Egg mimicry, not the sight of a common cuckoo, is the cue for parasitic egg rejection

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Abstract:

Many studies have found that if hosts observe a brood parasite at their nest, they use it as a cue to reject parasitic eggs. However, most previous work has simulated brood parasitic events by exposing a stuffed parasite near a host's nest. Responses to the presence of a real parasite have not yet been adequately studied under natural conditions. We therefore investigated whether great reed warblers (*Acrocephalus arundinaceus*) are more likely to reject a parasitic egg if they see a parasitizing common cuckoo (*Cuculus canorus*) at their nest than if the parasite approaches host nest unnoticed. Using video recordings of 70 nests we showed that spotting a cuckoo at the nest did not increase rejection rate of parasitic eggs, even if hosts saw the cuckoo repeatedly. Hosts instead used the level of mimicry in background colour for cuckoo egg rejection. Since not every visit by the cuckoo leads to parasitism, seeing the brood parasite may not be a reliable enough cue for the host. Therefore, our results suggest that the sight of a cuckoo at the nest may not have as severe consequences for it as previously thought.

Keywords: animal behaviour, brood parasitism, coevolution, egg rejection, mimicry, video recording

Introduction:

Coevolutionary interactions between species are considered key drivers of biological diversity through selection (Laine 2009; Yoder and Nuismer 2010). Such a relationship can be found for example in obligate brood parasitism, a remarkable reproductive strategy employed by some social insects (Brandt et al. 2005), fish (Sato 1986; Blažek et al. 2018) and approximately 1% of all bird species (Davies 2000; Mann 2017). Avian obligate brood parasites never build nests and have evolved deceptive adaptations to lay eggs into the nests of other bird species, their hosts (Davies 2000). Hosts are then obliged to care for a foreign parasitic young and consequently their reproductive success is lowered (Rothstein 1990; Davies 2000; Soler 2017). This exerts a selection pressure on them to evolve defensive counter-adaptations against brood parasitism of which one of the most notable is the ability to reject a parasitic egg (Davies and Brooke 1989; Rothstein 1990; Davies 2000; Soler 2017) with better mimetic parasite eggs being accepted more frequently than less mimetic eggs (Brooke and Davies 1988; Spottiswoode and Stevens 2010).

Another well-known example of brood parasitic adaptation is their rapid egg laying, which is traditionally viewed as an adaptation to escape aggressiveness, as some hosts can injure them (Gloag et al. 2013; Jelínek et al. 2021) or even cause their death (Šulc et al. 2020). Moreover, it has been suggested that parasitism is so swift because hosts may perceive the presence of a brood parasite at their nest as a cue that the nest has been parasitized and triggering the rejection response (Davies and Brooke 1988; Moksnes and Røskaft 1989; Moksnes et al. 1993; Moksnes et al. 2000; Bártol et al. 2002; Guigueno and Sealy 2011; Samaš et al. 2016). Studies that investigated this hypothesis in hosts of the brown-headed cowbird (*Molothrus ater*), the greater honeyguide (*Indicator indicator*), and the great spotted cuckoo (*Clamator glandarius*) have produced mixed results (Sealy 1995; Soler et al. 2000; Guigueno and Sealy 2011; Tong et al. 2015). On the other hand, hosts of the common cuckoo (hereafter cuckoo) significantly increased rejection rate of experimental eggs when exposed to a stuffed cuckoo near their nest (Davies and Brooke 1988; Moksnes and Røskaft 1989; Moksnes et al. 1993; Bártol et al. 2002). However in most published studies researchers used an experimental approach and presented either brood parasitic mounts or artificial eggs or both (Davies and Brooke 1988; Moksnes and Røskaft 1989; Moksnes et al. 1993; Sealy 1995; Lindholm 2000; Soler et al. 2000; Bártol et al. 2002; Guigueno and Sealy 2011; Tong et al. 2015; Strausberger and Hauber 2017; Tryjanowski et al. 2021), which might not initiate natural bird responses (Lahti 2015). Mounts are for example lacking the movement and vocalisation of a live bird (Guigueno and Sealy 2011). Even though some cuckoo hosts react to a stuffed and live cuckoo male in a similar manner (Tryjanowski et al. 2018), we know very little about how spotting a live brood parasitic female at their nest can influence responses towards her real parasitic egg. In aforementioned studies using brood parasitic mounts (Davies and Brooke 1988; Moksnes and Røskaft 1989; Moksnes et al. 1993; Sealy 1995; Lindholm 2000; Soler et al. 2000; Bártol et al. 2002; Guigueno and Sealy 2011; Tong et al. 2015; Strausberger and Hauber 2017; Tryjanowski et al. 2021) it was also never checked whether hosts encountered a live brood parasite at their nest prior to the experimental procedure, which could have possibly influenced their response. Moreover, previous studies often increased the presentation time of a parasitic mount to five minutes (Davies and Brooke 1988; Moksnes and Røskaft 1989; Moksnes et al. 1993; Lindholm 2000; Bártol et al. 2002; Tryjanowski et al. 2021) while real parasitism event usually lasts only few seconds depending on presence of the host but it is on average never longer than one minute (Moksnes et al. 2000; Gloag et al. 2013; Jelínek et al. 2021). Therefore, it remains an open question whether the findings of experimental studies will hold also under natural situations and show that spotting the parasite at the nest serves hosts as a cue for getting rid of the parasitic egg.

In only one previous study, the authors video recorded 13 reed warbler (*Acrocephalus scirpaceus*) nests during egg-laying and suggested that the sight of a cuckoo elicited rejection behaviour of this cuckoo host (Moksnes et al. 2000). However, as the video recording was not continuous, some cuckoo visits may have been missed. The authors also did not consider the effect of cuckoo egg mimicry. Since it has been shown that egg mimicry has a strong impact on host recognition and response (Honza and Cherry 2017; Samaš et al. 2021), we suggest that both the sight of the parasitizing cuckoo and the level of parasitic egg mimicry should be investigated simultaneously. Moreover, hosts seem to be able to react to brood parasites conditionally – they are able to alter their defence after repeated brood parasite encounters according to local parasitism risk (Davies et al. 1996; Øien et al. 1999; Čapek et al. 2010) and they are able to sum different stimuli – it was experimentally confirmed that if hosts saw a brood parasite at their nest and the parasitic egg was also less mimetic, they rejected it more often than if it had better mimicry (Moksnes et al. 1993; Bártol et al. 2002).

In the present study we used our continuous video recording dataset of the great reed warbler (hereafter GRW) nests to investigate the effect of spotting the cuckoo during the parasitism act on parasitic egg rejection. By analysing the appearance of cuckoo and host eggs we were also able to control for the effect of cuckoo egg mimicry and disentangle the influence of these two signals on host decision making under natural conditions. We predicted that catching the parasitizing cuckoo at the nest by hosts will increase the chance of the cuckoo egg being rejected and that less mimetic eggs will be rejected more often than better mimics. Since GRW hosts often reject parasitic eggs by abandoning their nest (Šulc et al. 2019), we presumed that spotting the cuckoo at the nest may trigger rejection by nest desertion even when the cuckoo egg is a good mimic (Šulc et al. 2019). Finally, as hosts seem to respond to brood parasites according to the frequency of their interactions with them (Davies et al. 1996; Øien et al. 1999; Čapek et al. 2010) we predicted that multiple host-parasite encounters would increase the likelihood of rejection of the parasitic egg.

Methods:

Data were collected from May to July in 2016-2020 (excluding 2017) in a fishpond area situated between Dubňany (48.9169383N, 17.0900417E) and Lužice (48.8409783N, 17.0710389E) in the Czech Republic. The average parasitism rate was 76.5 % during the years of the study (detailed information of parasitism rate during each year is available in Honza et al. 2022). The GRW population was mapped every year upon arrival and was colour ringed to ensure individual identification. Nests were usually found at the building stage and were checked daily until the last egg was laid. During incubation nests were checked less frequently (approximately every three days). At our study site cuckoos most often lay into host nests with one or two host eggs (Honza et al. 2020) and GRWs reject cuckoo eggs on average in two days (Šulc et al. 2019, our data from video recordings show they reject in 2.05 ± 1.74 days– details in Supplementary material). For a more detailed description of the fieldwork routine see (Honza et al. 2020).

Video recording of nests

We continuously video recorded available nests during the egg laying period. We usually found the nest at the building stage and then checked it in few days. When the GRW female started to line the nest and it was almost fully built, we placed a camera and started to record. The females then usually started to lay their eggs the next day or the day after and we continued recording until one day after clutch completion – recording usually lasted six or seven days. We used either cameras with an IR illumination (Carmedien STO-IR) connected to a miniature external video recorders (Mini DVR CH-HD0065) stored in a waterproof boxes or custom HD cameras without IR illumination with inbuilt recorders, both cameras did not record sound. All equipment was powered by 12V/100Ah gel batteries, and it was always properly camouflaged by reed and masked cloth to avoid nest desertion. The capacity of batteries was sufficient for about 7 days of continual recording, so in the majority of cases we did not need to change them during the entire recording period. For a detailed description of the video recording equipment and its installation see (Jelínek et al. 2021). If we recorded mobbing of cuckoo by GRWs we were sure they saw it. However, as these cameras only recorded a view within close vicinity of the nest (mostly < 0.5m), in cases in which we did not see GRW attacks, they could potentially see the cuckoo from a greater distance without having a direct encounter in camera's view. We therefore monitored the subset of nests in 2020 using cameras with a wider frame and with a smaller frame simultaneously. For this purpose, we used colour capture cameras with sound recording (Sony HDR-CX 240) powered by a power bank (GoGEN 20000 mAh) placed about 2 to 10 meters from the nest, depending on the density of the reeds. The power banks were changed every day during regular nest checks. These video recordings showed that if a cuckoo was spotted further from the nest, in most cases (13/14) GRWs immediately started to mob it and continued mobbing directly at the nest. We also additionally checked GRW behaviour directly after cuckoo encounters and in two cases where we did not record host presence during cuckoo parasitism but hosts returned to the nest very early after the cuckoo left the nest, and these hosts were apparently stressed (they had erected feathers on their head, showed nervous behaviour –

were jumping around and checking nest content multiple times), we additionally considered these as cases where the hosts spotted the cuckoo during parasitism (Jelínek et al. 2021).

Video recording analysis

To identify all events when a cuckoo appeared at the GRWs' nests, we watched the entire filmed footage. For each nest we recorded all types of events when the cuckoo approached the host nest, i.e., parasitism, egg predation, nest visit and successful defence of the GRWs, and whether at least one GRW parent was present during the event, because in GRWs only females are responsible for egg rejection (Požgayová et al. 2009). Unfortunately, when only one parent was present, it was not possible to distinguish whether the cuckoo was seen by a male or female host, as GRW is not a sexually dimorphic species, and the quality of the video recording was usually too low to identify parents by their colour rings. When the nest was parasitized, we classified host response as acceptance if the cuckoo egg remained in the nest until five days after the parasitism event, ejection when we observed it directly or when the cuckoo egg disappeared from the nest after the camera was removed, or desertion if the eggs were cold and no parental activity was observed around the nest.

Egg mimicry analysis

When a cuckoo egg was found in a GRW nest, it was photographed, and its background colour was immediately measured. After the last GRW egg was laid, all host eggs were then measured in the same manner. To estimate level of mimicry for each cuckoo egg, we calculated the similarity between the cuckoo egg and host eggs in background colour, spotting pattern and egg volume. Since there was usually more than one host egg in the clutch, we used the average of these characteristics calculated from all host eggs present in the nest at the time of host response.

Background colour was measured on 9 points on each egg using a spectrometer (Jaz spectrometer, Ocean Optics) in the 300–700 nm range to capture the full part of the spectrum perceived by the birds (Cuthill 2006). Measurements were relative to a white standard (WS-2, Ocean Optics) and to darkness. For subsequent analyses, the reflectance curve with the highest reflectance (lightest measured colour) was used because it best corresponds to the egg background colour (Šulc et al. 2019). To calculate colour differences between cuckoo egg and host eggs (mimicry) we used the *pavo* package (Maia et al. 2019) implemented in R (R Core Team 2020), using the bird vision model (Vorobyev and Osorio 1998). The model included information about the sensitivity of the bird's cones – using sensitivity of a blue tit (*Cyanistes caeruleus*) as GRW's closest relative with known values (Hart 2001; Hart and Vorobyev 2005), Weber fraction set to 0.05 and information about the ambient light conditions measured at open nests (Avilés 2008). The level of cuckoo egg mimicry in the background colour was expressed as chromatic contrast (ΔS) between the focal cuckoo egg and host eggs present in the nest and lower numbers of ΔS correspond to better mimicry.

Digital photographs were taken with a Canon Power Shot A3000 IS camera (in 2016) and a Canon EoS 700D camera with a Canon EF 40 mm lens (in 2018-2020) in RAW format to analyse spotting pattern and volume of eggs. To describe spotting pattern, we used granularity analysis implemented in the MICA toolbox (Troscianko and Stevens 2015; Berg et al. 2020) which is loosely based on our understanding of low-level neuro-physiological image processing in numerous vertebrates and invertebrates (Troscianko and Stevens 2015; Berg et al. 2020). The result of this analysis is a pattern energy curve that describes the intensity of spotting, and the dominant size of spots present on the eggshell. Pattern mimicry of a focal cuckoo egg was expressed as the difference between the pattern energy curve of the cuckoo egg and the average pattern energy curve calculated from the host eggs present in the nest (Šulc et al. 2019).

The volume of the eggs was calculated also using the MICA toolbox (Troscianko 2014; Troscianko and Stevens 2015; Berg et al. 2020). Volume mimicry was expressed as the absolute value of the difference between the volume of the cuckoo egg and the average volume of the host eggs present in the nest. More detailed information about measurements and analysis of used egg characteristics can be found in (Šulc et al. 2016; Šulc et al. 2019).

Host response dataset

In total, we video recorded 222 GRW nests (72, 64, 58 and 28 in 2016, 2018, 2019 and 2020). For all the subsequent analyses we however used only nests that met the following conditions: 1) the nest was parasitized, 2) the cuckoo and host eggs were photographed and the colour of eggs was measured by spectrometer so the cuckoo egg mimicry could be analysed, 3) there was only one cuckoo egg in the nest during host response (detailed explanation in Supplementary material), as multiple parasite eggs may affect the host response (Moskát et al. 2009; Manna et al. 2019; Šulc et al. 2019), 4) continuous video recording was taken throughout the whole host laying period, and 5) the nest was not deserted because of camera installation or predation before we recorded the host response towards cuckoo egg. After applying all these criteria, the final dataset contained 70 nests (28, 17, 14 and 11 nests in 2016, 2018, 2019 and 2020, Table 1).

Table 1: Number of nests where GRW rejected or accepted a parasitic egg in relation to sightings of a cuckoo at their nests

Host response	***Number of cuckoo encounters***		
	Never	***Once***	***Twice and more***
Acceptance	15	18	11
Rejection	6	15	5

Data analysis

We used generalised linear mixed models (GLMM) to investigate whether seeing the cuckoo female at the nest and cuckoo egg mimicry affect host rejection behaviour (binary; 0=acceptance, 1=rejection, Table 1). We checked the predictors for collinearity using Spearman correlation (all r_S were < 0.35) and contingency tables for categorical variables. We then tested whether hosts' response is affected by 1) the sight of a parasitising cuckoo at the host nest – (binary; 0=did not see the cuckoo, 1=saw the cuckoo); 2) how many times a cuckoo was seen parasitising at their nest (from 0 to 3). To both models we also added three mimicry characteristics: chromatic contrast (colour mimicry), volume mimicry, and pattern mimicry. We also included interactions between the variable of the sight of a cuckoo at the nest and mimicry characteristics, as it is possible that if GRWs catch a cuckoo at the nest, they might reject even a mimetic cuckoo egg. Since host response can vary between years and throughout the laying season, we included year (a categorical variable) laying date (a continuous variable) in the model. Mimicry characteristics and laying date were standardized using the *scale* function (by subtracting the mean and dividing by the standard deviation). Female identity (a categorical variable) was used as a random intercept as some females have been tested multiple times over the years and their responses may be consistent (Samaš et al. 2011) and, in GRWs, only females are responsible for egg rejection (Požgayová et al. 2009). We fit a number of candidate models that included interaction terms. However, the model with no interaction terms had the lowest Akaike information criterion value (corrected for small sample sizes) (Burnham and Anderson 2002) and thus we used a model without interactions as our final model. After that the final syntax of the models was as follows:

rejection behaviour ~ sight of a cuckoo at the nest + chromatic contrast + volume mimicry + pattern mimicry +laying date + year + (1|female ID).

Additionally, as GRWs might not be able to distinguish if the cuckoo female parasitized their nest or only visited it and therefore every sight of a cuckoo at the nest can be perceived as a threat by hosts, we created another two models operating with a *seen* variable considering not only spotting cuckoo during parasitism act but during any kind of event happening at the nest (i.e. visit, predation, or successful defence by GRWs). The rest of the tested variables remained the same as in previous two models. All data were analysed in R 3.6.1 (R Core Team 2020).

Results:

GRWs did not reject parasitic eggs more often if they saw a cuckoo on their nest (Table 2), nor did they reject more often when they caught a cuckoo on their nest more frequently (Table 3). These results remained the same in the additional models when we considered all host encounters with the cuckoo (results in Supplementary material, Table 5 and 6). In contrast, the results showed that the degree of mimicry of cuckoo eggs in the background colour has a significant effect on cuckoo egg rejection ($p = 0.02$). Cuckoo eggs that differed more in their background colour from the hosts' eggs were rejected more often than eggs that were more similar (Figure 1). The degree of egg pattern and volume mimicry did not affect rejection of GRWs.

Table 2: The effect of sight of a parasitizing cuckoo and egg mimicry characteristics on host response

Predictors	***Statistic***	***CI***	***p***
(Intercept)	-1.58	0.07 – 1.32	0.114
seen parasitizing [1]	0.64	0.43 – 5.23	0.520
pattern mimicry	0.05	0.55 – 1.88	0.963
volume mimicry	0.32	0.61 – 2.01	0.748
chromatic contrast	2.27	1.12 – 4.80	**0.023 ***
year [2018]	-0.07	0.17 – 5.09	0.945
year [2019]	0.55	0.31 – 8.11	0.581
year [2020]	1.01	0.41 – 15.84	0.312
laying date	-1.39	0.32 – 1.21	0.165

Table 3: The effect of multiple sightings of parasitizing cuckoos and egg mimicry characteristics on host response

Predictors	***Statistic***	***CI***	***p***
(Intercept)	-1.35	0.10 – 1.53	0.178
times seen parasitizing	0.17	0.49 – 2.32	0.867
pattern mimicry	0.05	0.55 – 1.88	0.960
volume mimicry	0.41	0.62 – 2.05	0.685
chromatic contrast	2.26	1.12 – 4.69	**0.024 ***
year [2018]	-0.10	0.16 – 5.22	0.923
year [2019]	0.54	0.31 – 8.09	0.586
year [2020]	0.95	0.39 – 14.99	0.343

laying date	-1.33	0.34 – 1.23	0.184

Figure 1: The effect of the degree of similarity of the cuckoo egg to the host eggs in the background colour (chromatic contrast - the greater the value, the lower the similarity) on the probability of rejection of the cuckoo egg by the host. The graph was generated based on the GLMM model (Table 2). Chromatic contrast is standardized (see Methods). The dark grey band shows standard errors.

Discussion:

In theory, it should be advantageous for hosts to understand the link between the sight of a laying brood parasite at their nest and the risk of parasitism. This understanding should then facilitate the decision-making process leading to a successful rejection of the parasitic egg. Hosts which are able to recognize parasitic egg in their clutch should eject it and even in cases when the parasitic egg is a good mimic, they can desert the whole clutch and subsequently renest (Šulc et al. 2019). However, contrary to these expectations, we found that rejection behaviour of our GRW hosts was not affected by this cue in natural situations.

Our finding is also in contrast with the results of most published studies investigating the relationship between the sight of brood parasite and rejection behaviour in cuckoo hosts (Davies and Brooke 1988; Moksnes and Røskaft 1989; Moksnes et al. 1993; Moksnes et al. 2000; Bártol et al. 2002). All but one (Moksnes et al. 2000) of these studies were experimental using mainly artificial eggs and stuffed parasite dummies. In most of these studies, the experimental period lasted at least 5 minutes (Davies and Brooke 1988; Moksnes and Røskaft 1989; Moksnes et al. 1993; Lindholm 2000; Bártol et al. 2002; Tryjanowski et al. 2021) in contrast with the real parasitism duration (Moksnes et al. 2000; Jelínek et al. 2021). Studies in which the brood parasite was removed immediately after the host response (Soler et al. 2000; Tong et al. 2015) or the timing of the exposure was similar to the natural cowbird parasitism events (Sealy 1995; Guigueno and Sealy 2011) might have simulated the real parasitic event better, and indeed, similarly to us three of these four studies did not find a relationship between the sight of a brood parasite at the nest and rejection of parasitic eggs (Sealy 1995; Soler et al. 2000; Tong et al. 2015). Moreover, the use of the stuffed brood parasite and researchers' presence because of the placement and retrieval of the mount and placement of an experimental egg in the nest may have led to different stress levels than those occurring during real parasitism, which may substantially affect host response to the parasitic egg (Abolins-Abols and Hauber 2018; Abolins-Abols and Hauber 2020).

When animals are exposed to a stressful situation, they change their behaviour (Cockrem and Silverin 2002; Tilgar et al. 2011) and their cognitive abilities may also be affected (Sapolsky et al. 2000). Therefore, it is also possible that repeated spotting of cuckoo females at the nest within a short time window (while stress hormone levels are still elevated) is required to induce rejection behaviour as corticosterone levels rise after a few minutes of exposure to a stressor (Romero and Reed 2005) and usually return to basal levels after about three hours (McCormick et al. 1998; Cockrem and Silverin 2002). However, GRWs also did not reject parasitic egg more often if they caught the cuckoo at their nest repeatedly during a particular nesting attempt.

One possibility of why the sight of a cuckoo on the nest is not a sufficient cue triggering the host rejection, is that hosts may not distinguish parasitism from non-parasitism events. Therefore, ejecting an egg or deserting the whole clutch immediately after seeing the brood parasite would not be beneficial and adaptive as brood parasites often only predate or visit host nests (Jelínek et al. 2021).

The results also hint that being caught at the nest does not have such negative consequences for cuckoos as previously thought. They very often lay in the presence of hosts, and cuckoo laying is therefore not highly secretive (Moksnes et al. 2000; Soler 2014; Jelínek et al. 2021). Moreover, female cuckoos that are not attacked by hosts spend longer time at the nest than would be necessary for a successful parasitism (Moksnes et al. 2000; Jelínek et al. 2021). Yet if spotting a brood parasite at hosts nest would increase the likelihood of the host rejecting their eggs, it would be most advantageous for cuckoos to leave as soon as possible. This suggests that by shortening the parasitism period, cuckoos are likely mainly trying to escape immediate host attacks, rather than reducing the chance of parasitic egg rejection because of being spotted (Jelínek et al. 2021).

The perception of a brood parasite at a nest and the subsequent response of hosts to a parasitic egg is likely to be a relatively complex behavioural act influenced by several factors. Therefore, in our study, we also controlled for the degree of mimicry of parasite eggs. We found that the mimicry signal is more important than the sight of a cuckoo at the nest in natural conditions when responding to a real cuckoo egg. GRWs rejected cuckoo eggs that were more different in background colour from their own more frequently. This is consistent with previously published work examining egg rejection in this host (Honza et al. 2011; Stoddard and Stevens 2011), but also in other host species (Honza and Cherry 2017; Samaš et al. 2021). However, we did not confirm that differences between cuckoo eggs and host eggs in pattern or size played a role in their rejection decision although these characteristics seem to play an important role in other species (Honza and Cherry 2017). It is possible that, in our studied population, cuckoo and host eggs are so similar in pattern and egg size that GRWs cannot use these cues during egg recognition.

We are aware we were not able to identify the sex of individual birds from the host pair in the video recordings (see Methods). If only the male saw the cuckoo female during parasitism, the GRW female who is responsible for egg rejection (Požgayová et al. 2009) may not have known about the cuckoo, which may have influenced her subsequent response to the parasite egg. However, our data from nests shot simultaneously by a camera with smaller and wider frame (see Methods), indicate that if we saw at least one parent in the small frame (N=6), there were always both GRW parents present. Thus, the warning calls of one parent alerts and attracts the other one and GRW female therefore most probably knew about cuckoo on her nest in a vast majority of cases.

Moreover, as we were not able record sound, we were also not able to assess the effect of cuckoo female bubbling call during parasitism on GRW behaviour as it was proposed by recent experimental studies as a part of a cuckoo trickery (Marton et al. 2021). Nevertheless, under natural conditions of parasitism, Honza et al. (2002) observed 26 events and Mikulica and Trnka (2022) observed 53 events of parasitism, all of which occurred without any vocalizations. Therefore, we believe that our results are not affected much by the absence of information on female cuckoo vocalizations because it is used during parasitism quite rarely.

To conclude, our work has shown that the sight of a cuckoo at the nest during natural parasitism does not affect the rate of cuckoo egg rejection by GRWs, and that the most important signal for GRWs is the colour mimicry between the host and parasitic egg. Our results suggest that the sight of a cuckoo at host nests does not have as severe consequences for it as previously presumed, we however stress the importance of future studies regarding this topic also in different brood parasite-host systems and on different localities. Furthermore, we did not find that repeated sightings of the cuckoo at the nest increased the chance of rejection of the parasite egg, suggesting that repeated encounters with the parasite either do not increase stress levels and/or that elevated stress levels do not elicit rejection behaviour in the GRW. Future studies should investigate the effect of spotting a brood parasite on host stress and explore whether previous experience with brood parasitism influences the use of this signal in the response towards parasite eggs.

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Data Availability Statement:

Analyses reported in this article can be reproduced using the data provided by Štětková et al. (2023).

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