

For simple bivariate comparisons of trials (reaction compared with no reaction), we applied categorical tests (Fisher’s exact and χ^2 tests). In turn, we used logistic, generalised linear models to study the relationship between behavioural variables that were considered fixed factors and occurrences of focal bird displays as the response variable. We applied the “binomial” family of distribution for occurrence data using the complementary loglog link function. This link function is asymmetric and will often produce different results from the logit and probit link functions. The complementary loglog corresponds to applications where we can detect either zero events (e.g. defects) or one or more event, where the number of events is assumed to follow the Poisson distribution (Van Horn 2015). We modelled these data with generalised linear models (GLM, Bolker et al. 2009) using the glm function in R 3.6.1 (R Core Team 2019) and the following explanatory variables in the evaluated models: (i) time-based variables (measured in seconds): time at first detection, time at closest detection, latency of calling, length of continuous calling; (ii) distance-based variables (measured in metres): distance at first

detection, closest distance; and (iii) meristic variables: number of calls, number of flights, number of birds. For the parameterisation of the most parsimonious model, we used a model selection information criterion (AICc) to rank the above models in terms of their ability to explain occurrences while accounting for the number of parameters estimated (Burnham and Anderson 2002). First, we fitted a full logistic model that included all explanatory variables mentioned above, and then we removed the variable with the least explanatory power, refitted the model, and repeated this process until we reached the optimal number of model parameters based on AICc (Bolker et al. 2009). In this way, a “best approximating” model was selected as the most parsimonious explanation of the data. We conducted this model parameterisation approach for each sex separately.

Behavioural and acoustic responses of female and male cuckoos were analysed separately using principal component analysis (PCA) in the program package SPSS ver. 17 (SPSS Inc., Chicago, IL, USA). PCAs were run on the correlation matrix of response variables, and components were retained where the corresponding eigenvalues were > 1.0 . No subsequent rotation on component loadings was applied.

Results

Responses of cuckoos to rufous and grey cuckoo models coupled with bubbling call playbacks

The simple categorisation (see “Study area and methods”) of the output of our trials revealed that both female and male common cuckoos showed consistently more responses toward the cuckoo playbacks and models than the control doves (Table 1). Cuckoos frequently responded to conspecific models and playbacks by approaching movements and calling behaviour. Female cuckoos responded to the rufous morph model and playbacks in 14/18 of trials, and male cuckoos responded in 16/18 cases (both sexes responded in 12/18 cases). Similar response frequencies were obtained when using the grey cuckoo model coupled with the playback: 15/17 responses by females and 15/17 responses by males, with 13/17 trials when both sexes responded.

Critically, the variation in wild cuckoo responses to the playbacks of female cuckoo calls coupled with either colour morph of the cuckoo model and the control dove presentations was significant for both cuckoo sexes (Fisher’s exact tests, both $P < 0.01$), but the responses to the two types of colour morph models were statistically similar (rufous vs. grey: $P = 0.658$ for females, and $P = 0.677$ for males). Similar patterns were found when acoustic responses were analysed separately from movement responses, and also when the numbers of flighted approaches toward the model were compared solely

Table 1 Summary of female and male responses of common cuckoos (*Cuculus canorus*) to vocal playbacks of female cuckoo calls coupled with presentations of different models (rufous female cuckoo, grey female cuckoo, and Eurasian collared dove (*Streptopelia decaocto*) used for control)

	Acoustic response or movement	Acoustic response	Flights	N
Female cuckoo responses to				
Rufous cuckoo model	14	13	13	18
Grey cuckoo model	15	13	13	17
Dove control model	4	4	4	17
Male cuckoo responses to				
Rufous cuckoo model	16	15	16	18
Grey cuckoo model	15	14	14	17
Dove control model	5	4	4	17

(Fig. 3). Both female and male cuckoos also responded vocally more intensively to cuckoo presentations than to the dove controls (all $P < 0.001$). In turn, cuckoos responded vocally and with number of flights equally to the two colour morphs (females: $P = 1.0$, males: $P = 1.0$).

Behaviour of female and male cuckoos during the experiment

Neither logistic regression models (Table 2) nor the PCAs (Table 3; Figs. 4a, b and 5a, b) revealed statistical differences

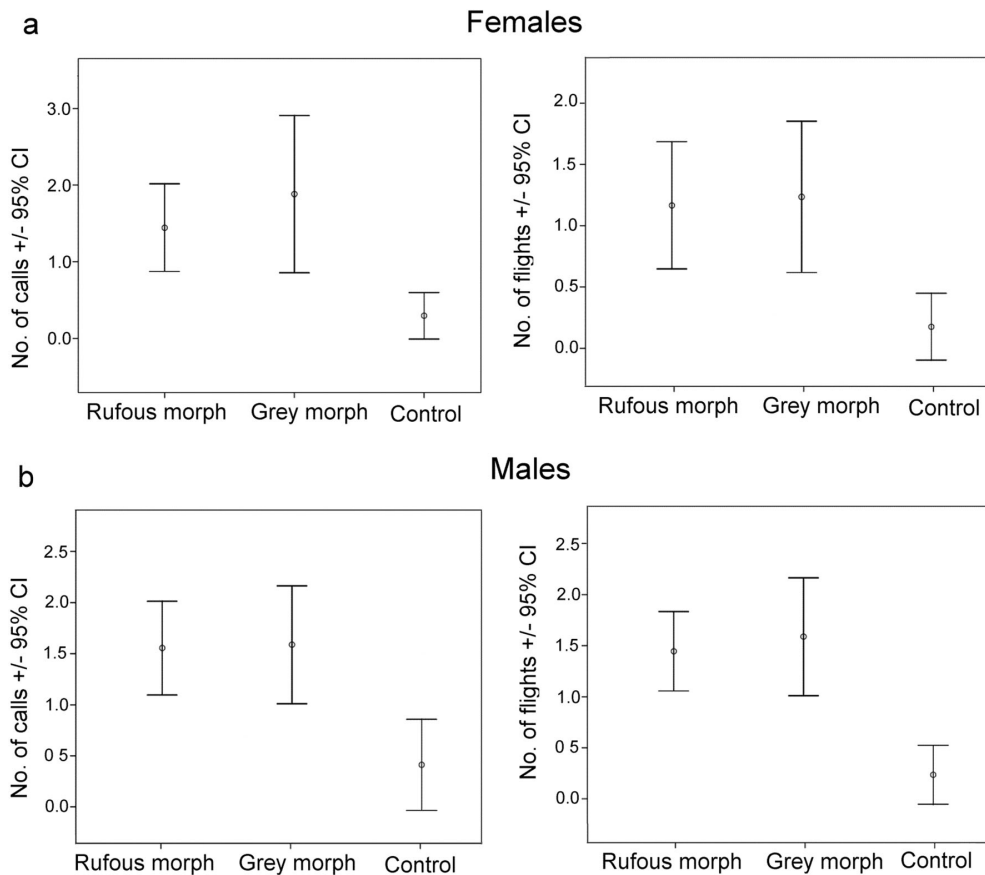


Fig. 3 Acoustic (number of calls) and movement (number of flights) responses of female (a) and male (b) common cuckoos (*Cuculus canorus*) to playback experimental trials where female cuckoo bubbling calls were played back with the demonstration of a rufous or grey model

of cuckoos, relative to controls (Eurasian collared dove (*Streptopelia decaocto*) calls with a collared dove model). Means and 95% confidence intervals are shown

Table 2 Responses of female and male common cuckoos (*Cuculus canorus*) to vocal playbacks of female cuckoos coupled with different colour morph models, based on a logistic generalized linear regression. The unit of measurement of each variable is indicated in parentheses

Focal birds' sex	Variable	Estimate	S.E.	z	p
Female	Intercept	0.039	1.005	0.039	0.969
	Time at closest detection (s)	− 0.002	0.004	− 0.535	0.592
	Latency of calling (s)	0.004	0.005	0.831	0.406
	Maximum length of continuous calling (s)	− 0.465	0.335	− 1.387	0.166
	No. of flights	0.308	0.304	1.015	0.310
Male	Intercept	0.025	0.533	0.048	0.962
	Distance at first detection (m)	− 0.020	0.033	− 0.590	0.552
	Closest distance (m)	0.002	0.037	0.060	0.952
	Time at first detection (s)	0.0004	0.004	0.100	0.915

in how female and male cuckoos responded to grey compared with rufous cuckoo models. Stepwise logistic regression retained the variables time of closest detection, latency of calling, maximal continuous calling, and the number of flights by females, and it retained distance in first detection, closest distance, and time of closest detection by males (Table 2). The PCAs also revealed sex-specific characteristics of cuckoo behaviour in our experiment. For example, in females, the variables of time at first detection and latency of calling showed the highest positive loadings for component 1, whereas distance at first detection and closest distance in males with positive signs and the number of flights with negative sign in males (Table 3).

Discussion

In our study area in Hungary, we did not find any behavioural or acoustic selectivity in adult cuckoo responses between the

presentations of either of the two colour morphs of conspecific models coupled with playbacks of female bubbling calls. Both males and females responded to the different morphs similarly. In an experiment performed in South Korea, male common cuckoos also treated the grey and rufous females similarly, except that they tried to copulate more frequently with the grey colour variant (Lee et al. 2019). However, the rufous morph was extremely rare in Korea and was practically lacking from many areas (e.g. it was represented by none of 18 females studied by Noh et al. 2016), and so, at that site, it probably represented an entirely novel colour variant when tested with male common cuckoos. Although male cuckoos in our study did not attempt to copulate with female models, the similar intensity of responses to both colour morphs of female adults was consistent with Lee et al.'s (2019) prediction based on their sexual harassment hypothesis for a population without any "rare" morphs present.

In general, plumage colour of birds is influenced by environmental and intrinsic factors; for example, plumage colour

Table 3 Component matrix of PCAs on female and male common cuckoos' (*Cuculus canorus*) responses to the cuckoo model presentations with playbacks of female cuckoo bubbling calls. The unit of measurement of each variable is indicated in parentheses

	Component						
	Females			Males			
	PC1	PC2	PC3	PC1	PC2	PC3	PC4
Distance at first detection (m)	− 0.424	0.221	0.762	0.773	− 0.228	0.185	0.438
Time at first detection (s)	0.858	0.042	0.090	0.534	0.609	0.107	0.071
Closest distance (m)	0.116	0.724	− 0.179	0.777	− 0.319	− 0.158	0.358
Time at closest detection (s)	0.590	0.137	0.569	0.376	0.024	0.873	0.043
No. of calls	− 0.461	− 0.377	0.111	− 0.403	0.577	0.337	0.427
Latency of calling (s)	0.820	0.372	− 0.024	0.530	0.551	− 0.013	− 0.232
Lengths of continuous calling (s)	− 0.563	0.530	0.361	0.175	− 0.656	0.530	− 0.410
No. of flights	0.193	− 0.525	0.104	− 0.758	0.094	0.500	0.046
No. of birds	0.410	− 0.457	0.450	− 0.513	− 0.422	− 0.044	0.526
Eigenvalue	2.684	1.639	1.302	2.939	1.779	1.480	1.009
Cumulative variance explained (%)	29.82	48.03	62.50	32.66	52.42	68.87	80.08

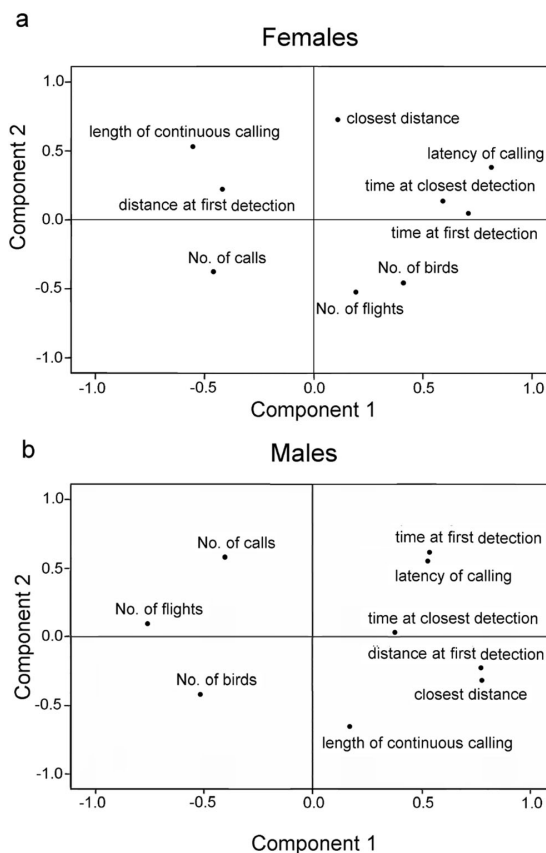


Fig. 4 PCA ordination plots of response variables of female (a) and male (b) common cuckoos (*Cuculus canorus*) to playbacks of female cuckoo bubbling calls and with grey and rufous colour morphs of model cuckoos. The component loadings are shown for the first two principal components

may change with advancing age, seasonally, due to parasite infection, and/or with variation in body condition (e.g. Badás et al. 2018; Delhey et al. 2006). In contrast, colour polymorphs are typically genetically encoded in birds, but see age-dependence in female tree swallow (*Tachycineta bicolor*) colouration (Hussell 1983) and delayed plumage maturation in many male birds (Hawkins et al. 2012). Frequencies of colour polymorphisms in a population seem to be relatively stable, and colour polymorphism often has adaptive value for reproduction for the rarer morph, its behaviour, and/or life history (Roulin 2004). For example, in the tawny owl (*Strix aluco*), female colour plumage polymorphism was associated with their reproductive strategy in that grey females produced offspring of higher quality than rufous females but they did not breed every year (Roulin et al. 2003). In contrast, in the present study, we revealed that the rufous morph of female cuckoos seemed to have no recognition advantage over the grey morph, at least from the viewpoint of territorial intrusions by conspecifics of either sex. However, we cannot exclude its

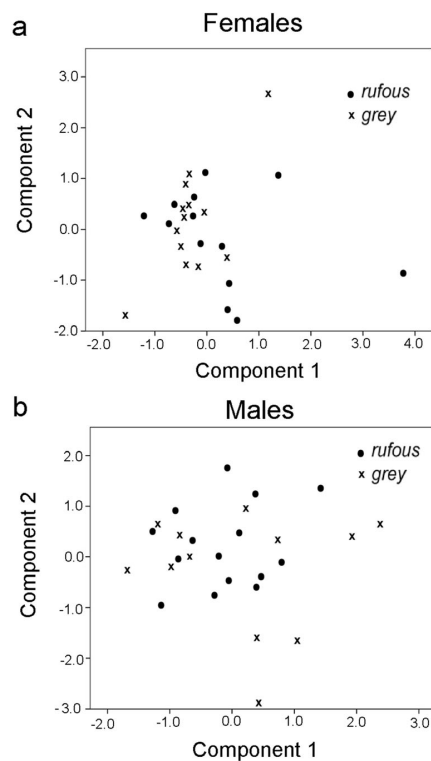


Fig. 5 PCA score plots of responses of female (a) and male (b) common cuckoos (*Cuculus canorus*) to playbacks of female cuckoo bubbling calls and with grey and rufous colour morphs of model cuckoos

potential role in other aspects of cuckoo breeding behaviour, which include host-evasion.

We showed that female common cuckoos pay close attention to intruding females in their territories and responded both acoustically with bubbling calls and visually by approaching the decoys. Previously there was only limited information on inter-female aggression in common cuckoos, which included scarce observations on direct female-female fights (Moskát and Hauber 2019). Riddiford (1986) observed that territory-holder female cuckoos expelled intruding non-territorial females. More recently, however, Lee et al. (2019) reported four cases when 3D female common cuckoo dummies were attacked by adult female cuckoos in a field experiment. After observing cuckoos equipped with radio transmitters, Dröscher (1988) stated that female cuckoos defended their laying territories from other females, especially in the morning. In our study population, we observed an arriving female cuckoo chased another female that was already perched on a tree. In another case, a flying female cuckoo was attacked in mid-air when it was in the proximity of another female cuckoo (C. Moskát pers. obs.). Probably there are many more such observations from different cuckoo populations, but there is a problem of identifying the sex of adult grey cuckoos in the

field accurately, except when these have been caught and marked with visible marks a priori, sexed morphologically or by DNA, and/or when radio telemetry or other tagging had been applied. Alternatively, whereas the sex of the rufous morph is clear (always female), the sex of the grey morph can still be identified when it produces male- or female-specific call types.

Our results demonstrated the importance of acoustic signals in inter-female recognition. In a recent study on the same population, we showed that female cuckoos responded to playback of females' bubbling calls (Moskát and Hauber 2019). In that experiment, about half of the female cuckoos moved closer to the speaker, which suggested that they were directing attention toward the simulated intruder. In the current study, the intensity or frequency of responses did not increase when a model cuckoo was placed at the speaker. In contrast to Lee et al. (2019), we did not observe any direct contact (e.g. mobbing or copulation attempt) with the cuckoo model, but the goals and the experimental protocols differed between the two studies. Lee et al. (2019) placed dummy cuckoos for a longer period at a site and left them for 20 min after the first positive response was observed. We studied female cuckoos' interest toward the appearance of new, unfamiliar conspecific females by following the protocol used in our previous study on female-female acoustic communications (Moskát and Hauber 2019). Our 4-min observational period was suitable to detect an acoustic response from male and female cuckoos and to attract their interest in the dummy cuckoo, which differed from the reactions toward the dove dummy used for control. Additionally, the type of dummy may result in a different intensity of responses toward the dummy. For example, Němec et al. (2015) revealed that red-backed shrikes (*Lanius collurio*) mobbed the dummy of the nest predator Eurasian jay (*Garrulus glandarius*) at various frequencies at their nests, which depended on the texture of the surface of the decoy presented. They behaved most aggressively toward a taxidermic mount, but the frequency of the attacks decreased toward a plush decoy, and the silicon decoy was attacked only when it was presented after the stuffed mount or the plush dummy.

Common cuckoos exhibit highly developed social lives (Davies 2000), although several details have not yet been described and understood. This species is thought to be polygynandrous (Marchetti et al. 1998; Wyllie 1975), where, in the simplest case, overlapping territories of 1–3 males encompass the laying area of a single female cuckoo. In our study area, we also found a similar spacing pattern of common cuckoos of overlapping territories of 1–3 males around an individual female cuckoo during their breeding season (Moskát and Hauber 2019). Male cuckoos that compete for females somewhat tolerate the presence of 1–2 additional (and presumably familiar) males in a female's laying area, but are intolerant toward non-familiar intruders (Moskát et al. 2017).

We agree with Gärtner (1981) that the most typical case of a female territorial system is where dominant females have more or less separate (partly overlapping) laying areas. In such a system, it would be crucial that female cuckoos recognise each other, advertise their claim for territories, and defend their territories. Because common cuckoos exhibit no parental care and defending an area with several host nests suitable for parasitism is costly, behavioural mechanisms to lower the intensity and costs of inter-female aggression are predicted to evolve. Female calling could be seen as such a mechanism because it elicits a quick response from male and female cuckoos locally. The difference revealed by PCAs for females and males was explained by the higher number of males than females at a trial site and by the more cryptic behaviour of the females in this species (Davies 2000). In essence, these characters explained the quicker responses of males than females to decoys coupled with the playback calls. Besides the latency and intensity of responses, the presumed behavioural functions of the two sexes when detecting and intercepting an intruder during the experimental trial were also different. Males were likely attempting to look for new mating possibilities, whereas females were trying to defend their existing resources (i.e. host nests).

Theory predicts that the typically rare cuckoo female colour morph (i.e. rufous) can be evolutionarily advantageous from several aspects (e.g. Mappes and Lindström 2012). However, we report statistically similar responses of female and male cuckoos toward rufous and grey models of cuckoos presented during playback experiments with female bubbling calls when the rufous morph was as common as the grey morph. Our study revealed that colour polymorphism did not affect territorial interactions amongst female cuckoos and social interactions with males. Therefore, we suggest that further studies should address the role of colour polymorphism in other socio-ecological contexts. This could include sexual selection, immunity trade-offs, and parasite loads (Ducrest et al. 2008; Arai et al. 2018). In brood parasite-host interactions, adaptations and counteradaptations from the two sides are of particular importance. Future studies should also focus on cuckoo-host interactions in the context of adult female colour polymorphism both at sites where the rufous morph is rare (most populations) and again in Hungary (where the rufous morph is common). For example, female cuckoos often parasitise host nests in the late afternoon (e.g. Davies and Brooke 1988; Honza et al. 2002), or even under dim light conditions at sunset (our observations in our study area), when the rufous morph could be less visible to hosts and, consequently, could be more advantageous for laying rufous cuckoos.

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Author contributions CM conceived and designed the experiment with input from ZE and MEH. CM, JR, and ZE performed the field experiments. AM and MB also participated in some of the fieldwork, and AM constructed 3D model birds. CM and ZE analysed data and visualised the results. CM wrote the manuscript, with contributions from MEH; all other authors edited and approved the final draft.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics statement The observations of cuckoos in this study were non-invasive. All international, national, and/or institutional guidelines for the care and use of animals were followed. Local ethical regulations and agreements were followed. All work complied with the Hungarian laws, and the Middle-Danube-Valley Inspectorate for Environmental Protection, 171900-3/20/2015).

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References

Aidala Z, Chong N, Anderson MG, Hauber ME (2012) Predicted visual sensitivity for short-wavelength light in the brood parasitic cuckoos of New Zealand. *Chin Birds* 3:295–301

Ajuira-Ibarra H, Reader T (2013) Reasons to be different: do conspicuous polymorphisms in invertebrates persist because rare forms are fitter? *J Zool* 290:81–85. <https://doi.org/10.1111/jzo.12034>

Arai E, Hasegawa M, Sato M, Sakai H, Ito S, Wakamatsu K (2018) Eumelanin levels in rufous feathers explain plasma testosterone levels and survival in swallows. *Ecol Evol* 9:2755–2764. <https://doi.org/10.1002/ece3.4946>

Badás EP, Martínez J, Rivero-de-Aguilar J, Ponce C, Stevens M, Serino S (2018) Colour change in a structural ornament is related to individual quality, parasites and mating patterns in the blue tit. *Sci Nat* 105: 17. <https://doi.org/10.1007/s00114-018-1539-z>

Bán M, Moskát C, Fülöp A, Hauber ME (2018) Return migration of common cuckoos (*Cuculus canorus*) between breeding grounds in Hungary and wintering grounds in Africa as documented by non-PTT GPS technology. *J Ornithol* 159:337–344. <https://doi.org/10.1007/s10336-017-1508-x>

Bártol I, Karcza Z, Moskát C, Röskaft E, Kisbenedek T (2002) Responses of great reed warblers *Acrocephalus arundinaceus* to experimental brood parasitism: the effects of a cuckoo *Cuculus canorus* dummy and egg mimicry. *J Avian Biol* 33:420–425. <https://doi.org/10.1034/j.1600-048X.2002.02945.x>

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MH, White JS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–135. <https://doi.org/10.1016/j.tree.2008.10.008>

Budka M, Matyjasiak P, Typiak J, Okoński M, Zagalska-Neubauer M (2019) Experienced males modify their behaviour during playback: the case of the chaffinch. *J Ornithol* 160:673–684. <https://doi.org/10.1007/s10336-019-01647-w>

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information theoretic approach. Springer, New York

Campobello D, Sealy SG (2010) Enemy recognition of reed warblers (*Acrocephalus scirpaceus*): threats and reproductive value act independently in nest defence modulation. *Ethology* 116:498–508. <https://doi.org/10.1111/j.1439-0310.2010.01764.x>

Carlson NV, Healy S, Templeton CN (2017) Hoo are you? Tits do not respond to novel predators as threats. *Anim Behav* 128:79–84. <https://doi.org/10.1016/j.anbehav.2017.04.006>

Caro T (2005) The adaptive significance of coloration in mammals. *BioScience* 55:125–136. [https://doi.org/10.1641/0006-3568\(2005\)055\[0125:TASOCI\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2005)055[0125:TASOCI]2.0.CO;2)

Chance EP (1940) The truth about the cuckoo. Country Life Ltd., London

Cuthill IC, Allen WL, Arbuckle K, Caspers B, Chaplin G, Hauber ME, Hill GE, Jablonski NG, Jiggins CD, Kelber A, Mappes J, Marshall J, Merrill R, Osorio D, Prum R, Roberts NW, Roulin A, Rowland HM, Sherratt TN, Skelhorn J, Speed MP, Stevens M, Stoddard MC, Stuart-Fox D, Talas L, Tibbetts E, Caro T (2017) The biology of color. *Science* 357:eaan 0221. <https://doi.org/10.1126/science.aan0221>

Davies NB (2000) Cuckoos, cowbirds and other cheats. Poyser, London

Davies NB, Brooke M de L (1988) Cuckoos versus reed warblers: adaptations and counteradaptations. *Anim Behav* 36:262–284. [https://doi.org/10.1016/S0003-3472\(88\)80269-0](https://doi.org/10.1016/S0003-3472(88)80269-0)

Davies NB, Welbergen JA (2008) Cuckoo–hawk mimicry? An experimental test. *Proc R Soc B* 275:1817–1822. <https://doi.org/10.1098/rspb.2008.0331>

Delhey K, Peters A, Johnsen A, Kempenaers B (2006) Seasonal changes in blue tit crown color: do they signal individual quality? *Behav Ecol* 17:790–798. <https://doi.org/10.1093/beheco/ari012>

Deng Z, Lloyd H, Xia C, Möller AP, Liang W, Zhang Y (2019) Components of variation in female common cuckoo calls. *Behav Process* 158:106–112. <https://doi.org/10.1016/j.beproc.2018.10.007>

Dröschler L (1988) A study on radio-tracking of the European cuckoo (*Cuculus canorus canorus*). In: van den Elzen R, Schuchmann K-L, Schmidt-Koenig K (eds) Proceedings of the International 100th Deutsche Ornithologische-Gesellschaft Meeting. Deutsche Ornithologen-Gesellschaft, Bonn, pp 187–193

Ducrest A-L, Keller L, Roulin A (2008) Pleiotropy in the melanocortin system, coloration and behavioural syndromes. *Trends Ecol Evol* 23:502–510. <https://doi.org/10.1016/j.tree.2008.06.001>

Dyrce A, Halupka L (2006) Great reed warbler *Acrocephalus arundinaceus* and reed warbler *Acrocephalus scirpaceus* respond differently to cuckoo dummy at the nest. *J Ornithol* 147:649–652. <https://doi.org/10.1007/s10336-006-0097-x>

Erritzøe J, Mann CF, Brammer FP, Fuller RA (2012) Cuckoos of the world. Bloomsbury, London

- Excoffier L, Foll M, Petit RJ (2009) Genetic consequences of range expansions. *Annu Rev Ecol Evol Syst* 40:481–501. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173414>
- Galeotti P, Rubolini D, Dunn PO, Fasola M (2003) Colour polymorphism in birds: causes and functions. *J Evol Biol* 16:635–646. <https://doi.org/10.1046/j.1420-9101.2003.00569.x>
- Gärtner K (1981) Das Wegnehmen von Wirtsvogeleiern durch den Kuckuck *Cuculus canorus*. *Ornithol Mitt* 33:115–131
- Gluckman TL, Mundy NI (2013) Cuckoos in raptors' clothing: barred plumage illuminates a fundamental principle of Batesian mimicry. *Anim Behav* 86:1165–1181. <https://doi.org/10.1016/j.anbehav.2013.09.020>
- Göth A, Hauber ME (2004) Ecological approaches to species recognition in birds through studies of model and non-model species. *Ann Zool Fenn* 41:823–842
- Hauber ME, Sherman PW, Paprika D (2000) Self-referent phenotype matching in a brood parasite: the armpit effect in brown-headed cowbirds (*Molothrus ater*). *Anim Cogn* 3:113–117. <https://doi.org/10.1007/s100710000071>
- Hawkins GL, Hill GE, Mercadante A (2012) Delayed plumage maturation and delayed reproductive investment in birds. *Biol Rev* 87:257–274. <https://doi.org/10.1111/j.1469-185X.2011.00193.x>
- Honza M, Taborsky B, Taborsky M, Teuschl Y, Vogl W, Moksnes A, Røskaft E (2002) Behaviour of female common cuckoos, *Cuculus canorus*, in the vicinity of host nests before and during egg laying: a radiotelemetry study. *Anim Behav* 64:861–868. <https://doi.org/10.1006/anbe.2002.1969>
- Honza M, Sicha V, Procházka P, Lezálová R (2006) Host nest defense against a color-dimorphic brood parasite: great reed warblers (*Acrocephalus arundinaceus*) versus common cuckoos (*Cuculus canorus*). *J Ornithol* 147:629–637. <https://doi.org/10.1007/s10336-006-0088-y>
- Horton BM, Hauber ME, Maney DL (2012) Morph matters: aggression bias in a polymorphic sparrow. *PLoS ONE* 7:e4875. <https://doi.org/10.1371/journal.pone.0048705>
- Hubbard JK, Uy JAC, Hauber ME, Hoekstra HE, Safran RJ (2010) Vertebrate pigmentation: from underlying genes to adaptive functions. *Trends Genet* 26:231–239. <https://doi.org/10.1016/j.tig.2010.02.002>
- Hurlbert SH (1984) Pseudoreplication and design of ecological field experiments. *Ecol Monogr* 54:187–211. <https://doi.org/10.2307/1942661>
- Hussell DJT (1983) Age and plumage color in female tree swallows. *J Field Ornithol* 54:312–318. <https://www.jstor.org/stable/4512839>
- Johansson K, Butlin RK (2017) What explains rare and conspicuous colours in a snail? A test of time-series data against models of drift, migration or selection. *Heredity* 118:21–30. <https://doi.org/10.1038/hdy.2016.77>
- Koleček J, Šulc M, Piálková R, Troscianko J, Požgayová M, Honza M, Procházka M (2019) Rufous common cuckoo chicks are not always female. *J Ornithol* 160:155–163. <https://doi.org/10.1007/s10336-018-1591-7>
- Kroodsmas DE (1989) Suggested experimental designs for song playbacks. *Anim Behav* 37:600–609. [https://doi.org/10.1016/0003-3472\(89\)90039-0](https://doi.org/10.1016/0003-3472(89)90039-0)
- Laczi M, Török J, Rosivall B, Hegyi G (2011) Integration of spectral reflectance across the plumage: implications for mating patterns. *PLoS ONE* 6:e23201. <https://doi.org/10.1371/journal.pone.0023201>
- Lee J-W, Kim H-N, Yoo S, Yoo J-C (2019) Common cuckoo females may escape male sexual harassment by color polymorphism. *Sci Rep* 9:7515. <https://doi.org/10.1038/s41598-019-44024-6>
- Lei F-M, Zhao H-F, Wang A-Z, Yin Z-H, Payne RB (2005) Vocalizations of the common cuckoo *Cuculus canorus* in China. *Acta Zool Sin* 51:31–37
- Li D, Wei H, Zhang Z, Liang W, Stokke BG (2015) Oriental reed warbler (*Acrocephalus orientalis*) nest defence behaviour towards brood parasites and nest predators. *Behaviour* 152:1601–1621. <https://doi.org/10.1163/1568539X-00003295>
- Liang W, Möller AP (2015) Hawk mimicry in cuckoos and anti-parasitic aggressive behavior of barn swallows in Denmark and China. *J Avian Biol* 46:216–223. <https://doi.org/10.1111/jav.00515>
- Louder MIM, Balakrishnan CN, Louder ANA, Driver RJ, London SE, Hauber ME (2019) An acoustic password enhances auditory learning in juvenile brood parasitic cowbirds. *Curr Biol* 29:1–7. <https://doi.org/10.1016/j.cub.2019.09.046>
- Lovási P, Moskát C (2004) Break-down of arms race between the red-backed shrike (*Lanius collurio*) and common cuckoo (*Cuculus canorus*). *Behaviour* 141:245–262. <https://doi.org/10.1163/156853904322890843>
- Ma L, Yang C, Liang W (2018) Hawk mimicry does not reduce attacks of cuckoos by highly aggressive hosts. *Avian Res* 9:35. <https://doi.org/10.1186/s40657-018-0127-4>
- Mappes J, Lindström L (2012) How did the cuckoo get its polymorphic plumage? *Science* 337:532. <https://doi.org/10.1126/science.1225997>
- Marchetti K, Nakamura H, Gibbs HL (1998) Host-race formation in the common cuckoo. *Science* 282:471–472. <https://doi.org/10.1126/science.282.5388.471>
- Marton A, Fülöp A, Ozogány K, Moskát C, Bán M (2019) Host alarm calls attract the unwanted attention of the brood parasitic common cuckoo. *Sci Rep* 9:18563. <https://doi.org/10.1038/s41598-019-54909-1>
- McGraw KJ, Safran RJ, Wakamatsu K (2005) How feather colour reflects its melanin content. *Funct Ecol* 19:816–821. <https://doi.org/10.1111/j.1365-2435.2005.01032.x>
- Mikulica O, Grim T, Schulze-Hagen K, Stokke BG (2017) The cuckoo: the uninvited guest. Wild Nature Press, Plymouth
- Moksnes A, Røskaft E, Braa AT, Korsnes L, Lampe HM, Pedersen HC (1991) Behavioural response of potential hosts towards artificial cuckoo eggs and dummies. *Behaviour* 116:64–89. <https://doi.org/10.1163/156853990X00365>
- Moskát C, Hauber ME (2019) Sex-specific responses to simulated territorial intrusions in the common cuckoo: a dual function of female acoustic signaling. *Behav Ecol Sociobiol* 73:60. <https://doi.org/10.1007/s00265-019-2665-0>
- Moskát C, Honza M (2000) Effect of nest and nest site characteristics on the risk of cuckoo *Cuculus canorus* parasitism in the great reed warbler *Acrocephalus arundinaceus*. *Ecography* 23:335–341. <https://doi.org/10.1111/j.1600-0587.2000.tb00289.x>
- Moskát C, Elek Z, Bán M, Geltsch N, Hauber ME (2017) Can common cuckoos discriminate between neighbours and strangers by their calls? *Anim Behav* 126:253–260. <https://doi.org/10.1016/j.anbehav.2017.02.013>
- Moskát C, Bán M, Fülöp A, Bereczki J, Hauber ME (2019) Bimodal habitat use in brood parasitic common cuckoos (*Cuculus canorus*) revealed by GPS telemetry. *Auk* 136:1–12. <https://doi.org/10.1093/auk/uky019>
- Mullen P, Pohland G (2008) Studies on UV reflection in feathers of some 1000 bird species: are UV peaks in feathers correlated with violet-sensitive and ultraviolet-sensitive cones? *Ibis* 150:59–68. <https://doi.org/10.1111/j.1474-919X.2007.2007.00736.x>
- Nakamura H, Miyazawa Y (1997) Movements, space use and social organization of radio-tracked common cuckoos during the breeding season in Japan. *Jpn J Ornithol* 46:23–54. <https://doi.org/10.3838/jjo.46.23>
- Nakamura H, Miyazawa Y, Kashiwagi K (2005) Behavior of radio-tracked common cuckoo females during the breeding season in Japan. *Ornithol Sci* 4:31–41. <https://doi.org/10.2326/osj.4.31>
- Němec M, Syrová M, Dokoupilová L, Veselý P, Šmilauer P, Landová E, Lišková S, Fuchs R (2015) Surface texture and priming play

- important roles in predator recognition by the red-backed shrike in field experiments. *Anim Cogn* 18:259–268. <https://doi.org/10.1007/s10071-014-0796-2>
- Noh H-J, Lee J-W, Yoo J-C (2016) Color morph variation in two brood parasites: common cuckoo and lesser cuckoo. *Ornithol Sci* 15:109–117. <https://doi.org/10.2326/osj.15.109>
- Payne RB (1967) Interspecific communication signals in parasitic birds. *Am Nat* 101:363–375. <https://doi.org/10.1086/282504>
- Payne RB (2005) The cuckoos. Oxford University Press, New York
- Payne RB, Payne LL, Woods JL, Sorenson MD (2000) Imprinting and the origin of parasite–host species associations in brood-parasitic indigobirds, *Vidua chalybeata*. *Anim Behav* 59:69–81. <https://doi.org/10.1006/anbe.1999.1283>
- R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>. Accessed 04 Sep 2019
- Riddiford N (1986) Why do cuckoos *Cuculus canorus* use so many species of hosts? *Bird Study* 33:1–5. <https://doi.org/10.1080/0006358609476883>
- Røskoft E, Moksnes A, Stokke BG, Bicik V, Moskát C (2002a) Aggression to dummy cuckoos by potential European cuckoo hosts. *Behaviour* 139:613–628. <https://doi.org/10.1163/15685390260136735>
- Røskoft E, Moksnes A, Stokke BG, Moskát C, Honza M (2002b) The spatial habitat structure of host populations explains the pattern of rejection behaviour in hosts and parasitic adaptations in cuckoos. *Behav Ecol* 13:163–168. <https://doi.org/10.1093/beheco/13.2.163>
- Roulin A (2004) The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biol Rev* 79:815–848. <https://doi.org/10.1017/S1464793104006487>
- Roulin A, Ducret B, Ravussin P-A, Altwegg R (2003) Female colour polymorphism covaries with reproductive strategies in the tawny owl *Strix aluco*. *J Avian Biol* 34:393–401. <https://doi.org/10.1111/j.0908-8857.2003.03139.x>
- San-Jose LM, Séchaud R, Schalcher K, Jüdes C, Questiaux A, Oliveira-Xavier A, Gémard C, Almasi B, Béziers P, Kelber A, Amar A, Roulin A (2019) Differential fitness effects of moonlight on plumage colour morphs in barn owls. *Nat Ecol Evol* 3:1331–1340. <https://doi.org/10.1038/s41559-019-0967-2>
- Sato NJ, Tanaka KD, Okahisa Y, Yamamichi M, Kuehn R, Gula R, Ueda K, Theuerkauf J (2015) Nestling polymorphism in a cuckoo-host system. *Curr Biol* 25:R1164–R1165. <https://doi.org/10.1016/j.cub.2015.11.028>
- Soler M (ed) (2017) Avian brood parasitism: behaviour, ecology, evolution and coevolution. Springer Int Pub, Cham
- Soler M, Soler JJ (1999) Innate versus learned recognition of conspecifics in great spotted cuckoos *Clamator glandarius*. *Anim Cogn* 2:97–102. <https://doi.org/10.1007/s100710050029>
- Sprau P, Roth T, Amrhein V, Naguib M (2014) Effects of previous intrusion pressure on territorial responses in nightingales. *J Ornithol* 155:111–119. <https://doi.org/10.1007/s10336-013-0993-9>
- Stoddard MC, Hauber ME (2017) Colour, vision and coevolution in avian brood parasitism. *Philos Trans R Soc B* 372:2016.0339. <https://doi.org/10.1098/rstb.2016.0339>
- Svensson EI, Abbott JA, Gosden TP, Coreau A (2009) Female polymorphisms, sexual conflict and limits to speciation processes in animals. *Evol Ecol* 23:93–108. <https://doi.org/10.1007/s10682-007-9208-2>
- Tanaka K (2016) Polymorphism in avian brood parasitism: a coevolutionary perspective. *Ornithol Sci* 15:133–140. <https://doi.org/10.2326/osj.15.133>
- Thorogood R, Davies NB (2012) Cuckoos combat socially transmitted defenses of reed warbler hosts with a plumage polymorphism. *Science* 337:578–580. <https://doi.org/10.1126/science.1220759>
- Thorogood R, Davies NB (2013) Hawk mimicry and the evolution of polymorphic cuckoos. *Chin Birds* 4:39–50
- Toral GM, Figuerola J, Negro JJ (2008) Multiple ways to become red: pigment identification in red feathers using spectrometry. *Comp Biochem Physiol Part B* 150:147–152. <https://doi.org/10.1016/j.cbpb.2008.02.006>
- Trnka A, Grim T (2013) Color plumage polymorphism and predator mimicry in brood parasites. *Front Zool* 10:25. <https://doi.org/10.1186/1742-9994-10-25>
- Trnka A, Trnka M, Grim T (2015) Do rufous common cuckoo females indeed mimic a predator? An experimental test. *Biol J Linn Soc* 116: 134–143. <https://doi.org/10.1111/bj.12570>
- Tryjanowski P, Morelli F, Kwieciński Z, Indykiewicz P, Möller AP (2018a) Birds respond similarly to taxidermic models and live cuckoos *Cuculus canorus*. *J Ethol* 36:243–249. <https://doi.org/10.1007/s10164-018-0554-z>
- Tryjanowski P, Morelli F, Osiejuk TS, Möller AP (2018b) Functional significance of cuckoo *Cuculus canorus* calls: responses of conspecifics, hosts and non-hosts. *PeerJ* 6:e5302. <https://doi.org/10.7717/peerj.5302>
- Van Horn KS (2015) Which link function — logit, probit, or cloglog? *Bayesian Analytics*. 2015-08-14. <https://bayesian.com/wp-content/uploads/2015/08/logit-probit-cloglog.pdf>. Accessed 04 Sep 2019
- Vesely P, Bursiková M, Fuchs R (2016) Birds at the winter feeder do not recognize an artificially coloured predator. *Ethology* 122:937–944. <https://doi.org/10.1111/eth.12565>
- Vogl W, Taborsky B, Taborsky M, Teuschl Y, Honza M (2004) Habitat and space use of European cuckoo females during the egg laying period. *Behaviour* 141:881–898. <https://doi.org/10.1163/1568539042265671>
- Voipio P (1953) The hepaticus variety and the juvenile types of the cuckoo. *Ornis Fenn* 30:97–117
- Welbergen JA, Davies NB (2011) A parasite in wolf's clothing: hawk mimicry reduces mobbing of cuckoos by hosts. *Behav Ecol* 22:574–579. <https://doi.org/10.1093/beheco/arn008>
- White TE, Kemp DJ (2016) Colour polymorphism. *Curr Biol* 26:R517–R518. <https://doi.org/10.1016/j.cub.2016.03.017>
- Williams LJ, King AJ, Mettke-Hofmann C (2012) Colourful characters: head colour reflects personality in a social bird, the Gouldian finch, *Erythrura gouldiae*. *Anim Behav* 84:159–165. <https://doi.org/10.1016/j.anbehav.2012.04.025>
- Wyllie I (1975) Study of cuckoos and reed warblers. *British Birds* 68: 369–378
- Wyllie I (1981) The cuckoo. Batsford, London
- Xia C, Deng Z, Lloyd H, Möller AP, Zhao X, Zhang Y (2019) The function of three main call types in common cuckoo calls. *Ethology* 125:652–659. <https://doi.org/10.1111/eth.12918>
- Yoo S, Kim N-N, Lee J-W, Yoo J-C (2019) Seasonal and diurnal patterns of population vocal activity in avian brood parasites. *Ibis* (in press). <https://doi.org/10.1111/ibi.12741>
- York JE, Davies NB (2017) Female cuckoo calls misdirect host defences towards the wrong enemy. *Nat Ecol Evol* 1:1520–1525. <https://doi.org/10.1038/s41559-017-0279-32>
- Zölei A, Bán M, Moskát C (2015) No change in common cuckoo *Cuculus canorus* parasitism and great reed warblers' *Acrocephalus arundinaceus* egg rejection after seven decades. *J Avian Biol* 46: 570–576. <https://doi.org/10.1111/jav.00673>

12. Study IV:

Call rate in common cuckoos does not predict body size and responses to conspecific playbacks

Elek, Z., Bán, M., Fülöp, A., Marton, A., Hauber, M. E. & Moskát, C.

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Abstract

The brood parasitic common cuckoo *Cuculus canorus* is best known for its two-note “cu-coo” call which is almost continuously uttered by male during the breeding season and can be heard across long distances in the field. Although the informative value of the cuckoo call was intensively investigated recently, it is still not clear whether call characteristic(s) indicate any of the phenotypic traits of the respective vocalising individuals. To fill this gap, we studied whether the call rate of male cuckoos (i.e., the number of calls uttered per unit of time) provides information on their body size, which might be a relevant trait during intrasexual territorial conflicts. We captured free-living male cuckoos and measured their body size parameters (mass, wing, tail and tarsus lengths). Each subject was then radio-tagged, released, and its individual “cu-coo” calls were recorded soon after that in the field. The results showed that none of the body size parameters covaried statistically with the call rates of individual male common cuckoos. In addition, we experimentally tested whether the “cu-coo” call rates affect behavioural responses of cuckoos using playbacks of either a quicker or a slower paced call than the calls with natural rates. Cuckoos responded similarly to both types of experimental playback treatments by approaching the speaker with statistically similar levels of responses as when presented with calls at the natural rate. We conclude that male common cuckoos do not advertise reliable information acoustically regarding their body size, and so, cuckoo calls are neither useful to characterize cuckoos’ phenotypic traits directly nor to indicate environmental quality indirectly.



Call rate in Common Cuckoos does not predict body size and responses to conspecific playbacks

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Abstract

The brood parasitic Common Cuckoo *Cuculus canorus* is best known for its two-note “cu-coo” call which is almost continuously uttered by male during the breeding season and can be heard across long distances in the field. Although the informative value of the cuckoo call was intensively investigated recently, it is still not clear whether call characteristic(s) indicate any of the phenotypic traits of the respective vocalising individuals. To fill this gap, we studied whether the call rate of male cuckoos (i.e., the number of calls uttered per unit of time) provides information on their body size, which might be a relevant trait during intrasexual territorial conflicts. We captured free-living male cuckoos and measured their body size parameters (mass, wing, tail and tarsus lengths). Each subject was then radio-tagged, released, and its individual “cu-coo” calls were recorded soon after that in the field. The results showed that none of the body size parameters covaried statistically with the call rates of individual male Common Cuckoos. In addition, we experimentally tested whether the “cu-coo” call rates affect behavioural responses of cuckoos using playbacks of either a quicker or a slower paced call than the calls with natural rates. Cuckoos responded similarly to both types of experimental playback treatments by approaching the speaker with statistically similar levels of responses as when presented with calls at the natural rate. We conclude that male Common Cuckoos do not advertise reliable information acoustically regarding their body size, and so, cuckoo calls are neither useful to characterize cuckoos’ phenotypic traits directly nor to indicate environmental quality indirectly.

Keywords Call rate · Body size · Playback · Territorial signalling

Zusammenfassung

Von der Rufrate des Kuckucks lässt sich weder auf die Körpergröße noch auf die Reaktionen von Artgenossen auf das Vorspielen der Rufe schließen

Der Brutparasit Kuckuck (*Cuculus canorus*) ist vor allem für seinen Ruf „gu-kuh“ bekannt, der während der Brutzeit von den Männchen fast ununterbrochen ausgestoßen wird und im Freiland über weite Entfernungen zu hören ist. Obwohl der Informationsgehalt des Kuckuckrufs in jüngster Zeit intensiv untersucht wurde, ist immer noch nicht klar, ob die Rufcharakteristik(en) auf irgendwelche phänotypischen Merkmale des rufenden Individuums hinweisen. Um diese Lücke

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zu schließen, untersuchten wir, ob die Rufrate männlicher Kuckucke (d.h. die Anzahl der pro Zeiteinheit geäußerten Rufe) Informationen über ihre Körpergröße liefert, was innerhalb des gleichen Geschlechts ein für Revierkonflikte relevantes Merkmal sein könnte. Wir fingen freilebende männliche Kuckucke und maßen ihre Körpergrößenparameter (Masse, Flügel-, Schwanz- und Tarsuslänge). Jedes Tier wurde dann mit einem Sender versehen, wieder freigelassen und seine individuellen Rufe kurz darauf im Freiland aufgezeichnet. Die Ergebnisse zeigten, dass keiner der Körpergrößenparameter statistisch mit den Rufraten einzelner männlicher Kuckucke kovarierte. Zusätzlich testeten wir experimentell, ob die „gu-kuh“-Rufraten die Reaktionen anderer Kuckucke beeinflussten, indem wir die Wiedergabe der Rufe entweder schneller oder langsamer als mit den natürlichen Rufraten abspielten. Die Kuckucke reagierten im Experiment auf beide Wiedergabegeschwindigkeiten ähnlich, indem sie sich dem rufenden Vogel mit statistisch ähnlichen Rufraten näherten. Wir schließen daraus, dass männliche Kuckucke auf akustischem Weg keine verlässlichen Informationen über ihre Körpergröße abgeben und ihre Rufe daher nicht geeignet sind, direkt auf phänotypische Merkmale schließen zu lassen oder indirekt Informationen über die Umweltqualität zu geben.

Introduction

Acoustic communication is fine-tuned across diverse avian lineages. Auditory signals, including songs and calls, can carry information both about and for conspecifics and/or heterospecifics (Bradbury and Vehrencamp 2011). Typically, learned songs offer complex ways to express vocal signals, but are restricted to a handful, species-rich lineages, including songbirds, hummingbirds, and parrots. Many other bird taxa communicate with simpler and non-learned vocalisations, termed calls (Brenowitz 1991; Kumar 2003). The calls and songs of individual birds often show variation within a population (Catchpole and Slater 2008), depend on several factors (e.g., age, sex, body size, and nutritional condition), and may function as honest signals of the individual quality of the signaller (Galeotti et al. 1997; Redpath et al. 2000; Murphy et al. 2008). For example, body size of birds may reliably affect songs' or calls' acoustic characteristics (Hall et al. 2013; Derryberry et al. 2018; but also see Brumm 2009; Patel et al. 2010; Budka and Osiejuk 2013).

Common Cuckoos *Cuculus canorus* (hereafter: cuckoos) are obligate brood parasites, utilising other avian species for incubating, feeding, and rearing their offspring (Makatsch 1955; Payne 2005; Erritzoe et al. 2012). Cuckoos exhibit complex social lives (Davies 2000), probably as the consequence of being emancipated from several costly and time-consuming forms of parental care. The vocalisation of cuckoos is known to play an important part in conspecific communication, for example, in territorial disputes both between males (Moskát et al. 2017; Yoo et al. 2020), but also between females (Deng et al. 2019a; Moskát and Hauber 2019; Xia et al. 2019).

Male cuckoos utter almost continuously their characteristic “cu-coo” calls during the breeding season (Wyllie 1981); therefore, they can be detected relatively easily. Previous studies attempted to use the cuckoos' presence in an area as an indicator of the naturalness (i.e., the opposite of anthropogenic disturbance) of the habitat, including as a predictor

avian species richness (Morelli et al. 2015) and diversity (Tryjanowski and Morelli 2015). Although several factors may affect cuckoo calling behaviour (Benedetti et al. 2018), this line of studies treated the length of continuous calling as a positive predictor of avian species richness (Benedetti et al. 2018) or individual cuckoo quality (Møller et al. 2016). As vocal production is metabolically costly (e.g., Oberweger and Goller 2001), individuals may advertise their higher quality by decreasing the amount of time elapsed between two calls (i.e., increasing call rate), or increasing the speed of the uttered calls, both of which result in higher number of calls per unit of time. Call rate is an honest indicator of body size and condition in several bird species (e.g., Redpath et al. 2000; Garamszegi et al. 2004); therefore, it is also expected to be a suitable signal to advertise individual quality (i.e., phenotype) in cuckoos.

Although body size or condition may affect acoustic parameters of calling also in cuckoos, no study analysed this potential relationship previously in this particular species. Larger body size might have advantages for cuckoos, as larger-bodied birds tend to have larger territories and are capable of producing louder acoustic signals (Catchpole and Slater 2008). Here we compared body size parameters (mass, and tarsus, wing, and tail sizes) and an estimated body condition index with the call rate in free-living male Common Cuckoos, tagged with telemetry transmitters. We hypothesised that larger individuals (e.g., heavier in weight or with greater body length parameters) produced calls more dynamically, i.e., with higher call rates than others that are smaller. Previous studies suggested indirectly that body condition may affect the syllabus production rate of cuckoos (e.g., Tryjanowski et al. 2018), and therefore, to test if the call rates have a signalling value between male cuckoos, we carried out a field experiment, by playing back calls at natural or modified (i.e., quicker and slower) rates. Here we predicted that territorial males would respond more aggressively to the playbacks with higher call rates (i.e., quicker) than to those of lower call rates (i.e., slower variant).

Materials and methods

Study area

Field work was conducted in a 20×30 km area around Apaj, central Hungary (47° 6′ 53.9″ N; 19° 5′ 21.2″ E), ca. 50 km south of Budapest, in May between 2016 and 2019. Here Common Cuckoos parasitize Great Reed Warblers *Acrocephalus arundinaceus* in the reedbeds of a network of small irrigation and flood relief canals, connected with the river Danube. This host species prefers reedbed edges and other edge-like habitats along the canals for breeding (Moskát and Honza 2002; MÉRŐ et al. 2015). Common Cuckoos typically perch on and move between the trees along the banks of the canals, holding partly overlapping territories (Moskát et al. 2019).

Field procedures

We captured Common Cuckoos with mist-nets using playbacks of male and female cuckoos to attract them. Upon capture, we sexed cuckoos by morphological characters (Svensson et al. 2010; also confirmed by DNA analysis in 2016 and 2017; Moskát et al. 2019), then we measured the following body size metrics: *body mass* (g; to the nearest 1 g with a Pesola spring scale), *wing and tail length* (mm; to the nearest 1 mm, with a ruler), and *tarsus length* (to the nearest 0.1 mm; with a calliper). Following measurements, we tagged each cuckoo with a 1.2 g (1% of the body mass) radio transmitter (Pip3, type 392 by Biotrack Ltd; for more details, see Moskát et al. 2017) and released them at the site of capture.

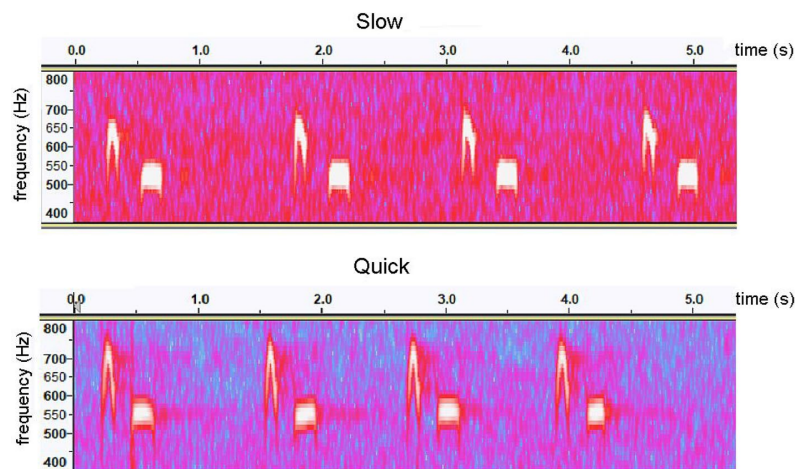
In the field we followed the tagged cuckoos and identified them individually using a Sika receiver (Biotrack Ltd.) and

a flexible 3-element Yagi antenna (Biotrack Ltd.). Calls of male cuckoos were recorded 2–5 days after they had been captured (mean ± SD = 3.21 ± 2.05) and tagged, using a Telinga Universal parabola dish with Rycota Hi Wind Cover, a Marantz PMD-620 MKII sound recorder (48 kHz sampling rate, 24-bit quality, wav format), a Sennhesiser ME62 microphone, with a K6 powering module and a FEL MX mono preamp.

We calculated the natural call rate of cuckoos by measuring the number of continuously uttered calls divided by the length in sec. To obtain a representative sample, we chose sound recordings randomly ($n = 18$), where the calling sequence was complete, used for assessing our call rate measurements on radio-tagged cuckoos ($n = 23$). As call rate could be measured in several ways, e.g., by dividing the number of calls per time for a full recording, or a section of continuous calling including short pauses and/or other call types than “cu-coo” (e.g., the 3-note “cu-cu-coo”, c.f. Xia et al. 2019), we chose complete sequences with no pause and containing only the “cu-coo” call type (Fig. 1). We also took care not to sample just after a female conspecific’s bubbling call (Chance 1940; Wyllie 1981) or avoiding any other potential effect might impact the tempo of calling (e.g., the arrival of a new male or female cuckoo).

Wyllie (1981) mentioned that this species’ males uttered 10–20 (up to 270) calls in uninterrupted series, with short inter-series intervals. Møller et al. (2017) measured a range of 1–45 repeats (mean ± SE: 15.6 ± 2.0) in 24 male cuckoos. We did not analyse the maximum number of syllables uttered continuously, which had been suggested to be an indicator of individual quality in male Common Cuckoos (Møller et al. 2016; Tryjanowski et al. 2018). We did not opt for this approach, because cuckoos live at high densities in our study area (Moskát and Honza 2002), and many conspecifically initiated social interactions disrupt continuous callings of individual

Fig. 1 Representative examples of quicker and slower call rates of free-living male Common Cuckoos. Both contained complete calling series of four “cu-coo” calls



male cuckoos at our study site (CM pers. obs.). Nonetheless, we observed the longest calling sequences from solitary, newly arriving male cuckoos (over 100 calls per series, where similar series were repeated several times after a pause of few seconds). Consequently, the numbers of syllables uttered continuously are unlikely to function as honest indicators of body size or condition (sensu Maynard Smith and Harper 2003) in our study area with high cuckoo density. Instead, here we used the temporal frequency of the calls uttered during unit of time (i.e., call rate) as a proxy for this metric (e.g., Yorzinski and Vehrencamp 2009; Carlson et al. 2017). We also conducted a playback experiment manipulating this trait to elicit behavioural responses of territorial male cuckoos (see below).

Playback experiment

We carried out a field experiment to test the function of one specific acoustic variable (call rate, i.e., the number of calls uttered per unit of time (s)) expected to be associated with body size and/or condition (Podos 1997; Martin et al. 2011; Weiss et al. 2012; Nishida and Takagi 2018), in a territorial context. We manipulated original cuckoo call recordings either by reducing or increasing the length of pauses among syllables, producing “quicker” or “slower” audio files for playbacks (Fig. 1), and we also used behavioural response data to cuckoo call sequences played back at the natural, unmanipulated speed (“normal”) from 2016. As the “cu-coo” call’s main function is territorial defence (Moskát et al. 2017; Tryjanowski et al. 2018), we also expected that the rate of cuckoo calls affected territorial display efficiency. Consequently, the playback file with higher or lower call rates may attract more or fewer conspecific males, respectively.

Here, we utilised the file structure we already used in previous experiments (2-min audio files, containing 3×30 s sequence of syllables, and 15 s pauses among the sequences; see more details of the basic call playback file structure in Moskát et al. 2017). The playback files with “normal” speed contained 20.4 ± 0.76 (mean \pm SE; range: 9–24) calls in the basic, 30 s, unit of the playback files, the “quicker” files had 28.0 ± 1.3 calls (range: 18–36), and the “slower” files contained 15.2 ± 0.69 calls (range: 9–18). The number of calls per unit of time differed both in the “quicker” and “slower” files when compared to the calling frequency in the “normal” file (Mann–Whitney U test: quicker vs. normal: $z_{17,15} = -3.888$, $P < 0.001$; slower vs. normal: $z_{16,15} = -3.959$, $P < 0.001$). Playback files were constructed with the Audacity 2.1.0. program, and we manipulated call rate by reducing or increasing inter-call intervals. The “quicker” vs. “slower” comparison of call numbers was, of course, also highly significant ($z_{17,16} = -4.843$, $P < 0.001$).

For the call rate experiment we searched for playback sites along the wooded parts of irrigation canals inhabited by host

Great Reed Warblers in a slow-moving car. Experimental trials were initiated at sites, where a male cuckoo was heard and seen within 80 m. For playback, we used a JBL Xtreme (40 W) loudspeaker, connected to a Lenovo TAB 2 A7 tablet with a 20 m audio cable (see more technical details of the playback in Moskát et al. 2017). The loudspeaker was placed on a tree ~1.5 m height, and two observers handled the equipment and observed wild cuckoos while hiding behind a bush. Observations on cuckoos were dictated onto a Tascam dr-05 ver2 sound recorder. To avoid pseudoreplication (sensu Hurlbert 1984; Kroodsmas 1989) we played a sound file only once, and selected the consecutive trial sites for playback at least 1 km distance from each other along the canals to use an individual focal cuckoo only once (e.g., Moskát et al. 2017). Playback experiments with quicker and slower treatments were carried out between May 6 and 11, 2018, in the early hours of the day (between 6 and 11 h). We also used data from playbacks of normal-speed cuckoo calls as a control for the speed manipulation specifically, and a harmless, similarly sized sympatric species, the Eurasian Collared Dove (*Streptopelia decaocto*) from May 2016 (Moskát et al. 2017) as a positive control, following a similar protocol to the experimental trials described above. All playbacks were conducted under good weather conditions, avoiding rainy, windy, or hot periods of the days. The sex of cuckoos was identified by their sex-specific calls and the partially sexually dichromatic plumage characteristics in this species (e.g., Moskát et al. 2020).

We analysed the following behavioural variables to characterize the cuckoos’ responses during the experimental playback trials in two sets of tests:

(i) A robust comparison of the effects of call rate for the categories “quicker”, “normal”, and “slower”, together with the dove calls used for general control, as these have proven to be the most important responses to playbacks in previous experiments on the Common Cuckoo (e.g., Moskát et al. 2017; Moskát and Hauber 2019).

Movements: A binary variable expressing if the focal cuckoo approached the speaker during the 2-min playback (Y/N).

Closest distance (m): The closest value of distance when the focal bird approached the speaker during a playback trial, either by flying or sitting on a nearby tree.

(ii) A more detailed comparison of call rate modulation (for the categories “quicker” and “slower”) to reveal fine-tuned differences in Common Cuckoos’ responses:

Distance to first detection (m): The distance of a cuckoo from the speaker when it appeared or called in the vicinity of the speaker.

Latency of first detection (s): Time spent from the start of playback until the first visual or vocal appearance of the cuckoo in the vicinity of the speaker.

Closest distance (m): The shortest distance between the cuckoo and the speaker observed during the full playback period. Approaching the speaker closely can be regarded the most important variable indicating positive response to cuckoo playbacks (Moskát et al. 2017).

Latency of closest detection (s): Time spent from the start of playback until the closest appearance of the cuckoo to the speaker during the observational period.

Latency of calling (s): Time spent from the start of playback until the male cuckoo started calling.

Length of continuous calling (s): The longest continuous calling sequence within the observational period.

Number of flights: Number of flights of the focal bird during the observational period. Cuckoos often flew away or towards the speaker, above it, or flew circle-like routes around the speaker, then sat on a tree. Some of them later repeated the same movement(s) once or several times.

Number of birds: The number of male cuckoos observed in the vicinity of the speaker during the playback.

Statistical analyses

In addition to using body size metrics, to characterise body condition, we used the residual index (Gould 1975), where the body mass is regressed on body size, and the residuals provide an estimate of condition (e.g., Jakob et al. 1996). Using this approach, we offset the size effect per se in the estimation of the physiological condition state of each subject, thus this index reflects to the true body mass of an individual without size constraints; we further we refer as physiological condition index (PCI).

We compared the two sets of call rates with Mann–Whitney *U* test in SPSS ver. 17.0 (SPSS Inc., Chicago, IL). We also used the glm function in the R 3.6.1 package (R core team 2019) for generalised linear model with quasi-Poisson error term (glm, Bolker et al. 2009), where call rate was the dependent variable, year was the covariate, and body size parameters (mass, PCI, tarsus, wing, and tail lengths) as fixed effects. Year and the linear predictor were adjusted by the number of birds/year. Data on body sizes collected in 2016 ($n=6$ from the total 29) were omitted from glm analyses, due to the lack of tarsus data from that year. We also included second order interaction terms in the model, i.e., years \times body size parameters.

We also used binary logistic regression to compare cuckoos' behaviour to the playbacks with call rate manipulation. In the model we used the playback type ("quicker" / "slower") as dependent variable, and eight variables as independent variables (see list of variables in Table 2). The SPSS Statistics 17.0 program package was used for binary logistic analysis, selecting the method enter. This package was also used for calculating other statistical properties and parametric unpaired *t* test and non-parametric Kruskal–Wallis test. Principal component analysis (PCA) was used to analyse behavioural response variables of cuckoos to playback. PCA was started from the correlation matrix, and a component was retained if the corresponding eigenvalue was greater than 1.0. For ordination plots the first two components were used with no rotation on component loadings.

Fisher's exact tests were carried out by the "vassarstats" online calculator (<https://vassarstats.net/index.html>; accessed on December 22, 2020).

Results

Call rate, body size and condition

We measured the call rate in vocalisation series uttered by radio-tagged cuckoos (call rate (no. of calls/sec): mean \pm SD: 0.772 ± 0.084 , $n=23$). These values did not differ from values in a control data set on non-experimental and not radio-tagged birds (call rate (no. of calls/sec): mean \pm SD: 0.786 ± 0.109 , $n=18$; Mann–Whitney *U* test: $z=-0.775$,

Table 1 Parameter estimates of the generalised linear model with quasi-Poisson error term on call rate of male cuckoos (no. of calls/sec), where body size parameters (body mass (g), wing (mm), tarsus (mm), and tail length (mm)) as fixed factor, and year as covariate and the linear predictor were adjusted by the number of birds/year

Variables	Estimate	SE	<i>T</i>	<i>P</i>
Intercept	− 2.21	1.55	− 1.427	0.184
Tarsus length \times year 2017	0.113	0.077	1.473	0.172
Tarsus length \times year 2018	− 0.039	0.057	− 0.681	0.511
Tarsus length \times year 2019	0.011	0.068	0.156	0.879
Wing length \times year 2017	− 0.008	0.009	− 0.93	0.374
Wing length \times year 2018	0.002	0.009	0.27	0.792
Wing length \times year 2019	− 0.0000	0.010	− 0.009	0.993
Body mass \times year 2017	− 0.007	0.005	− 1.576	0.146
Body mass \times year 2018	− 0.005	0.008	− 0.664	0.522
Body mass \times year 2019	− 0.001	0.005	− 0.29	0.778
Tail length \times year 2017	− 0.002	0.011	− 0.148	0.885
Tail length \times year 2018	0.005	0.013	0.387	0.707
Tail length \times year 2019	0.001	0.008	0.097	0.925

$P=0.438$). Then, we tested if body size and condition metrics of cuckoos were associated with the call rate. Our generalised linear model revealed no such effects. Although cuckoo body mass, but not the other body size measures (wing, tail and tarsus length), showed a significant correlation with call rate in the full model, this effect was not stable and disappeared from the reduced models (Table 1, Table SM1, Supplementary Material).

Playback experiment

For the call rate experiment, we conducted 62 playbacks, including 17 trials with the quicker cuckoo call playbacks, 15 with normal rate, 16 of the slower call playbacks, and 14 Collared Dove controls. Male cuckoos responded to the playbacks of each of the conspecific male playback type in 94% and 100% of trials in the “slower” and “quicker” categories, respectively, and also in 100% in the “normal” category (Fisher’s exact test: $P=0.646$), but only in 14% of trials to the dove controls (all cuckoos vs. dove controls, $P<0.001$;

Fig. 2a). We detected a similar pattern for the “closest distance” response variable (Fig. 2b): cuckoos approached the speaker closely in both the quicker and slower playback types, and when also calling sequence with the “normal” call rate was played (Kruskal–Wallis test: $\chi^2_{17,15,16}=2.415$, $P=0.299$). Cuckoo responses to all cuckoo playback files (“quicker”, “normal” and “slower”) combined for closest distance significantly differed from cuckoo responses to control playbacks with dove calls (Mann–Whitney U test: $z_{48,14}=-4.940$, $P<0.001$).

We also compared the two focal call rate modulation treatments, the “quicker” and “slower” playbacks, in detail. We did not detect any significant effect among the independent variables related to the “quicker” and “slower” trials in the binary logistic model as it showed that cuckoos responded to the two playback files similarly (Table 2). A PCA on the response variables revealed that the first two components accounted for the 58% of the total variance (Table 3), and the response variables were separated well along the two axes (Fig. 3). However, the “quicker” and the

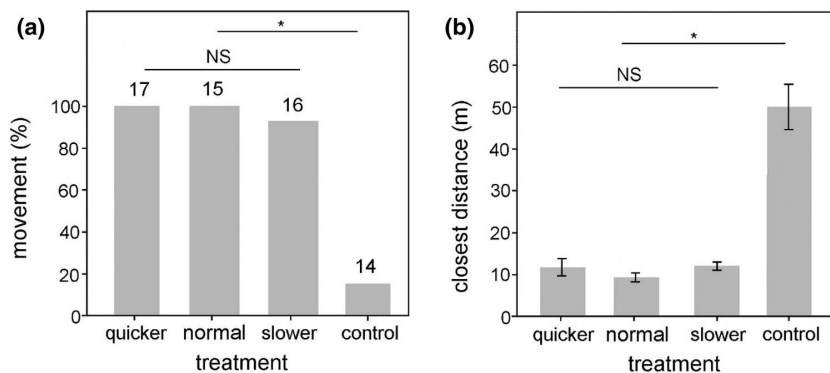


Fig. 2 **a** Percentage of playback experimental trials, where Common Cuckoos approached the speaker in response to four types of playbacks: “quicker”, “normal” or “slower” male Common Cuckoo male cu-coo calls and calls of Eurasian collared doves (“control”). Sample sizes are shown above the bars. **b** Closest distances of male

Common Cuckoos approaching the speakers during the four types of playbacks (as above). Significant differences, tested by unpaired t test, are shown by asterisk ($P<0.001$), or NS, when the test was not significant ($P>0.05$). Standard errors of the means (SE) are shown by the whiskers

Table 2 Parameter estimates of a binary logistic regression model of male Common Cuckoo responses to two types of experimental playback treatments of “cu-coo” call sequences of male Common Cuckoos, differing in call rates (“quicker” or “slower”)

Variables	<i>B</i>	SE	Wald	<i>Df</i>	<i>P</i>	Exp(<i>B</i>)
Distance to first detection (m)	− 0.010	0.035	0.076	1	0.783	0.990
Latency of first detection (s)	− 0.016	0.019	0.720	1	0.396	0.984
Closest distance (m)	0.033	0.056	0.356	1	0.551	1.034
Latency of closest detection (s)	− 0.002	0.007	0.084	1	0.772	0.998
Latency of calling (s)	0.001	0.009	0.007	1	0.931	1.001
Length of continuous calling (s)	0.007	0.006	1.373	1	0.241	1.007
Number of flights	− 0.169	0.293	0.335	1	0.563	0.844
Number of birds	0.952	0.518	3.378	1	0.066	2.592
Constant	− 1.217	2.173	0.313	1	0.576	0.296

Table 3 Component matrix of PCA on male Common Cuckoo responses to male Common Cuckoo calls (the general and most common type, the “cu-coo”), where call rate was manipulated (quicker in 17 cases, and slower in 16 cases)

Response variables	PC1	PC2	PC3
Latency of first detection (s)	0.844	0.103	0.117
Closest distance (m)	0.665	0.263	− 0.172
Latency of closest detection (s)	0.266	0.533	0.705
Latency of calling (s)	0.687	− 0.274	− 0.323
Length of continuous calling (s)	− 0.065	− 0.633	0.602
Number of flights	− 0.715	0.204	− 0.115
Number of birds	− 0.163	0.678	− 0.039
Eigenvalue	2.241	1.342	1.023
Variance explained	32.0%	19.2%	14.6%

The units of measurements of the variables are shown in parentheses

“slower” groups did not separate from each other in the PCA space when component scores were considered (independent sample *t* test, PCI: $t_{31}=0.495$, $P=0.624$; PCII: $t_{31}=− 0.462$, $P=0.647$).

Discussion

Our study did not reveal any statistical relationships between the calling rate of individually tagged male Common Cuckoos and their body size parameters (mass, tarsus, wing and tail lengths, or PCI). Although avian song rate and repertoire size can positively covary with and/or

signal individual quality (e.g., Nishida and Takagi 2018; reviewed in Catchpole and Slater 2008), these relationships do not hold in generality (e.g., Patel et al. 2010; Cardoso 2011; Mason and Burns 2015). Vocalisations of the cuckoos, and of many other brood parasitic species belonging to Cuculiformes, are simple and thought to be non-learned, i.e., inherited (Brenowitz 1991; Jarvis et al. 2000). However, call rate may be a vocal motor performance related trait, allowing a narrow range of acoustic variation to be affected by body condition. Van Hout et al. (2012) reported that in the European Starling *Sturnus vulgaris* it was not body condition but nutritional state that predicted vocalisation traits. Our anecdotal observations may support this idea in that during rainy, windy, and cold weather cuckoos at our study site preferred to stay in nearby forest, used for feeding or resting areas (Moskát et al. 2019), where they typically remained silent under these poor weather conditions (pers. obs.). Therefore, acute nutritional status may likely not have affected the direction and pattern of variations detected in our call measurements, as these calls had been recorded mostly under fair, sunny weather conditions. Future studies may reveal how diseases, for example bacterial, viral, or fungal infections, as well as any other health ailments, especially those affecting sound-producing organs (e.g., syringeal muscles), may modify cuckoos’ acoustic displays. Ecto-parasite load could be one of the most important factors, which is known to affect singing behaviour in diverse other bird species (e.g., Buchanan et al. 1999; Redpath et al. 2000), and can drive both visual and acoustic signalling (Garamszegi et al. 2004).

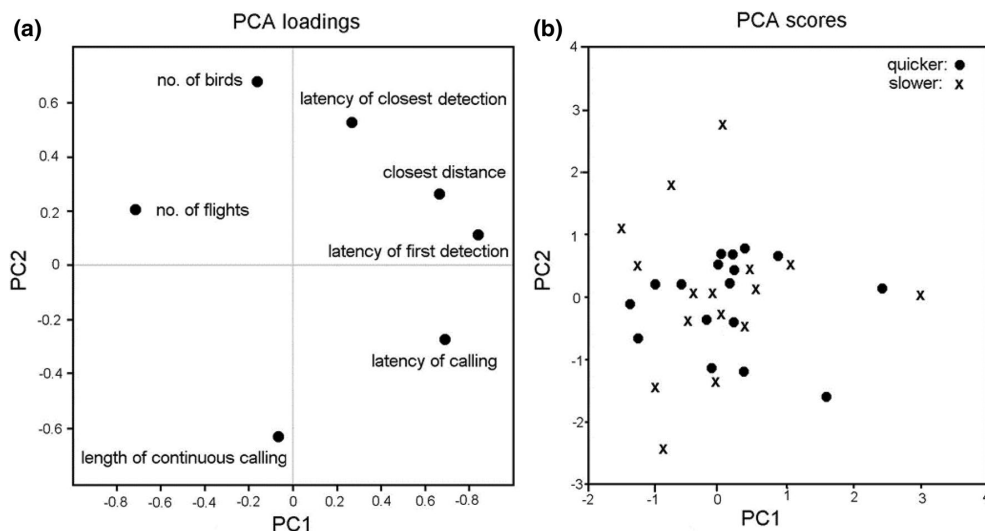


Fig. 3 PCA loadings and scatterplot of scores of 33 male Common Cuckoos responded to playbacks of male Common Cuckoos’ “cu-coo” calls, where the call rate was manipulated (increased, denoted as “quicker”, or decreased, denoted as “slower”)

Our experiment with manipulated call rates also revealed no difference in how territorial male cuckoos responded to quicker, normal, or slower sequences of calls. This reflects a high inherent intrasexual signalling value of the male cuckoos' characteristic cu-coo calls. It also suggests that, if any at all, signals other than acoustic displays may communicate body conditions better. The male cuckoos' swift flights within their territories, physical fighting abilities, and visual displays of their prominent tail spots are among the potential signalling cues which could express cuckoos' body condition and advertise it for nearby males as competitors and females as potential mating partners.

Previous studies revealed that Pale-winged Starlings *Onychognathus nabouroup* are able to modulate calling rate and use it for signalling on intending flight departure when foraging in pairs (Hausberger et al. 2020). Our results instead revealed that Common Cuckoos' calling rates seem to be stable at a time. We also showed that call rate was not correlated with any of the body size parameters. In the playback experiment with quicker, normal, and slower tempo of "cu-coo" calls we revealed no statistical difference in responses to simulated territorial intrusions by playback type. Although these results are straightforward, we are aware that our sample sizes are relatively small, in terms of individual subjects. Previous studies (Zsebők et al. 2017) from the same area revealed that the potential sample size for individual parasitic subjects is constrained due to the limited number of available host nests, thus any research can be restricted, regardless of the fact that this area consistently had some of the highest rates of host-parasitism by Common Cuckoos across its distribution (Moskát and Honza 2002). As cuckoos' vocal activity may change daily and seasonally during their breeding season (Deng et al. 2019b; Yoo et al. 2020), we suggest that further studies should collect longer calling sequences at the different reproductive stages, accordingly. Apart from call rate, rhythm patterns may also have intraspecific signalling content, as was found in the Corn-crake (*Crex crex*; Budka and Osiejuk 2013).

We conclude that male cuckoos do not acoustically advertise reliable information content of body size (probably linked to individual quality, condition and health state), and so the rate of cuckoo calls cannot be regarded as an honest signal of their body size and state or indicators of the cuckoo's species-specific environmental quality.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Local ethical regulations and agreements were followed for fieldwork. All field work complied with the Hungarian laws, and the Middle-Danube-Valley Inspectorate for Environmental Protection, Nature Conservation and Water Management, Budapest, provided permission for research (permit no. PE/KTF/17190-3/2015).

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References

- Benedetti Y, Slezak K, Møller AP, Morelli F, Tryjanowski P (2018) Number of syllables in cuckoo *Cuculus canorus* calls: A test using a citizen science project. *Sci Rep* 8:12872. <https://doi.org/10.1038/s41598-018-31329-1>
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MH, White JS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–135. <https://doi.org/10.1016/j.tree.2008.10.008>
- Bradbury JW, Vehrencamp SL (2011) Principles of animal communication, 2nd edn. Sinauer Associates Inc., Sunderland
- Brenowitz EA (1991) Evolution of the vocal control system in the avian brain. *Sem Neurosci* 3:339–407. [https://doi.org/10.1016/1044-5765\(91\)90030-R](https://doi.org/10.1016/1044-5765(91)90030-R)
- Brumm H (2009) Song amplitude and body size in birds. *Behav Ecol Sociobiol* 63:1157–1165. <https://doi.org/10.1007/s00265-009-0743-4>
- Buchanan KL, Catchpole CK, Lewis JW, Lodge A (1999) Song as an indicator of parasitism in the sedge warbler. *Anim Behav* 57:307–314. <https://doi.org/10.1006/anbe.1998.0969>
- Budka M, Osiejuk TS (2013) Formant frequencies are acoustic cues to caller discrimination and are a weak indicator of the body size of Corn-crake males. *Ethology* 119:960–969. <https://doi.org/10.1111/eth.12141>
- Cardoso GC (2011) Paradoxical calls: the opposite signaling role of sound frequency across bird species. *Behav Ecol* 23:237–241. <https://doi.org/10.1093/beheco/arr200>
- Carlson NV, Healy SD, Templeton CM (2017) A comparative study of how British tits encode predator threat in their mobbing calls.

- Anim Behav 125:77–92. <https://doi.org/10.1016/j.anbehav.2017.01.011>
- Catchpole CK, Slater PJB (2008) Bird song: biological themes and variations, 2nd edn. Cambridge University Press, Cambridge
- Chance EP (1940) The truth about the cuckoo. Country Life Ltd., London, UK
- Davies NB (2000) Cuckoos, cowbirds and other cheats. Poyser, London
- Deng Z, Lloyd H, Xia C, Møller AP, Liang W, Zhang Y (2019a) Components of variation in female common cuckoo calls. Behav Process 158:106–112. <https://doi.org/10.1016/j.beproc.2018.10.007>
- Deng Z, Lloyd H, Xia C, Li D, Zhang Y (2019b) Within-season decline in call consistency of individual male Common Cuckoos (*Cuculus canorus*). J Ornithol 160:317–327. <https://doi.org/10.1007/s10336-019-01631-4>
- Derryberry EP, Seddon N, Derryberry GA, Claramunt S, Seeholzer GF, Brumfield RB, Tobias JA (2018) Ecological drivers of song evolution in birds: disentangling the effects of habitat and morphology. Ecol Evol 8:1890–1905. <https://doi.org/10.1002/ece3.3760>
- Erritzøe J, Mann CF, Brammer FP, Fuller RA (2012) Cuckoos of the world. Christopher Helm Publishers Ltd, London, UK
- Galeotti P, Saino N, Sacchi R, Møller AP (1997) Song correlates with social context, testosterone and body condition in male barn swallows. Anim Behav 53:687–700. <https://doi.org/10.1006/anbe.1996.0304>
- Garamszegi LZ, Møller AP, Török J, Michl G, Péczely P, Richarde M (2004) Immune challenge mediates vocal communication in a passerine bird: an experiment. Behav Ecol 15:148–157. <https://doi.org/10.1093/beheco/arg108>
- Gould S (1975) Allometry in primates, with emphasis on scaling and evolution of the brain. Contrib Primatol 5:244–292
- Hall ML, Kingma SA, Peters A (2013) Male songbird indicates body size with low-pitched advertising songs. PLoS ONE 8:e56717. <https://doi.org/10.1371/journal.pone.0056717>
- Hausberger M, Giacalone A, Harmand M, Craig AJFK, Henry L (2020) Calling rhythm as a predictor of the outcome of vocal interactions: flight departure in pale-winged starling pairs. Sci Nat 107:2. <https://doi.org/10.1007/s00114-019-1658-1>
- Hurlbert SH (1984) Pseudoreplication and design of ecological field experiments. Ecol Monogr 54:187–211. <https://doi.org/10.2307/1942661>
- Jakob EM, Marshall SD, Uetz GW (1996) Estimating fitness: a comparison of body condition indices estimating fitness: a comparison of body condition indices. Oikos 77:61–67
- Jarvis ED, Ribeiro S, da Silva ML, Ventura D, Vielliard J, Mello CV (2000) Behaviourally driven gene expression reveals song nuclei in hummingbird brain. Nature 406:628–632. <https://doi.org/10.1038/35020570>
- Kroodsmas DE (1989) Suggested experimental designs for song playbacks. Anim Behav 37:600–609. [https://doi.org/10.1016/0003-3472\(89\)90039-0](https://doi.org/10.1016/0003-3472(89)90039-0)
- Kumar A (2003) Acoustic communication in birds. Resonance 8:44–55. <https://www.ias.ac.in/article/fulltext/reso/008/06/0044-0055>
- Makatsch W (1955) Der Brutparasitismus in der Vogelwelt. Neumann Verlag, Radebeul und Berlin
- Martin JP, Doucet SM, Knox RC, Mennill DJ (2011) Body size correlates negatively with the frequency of distress calls and songs of Neotropical birds. J Field Ornithol 83(2):59–268. <https://doi.org/10.1111/j.1557-9263.2011.00329.x>
- Mason NA, Burns KJ (2015) The effect of habitat and body size on the evolution of vocal displays in Thraupidae (tanagers), the largest family of songbirds. Biol J Linn Soc 114:538–551. <https://doi.org/10.1111/bij.12455>
- Maynard Smith J, Harper D (2003) Animal signals. Oxford University Press, Oxford, UK
- Mérő TO, Zuljević A, Varga K, Lengyel S (2015) Habitat use and nesting success of the Great Reed Warbler *Acrocephalus arundinaceus* in different reed habitats in Serbia. Wilson J Ornithol 127:477–485. <https://doi.org/10.1676/13-200.1>
- Møller AP, Morelli F, Mousseau TA, Tryjanowski P (2016) The number of syllables in Chernobyl cuckoo calls reliably indicate habitat, soil and radiation levels. Ecol Ind 66:592–597. <https://doi.org/10.1016/j.ecolind.2016.02.037>
- Møller AP, Morelli F, Tryjanowski P (2017) Cuckoo folklore and human well-being: cuckoo calls predict how long farmers live. Ecol Ind 72:766–768. <https://doi.org/10.1016/j.ecolind.2016.09.006>
- Morelli F, Jiguet F, Reif J, Plexida S, Valli AS, Indykiewicz P, Simova P, Tichit M, Moretti M, Tryjanowski P (2015) Cuckoo and biodiversity: testing the correlation between species occurrence and bird species richness in Europe. Biol Conserv 190:123–132. <https://doi.org/10.1016/j.biocon.2015.06.003>
- Moskát C, Hauber ME (2019) Sex-specific responses to simulated territorial intrusions in the common cuckoo: a dual function of female acoustic signaling. Behav Ecol Sociobiol 73:60. <https://doi.org/10.1007/s00265-019-2665-0>
- Moskát C, Honza M (2002) European cuckoo *Cuculus canorus* parasitism and host's rejection behaviour in a heavily parasitized Great Reed Warbler *Acrocephalus arundinaceus* population. Ibis 144:614–622. <https://doi.org/10.1046/j.1474-919X.2002.00085.x>
- Moskát C, Elek Z, Bán M, Geltsch N, Hauber ME (2017) Can Common Cuckoos discriminate between neighbours and strangers by their calls? Anim Behav 126:253–260. <https://doi.org/10.1016/j.anbehav.2017.02.013>
- Moskát C, Bán M, Fülöp A, Bereczki J, Hauber ME (2019) Bimodal habitat use in brood parasitic Common Cuckoos (*Cuculus canorus*) revealed by GPS telemetry. Auk 136:1–12. <https://doi.org/10.1093/auk/uky019>
- Moskát C, Hauber ME, Růžicková J, Marton A, Bán M, Elek Z (2020) Female-female aggression and male responses to the two colour morphs of female Common Cuckoos. Sci Nat 107:28. <https://doi.org/10.1007/s00114-020-01680-3>
- Murphy MT, Sexton K, Dolan AC, Redmond LJ (2008) Dawn song of the eastern kingbird: an honest signal of male quality? Anim Behav 75:1075–1084. <https://doi.org/10.1016/j.anbehav.2007.08.020>
- Nishida T, Takagi M (2018) Song performance is a condition-dependent dynamic trait honestly indicating the quality of paternal care in the Bull-headed Shrike. J Avian Biol 49:e01794. <https://doi.org/10.1111/jav.01794>
- Oberweger K, Goller F (2001) The metabolic cost of birdsong production. J Exp Biol 204:3379–3388
- Patel R, Mulder RA, Cardoso GC (2010) What makes vocalisation frequency an unreliable signal of body size in birds? A study on Black Swans. Ethology 116:554–563. <https://doi.org/10.1111/j.1439-0310.2010.01769.x>
- Payne RB (2005) The cuckoos. Oxford University Press, New York
- Podos J (1997) A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes: Emberizidae). Evolution 51:537–551. <https://doi.org/10.1111/j.1558-5646.1997.tb02441.x>
- R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>. Accessed 04 Sep 2019

- Redpath SM, Appleby MB, Petty SJ (2000) Do male hoots betray parasite loads in tawny owls? *J Avian Biol* 31:457–462. <https://doi.org/10.1034/j.1600-048X.2000.310404.x>
- Svensson L, Mullarney K, Zetterstrom D, Grant PJ (2010) *Collins Bird Guide*, 2nd edn. Harper Collins, London
- Tryjanowski P, Morelli F (2015) Presence of cuckoo reliably indicates high bird diversity: a case study in a farmland area. *Ecol Ind* 55:2–58. <https://doi.org/10.1016/j.ecolind.2015.03.012>
- Tryjanowski P, Morelli F, Osiejuk TS, Møller AP (2018) Functional significance of cuckoo *Cuculus canorus* calls: responses of conspecifics, hosts and non-hosts. *PeerJ* 6:e5302. <https://doi.org/10.7717/peerj.5302>
- Van Hout AJ-M, Pinxten R, Geens A, Eens M (2012) Non-breeding song rate reflects nutritional condition rather than body condition. *PLoS ONE* 7:e36547. <https://doi.org/10.1371/journal.pone.0036547>
- Weiss M, Kiefer S, Kipper S (2012) Buzzwords in females' ears? The use of buzz songs in the communication of Nightingales (*Luscinia megarhynchos*). *PLoS ONE* 7:e45057. <https://doi.org/10.1371/journal.pone.0045057>
- Wyllie I (1981) *The Cuckoo*. Batsford, London
- Xia C, Deng Z, Lloyd H, Møller AP, Zhao X, Zhang Y (2019) The function of three main call types in Common Cuckoo. *Ethology* 125:652–659. <https://doi.org/10.1111/eth.12918>
- Yoo S, Kim H-N, Lee J-W, Yoo J-C (2020) Seasonal and diurnal patterns of population vocal activity in avian brood parasites. *Ibis* 162:1001–1011. <https://doi.org/10.1111/ibi.12741>
- Yorzinski JL, Vehrencamp SL (2009) The effect of predator type and danger level on the mob calls of the American Crow. *Condor* 111:159–168. <https://doi.org/10.1525/cond.2009.080057>
- Zsebők S, Moskát C, Bán M (2017) Individually distinctive vocalization in Common Cuckoos (*Cuculus canorus*). *J Ornithol* 158:213–222. <https://doi.org/10.1007/s10336-016-1376-9>

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¹ The male common cuckoo call displayed on Fig. 2a was recorded by Dr. Csaba Moskát and uploaded to the xeno-canto.org webpage, where it can be accessed using the following link: <https://xeno-canto.org/380974>

² The female common cuckoo bubbling call displayed on Fig. 2b was recorded by Dr. Csaba Moskát and uploaded to the xeno-canto.org webpage, where it can be accessed using the following link: <https://xeno-canto.org/422426>

³ The call of the sparrowhawk displayed on Fig. 2c was recorded by Franz Lindinger and uploaded to the xeno-canto.org webpage, where it can be accessed using the following link: <https://xeno-canto.org/721201>

⁴ The song of the great reed warbler displayed on Fig. 3a was recorded by Ricardo Leoncini and uploaded to the xeno-canto.org webpage, where it can be accessed using the following link: <https://xeno-canto.org/473249>

⁵ The alarm call of the great reed warbler displayed on Fig. 3a was recorded by Attila Marton and uploaded to the xeno-canto.org webpage, where it can be accessed using the following link: <https://xeno-canto.org/732233>

⁶ A video published as a supplementary material to Marton et al. 2019 is uploaded to Attila Marton's personal YouTube channel and can be accessed using the following link: <https://youtu.be/gzgeHh3rcHY>

⁷ A video of the reaction of an exceptionally aggressive great reed warbler pair and another great reed warbler against a rufous cuckoo decoy used in the experimental setup is uploaded to Attila Marton's personal YouTube channel and can be accessed using the following link: <https://youtu.be/eleMr8b013Q>