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Egg rejection based on egg size recognition as a specific strategy against parasitic cuckoos

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Abstract

In the coevolutionary interactions between avian brood parasites and their hosts, egg recognition based on color and/or pattern is a common and effective defense to counter parasitism. However, for egg recognition based on size, only a few studies have found affirmative results, and they do not provide unambiguous evidence that egg size recognition in hosts has evolved as an important and specific anti-parasite adaptation against parasite eggs. We studied the brood parasite system between the Asian emerald cuckoo *Chrysococcyx maculatus* and its host, the chestnut-crowned warbler *Phylloscopus castaniceps*. The cuckoo parasitizes the warbler using non-mimetic and larger eggs at a parasitism rate of 12.9%. The warbler nests used in this experiment were built in a dark environment with the nest illuminance near 0 lux. Experiments with 2 types of model eggs with colors and patterns resembling cuckoo eggs of different sizes (cuckoo egg size or host egg size) showed that the warblers were able to reject 63.6% of cuckoo model eggs under these dim light conditions. However, model eggs with the same color and pattern similar to the warbler egg size were always accepted. This study provides strong evidence supporting the theory that egg size recognition can be evolved in hosts as a specific anti-parasite adaptation against cuckoos. We suggest that the egg size recognition of the warbler is an outcome of the tradeoff between the costs of violating the parental investment rule and suffering cuckoo parasitism.

Key words: egg discrimination, egg mimicry, nest illuminance, nest light condition, visual modeling.

Obligate avian brood parasites account for approximately 1% of bird species around the world. They do not build their own nests but, instead, lay their eggs in the nests of other species (hosts) (Davies 2000). Generally, these parasites impose a high reproductive cost on their hosts by transferring parental care and thus reduce the host's fitness. As a response, hosts have evolved defensive strategies to minimize the reproductive costs of parasitism (Rothstein 1990; Davies 2011). These strategies, in turn, have triggered counter-adaptations in the parasites, and this has resulted in a coevolutionary race between the hosts and their parasites (Davies and Brooke 1989; Soler 2014). Egg discrimination, which refers to recognizing and rejecting a parasite egg in a host nest, is one of the most effective and widespread anti-parasite strategies in hosts (Rothstein 1990; Soler 2014). As such, egg discrimination has been the focus of most previous studies of parasite–host systems.

Some of the most important aspects of egg discrimination are the host's reaction to parasitic eggs and the mechanism of egg recognition in hosts (Avilés et al. 2010). Many parasites have evolved a variety of mimetic eggs to counter egg recognition (Brooke and Davies 1988), and different host species exhibit variation in their ability to recognize parasitic eggs (Davies and Brooke 1989; Moskát and Honza 2002). Part of the reason for this variation is the difference in the host's ability to perceive the morphological contrast between parasite eggs and

their own eggs (Kilner 2006). The perception of color or/and pattern contrasts is important for hosts, and this ability has received the most attention in previous studies (e.g. Holveck et al. 2010; Honza and Cherry 2017). However, the ability to discriminate using other traits, such as egg size, has received less research attention. A total of 18 studies have tested egg discrimination based on egg size in 13 host species (Table 1), and half of these (9 of 18) documented egg size recognition. Furthermore, among these studies, 66.7% (6 out of 9) have involved host species that build semi-open or closed nests. For the 3 studies involving open nests, Segura et al. (2016) found that egg color was still the most important cue for egg recognition by the red-crested cardinal *Paroaria coronata*, a host of the shiny cowbird *Molothrus bonariensis*. Egg width was used as an extra cue only if the color of foreign eggs was too similar to be distinguished. Two other studies were conducted on hosts of the brown-headed cowbird *M. ater*, including the yellow warbler *Setophaga petechia* and American robin *Turdus migratorius*. However, both of these studies indicated that the egg color and size had mixed effects on egg recognition by hosts (Guigueno et al. 2014; Luro et al. 2018). In another 6 studies on hosts building semi-open or closed nests, unambiguous evidence for the evolution of egg size recognition as a specific anti-parasite adaptation is lacking. For example, Marchetti found that the host's rejection decision was

Table 1. Summary of previous studies that tested egg size recognition in hosts

Parasites	Hosts	Host nest types	Recognition by egg size	References
Common cuckoo (*Cuculus canorus*)	Oriental reed warbler *Acrocephalus orientalis*	Open	No	Li et al. (2020)
	Marsh warbler *Acrocephalus palustris*	Open	No	Antonov et al. (2006)
	Reed warbler *Acrocephalus scirpaceus*	Open	No	Stokke et al. (2010)
	Rufous bush chat *Cercotrichas galactotes*	Open	No	Alvarez (2000)
	Western Bonelli's warbler *Phylloscopus bonelli*	Semi-open	Yes	Roncalli et al. (2016)
Oriental cuckoo *Cuculus optatus*	Yellow-browed warbler *Phylloscopus inornatus*	Semi-open	Yes	Meshcheryagina et al. (2020)
Shiny cowbird *Molothrus bonariensis*	Rufous hornero *Furnarius rufus*	Closed	Yes	Mason and Rothstein (1986) Tosi-Germán et al. (2020)
	Red-crested cardinal *Paroaria coronata*	Open	Yes	Segura et al. (2016)
Shiny cowbird *Molothrus bonariensis*	Brown-and-yellow marshbird *Pseudoleistes virescens*	Open	No	Mermoz et al. (2013)
Brown-headed cowbird *Molothrus ater*	Yellow warbler *Setophaga petechia*	Open	Yes	Guigueno et al. (2014)
	American robin *Turdus migratorius*	Open	Yes No	Luro et al. (2018) Igic et al. (2015) Rothstein (1982) Carmody et al. (2016)
	Warbling vireo *Vireo gilvus*	Open	No	Underwood and Sealy (2006)
Unknown	Yellow-browed leaf warbler *Phylloscopus humei*	Semi-open	Yes	Marchetti (1992) Marchetti (2000)

based on the relative egg size of a clutch, although egg color contrast also influenced recognition (Marchetti 1992, 2000). Therefore, the hosts used the relative size of the eggs, rather than the cuckoo egg size, for recognition. This means that this strategy did not evolve specifically against cuckoo egg size. Nevertheless, no parasitic species have been found to use this host species, which limits our understanding of the adaptation of egg size recognition under the coevolutionary context of a brood parasite–host system. Meshcheryagina et al. (2020) used heterospecific real eggs with different colors/patterns to artificially parasitize Yellow-browed warblers *Phylloscopus inornatus*, a host of the Oriental cuckoo *Cuculus optatus*. They showed that egg width influenced egg recognition. Roncalli et al. (2016) studied an unparasitized population of the Western Bonelli's warbler *Phylloscopus bonelli*, a host of the common cuckoo *Cuculus canorus*, and found that egg size influenced the rejection types because larger eggs (but not smaller eggs) triggered desertion. However, the rejection frequency did not differ between the different egg sizes. The other 2 studies came from rufous hornero *Furnarius rufus*, a host of shiny cowbirds *M. bonariensis*. The results showed that both egg size and egg color/pattern influenced egg recognition. Egg width influenced egg recognition, while model eggs mimicking cowbird eggs were rejected more frequently than model eggs mimicking host eggs (Mason and Rothstein 1986; Tosi-Germán et al. 2020). In summary, these previous studies mostly focused

on cowbird–host systems. Cuckoo–host systems have received less attention, while both the egg size and egg color/pattern have mixed effects on egg recognition in most studies. The importance of egg size recognition as a specific anti-parasite adaptation in parasite–host systems is still unclear.

We studied egg size recognition by hosts in the coevolutionary system between the Asian emerald cuckoo *Chrysococcyx maculatus* and its host, the chestnut-crowned warbler *P. castaniceps*. To test egg size recognition in the warbler, we used model eggs that are different in size (host-sized and cuckoo-sized) but consistent in color and pattern (simulating cuckoo egg color and pattern) to artificially parasitize warbler nests. We determined whether the chestnut-crowed warbler has evolved egg size recognition as a specific anti-parasite adaptation against the larger eggs laid by Asian emerald cuckoos. We predicted that the warbler may primarily use egg size as a cue to reject cuckoo-sized model eggs but accept host-sized model eggs.

Materials and Methods

Study area and species

This study was conducted at the Kuankuoshui National Nature Reserve, Guizhou Province, southwestern China (28°10′N, 107°10′E). The field area is situated in a subtropical moist broadleaved and mixed forest at an altitude of about 1,500 m. The average annual temperature is 13.6 °C, and the

average annual total precipitation is 1,210 mm (Yang et al. 2019). According to our observations and previous literature, the chestnut-crowned warbler *P. castaniceps* is a small passerine bird that breeds mainly from May to July and builds side-entrance nests that are concealed by vegetation and a soil ridge (Shao et al. 2016; Figure 1). The light condition inside their nests is dim (0.019 ± 0.002 lux, *n* = 22), and the interior can only be observed under flashlight illumination. The average clutch size is 4 to 5 eggs (Shao et al. 2016). In the study area, the warbler is a common host of the small-sized parasite, the Asian emerald cuckoo *Chrysococcyx maculatus* (Yang et al. 2012). The warbler is parasitized by the cuckoos at a parasitism rate of 12.90% (*n* = 31).

Quantification of egg appearance

A spectrophotometer (Avantes-2048; Avantes, Apeldoorn, the Netherlands) was used to measure the reflectance of egg ground color and pattern. For each egg, the reflectance of ground color or pattern was scored by taking an average of 6 randomly selected measurement points (2 at the blunt end, 2 at the middle, and 2 at the sharp end of the egg) from the ground color or pattern, respectively. The measurement points were circular with a diameter of 1 mm, and the measured angle of the probe was 90 degrees. We calculated the chromatic just noticeable differences (JNDs) in Vorobyev–Osorio models (Vorobyev and Osorio 1998) with average spectral sensitivity curves for ultraviolet-visible-type avian retinas using the AVICOL program (Gomez 2006). JNDs are the units for the perceptual difference between 2 colors, and they increase as their discrimination increases (Vorobyev and Osorio 1998). According to Cassey et al. (2009), the chromatic contrast between 2 colors is undetectable by birds when JNDs < 1 and is difficult to distinguish even under favorable light conditions when JNDs < 3. Therefore, we consider color mimicry to be high if the JNDs < 3. For the egg pattern, we photographed the eggs on an 18% neutral gray card using a camera (Samsung NX1000, Gyeonggi, South Korea) with a vertical distance of 18 cm from the eggs to the camera lens and then quantified the egg markings using granularity analysis (Stoddard and Stevens 2010), in which the markings were calculated by the normalized energies of 7 spatial scales corresponding to 7 filter sizes (1, 2, 4, 8, 16, 32, and 64). The filters were octave-wide, isotropic band-pass filters that functioned like sieves that captured information at different spatial scales (differently sized markings). The filter sizes negatively correlate with the egg markings, and larger and smaller filter sizes refer to smaller and larger egg markings, respectively. For more details on the JNDs and granularity analyses, see Vorobyev et al. (1998) and Stoddard and Stevens (2010).

Figure 1. Coevolution between the Asian emerald cuckoo and its host, the chestnut-crowned warbler: photos of host nest, host egg, and cuckoo egg.

Investigation of light condition and egg mimicry under nest illumination

Firstly, we used an illuminometer (ST-80C; lux as unit; Photoelectric Instrument Factory of Beijing Normal University, China) to measure the light conditions of 3 positions: 1) nest cup light, or the illuminance in the host nest; 2) nest surrounding light, or the illuminance around the nest and covered by vegetation and soil; and 3) ambient light, or the illuminance near the nest outside the vegetation and soil. Secondly, we measured the nest light or ambient light conditions at the wavelength of 300–700 nm by a spectrophotometer. The light conditions were then included in the Vorobyev–Osorio models to assess the model egg mimicry on host eggs under these 2 light conditions by calculating the chromatic JNDs and normalized energy contrasts between model eggs and host eggs. Therefore, the measurements obtained by an illuminometer were used to present the illuminance of the 3 positions above, whereas the measurements obtained using a spectrophotometer were used for JNDs calculations. The latter provides a better understanding of how the mimicry changes from normal illuminance to the nest illuminance that is actually perceived by the hosts.

Parasitism experiment

To test the effect of egg size on egg recognition while controlling for egg mimicry, we used 2 sizes of model eggs whose color and pattern mimic the eggs of the Asian emerald cuckoo (see the Results section for the mimicry) to artificially parasitize the nests of the chestnut-crowned warbler. The model eggs were made of polymer clay, and the 2 sizes of model eggs (0.90 and 1.38 cm^3) referred to the natural egg sizes of warblers and cuckoos, respectively. The experimental treatment included 2 trials (one trial for each nest): 1) the host-sized model egg trial (n = 11) and 2) the cuckoo-sized trial (n = 11), in which the 2 model egg sizes corresponded to the egg sizes of the warbler and the cuckoo, respectively, but their color and pattern (painted with a marker pen) both simulated the cuckoo eggs. For the parasitism experiment, one model egg was placed in one host nest during the early incubation stage (i.e., within 3 days after the clutch completion) and was then monitored for 6 days at a standard frequency of 3 times (1, 3, and 6 days after placement). When the model egg was ejected, buried, or deserted within 6 days, it was considered as rejected by the hosts. On the contrary, if the model egg continued to be incubated after 6 days, it was regarded as accepted by the hosts. The warbler is a small-sized host with a small bill that could be a puncture-ejecter (rejecting parasite eggs by puncturing) rather than a grasp-ejecter (rejecting parasite eggs by grasping). However, a small host similar to the warbler would recognize a model egg and puncture it during rejection but would fail to eject it (Antonov et al. 2009) because the model egg is solid. Therefore, we checked the model egg for possible pecking marks during investigation and considered a model egg with heavy pecking marks as a case of rejection in the experiment. Therefore, these cases with pecking marks were counted as a rejection in the statistical analyses. A control trial (n = 11) followed the procedure of the 2 experimental trials above, except that the insertion of a model egg was used to control for the disturbance of manipulation. No occurrence of egg rejection or nest desertion was found in the control trial. To minimize the risk of pseudo-replication by repeated sampling of the same individual during the parasitism experiment, when 2 nests were found with a distance between them of less than 400 m, only one of the nests was used for the experiment. A breeding cycle refers to the period from the beginning of nest building to the end of nestling until it fledged. Generally, the territory size of *Phylloscopus* warblers is only about 1 ha with an average distance of roughly 150 m between territories (Arvidsson and Klaesson 1986; Herremans 1993). Therefore, avoiding the sampling of 2 nests that were less than 400 m from each other would avoid pseudo-replication.

Justification of model egg mimicry

The chromatic JNDs between cuckoo-sized and host-sized model eggs for both background and pattern colors were smaller than 1, and the normalized energies of pattern markings between cuckoo-sized and host-sized model eggs were similar (filter size 1: t = 0.215, P = 0.832; filter size 2: t = −0.530, P = 0.602; filter size 4: t = −0.527, P = −0.604; filter size 8: t = 0.228, P = 0.822; filter size 16: t = 0.395, P = 0.697; filter size 32: t = −1.729, P = 0.099; filter size 64: t = −1.623, P = 0.120, df = 20 for all, Student's t-test). This indicates that both the color and pattern were consistent between the cuckoo-sized and host-sized model eggs; thus, we used both of them to represent the total color and pattern of model eggs used in the experiment (i.e. regarding each sample for either cuckoo-sized or host-sized models as an independent sample). Then, we verified the model egg color and pattern by calculating the JNDs and the contrasts of normalized energies between model eggs and host eggs. The color and pattern of a model egg were quantified and compared with a host egg that was randomly selected from a nest that received the model egg for the experiment. We also calculated these values for the comparison between the cuckoo egg and the host egg. Because the cuckoo eggs were few and because avoiding pseudo-replication was necessary, we used the color and pattern of 2 cuckoo eggs to compare with each host egg. Our intent was to justify the use of model eggs as a substitute for cuckoo eggs. Two replicates of cuckoo eggs are consistent in their comparison with the model eggs. Firstly, the chromatic JNDs of background color between cuckoo eggs and model eggs were consistent between 2 replicates of cuckoo eggs (W = 99, P = 0.381, Wilcoxon signed-rank test). Secondly, the chromatic JNDs of pattern color between cuckoo eggs and model eggs were also consistent between 2 replicates of cuckoo eggs (W = 116, P = 0.745, Wilcoxon signed-rank test). Finally, the contrasts of normalized energies of pattern markings for all filter sizes between cuckoo eggs and model eggs were similar between 2 replicates of cuckoo eggs (filter size 1: t = −0.456, P = 0.653; filter size 2: t = −0.342; P = 0.736; filter size 4: t = −0.719; P = 0.480; filter size 8: t = 0.069; P = 0.946; filter size 16: t = −0.281; P = 0.782; filter size 32: t = −0.784; P = 0.442; filter size 64: t = −0.484; P = 0.633, df = 21 for all, paired samples t-test). The results of this justification showed that both the color and pattern of model eggs highly simulated the cuckoo eggs. Firstly, the chromatic JNDs of background color between cuckoo and model eggs were 1.78 ± 0.06 (mean ± standard error [SE]). Secondly, the chromatic JNDs of pattern color between cuckoo and model eggs were 1.86 ± 0.06 (mean ± SE) (Figure 2). Finally, the normalized energies of pattern markings were similar between the cuckoo and model eggs (filter size 1: t = −0.541, P = 0.591; filter size 2: t = −0.878, P = 0.385; filter size 4: t = 1.031, P = 0.348; filter size 8: t = 1.183, P = 0.243; filter size 16: t = 0.342, P = 0.734; filter size 32: t = −0.787, P = 0.435; filter size 64: t = 1.312, P = 0.197, df = 43 for all, Student's t-test; Figure 3). In contrast, the model eggs are non-mimetic to the host eggs in both color and pattern (Figures 2 and 3).
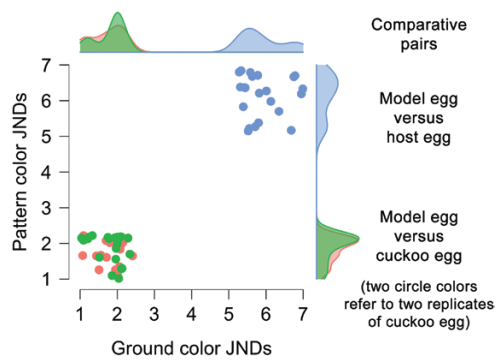
Figure 2. Scatter plots and density distribution of chromatic JNDs between eggs.
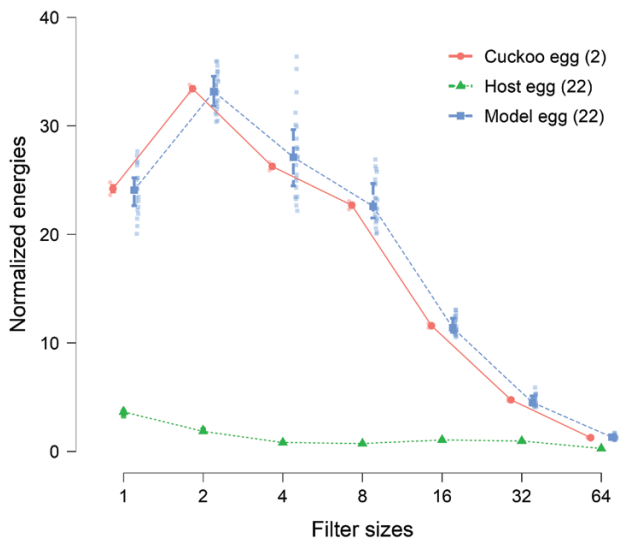

Figure 3. Normalized energies of egg pattern markings quantified by granularity analysis. Data are presented as scatter plots with mean and SE indicated.

Statistical analyses

Analysis of variance (ANOVA) was used to compare the illumination among the 3 light conditions (nest cup light, nest surrounding light, and ambient light). A paired samples t-test was used to compare the chromatic JNDs or normalized energy contrasts (between model eggs and host eggs) of egg pattern markings under ambient light with nest cup light. A generalized linear mixed model (GLMM) by Markov chain Monte Carlo (MCMC) technique was used to analyze the host reaction toward model eggs. The response variable was the host reaction toward model eggs (coded as a binomial variable, with 1 = rejection and 0 = acceptance); the fixed effects included treatment (host-sized model egg trial or cuckoo-sized model egg trial), clutch size, and egg-laying date, while the nest identity was the random effect. The ANOVA and paired samples t-test were performed using JASP (version 0.15) for Windows (University of Amsterdam, the Netherlands), and GLMM was performed by using the *MCMCglmm* package in R (Version 4.1.0) for Windows (https://www.r-project.org/). All tests were 2-tailed, data were presented as mean ± SE, and the significance level was $P = 0.05$.

Results

The chestnut-crowned warbler is parasitized by the Asian emerald cuckoo at a parasitism rate of 12.90% (4 out of

31 host nests during the 2010–2013 study). The warbler lays a small unmarked white egg (mean ± SE in size: $0.901 ± 0.008$ cm^3, $n = 22$), while the cuckoo lays a larger beige-colored egg with brown markings (mean ± SE in size: $1.375 ± 0.035$ cm^3, $n = 2$) (Figure 1). One out of 4 parasitism cases was rejected by the warbler, with a rejection rate of 25%. No other cuckoo species was recorded as a parasite. The light conditions in warbler nests were dim and near 0 lux ($0.019 ± 0.002$ lux, $n = 22$), which may be responsible for the low parasitism rate (Muñoz et al. 2007). The illuminance was found to be significantly different among the 3 light conditions. The illuminance of ambient light, nest surrounding light, and nest cup light was $3,190.909 ± 197.595$ lux ($n = 22$), $2.296 ± 0.172$ lux ($n = 22$), and $0.019 ± 0.002$ lux ($n = 22$), respectively ($F = 6,129.28$, df = 2, $P < 0.001$, ANOVA; Figure 4). The chromatic JNDs of background and pattern colors between model eggs and host eggs were $5.918 ± 0.122$ JND and $6.136 ± 0.125$ JND, respectively, under ambient light but dramatically decreased to $1.992 ± 0.041$ JND ($t = 48.298$, df = 21, $P < 0.001$, paired samples t-test) and $2.066 ± 0.042$ JND ($t = 49.329$, df = 21, $P < 0.001$, paired samples t-test), respectively, under nest cup light (Figure 5A). For the pattern markings, the normalized energies of all filter sizes significantly decreased from ambient light to nest cup light ($P < 0.001$ for all filter sizes, paired samples t-test; Figure 5B; Table 2). However, although the egg color and pattern were undetectable under nest illuminance, the parasitism experiment indicated that the warblers rejected 63.6% of cuckoo-sized model eggs ($n = 11$) but accepted all host-sized model eggs ($n = 11$). The rejection rate of cuckoo-sized model eggs did not differ in regard to real cuckoo eggs ($\chi^2 = 1.759$, $P = 0.282$, Fisher's exact test). For the 7 cases of rejection of cuckoo-sized model eggs, pecking marks were detected in 5 cases (71.4%), while another 2 cases involved desertion. For the 5 cases with pecking marks, 3 were deserted and 2 maintained incubation after 6 days. This result indicates that the warbler is a puncture ejector and may thus be unable to reject model eggs by ejection. Neither desertion nor pecking marks were found in host-sized model trials. The result of the GLMM also indicates that only the treatment significantly predicted the host reaction toward model eggs (P-MCMC < 0.001, GLMM; Table 3).

Discussion

In this study, our aim was to investigate the role of egg size recognition in parasite–host systems. As we predicted, the result indicated that the chestnut-crowned warbler rejects cuckoo-sized model eggs but accepts host-sized model eggs. This demonstrates that the warbler uses egg size as a cue for discrimination. Although a number of previous studies have investigated the impact of nest light conditions on egg discrimination, their focus has been on egg color recognition rather than egg size recognition (Antonov et al. 2011; Honza et al. 2011; Avilés et al. 2015; Medina and Langmore 2019; Rutledge et al. 2021). Although several studies found that egg size was linked to egg rejection, they were unable to convincingly demonstrate that egg size recognition was used as a specific adaptation against brood parasites. For example, these studies either found mixed effects due to egg size and egg color/pattern on egg recognition (Mason and Rothstein 1986; Tosi-Germán et al. 2020), failed to confirm the host's coevolutionary contact with any parasites (Marchetti 1992, 2000), or illustrated that egg size

influenced the types of rejection but not the egg recognition itself (Roncalli et al. 2016). In this study, the chestnut-crowned warbler, a host of the Asian emerald cuckoo, accepted foreign eggs of their own egg size but rejected those of cuckoo egg size. This indicates that warbler hosts are sensitive to cuckoo egg size and have evolved recognition based on egg size as a specific defense to parasitism during their coevolution with the cuckoo.

It is worth noting that recognition based on egg size has rarely been found in hosts, even considering the positive result of this study. It is obvious that recognition based on egg color and pattern rather than egg size plays an important role in the anti-parasitism defenses of many hosts. Why did egg size recognition evolve in so few cases during the coevolution between brood parasites and hosts? In our opinion, there are 2 mutually non-exclusive explanations for this phenomenon. Firstly, the egg size of parasites is generally larger than that of the hosts, but rejecting a larger egg is maladaptive because it contradicts the law of

parental investment theory. According to this theory, parental birds prefer larger eggs (Tinbergen 1951; Vidya 2018) and feed larger offspring (Soler et al. 1995; Bortolato et al. 2019) because they have a higher survival rate (Perrins 1965; Trivers 1972). Secondly, color/pattern is an easy-to-detect visual signal, and it is reasonable to expect color/pattern recognition to be a dominant anti-parasite recognition mechanism that develops during evolution. However, despite these factors, the chestnut-crowned warbler evolved egg size recognition to reject larger eggs. Correspondingly, we propose that there may be 2 necessary conditions to explain this special case: 1) the parasitism pressure must be high enough and 2) the nest must be dark enough. These 2 conditions may act together to mutually promote the evolution of egg size recognition in the warbler. In other words, the outcome may be a result of the tradeoff between the costs of violating parental investment theory and suffering parasitism in low light conditions. We considered the warbler to have been under high parasitism pressure in the past, which led to its sensitivity to the size of cuckoo eggs. The low parasitism rate in warblers in the present should not be used to evaluate the level of interaction between warblers and cuckoos because it does not reflect the situation in the past during coevolution. For example, a high level of coevolution has been proposed between the ashy-throated parrotbill *Sinosuthora alphonsiana* and common cuckoo *C. canorus* because both species have evolved polymorphic eggs as an anti-parasitism adaptation and counter-adaptation, respectively (Yang et al. 2010, 2015). However, the parasitism rate, in that case, was only 4.3%, which was less than that identified in this study (12.9%). For the nest light conditions, although the nests built by the warbler are side-entranced, they cannot be seen from the outside because they are concealed by vegetation and soil, with the entrances facing the soil wall (Figure 1). This makes the light condition in nests extremely low and near 0 lux. In such special nest location environments, we consider the warbler nests to be a type of closed nest rather than a semi-open nest. We hypothesized that egg color and pattern are not used as recognition cues by the warblers because the
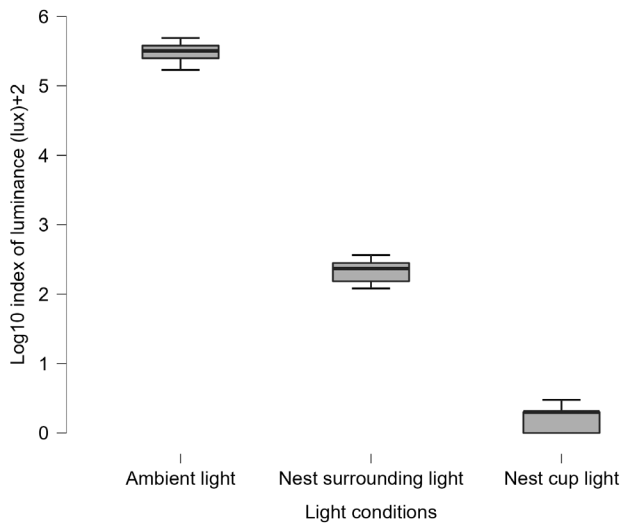

Figure 4. Illuminance boxplots (median, quantiles, and 5th and 95th percentiles) of 3 positions for the nest of the chestnut-crowned warbler.
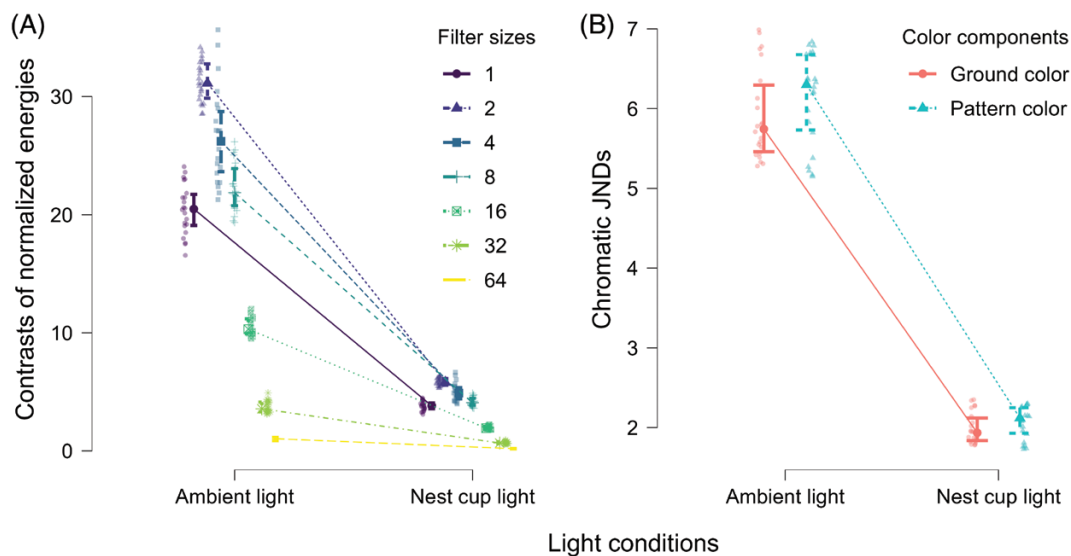

Figure 5. Contrasts of egg pattern (normalized energies; A) and color (chromatic JNDs; B) between parasite and host eggs under ambient light and nest cup light conditions. Data are presented as scatter plots with mean and SE.

Table 2. Contrasts of normalized energies of pattern markings between model eggs and host eggs and comparisons using the paired samples *t*-test

Filter sizes	Ambient light	Nest cup light	t	df	P
1	20.441 ± 0.453	3.813 ± 0.084	45.066	21	< 0.001***
2	31.265 ± 0.367	5.833 ± 0.069	85.245	21	< 0.001***
4	26.641 ± 0.847	4.970 ± 0.158	31.457	21	< 0.001***
8	22.317 ± 0.437	4.163 ± 0.082	51.015	21	< 0.001***
16	10.566 ± 0.172	1.972 ± 0.032	61.387	21	< 0.001***
32	3.730 ± 0.106	0.697 ± 0.020	35.215	21	< 0.001***
64	1.040 ± 0.043	0.194 ± 0.008	24.127	21	< 0.001***

***$P < 0.001$.

Table 3. The result of GLMM for the parasitism experiment

	Posterior mean	Lower 95% CI	Upper 95% CI	P-MCMC
Intercept	–0.8914	–2.669378	0.76754	0.298ns
Treatment	0.647927	0.334627	0.969278	< 0.001***
Clutch size	0.066214	–0.227678	0.467632	0.716ns
Egg-laying date	–0.002968	–0.013429	0.006566	0.544ns

ns$P \geq 0.05$; ***$P < 0.001$. CI: confident interval.

light in their nests is too dim for color or pattern detection. Despite that the cuckoo/model eggs are highly non-mimetic to warbler eggs under ambient light conditions (Figures 2 and 3), both the color and pattern contrasts between them dramatically decrease to a level that may be below the level detectable by warblers under nest light conditions (Figures 4 and 5). Therefore, together with the results of some previous studies, our results suggest that a dim light condition may act as a precondition favoring cuckoo hosts to evolve recognition based on egg size. An extremely dim light condition may block the light-dependent perception of egg color and pattern, which allows the natural selection from parasitic pressure to focus on detectable traits such as egg size. Correspondingly, for those parasites that utilize hosts nesting in conditions with very dim light, laying mimetic eggs is unnecessary because natural selection for egg mimicry is lacking. This explains why the chestnut-crowed warbler is sensitive to the egg size of the Asian emerald cuckoo, and why the cuckoo lays non-mimetic eggs for parasitism. Although the possibility that the non-mimetic cuckoo eggs are an adaptation to other hosts cannot be completely excluded, it is an unlikely explanation because the evolution of egg size recognition in warblers would violate the key rule of parental investment theory (see the discussion above). Additionally, it is worth mentioning that 2 of our researchers (Y.C. and C.Y.) witnessed a female Asian emerald cuckoo hastily targeting a chestnut-crowned warbler nest for parasitism. In this observation, the female cuckoo appeared to be searching for something in the warbler nest habitat. It was observed that she eventually settled in one location and remained there for a period of time after searching. After the cuckoo left, we examined the location where the cuckoo remained and found that it was a new warbler nest. The female cuckoo did not lay an egg in the nest because it was a newly completed nest without host eggs. Considering that the warbler nest is built in a special

habitat (Figure 1) and no other cuckoo host species were found to build nests in such habitat in the study area, the specific behavior of the female Asian emerald cuckoo in relation to the chestnut-crowned warbler nest may also provide evidence that the warbler is a regular host of this cuckoo species.

Overall, this study suggests that the warbler has evolved egg size recognition as a key and specific anti-parasite adaptation to counter parasitism from the Asian emerald cuckoo. Although the sample size of this study was relatively small because we limited the sampling to avoid pseudo-replication, the results establish that, under very dim light nest illuminance, the warbler is able to distinguish foreign eggs of cuckoo size from eggs of their own size and rejects the cuckoo-sized eggs. This study, therefore, provides rare and unambiguous evidence that a cuckoo host can evolve egg size recognition as a specific anti-parasite adaptation against cuckoos. It also illustrates the important role of egg size recognition in host defenses and helps us to understand the evolution of host egg recognition under dim light conditions.

Authors' Contributions

C.Y. designed the study; P.Y., Y.C., and C.Y. performed field experiments; C.Y. carried out laboratory and statistical analyses. P.Y. wrote the draft manuscript, and C.Y. improved the manuscript. N.W., X.Y., G.L., and W.L. helped assist in the fieldwork. All authors approved the final submission.

Ethical Note

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Conflict of Interest Statement

The authors declare that they have no competing interests.

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